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DEVELOPMENT AND VALIDATION OF SPECTROPHOTOMETRIC METHODS FOR SIMULTANEOUS ESTIMATION OF OLANZAPINE AND FLUOXETINE FROM BULK AND TABLET DOSAGE FORM BY Q-ABSORBANCE RATIO METHOD

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Keywords:

Olanzapine, Fluoxetine, UV-Visible spectroscopy, Q-Absorbance Ratio method

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ABSTRACT: Depression is a common mental disorder that presents with depressed mood, loss of interest or pleasure, decreased energy, feelings of guilt or low self-worth, disturbed sleep or appetite and poor concentration with anxiety. Olanzapine is a second-generation antipsychotic (SGA) or atypical antipsychotic. It has attracted considerable interests due to its wide range of therapeutic and pharmacological properties and works in the brain to treat schizophrenia disorder, and may also be used in bipolar disorder and Schizophrenia. Fluoxetine is a class of selective serotonin reuptake inhibitors (SSRI) and works by blocking the absorption of the neurotransmitter serotonin in the brain. Regulating the amount of serotonin helps brain cells transmit messages to each other. This results in a better and more stable mood. For development and validation of Spectrophotometric Methods for Simultaneous estimation of Olanzapine and Fluoxetine from Bulk and Tablet Dosage Form was used Q-Absorbance Ratio method. Using the double beam instrument of Shimadzu –UV1900, as UV/Visible spectrophotometer method developed for simultaneous estimation of Q-Absorbance Ratio method. For the Q-Absorbance Ratio method Olanzapine at 288 nm for Olanzapine (λ_{max}) and 232.5 nm (Isosbestic point) for Olanzapine and Fluoxetine, at 288 nm Fluoxetine showed zero absorbance, and at 288 nm Olanzapine showed significant absorbance. The developed methods can be successfully applied for the determination of the amount of drugs present in tablet formulation.

INTRODUCTION: "Depression is a common mental disorder that presents with depressed mood, loss of interest or pleasure, decreased energy, feelings of guilt or low self-worth, disturbed sleep or appetite, and poor concentration.

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Moreover, depression often comes with symptoms of anxiety [1-3]. Olanzapine is a second-generation antipsychotic (SGA) or atypical antipsychotic, 2-methyl-4-(4-methylpiperazin-1-yl)-10H-thieno[2,3-b][1,5]benzodiazepine, has attracted considerable interests due to its wide range of therapeutic and pharmacological properties and works in the brain to treat schizophrenia disorder and may also use in bipolar disorder and Schizophrenia [3-5]. Fluoxetine, methyl ({3-phenyl-3-[4-(trifluoromethyl) phenoxy] propyl})amine Fluoxetine is a class of selective serotonin reuptake inhibitor (SSRI) and works by

blocking the absorption of the neurotransmitter serotonin in the brain. Regulating the amount of serotonin helps brain cells transmit messages to each other. This results in a better and more stable mood [6, 7]. The absorbance ratio method is a modification of the simultaneous equations procedure. It depends on the property that, for a substance, which obeys Beer's law at all wavelengths. Q-analysis is based on the relationship between absorbance ratio value of a binary mixture and relative concentrations of such a mixture. The ratio of two absorbance determined on the same solution at two different wavelengths is constant. This constant was termed as "Hufner's Quotient' or Q-value which is independent of concentration and solution thickness e.g., two different dilutions of the same substances give the same absorbance ratio A1/A2. In the USP this ratio is referred to as a Q value. In the quantitative assay of two components in admixture by the absorbance ratio method, absorbance is measured at two wavelengths, one being the λ_{max} of one of the components (λ2) and the other being a wavelength of equal Absorptivity of the two components (λ1), an iso-absorptive point.

$$CX = [(Qm – Qy) / (Qx – Qy)] X A1/ ax1$$
$$CY = [(Qm – Qx) / (Qy – Qx)] X A2/ ax1$$

The equation gives the concentration of X in terms of absorbance ratios, the absorbance of the mixture and the absorptivity of the compounds at the iso-absorptive wavelengths. Accurate dilutions of the sample solution and of the standard solutions of X and Y are necessary for the accurate measurement of A1 and A2, respectively [8, 9].

MATERIALS AND METHODS:

Instrumentation:

Make: Shimadzu

Model: UV-1900

Spectral Bandwidth: 1 nm

Wavelength Accuracy: +0.3 nm

Photometric Range: Absorbance: -4 to 4, Transmittance: 0% to 400%

Photometric Accuracy: ± 0.002 Abs at 0.5 Abs± 0.004 Abs at 1.0 Abs± 0.006 Abs at 2.0 Abs

Scanning Range: 190 – 1100 nm

Detector: Silicon photodiode

Reagents and Materials: Olanzapine, Fluoxetine, Methanol.

Preparation of Standard Stock Solution: 10 mg and 20 mg each of Olanzapine and Fluoxetine were weighed separately and transferred in two different 100 ml volumetric flasks. Both the drugs were dissolved in Methanol by vigorous shaking, and then the volume was made up to the mark with a Methanol to obtain a final concentration of 100 µg/ml and 200 µg/ml of each component.

Selection of Wavelength: Using appropriate dilutions of the standard stock solution, the solutions were scanned separately in the wavelength region of 200 - 400 nm. The absorbance spectra obtained were derivatized to remove the interference of absorbing species. The two wavelengths selected should be such that at each wavelength, the absorbance difference between the components should be as large as possible. From the examination of a Q-Absorbance ratio spectrophotometric method of olanzapine and fluoxetine at 232.5 nm (Isosbestic point) and 288 nm (λ_{max}) were selected as working wavelengths for the Q-Absorbance ratio spectrophotometric method **Fig. 17**, as at 288 nm Fluoxetine was shown zero absorbance and at 288 nm Olanzapine showed maximum absorbance.

The concentration of 20 µg/ml of Olanzapine and 40 µg/ml of Fluoxetine were used as standard 1 and standard 2. The Q values and absorptivities for both the drugs were calculated as follows:

$$a = A / bc$$

Where,

A = Absorbance at the selected wavelength.
b = path length (in cm).
c = concentration of the drug (mol/l).
Q_{OLA}= Absorptivity of OLA at 288 nm/ Absorptivity of OLA at 232.5 nm.
Q_{FLU}=Absorptivity of FLU at 288 nm/Absorptivity of FLU at 232.5 nm
a_{OLA} =Absorbance of OLA at 232.5 nm/Concentration of OLA in gms/lit

a_{FLU} = Absorbance of FLU at 232.5nm / Concentration of FLU in gms/lit.

Where, Q_{OLA} and Q_{FLU} are Q values, a_{OLA} and a_{FLU} are absorptivities at the isosbestic point for Olanzapine and Fluoxetine, respectively. These values were found to be, $Q_{OLA} = 0.0$, $Q_{FLU} = 0.6377$, $a_{OLA} = 61.15$, $a_{FLU} = 30.57$

$$C_{FLU} = Q_M - Q_{FLU} / Q_{OLA} - Q_{FLU} \times A / a_{FLU}$$

$$C_{OLA} = Q_M - Q_{FLU} / Q_{FLU} - Q_{OLA} \times A / a_{OLA}$$

Where, C_{OLA} and C_{FLU} are the concentration of Olanzapine and Fluoxetine.

Q_M=Absorbance of the mixture at 288 nm/Absorbance of the mixture at 232.5nm.

$Q_M = 0.3188$

A= Absorbance of the mixture at 232.5 nm

A standard equation can be obtained by putting the values in the above equations-(1) and (2)

$$C_{FLU} = 0.3188 - 0.0 / 0.6377 - 0.0 \times A/30.57$$

$$C_{OLA} = 0.3188 - 0.0 / 0.0 - 0.6377 \times A / 61.15$$

A standard equation can be obtained by putting the values in the above equations.

Validation of Analytical Methods: [10]

Linearity: From the Olanzapine standard stock solution (100 µg/ml), 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 ml of solution were pipetted out into 6 different 10 ml volumetric flask and volume were made up to 10 ml with methanol to obtain solution 5, 10, 15, 20, 25, and 30 µg/ml solutions. From the Fluoxetine standard stock solution (200 µg/ml), concentration of 10, 20, 30, 40, 50, and 60 µg/ml of Fluoxetine were prepared by taking 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 ml of stock solution respectively. All the above-obtained solutions were scanned in the range of 200-400 nm in UV spectrophotometer.

The set of 5, 10, 15, 20, 25, and 30 µg/ml of Olanzapine and 10, 20, 30, 40, 50 and 60 µg/ml of Fluoxetine were chosen as concentration range as it showed linearity up to 30 µg/ml Olanzapine and 60 µg/ml Fluoxetine. The working curve equation was obtained by measuring absorbance at isosbestic point of both the drug at 232.5 nm and λ_{max} for Olanzapine at 288. The standard calibration curve of both drugs at the selected wavelength was plotted for absorbance *v/s* concentration. The standard calibration curve of both drugs at the selected wavelength was plotted for absorbance *vs.* concentration. **Table 1** Statistical validation of linear regression data is given in **Table 2**.

Limit of Detection and Limit of Quantitation: Calibration curve was repeated six times and the standard deviation of the intercepts was calculated. Then LOD and LOQ were calculated as follow:

$$LOD = 3.3 \times SD / \text{Slope of calibration curve}$$

$$LOQ = 3.3 \times SD / \text{Slope of calibration curve}$$

Where, SD = Standard Deviation of intercepts of calibration curves.

Analysis of Powder Mixture: The mixture containing 20 µg/ml Olanzapine and 40 µg/ml Fluoxetine were analyzed using UV spectrophotometry (Q-Absorbance Ratio method). The absorbance of the solution at 232.5 nm and 288 nm were measured. The values were substituted in the above equation to get a concentration of Olanzapine and Fluoxetine, respectively. The data for statistical validation of analysis of powder mixture are given in **Table 4**.

Analysis of Tablet Formulation: Twenty tablets of Olanzapine and Fluoxetine in combination were weighed and their average weight was determined and the tablets were crushed to powder sample. From the triturate, 10 mg tablet powder (*i.e.*, equivalent to 10 mg of Olanzapine and 20 mg of fluoxetine) was weighed and transferred to 100 ml volumetric flask and dissolved in methanol, and the content was kept in ultra-sonicator for 25 min. Finally, the volume was made up to the mark with methanol. The solution was filtered through Whatman filter paper no. 41.

This tablet solution was further diluted to obtain 20 µg/ml for Olanzapine and 40 µg/ml for Fluoxetine. The mixed sample solutions were analyzed to obtain spectra, and absorbance values at 232.5 nm (λ2) and 288 nm (λ1) were noted. The concentration of Olanzapine and Fluoxetine was calculated from the equation (1) and (2). The results of the analysis of tablet formulation are reported in **Table 4** and data for statistical validation are given in **Table 4**.

Recovery: The Recovery studies were carried out by applying the method to determine drug sample present in the tablet formulation to which known amount of Olanzapine and Fluoxetine corresponding to 80, 100, 120% of label claim was added (standard addition method).

In the 80% recovery study amount of standard added is 8 mg of Olanzapine and 16 mg of Fluoxetine (*i.e.,* 80% addition).

In 100% recovery study, the amount of standard added is 10 mg of Olanzapine and 20 mg of Fluoxetine (*i.e.,* 100% addition). In a 120% recovery study, the amount of standard added is 12 mg of Olanzapine and 24 mg of Fluoxetine (*i.e.,* 120% addition).

For 100% recovery study, 10 mg of Olanzapine and 20 mg of Fluoxetine were added to the tablet powder. Powder equivalent to 10 mg of olanzapine wascalculated and added into a 100 ml volumetric flask and volume was made up with methanol up to 100 ml.

From it 2 ml and 4 ml of the above solution was pipetted out into a 10 ml volumetric flask, and volume was made up with 10 ml with methanol to obtain the solution of concentration 20 µg/ml Olanzapine and 40 µg/ml Fluoxetine.

The resulting solution obtained was in the linearity range and of concentration 20 µg/ ml Olanzapine and 40 µg/ ml Fluoxetine.

In a similar manner, serial dilution was done as per the calculation, and the results corresponding to 80% and 120% recovery study were obtained.

At each level, three determinations were performed, and results obtained were compared with expected results. The results for recovery studies and statistical evaluation data are shown in **Table 5**.

Precision:

I. Repeatability: Standard solution containing Olanzapine (20 µg/ml) and Fluoxetine (40 µg/ml) were measured, and % RSD was calculated. The validation data are shown in **Table 4**.

II. Interday Precision: Standard solution containing Olanzapine (20 µg/ml) and Fluoxetine (40 µg/ml) were analyzed six times on different day, and % RSD was calculated. The validation data are shown in **Table 4**. **III. Intraday Precision:** Standard solution containing Olanzapine (20 µg/ml) and Fluoxetine (40 µg/ml) were analyzed six times on the same day, and % RSD was calculated. The validation data are shown in **Table 4**.

Robustness: The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage. A robustness study was performed by changing the scanning speed. The dilute solution obtained by dilution of stock solution as mentioned in the procedure for selection of analytical concentration range.

A mixed solution of final concentration 20 µg/ml of Olanzapine and 40 µg/ml of Fluoxetine respectively were scanned in the region of 200-400 nm with the help of UV-visible spectrophotometer by changing the scanning speed, *i.e.,* fast, medium, and slow.

For fast, medium and slow scanning speed, the reading were obtained two times for each factor, and then further calculation were done by taking the mean for each factor. The solutions obtained were scanned in the region of 200 - 400 nm, and further calculations were done to obtain SD, % RSD and standard error. The results are shown in **Table 6** and **7**.

Ruggedness: A ruggedness study was carried out by the degree of reproducibility of test results obtained by the analysis of the same sample by different analysts.

The solution of concentration 20µg/ml of Olanzapine and 40 µg/ml of Fluoxetine respectively were prepared by diluting and preparing the stock and diluted solution by using methanol as per procedure mentioned in selection of analytical range.

Two different analysts did the above procedure, and the results obtained by both the analyst were tabulated, and calculations were done to obtain SD, % RSD, and Standard error. The results obtained are tabulated in **Tables 6** and **7**.

RESULTS AND DISCUSSION:

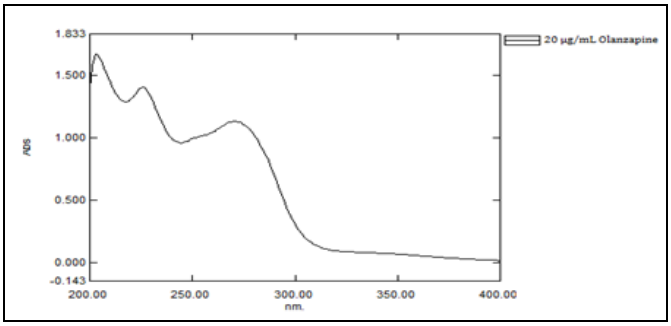

FIG. 1: NORMAL SPECTRUM OF OLANZAPINE

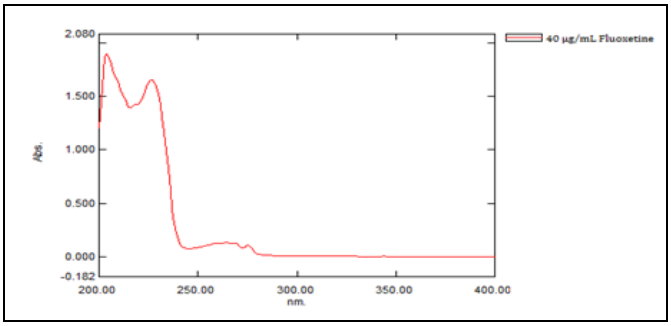

FIG. 2: NORMAL SPECTRUM OF FLUOXETINE

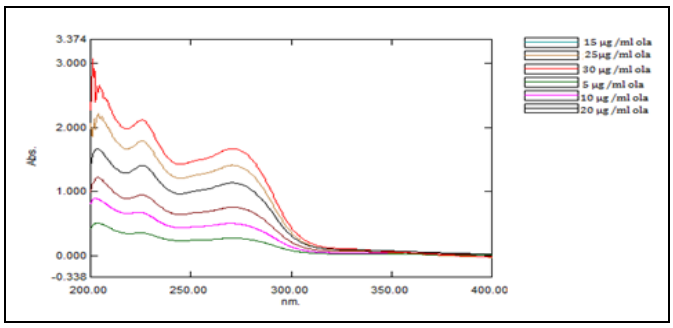

FIG. 3: LINEARITY OF OLANZAPINE

TABLE 1: CALIBRATION TABLE FOR OLANZAPINE

S. no.	For Olanzapine			For Fluoxetine	
	Concentration (µg /ml)	Absorbance at 288 nm *(λ_1)	Absorbance at 232.5 nm *(λ_2)	Concentration (µg /ml)	Absorbance at 232.5 nm*
1	5	0.192	0.312	10	0.302
2	10	0.363	0.612	20	0.613
3	15	0.583	0.913	30	0.918
4	20	0.766	1.193	40	1.223
5	25	0.992	1.529	50	1.559
6	30	1.186	1.848	60	1.812

*n=6

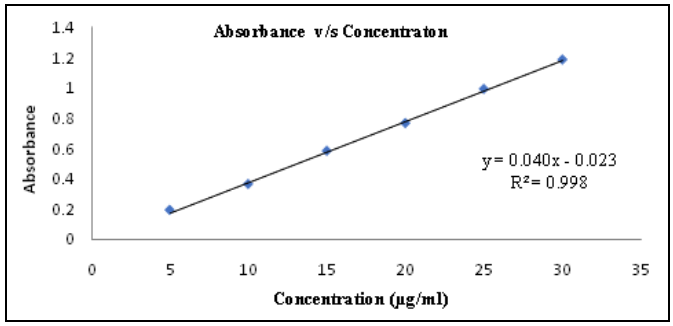

FIG. 4: CALIBRATION CURVE OF OLANZAPINE AT 288 nm

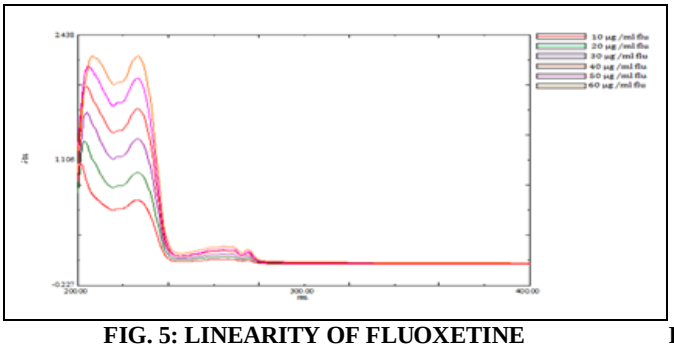

FIG. 5: LINEARITY OF FLUOXETINE

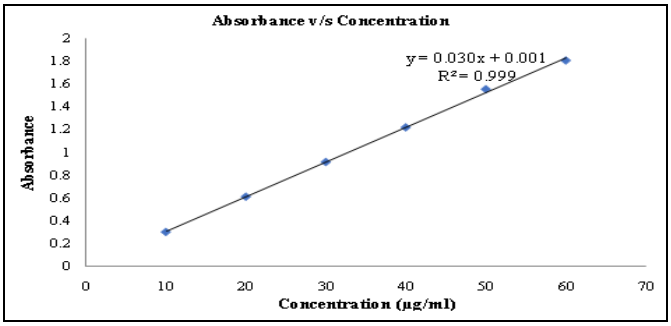

FIG. 6: CALIBRATION CURVE OF FLUOXETINE AT 232.5 nm

TABLE 2: STATISTICAL VALIDATION OF LINEAR REGRESSION DATA

Parameter	Mean*		Standard Deviation*		%Relative Standard Deviation*		Standard Error*	
	OLA	FLU	OLA	FLU	OLA	FLU	OLA	FLU
Slope	0.060	0.030	0.0012	$2.9*10^{-4}$	1.97	0.965	$4.9*10^{-4}$	$1.2*10^{-4}$
Intercept	0.023	0.006	0.685	$1.1*10^{-4}$	0.594	1.793	$1.1*10^{-4}$	$4.0*10^{-4}$
Regression coefficient	0.994	0.998	0.357	$1.1*10^{-4}$	0.354	0.115	$3.4*10^{-4}$	$4.7*10^{-4}$

*n=6

TABLE 3: LOD AND LOQ DATA FOR OLANZAPINE AND FLUOXETINE

Drug	LOD(µg /ml)	LOQ (µg /ml)
Olanzapine	0.0350	0.1061
Fluoxetine	0.0126	0.0382

Analysis of Powder Mixture and Tablet Powder:

TABLE 4: STATISTICAL VALIDATION FOR POWDER MIXTURE AND TABLET POWDER

Validation Parameter	Drug	% Mean Recovery*	Standard Deviation*	Co-efficient of Variation* (% R.S.D.)	Standard Error*
Repeatability	OLA	100.08	0.410	0.410	0.168
	FLU	100.35	0.457	0.456	0.187
Inter – Day Precision	OLA	100.20	0.442	0.441	0.181
	FLU	100.61	0.327	0.325	0.134
Intra – Day Precision	OLA	100.13	0.553	0.553	0.226
	FLU	100.39	0.558	0.556	0.229
Tablet Formulation Analysis	OLA	100.11	0.511	0.510	0.209
	FLU	100.40	0.475	0.473	0.195

*n = 6

Recovery Study:

TABLE 5: STATISTICAL VALIDATION FOR RECOVERY STUDIES

Level of % Recovery	% Mean Recovery*		Standard Deviation*		Co-efficient of Variation* (% R.S.D.)		Standard Error*	
	OLA	FLU	OLA	FLU	OLA	FLU	OLA	FLU
80	100.12	100.38	0.558	0.568	0.558	0.572	0.323	0.332
100	100.11	100.37	0.375	0.375	0.374	0.373	0.216	0.216
120	101.04	100.37	0.725	0.656	0.719	0.654	0.416	0.379

*n = 3

The results have shown that the recovery of Olanzapine and Fluoxetine was in the range of 98.00% to 102.00%. The relative standard deviation was less than 2%. These results demonstrate that this method was repeatable and precise.

Robustness and Ruggedness Study:

TABLE 6: DATA FOR ROBUSTNESS AND RUGGEDNESS STUDY

Validation Parameter	Variation and Level		Concentration (µg/ml)		Amount Found		% Assay	
			OLA	FLU	OLA	FLU	OLA	FLU
Robustness study	Change in scanning speed	Fast	20	40	19.94	39.99	99.70	99.97
		Medium	20	40	19.99	40.09	99.95	100.22
		Slow	20	40	20.10	40.30	100.50	100.75
Ruggedness Study	Different analyst	Analyst 1	20	40	19.94	39.99	99.73	99.98
		Analyst 2	20	40	20.08	40.27	100.40	100.67

TABLE 7: STATISTICAL VALIDATION FOR ROBUSTNESS STUDY

Validation Parameter	Variation	Mean* (%)		Standard Deviation*		Co-efficient of Variation* (% R.S.D.)		Standard Error*	
		OLA	FLU	OLA	FLU	OLA	FLU	OLA	FLU
Robustness study	Change in scanning speed	100.05	100.31	0.409	0.398	0.409	0.397	0.236	0.230
Ruggedness study	Change in analyst	100.06	0.473	0.473	0.273	100.06	0.473	0.473	0.273

*n = 3

CONCLUSION: The present work described about Q-Absorbance Ratio method for simultaneous determination of olanzapine and fluoxetine from bulk and tablet dosage. The method is developed by using solvent as a methanol.

The Developed method was found to be linear, accurate, sensitive and precise within the range of 5 - 30 µg/ml olanzapine and 10 - 60µg/ml fluoxetine. It can now transfer to use it for routine laboratory analysis of both drugspharmaceutical dosage form.

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CONFLICTS OF INTEREST: The authors declare that they have no conflict of interest.

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