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METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF FLUOXETINE HYDROCHLORIDE AND OLANZAPINE IN A PHARMACEUTICAL FORMULATION BY RP-HPLC METHOD

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ABSTRACT

An isocratic Simultaneous estimation by RP-HPLC Method were developed and validated for the quantification of Fluoxetine Hcl and Olanzapine in tablet dosage form. Quantification was achieved by using a reversed-phase C18 column (HYPERSIL 3V ODS Column, 5µ, 250 mm × 4.6 mm) at ambient temperature with mobile phase consisting of Mixed Phosphate buffer (KH_2PO_4 and K_2HPO_4): Acetonitrile (55:45) pH-5.8. The flow rate was 1.0 ml/min. Measurements were made at a wavelength of 261nm. The average retention time for Fluoxetine Hcl and Olanzapine were found to be 2.427 min and 3.427. The proposed method was validated for selectivity, precision, linearity and accuracy. The assay methods were found to be linear from 72-168 µg/ml for Fluoxetine Hcl and 18 to 48µg/ml for Olanzapine. All validation parameters were within the acceptable range. The developed methods were successfully applied to estimate the amount of Fluoxetine Hcl and Olanzapine in tablet dosage form.

KEYWORDS

Fluoxetine Hcl, Olanzapine, RP-HPLC method, HYPERSIL 3V ODS, Acetonitrile, KH_2PO_4, K_2HPO_4, Ortho phosphoric acid and Validation.

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INTRODUCTION

Fluoxetine Hcl (Figure No.1) is an antidepressant of the selective serotonin reuptake inhibitor (SSRI) class[1, 2]. Fluoxetine is approved for the treatment of major depression (including pediatric depression), obsessive-compulsive disorder (in both adult and pediatric populations), bulimia nervosa, panic disorder and premenstrual dysphoric disorder.

In addition, fluoxetine is used to treat trichotillomania if cognitive behaviour therapy is unsuccessful. In combination with olanzapine it is known as Symbyax[1, 2].

Mechanism of action

Fluoxetine's mechanism of action is predominantly that of a serotonin reuptake inhibitor. Fluoxetine delays the reuptake of serotonin, resulting in serotonin persisting longer when it is released. Fluoxetine may also produce some of its effects via its weak 5-HT2C receptor antagonist effects. In addition, fluoxetine has been found to act as an agonist of the σ1-receptor, with a potency greater than that of citalopram but less than that of fluvoxamine. However, the significance of this property is not fully clear[2].

Medical uses

Fluoxetine is frequently used to treat major depression, obsessive compulsive disorder, post-traumatic stress disorder, bulimia nervosa, panic disorder, body dysmorphic disorder, premenstrual dysphoric disorder, and trichotillomania. Caution should be taken when using any SSRI for bipolar disorder as this can increase the likelihood of mania; however, fluoxetine can be used with an antipsychotic (such as quetiapine) for bipolar. It has also been used for cataplexy, obesity, and alcohol dependence, as well as binge eating disorder[1, 2].

Side Effects

Sexual dysfunction is a common side effect with SSRIs. Specifically, side effects often include difficulty becoming aroused, erectile dysfunction, lack of interest in sex, and anorgasmia (inability to achieve orgasm). Genital anesthesia, loss of or decreased response to sexual stimuli, and ejaculatory anhedonia are also possible[1, 2].

Olanzapine

Olanzapine (Figure No.1) is an atypical antipsychotic drug[3, 4].

Categories

Anti-emetics, Antipsychotics, Antipsychotic Agents, Serotonin Uptake Inhibitors[4].

Mechanism of Action

Olanzapine's antipsychotic activity is likely due to a combination of antagonism at D2 receptors in the mesolimbic pathway and 5HT2A receptors in the frontal cortex. Antagonism at D2 receptors relieves positive symptoms while antagonism at 5HT2A receptors relieves negative symptoms of schizophrenia[4].

Pharmacodynamics

Olanzapine, an atypical antipsychotic agent, is used to treat both negative and positive symptoms of schizophrenia, acute mania with bipolar disorder, agitation, and psychotic symptoms in dementia. Future uses may include the treatment of obsessive-compulsive disorder and severe behavioral disorders in autism. Structurally and pharmacologically similar to clozapine, olanzapine binds to alpha(1), dopamine, histamine H1, muscarinic, and serotonin type 2 (5-HT2) receptors[3, 4].

Route of elimination

It is eliminated extensively by first pass metabolism, with approximately 40% of the dose metabolized before reaching the systemic circulation. Following a single oral dose of 14C labeled olanzapine, 7% of the dose of olanzapine was recovered in the urine as unchanged drug, indicating that olanzapine is highly metabolized[3, 4].

MATERIAL AND METHOD

Instruments the chromatographic technique performed on a Shimadzu LC20-AT Liquid chromatography with SPD-20A prominence UV-visible detector and Spinchrom software, reversed phase C18 column (HYPERSIL 5µ, 250 mm × 4.6 mm) as stationary phase, Electron corporation double beam UV-visible spectrophotometer (vision pro-software), Ultrasonic cleaner, Shimadzu analytical balance AY-220, Vacuum micro filtration unit with 0.45µ membrane filter was used in the study.

MATERIALS

Pharmaceutically pure sample of Fluoxetine Hcl and Olanzapine were obtained as gift samples from Chandra lab, Prashanthinagar, Kukatpally, Hyderabad, India. The purity of the drug was evaluated by obtaining its melting point and ultraviolet (UV) and infrared (IR) spectra. No

impurities were found. The drug was used without further purification.

HPLC-grade Acetonitrile and THF ware from standard reagents pvt ltd. KH_2PO_4 and K_2HPO_4 (AR grade) was from Merck.

A tablet formulation of Fluoxetine Hcl and Olanzapine (20 mg and 5mg label claims) were procured from local market (FOSTERA-5, INTAS COMPANY, India).

METHOD[5-11]

Determination of Working Wavelength (λmax)

10 mg of the Fluoxetine Hcl standard drug is taken in a 10 ml volumetric flask and dissolved in methanol and volume made up to the mark, from this solution 0.1ml is pipetted into 10 ml volumetric flask and made upto the mark with the methanol to give a concentration of 10 µg/ml. The above prepared solution is scanned in UV between 200-400nm using methanol as blank. The λmax was found to be 247nm (Figure No.2).

10 mg of the Olanzapine standard drug is taken in a 10 ml volumetric flask and dissolved in methanol and volume made up to the mark, from this solution 0.1ml is pipetted into 10 ml volumetric flask and made upto the mark with the methanol to give a concentration of 10 µg/ml. The above prepared solution is scanned in UV between 200-400 nm using methanol as blank. The λmax was found to be 276nm (Figure No.2.1).

The iso bestic point of Fluoxetine Hcl and Olanzapine were found to be 261nm (Figure No.2.2).

Preparation of mobile phase

Buffer Preparation

Weigh accurately about 1.625 gms of Potassium Di Hydrogen Ortho Phosphate and 0.3 gms of Di Potassium Hydrogen Ortho Phosphate and dissolve with 200ml of HPLC Grade water than make up to 550 ml with HPLC grade water then adjust the pH: 5.8 with Ortho phosphoric acid.

Mobile phase

Then add 55 volumes of buffer, 45 volumes of Acetonitrile and sonicated for 15 min and filtered through a 0.45 µ membrane filter.

Analysis of formulation

Preparation of standard solution

A 120mg of standard Fluoxetine Hcl and 32 mg Olanzapine ware weighed and transferred to 100 ml of volumetric flask and dissolved in mobile phase. The flask was shaken and volume was made up to mark with mobile phase to give a primary stock solution containing 1200µg/ml Fluoxetine Hcl and 320µg/ml of Olanzapine. From the above solution 5ml of solution is pipetted out into a 50 ml volumetric flask and volume was made up to mark with mobile phase to give a solution containing 120µg/ml Fluoxetine Hcl and 32µg/ml of Olanzapine.

Preparation of sample solution

For the estimation of the drug in tablet formulation twenty tablets were weighed and their average weight was determined. The tablets were then finely powdered. Appropriate quantity equivalent to 120mg Fluoxetine Hcl and 32mg Olanzapine ware accurately weighed and The powder was transferred to 100 ml volumetric flask and shaken vigorously with mobile phase and sonicated for 15 min and volume made up to the mark with mobile phase. The solution was shaken vigorously and filtered by using whatmann filter no.41. from the above filtered clear solution 5ml of sample pipetted out into a 100 ml volumetric flask volume made up to the mark with mobile phase to give a solution containing 120µg/ml Fluoxetine Hcl and 32µg/ml of Olanzapine.

Calculation 5 replicates of each of sample and standard solutions were injected and their average peak areas were taken.

The amount of Fluoxetine Hcl and Olanzapine present in the formulation by using the formula given below and results shown in above Table No.6.

$$\%\ \text{Assay} = \frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \frac{AW}{LC} \times 100$$

Where,

AS: Average peak area due to standard preparation

AT: Peak area due to assay preparation

WS: Weight of standard drug taken

WT: Weight of sample in assay preparation

DT: Dilution of assay preparation
DS: Dilution of standard preparation
AW: Average weight of 20 tablets
LC: Label claim
P: Purity of standard drug.

METHOD VALIDATION

Linearity

Linearity was studied by analyzing five standard solutions covering the range of 76-168 µg/ml for Fluoxetine Hcl and 18-48µg/ml for Olanzapine of the drug. From the primary stock solution 0.6ml, 0.8ml, 1.0ml, 1.2ml, 1.4 ml of aliquots are pipetted into 10 ml volumetric flasks and made up to the mark with the mobile phase to give a concentrations of 72µg/mL, 96µg/mL, 120µg/mL, 144µg/mL and 168 µg/mL of Fluoxetine Hcl and 18µg/mL, 24µg/Ml, 32µg/mL, 40µg/mL, 48µg/mL of Olanzapine (Table No.1 and 1.1).

Calibration curve (Figure No.3 and 3.1) with concentration verses peak areas was plotted by injecting the above prepared solutions and the obtained data were subjected to regression analysis using the least squares method (Figure No.4).

Method precision (repeatability)

The precision of the instrument was checked by repeated injections and measurement of peak areas and retention times of solutions (n = 6) for, 120µg/ml of Fluoxetine Hcl and 32µg/ml of Olanzapine without changing the parameter of the proposed chromatographic method.

Intermediate precision (reproducibility)

The intraday and interday precision of the proposed method was determined by analyzing the corresponding responses 3 different days over a period of 1 week for 120µg/ml and 32µg/ml concentrations of standard solutions of Fluoxetine Hcl and Olanzapine. The result was reported in terms of relative standard deviation (% RSD).

Limit of detection and limit of quantification

The limit of detection (LOD) and limit of quantification (LOQ) (Table No.2, 2.1 and 2.2) were separately determined based on standard deviation of the y-intercept and the slope of the calibration curve by using the equations (2) and (3), respectively.

$$LOD = 3.3\ \delta/S \quad \text{…… (3)}$$

$$LOQ = 10\ \delta/S \quad \text{…….. (4)}$$

Where,

σ = the standard deviation of the response
S = the slope of the calibration curve

The slope S may be estimated from the calibration curve of the analyte.

Accuracy (recovery study)

The accuracy of the method was determined by calculating the recoveries of Fluoxetine Hcl and Olanzapine by the standard addition method. Known amounts of standard solutions of Fluoxetine Hcl and Olanzapine were added at 20% concentration to pre quantified sample solutions of Fluoxetine Hcl (120,144, 168µg/ml) and Olanzapine (30, 36, 48µg/ml) (Figure No.5 and 6). The amount of Fluoxetine Hcl and Olanzapine recovered was estimated by using the following formulas (Table No.3).

$$\% \text{ Recovery} = \frac{\text{Amount found}}{\text{Amount added}} \times 100$$

$$\text{Amount Found (mcg/ml)} = \frac{\text{Mean test area}}{\text{Mean standard area}} \times \text{Standard concentration}$$

Specificity

In an assay, demonstration of specificity requires that it can be shown that the procedure is unaffected by the presence of impurities or excipients. In practice, this can be done by spiking the drug substance or product with appropriate levels of impurities or excipients and demonstrating that the assay results are unaffected by the presence of these extraneous materials. There should be no interference of the diluents, placebo at retention time of drug substances (Figure No.7).

Robustness

Robustness is the measure of a method remain unaffected by small, deliberate changes in method parameters like flow rate and detection wavelength on assay of the analyte of interest. Here the detection wavelength varied ±2nm and flow rate was varied ±0.2 ml/min. The results were shown in (Table No.4).

Ruggedness

The ruggedness of the method was studied by analyzing the sample and standard preparations by two analysts. The % RSD assay values between two analysts was calculated i.e., (limit <2%). This indicates the method was rugged. The results were shown in Table No.5.

RESULTS AND DISCUSSION

In RP-HPLC method, the primary requirement for developing a method for analysis is that the using different solvents and buffers and columns to get better retention time and theoretical plates, and better cost effective and time saving method than the previously developed methods. The Iso bestic Point of Fluoxetine Hcl and Olanzapine were found to be 261nm (Figure No.2.2) by scanning in UV region. The chromatographic method was optimised with mobile phase consisting of Mixed Phosphate Buffer: Acetonitrile (55:45) and C18 HYPERSIL 3V ODS column. All the validation parameters were studied at a the wavelength 261nm. Accuracy was determined by calculating the recovery (Table No.3) and the results were in acceptable range (limit 98-102%). The method was successfully used to determine the amount of Fluoxetine Hcl and Olanzapine present in the Tablet. The results obtained were in good agreement with the corresponding labeled amount (Table No.3). The method was linear in the concentration range of 72 to 168 µg/ml for Fluoxetine Hcl and 18 to 48 µg/ml for Olanzapine (Figure No.3 and 3.1, Table No.1 and 1.1). Precision was calculated as repeatability and intra and inter day variations (% RSD) for the drug (Table No.7 and 8). Robustness and ruggedness results were in acceptable range (Table No.4 and Table No.5). Summary of all validation parameters for method is given in Table No.9. By observing the validation parameters, the method was found to be simple, sensitive, accurate and precise. Hence the method can be employed for the routine analysis Fluoxetine Hcl and Olanzapine in tablet dosage form.

Table No.1 and 1.1: Linearity

S.No	Fluoxetine Hcl	
	Mcg	Area
1	72	2069.276
2	96	2691.879
3	120	3303.04
4	144	4117.443
5	168	4712.721

S.No	Olanzapine Hcl	
	Mcg	Area
1	18	715.354
2	24	994.378
3	30	1201.435
4	36	1501.708
5	42	1741.482

Table No.2, 2.1 and 2.2: LOD and LOQ values from calibration curve

S.No	Fluoxetine Hcl	
	Mcg	**Area**
1	72	2069.276
2	96	2691.879
3	120	3303.04
4	144	4117.443
5	168	4712.721
Std dev	**37.9**	**1063**
Slope	**62.9**	

S.No	Olanzapine	
	Mcg	**Area**
1	18	715.354
2	24	994.378
3	30	1201.435
4	36	1501.708
5	42	1741.482
Std dev	**9.48**	**405**
Slope	**44.92**	

S.No	Fluoxetine Hcl			S.No	Olanzapine	
		Mcg	**Area**		**mcg**	**Area**
1	**LOD**	1.99	55.76	1	0.70	29.76
2	**LOQ**	6.03	168.98	2	2.11	90.20

Table No.3: Recovery data

S.No	Level	Amount of Sample taken (%)	Amount of Standard Spiked (%)	% Recovery of Fluoxetine Hcl	% Recovery of Olanzapine
1	I	80	20	99.45%	100.23%
		80	20		
		80	20		
2	II	100	20	98.45%	100.00%
		100	20		
		100	20		
3	III	120	20	100.01%	100.71 %
		120	20		
		120	20		

Table No.4: Results of Robustness study

S.No	Parameter	Rt of Fluoxetine Hcl	Tailing factor	Peak Area	% Assay
1	Flow Rate(0.8ml)	2.710	1.647	3694.353	98.09%
2	1.2ml	2.207	1.607	3464.085	
3	1.0ml	2.520	1.308	3544.463	
4	Wave Length259nm	2.447	1.531	3419.115	100.21
5	261nm	2.441	1.512	3454.896	
6	263nm	2.448	1.613	3440.738	

S.No	Parameter	Rt of Olanzapine	Tailing factor	Peak Area	% Assay
1	Flow Rate(0.8ml)	3.837	1.375	1376.990	98.91%
2	1.2ml	3.117	1.424	1293.728	
3	1.0ml	3.457	1.452	1304.121	
4	Wave Length259nm	3.450	1.417	1338.756	98.84%
5	261nm	3.440	1.301	1339.214	
6	263nm	3.450	1.389	1301.608	

Table No.5: Results of Ruggedness

S.No	Analyst	Drug	Std Area	Spl Area	% Assay	% RSD
1	Analyst-1	Fluoxetine Hcl	3396.887	3410.013	100.24%	0.24%
2	Analyst-2		3384.712	3404.891	100.392%	
3	Analyst-1	Olanzapine	1267.424	1269.233	99.83%	1.18%
4	Analyst-2		1299.621	1269.367	98.33%	

Table No.6: Assay Results

S.No	Fluoxetine Hcl			Olanzapine	
1	Standard Area	1	3401.051		1298.347
		2	3402.768		1266.878
		3	3384.712	---	1267.424
		4	3393.092		1258.842
		5	3404.891		1269.233
		Average	3396.177	Average	1272.145
2	Sample area	1	3421.229		1277.251
		2	3393.092		1258.842
		3	3404.765	---	1273.900
		4	3410.013		1269.367
		5	3396.887		1299.621
		Average	3405.197	Average	1275.796
3	Tablet average weight		60.2 mg		60.2 mg
4	Standard weight		25 mg		2.5 mg
5	Sample weight		300.5 mg		300.2 mg
5	Label amount		5 mg	---	0.5 mg
6	Std.purity		99.6 %		99.7 %
6	Cal.:		5.00 mg		0.50 mg
	---	% Assay	100.03 %		100.25 %

Table No.7: Method Precision (Repeatability)

S.No	Fluoxetine Hcl		Olanzapine	
	Rt	Area	Rt	Area
1	2.443	3345.136	3.480	1265.773
2	2.44	3438.110	3.477	1285.365
3	2.433	3422.085	3.467	1276.575
4	2.42	3401.776	3.447	1252.495
5	2.42	3396.296	3.450	1267.887
6	2.423	3410.543	3.453	1259.386
Avg	2.4298	3402.324	3.462	1267.914
St.dev	0.0103	31.767	0.014	11.786
% RSD	0.42	0.93	0.41	0.93

Table No.8: Intermediate Precision

Method Precision (day1)

S.No	Fluoxetine Hcl		Olanzapine	
	Rt	Area	Rt	Area
1	2.444	3346.254	3.480	1265.258
2	2.44	3462.258	3.477	1261.325
3	2.441	3425.258	3.467	1260.504
4	2.442	3441.250	3.447	1254.258
5	2.44	3440.152	3.450	1262.054
6	2.424	3441.258	3.453	1260.25
Avg	2.4385	3426.072	3.462	1260.608
St.dev	0.0073	40.842	0.014	3.597
% RSD	0.30	1.19	0.41	0.29

Method Precision (day2)

S.No	Fluoxetine Hcl		Olanzapine	
	Rt	Area	Rt	Area
1	2.404	3424.258	3.475	1262.252
2	2.432	3421.251	3.457	1264.58
3	2.441	3431.250	3.467	1268.254
4	2.439	3421.646	3.441	1264.258
5	2.44	3425.258	3.417	1261.243
6	2.44	3425.879	3.417	1267.895
Avg	2.4327	3424.924	3.446	1264.747
St.dev	0.0144	3.626	0.025	2.863
% RSD	0.59	0.11	0.72	0.23

Method Precision (day3)

S.No	Fluoxetine Hcl		Olanzapine	
	Rt	Area	Rt	Area
1	2.451	3425.258	3.475	1236.254
2	2.441	3415.268	3.486	1234.582
3	2.417	3412.358	3.463	1247.256
4	2.475	3475.874	3.486	1275.258
5	2.447	3412.256	3.475	1236.369
6	2.441	3426.325	3.412	1278.214
Avg	2.4453	3427.890	3.466	1251.322
St.dev	0.0187	24.318	0.028	20.216
% RSD	0.77	0.71	0.80	1.62

Table No.9: Validation parameters of evaluated method

S.No	Parameter	Value Obtained of Fluoxetine Hcl	Value Obtained Olanzapine
1	Accuracy (% Recovery)	98.45-100.01%	100.00-100.71%
2	Linearity concentrations Range (µ g/mL) Regression coefficient (R2 value)	72-168 µ g/mL 0.997	18-48 µ g/mL 0.997
3	LOD	1.99	0.70
4	LOQ	6.09	2.11
5	Precision (% RSD) Method precision (Repeatability) (% RSD, n = 6)	0.42-0.93%	0.41-0.93%
6	Intermediate Precision	0.11-1.19%	0.23-1.69%
7	Robustness (%assay)	98.09-99.21%	98.84-99.71%
8	Ruggedness (% RSD analyst to analyst variation)	98.19-100.21%	98.84-98.91%

aSD=Standard deviation, bLOD = Limit of detection, cLOQ = Limit of quantification,

dRSD = Relative standard deviation.

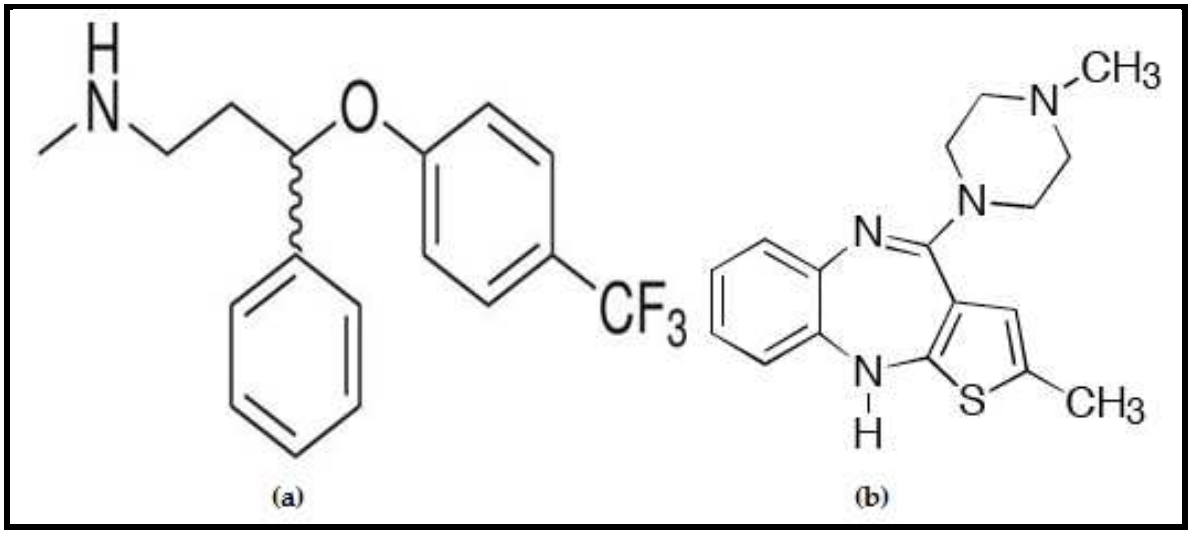

Figure No.1: Structure of Fluoxetine Hcl (a) and Olanzapine (b)

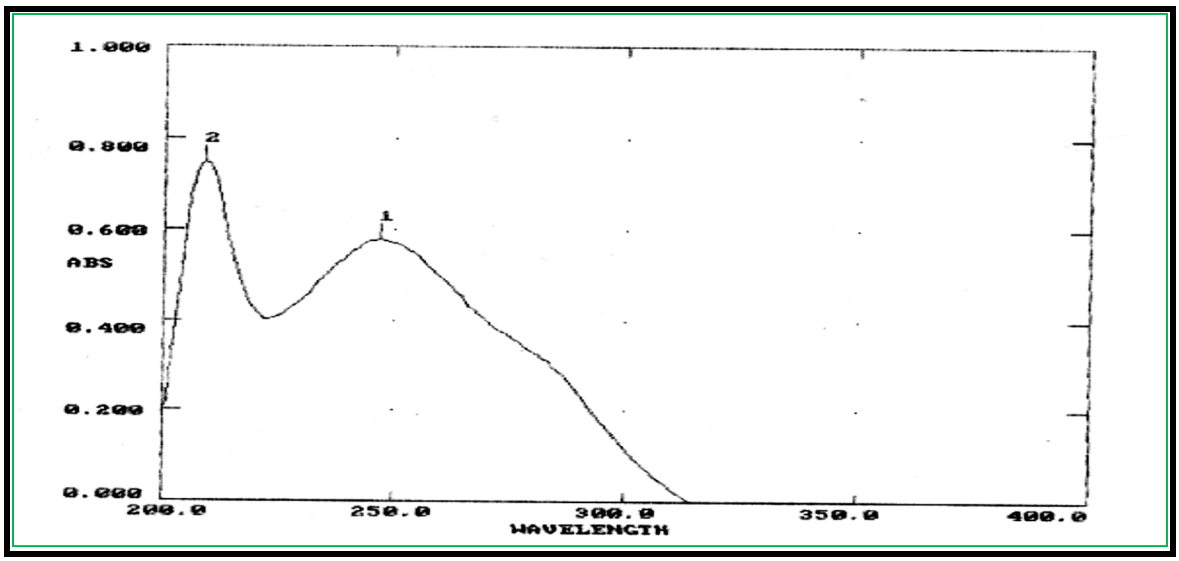

Figure No.2: Determination of Working Wavelength (λmax) of Fluoxetine Hcl

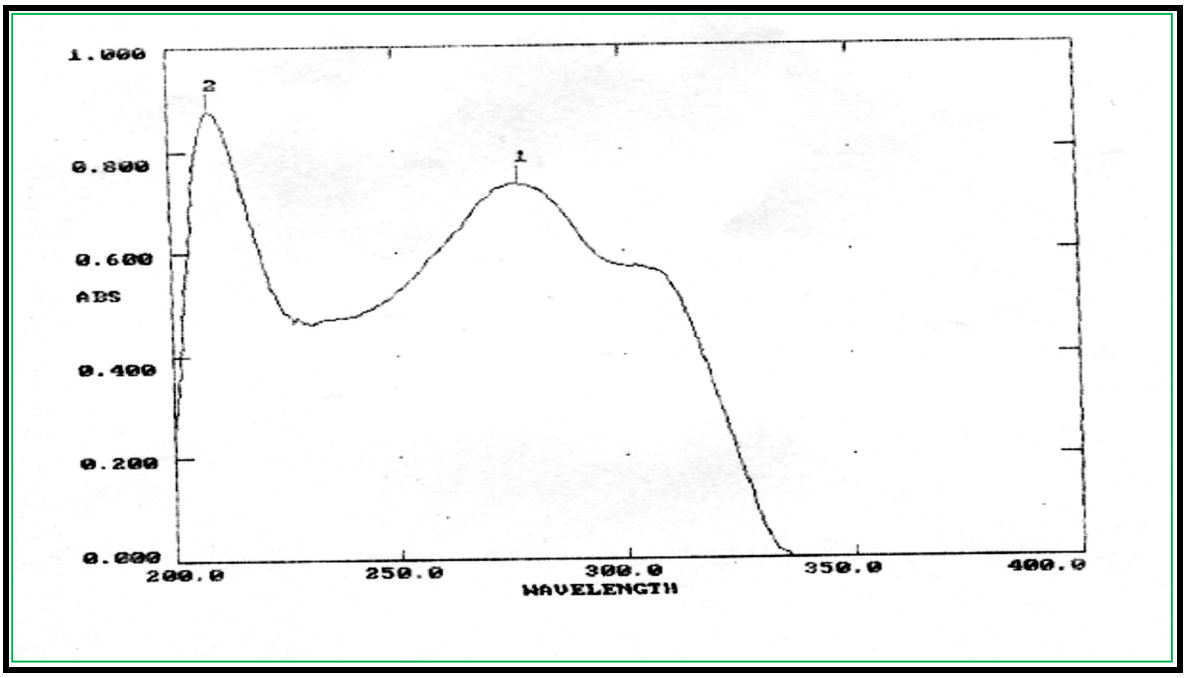

Figure No.2.1: Determination of Working Wavelength (λmax) of Olanzapine

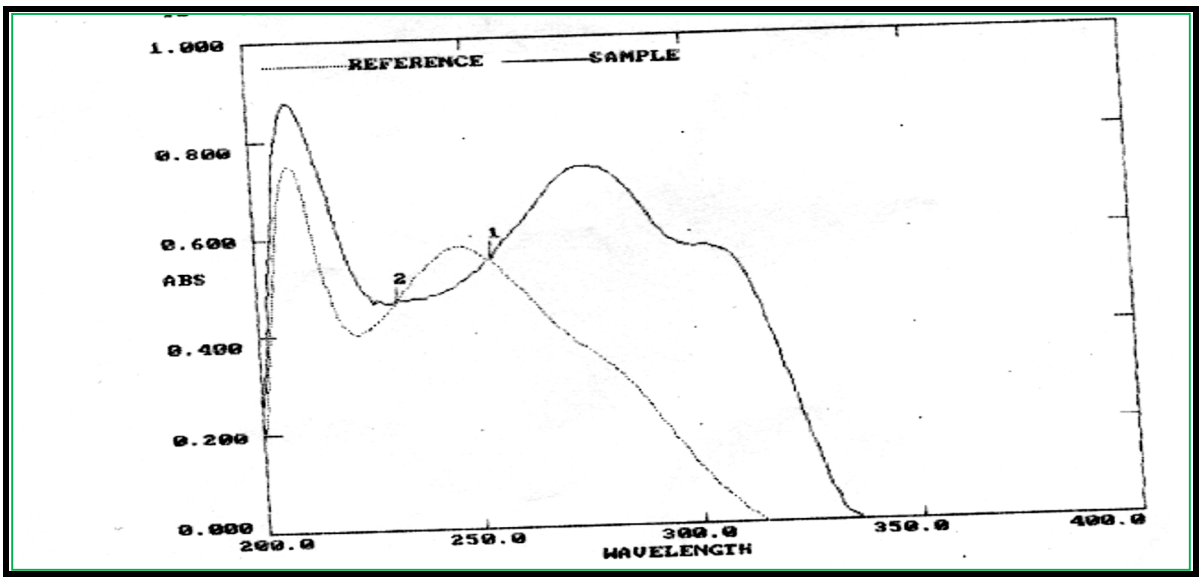

Figure No.2.2: Determination of iso bestic Point

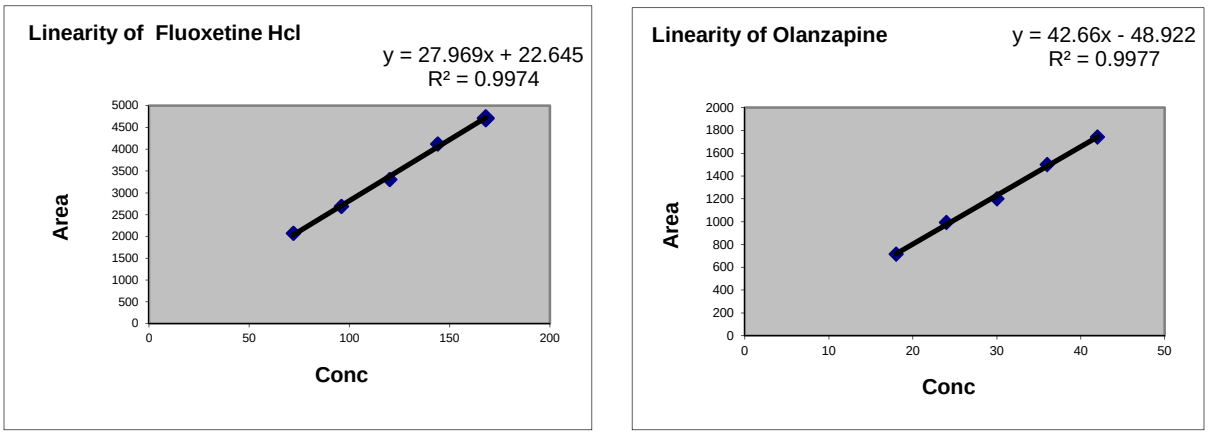

Figure No.3 and 3.1: Linearity (calibration) curve of Fluoxetine Hcl and Olanzapine

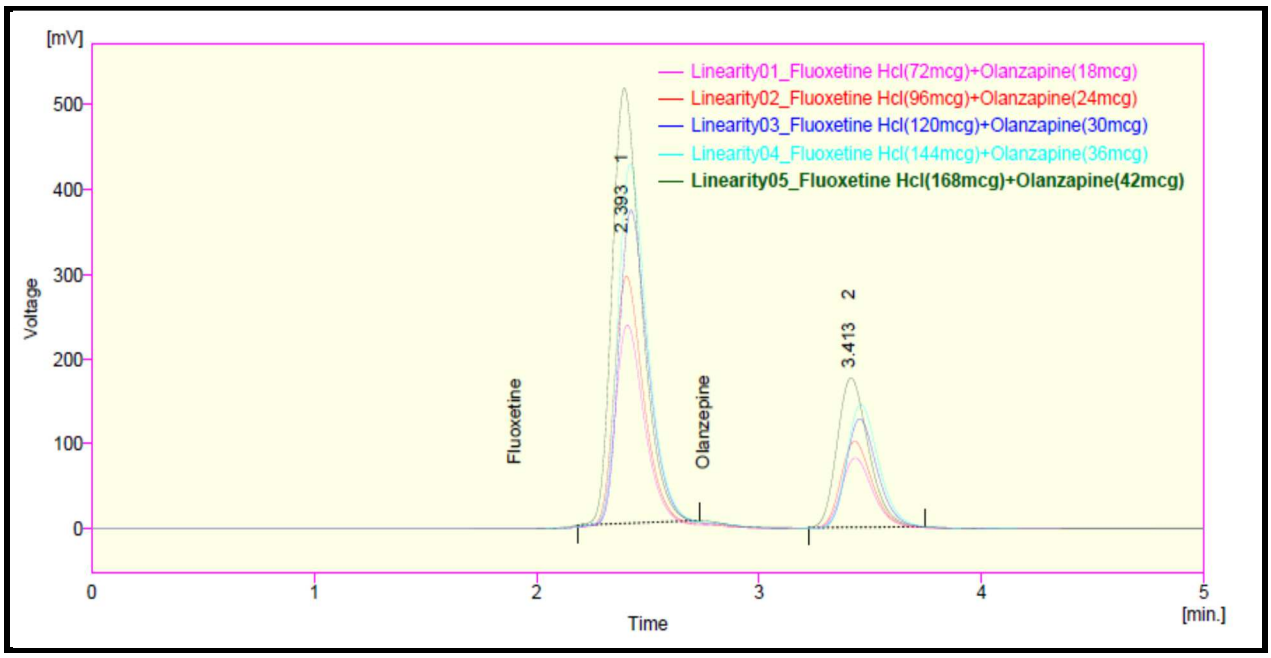

Figure No.4: Overlain chromatograms of Linearity

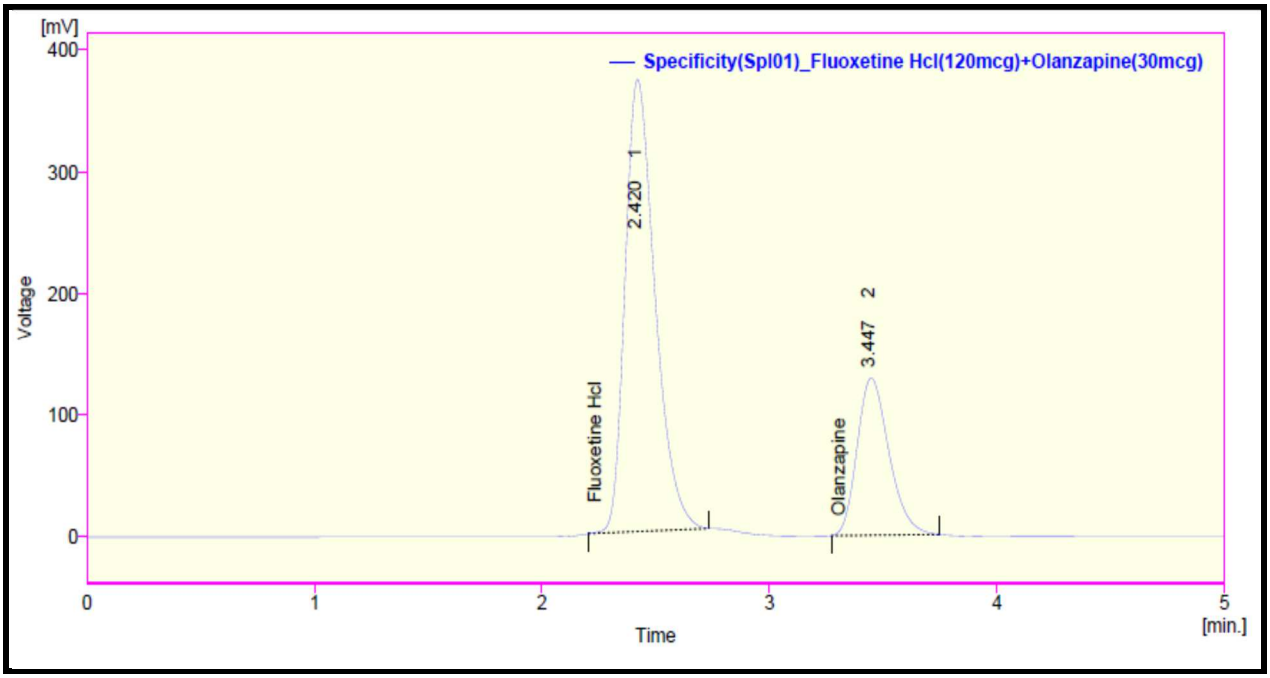

Figure No.5: Chromatogram of Assay sample preparation

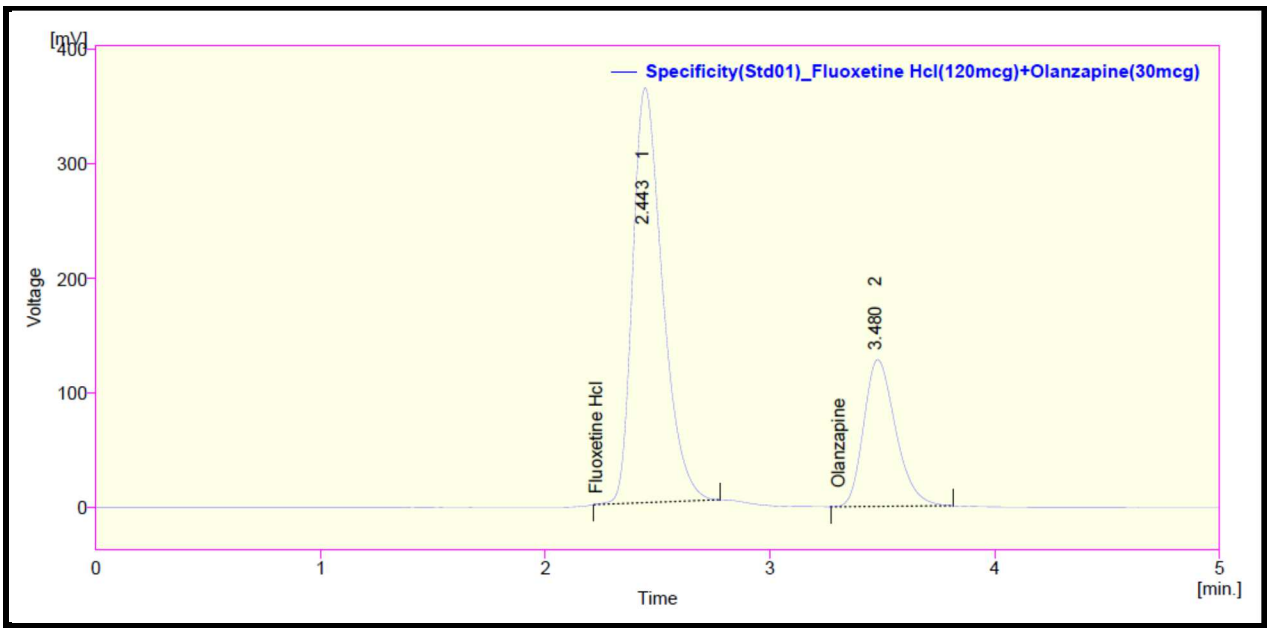

Figure No.6: Chromatogram of assay standard preparation

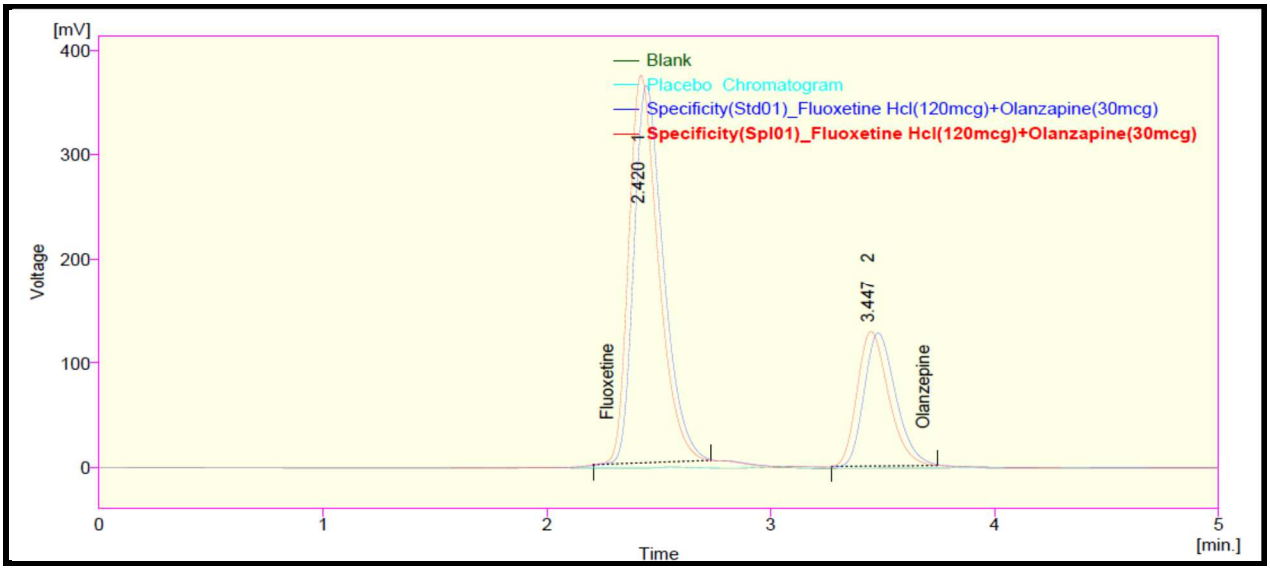

Figure No.7: Overlain chromatograms of Specificity (placebo, blank, sample and standard preparations)

CONCLUSION

The proposed Simultaneous Estimation by RP-HPLC method was found to be simple, sensitive, accurate and precise for determination of Fluoxetine Hcl and Olanzapine in tablet. The method utilizes easily available and cheap solvent for analysis of Fluoxetine Hcl and Olanzapine hence the method was also economic for estimation of Fluoxetine Hcl and Olanzapine from Tablet. The common excipients and other additives are usually present in the Tablet mixture do not interfere in the analysis of Fluoxetine Hcl and Olanzapine, hence it can be conveniently adopted for routine quality control analysis of the drug in pharmaceutical formulation.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

REFERENCES

1. www.wikipedia.org/wiki/Fluoxetine Hcl.
2. http://www.drugbank.ca/drugs/DB00335.
3. www.wikipedia.org/wiki/Olanzapine.
4. http://www.drugbank.ca/drugs/DB00528.
5. ICH, Q2A validation of analytical procedure: Methodology International Conference on Harmonization, Geneva, 1994.
6. ICH, Q2B Validation of analytical procedure: Methodology International Conference on Harmonization, Geneva, 1996.
7. http://www.medlineindia.com.
8. Patel S and Patel N J. Developed Simultaneous Estimation of Fluoxetine Hcl and Olanzepine in Combined Dosage Form by RP-HPLC Method,
9. Eshwarudu M M, Anitha M, Gayathri N. Developed Simultaneous Estimation of Fluoxetine Hcl and Olanzepine in Combined Dosage Form by RP-HPLC Method,
10. http://www.ncbi.nlm.nih.gov/pubmed/20502563
11. http://www.irjponline.com/admin/php/uploads/1029_pdf.pdf.

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