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

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ABSTRACT

A simple, sensitive, accurate and economic reverse phase high performance liquid chromatographic method has been developed and validated for the simultaneous determenation of Fluoxetine hydrochloride and Olanzapine in pharmaceutical dosage forms. The mobile phase consisted of degassed Methanol: Acetonitrile in the ratio of 90:10 v/v delivered at a flow rate of 1.0ml/min and wavelength of detection at 233nm. The retention times of Fluoxetine and Olanzapine were 2.95min and 3.53min respectively. According to ICH guidelines the method was developed and validated. The proposed method can be used for estimation of these drugs in combined pharmaceutical dosage forms.

KEYWORDS

Fluoxetine hydrochloride, Olanzapine, RP- HPLC and Validation.

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INTRODUCTION

Fluoxetine hydrochloride (FLUOX) is chemically known as, N-Methyl-γ-[4- (trifluoromethyl) phenoxybenzenpropanamine hydrochloride[1]. Fluoxetine hydrochloride is an antidepressant with non-sedating properties through selective serotonin reuptake inhibiton. Olanzapine (OLANZ) is chemically known as, 2- methyl-4-(4-methyl-1-piperazinyl)-10H-thieno [2, 3-b] [1, 5]-benzodiazepine[1]. Olanzapine is an antipsychotic agent, used in schizophrenia[2]. FLX is official in BP and USP and both describe an LC method for the estimation of Fluoxetine[3,4]. A literature survey

showed spectrophotometric methods of FLUOX in formulations[5-7]; HPLC[8-10] and LC-MS[11] methods of Fluoxetine with Norfluoxetine in plasma. Literature survey also indicated HPLC[12], HPLC-MS/ESI[13], capillary GC[14] methods for simultaneous estimation of FLX in pharmaceutical formulation with drugs like Fluvoxamine, Clomipramine, Citalopram and Paroxetine. OLANZ is official in IP[15], which described an HPLC method for its estimation. Literature survey indicated spectrophotometric[16,17], derivative spectroscopy[18,19] and solid phase extraction[20] methods for estimation of OLANZ. Literature survey also indicated HPLC [21,22] and LC-MS[23,24] methods for determination of OLANZ in biological fluids. FLUOX and OLANZ are formulated together in the form of a tablet. Literature survey revealed four HPLC methods[25-29] for simultaneous determination of these two drugs. The method currently developed have the advantage of being more sensitive to determine both drugs concurrently by simple, accurate, rapid and precise RP-HPLC assay for routine analysis.

MATERIAL AND METHODS

Instrumentation, Chemicals and Reagents

High performance liquid chromatograph, Waters model e2695, inertsil C18 ODS column, pump LC-10AT VP equipped with Rheodyne injector with 20µl fixed loop, electronic balance (Sartorious), Sonicator and Empower software was used. Pure FLUOX and OLANZ were procured as gift samples from Dr. Reddy's Laboratories Limited (Hyderabad, India). Olanex F tablets (containing FLUOX 20 mg and OLANZ 5 mg per tablet) were manufactured by Ranbaxy Laboratories Ltd. (New Delhi, India) were purchased from local market. Acetonitrile HPLC grade, Methanol HPLC grade, Purified Water HPLC grade were purchased from E.Merck (Mumbai).

Chromatographic conditions of method

Inertsil C18 ODS column (250-4.6mm), 5µ particle size was used at ambient temperature. The mobile phase consist of Methol: Acetonitrile (90:10) v/v. It was pumped at flow rate of 1ml/min. The mobile phase was passed through nylon 0.45µm membrane filters and degassed before use. The elution was monitored FLUOX and OLANZ at 233nm and the injection volume was 20µl.

Preparation of Stock solution

10mg of Fluoxetine HCL and Olanzapine RS drug was weighed and dissolved in 10ml of mobile phase and taken in 10ml of volumetric flask, sonicated for 20min to get 1000ppm and 2 ml was taken from this and diluted to 10ml with mobile phase.

Preparation of Working Standard

Stock solution equivalent to 20µg to 80µg were prepared, sonicated and filtered through 0.45µ membrane.

Preparation of sample solution

20 tablets were taken and their average weight was determined, they were crushed to fine powder. The powder equivalent to 20mg of Fluoxetine and 5mg Olanzapine was weighed and dissolved in 20ml of mobile phase with the aid of ultra-sonication for 20min. The content was diluted to 50 with mobile phase to furnish a stock test solution. The stock solution is filtered through a 0.5µm nylon syringe filter and 10ml of filtrate was diluted in to a 50ml volumetric flask to give a test solution containing 80µg/ml fluoxetine HCL and 20µg/ml Olanzapine.

UV Spectra's of Fluoxetine and Olanzapine

Absorbance maxima of Fluoxetine Hydrochloride and Olanzapine were detected at 251nm (λ1) and 224nm (λ2), respectively. Both the spectra's were overlapped at 233nm. Both the drugs showed linearity with absorbance in the range 20-80µg/ml and 20-80µg/ml respectively, when measured at 224nm and 251nm. Calibration curves were plotted from the absorbance values at these wavelengths.

RESULTS AND DISCUSSION

To optimize the HPLC parameters, several mobile phase compositions were tried. A satisfactory separation of Fluoxetine and Olanzapine with good peak symmetry and steady baseline was obtained with mobile phase Methanol: Acetonitrile (90:10). Quantitation was achieved with UV detection at 233nm based on peak area. Complete resolution of the peaks with clear baseline separation was obtained (Figure No.4).

Linearity

Linear correlation was obtained between peak areas and concentration of Fluoxetine and Olanzapine in the range of 20-80µg/ml and 20-80µg/ml respectively. Data of the regression analysis are summarized in (Table No.1, 2)

Accuracy

The recovery experiments were performed by standard addition method. The recoveries obtained were 100.01-100.16% and 99.98-100.08% for FLUOX and OLAZ respectively. The result of recovery study is presented in (Table No.3, 4).

Method precision

The RSD values for Fluoxetine and Olanzapine were found to be 0.11 % and 0.31% respectively (Table No.5, 6).

LOD and LOQ

LOD values for Fluoxetine and Olanzapine were found to be 0.41 and 0.16µg/ml respectively. LOQ values for Fluoxetine and Olanzapine were found to be 1.25 and 0.48µg/ml respectively (Table No.7).

System suitability

Parameters calculated for system suitability was a number of theoretical plates, tailing factor, resolution, retention time, and area. Results are shown in (Table No.8).

Assay of the tablet dosage form

(Fluoxetine 20mg/tab and Olanzapine 5mg/tablet)

The proposed validated method was successfully applied to determine Fluoxetine and Olanzapine in tablet dosage form. The result obtained for Fluoxetine and Olanzapine were comparable with corresponding labeled amounts (Table No.9).

Table No.1: Fluoxetine hydrochloride (Linearity)

S.No	Concentration(µg/ml)	Area
1	20	366469
2	30	549948
3	40	732444
4	50	916214
5	60	1098544
6	70	1281360
7	80	146465

Table No.2: Olanzapine (Linearity)

S.No	Concentration(µg/ml)	Area
1	20	108506
2	30	162477
3	40	216456
4	50	270647
5	60	325353
6	70	379178
7	80	432497

Table No.3: Fluoxetine hydrochloride (Accuracy)

S.No	Spike Level	µg/ml added	µg/ml found	% Recovery	mean % recovery
1	50%	20	20.06	100.31	100.11%
2	50%	20	19.95	99.75	
3	50%	20	20.05	100.29	
1	100%	40	40.06	100.15	100.01%
2	100%	40	39.87	99.68	
3	100%	40	40.08	100.20	
1	150%	60	59.87	99.75	100.16%
2	150%	60	0.22	100.37	
3	150%	60	60.20	100.34	

Table No.4: Olanzapine (Accuracy)

S.No	Spike Level	µg/ml added	µg/ml found	%recovery	mean % recovery
1	50%	20	20.07	100.40%	100%
2	50%	20	19.93	99.65%	
3	50%	20	19.99	99.95%	
1	100%	40	40.05	100.12%	100.08%
2	100%	40	39.97	99.93%	
3	100%	40	40.07	100.19%	
1	150%	60	59.97	99.94%	99.98%
2	150%	60	60.01	100.02%	
3	150%	60	59.99	99.99%	

Table No.5: Method Precision of Fluoxetine hydrochloride

S.No	Retention Time(min)	Area
1	2.955	733495
2	2.955	732045
3	2.954	733992
4	2.953	732590
5	2.954	731085
Average	2.9542	733030
S.D	0.000837	876.64
%R.S.D	0.028321	0.119

Table No.6: Method Precision of Olanzapine

S.No	Retention Time(min)	Area
1	3.538	216925
2	3.532	215907
3	3.529	217042
4	3.526	215465
5	3.525	216567
Average	3.530	216381
S.D	0.005244	676.6064
%R.S.D	0.148556	0.312

Table No.7: LOD and LOQ

S.No	Parameter	Fluoxetine	Olanzapine
1	LOD	0.41µg/ml	0.16µg/ml
2	LOQ	1.25 µg/ml	0.48 µg/ml

Table No.8: System Suitability Parameters

S.No	Parameter	Acceptance criteria	Observed value
Theoritical plates			
1	Fluoxetine HCl	(Not less than 3000)	10589.08
	Olanzapine		9548.33
Tailing factor			
2	Fluoxetine HCl	(Not more than 2)	1.59
	Olanzapine		1.10
Repeatability			
3	Fluoxetine HCl	(RSD <1% for N>5)	0.11
	Olanzapine		0.31
4	Resolution(Rs)	(Rs>2)	14.41

Table No.9: Assay

S.No	Drug	%Assay	Amount Present
1	Fluoxetine HCl	99.80	19.98 mg/tab
2	Olanzapine	99.90	4.95 mg/tab

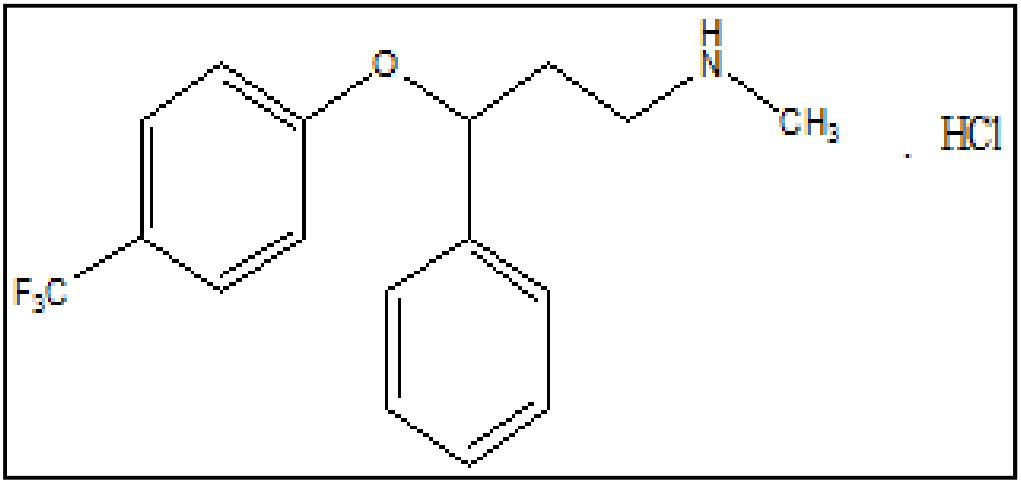

Figure No.1: Molecular structure of Fluoxetine hydrochloride

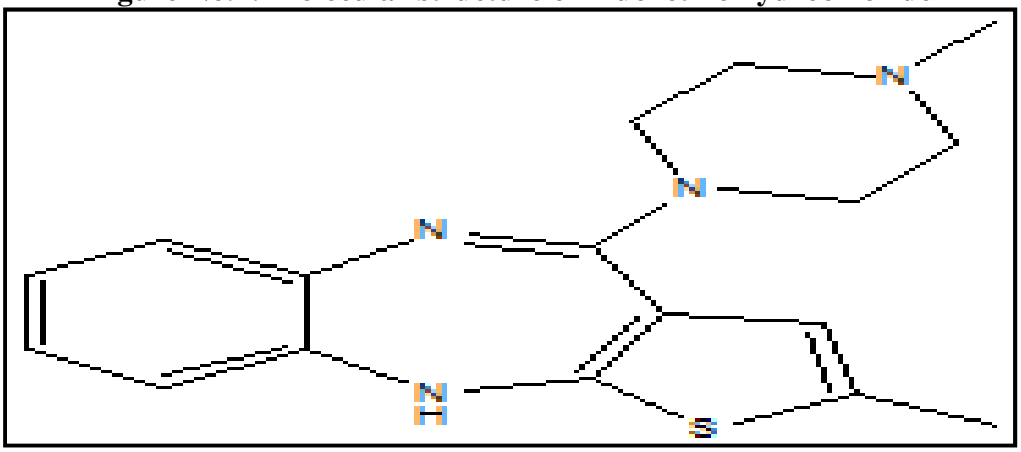

Figure No.2: Molecular structure of Olanzapine

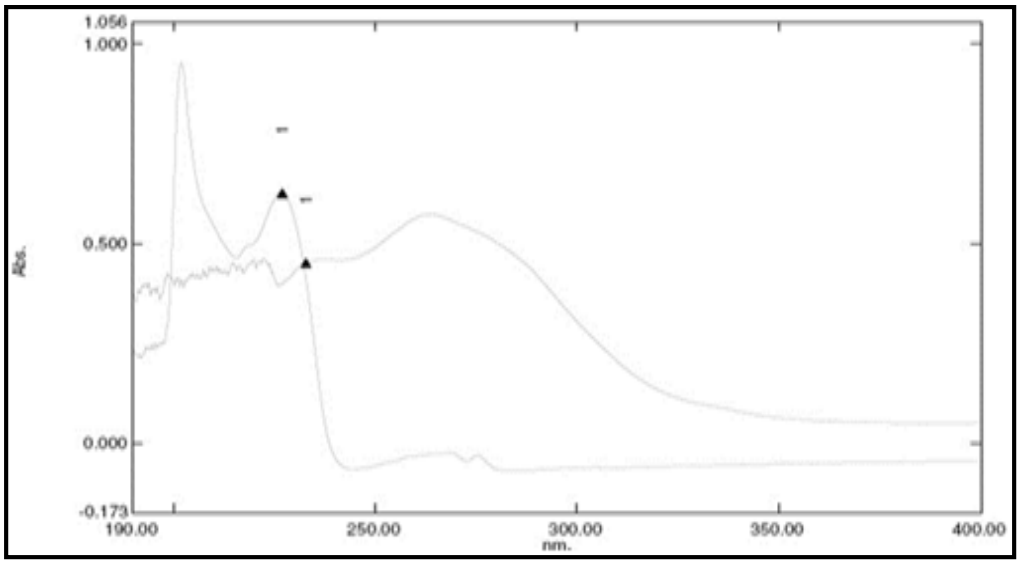

Figure No.3: UV Spectra Fluoxetine Hydrochloride and Olanzapine

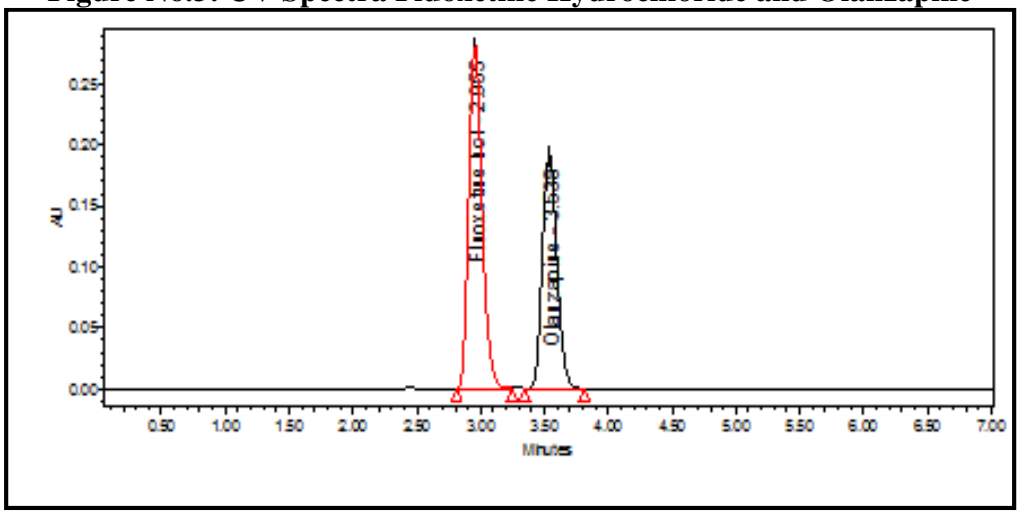

Figure No.4: High performance liquid chromatogram of FLUOX and OLAZ with detection at 233 nm

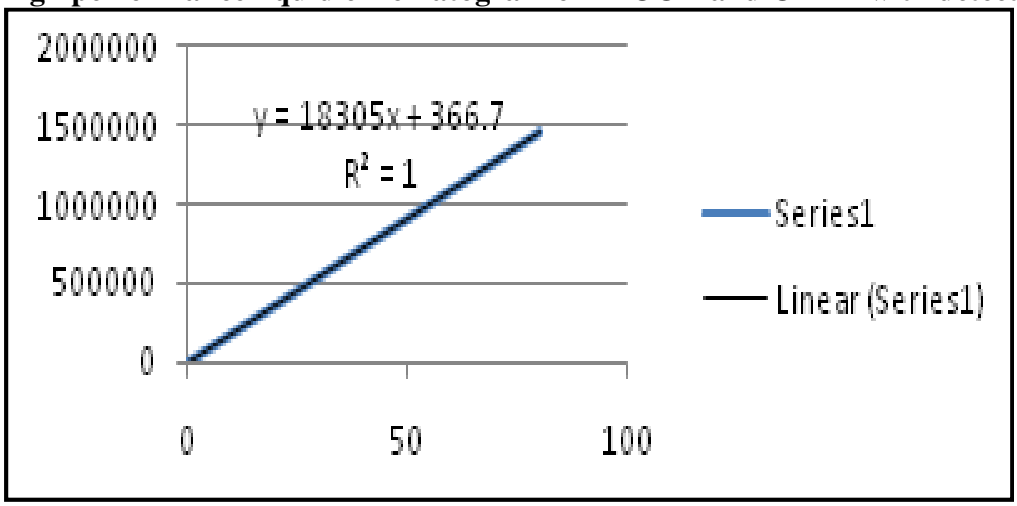

Figure No.5: Linearity graph of Fluoxetine hydrochloride

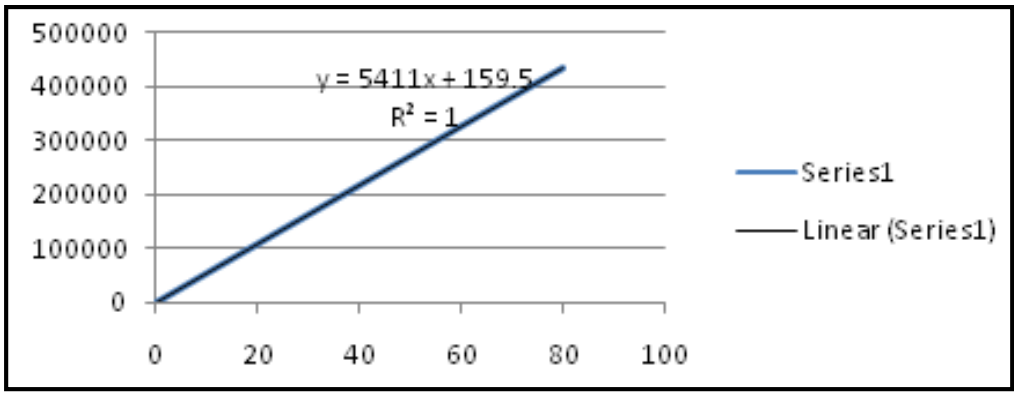

Figure No.6: Linearity graph of Olanzapine

CONCLUSION

The proposed method has advantage of simplicity and convenience for the separation and quantitation of Fluoxetine HCl and Olanzapine in the combination and can be used for the assay of their dosage form. Also, very short analytical run time and low quantity of solvent utilization and lead to environmentally simple chromatographic technique. The method is accurate, precise, rapid and selective for simultaneous estimation of Fluoxetine HCl and Olanzapine in tablet dosage form. Hence the method can be easily adopted for routine analysis.

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CONFLICT OF INTEREST

Authors declare no conflict of interest.

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