Analytical Method Validation For The Determination Of Fumaric Acid Content In Quetiapine Hemi Fumarate By RP-HPLC

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ABSTRACT

A new, simple, rapid, selective, precise and accurate isocratic reverse phase high performance liquid Chromatography assay method has been developed for estimation of Fumaric acid in Quetiapine hemi fumarate drug substance. The separation was achieved by using column Hypersil C18 (250×4.6mm, 5µm), in mobile phase consisted of acetonitrile and pH 3.0 phosphate buffer, adjusted to pH 3.0 with the help of dilute orthophosphoric acid in the gradient elution. The flow rate was 1.0 mL/min^{-1} and the separated Fumaric acid was detected using UV detector at the wavelength of 210 nm. Column temperature 25°C and sample temperature ambient and injection volume 20µl. The retention time of Fumaric acid, was noted to be 3.65 min respectively, indicative of rather shorter analysis time. The method was validated as per ICH guidelines. The proposed method was found to be accurate, reproducible, and consistent.

Keywords: Liquid Chromatography, Fumaric acid and, Quetiapine hemi fumarate and Validation

INTRODUCTION

Quetiapine fumarate chemically known as {2-(2-(4-dibenzo [1, 4] thiazepine-11-yl-1-piperazinyl) ethoxyethanol, fumaric acid, molecular formula $C_{29}H_{33}N_3O_{10}S$, molecular weight: 615.66 dibenzothiazepine derivative, is one of the most recent antipsychotic drugs. An oral antipsychotic drug that acts as an antagonist of multiple neurotransmitters including serotonin and nor epinephrine is used in the treatment of schizophrenia. It is a selective monoaminergic antagonist with high affinity for the serotonin type 2 (5HT2) and dopamine type 2 (D2) receptors. QTF belongs to the same family as clozapine and olanzapine, which are classified as a typical antipsychotic and do not cause major extra pyramidal side effects. The generic name of quetiapine hemifumarate is Seroquel; it is prescribed for the treatment of schizophrenia, a mental disorder marked by delusions (false beliefs), hallucinations, disrupted thinking, and loss of contact with reality. It is also used for the short-term treatment of mania associated with bipolar disorder. Seroquel is the first in a new class of antipsychotic medications. Researchers believe that it works by diminishing the action of dopamine and serotonin, two of the brain's chief chemical messengers. It is white or almost white powder, moderately soluble in water and soluble in methanol and 0.1 N HCl. It is available in tablets form in dosage level of 25 mg, 50 mg, 100 mg, 200 mg, 300 mg, and 400 mg. Maximum daily dosage is 800 mg in adults. This drug is rapidly absorbed after oral administration with peak plasma concentration attained within 1.50 hrs. Bioavailability of tablet formulation is 100% relative to an oral solution, which may be marginally affected by food. Plasma protein binding of quetiapine is 83%. The drug is extensively metabolites, principally through CYP3A4. The drug is having half-life period of approximately 6 hours. Several methods have been reported for the quantitative determination of quetiapine in bulk, and pharmaceutical and biological samples. These methods include UV-Visible Spectrophotometric [1–3], UV-derivative and extraction-free methods [4], HPTLC [5], Capillary zone electrophoretic method [6], HPLC-UV-detector [7–11], HPLC with solid phase extraction [12], UPLC with mass-spectrophotometer detector [13], HPLC with column switching method [14], gas-chromatography-liquid chromatography-mass spectrophotometry [15], and hyphenated techniques such as LC-MS [16], HPLC-electrospray mass ionization mass spectrometry [17], HPLC-Tandem-mass spectrometry [18], and HPLCMS- MS method [19].

Literature survey revealed that only few internal standard methods have been reported for the quantification of quetiapine fumarate in bulk and pharmaceutical formulations. The present work describes a simple, stability indicating HPLC method for the determination of Fumaric acid in Quetiapine hemi fumarate in bulk and tablet dosage form according to ICH guidelines.
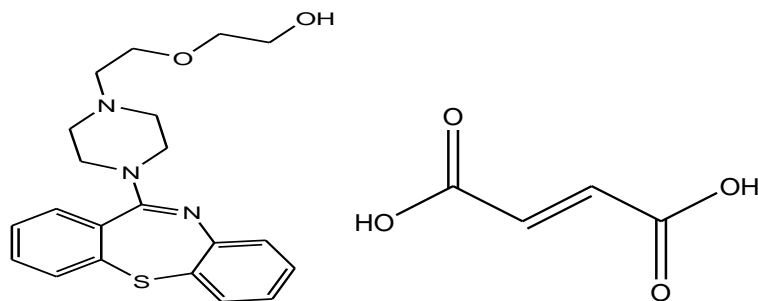
Figure 1: Quetiapine Hemifumarate structure

MATERIALS AND METHOD

Chemicals and Reagents

Analytical-grade potassium dihydrogen orthophosphate, orthophosphoric acid, Fumaric acid , were from Merck Chemicals Mumbai, India. Acetonitrile and Water, both HPLC-grades, were from Merck Chemicals. Mumbai, India.

Instrumentation

Agilent 1200 series, open lab software, Bandelin ultrasonic bath, pH Meter (Thermo Orion Model), Analytical Balance (Metller Toledo Model).

Preparation phosphate Buffer pH 3.0:

Accurately measured quantity of 3.4 gm of potassium dihydrogen orthophosphate anhydrous in 1000 ml of HPLC grade water and pH was adjusted to 3.0 with dilute orthophosphoric acid and degassed. The solution was filtered through 0.45μ filter paper and degassed.

Mobile phase preparation

The mobile phase consisted of pH 3.0 phosphate buffer and Acetonitrile in the gradient mode.

Diluent preparation

Mixed pH 3.0 Phosphate buffer and Acetonitrile in the ratio of (95:5 %v/v).

Standard preparation:

Weighed accurately and transferred about 40mg of Fumaric acid working standard into a 100ml volumetric flask and dissolved in 50ml diluent by sonicating 10 min. and was made up to the volume with diluent. Further transfer 5ml of this solution into a 50ml and was made up to the volume with diluent.

Sample preparation:

Weighed accurately and transferred about 30mg of test sample into a 100ml volumetric flask and dissolved in 50ml diluent by sonicating 10 min. and was made up to the volume with diluent.

Chromatographic conditions

Chromatographic analysis was performed on Hypersil C18 (250×4.6mm, 5µm) (Make: Thermo) column. The mobile phase consisted of pH 3.0 phosphate buffer and Acetonitrile in the gradient mode. The flow rate was 1.0 mL/min, column oven temperature 25°C, the injection volume was 20µL, and detection was performed at 210 nm using a photodiode array detector (PDA).

RESULTS AND DISCUSSION

Method development

Spectroscopic analysis of compound Fumaric acid showed that maximum UV absorbance (λmax) at 210 nm respectively. To develop a suitable and robust LC method for the determination of Fumaric acid content in Quetiapine hemi fumarate, different mobile phases were employed to achieve the best separation and resolution. The method development was started with Agilent Zorbax AQ C18 with the following different mobile phase compositions like that Buffer and acetonitrile in the ratio of 40:60 v/v 50:50 v/v & 55:45. It was observed that when fumaric acid was injected, Peak Tailing, not satisfactory.

For next trial Hypersil C18 (250×4.6mm, 5µm) column used and the mobile phase composition were changed slightly. The mobile phase composition was buffer and acetonitrile in the gradient mode. respectively as eluent at flow rate 1.0 mL/min. UV detection was performed at 210nm. The retention time of fumaric acid is 3.65 minutes and the peak shape was good. The chromatogram of fumaric acid standard using the proposed method is shown in (Fig: 2) system suitability results of the method are presented in Table 1.

Chromatogram

Peak Results

VWD: Signal A,
210 nm Results

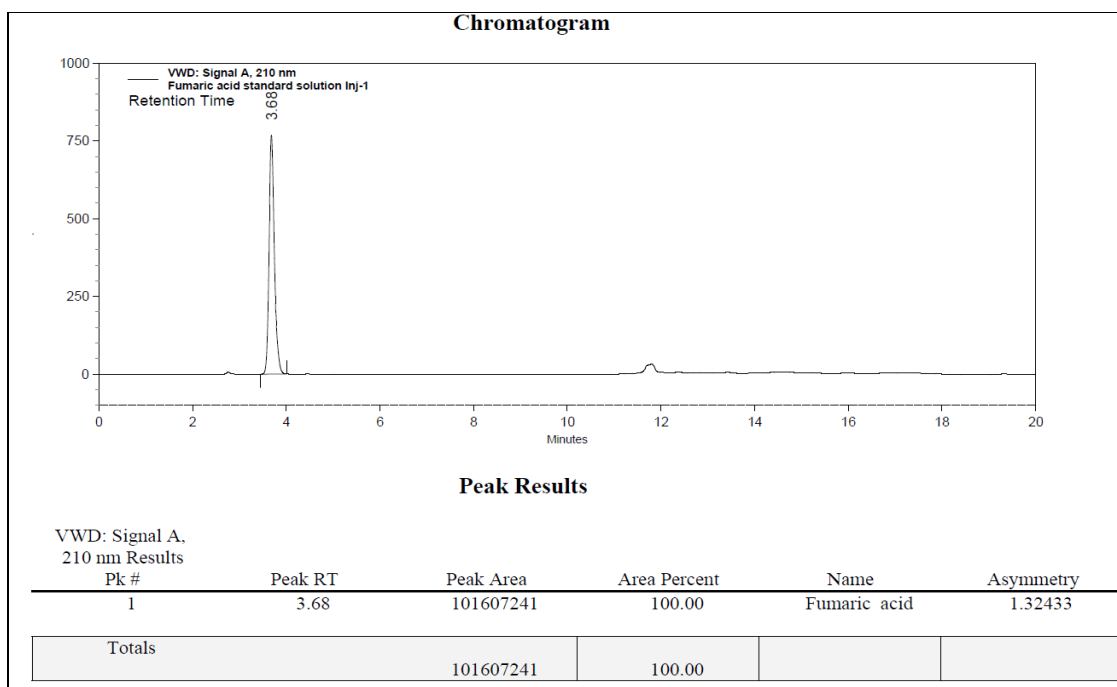| Pk #	Peak RT	Peak Area	Area Percent	Name	Asymmetry
1	3.68	101607241	100.00	Fumaric acid	1.32433
Totals		101607241	100.00		

Figure 2: Chromatogram showing the peak of Fumaric acid

Method validation

The developed RP-LC method extensively validated for Fumaric acid content in Quetiapine hemi fumarate using the following parameters.

Specificity

Preparation of blank solution:

Mix pH 3.0 phosphate buffer and Acetonitrile in the ratio of (95:5 %v/v) and Sonicated for about 5 minutes for degas the diluent.

Blank interference

A study to establish the interference of blank was conducted. Diluent was injected into the chromatograph in the defined above chromatographic conditions and the blank chromatogram was recorded. Chromatogram of blank solution (Fig 3) showed no peak at the retention time of fumaric acid peak. This indicates that the diluent solution used in sample preparation do not interfere in estimation of Fumaric acid content in Quetiapine hemi fumarate.
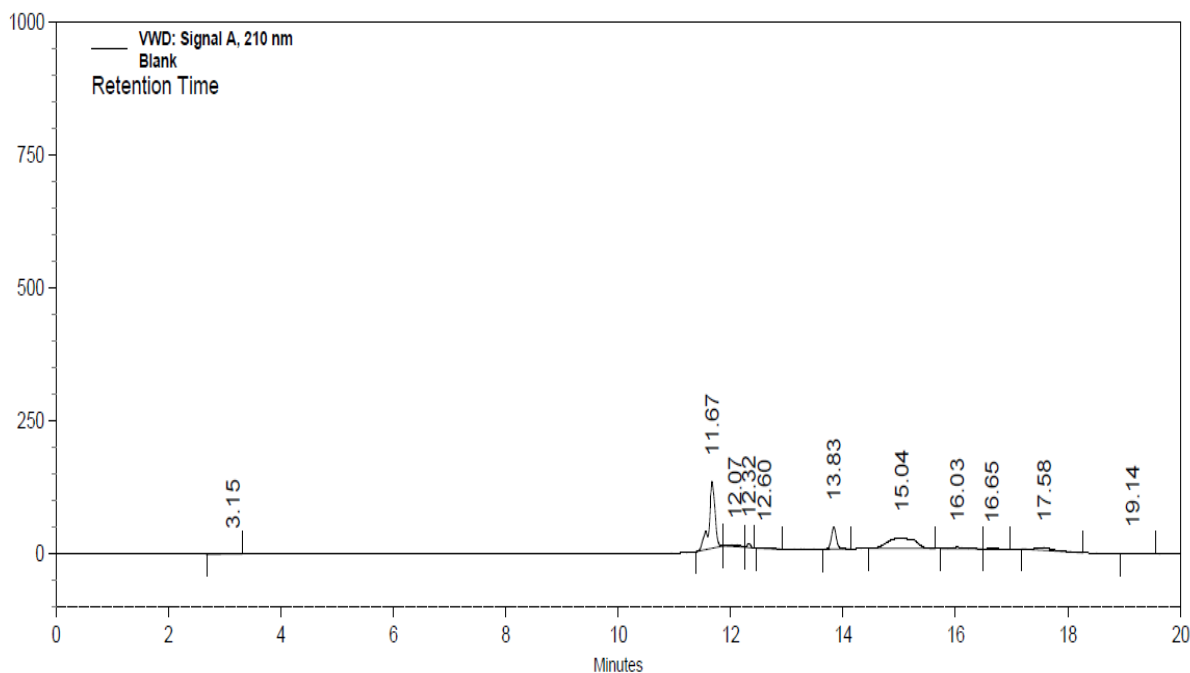

Figure 3: Chromatogram showing the no interference of diluent for fumaric acid
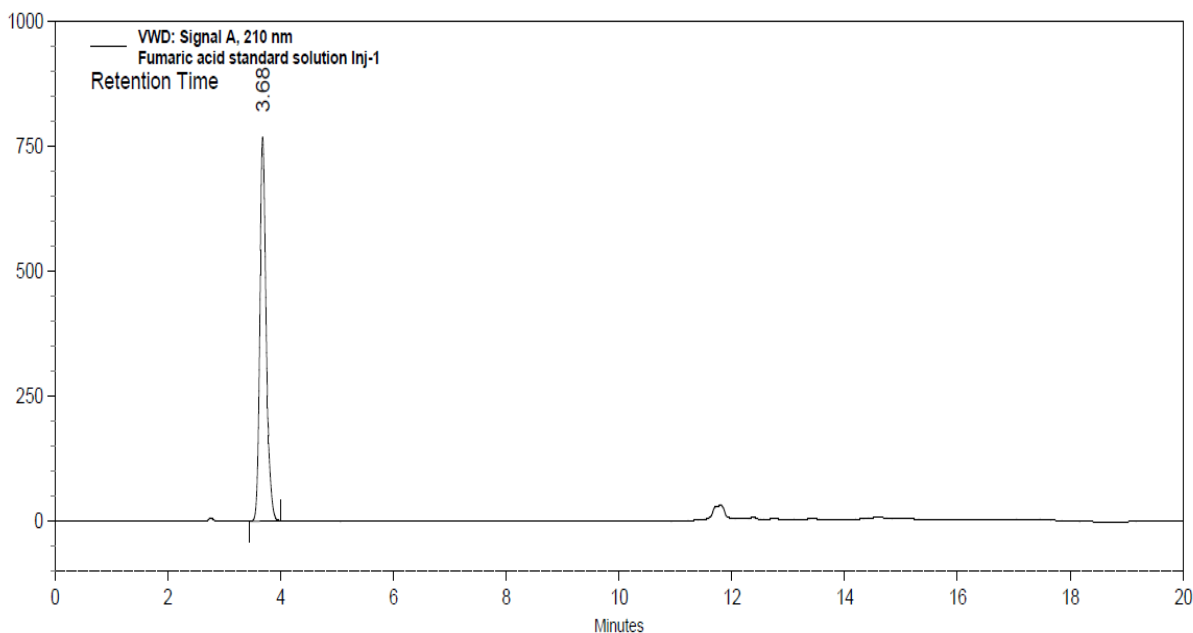
Figure 4: Typical Chromatogram of fumaric acid standard solution

Table 1: System suitability parameters for fumaric acid by proposed method

Name of the Compound	Retention Time	Theoretical plates	Tailing factor
Fumaric acid	3.68	15085	1.32

Method precision:

The precision of test method was evaluated by doing content of fumaric acid for six samples of as per test method. The content of fumaric acid in Quetiapine hemi fumarate for each of the test preparation was calculated. The average content of the six preparations and % RSD for the six observations were calculated. The data were shown in Table 2.

Table 2: Method precision data for Fumaric acid

No. of injections	Content of Fumaric acid in (%)
Preparation 1	12.75
Preparation 2	12.73
Preparation 3	12.71
Preparation 4	12.70
Preparation 5	12.70
Preparation 6	12.72
Average	12.70
%RSD	**0.15**

Linearity of detector response

The linearity of an analytical method is its ability to obtain test results which has a definite mathematical relation to the concentration of analyte. The linearity of response for fumaric acid was determined in the range of LOQ to 150 %. The calibration curve of analytical method was assessed

by plotting concentration versus peak area and represented graphically. The correlation coefficient [r2] was found to be 1.000. Therefore the HPLC method was found to be linear standard curve were calculated and given in Figure 4 to demonstrate the linearity of the proposed method. From the data obtained which given in Table 3 the method was found to be linear within the proposed range.

Table 3: Linearity studies for fumaric acid by proposed method

S.No	Fumaric acid Linearity concentration	Concentration (mg / ml)	Average area response
1	LOQ	0.000024	132779
2	25%	0.01	24640605
3	50%	0.02	48443247
4	75%	0.03	72365475
5	100%	0.04	97101520
6	125%	0.05	121447667
7	150%	0.06	144705811
Correlation coefficient:			1.000
R^2 Value			1.000
% Y-intercept			0.24
Slope (m):			2414874914
Intercept (y):			236488

The chart shows: **Linearity of Fumaric acid**, with equation $y = 2{,}414{,}874{,}914x + 236{,}488$ and $R^2 = 1$. X-axis: Concentration mg/ml. Y-axis: Area.
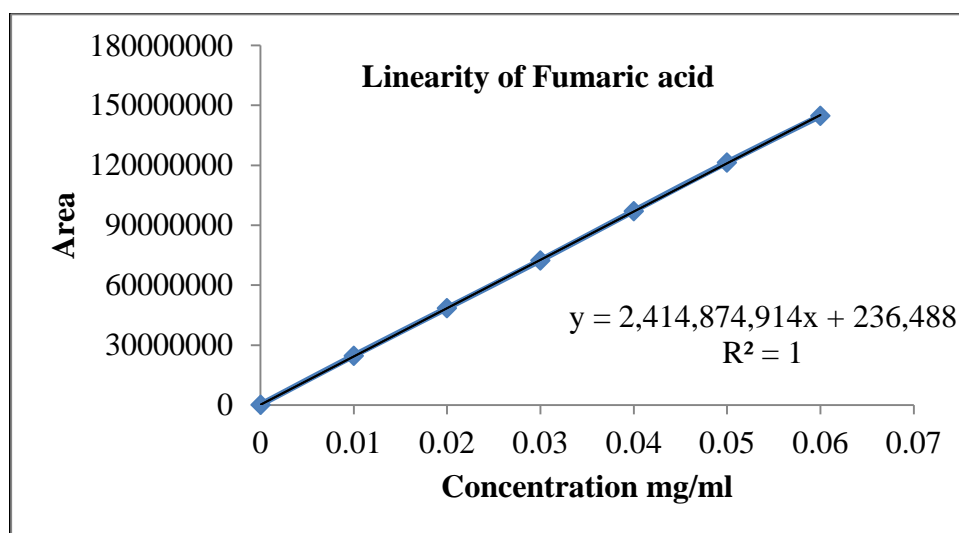
Figure 4: Calibration curve for Fumaric Acid

Accuracy

The accuracy of the method was determined on three concentration levels by recovery experiments. The recovery studies were carried out in triplicate preparations, analyzed as per the proposed method. The mean percentage recovery for LOQ, 100%, 150% level was found to be 101.24, 98.52 and 98.17. %RSD was found to be 0.07, 0.03 and 0.05 respectively. They are within the acceptance limits. Therefore, the HPLC method for the determination of fumaric acid content in Quetiapine

fumarate drug substance was found to be accurate. The data obtained which given in Table 4 the method was found to be accurate.

Table 4: Recovery studies for Fumaric acid by proposed method

	% Recovery of Fumaric acid		
	(LOQ)	(100%)	(150%)
Injection-1	101.24	98.50	98.18
Injection-2	101.32	98.51	98.11
Injection-3	101.17	98.55	98.21
Mean	101.24	98.52	98.17
SD	0.075	0.026	0.051
%RSD	0.07	0.03	0.05

LOD:

A solution containing 0.024 µg/ml of fumaric acid standard was injected three times. The worst found signal to noise ratio for each peak was greater than 3 in each injection. All the peaks were detected in all the three injections.
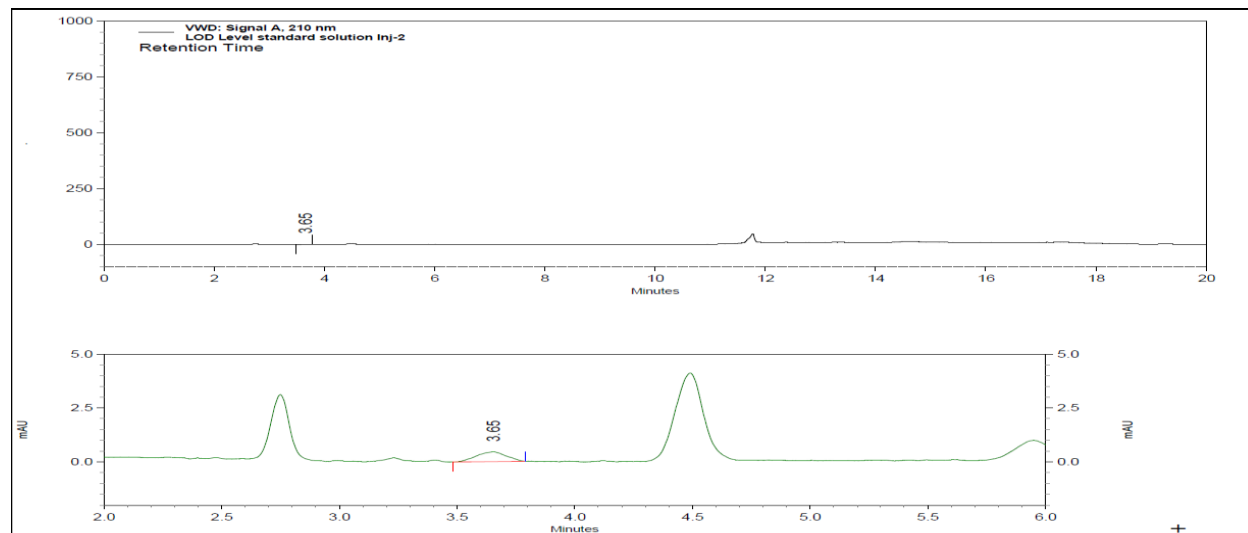

Figure 5: Typical Chromatogram of fumaric acid LOD solution

Table 5: Limit of detection (LOD) for fumaric acid

Name	Inj-1		Inj-2		Inj-3		Mean	Mean
	Area	S/N	Area	S/N	Area	S/N	Area	S/N
Fumaric acid	58569	3.96	65871	3.13	67566	3.37	**64002**	**3.49**

LOQ:

A solution containing 0.08 µg/mL of Fumaric acid standard was injected six times. The %RSD of areas, deviations of each six replicates from the linear regression curve and average deviation for each standard were calculated. The worst found signal to noise ratio for each peak was greater than 10 in each injection. The results are presented in the following tables:
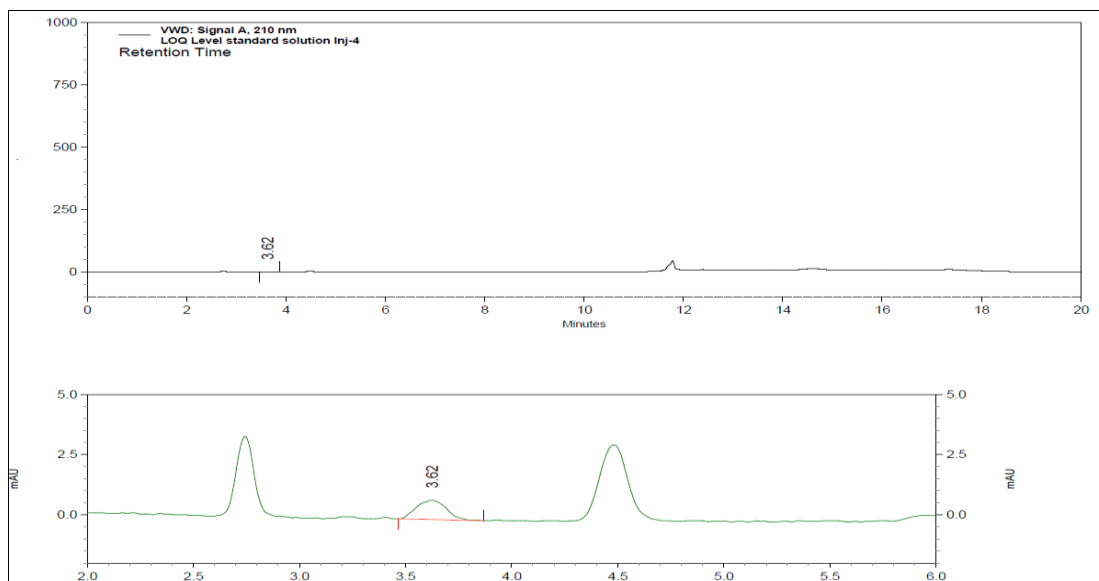
Figure 6: Typical Chromatogram of fumaric acid LOQ solution

Table 6: Areas of LOQ level mixed standard Fumaric acid

Component	Inj-1	Inj-2	Inj-3	Inj-4	Inj-5	Inj-6	Avg.	%RSD
Fumaric acid	134339	133564	130434	132424	131807	134275	132807	**1.16**

Table 7: S/N of LOQ level mixed standard Fumaric acid

Component	Inj-1	Inj-2	Inj-3	Inj-4	Inj-5	Inj-6	Avg.
Fumaric acid	12.01	12.12	11.80	10.54	11.52	10.42	**11.40**

Robustness:

Effect of variation in flow rate:

As the flow rate in the proposed method was 1ml/min, the flow rate was changed between 0.8 ml/min to 1.2 ml/min. After equilibration of mobile phase with stationary phase, standard solution was injected and the chromatograms were recorded. The results were shown in Table 8.

Table 8: System suitability data for Flow rate variation

System suitability parameters	Parameters and Results	
	(0.8 ml/min)	(1.2 ml/min)
% RSD for area count of five replicate injections of standard.	0.05	0.03
Tailing factor	1.01	1.01
Theoretical plates	16875	15985

Effect of variation in pH:

Prepared and injected standard and check standard solution as per the test method into HPLC system with PH variation of ± 0.5 units and evaluated system suitability parameters. The results were shown in Table 9.

Table 9: System suitability data for pH variation

System suitability parameters	Parameters and Results	
	(pH 2.50)	(pH 3.50)
% RSD for area count of five replicate injections of standard.	0.10	0.04
Tailing factor	1.05	1.04
Theoretical plates	17368	17524

Effect of variation in mobile phase composition:

Prepare two Isocratic programs, injected standard solution as per the test method and evaluated system suitability parameters. System suitability parameters are within the specified limits as per test method. The results were shown in Table 10.

Table: 1.10 System suitability data for Mobile phase variation

Parameters	Mobile phase variation (92.5:7.5)	Mobile phase variation (97.5:2.5)
% RSD for area count of five replicate injections of standard.	0.18	0.10
Tailing factor	1.06	1.05
Theoretical plates	15749	15986

CONCLUSION

An RP-HPLC method for estimation of Fumaric acid content in Quetiapine hemi fumarate was developed and validated as per ICH guidelines. A simple, accurate and reproducible reverse phase HPLC method was developed for the estimation of Fumaric acid in Quetiapine hemi fumarate bulk drugs. The optimized method consists of mobile phase pH 3.0 phosphate buffer and Acetonitrile in the gradient mode with Hypersil C18 250×4.6mm, 5µm column. The retention time of Fumaric acid was found to be 3.65min. The developed method was validated as per ICH Q2A (R1) guideline. The proposed HPLC method was linear over the range of 0.000024 to 0.06 mg/ml, the correlation coefficient was found to be 1.000. Relative standard deviation for method precision was found to be 0.15%.

The limit of detection (LOD) and limit of quantitation (LOQ) for Fumaric acid standard 0.024 & 0.08µg/mL respectively. The linearity results for Fumaric acid standard in the specified concentration range are found satisfactory, with a correlation coefficient greater than 0.99.Calibration curve was plotted and correlation co-efficient for fumaric acid found to be 1.000 respectively.

The accuracy studies were shown as % recovery for fumaric acid at LOQ to 150% level. The limit

of % recovered shown is in the range of 101.24% to 98.17% and the results obtained were found to be within the limits. The relative standard deviation values of recoveries 0.07% to 0.05%.Hence the method was found to be accurate.

We have developed a fast, simple and reliable analytical method for determination of Fumaric acid content in Quetiapine hemi fumarate pharmaceutical preparation using RP-LC. As there is no interference of blank at the retention time of Fumaric acid. It is very fast, with good reproducibility and good response. Validation of this method was accomplished, getting results meeting all requirements. The method is simple, reproducible, with a good accuracy and Linearity. It allows reliably the analysis of Fumaric acid in its different pharmaceutical dosage forms.

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