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Analytical method development for the simultaneous estimation of olanzapine and fluoxetine by RP-HPLC method

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Abstract: A simple, rapid, accurate, precise and reproducible simultaneous RP-HPLC method for the estimation of Olanzapine and Fluoxetine in bulk and tablet dosage form was developed and validated as per ICH guidelines. The separation was done using BDS Eqisil C_{18} 250 x 4.6 mm, 5 µ column. The mobile phase (Phosphate Buffer pH 4.9 and Acetonitrile 50:50% v/v) was pumped at 1.0ml/min and effluent was detected at 233nm using a UV detector. The retention times for Fluoxetine and Olanzapine were 3.641 ± 0.1 min and 6.3332 ± 0.1 min and the method produced linear response in the concentration range of 2-20 µg/ml for Fluoxetine and 5-30 µg/ml for Olanzapine (r^2 - 0.999). In recovery studies, % RSD from reproducibility was found to be below 2%. LOD and LOQ were 0.726 µg/ml and 2.2 µg/ml for Fluoxetin and 1.056 µg/ml and 3.2 µg/ml for Olanzapine respectively. The developed RP-HPLC method was found to be effective, sensitive and specific for the simultaneous estimation of Fluoxetine and Olanzapine in bulk and tablet dosage form.

Key words: Fluoxetine; Olanzapine; RP-HPLC; Validation

Introduction

Olanzapine (fig.1) is a second-generation antipsychotic drug used to treat schizophrenia, acute mania and the prevention of relapse in bipolar disorder [1]. It antagonizes the binding affinity to serotonin $5HT_{2A/2C}$, 5HT6, Dopamine D_{1-4}, Histamine H_1 and adrenergic (α_1) receptors [2]. It has an estimated prevalence of 1.6 % – 3.7 %, and is an episodic illness interspersed with erratic cycles of mania and depression or mixed episodes [1]. Olanzapine is chemically, 2-methyl-4-(4-methyl1-piperazinyl)-10H-thieno- [2, 3b], [1, 5] benzodiazepine and is practically insoluble in water [3,4].

Fluoxetine (fig.2) is the first selective serotonin reuptake inhibitor (SSRI) used for treatment of depression [5]. It works by inhibiting the uptake of serotonin by the neurons in the brain and enhances serotonin neurotransmission through action on 5HT2ain particular 5HT2c receptors [6]. Fluoxetine is chemically, (*R*, *S*)-*N*-methyl-3-phenyl-3-(4-(trifluoromethyl) phenoxy) propan-1-amine and is soluble in water [5].

Literature survey revealed that several RP-HPLC based methods [7-14] have been reported for thesimultaneous estimation of Fluoxetine and Olanzapine, but there is no method reported with Phosphate buffer: Acetonitrile (50:50% v/v) as mobile phase. The aim of the present work was to develop simple, rapid, sensitive, specific, accurate, precise, economic and reliable RP-HPLC method for the simultaneous estimation of Fluoxetine and Olanzapine in bulk and tablet dosage form suitable for quality control analysis.

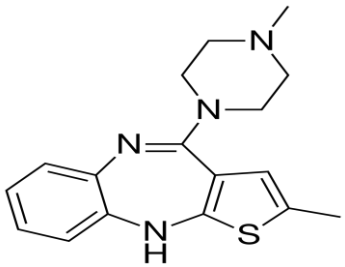

Fig. 1: Structure of Olanzapine

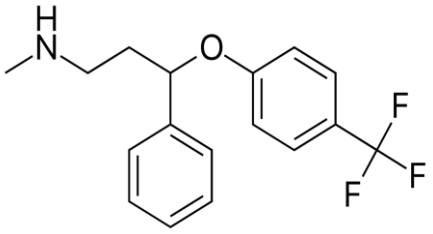

Fig. 2: Structure of Fluoxetine

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Materials and Methods

Chemicals

Olanzapine and Fluoxetine working standards were received as gift sample from Chandra labs and Chem Labs, Hyderabad and sample tablets (Label claim: 20mg Olanzapine and 5mg Fluoxetine tablets) were procured from a local medical shop. HPLC grade acetonitrile, water and AR grade Sodium dihydrogen phosphate were purchased from Merck Specialities Private Ltd., Mumbai and Sd Fine Chem. Limited, Mumbai.

Chromatographic Conditions

HPLC-Shimadzu prominence system with UV detector was used for the method development. The output signal was monitored and processed using Epichrom solution software. Chromatographic separation was performed on Eqisil C_{18} Column (250* 4.6mm, 5µ) at 25°C. The mobile phase containing phosphate buffer pH 4.9 and acetonitrile in the ratio of 50:50 (v/v) was pumped at 1.0 ml/min and detection was carried out at 233 nm. The injection volume for standard and sample was 20µl (fixed loop) and the total run time was 8 min [table 1].

Diluent: Mobile Phase

Preparation of standard stock solution

About 1 g of Olanzapine and 1g of Fluoxetine working standards were accurately weighed and transferred into 100 ml volumetric flasks, dissolved in diluent. The solution was filtered through 0.45 µm Ultipor N66 nylon filter and the volume was made up to the mark with the diluent to get 100 µg/ml of Olanzapine and Fluoxetine.

Preparation of standard solution

1ml of Olanzapine and 2 ml of Fluoxetine from the above standard solutions were transferred to a 10ml volumetric flask and made upto the volume with diluent to get 10 µg/ml and 20 µg/ml concentrations of Olanzapine and Fluoxetine and filtered through 0.45 µm Ultipor N66 nylon filter. Accurately 20 µl was injected into the HPLC system and chromatogram was recorded.

Preparation of Sample solution

Twenty tablets were weighed, average weight determined and finely powdered. An accurately weighed quantity of powder equivalent to 1g of Olanzapine was transferred into a 100 ml volumetric flask. The tablet powder was dissolved in sufficient volume of diluent, sonicated for 20 minutes and degassed. The volume was made up to the mark with the diluent and the sample solution was filtered through 0.45 µm nylon filter. From this sample solution appropriate aliquot was prepared using the diluent. Accurately 20 µl was injected into the HPLC system and the peak area was recorded at 233nm.

Validation of the developed method

The method developed was validated as per ICH guidelines [15] for linearity, accuracy, precision, LOD, LOQ, ruggedness and specificity.

Linearity

The linearity of the developed method was demonstrated over the concentration range of 2-20 µg/ml of Fluoxetine and 5-30 µg/ml of Olanzapine prepared from the stock solution. A calibration curve of the drug was plotted for concentration v/s peak area. The regression equation of calibration curve was y=3563x+937.26 and R^2= 0.9991 for Fluoxetine and y=1960.8x+282.18 and R^2= 0.9993 of Olanzapine.

Accuracy

The accuracy of the method was determined by recovery studies in triplicate for each level. Fixed amount of sample was taken and Olanzapine and Fluoxetine equivalent to 80, 100 and 120 % of the standard was injected into the HPLC system. The method was repeated three times for each level. The average % recovery was calculated.

Precision

The precision of the method was studied by estimation of multiple samplings from the homogeneous sample of the drug at three different concentrations on the same day and on three different days. The precision was expressed as %RSD and was calculated for intra day and inter day precision.

Limit of detection (LOD) and limit of quantitation (LOQ)

The calibration curve of the drug was prepared using 2-20 µg/ml and 5-30 µg/ml concentrations of Fluoxetine and Olanzapine. The Standard deviation of Y intercepts of regression lines were determined and substituted in the following equation for the determination of LOD and LOQ. Limit of Detection (LOD) =3.3σ /S and Limit of quantitation (LOQ)= 10σ/S. In this equation, σ is the standard deviation of Y intercept of regression lines and **S** is the slope of calibration curve. The LOD for Fluoxetine and Olanzapine were found to be 0.726 and 1.056 µg/ml and LOQ for Fluoxetine and Olanzapine were found to be 2.2 and 3.2 µg/ml respectively.

Robustness
Robustness of the method was determined by making slight changes in the composition of mobile phase ± 2 %, flow rate by ± 0.1 ml/min and temperature by ± 2° C. Retention time and chromatograms were determined for the drugs.

Specificity
Commonly used excipients such as starch, lactose and magnesium stearate were spiked into weighed quantity of the drug. The chromatograms were recorded by making suitable dilutions and the amount of drug present in the sample was determined.

Stability
Stability of both the standard and sample solutions was tested during analysis up to 24 hours at room temperature.

Results and Discussion

In the present study, RP-HPLC method developed for the simultaneous estimation of Fluoxetine and Olanzapine in bulk and tablet dosage form using Eqisil C_{18} column (250 mm x 4.6 mm x 5 µ particle size) at 25° C. To develop an effective method for the simultaneous estimation of Fluoxetine and Olanzapine, conditions such as detection wavelength, ideal mobile phase and concentration of the standard were optimized in preliminary trials. Fluoxetine and Olanzapine standard concentrations were scanned in UV- region between 200-400 nm. Optimized wavelength of Fluoxetine and Olanzapine was found to be at 233nm [fig. 3 & 4]. The Fluoxetine and Olanzapine peaks in the sample were identified by comparing with the Fluoxetine and Olanzapine standards and the retention time was found to be around 3.641 ± 0.1minutes for Fluoxetine and 6.331 ± 0.1 minutes for Olanzapine [fig. 5 & 6].

The estimation of Fluoxetine and Olanzapine tablets was carried out by RP-HPLC using mobile phase, Phosphate buffer pH 4.9 and Acetonitrile in the ratio of 50:50%v/v with flow rate of 1.0 ml/min. The retention time was found to be 3.641 ± 0.1 minutes for Fluoxetine and 6.331 ± 0.1 minutes for Olanzapine. System suitability parameters such as RSD for six replicate injections were carried out on freshly prepared standard solution and parameters were given in [table No 2]. % RSD found to be less than 2%, theoretical plates 7333 for Fluoxetine and 6319 for Olanzapine, and tailing factor 1.129 for Fluoxetine and 1.667 for Olanzapine indicating the suitability of the system for the estimation of the drug.

The typical standard chromatogram of Fluoxetine and Olanzapine is shown in figure 1. The calibration curve of the drugs was constructed by plotting peak area of the drug (Y-axis) and concentration of the drug on (x-axis). A good linear relationship was observed between concentration of the drugs and the respective ratio of peak areas in the range of 2-20mcg/ml for Fluoxetine and 5-30mcg/ml for Olanzapine with a correlation coefficient of 0.9991 for Fluoxetine and 0.9993 for Olanzapine reflecting that good correlation exists between peak area and the concentration [fig. 7 & 8].

The quantitative estimation of the drugs in tablet were determined by taking concentration of the drug same to that of standard solution and the assay result was found to be 100.4 % for Fluoxetine and 99.7 % for Olanzapine [table 3]. The acceptance criterion of repeatability is RSD, and should not be more than 2.0 %. The method repeatability was 0.419% for Fluoxetine and 0.627 % for Olanzapine shows that the method was precise. The developed method was validated for its intra-day and inter-day precision. The results obtained were within the acceptable limit (table 3). Estimation of the drug by the developed RP-HPLC method for finding out intra and inter day variations show low coefficient of variation values which indicate that the developed method is highly precise.

By spiking various concentrations of the drug ranging from 80-100-120 % into previously analyzed samples the amount of the drugs recovered were calculated and the results were shown in table 4. The Accuracy limit was the % recovery and was in the range of 100.02 % to 100.35 % for Fluoxetine and 99.88 % to 100.85% for Olanzapine. From the validation of the developed method, the accuracy was within the limit, indicating that the proposed RP-HPLC method was highly accurate. LOD 0.726µg/ml for Fluoxetine and 1.056 µg/ml for Olanzapine, LOQ 2.2 µg/ml for Fluoxetine and 3.3 µg/ml for Olanzapine [table 2] of the drug suggest that low concentration of the drugs can be estimated accurately.

Robustness of the method was studied by changing the chromatographic conditions slightly and results were interpreted. From the method developed it was observed that there were no significant changes in the retention time and area of the chromatograms by making slight alterations in temperature, composition and flow rate of the mobile phase. The % RSD was less than 1%, which

demonstrated that the RP-HPLC method developed was robust.

The RP-HPLC method developed in the present study was used to quantify Fluoxetine and Olanzapine in bulk and tablet dosage form and the results were comparable with the corresponding labeled quantity (table 3). High recovery values and no additional peaks in the chromatogram indicate that the developed method was free from interference of the commonly used excipients in the tablet dosage form. In stability studies the peak area and retention time of the drugs remained almost unchanged and no significant degradation was observed up to 24 hours indicating stability of the drugs.

Table 1: Optimized chromatographic conditions

Parameter	Optimized condition
Chromatograph	HPLC (Shimadzu prominence with UV detector)
Column	Eqisil C_{18} 250mm x 4.6mm, 5µ
Mobile Phase	Phosphate Buffer pH 4.9 and Acetonitrile (50:50)
Flow rate	1.0 ml/min
Detection wavelength	UV at 233 nm
Injection volume	20µl
Column temperature	25° C

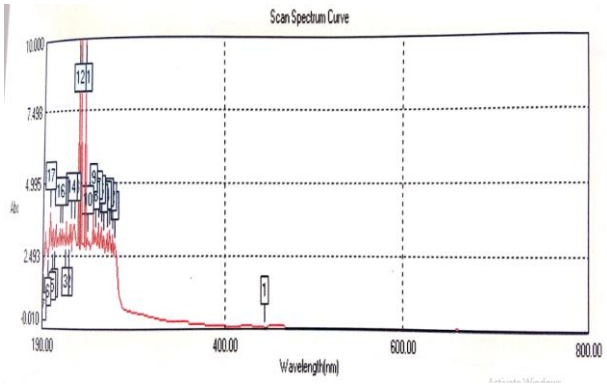

Fig.3. UV Spectrum of Fluoxetine

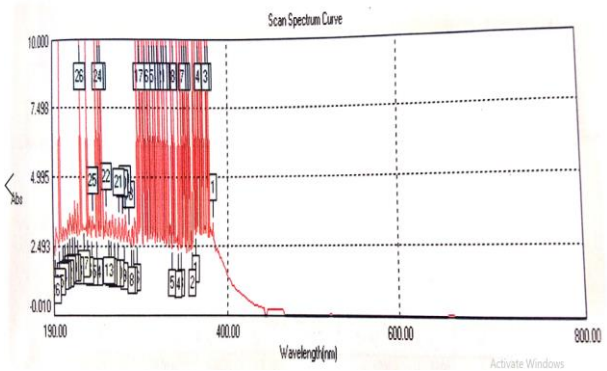

Fig.4. UV Spectrum of Olanzapine

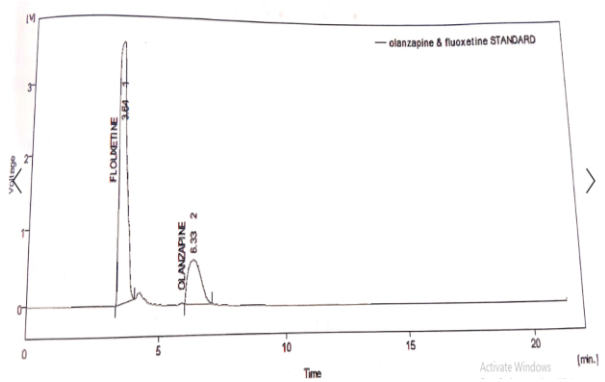

Fig.5. Chromatogram of standard solution of Fluoxetine and Olanzapine

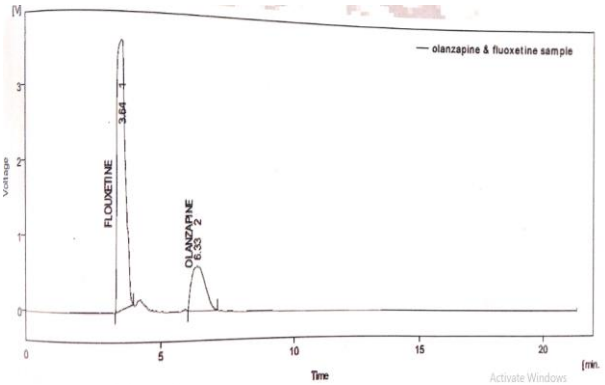

Fig.6. Chromatogram of sample solution of Fluoxetine and Olanzapine

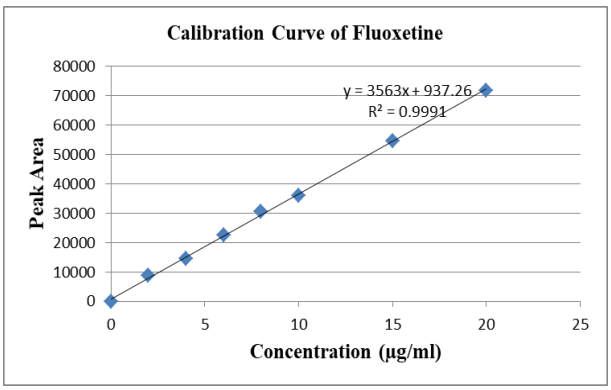

Fig.7. Calibration curve of Fluoxetine

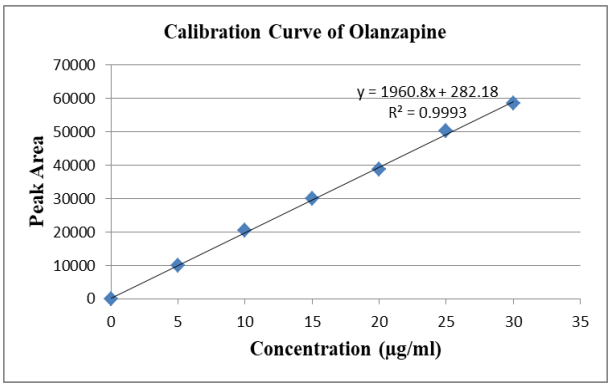

Fig.8. Calibration curve of Olanzapine

Table 2: System suitability and validation parameters of the developed method

Parameter	Fluoxetine	Olanzapine
Theoretical plates	7333	6319
Tailing factor	1.129	1.636
Retention time (min)	3.639	6.329
Linearity range (µg/ml)	2-20µg/ml	5-30µg/ml
Regression equation Y=mx=c	y=3563x+937.26	y=1961x+282.18
Slope (m)	3563	1961
Intercept (c)	937.26	282.18
Correlation coefficient	0.9991	0.9993
Percent RSD	< 2	<2
Precision Intra day (n=6)	0.419	0.627
Precision Inter day (n=6)	0.243	0.781
LOD (µg/ml)	0.726	2.2
LOQ (µg/ml)	1.056	3.2

Table 3: Results of Analysis of the tablet dosage form

Formulation	Label claim	Amount Found ± SD (n=5)	% recovery	% RSD
Fluoxetin	5mg	5.02mg ± 0.0071 **Intra day** Session- 1 Session- 2 Session- 3 **Inter day** Day 1 Day 2 Day 3	100.4% — —	0.141 0.383 0.435 0.169 0.201 0.648 1.426
Olanzapine	20mg	19.94mg ± 0.0187 **Intra day** Session- 1 Session- 2 Session- 3 **Inter day** Day 1 Day 2 Day 3	99.7% — —	0.094 0.155 0.501 0.924 0.198 0.562 0.914

*Average of 6 determinations

Table 4: Recovery studies of the developed method

Sample	Preanalysed Sample Conc (µg/ml)	Recovery Level	Amount Added (µg/ml)	Total Amount Found (µg/ml)	% Recovery
Fluoxetin	8	80%	6.4	14.45	100.35%
		100%	8	16.05	100.31%
		120%	9.6	17.604	100.02%
Olanzapine	15	80%	12	27.23	100.85%
		100%	15	30.18	100.6%
		120%	18	32.96	99.88%

Conclusion

The developed new RP-HPLC method in the present study was found to be simple, rapid, specific, accurate, precise, linear and robust. Thus, the method is suitable for the simultaneous estimation of Fluoxetine and Olanzapine in raw material and tablet formulation in quality control with a high degree of Accuracy and Precision.

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