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DEVELOPMENT & VALIDATION OF STABILITY INDICATING RP-HPLC METHOD FOR QUANTITATIVE ESTIMATION OF QUETIAPINE FUMARATE AND ITS IMPURITIES IN DRUG SUBSTANCE AS PER ICH GUIDELINES

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ABSTRACT
The analysis of improved RP-HPLC method for the separation and quantification of Quetiapine fumarate and its impurities are described. Samples are analysed by means of reverse phase (RP-HPLC) using an Zodiac C18, 100 x 4.6 mm, 3µm, and the mobile phase consists of two Channels A and B. Channel-A pH 6.50 buffer: methanol (500:500%v/v) and Channel-B: pH 6.50 buffer: methanol (150:850%v/v),. The flow rate is 1.0 ml/min. The column temperature was maintained at 45°C and sample temperature was maintained at ambient (25°C) and wavelength fixed at 252nm UV-detection. It is found that the method of RP-HPLC with UV-detection system for the analysis of Quetiapine fumarate impurities are straight forward and applied in qualitative and quantitative analysis. The developed LC method was validated with respect to specificity, precision, linearity, ruggedness and robustness. Validation study compared as per ICH guideline.

KEYWORDS: Quetiapine fumarate, estimation of related substances, liquid chromatography.

1.0 INTRODUCTION
Quetiapine fumarate is dibenzothiazepine atypical antipsychotic. It is used in the treatment of schizophrenia and of bipolar disorder. Chemically it is 2-[2-(4-Dibenzo [b, f] [1, 4] thiazepin-11-yl-1-piperazinyl) ethoxy] ethanol fumarate (2:1) salt. It is reported to have affinity for serotonin (5-HT2), histamine (H1), and adrenergic (a1 and a2) receptors as well as dopamine D1 and D2 receptors.[1,2] The chemical structure of Quetiapine fumarate shown in **(Fig. 1.1).**

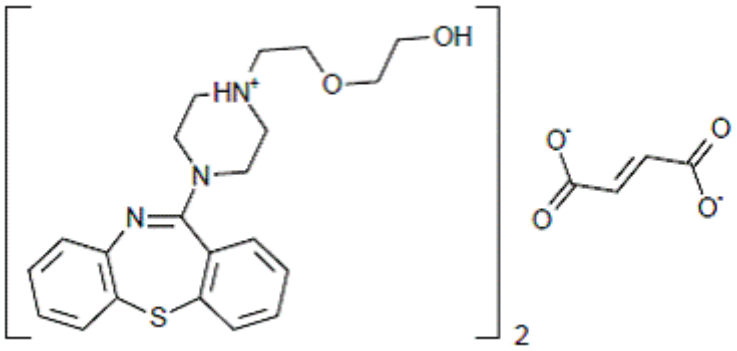

Figure 1.1.: Chemical Structure of Quetiapine fumarate.

A few analytical methods have been reported for the determination of Quetiapine fumarate in pure drug, pharmaceutical dosage forms and biological samples using spectrophotometry[3,4], liquid chromatography[5-19], high performance thin layer chromatography[20,21], gas chromatography[22], electrophoresis[23,24] and polarography.[25]

The objective of the present work is to develop a stability indicating HPLC method and validated as per ICH and USP validation guidelines[26] for the estimation of Quetiapine fumarate in applied for routine analysis in laboratories and is suitable for the quality control of the raw materials.

Impurity profiling of active pharmaceutical ingredients (API) in both bulk material and formulations is one of the most challenging tasks. The presence of unwanted or in certain cases unknown chemicals, even in small amounts, may influence not only the therapeutic efficacy but also the safety of the pharmaceutical products. For these reasons, all major international pharmacopoeias have established maximum allowed limits for related compounds for both bulk and formulated APIs. As per the requirements of various regulatory authorities, the impurity profile study of drug substances and drug products has to be carried out using a suitable analytical method in the final product.

2.0 EXPERIMENTAL

2.1 Reagents and chemicals

Potassium dihydrogen orthophosphate, Di-potassium orthophosphate, Orthophosphoric acid, Methanol was procured from Merck. Water (Milli-Q). All chemicals were of an analytical grade and used as received.

2.2 Instrumentation

Chromatographic separation was achieved by using an Agilent-1200, Open-lab software using, Zodiac C18, 100 x 4.6 mm, 3µm column with eluent-A: pH 6.50 buffer: methanol (500:500% v/v) and eluent-B: pH 6.50 buffer: methanol (150:850% v/v) as mobile phase at a flow rate of 1.0 mL/min. with UV detection at 252 nm. Column maintained at temperature 45ºC, sample temperature ambient. The overall run time was 50 minutes. 10 µl of sample was injected into the HPLC system. Retention times of impurities were 8.77 for impurity-A, 11.95 for Impurity-B, 19.40 for Impurity-C and 23.06 for Quetiapine Hemi fumarate.

2.3 Preparation of mobile phase and standard and sample solution

2.3.1 Preparation of Buffer

Weigh accurately about 1.52g of potassium dihydrogen orthophosphate and 2.41g of di-potassium orthophosphate and transfer in to 2000mL beaker dissolve and dilute to volume with 1000mL milli-Q or HPLC grade water. Adjust the pH to 6.5 ± 0.05 with dilute orthophosphoric acid.

2.3.2 Mobile phase-A: Transfer 500mL of buffer and 500mL of methanol into 1000mL beaker mixed well. Filter through 0.45µ membrane filter and degas.

2.3.3 Mobile phase-B: Transfer 150mL of buffer and 850mL of methanol into 1000mL beaker mixed well. Filter through 0.45µ membrane filter and degas.

2.3.4 Diluent preparation: Mixed buffer and methanol in the ratio of (15:85 v/v).

2.3.5 Preparation of Test solution

Weigh accurately and transfer about 100mg of test sample into a 50ml volumetric flask, dissolve in and dilute to the volume with diluent.

2.3.6 System suitability stock solution

Weigh accurately and transfer about 25mg of Impurity-A, 25mg of Impurity-B and 25mg of Impurity-C into a 50ml volumetric flask, dissolve in and dilute to the volume with methanol.

2.3.7 Preparation of System suitability solution

Weigh accurately and transfer about 100mg of quetiapine hemi fumarate working standard transfer into a 50 ml volumetric flask, add to it 0.5mL of system suitability stock solution, dissolve in and makeup to volume with diluent.

3.0 RESULTS AND DISCUSSION

3.1 Method optimization parameters

An understanding of the nature of API (functionality, acidity, or basicity), the synthetic process, related impurities, the possible degradation pathways and their degradation products are needed for successful method development in reverse-phase HPLC. In addition, successful method development should result a robust, simple and time efficient method that is capable of being utilized in manufacturing setting.

3.2 Selection of wavelength

The sensitivity of the HPLC method depends upon the selection of detection wavelength. An ideal wavelength is one that gives good response for related substances and the drugs to be detected. The wavelength for measurement was selected as 252 nm from the absorption spectrum.

3.3. Selection of stationary phase

Proper selection of the stationary phase depends up on the nature of the sample and chemical profile. The drug selected for the present study was polar compound and could be separated either by normal phase chromatography or reverse phase chromatography. From literature survey, it was found that different C18 columns could be appropriately used for the separation of related substances for Quetiapine fumarate.

3.4. Selection of mobile phase

Different mobile phase and stationary phases were employed to develop a suitable LC method for the quantitative determination of impurities in Quetiapine fumarate. A number of column chemistries supplied by different manufacturers and different mobile phase composition were tried to get good peak shapes and selectivity for the impurities present in Quetiapine fumarate.

Poor peak shape and resolution was observed when Hypersil BDS C18 (100mm x 4.6mm, 3µ) and gradient mobile phase programmed of Mobile Phase: A 0.1% OPA in water and Mobile Phase: B 0.1% OPA in Acetonitrile. There was no proper resolution of impurities and analyte peak and efficiency of the peak is also not achieved and peak interferences are present.

In second attempt made using Zodiac C18, 100 x 4.6 mm, 3µm eluent-A: pH 6.50 buffer: methanol (500:500% v/v) and eluent-B: pH 6.50 buffer: methanol (150:850% v/v). The resolution of both drug and impurities was achieved. These chromatographic conditions were selected for validation studies.

4.0 Method Validation

4.1 Specificity

Blank interference

A study to establish the interference of blank was conducted. Diluent was injected as per the test method.

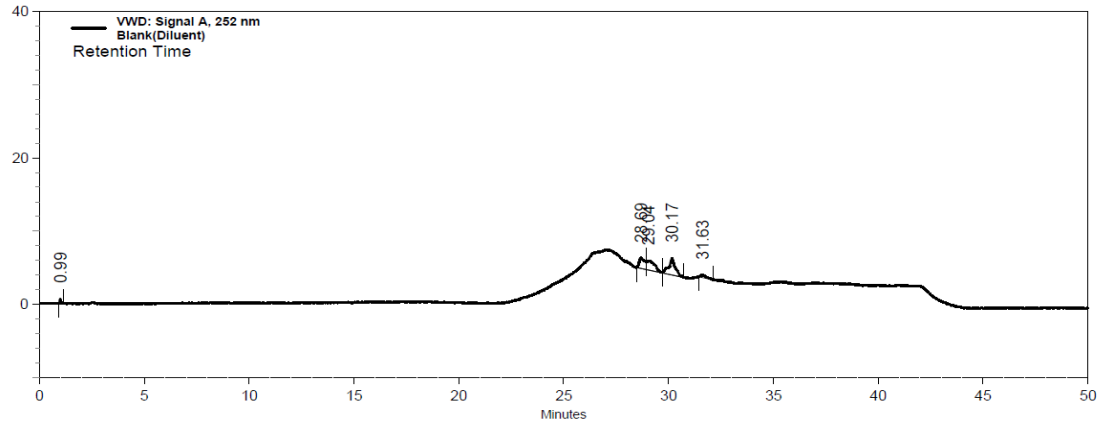

Figure 1.2: Typical chromatogram of Blank.

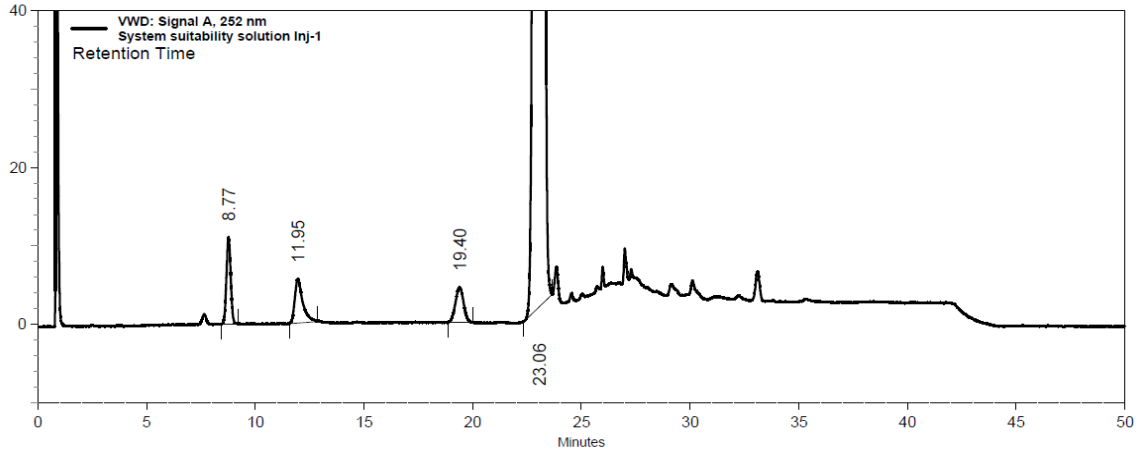

Figure 1.3: Typical chromatogram Spiked Sample.

Table 1.1: Impurity interference data.

Peak Name	Retention Time	Relative retention time(RRT)
Impurity-A	8.77	0.38
Impurity-B	11.95	0.52
Impurity-C	19.40	0.84
Quetiapine Hemi fumarate	23.06	1.00

It was observed that known impurities are not co eluting with each other and main analyte peak. Quetiapine fumarate standard solution preparation and in spiked test preparation was calculated and found to be within the acceptable limit.

4.2 Precision

4.2.1 System Precision

System precision

Perform the analysis of reference solution (Diluted standard) six times and determine the percentage relative standard deviation of peak area of replicate injections of Quetiapine Hemi fumarate.

Table 1.3: System Precision data for Quetiapine fumarate.

Injection No	Quetiapine Hemi fumarate
1	696974
2	727021
3	722426
4	705485
5	715489
6	718561
Mean area	**714326**
%RSD	**1.57**

The %RSD of peak area for Quetiapine fumarate was found to be 1.57% which is below 5.0% indicates that the system gives precise result.

4.2.2 Method Precision

Precision of the impurities and degradants method was determined by injecting six sample solutions spiked with impurities (Impurity-A, B and C) at specification level. The samples were prepared as per the method and the result for precision study is tabulated in **Table: 1.4.**

Table 1.4: Results of method precision.

Inj. No	Impurity-A	Impurity-B	Impurity-C	Impurity at RRT about 1.5	Individual Unknown impurity
1	0.256	0.244	0.261	0.06	0.06
2	0.247	0.255	0.247	0.06	0.06
3	0.241	0.249	0.263	0.06	0.06
4	0.255	0.238	0.251	0.06	0.06
5	0.253	0.261	0.254	0.06	0.06
6	0.261	0.254	0.252	0.06	0.06
Mean (%)	0.25	0.25	0.25	0.06	0.06
% RSD	**2.82**	**3.31**	**2.42**	**0.00**	**0.00**

The method precession was performed with six replicate solutions of standard solutions prepared and the system suitability parameters found were within the acceptance criteria

4.3 Limit of detection (LOQ) & Limit of Quantitation (LOD)

A solution containing 0.15 µg/ml of Quetiapine Hemi fumarate standard, 0.06 µg/ml of impurity-A, 0.25 µg/ml of impurity-B and 0.23 µg/ml of impurity-C 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.

Table 1.6: LOD for Quetiapine fumarate and impurities.

Name	Inj-1		Inj-2		Inj-3		Mean Area	Mean S/N
	Area	S/N	Area	S/N	Area	S/N		
QHF	109153	4.01	104883	3.03	114059	3.54	109365	**3.53**
Impurity-A	110779	3.90	101094	3.30	87140	2.98	99671	**3.39**
Impurity-B	183931	4.00	205971	3.14	167144	3.27	185682	**3.47**
Impurity-C	186402	4.38	186642	3.46	169924	3.22	180989	**3.69**

A solution containing 0.513 µg/mL of Quetiapine Hemi fumarate standard, 0.2056µg/ml of impurity-A, 0.8232 µg/mL of impurity-B and impurity-C 0.8352 µg/mL 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 results are presented in the following tables:

Table 1.7: LOQ for Quetiapine fumarate and impurities.

Component	Inj-1	Inj-2	Inj-3	Inj-4	Inj-5	Inj-6	Avg	%RSD
Impurity–A	230115	213633	227930	229786	220121	222208	223966	**2.91**
Impurity–B	490576	499201	491854	478521	488436	514717	493884	**2.47**
Impurity–C	433360	453015	446631	432951	454089	449901	444991	**2.14**
QHF	333803	323331	324325	337402	347716	353604	336697	**3.63**

The limit of limit of quantitation and detection of quantitation values obtained for each impurity and Quetiapine fumarate are within the acceptance criteria.

4.4 Linearity and Range

The linearity is determined by injecting the solutions in duplicate containing known impurities and Quetiapine Hemi fumarate and impurities ranging from LOQ to 150% of the specified limit. Perform the regression analysis and determine the correlation coefficient and residual sum of squares. Determine the response factor for each impurity with respect to Quetiapine Hemi fumarate. Report the linearity range as the range for determining the impurities. Results obtained are in the tables & figures show the line of best fit for peak area versus concentration for each impurity. (**Table 1.8, Table 1.9, Table 1.10 and Table 1.11) &** figures show the line of best fit for peak area versus concentration for each impurity.

Table 1.8: Linearity of detector response Impurity-A.

Level	Concentration (%)	Mean Area
LOQ	0.02	223893
25%	0.0627	654091
50%	0.1254	1361896
75%	0.1880	2041352
100%	0.2507	2667732
125%	0.3134	3339720
150%	0.3761	3994990
Correlation coefficient		0.9999
R^2 Value		1.000
% Y-intercept		0.54
Slope		10613253
Intercept		14455

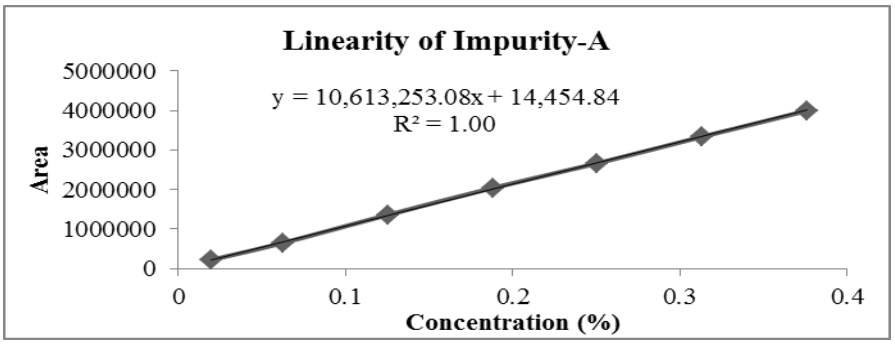

Fig. 1.4: Linearity of detector response for Impurity-A.

Table 1.9: Linearity of detector response Impurity-B.

Level	Concentration (%)	Mean Area
LOQ	0.08	493877
50%	0.1253	871375
75%	0.1879	1366208
100%	0.2505	1842463
125%	0.3131	2329785
150%	0.3758	2676365
Correlation coefficient		0.9985
R^2 Value		0.9970
% Y-intercept		-3.49
Slope		7478151
Intercept		-64218

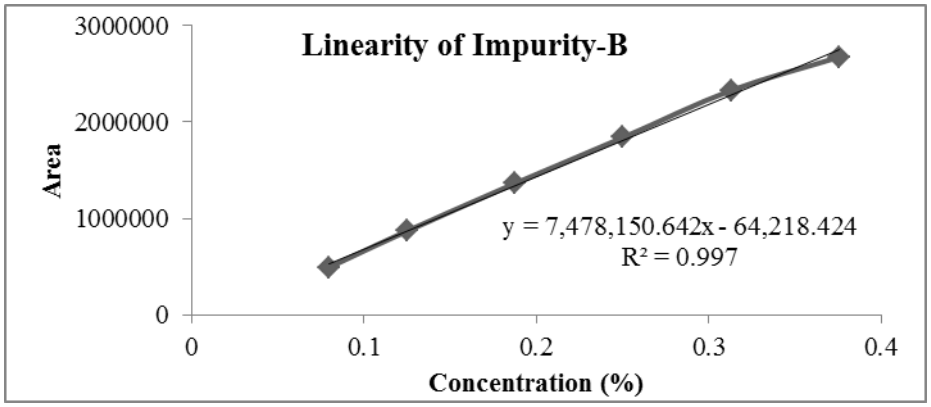

Fig. 1.5: Linearity of detector response for Impurity-B.

Table 1.10: Linearity of detector response Impurity-C.

Level	Concentration (%)	Mean Area
LOQ	0.08	444335
50%	0.1252	708961
75%	0.1878	1061341
100%	0.2504	1431668
125%	0.3130	1851474
150%	0.3750	2166111
Correlation coefficient		0.9996
R^2 Value		0.9991
% Y-intercept		-2.33
Slope		5906674
Intercept		-33376

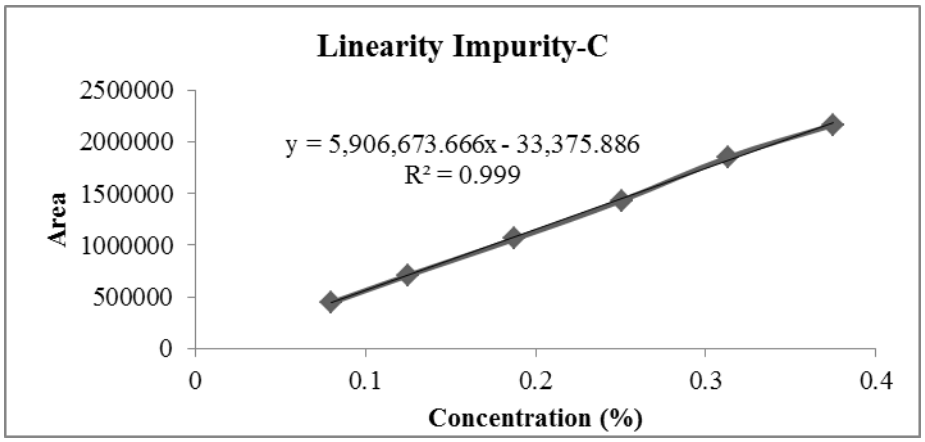

Fig. 1.6: Linearity of detector response for Impurity-C.

Table 1.11: Linearity of detector response Quetiapine fumarate.

Level	Concentration (%)	Mean Area
LOQ	0.050	327153
75%	0.0755	531753
100%	0.1007	721939
125%	0.1259	868749
150%	0.1511	1062292
Correlation coefficient		0.9987
R^2		0.9975
% Y-intercept		-2.46
Slope		7155294
Intercept		-17732

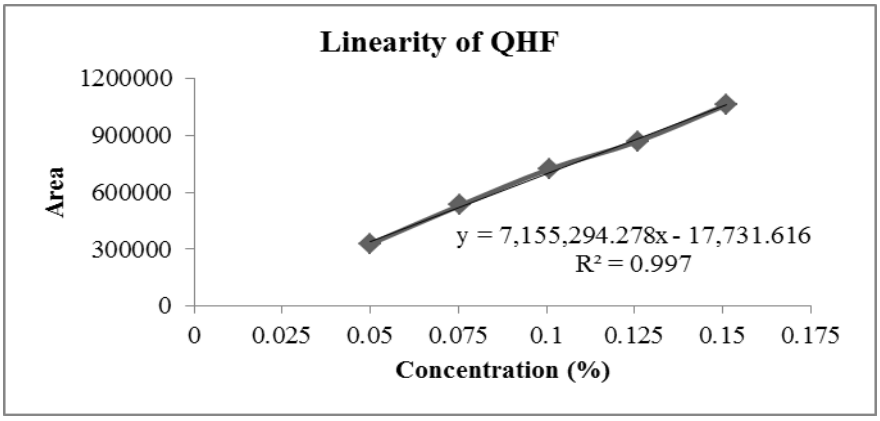

Fig. 1.7: Linearity of detector response for Quetiapine fumarate.

The linearity results for Quetiapine Hemi fumarate and all the impurities in the specified concentration range are found satisfactory, with a correlation coefficient greater than 0.99.

4.5 Accuracy

Recovery of Quetiapine fumarate impurities in Quetiapine fumarate was performed. The sample was taken and varying amounts of Quetiapine fumarate impurities representing 50 to 150% of specification level were added to the flasks. The spiked samples were prepared as per the method and the results are tabulated in **Table 1.12.**

Table 1.12: Accuracy study of Quetiapine fumarate.

S.No.	Theoretical (%)	% Mean Recovery		
		Impurity-A	Impurity-B	Impurity-C
1	50	99.31	101.83	99.99
2	100	101.23	99.85	101.07
3	150	100.0	98.21	100.59

5.0 RESULTS AND DISCUSSION

A simple, economic, accurate and precise HPLC method was successfully developed. In this method it was carried out by using Zodiac C18, 100 x 4.6 mm, 3µm**.** Injection volume of 10µl is injected and eluted with the mobile phase eluent-A: pH 6.50 buffer: methanol (500:500%v/v) and eluent-B: pH 6.50 buffer: methanol (150:850%v/v), which is pumped at a flow rate of 1.0 ml/min. Column temperature 45°C and sample temperature 25°C. Detection was carried out at 252 nm. The results obtained were accurate and reproducible. The method developed was statistically validated in terms of Selectivity, accuracy, linearity, precision, robustness, and stability of solution.

For Selectivity, the chromatograms were recorded for standard and sample solutions of Quetiapine Hemi fumarate and its related substances. Selectivity studies reveal that the peak is well separated from each other. Therefore the method is selective for the determination of related substances in Quetiapine Hemi fumarate. There is no interference of diluent at QHF and impurities peaks. The elution order and the retention times of Impurities and QHF obtained from individual standard preparations and mixed standard Preparations are comparable.

The limit of detection (LOD) and limit of quantitation (LOQ) for Quetiapine Hemi fumarate standard 0.15 & 0.51µg/mL, impurity-A 0.06 & 0.2056µg/mL, impurity-B 0.025 & 0.8232 µg/mL and impurity-C 0.23 & 0.8352 µg/mL respectively.

The linearity results for Quetiapine Hemi fumarate and all the impurities 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 Quetiapine Hemi fumarate and its impurities found to be 0.9987, 0.9999, 0.9985 and 0.9996 respectively.

The accuracy studies were shown as % recovery for Quetiapine Hemi fumarate and its impurities at specification level. The limit of % recovered shown is in the range of 90 and 110% and the results obtained were found to be within the limits. Hence the method was found to be accurate.

The relative standard deviation values of recoveries obtained for all impurities are in the range of 0.28%-1.80%.

For Precision studies six (6) replicate injections were performed. %RSD was determined from the peak areas of Quetiapine Hemi fumarate and its impurities. The

acceptance limit should be not more than 10, and the results were found to be within the acceptance limits.

Hence, the chromatographic method developed for Quetiapine Hemi fumarate and its related substances are rapid, simple, sensitive, precise, and accurate. Therefore, the proposed method can be successfully applied for the routine analysis of the active pharmaceutical ingredients for assurance of its quality during its formulation.

6.0. CONCUSIONS

The new HPLC method developed and validated for determination of Quetiapine Hemi fumarate pharmaceutical dosage forms and assured the satisfactory precision and accuracy and also determining lower concentration of drug in its solid dosage form by RP-HPLC method. The method was found to be simple, accurate, economical and rapid and they can be applied for routine analysis in laboratories and is suitable for the quality control of the raw materials.

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