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RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF FLUOXETINE HYDROCHLORIDE AND QUETIAPINE FUMARATE

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ABSTRACT

Objectives: The objective of the existing study was to develop a simple, precise, accurate, rapid, and economical UV Spectrophotometric and isocratic reversed-phase high performance liquid chromatography (RP-HPLC) method for the simultaneous estimation of fluoxetine HCl and quetiapine fumarate in synthetic mixture. **Methods:** In UV Spectrophotometric method 0.1N HCl is used as solvent. Method I is based on simultaneous equation method, known as Vierodt's method. Method II is based on principle of Q-analysis, known as absorbance ratio method. Isocratic RP-HPLC separation was achieved on an Hibar R 250 × 4.6 mm HPLC column Purosphens R STAR RP-18, using a mobile phase of phosphate buffer (KH_2PO_4 and K_2HPO_4):acetonitrile (55:45v/v) at a flow rate of 1.0ml/min. The method was used successfully for the simultaneous determination of fluoxetine HCl and quetiapine fumarate in synthetic mixture. **Result:** In method I Fluoxetine HCl and quetiapine fumarate show absorbance maxima at 228 nm and 254 nm. In method II both drugs was measured at 233 nm (Isobestic point) and 254 nm (λmax of Quetiapine fumarate). In this method absorbance of Fluoxetine HCl and Quetiapine fumarate obeys Beer's law in the concentration range of 5 to 30µg/ml. The RP-HPLC method results in excellent separation with good resolution between the two analyte. The retention times of fluoxetine HCl and quetiapine fumarate was found to be 6.667 and 4.458 mins. **Conclusion:** The developed methods are precise, accurate, rapid, simple, reproducible and economical for simultaneous estimation of fluoxetine HCl and quetiapine fumarate in synthetic mixture.

KEYWORDS: Fluoxetine HCl, Quetiapine fumarate, Simultaneous estimation method, Absorbance ratio method, Validation, RP-HPLC.

INTRODUCTION

Fluoxetine HCl (FLX) {(RS)-N-methyl-3-phenyl-3-[4-(trifluromethyl) phenoxy] propan-1-amine} hydrochloride (Fig.1) is used as an antidepressant of the selective serotonin reuptake inhibitor (SSRI).[1] It is approved for treatment of major depression (including pediatric depression), obsessive compulsive disorder, posttraumatic stress disorder, panic disorder, body dysmorphic disorder, premenstrual dysphoric disorder, bulimia nervosa and trichotillomania.[2] Fluoxetine can be used with an antipsychotic for bipolar. Caution should be taken when using any SSRI for bipolar disorder as this can increase the like hood of mania. The mode of action of fluoxetine is predominantly that of a serotonin reuptake inhibitor. Fluoxetine is delays the reuptake of serotonin, resulting in serotonin persisting longer when it is released. Fluoxetine may also produce some of its effects via its weak 5-HT_2C receptor antagonist effects. Fluoxetine acts as an agonist of the σ_1-receptor, with potency greater than that of citalopram, but less than that of fluvoxamine.[2]

Quetiapine fumarate (QTF) 2-(2-(4-dibenzo [b, f] [1,4]thiazepine-11-yl1piperazinyl)ethoxy) ethanol fumaric acid (Fig.2) is a dibenzothiazepine class of derivative used as a antipsychotic.[3] The mode of action of Quetiapine fumarate, as with other drug used to treat schizophrenia is unknown. Drug having efficacy in treatment of schizophrenia and bipolar disorder is mediated through a combination of dopamine type 2(D2) and serotonin type 2 ($5HT_2$) antagonisms.[4] Antagonism at receptor other than dopamine and serotonin may explain some other effects of its due to antagonism at histamine (H_1) receptor and antagonism at adrenergic (α_1b) receptor which results in orthostatic hypotension whereas antagonism at muscarnic (M_1) receptor shows anticholinergic effects.[5]

The review of literature revealed that several methods are available for the determination of Fluoxetine and QTF individually. Reported methods for estimation of Fluoxetine were Spectrophotometric[6], HPLC[7,8], HPTLC[9], and LC-MS[10] and for QTF were Spectrophotometric[11], HPLC[11] and HPTLC.[12] But there is no any analytical method yet reported for simultaneous estimation of these drugs in combination.

UV Spectrophotometric method

MATERIALS AND METHODS

UV-Visible double beam spectrophotometer, (Jasco model 2201) with spectral bandwidth of 1 nm, wavelength accuracy of ± 0.3 nm and a pair of 1mm matched quartz cell was used. The commercially available fluoxetine HCl and quetiapine fumarate was procured from local market.

Preparation of standard stock solution and calibration curve

The standard stock solution of FLX and QTF were prepared by dissolving 10 mg of FLX and QTF in 0.1 N HCl in a separate 100 ml volumetric flask and final volume was adjusted with the same solvent in 100 ml of volumetric flask to get a solution containing 100 µg/ml of FLX and 100 µg/ml of QTF respectively.

Working standard solutions of 10µg/ml for FLX and QTF were scanned in the entire UV range of 200-400 nm to determine their λmax. The λmax of FLX and QTF is found to be 228 nm and 254 nm respectively and isobestic point is at 233 nm from overlain spectra as shown in Fig.3. Six working standard solutions with concentration 5, 10, 15, 20, 25, 30µg/ml for FLX and QTF were prepared in 0.1N HCl from stock solution. The absorbance of resulting solutions were measured at their respective λmax and isobestic point and plotted a calibration curve to get the linearity and regression equation as shown in Fig. 4 and 5.

Method I (Simultaneous equation method)

Simultaneous equation method of analysis is based on the absorption of both drugs at their wavelength maximum. Two wavelengths selected for the development of the simultaneous equation are 228 nm and 254 nm. The absorptivity values were determined for FLX are 0.14621(ax_1), 0.08121 (ax_2) and for QTF are 0.07191 (ay_1), 0.1771 (ay_2) at 228 nm and 254 nm respectively. These values are means of six estimations. The absorbances and absorptivity at these wavelengths were substituted in equation 1 and 2 to obtain the concentration of these drugs.

$$C_{FLX} = \frac{(A_2 \times ay_1) - (A_1 \times ay_2)}{ax_2 \times ay_1 - ax_1 \times ay_2} \quad \text{.....Eqn.1}$$

$$C_{QTF} = \frac{(A_1 \times ax_2) - (A_2 \times ax_1)}{ax_2 \times ay_1 - ax_1 \times ay_2} \quad \text{.....Eqn.2}$$

Where C_{FLX} and C_{QTF} are concentration of FLX and QTF respectively in µg/ml. A_1 and A_2 were the absorbance of the sample at 228 nm and 254 nm respectively.

Method II (Absorbance ratio method)

Absorbance ratio method of analysis is based on the absorbance at two selected wavelengths, one of which is an isobestic point and the other being the absorption maximum of one of the two drugs. From overlain spectra (**Fig.3**) 233 nm (isobestic point) and 254 nm (λmax of QTF) are selected for the formation of Q absorbance equation (Eqn.3 and 4). The absorptivity values determined for FLZ are 0.08121 (ax_1), 0.04958 (ax_2) and for QTF are 0.17710 (ay_1), 0.17794 (ay_2) at 254 nm and 233 nm respectively. These values are means of six estimations. The absorbance and absorptivities at these wavelengths were substituted in equation 3 and 4 to obtain the concentration of these drugs.

$$C_{FLX} = \frac{Q_M - Q_Y}{Q_Y - Q_X} \times \frac{A_1}{ax_1} \quad \text{..... Eqn.3}$$

$$C_{QTF} = \frac{Q_M - Q_X}{Q_X - Q_Y} \times \frac{A_1}{ay_1} \quad \text{..... Eqn.4}$$

Q_M, Q_X and Q_Y were obtained from Eqn.no.5, 6, 7 respectively.

$$Q_M = \frac{A_2}{A_1} \quad \text{..... Eqn.5}$$

$$Q_X = \frac{ax_2}{ax_1} \quad \text{..... Eqn.6}$$

$$Q_Y = \frac{ay_2}{ay_1} \quad \text{..... Eqn.7}$$

Where C_{FLX} and C_{QTF} are concentration of FLX and QTF respectively in µg/ml. A_1 and A_2 were the absorbance of the sample at 233 nm and 254 nm respectively.

Validation of developed methods[13]

Linearity

For each drug, appropriate dilutions of standard stock solutions were assayed as per the developed method. For method I and II, the Beer-Lambert's concentration range was found to be 5-30 µg/ml for both FLX and QTF. The linearity data of both methods are presented in **Table.1.**

Accuracy

To check the accuracy of the proposed methods, recovery studies were carried out at 80, 100 and 120% of the test concentration as per ICH guidelines. The recovery study was performed three times at each level. The results of the recovery studies are shown in **Table.2.**

Repeatability

To check the degree of repeatability of these methods, suitable statistical evaluation was carried out. Repeatability was performed for six times with synthetic mixture. The standard deviation and coefficient of variation were calculated. The results of statistical evaluation are given in **Table.2.**

Intermediate Precision (Interday and Intraday precision)

The Interday and intraday precision was determined by assay of the sample solution on the same day and on different days at different time intervals respectively. The results of the same are presented in **Table.3**.

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

The LOD and LOQ of Fluoxetine HCl and Quetiapine fumarate by proposed methods were determined using calibration standards. LOD and LOQ were calculated as 3.3 σ/S and 10 σ/S respectively. Where S is the slope of the calibration curve and σ is the standard deviation of response. The results of the same are quoted in **Table.3**.

RP- HPLC method

Instrument

HPLC system (PU2080HPLC2000, JASCO, Power requirement: 230V, 50Hz) with Jasco PU-2080 Plus (intelligent HPLC Pump), Jasco UV-2075 Plus Intelligent UV/Vis detector wit column was employed. BROWIN CHROMATOGRAPHY SOFTWARE was used for data acquisition and processing.

Analytical column

Fluoxetine HCl and quetiapine fumarate was analyzed by reverse phase-HPLC analysis using HiQ sil C18 HS size 4.6 mm inner diameter 250 mm length, No.OH500218.

Chemical and Reagents: All analytical grade reagents were used.

Chromatographic Conditions

A mixture of phosphate buffer and acetonitrile in the ratio of (55:45 v/v) was used as mobile phase and pH 5.8 adjusted with ortho-phosphoric acid. It was filtered through 0.45 µ membrane filter. The flow rate used was 1 ml/min. The injected volume was 20 µl. Run time used was 20 min.

Preparation of mobile phase

Buffer preparation

Weigh accurately about 1.625 gms of potassium di hydrogen ortho phosphate and 0.3 gms di potassium hydrogen ortho phosphate and dissolve with 200 ml of HPLC grade water than make up to 550 ml with HPLC grade water than adjust the pH 508 with Ortho Phosphoric Acid.

Mobile phase

Then add 55 volume of buffer, 45 volume of acetonitrile and sonicated for 15 min and filtered through a 0.45 µ membrane filter.

Preparation of standard stock solution

Standard stock solutions for each drug were prepared separately by dissolving 25 mg of drugs in mobile phase up to 25 ml. The volumetric flasks having 10 ml of mobile phase with the drugs were shaken, sonicated for 5 min and finally volume was made up to get a concentration of 1000 µg/ml. Standard drugs solutions were filtered through a 0.45 µ membrane filter.

Working standard solution

Working standard solutions were prepared in the range of 100-400 µg/ml for both Fluoxetine hydrochloride and Quetiapine fumarate.

RESULTS AND DISCUSSION

Linearity of FLX and QTF is upto 30 µg/ml at respective selected wavelengths. The coefficient of correlation for FLX at 228 nm and for QTF at 254 nm is 0.999 and 0.998 respectively. Both drugs showed good regression values at their respective wavelengths and the results of recovery study revealed that any small change in drug concentration in the solution could be accurately determined by the proposed methods. The validity and reliability of proposed methods were assessed by recovery studies. Sample recovery for both these methods is in good agreement with their respective label claims, which suggest non interference of formulation additives in estimation as shown in **Table.2.**

Precision was determined by studying the repeatability and intermediate precision. Repeatability result indicates the precision under the same operating condition over a short interval of time and interassay precision. Intermediate precision study expresses within

laboratory variation in different days. In both intra and inter day precision study for both the methods % COV is not more than 2.0 % indicates good repeatability and intermediate precision as shown in Table 3. The LOD and LOQ values for FLX and QTF for method I and method II were calculated and are quoted in Table 3. Low values of LOD and LOQ indicates good sensitivity of proposed methods.

Method development and optimization

Method development process was carried out by examining conditions like flow rate. A flow rate of 1 ml/min gave an optimal signal to noise ratio with a reasonable separation time. Mobile phase compositions like phosphate buffer: acetonitrile and ratio (55:45v/v) was used. The drugs FLX and QTF were found showing a significant UV absorbance at 254 nm in phosphate buffer: acetonitrile (55:45v/v), so this wavelength was chosen for UV detection. By use of a C18 column it was found the mobile phase consisting of phosphate buffer: acetonitrile (55:45v/v) provided well defined peak shape with good resolution. The retention times for FLX and QTF was found to be 6.667 and 4.458 min respectively. The representative chromatograms of pure drug and combined drug product are shown in Fig. 6, 7 and 8 respectively. The chromatographic data of both pure drug and combined drug product shown in Table 4, 5,and 6 respectively.

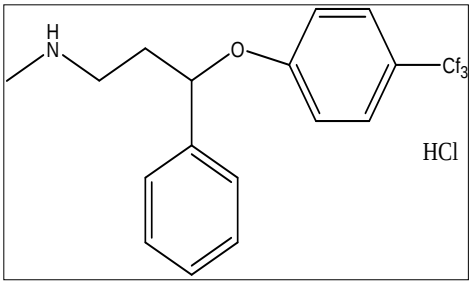

Figure 1: Fluoxetine hydrochloride.

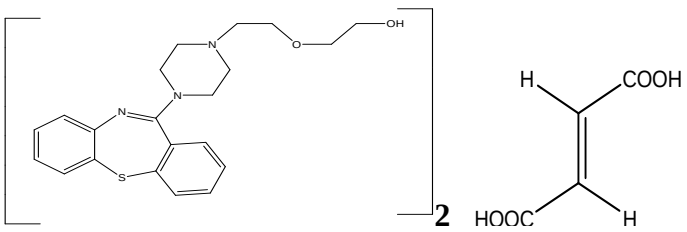

Figure 2: Quetiapine fumarate.

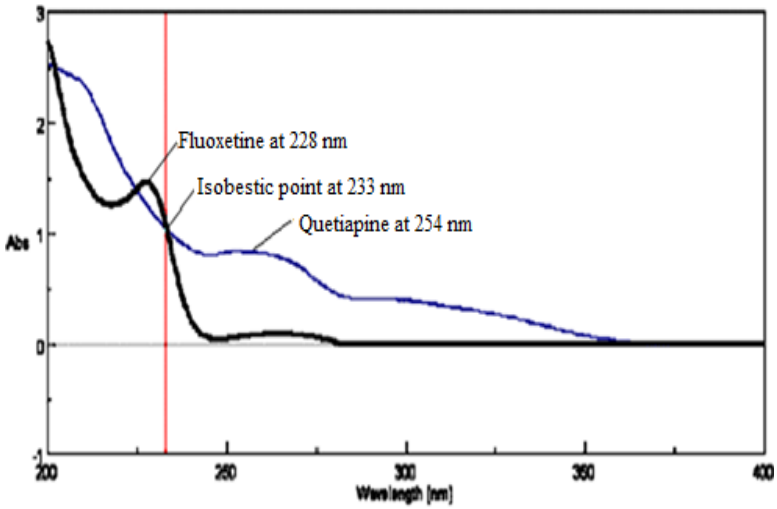

Fig 3: Overlain spectra of Fluoxetine HCl and Quetiapine fumarate.

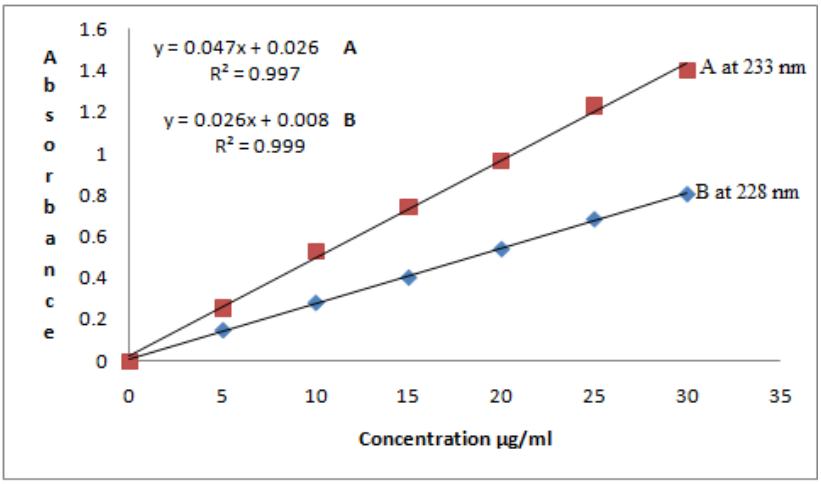

Fig 4: Calibration curve and regression equation of Fluoxetine in 0.1N HCl.

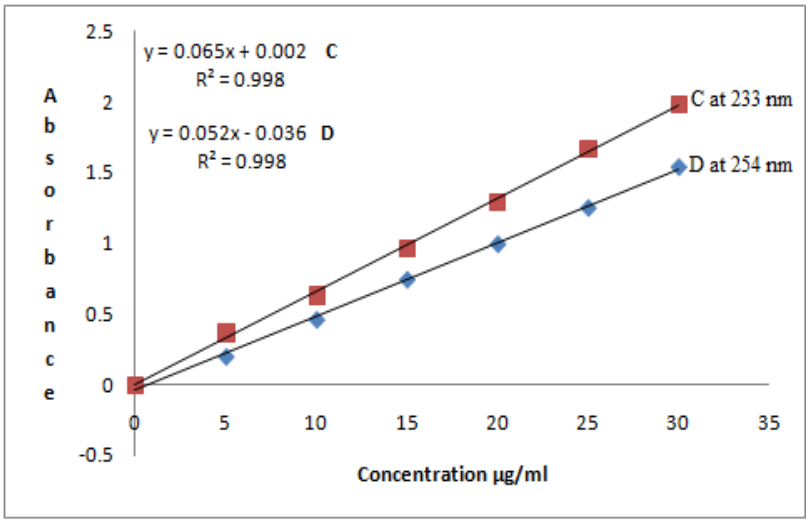

Fig 5: Calibration curve and regression equation of Quetiapine in 0.1N HCl

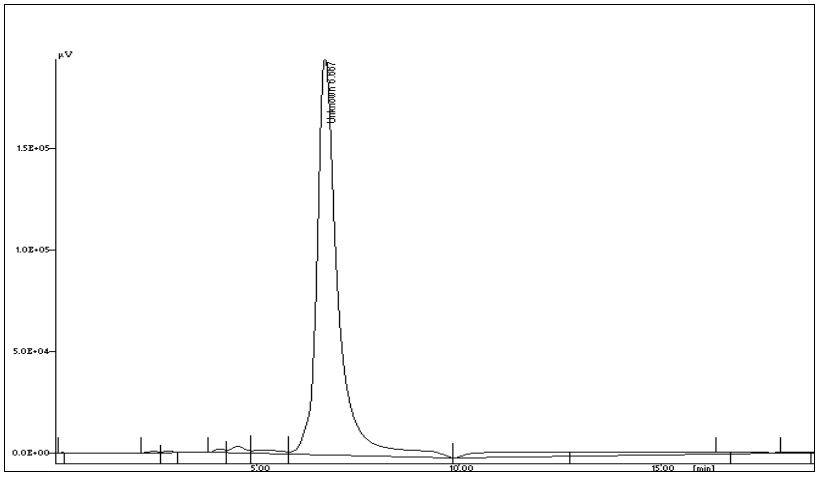

Figure 6: Representative chromatogram for Fluoxetine HCl (retention time =6.667).

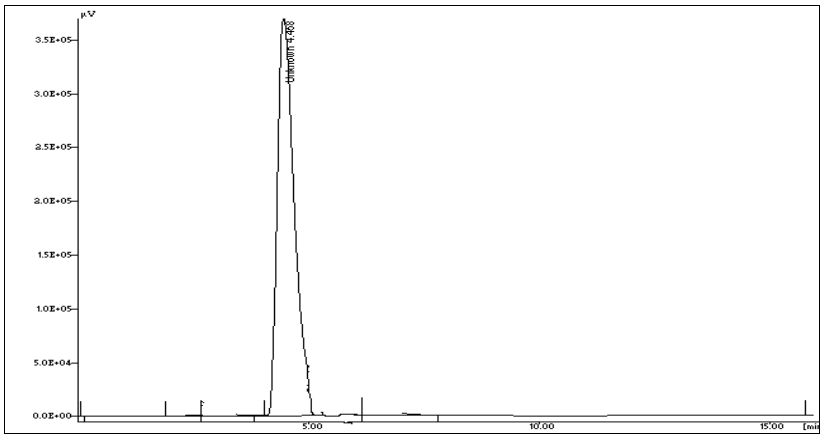

Figure 7: Representative chromatogram for Quetiapine fumarate (retention time = 4.458).

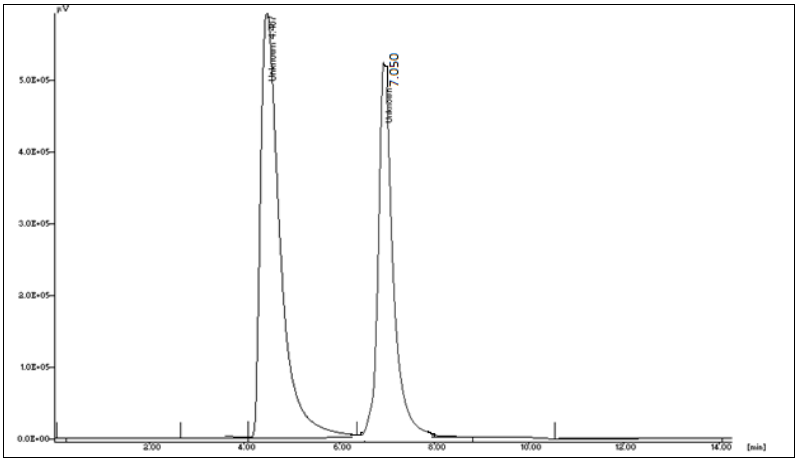

Figure 8: Representative chromatogram for Fluoxetine HCl (retention time = 7.050) and Quetiapine fumarate (retention time = 4.458).

Table 1: Optical Characteristics for Fluoxetine HCl and Quetiapine fumarate.

Parameters	FLX		QTF	
Working wavelength	**228nm**	**233nm**	**254nm**	**233nm**
Beer's law limit(µg/ml)	5-30	5-30	5-30	5-30
Absorptivity*	0.1462	0.0495	0.1771	0.1779
Correlation coefficient*	0.999	0.997	0.998	0.998
Intercept*	0.008	0.026	0.036	0.002
Slope*	0.026	0.047	0.052	0.065

**Average of six estimations.*

Table 2: Recovery studies.

Method	Level of recovery (%)	% Recovery ±S.D.#	
		FLX	QTF
I	80	98.5±0.45	99.45±0.82
	100	99.42±0.60	100.5±0.45
	120	99.60±0.97	101.2±0.54
II	80	98.56±0.54	100.54±0.23
	100	99.45±0.65	101.2±0.12
	120	100.56±0.78	99.60±0.45

#mean of three determinations, SD: Standard Deviation.

Table 3: Validation Parameters.

Method	Drug	LOD* µg/ml	LOQ* µg/ml	Precision (%COV)			
				Intraday n=3	Interday*		
					First day	Second day	Third day
I	FLX	0.25	0.24	0.4512	0.1547	0.9654	0.5498
	QTF	0.12	0.15	0.2314	0.2587	0.8954	0.7815
II	FLX	0.20	0.17	0.6589	0.1258	0.4587	0.2563
	QTF	0.22	0.19	0.7845	0.4568	0.7823	0.2541

*COV: Coefficient of Variation,*Average of six determination.*

Table 4: Chromatographic data for Fluoxetine HCl.

Name	RT	Area [µV.Sec]	Resolution	Plates	Capacity	Asymmetry
Fluoxetine HCl	6.667	6741923.220	1.84	1317.33	799.00	1.47

Total Area of Peak = 7359593.916 [µV.Sec]

Table 5: Chromatographic data for Quetiapine fumarate.

Name	RT	Area [µV.Sec]	Resolution	Plates	Capacity	Asymmetry
Quetiapine fumarate	4.458	9548053.938	2.66	836.60	534	1.81

Total Area of Peak = 10361730.697 [µV.Sec]

Table 6: Chromatographic data for Fluoxetine HCl and Quetiapine fumarate

Name	RT	Area [µV.Sec]	Resolution	Plates	Capacity	Asymmetry
Fluoxetine HCl	7.050	1809841.386	3.64	1347.38	845.00	1.91
Quetiapine fumarate	4.458	16269227.022	2.92	749.19	535.00	2.21

Total Area of Peak = 19501734.500 [µV.Sec]

CONCLUSION

The developed UV Spectrophotometric and RP-HPLC methods are found to be fast, sensitive, precise, and reproducible for simultaneous estimation of FLX and QTF. These methods are validated as per the ICH Guidelines.

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Conflict of Interest

Nil.

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