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Bioanalytical Estimation of Quetiapine in Human Plasma By RP-HPLC

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Abstract:

Based on RP-HPLC, a bioanalytical method for the estimation of Quetiapine (QTP)in human plasma was developed and validated. The separation is carried out by Sunfire C18 Column. The optimized batch was carried out by HPLC gradient system with auto-injector using UV (DAD) detector which had C18 quaternary gradient column at room temperature. Separation is done by using mobile phase MEOH & 0.05% OPA in the ratio of 45:55 with a flow rate of 0.7 ml/min. The sample was detected at a wavelength of 296 nm and the sample size was 20µl. According to ICH guidelines, the purpose of method validation is to demonstrate the acceptability of an analytical technique for its intended purpose. The standard calibration plot was found linear over a range of 5 to 25µg/ml and the coefficient of correlation was found to be (r2 =0.999). The % RSD value of intraday and interday precision. The LOD and LOQ were found to be 0.267664 and 0.811103 for quetiapine fumarate. The developed method was eventually applied for quantification of the marketed formulation satisfactory result was obtained.

Keywords: Bioanalytical method development, Quetiapine, RP-HPLC, Validation, Bioanalysis

Introduction:

Bioanalysis is the method used to determine the concentration of drugs, their metabolites, and/or endogenous substances in biological matrices such as blood plasma, serum, cerebrospinal fluid, urine, and saliva.[1] Bioanalytical methods are widely used to quantify drugs and their metabolites in the physiological matrices. The methods could be applied to human clinical pharmacology and non-human pharmacology/toxicology studies.[2] The bioanalytical method employed for the quantitative determination of drugs and their metabolites in biological fluids plays a significant role in evaluating and interpreting bioequivalence, pharmacokinetics, and toxic kinetic studies.[3] It helps in carrying out studies like pharmacodynamics, toxicology, pharmacokinetics, bioequivalence, therapeutic drug monitoring (TDM), and clinical studies. These studies' initial stages are done only to find out over dosage conditions and in toxicological studies.[4] When the concentration of the drug in the biological matrix is known, then pharmacokinetic parameters are calculated from that. Drug development and discovery benefit greatly from bioanalytical studies.[5]

The therapeutic efficacy of the particular drug can be known by bioanalysis. In-pharma field bioanalysis plays a significant role.

Bioanalysis involves the following steps.[4]

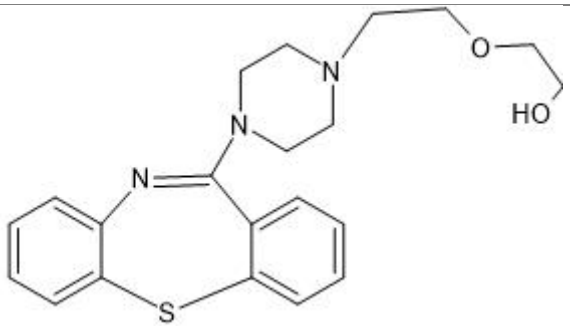
- Selection and collection of biological fluid.
- Preparation of sample –Analyte extraction from the biological matrix.
- Analyte detection done by various methods.

The powder form of quetiapine is white to off-white. A drug's ability to treat schizophrenia and bipolar illness is mediated by a concoction of serotonin type 2 (5HT2) and dopamine type 2 (D2) antagonists. An atypical antipsychotic called quetiapine fumarate (2-[2-(4-dibenzo [b, f] [1, 4] thiazepin-11-yl-1-piperazinyl) ethoxy] ethanol fumarate (2:1 salt)) has a distinct receptor-binding profile and belongs to a new chemical class called dibenzothiazepine derivatives.[6]

The research's objective was to create a new RP-HPLC method for determining quetiapine in human plasma and apply the results in the fastest, most efficient way possible to analyse pharmaceutical drugs. The RP HPLC approach is advantageous because, to our knowledge, there aren't many analytical papers accessible for the determination of the medication in plasma.

Drug Profile:

Table No. 1 Drug Profile

Sr.No.	Name of Drug:	Quetiapine (QTP)
1	Structure	
2	Molecular Formula	$C_{21}H_{25}N_3O_2S$
3	Molecular Weight	383.5 g/mol
4	Category	Antipsychotic drugs
5	Chemical Name	(2-[2-(4-dibenzo [b, f] [1, 4] thiazepin-11-yl-1- piperazinyl) ethoxy] ethanol fumarate
6	Description	a white to off-white crystalline powder.
7	Melting Point	172-176 °C
8	Solubility	Sparingly soluble in methanol, slightly soluble in water &in ethanol (99.5)
9	Mode of Action	improves the positive and negative symptoms of schizophrenia and major depression by acting on various neurotransmitter receptors, such as the serotonin and dopamine receptors.

Materials & method :

1. Chemical and Reagent Used

The following chemicals have been procured for the process

- Water [HPLC Grade]
- Quetiapine [Working Standards]
- Methanol [HPLC Grade]
- Orthophosphoric acid

All the chemicals are procured from STANDARD SOLUTIONS and the tablets [100mg Label Claim] were collected from the Local market the manufacturer was Intas Pharma and the brand name of the Tablet was QUTAN 100 mg (Intas Pharma)

2. Apparatus and Chromatographic Conditions Equipment :

Equipment:

- High-performance liquid chromatography equipped with Auto Sampler and DAD or UV detector. (Waters, Alliance 2695 Separation Module with 2487 UV Vis Detector)
- Column: Symmetry C18 (4.6 x 150mm, 5 µm Make: Thermo)
- Flow rate: 1.0mL per min
- Wavelength: 290 nm
- Injection volume: 20 µl
- Temperature: Ambient
- Run time: 6.0 min

3. Method development and optimization of chromatographic condition

I. Preparation of Phosphate buffer :

The Buffer Solution was prepared by weighing accurately and transferring 2.5 grams of sodium dihydrogen phosphate into a 1000ml volumetric flask, dissolved and diluted to 1000ml with HPLC water. The pH of the buffer was adjusted to 4.0 by using Orthophosphoric acid.

II. Selection of wavelength:

The spectrum was recorded by scanning dilutions of standard solutions in the range of 200 nm. by using UV spectrophotometry. The wavelength of detection is 296 nm, the method showed good linearity in this range.

III. Preparation of mobile phase:

The mobile phase was prepared by mixing the above buffer 450mL (45%) and 550 mL of Methanol [HPLC grade] (55%) and degassed in an ultrasonic water bath for 5 minutes. Then the solution was filtered through 0.45 µ filter under vacuum filtration.

IV. Selection of stationary phase:

Sunfire C18 (Agilent)2 stationary phases are used for separation because of its hydrophobic interaction. as solutes the in mobile phase travel through silica pores they can be attracted and held by hydrocarbons through the stationary phase was sunfireC18(Agilent). The mobile phase was methanol and 0-0.5% OPA in a ratio 45:55 wavelength selected for detection was 296nm; the flow rate for separation was 0.7ml/min. the temperature of the column was 15 °C and the particle size was 20ul.

V. Diluent Preparation:

The Mobile phase was used as diluent.

VI. Selection of flow rate:

0.7 ml/min flow rate was selected for a high flow rate to reduce retention time so component molecules have little time to interact with the stationary phase as they pass quickly through the column.

4. Preparation of the Quetiapine Standard & Sample Solution

Standard Solution Preparation: The Standard Stock Solution was prepared by weighing accurately and transferring 5mg of Quetiapine [Working standard] in 10 ml Methanol into a 10 mL volumetric flask. Initially about 7 mL of diluent was added and sonicated to dissolve it completely and the volume was made up to the mark with the same solvent. Further from the above Stock Solution pipette out 0.1 ml solution into a 10 ml volumetric flask and diluted up to the mark with diluent. The resultant solution was mixed thoroughly and filtered through a 0.45µm filter.

Spiking of Quetiapine to Plasma and Extraction of Quetiapine from plasma: The serial dilutions of analyte were prepared in the mobile phase and 0.5ml of each dilution was spiked into 0.5ml of plasma in a polypropylene tube. Then all the tubes were cyclo mixed for 5 min. Then 1 ml of acetonitrile was added and centrifuged for 20 min at 3000 rpm. Further, the supernatant liquids were collected in another Eppendorf tube and 20µL supernatant was injected into the analytical column.

5. Validation:[7,8]

The process of determining if a quantitative analytical method is appropriate for biochemical applications is known as bioanalytical method validation (BMV). A bioanalytical procedure should be validated in order to show the method's effectiveness and dependability, which will increase the level of trust that can be placed in the results.

The Basic parameters involved in bio-analytical validation are:

A. **Linearity:**

Linearity evaluates the facility of the bioanalytical method to obtain test results that are directly proportionate to the concentration of the analyte in the sample. FDA regulations state that the standard curve's linear range should have at least five standard points.

Admission Criteria: The calibration's correlation coefficient must be at least 0.99.

B. Accuracy:

The degree of similarity between the nominal or known real concentration and the measured concentrations. Relative error (% RE) is the most used unit of measurement. An accurate measurement depends on a number of variables, including precision and specificity.

$$\text{Abs \%} = \frac{\text{Measured value - True value}}{\text{True value}} \times 100$$

Accuracy should be expressed as a percentage recovery from the assay of the sample's known additional analyte concentration. Agree with the requirements for RSD 2%

C. Precision:

A bioanalytical method's accuracy is a measurement of random error and is determined by how closely a set of measurements obtained from multiple sampling of the same homogenous sample under the given conditions agree with one another.

By injecting a series of standards or by evaluating a series of samples taken from a homogeneous lot, precision can be determined. Precision is calculated as the relative standard deviation (% RSD) from the measured standard deviation (SD) and mean values.

RSD or CV =SD/Mean % 100

RSD of the mean concentration of five readings must be less than 15% for the bioanalytical method to be accepted

D. Repeatability:

Measuring repeatability describes the similarity of results acquired over a brief period of time with the same sample (or subsamples of the same sample) using the same measurement process, same operators, same measuring device, and same location

E. Robustness:

According to ICH criteria, an analytical procedure's robustness is determined by its ability to stay unaffected by minor, intentional changes to the technique parameters and to demonstrate its dependability in typical conditions.

In a robustness research approach, the parameters are purposefully changed to examine if the results are impacted. as in:

- Modification of the composition of the mobile phase
- Modification of wavelength
- Modification in flow rate

F. Limit of detection (LOD):[9]

The limit of detection is the lowest possible concentration at which the method can be detected (but not quantity) usually a limit of detection is determined only for qualitative determination.

G. Lower Limit of Quantification (LLOQ):[9]

It is the lowest amount of analyte in a sample that can be detected but necessarily quantitated under the stated experimental conditions with acceptable accuracy and precision.

6) Results and discussion

a) Development and optimization of Bioanalytical RP- HPLC method development

The present study was carried out to develop a sensitive, precise, and accurate RP-HPLC method for the analysis of the drug Quetiapine fumarate in Human Plasma. In order to method development under isocratic conditions, mixtures of Sodium Dihydrogen Phosphate Buffer with pH 4.0 adjusted with Orthophosphoric acid and Methanol HPLC grade in different combinations were tested as mobile phase on a Symmetry C18 (4.6 x 250 mm, 5 µm, Make: Thermo) column. A binary mixture of Sodium Dihydrogen Phosphate Buffer [pH 4.0] and Methanol [HPLC Grade] in a 45:55 v/v proportion was proved to be the most suitable of all combinations since the chromatographic peaks were better defined and resolved and almost free from the tailing. The retention times obtained for Quetiapine fumarate were around 2.867min. A model chromatogram was shown in Figures no.1 & 3

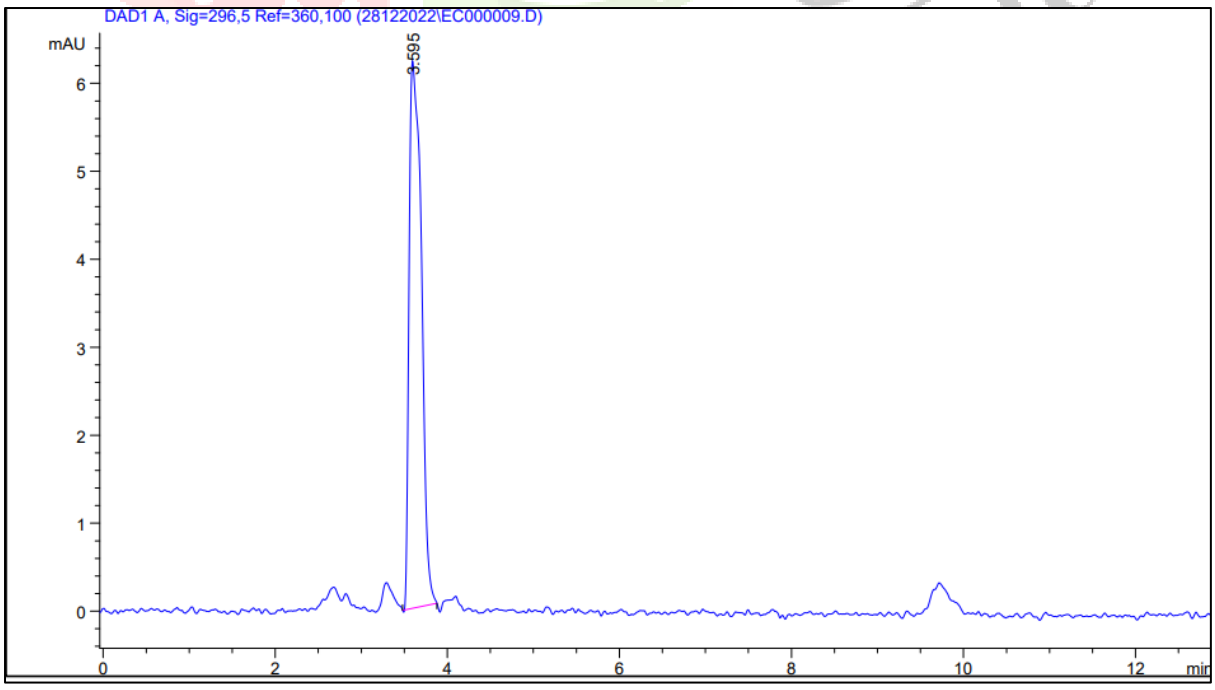


Figure No.1 Typical Chromatogram of Blank Plasma

UV spectroscopy:

Selection of wavelength and calibration curve UV absorption of 10 mg of QUET was transferred in 100 ml with a sufficient quantity of MeOH generated and absorbance was taken in the range of 200-400 nm. λ max of Quetiapine Fumarate in Me was found to be 296 nm.

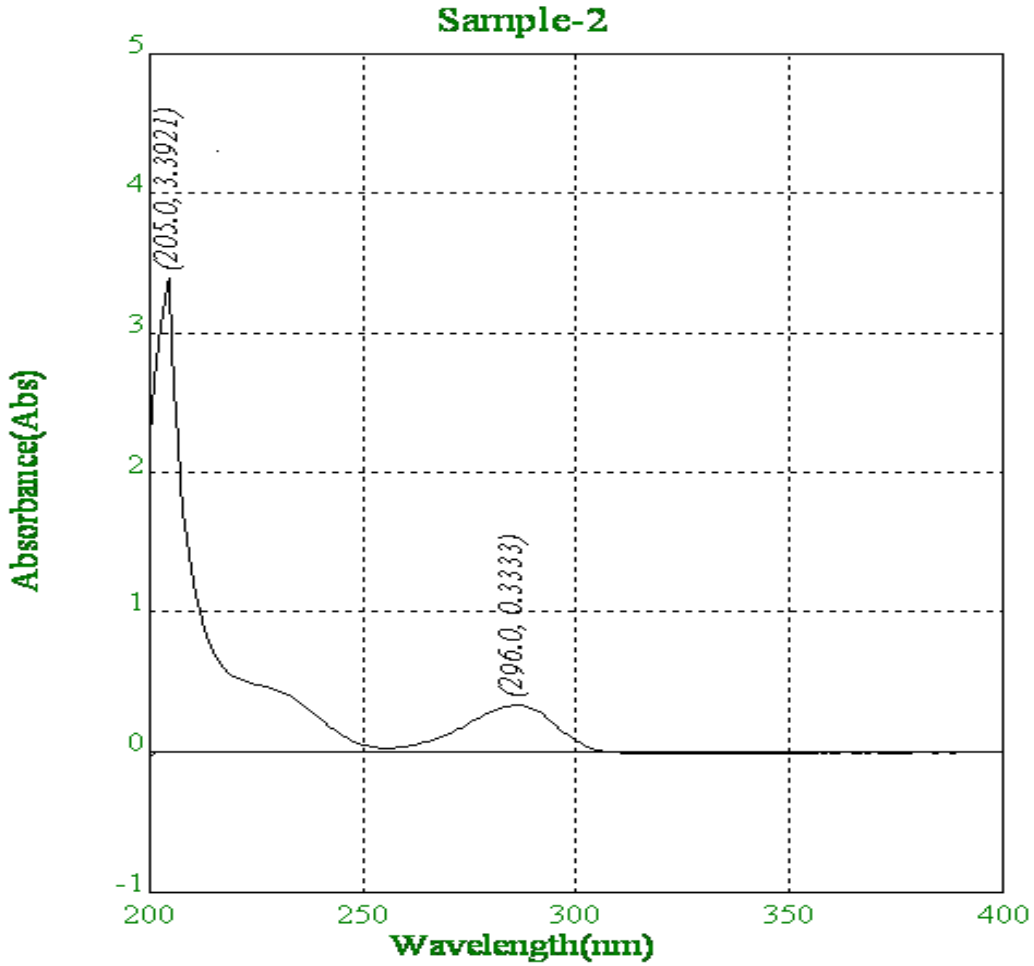


Figure No.2 UV Visible spectra of quetiapine Fumarate in Methanol

The following chromatographic conditions were established by trial and error and were kept constant throughout the experimentation.

Table 2: Chromatographic condition

Sr. No	Parameter	Description
1	HPLC	AGILENT(1100)
2	FORMT ware	CHEMSTATION
3	Column	-id 4.6*250mm length
l5	Mobile Phase	0.05 % OPA= 45:55
6	Detection wavelength	296 nm
7	Flow rate	0.7 ml/min
8	Temperature	25 °C
9	Sample size	20 µl
10	Buffer	0.05 % OPA

OPTIMIZED CHROMATOGRAPHIC BATCH:

Table.3 : Optimized Chromatographic Conditions

Sr. No	Parameter	Description
1	HPLC	Agilent Tech. Gradient System with Autoinjector
3	Detector	UV (DAD) G13148 S.NO. DE71365875
4	Pump	Quaternary Gradient
5	Column	4.6 x 250 mm
6	Stationary Phase	Sunfire C18
7	Mobile Phase	MEOH + 0.1 % OPA (45:55 % v/v)
8	Detection Wavelength	296 nm.
9	Sample size	20 µl
10	Flow rate	0.7 ml/min

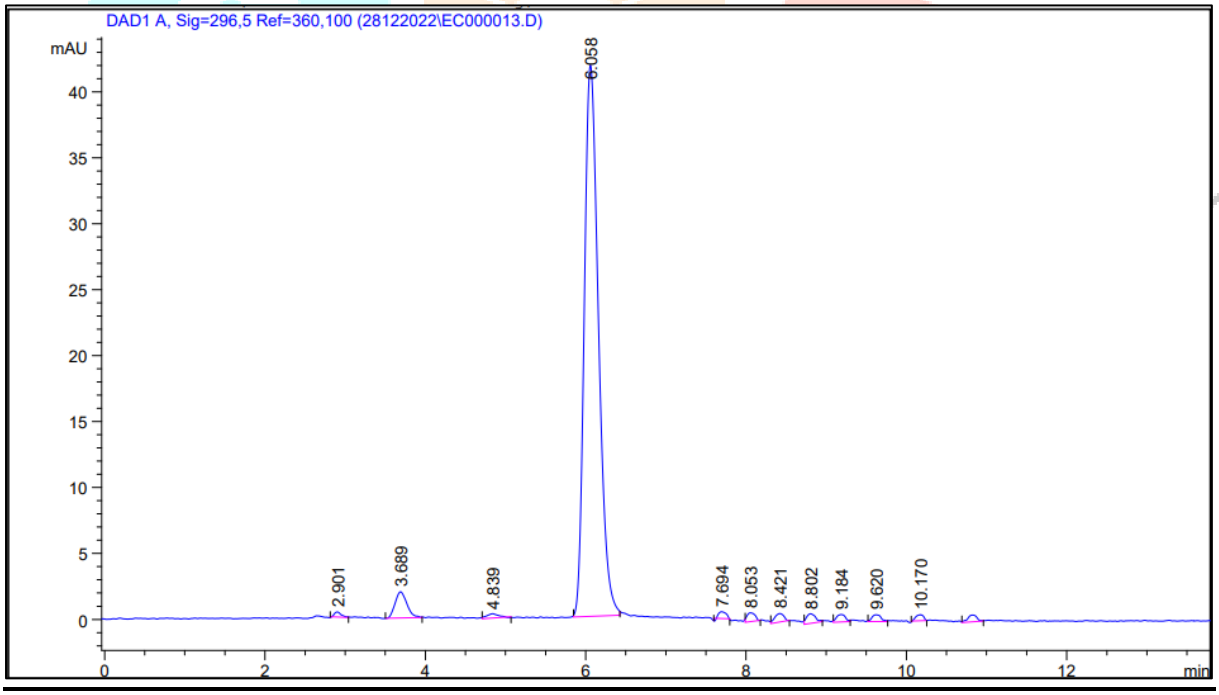


Figure No.3: Optimized Chromatogram of Quetiapine fumarate in Plasma

Table 4. Optimized method for RP-HPLC

Retention time (min)	Area	Height	Symmetry	Width	Plates	Resolution	selectivity
10.825	4.42050	5.28333	1.12	0.1200	45078	3.26	1.06

6. Method Validation Results:

The developed method was evaluated using ICH guidelines for specificity, linearity, range, accuracy, precision, LOD, LOQ, and robustness.

A. Linearity

As the concentration of the drug increases area under the curve also increases.

Table 5 : Linearity results

Concentration (ug/ml)	Area	Mean	SD	%RSD
5	173.73	185.20	0.11	0.06
10	362.80	374.27	3.03	0.81
15	557.26	568.73	4.82	0.85
20	749.57	761.04	1.66	0.22
25	949.57	961.04	6.10	0.63

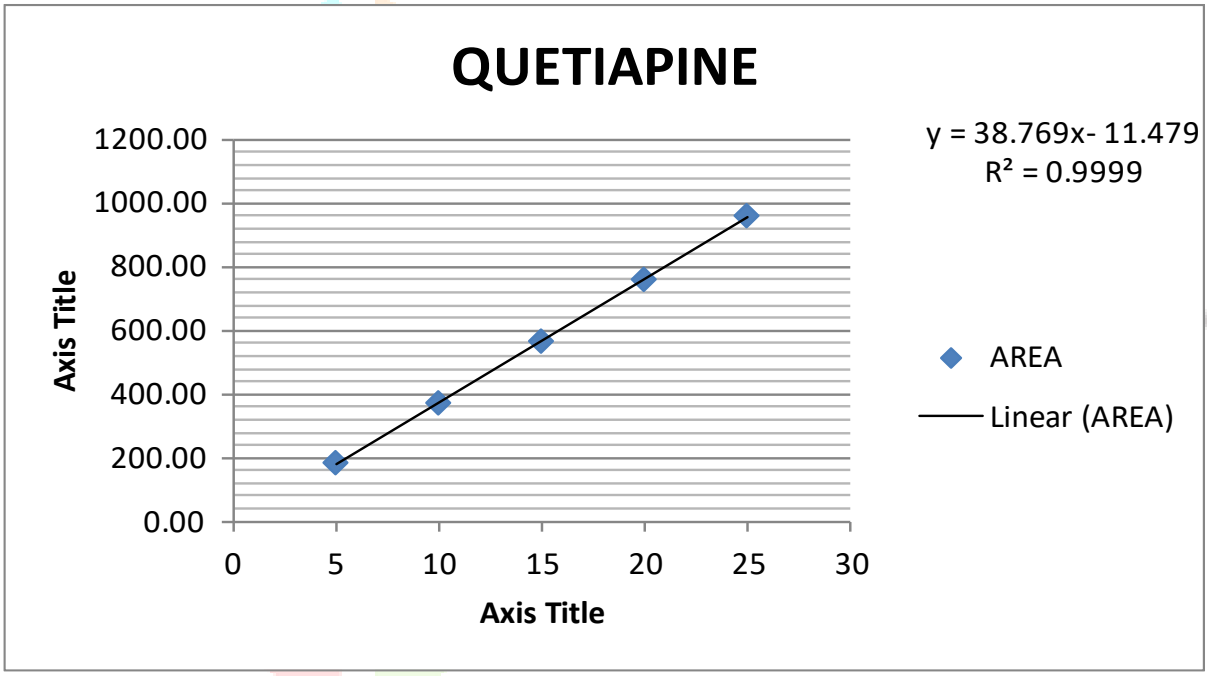


Fig No.3 Calibration Curve for Quetiapine fumarate

B. Accuracy:

As per Q2(R1), The accuracy for assay of a drug substance can be studied from 80 to 120% of the test solution.

Table 6 : Accuracy results

Method	Drug	Level (%)	Amt. taken (ug/ml)	Amt. taken (ug/ml)	Absorbance (Mean* ± S.D.)	Amt. recovered (Mean *±S.D.)	%Recovery (Mean *± S.D.)
RP-HPLC METHOD	QUET	80%	5	4	9.00±0.039	4±0.039	100.08±0.99
		100%	5	5	9.96±0.014	4.96±0.014	99.21±0.28
		120%	5	6	10.98±0.004	5.98±0.004	99.62±0.07

% Recovery = 100.037%, Acceptance Criteria 98%- 102%

C. Precision

Table 7: Precision results

Concentration(µg/ml)	Area	%Amt Found	% RSD
15	586.84	100.94	0.0020
20	779.92	100.61	0.0966
25	971.33	100.24	0.1374

Acceptance Criteria: Assay Value= 90-110%, % RSD=not more than 2

D. Robustness:

Table 8 : Robustness results

Parameters	Conc. (µg/ml)	Amt. of detected (mean± SD)	% RSD
Chromatogram of flow change 0.6 ml	15	13.78 ± 1.54	0.11
Chromatogram of flow change 0.8 ml	15	507.66±0.82	0.16
Chromatogram of comp change wavelength change 295 nm	15	725.1±0.24	0.03
Chromatogram of comp change wavelength change 297 nm	15	1244.41±0.73	0.06
A Chromatogram of mobile phase change 44+56 ml	15	577.5±0.57	0.57
Chromatogram of mobile phase change 46+54 ml	15	575.90±1.40	0.24

E. Repeatability

Table 9: Repeatability results

Concentration(µg/ml)	Area	%Amt Found	% RSD
10	386.88	99.810	0.27

F. LOD and LOQ

Standard deviation (σ) = 3.14, S = 38.76 (Slope)

LOD = 3.3 X Avg.SD/ Slope **LOQ** = 10 X Avg.SD/Slope

LOD = 0.267664 **LOQ = 0.811103**

slope- 38.76 **Inercept-11.47** **Regression-0.999**

7. Conclusion

This developed RP- HPLC bioanalytical method for QTP in human plasma was validated according to ICH guidelines in terms of linearity, precision, accuracy, robustness, and repeatability. All validation parameters were found to be within the allowed limit according to ICH guidelines. The approach created was exact, repeatable, precise, and particular. The approach is also used to test product stability and quality in a variety of physical lab combinations.

8. Acknowledgement:

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9. References:

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