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Simultaneous Estimation of Fluoxetine HCl and Olanzapine in Bulk Drug and Pharmaceutical Formulation by Using UV-Visible Spectroscopy Method

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ABSTRACT
Present work is to carry out an analytical method development and validation of Fluoxetine HCl (FLU) and Olanzapine (OLZ) in bulk drug and pharmaceutical dosage form. The developed method is based upon simultaneous equations (Vierodt's) method by using UV/Visible spectroscopy. Both drugs come in the categories of anti- depressant and anti-psychotic agent. The developed method can be used for the simultaneous estimation of FLU and OLZ in pharmaceutical dosage form without separating from each other or from the excipients. Primarily the λ max of Fluoxetine HCl (FLU) and Olanzapine (OLZ) was determined as 226 and 258 nm respectively**.** The suggested method is validated by using ICH validation parameters like accuracy, precession, linearity and LOD and LOQ respectively. Accuracy study showed percentage recovery in the range of 97-102% w/w respectively. Precision studies were carried out for 6 successive absorbance and studied for their percentage relative standard deviation (%RSD) was < 2%, LOD and LOQ was studied and the limit of detection and limit of quantification were found to be was 1-100 µg/ml for Olanzapine and Fluoxetine HCl, the slope of interception Y=0.23x6+0.054 (R^2 0.993) and Y=0.222x6-0.014 (R^2 0.995) respectively. Relative standard deviation for Fluoxetine hydrochloride and Olanzapine were 0.4904 and 0.53969, the co-relation coefficient were 0.997 and 0.825 respectively. This procedure was applied successfully for the analysis of FLU and OLZ in bulk drug and Pharmaceutical preparations.

Keywords: Fluoxetine HCl (FLU), Olanzapine (OLZ), Simultaneous equation.

INTRODUCTION

Fluoxetine Hydrochloride is an anti-depressant drug, chemically called as Benzenepropanamine, N-methyl-gamma-[4-(trifluoromethyl) phenoxy]-, Hydrochloride, or (+-)-N-Methyl-3-phenyl-3 [(alpha, alpha, alpha-trifluoro-p-tolyl) oxy] propylamine hydrochloride. In the early 1970s, evidence of the role of serotonin (5-hydroxytryptamine or 5-HT) in depression began to emerge and the hypothesis that enhancing 5-HT neurotransmission would be a viable mechanism to mediate antidepressant response was put forward. On the basis of this hypothesis, efforts to develop agents that inhibit the uptake of 5-HT from the synaptic cleft were initiated. These studies led to the discovery and development of the selective serotonin-reuptake inhibitor Fluoxetine hydrochloride (Prozac; Eli Lilly), which was approved for the treatment of depression by the US FDA in 1987. [1]

Olanzapine is an anti-psychiatric drug, chemically called as 2-Methyl-4-(4-methyl-1-piperazinyl)-10H-thieno [2, 3-b] [1, 5] benzodiazepine, first synthesized Olanzapine (Eli Lilly), in the United Kingdom in 1982. Lilly filed the 382 patent applications on May 22, 1992. The patent issued on July 20, 1993. The United States Food and Drug Administration (FDA) approved Olanzapine, sold by Lilly under the trademark Zyprexa®, in late 1996. By filing an ANDA, the defendants stipulate to infringement if the 382 patent is valid and enforceable. Olanzapine is an antipsychotic medication. It used to treat symptoms of psychotic conditions such as schizophrenia and manic depression. It works by changing the actions of chemicals in the brain. However, Olanzapine is

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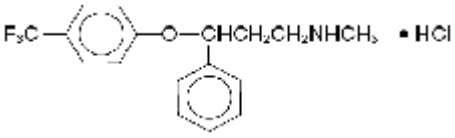
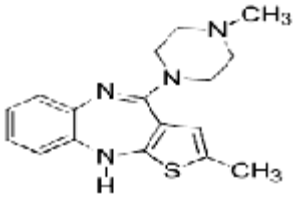
not for use in psychotic conditions that are related to dementia. It has caused fatal heart attack and stroke in older adults with dementia-related conditions.

As the literature reveals that many analytical methods are specified for the determination of Fluoxetine HCl and Olanzapine as individual and combined dosage form with other combination of drugs. FLU is official in BP and USP and both describe an LC method for the estimation of Fluoxetine [2-3], UV-Visible spectroscopy [4], HPLC [5-7], HPTLC, Flourimetery methods [8], non-aqueous capillary electrophoresis (NACE) [9], liquid chromatographic – tandem mass spectrometric method (LC-MS/MS). [10] OLZ is official in IP [11], UV-Visible spectroscopy [12-13], quadrupole MS using both (ESI and APCI) [14], HPLC [15, 16], only few literature reported for the simultaneous determination of FLU and OLZ in combined dosage form by HPLC and HPTLC. [16-19] In the present work an attempted has been made to develop an analytical method development for the simultaneous estimation of FLU and OLZ in the combine dosage form by using simultaneous equation method by UV spectroscopy.



Fluoxetine Hydrochloride



Olanzapine

MATERIALS AND METHOD

Chemical and reagents

Fluoxetine HCl was obtained as a gift sample from (SUN pharmaceuticals), and Olanzapine obtained as a gift sample from (Aurobindo Pharmaceutical), Methanol analytical grade solvent was obtained from Merck (Mumbai, India), Hydrochloric acid (SD- Fine chemicals).

Instrumentation

Spectroscopic analysis was carried out using Elico SL-198 UV/Vis-Double beam spectrophotometer with Spectra treaties software. Spectrophotometer with spectral width 2nm, wavelength accuracy of 0.5nm and a pair of 10mm matching quartz cells was used to measure absorbance of the resulting solutions.

Analytical method development

Preparation of standard stock solution

Weigh accurately about 100mg of Fluoxetine hydrochloride and Olanzapine and add 30 ml of methanol until the substance completely dissolves and make the volume up to 100 ml with 1M HCl in 100 ml volumetric flask and further dilutions was made with water to get 10µg/ml of Fluoxetine hydrochloride (standard stock solution A) and 10µg/ml of olanzapine (standard stock solution B) in a separate volumetric flask. A mixed standard stock solution was prepared from standard stock solution (A and B) to obtain a mixed standard solution, all the stock was scanned from 200-400 nm (Fig. 1-3) respectively.

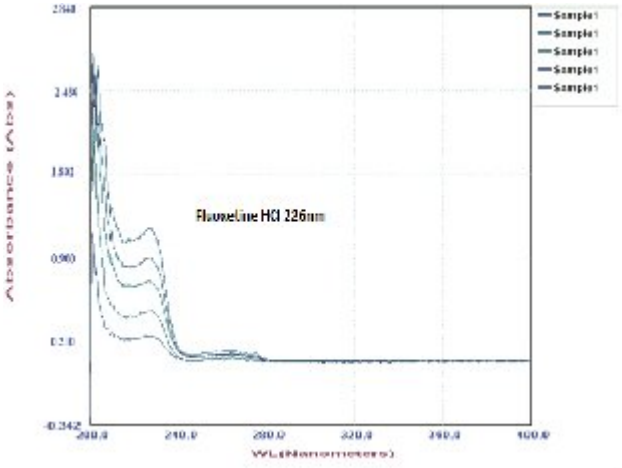


Fig. 1: Linear Spectra of Fluoxetine HCl

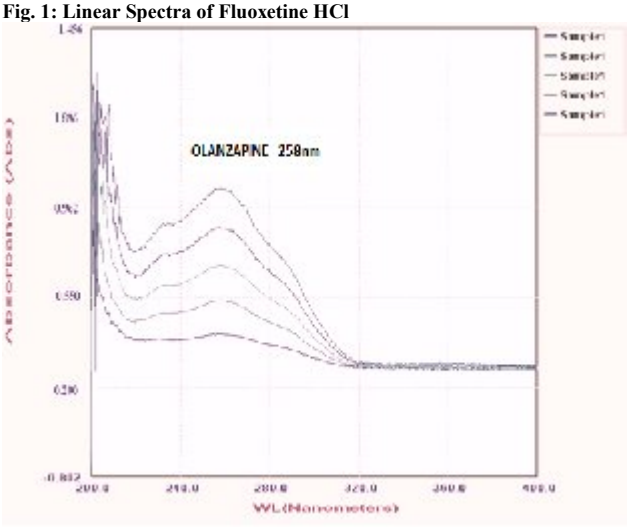


Fig. 2: Linear Spectra of Olanzapine

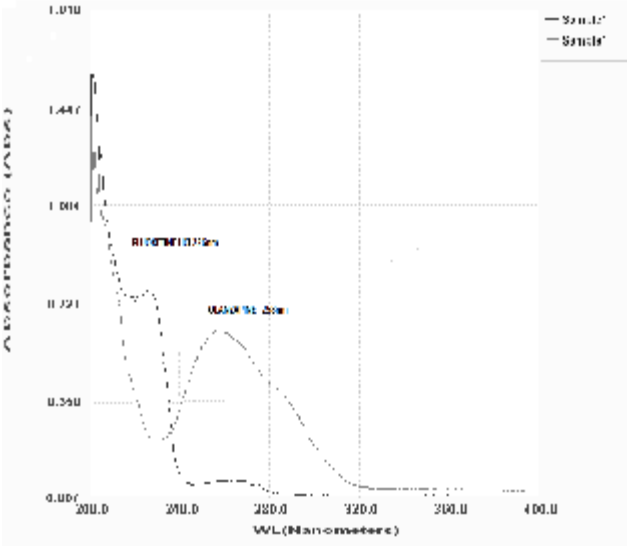


Fig. 3: Overlapping Spectrum showing Fluoxetine HCl and Olanzapine

Preparation of Sample Solution for Combined Dosage Form

Weigh accurately about 20 tablets and triturate in mortar and pestle. Then weigh accurately about 100mg equivalent weight of Fluoxetine hydrochloride and Olanzapine and add 30 ml of methanol until the substance is completely dissolved and make up the volume up to 100 ml with 1M HCL in

100ml volumetric flask, the resulting solution was filtered through whatmann filter paper No: 1 and filtrate is centrifuged and this solution was used for further dilutions with water to get 10µg/ml of drug. The above solutions were scanned between 200nm to 400nm in UV visible spectrophotometer (Fig. 4) respectively.

Table 1: UV-Spectrophotometric parameters for Fluoxetine HCl and Olanzapine

S. No	Parameters Used	Fluoxetine Hydrochloride	Olanzapine
1.	λ max	201 and 226nm	205 and 258nm
2.	Selected wavelength for simultaneous estimation	226nm	258nm
3.	Beer's Lambert Law	10-100 µg/ml	10-100 µg/ml
4.	Molar absorptivity	25.9125	19.032
5.	Assay Percentage	101.1%w/w	125.5%w/w
6.	Mean	0.744	0.652
7.	Standard Deviation	0.36487	0.26588
8.	% RSD	0.4904	0.53969
9.	Slope	Y=0.23x6+0.054 R^2 0.993	Y=0.222x6-0.014 R^2 0.995
10.	Correlation-coefficient	0.997	0.825
11.	Precision (Method and System precision)	1.485	1.850
12.	Linearity and Range	10-100 µg/ml	
13.	LOD	1-10 µg/ml	
14.	LOQ	10-50 µg/ml	

Table 2: The Accuracy studies for the simultaneous estimation of the Fluoxetine HCl and Olanzapine

S. No	Name of the compound	Percentage of the drug substance added (%)	Label clime (mg)	Amount added (mg)	% recovery (mg)	% purity
1	Fluoxetine HCl	80%	16	0.016	0.0159	99.37%
			16	0.016	0.016	100%
			16	0.016	0.0162	101.2%
		100%	20	0.020	0.0199	100.7%
			20	0.020	0.0197	98.5%
			20	0.020	0.020	100%
		120%	24	0.024	0.024	100%
			24	0.024	0.0239	99.5%
			24	0.024	0.024	100%
2	Olanzapine	80%	4	0.004	0.0041	102.5%
			4	0.004	0.0039	97.5%
			4	0.004	0.004	100%
		100%	5	0.005	0.005	100%
			5	0.005	0.0049	98.1%
			5	0.005	0.005	100%
		120%	6	0.006	0.006	100%
			6	0.006	0.0061	101.66 %
			6	0.006	0.006	100%

Simultaneous Equation Methods

Working standard solutions was scanned in the range of 200 to 400 nm to determine the λ max of both drugs using methanol and 1N HCl as a blank. The λ max of FLU and OLZ were found to be 201 and 226 nm and 205 and 258nm (Fig. 3) respectively. For the simultaneous equation method 226 and 258 nm were take as the maximum absorbance of FLU and OLZ respectively. A concentration ranges from 10-50µg/ml of FLU and OLZ was prepared by using the stock solution (0.1gm/100ml). The absorbance of the resulting solution was measured at 226 nm and 258 nm respectively and a calibration curve was plotted. The absorptivity coefficients of these two drugs were determined using calibration curve equation. Two simultaneous equations were formed using these absorptivity coefficient values. A_1= 750 C_{FLU}+15C_{OLZ} at 226 nm (i), A_2=231C_{FLU}+610C_{OLZ} at 258 nm (ii). Where 750 and 231 are the mean absorptivity of Fluoxetine HCl at λ_1 and λ_2 respectively and 15 and 610 are the mean absorptivity of Olanzapine at λ_1 and λ_2 respectively. The concentration of C_{FLU} and C_{OLZ} in mixed standard and the sample solution can be obtained by solving equation (i) and (ii). From the equation, concentration of C_{FLU} was found to be 0.557 gm and concentration C_{OLZ} was found to be 0.0173 gm.

Validation of proposed method

The proposed method was validated by studying several parameters such as accuracy, precision, LOD, LOQ and linearity.

Precision

The repeatability of the sample application was calculated by repeating the assay six times for each concentration. Intraday precision were performed by analyzing sample solution on the same days on the different days at specific time intervals.

Accuracy

To check the accuracy of the proposed method, recovery studies were carried out at 80, 100 and 120% of the test concentration as per ICH guidelines. The recovery studies were performed three times at each level. Results of the formulation analysis recovery studies along with its statistical validation data are given in Table 3.

Linearity

The linearity of the measurement was evaluated by analyzing different concentration of the solution of FLU and OLZ. For the simultaneous equation method the Beer-Lambert's concentration ranges was found to be from 10-50 µg/ml for curcuminoids and ascorbic acid respectively. The standard calibration curve of FLU and OLZ linear calibration graph showed in Fig. 5 and 6 respectively.

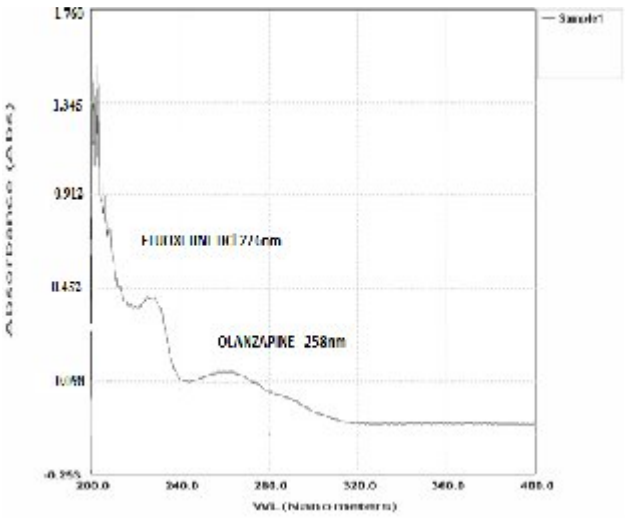


Fig. 4: Marketed formulation of Fluoxetine HCl and Olanzapine

RESULTS AND DISCUSSION

The present work reported is a new analytical method for the simultaneous estimation of Fluoxetine hydrochloride and Olanzapine in bulk drug and tablet dosage form. The method is developed by using methanol and 1M Hydrochloric acid to get a concentration of 10µg/ml. These solutions were scanned in UV-Visible region. It is found that Fluoxetine hydrochloride showed a maximum absorbance at 201 nm and 226 nm, Olanzapine showed at 205 nm and 258 nm. For the

study, the λ_{max} at 226 nm and 258 nm of Fluoxetine hydrochloride and Olanzapine is taken for the study using Simultaneous Equations method respectively. The simultaneous equation is obtained by using C_x and C_y were determined by using Vierodt's method.

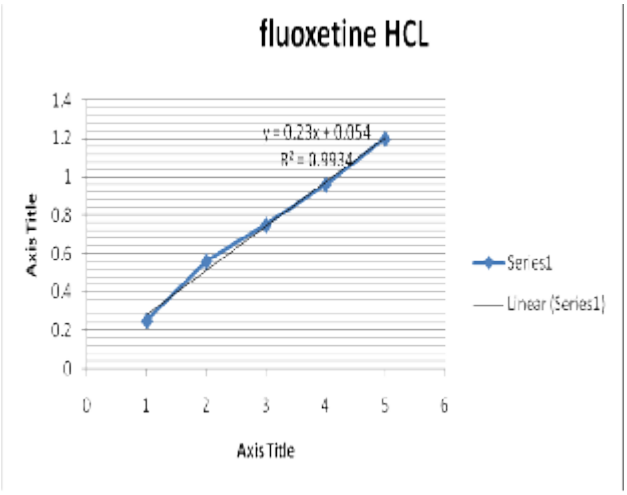


Fig. 5: Linear of Fluoxetine HCl

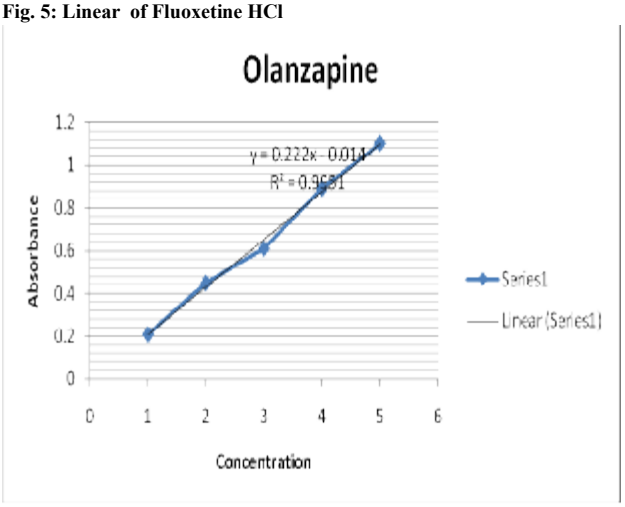


Fig. 6: linear graph of Olanzapine

The method was validated by using ICH guidelines for the following parameters: Accuracy, Precision, Linearity and Range, LOD and LOQ. Accuracy study was carried for the concentration of 80%, 100%, and 120% and the percentage recovery for Fluoxetine HCl and Olanzapine was found to be showing the percentage recovery in the range of 97-102% w/w respectively. Precision studies were carried out for the system and method precision where a mixed standard solution of Fluoxetine HCl and olanzapine is taken for 6 successive absorbance and their Relative Standard Deviation (%RSD) were 1.485 and 1.850 for Fluoxetine hydrochloric acid and Olanzapine respectively. It was found to be it is within the acceptance criteria (2%). LOD and LOQ were studied and the limit of detection and limit of quantification were found to be was 1-100 µg/ml for Olanzapine and Fluoxetine HCl, the slope of interception Y=0.23x6+0.054 (R^2 0.993) and Y=0.222x6-0.014 (R^2 0.995) respectively. Linearity and range was 10-100 µg/ml and % Relative standard deviation for Fluoxetine hydrochloride and olanzapine were 0.4904 and 0.53969, the co-relation coefficient were 0.997 and 0.825 respectively. The result is tabulated in Table 1 & 2 respectively. Hence the proposed method can be used for the routine quantitative analysis of Fluoxetine HCL and Olanzapine in pure and tablet dosage form.

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