IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Human Journals
Research Article
March 2023 Vol.:26, Issue:4

Development and Validation of Analytical Method for Simultaneous Estimation of Olanzapine and Fluoxetine Using UV-Visible Spectroscopy

IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Malathi.S, Ranjith.M.*

Department of Pharmaceutical Analysis, PSG College of Pharmacy, Peelamedu, Coimbatore-641004, Tamilnadu, India.

Submitted: 22 February 2023
Accepted: 28 February 2023
Published: 30 March 2023

Keywords: Olanzapine, Fluoxetine, Simultaneous equation, Validation.

ABSTRACT

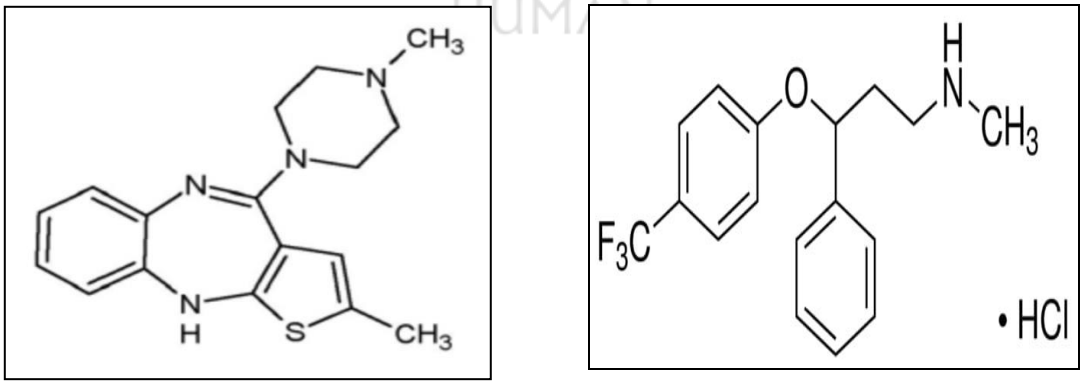
A simple, specific, accurate and precise UV spectrophotometric method has been developed for the simultaneous estimation of olanzapine and fluoxetine in pharmaceutical dosage form. The absorption maxima of the olanzapine and fluoxetine were found to be 272 nm and 227 nm respectively using methanol as a solvent. This method obeys Beer's law in the employed concentration range of 1-5 µg/ml and 4-20 µg/ml of olanzapine and fluoxetine respectively. Different analytical performance parameters such as linearity, precision, accuracy, limit of detection (LOD), limit of quantitation (LOQ) were determined according to ICH guidelines. For development and validation of spectrophotometric methods for simultaneous estimation of olanzapine and fluoxetine from bulk and tablet dosage form was used simultaneous equation method. Using the double beam instrument of Shimadzu–UV1650PC, as UV/Visible spectrophotometer method developed for simultaneous equation method.

INTRODUCTION

Olanzapine is an atypical antipsychotic drug, approved by the FDA for the treatment of schizophrenia and bipolar disorder. It is chemically designated as 2-methyl-4-(4-methyl-1-piperazinyl)-10*H*-thieno(2,3-*b*)(1,5) benzodiazepine. It has a higher affinity for 5-HT_2 serotonin receptors than D_2 dopamine receptors.

Fluoxetine, methyl ({3phenyl-3[4(trifluoromethyl) phenoxy] propyl}) amine, 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.

Analytical method development and validation play important roles in the discovery, development and manufacture of pharmaceuticals. The goal of the analytical method is to separate, quantify the main active drug, any reaction impurities, all available synthetic intermediates and any degradants. Analytical methods are used to ensure the identity, purity, potency, and safety of drug products. In present study, all validation parameters for quantitative analysis of olanzapine and fluoxetine tablets were tested and data were evaluated according to their acceptance criteria.



(A) **(B)**

FIGURE NO. 1: CHEMICAL STRUCTURE OF (A) OLANZAPINE (B) FLUOXETINE

MATERIALS AND METHODS

Instruments

A UV-Visible double beam spectrophotometer Shimadzu 1650 PC with 10mm quartz cells is used. Shimadzu balance model ELB-300 is used for weighing.

Materials

Pure drug sample of Olanzapine and Fluoxetine were purchased from Yarow chemicals.

Chemicals and Reagents

Acetonitrile, Methanol

PROCEDURE

Selection of Solvent and Wavelength

Olanzapine (3 µg/ml) and Fluoxetine (12 µg/ml) were dissolved in acetonitrile and methanol, respectively. Olanzapine and Fluoxetine spectra in the solutions were recorded with the UV spectrum. Methanol has been selected as a common solvent for developing spectral characteristics.

The selection of wavelengths for the analysis of Olanzapine and Fluoxetine were selected from the UV spectrum of drugs by scanning in the range of 200-400nm. The absorbance of both the drugs was higher and gave good sharp peak in methanol, so it was decided to prepare drug solution in methanol for further studies. The lambda max of Olanzapine and Fluoxetine were found to be 272 nm and 227 nm respectively. (Fig. 2, 3.). The overlay of the UV spectrum of both olanzapine and fluoxetine were shown in (Fig. 4).

Preparation of standard stock solution and study of Beer-Lambert's law

The Stock solutions of 1000 µg/ml of Olanzapine and Fluoxetine were prepared in methanol. From that solutions of 100 µg/ml of Olanzapine and Fluoxetine were prepared in methanol. The different concentration of Olanzapine (1-5µg/ml) and Fluoxetine (4-20µg/ml) were prepared from respective stock solution and scanned in UV region. The absorbance was noted at selected wavelength of 272nm and 227nm.

Simultaneous Equation Method

If a sample contains two absorbing drugs, each of which absorbs at the λmax of the other, it may be possible to determine both drugs simultaneously using multicomponent analysis UV Spectrophotometric 'Simultaneous Equation Method.'

Two wavelengths selected for the development of the simultaneous equations are 272 nm and 227 nm. The absorptivity values determined for olanzapine are 0.068 (ax1), 0.085 (ax2) and for fluoxetine are 0.005 (ay1), 0.050 (ay2) at 272 nm and 227 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 both drugs.

$$Cx = \frac{A2ay1 - A1ay2}{ax2ay1 - ax1ay2} \ldots\ldots\ldots 1$$

$$Cy = \frac{A1ax2 - A2ax1}{ax2ay1 - ax1ay2} \ldots\ldots\ldots 2$$

Where Cx and Cy are concentration of olanzapine and fluoxetine respectively in µg/ml. 0.275(A1) and 0.853(A2) are the absorbances of the mixture at 272 nm and 227nm respectively.

METHOD VALIDATION

Linearity and Range

The linearity of an analytical method is its ability to elicit test results that are directly proportional to the concentration of analyte in the sample within a given range.

The linearity of method was established by analyzing different concentrations of Olanzapine and Fluoxetine at 272 nm and 227 nm. Olanzapine in the concentration range 1-5 µg/ml obeyed Beer's Law at 272 nm and 227 nm. Linearity equation is y = 0.063x + 0.014 with r^2 =0.999. Fluoxetine in the concentration range 4-20 µg/ml obeyed Beer's Law at 272 nm and 227 nm. Linearity equation is y = 0.046x + 0.055 with r^2 =0.998.

Precision

The precision of an analytical method is the degree of agreement among individual test results when the method is applied repeatedly to multiple samplings of homogenous samples.

It provides an indication of random error results and was expressed as relative standard deviation (coefficient of variation).

Procedure for the Determination of Intra-day Precision

Intraday precision was found out by carrying out analysis of standard drug solution at three different concentrations (1 - 3 µg/ml) for Olanzapine and (4 – 12 µg/ml) for Fluoxetine in the linearity range for three times on the same day and % RSD was calculated.

Procedure for the Determination of Inter-day Precision

Interday precision was found out by carrying out analysis of standard drug solution at three different concentrations (1 - 3 µg/ml) for Olanzapine and (4 – 12 µg/ml) for Fluoxetine in the linearity range for three day over a period of one week and % RSD was calculated.

Procedure for the Determination of Repeatability

Standard solution of the same concentrations of (3µg/ml) for Olanzapine and (12µg/ml) for Fluoxetine were measured six times and its % RSD was calculated.

Detection and quantification limit

The limit of detection and limit of quantification was calculated by using the average value of standard deviation and slope. The LOD and LOQ were determined from the linearity studies and the values were tabulated.

$$LOD = 3.3*(SD/Slope)$$

$$LOQ = 10*(SD/slope)$$

RESULTS AND DISCUSSION

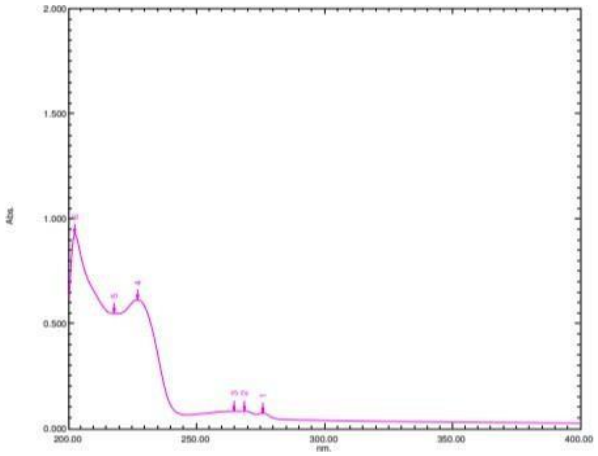


FIGURE NO. 2: UV SPECTRUM OF OLANZAPINE (3µG/ML)

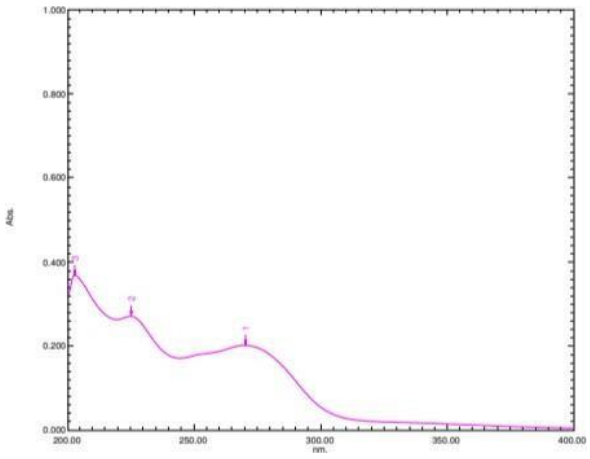


FIGURE NO. 3: UV SPECTRUM OF FLUOXETINE (12µG/ML)

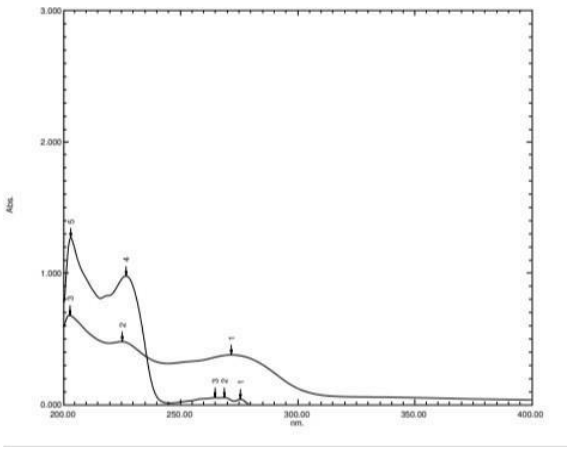


FIGURE NO. 4: OVERLAY SPECTRUM OF OLANZAPINE AND FLUOXETINE

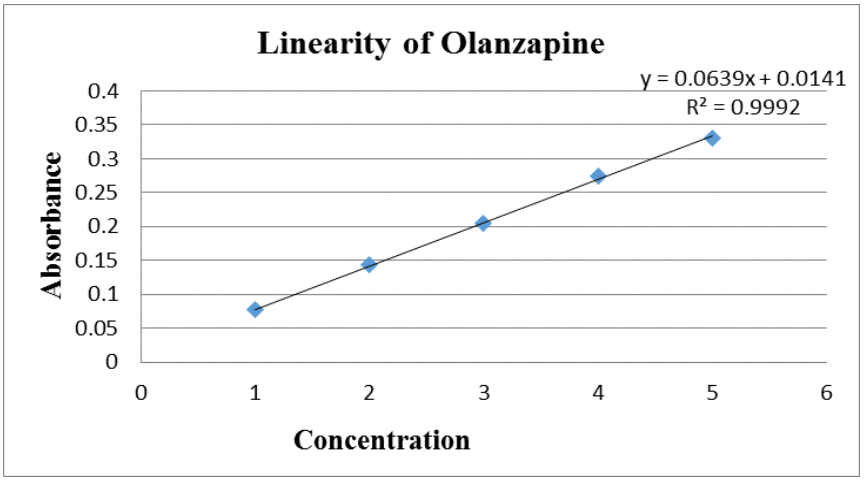


FIGURE NO. 5: LINEARITY GRAPH OF OLANZAPINE AT 272 NM

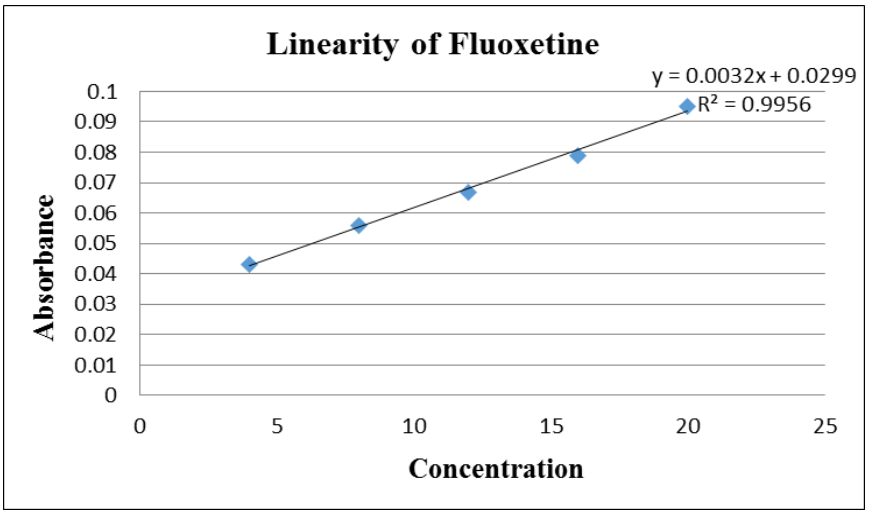


FIGURE NO. 6: LINEARITY GRAPH OF FLUOXETINE AT 272 NM

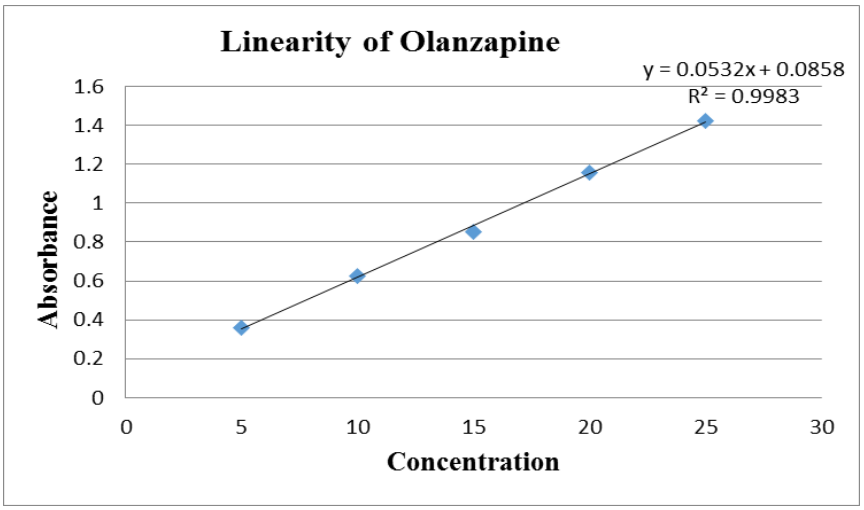


FIGURE NO. 7: LINEARITY GRAPH OF OLANZAPINE AT 227 NM

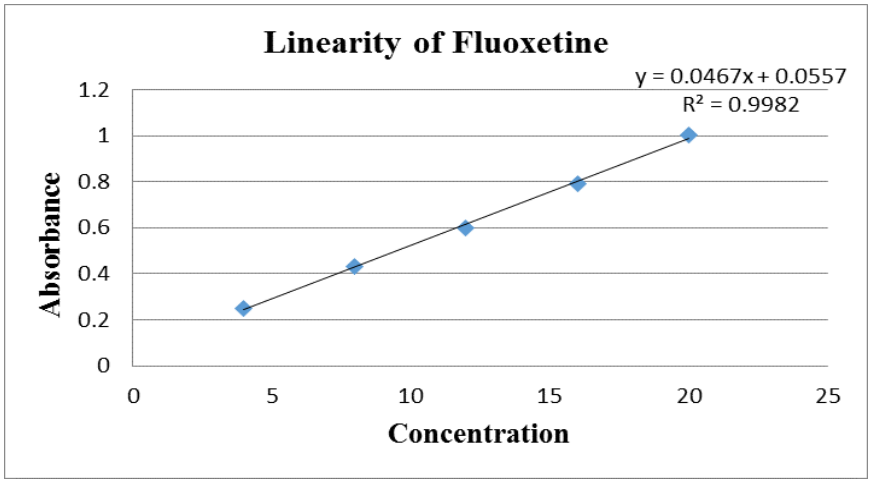


FIGURE NO. 8: LINEARITY GRAPH OF FLUOXETINE AT 227 N

TABLE NO. 1: LINEARITY RESULT OF OLANZAPINE AND FLUOXETINE AT 272 NM

OLANZAPINE		FLUOXETINE	
Conc (µg/ml)	Absorbance	Conc (µg/ml)	Absorbance
1	0.077	4	0.043
2	0.143	8	0.056
3	0.204	12	0.067
4	0.274	16	0.079
5	0.331	20	0.095

TABLE NO. 2: LINEARITY RESULT OF OLANZAPINE AND FLUOXETINE AT 227 NM

OLANZAPINE		FLUOXETINE	
Conc (µg/ml)	Absorbance	Conc (µg/ml)	Absorbance
1	0.102	4	0.251
2	0.195	8	0.431
3	0.256	12	0.601
4	0.355	16	0.792
5	0.419	20	1.004

TABLE NO. 3: LINEARITY RESULT MIXTURE AT 272 NM AND 227 NM

Precision

MIXTUREAT 272 nm		MIXTUREAT 227 nm	
Conc. (µg/ml)	**Absorbance**	**Conc. (µg/ml)**	**Absorbance**
5	0.123	5	0.359
10	0.199	10	0.626
15	0.275	15	0.853
20	0.358	20	1.158
25	0.426	25	1.423

TABLE NO. 4: RESULTS OF PRECISION STUDIES

Drug	**Amount taken (µg/ml)**	**Intra day**		**Inter day**	
		Absorbance	**%RSD**	**Absorbance**	**%RSD**
Olanzapine	3	0.342	0.60	0.345	0.44
		0.346		0.343	
		0.345		0.342	
Fluoxetine	12	0.625	0.48	0.625	0.27
		0.631		0.625	
		0.627		0.628	

TABLE NO. 5: REPEATABILITY

Concentration (µg/ml)		**No. of samples**	**Absorbance**		**% RSD**	
Olanzapine	**Fluoxetine**		**Olanzapine**	**Fluoxetine**	**Olanzapine**	**Fluoxetine**
3	12	1	0.342	0.615	0.84	0.94
		2	0.338	0.623		
		3	0.343	0.626		
		4	0.345	0.627		
		5	0.343	0.629		
		6	0.338	0.632		

$$\%RSD = \frac{S.D}{Mean} \times 100$$

TABLE NO. 6: LOD & LOQ

DRUGS	Parameters	
	LOD	LOQ
Olanzapine	0.88	3.9
Fluoxetine	3.00	5.5

$$\text{LOD} = \frac{3.3 \times \sigma}{S} \qquad \text{LOQ} = \frac{10 \times \sigma}{S}$$

CONCLUSION

The proposed spectrophotometric method is simple, rapid, accurate, precise, and economic and validated in terms of linearity and range, precision, LOD and LOQ. This method can be successfully used for simultaneous estimation of Olanzapine and Fluoxetine in pure and tablet dosage form. The described method gives accurate and precise results for determination of Olanzapine and Fluoxetine mixture in marketed formulation.

REFERENCES

1. Beckett A H, Stenlake J B, editors. Practical Pharmaceutical Chemistry. 4th ed. Vol 2. New Delhi: CBS Publishers and Distributers; 2005; 1-7, 275-277, 358-361.
2. Gurudeep. R. Chatwal, Sham. K. Anand. Instrumental methods of chemical analysis, Himalaya publishing house: Mumbai; 2002. 1.1-1.8, 2.14-2.15.
3. Ashutosh Kar. Pharmaceutical Drug Analysis, Revised 2nd Edition, New Age International (p) Limited, New Delhi, 301-303.
4. Skoog D A, Holler J F, Nieman T A. Principles of Instrumental Analysis. 5th ed. Singapore: Thomson Learning Inc; 1998; 110-300.
5. Michael E. Swartz, Ira S. Krull. Analytical method development and validation, Marcel Dekker, INC. New York, 53-77.
6. John. C. Lindon, George. E. Tranter, John. L. Holmes. Encyclopedia of Spectroscopy and Spectrometry, Part-I, Academic Press, 340,397.
7. Mahmoud A. Tantawy, Nagiba Y. Hassan, Nariman A. Elragehy, Mohamed Abdelkawy; Simultaneous determination of olanzapine and fluoxetine hydrochloride in capsules by spectrophotometry, TLC-spectrophotometry and HPLC, Journal of Advanced Research , 2013,4,173-180.
8. Vekaria H. J and Parmar V. J, Development and validation of spectrophotometric methods for simultaneous estimation of olanzapine and fluoxetine from bulk and tablet dosage form by q-absorbance ratio method, IJPSR 2021, 12(10), 5538-5544.
9. Ramarao Bandlamuri, Prasada Rao. M, Mubeena S. K, Samba Siva. A, Hanuma Naik. J, and Ramya M., A Modified uv-spectrophotometric method development and validation for the quantitative estimation of sildenafil and fluoxetine in pure and marketed formulations, WJPR, 2020, 9(5), 213-222.
10. Vijaykumar K. Parmar, Jatin N. Patel, Girish K. Jani, Laxman M. Prajapati and Jagbir Gagoria , First Derivative Spectrophotometric Determination of Fluoxetine Hydrochloride And Olanzapine In Tablets, IJPSR, (2011); 2(11): 2996-3001.