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DEVELOPMENT AND VALIDATION OF SIMPLE HPTLC – UV ASSAY METHOD FOR DETERMINATION OF QUETIAPINE FUMARATE CONCENTRATIONS IN SIMULATED PLASMA FLUID

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ABSTRACT

Background: Quetiapine fumarate (QTF) is a high-affinity monoaminergic antagonist selective for serotonin Type 2 (5HT2) and dopamine Type 2 (D2) receptors. In this paper, we formulated and validated a simple, reproducible, and convenient procedure for determining QTF concentration in Simulated Plasma Fluid using HPTLC-UV. **Methodology:** Simulated plasma samples do not require deproteinization. A simulated plasma fluid sample was prepared using a one-step filtration method with a 0.45 µm nylon syringe filter. HPTLC chromatographic separation of test plasma samples was achieved by TLC silica gel aluminium plates 60 F254, which served as the stationary phase. **Results and Discussion:** The mobile phase consisted of a mixture of methanol and acetonitrile (3:7 v/v), followed by densitometric detection at 296 nm. Well, separated peaks have been noted with retardation factors (Rf) of 0.62. Calibration plots were found to be highly linear (Correlation coefficient $r^2 > 0.99$) in the concentration interval of 20–120 ng/mL. Inter and intraday assay precision and accuracy were below 2%. The proposed method avoided the use of a buffer and employed low volumes of simulated plasma samples with plain mobile phase composition. **Conclusion:** The developed HPTLC–UV assay method was found to be simple, accurate, and reproducible for determining Quetiapine Fumarate in simulated plasma fluid. Validation results confirmed the method's specificity, linearity, precision, and robustness as per ICH guidelines. This method can be effectively applied in routine bioanalytical studies and drug monitoring.

INTRODUCTION

Quetiapine fumarate (QTF), 2-[2-(4-benzo[b] [1,4] benzothia-zepin-6-ylpiperazin-1-yl) ethoxy] ethanol;(E)-but-2-enedioic acid, is an atypical antipsychotic medication in a second generation of chemicals with a particular receptor-binding profile. That is utilized for schizophrenia as well as for acute manic episodes of bipolar disorder, either as monotherapy or in combination with other drugs (Figure 1) [1,2]. After the ingestion of an oral dose, it is rapidly and effectively absorbed. Quetiapine fumarate is 83% bound to protein and is metabolized in the liver. Oxidation and sulfoxidation are the major metabolic pathways of this drug. The drug was excreted through urine in

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approximately 73% and faeces in approximately 20% [3]. Simulated biofluids (such as simulated plasma fluid) are a possible technique for assessing the solubility of a dosage form in vitro conditions. They can determine dosage form's stability (biodurability), release mechanisms (bioaccessibility), and potentially even forecast the behavior in vivo [4]. Various reported techniques for determining QTF in human and rat plasma samples have been used, including HPLC [5], UHPLC [6], and LC-MS/MS [7]. There is no reported method for HPTLC method development and validation of QTF in simulated plasma samples in the vast literature survey. Therefore, quantification of QTF in simulated plasma fluid concentrations is essential for therapeutic drug monitoring. In this case, we established a reproducible, easy, and sensitive ultraviolet detection high-performance thin-layer chromatography (HPTLC-UV) method for quantifying QTF in simulated plasma fluid samples, making this analytical tool suitable for pharmacokinetic and research investigations. Our procedure employed a single step of filtration, a small simulated plasma volume (10 µl), compared to the 0.5-1 ml sample volume used in most procedures [8-11]. Additionally, a simple methanol/acetonitrile mixture served as the mobile phase, in contrast to the buffers typically used in most procedures [12-13]. In addition, this paper includes sections on materials and methods, results, and discussion. Finally, it concludes with successful results.

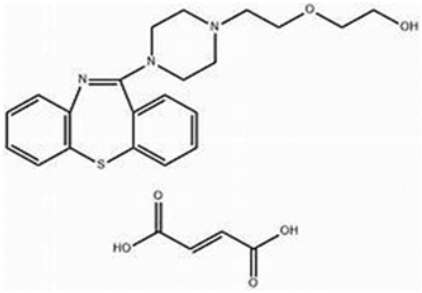


Figure 1: Quetiapine fumarate chemical structure

MATERIALS AND METHODS

Chemicals and Reagents

Quetiapine fumarate (purity > 98%) was provided by Torrent Pharmaceuticals (Ahmedabad, Gujarat, India), methanol, acetonitrile (HPLC grade), water, toluene, sodium chloride, sodium bicarbonate, potassium chloride, potassium phosphate dibasic trihydrate, magnesium chloride hexahydrate, hydrochloric acid (1 M), calcium chloride, sodium sulphate and of tris(hydroxymethyl) aminomethane was received from Central Drug House (P) Ltd. (Mumbai, India).

Preparation of simulated human blood plasma pH 7.4

The simulated human blood plasma pH 7.4 was prepared by dissolving 8.035 g of sodium chloride, 0.355 g of sodium bicarbonate, 0.225 g of potassium chloride, 0.231 g of potassium phosphate dibasic trihydrate, 0.311 g of magnesium chloride hexahydrate, 39 ml of 1 M hydrochloric acid, 0.292 g of calcium chloride, 0.072 g of sodium sulphate and 6.118 g of tris(hydroxymethyl) aminomethane and distilled water was added 1000 ml until a final volume achieved [14].

Instrument

The experiment was performed on a HPTLC CAMAG TLC system (CAMAG, Muttenz, Switzerland) including CAMAG Linomat V Sample Applicator connected to CAMAG microliter Syringe, CAMAG twin trough developing chamber, TLC visualizer 2, and scanning with TLC scanner 3. The HPTLC chromatographic separation was done on a silica gel G plate (plate size: 10 × 20 cm) pre-coated with normal-phase silica gel (particle size: 5 µm) 60F 254 plates. Vision CAT version 3.2 was employed in data acquisition and integration of analysis.

Chromatographic Analysis

A mixture of methanol and acetonitrile (3:7, v/v) was used as the mobile phase for QT F-separation. The rate of application for estimating QTF was set at 160 nL/s. HPTLC plates were developed in-line in ascending mode with an 80 µm spacing. The development chamber was saturated with vapours of the respective mobile phases for 20 min at 25 °C. Detection of QTF was carried out using a 296 nm wavelength. The scanningspeedand slitsizewere bothfixedat20 mm/s and 5 × 0.45 mm^2, respectively. 3 or 6 replicates were used for each estimate.

Preparation of Standard and Working Solutions

QTF was dissolved in methanol to obtain a 1 mg/mL stock standard solution. Working solutions of 50 ng/mL were obtained by further dilution of the stock solutions using HPLC-grade methanol. The stock and working solutions were freshly prepared daily.

Preparation of Calibration Concentrations and Quality Control (QC) Samples

We made serial dilutions of QTF concentrations in blank simulated plasma to produce calibration curves. QTF concentrations were 20-120 ng/band. We prepared four QC samples for method validation: a low limit of quantification sample (LLOQ), a low-level QC sample (LQC), a middle-level

QC sample (MQC, within the middle range of calibration concentration), and a high-level QC sample (HQC, near the upper end of calibration concentration).

Sample Preparation

The simulated plasma fluid was prepared. QTF was dissolved in simulated plasma with the help of vortexing for 1 min. The solution was filtered through a 0.45 µm nylon syringe filter. Hence, a working solution was obtained by further dilution of the stock solutions using HPLC-grade methanol.

Method Validation

The developed procedure was validated in accordance with the International Council for Harmonisation guidelines on bioanalytical method validation (ICH, 2022) [15]. We tested the linearity, sensitivity, precision, accuracy, and stability of the method.

Linearity

For method linearity evaluation, calibration curves were prepared by plotting the peak area ratios (QTF) against the calibration standard concentration of 0.05 mg/mL, using volumes ranging from 0.4 to 2.4 µL. Linear regression was employed to find the calibration curve parameters: slope, intercept, and the correlation coefficient (r^2).

Selectivity

We validated the selectivity of the method by observing the absence of any peaks at the analyte when blank plasma samples were injected. The LLOQ is the lowest concentration on the calibration curve that has precision of ≤ 20%, accuracy within ± 20%, and a signal at least 5 times greater than that of the blank plasma.

Sensitivity

Sensitivity of QTF in SPF was assessed in terms of Limit of Detection (LOD) and Limit of Quantitation (LOQ). LOD = 3.3× SD/S and LOQ = 10 × SD/S, where SD is the standard deviation of the response and S is the slope of the calibration curve.

Precision

To study the inter-day and intra-day precision of the method, six replicates of the mixed standards of the analytes at three QC levels (40, 80, and 120 ng/band) were analysed on the same day and on three consecutive days, respectively. The %RSD for all the levels must be ≤ 2.0%.

Stability

We established sample stability by comparing the values of newly prepared samples (80 ng/band, n = 6) analyzed before and after exposure to various conditions. Stability conditions were simulated to ensure equivalent conditions for sample handling, storage, and analysis. We evaluated the stability of QTF in simulated plasma at room temperature and at -20°C over a 30-day period. We also assessed the stability of the sample after three freeze–thaw cycles. Every cycle consisted of a 24-hour freeze and subsequent thaw at room temperature, and the cycle was repeated. Storage stability of the stock solution was also tested at 4°C and -20°C. An acceptable stability-to-reference samples ratio was between 85% and 115%, and an acceptable percent error was within ±15%.

Accuracy

The accuracy of the proposed method was determined using the standard addition method, which involved calculating the percentage recovery of the drug. The accuracy was evaluated in 6 replicates at three different concentration levels, i.e., 80%, 100%, and 120% of the active ingredients, by adding various concentrations of quetiapine fumarate standard to a known amount of sample and calculating recovery and %RSD for the drug.

RESULTS AND DISCUSSION

Optimization of Chromatographic Conditions

To maximize our method, we tested the effectiveness of varying solvent volumes, different detection wavelengths, and various mobile phase compositions. To enhance QTF peak separation, mobile phase compositions of 8:2, 7:3, 6:4, 5:5, and 3:7 v/v methanol: acetonitrile were tested. The composition of 3:7 methanol: acetonitrile v/v produced the optimum separation and peak intensity of QTF at Rf 0.62 ± 0.02, as seen in Figure 2. Plasma non-interfering peaks, however, were also visible in the chromatogram. But good linearity calibration curves were obtained. To further increase peak intensity, we tested five detection wavelengths: 290, 293, 292, 296, and 294 nm. Among them, the 296 nm produced the maximum peak intensity for QTF simulated plasma samples.

Method Validation: Linearity

Method linearity assessment was carried out by analysing simulated plasma and pure samples across the QTF calibration ranges. Peak area (AU) towards concentration fitted well to a

straight line for calibration curves that demonstrated high linearity, r2 = 0.998, for the concentration range of 20 – 120 ng/band, and the regression equation: $y = 1.513 \times 10^{-8} + 1.597 \times 10^{-4}$ was found. RSD% % of slop and RSD% of intercept were found to be 2.08 and 1.03, respectively, as shown in Table 1.

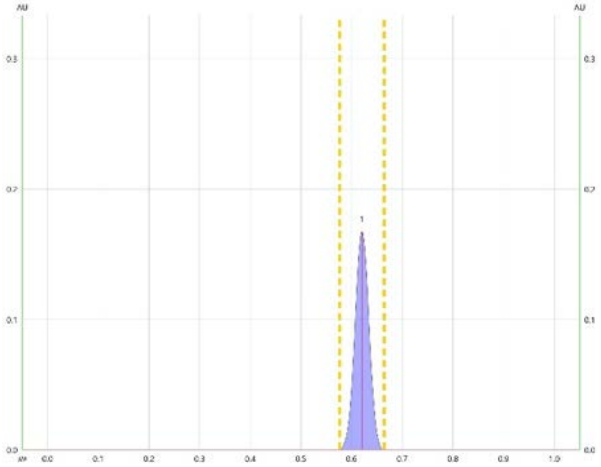


Figure 2: A typical chromatogram of QTF

Table 1: Linearity study of QTF in SPF

S.No.	Parameter	Observation
1.	Linearity range	20 – 120 ng/band
2.	Regression equation	y = 2E-05x + 2E-05
3.	Coefficient of variation	1.73 %
4.	Correlation coefficient	0.998
5.	RSD % of slop	2.08
6.	RSD % of intercept	1.03

n=6

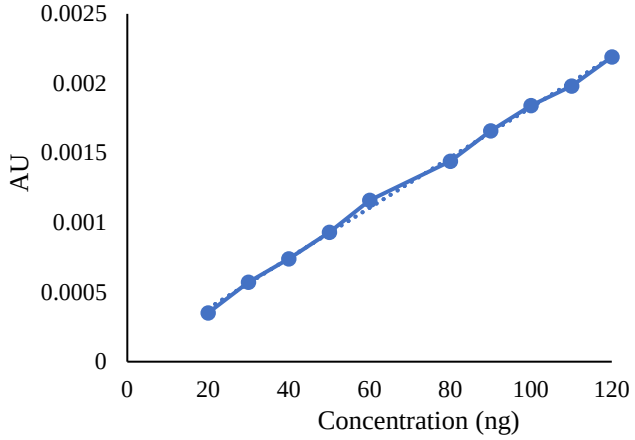
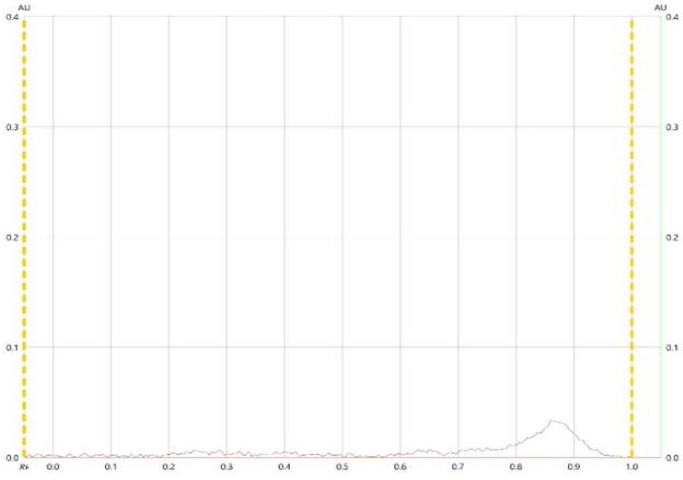


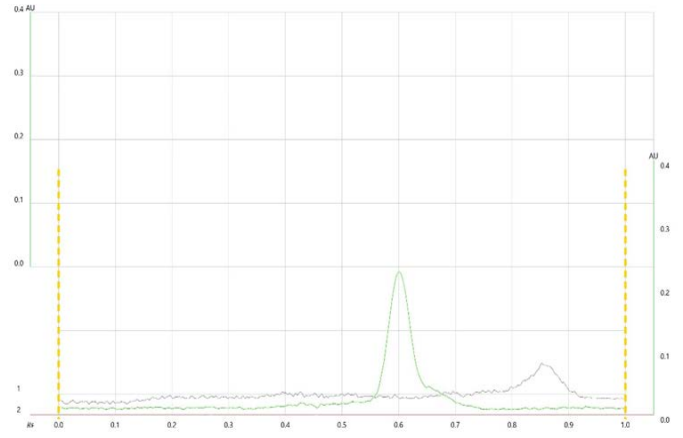
Figure 3: A representative calibration curve of Quetiapine fumarate throughout the range 20–120ng/band.

Selectivity

The specificity of the method was ascertained by analyzing a drug sample (tablet formulation) of quetiapine fumarate and simulated plasma fluid. The results suggested that the proposed method is specific; the excipients present in the formulation and components of simulated plasma fluid do not affect the outcome. The chromatogram was taken by running the drug sample (tablet formulation) and simulated plasma fluid.



(A)



(B)

Figure 4: (A) A simulated blank plasma fluid chromatogram; (B) a chromatogram showing a sample of simulated blank plasma fluid spiked with 80 ng/band Quetiapine fumarate (QTF). Simulated blank plasma fluid & QTF Rf value were found to be 0.85 and 0.62, respectively.

Sensitivity

Sensitivity in terms of LOD and LOQ was found to be 5.86 ng and 14.92 ng for QTF in SPF.

Precision

The precision of the developed method was determined by replicate analyses (n = 6) of quality control samples containing 40, 80, and 120 ng/band of QTF in SPF. The % RSD of QTF in SPF was found to be 1.33 – 1.59 % for intraday precision and 1.13 – 1.67 % for interday precision at 3 different concentrations. Results are shown in the table. 2.

Table 2: Intraday and interday precision for Quetiapine Fumarate.

Intraday			Interday	
Concentration (ng/band)	**Mean ± SD**	**% RSD**	**Mean ± SD**	**% RSD**
40	0.00066 ± 0.00001	1.51	0.00088 ± 0.00001	1.13
80	0.00150 ± 0.00002	1.33	0.00158 ± 0.000026	1.67
120	0.00215 ± 0.00003	1.39	0.00228 ± 0.00003	1.53

(n=6)

Stability

Stability results are presented in Table 3. QTF stock solutions were found to be stable for at least 2 months at -20°C, indicating that new stock solutions can be prepared every 2 months. In addition, room temp. Stability was established that QTF samples simulating plasma fluid remained stable at room temperature for 12 hours with an accuracy of ±15%, ensuring minimal breakdown of QTF over this period. After three freeze-thaw cycles, the accuracy and precision of QTF concentrations were also within ±15% of the nominal concentration. In addition, QTF extracted imitated plasma samples remained stable for a minimum of 1 month at -20°C or room temp. All data are shown in Table 3.

Table 3: Quetiapine fumarate stability testing

Stability Test	Conc.(ng/band)	Mean Calculated Conc. (ng/band) ± SD	CV (%)	% Error
Stock solution stability at -20°C (1 month)	80	76.39 ± 1.72	2.26	4.71
Stock solution stability at -20°C (2 months)	80	81.06 ± 1.90	2.34	1.32
Stability testing at room temperature (Post Preparative temp)	80	81.44 ± 2.81	3.45	1.80
Long-term stability at -20°C (3 months)	80	78.99 ± 3.63	4.59	1.27
Freeze-thaw stability	80	79.04 ± 3.90	4.93	1.21
Bench-top stability	80	80.80 ± 1.68	2.08	1.00

n=6, CV = coefficient of variation

Accuracy

Accuracy was determined by the standard addition method. This parameter was studied by adding a standard drug solution at three different levels of QTF (32, 40, 48 ng/band) to the pre-analyzed sample solution in six replicates. Chromatograms were obtained, and peak areas were assessed. The concentration of each drug and, thereby, the recovery was calculated from respective calibration curves. The %RSD for all the levels must be ≤ 2.0% [15]. The average percentage recovery ranged from 99.41% to 99.65%, as shown in Table 4.

Table 4: Results of accuracy studies of QTF in SPF

%Recovery	Target Conc.(ng/band)	Spiked Conc. (ng/band)	Final Conc. (ng/band)	Conc. Obtained (ng/band)	% Recovery	SD	%RSD
80 %	40	32	72	71.75	99.65	0.48	0.49
100 %	40	40	80	79.53	99.41	0.56	0.57
120 %	40	48	88	87.52	99.44	0.44	0.44

n=6

In the present study, we have described a simple, reproducible, and selective technique for quantitatively determining QTF in simulated plasma fluid samples by HPTLC-UV. The analysis of simulated plasma fluid samples can be carried out within 10 min, which is comparable or even better than the reported method, which has employed LC-MS and HPLC. Drug quantitation in biofluid samples requires sample pretreatment to eliminate interfering substances & proteins before quantification. Initial experiments were conducted to select a deproteinizing solvent, as its volume was determined. However, in the case of simulated plasma fluid samples, they do not require a deproteinizing solvent. In this procedure, a 0.45 µm nylon syringe filter step is used, ensuring adequate recovery. These procedures are easy, reproducible, and save time for sample preparation compared to human plasma samples. Additionally, a key benefit of the established method over the published HPLC methods is the seemingly reduced volume of simulated plasma samples required (10 µl compared to 0.5-1 ml in most methods) [8-11] and even mobile phase volume (10 ml), making this chromatographic procedure viable for regular monitoring of QTF in simulated plasma fluid samples. We also used a basic isocratic blend of methanol and acetonitrile without buffers (in

contrast to most reported procedures), which contributed to the ease of our procedure and had no impact on the separation performance. The use of buffers has several drawbacks, as altering the environment can be hazardous with frequent utilization, thereby increasing the complexity and expense of the procedure. In the HPLC technique, due to the time required for a long column wash after analysis, a column wash is necessary. However, in the HPTLC-UV technique, no column wash is needed, and the advantage is the ability to perform multiple sample analyses within a single time frame. QTF remained stable for at least 1 month in both stock solution and simulated plasma samples at room temperature, in the refrigerator, and at -20°C, ensuring safe handling, storage, and analysis of QTF using our validated method.

CONCLUSION

In the present research, a highly validated chromatographic HPTLC-UV technique was described for the accurate quantification of QTF in spiked plasma samples. The proposed method, which was developed, was straightforward, reproducible, and sensitive, respectively. It was concise, as we employed a single mobile phase composition containing no buffers and a single filtration step. This, aside from the minimal sample volume needed in analysis. Together, the ease of the technique reduces the expense and analysis time.

ABBREVIATION

QTF - Quetiapine fumarate, SPF – Simulated plasma fluid, TDM – Therapeutic drug monitoring

FINANCIAL ASSISTANCE

NIL

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTION

Aakanksha Sinha and S.J. Daharwal conceptualized the whole work. They contributed to experimental work. Aakansha Sinha did the analysis and wrote the original draft of the manuscript.. S. J. Daharwal contributed to the review and editing of the original draft of the manuscript.

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