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RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF OLANZAPINE AND FLUOXETINE IN TABLET DOSAGE FORM

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ABSTRACT

A new precise, accurate, reliable validated method for the determination of Olanzapine and Fluoxetine has been developed by using reverse phase high performance liquid chromatography (RP-HPLC) in pharmaceutical dosage form. Chromatographic separation was carried out by using mobile phase 0.01M Phosphate buffer $_{p}$H 5.8: Acetonitrile (55:45v/v, $_{p}$H-2.6 adjusted with Orthophosphoric acid) on HYPERSIL ODS C_{18} (250 x 4.6 mm, 5µ) at a flow rate 1ml/min with UV detection at 261nm.The retention times for Olanzapine and Fluoxetine were 3.480 and 2.597 min respectively and both drugs showed good linearity in the range of 18-42µg/ml and 72-168µg/ml. The proposed method has been successfully applied to marketed formulation and was validated according to ICH guidelines and method showed good precision with percentage relative standard deviation less than 2%. The percentage recovery for Olanzapine and Fluoxetine was found between 100.3 and 99.3 respectively indicating the proposed method was accurate and precise.

Keywords: Olanzapine(OLZ) ,Fluoxetine(FLU), RP-HPLC, Simultaneous estimation.

INTRODUCTION

Olanzapine (OLZ) is a benzodiazepine chemically named as 2-methyl-4-(4-methylpiperazin-1-yl)-5H-thieno [3,2-c][1,5] benzodiazepine. It is used in the treatment of depression. It improves hepatic and peripheral tissue sensitivity to insulin without the problem of serious lactic acidosis. It decreases the gluconeogenesis while increasing the glucose uptake by muscles and fat cells. Fluoxetine (FLU) is chemically 2-methyl-4-(4-methylpiperazine 1-yl)-5H-thieno [3,2-c] benzodiazepine.

Literature survey revealed that few analytical techniques are available for estimation of OLZ alone as well as in combine dosage form such as UV, HPLC [5-8].Similarly few analytical methods are available for estimation of FEN alone and its combination with drugs such as UV and HPLC [9-12]. Keeping this objective in mind an attempt has been made to develop and validate the RP-HPLC method for the simultaneous estimation of Olanzapine and Fluoxetine which would be highly sensitive having good resolution reproducible and cost effective. Various validation aspects of the analysis accuracy, precision, recovery, the limits of detection and quantification etc have been measured as per ICH guidelines.

MATERIALSANDMETHOD

Equipments

Chromatographic separation was performed on HPLC system - Water's alliance 2695 with 2996 module Photo Diode Array (PDA) detector equipped with a solvent delivery pump, automatic sample injector and column thermostats. Waters Empower2 software was applied for data collecting and processing.

Chemicals andReagents

Water, Acetonitrile (HPLC grade) was used. Buffer used was Potassium dihydrogen or thiophosphate. Reference standards Olanzapine and Fluoxetine were obtained from CHANDRA LABS. OLANZ PLUS Tablets of OLZ (30mg) and FLU (120mg) manufactured by sun

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pharmaceuticals Ltd were procured from local market.

Preparation ofstandardsolutions

Accurately weighed 30 mg of Olanzapine and 120 mg of Fluoxetine each was transferred into a clean and dry 25ml volumetric flask, dissolved with sufficient volume of diluent and sonicate for 5min. The volume made up to 25ml with diluent to obtain 300µg/ml of Olanzapine and 1200µg/ml of Fluoxetine stock solutions. 0.5ml of standard stock solution of Olanzapine(300µg/ml) and 0.5ml of standard stock solution of Fluoxetine(1200µg/ml) are transferred in to a 10 ml volumetric flask and the volume made with diluent. The resulting solution was sonicated for 10 min.

Preparation of sample solution

5 tablets were weighed and crushed into powder, in order to calculate the average weight of each tablet. From that powder weight equivalent to 30mg of Olanzapine and 120mg of Fluoxetine were transferred into a 500 ml volumetric flask, 300mL of methanol added and sonicated for 25 min, further the volume made up with methanol and filtered.

Working sample solution: 1 ml of sample stock solution containing Olanzapine (300µg/ml) and Fluoxetine (1200µg/ml) is transferred in to a 10 ml volumetric flask and the volume made with methanol. The resulting solution was sonicated for 10 min

Preparationofbuffer

Accurately weighed 1.625gm of Potassium dihydrogen orthophosphate was transferred into a 1000ml of Volumetric flask, about 550ml of Milli-Q water was added and to sonicate to degassed and finally make up the volume with water

Optimizedchromatographicconditions:

Column :HYPERSIL ODS C18(250mmm x4.6 mm ID) 5µm

Mobile phase : Phosphate Buffer pH 5.8: ACN (55:45) v/v

Flow rate	:	1.0 ml/min
Detection wavelength	:	261 nm
Injection volume	:	20 µL
Run time	:	5 min

METHODVALIDATION

Systemsuitabilitytest

This parameter was evaluated before each stage of validation. Six replication injections of standard preparation were injected. Asymmetry, number of theoretical plates and relative standard deviation of peak area were determined.

Linearity

Solutions were prepared containing 500µg/ml,750µg/ml, 1000µg/ml, 1500µg/ml, 2000µg/ml concentrations of Olanzapine and 30µg/ml,45µg/ml, 60µg/ml, 90µg/ml, 120µg/ml concentrations of Fluoxetine which corresponding to 50, 75, 100, 150 and 200% respectively of the test solution concentration. Each solution was injected, linearity was evaluated by linear-regression analysis.

Accuracy

Accuracy was determined by the recovery studies at three different concentrations (corresponding to 50, 100 and 150% of the test solution concentration) by addition of known amounts of standard to pre-analysed sample preparation. For each concentration, three sets were prepared and injected.

Precision

Intraday and inter day variations were determined by using six replicate injections of one concentration and analyzed on the same day and different days. Precision of an analytical method is usually expressed as the standard deviation or relative standard deviation (coefficient of variation) of a series of measurements.

Robustness

The robustness was evaluated by assaying test solutions after slight but deliberate changes in the analytical conditions. The factors choosen for this study were the flow rate (±0.1ml/min),mobile phasecomposition (buffer: acetonitrileby2%).

Limit ofdetection (LOD) and Limit of quantification (LOQ)

LOD and LOQ was calculated from linear curve using formulae

LOD=3.3*σ /slope, LOQ=10*σ /slope(Where σ=the standard deviation of the response and S=Slope of calibration curve).

Specificity

Specificity wascheckedforthe interferenceof impuritiesin theanalysisof blank solutionandinjecting samplesolutionunder optimized chromatographic conditions to demonstrate separation of both OLZ and FLU from impurities.

RESULTS AND DISCUSSION

Several mobile phase compositions were tried to resolve the peak of OLZ and FLU. The mobile phase containing buffer: Acetonitrile in proportion of 55:45v/v was found ideal to resolve the peak of OLZ and FLU satisfactory. Retention time of FLU and OLZ were 2.407 and 3.433 min respectively. The proposed method was found to be linear in concentration range 500-2000µg/ml for OLZ and 30-120µg/ml for FLU. The mean percentage recovery for FLU and OLZ was found to be between 99.3 and 100.3% respectively, which are well within the limit

and hence the method was found to be accurate . LOD and LOQ values were found to be 0.70,2.11µg/ml for Olanzapine and 1.99,6.03µg/ml for Fluoxetine 9.12µg/ml and 27.65µg/ml. Results of intraday and interday precision were shown. The robustness of the method was investigated by varying experimental conditions such as changes in flow rate, mobile phase composition and temperature.

Table1.Analysis data of formulation

Injection	Label claim(mg)	Assay (%)
OLZ	30	99.9
FLU	120	99.8

Table 2.Resultof Linearity

S. no	Olanzapine		Fluoxetine	
	Conc. (µg/ml)	Peak area	Conc. (µg/ml)	Peak area
1	18	715.354	72	2069.276
2	24	994.378	96	2691.879
3	30	1201.435	120	3303.04
4	36	1501.708	144	4117.443
5	42	1741.482	168	4712.721

Table 3. System suitability studies

Parameters	Olanzapine	Fluoxetine	Acceptance criteria
Theoretical plates	4929	2906	Not less than 2000
Tailing factor	1.567	1.655	Not more than 2
%RSD	0.64	0.50	Not more than 2

Table 4.Recoverystudies forOlanzapine and Fluoxetine

DRUG	Spiked level%	Amount taken (µg/ml)	Amount found (µg/ml)	Percent recovery n=4	% RSD
OLZ	20	200	19.98	99.88	0.23
	24	240	24.61	102.56	0.24
	28	280	27.80	99.29	1.04
FLU	96	120	119.38	99.48	0.65
	120	144	146.30	101.59	1.05
	144	168	167.8	99.89	1.53

n- Number of replicate injections

Table 5.LOD and LOQfor Olanzapine and Fluoxetine

DRUG	LOD (µg/ml)	LOQ (µg/ml)
Olanzapine	0.70	2.11
Fluoxetine	1.99	6.03

Table 6(a).Results of Intraday Precision

DRUGS	Conc. (µg/ml)	Peak area (n=6)	% RSD
OLZ	20	4051.848	0.66
FLU	120	4748.148	0.54

n- Number of replicate injections

Table 6(b).Results of Interday Precision

DRUG	Conc. (µg/ml)	Peak area (n=6)	% RSD
OLZ	20	5045.842	0.57
FLU	120	5728.127	0.10

Figure 1. Chromatogram of FLU and OLZ standard

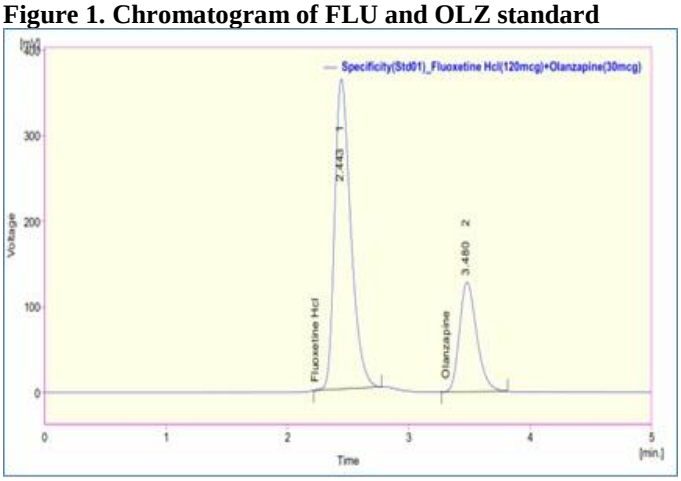


Figure 2. Chromatogram of FLU and OLZ in sample

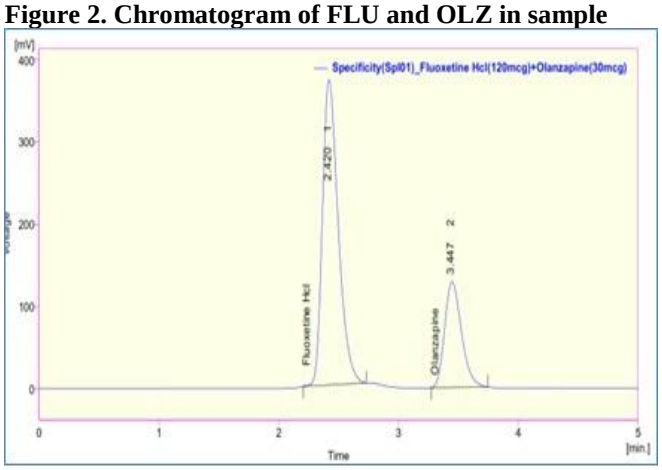


Figure 3. Calibration Curve for Fluoxetine

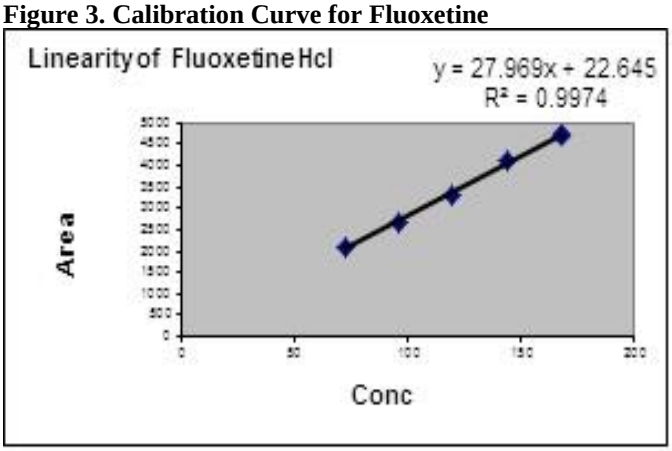
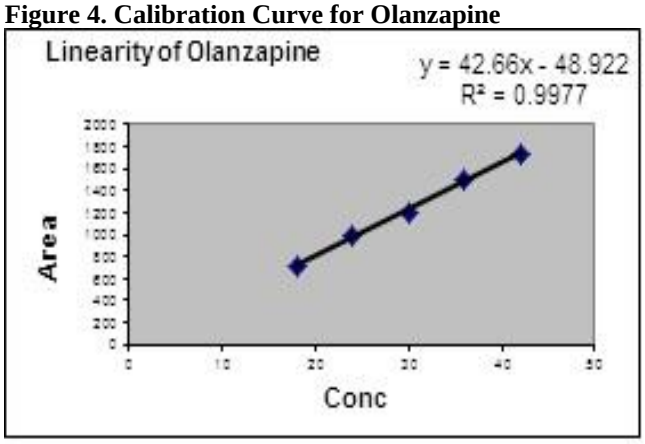


Figure 4. Calibration Curve for Olanzapine



CONCLUSION

The proposed RP-HPLC method was validated as per International conference on harmonization (ICH) guidelines, and found to be applicable for routine quality control analysis for the simultaneous estimation of OLZ and FLU using isocratic mode of elution. The results of linearity, precision, accuracy and specificity, proved to be within the limits. The proposed method is highly sensitive, reproducible, reliable, rapid and specific.

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