Research Article

DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF BREXPIPRAZOLE AND FLUOXETINE IN DRUG SUBSTANCE BY RP-HPLC

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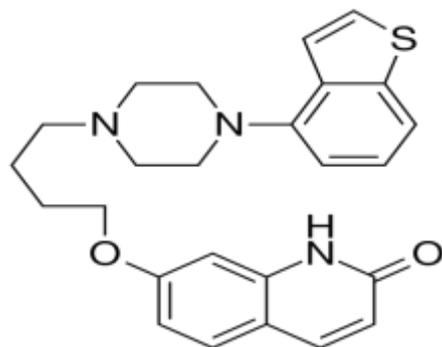
ABSTRACT

New Analytical method was developed for the estimation of Brexpiprazole and Fluoxetine in drug product by liquid chromatography. The chromatographic separation was achieved on C18 column (Inertsil ODS 3V 250*4.6mm) at ambient temperature. The separation achieved employing a mobile phase consists of 0.1%v/v Formic acid in water: Methanol(350:650). The flow rate was 0.8ml/ minute and ultra violet detector at 263nm. The average retention time for Brexpiprazole and Fluoxetine found to be 2.742 min and 3.410 min. the proposed method was validated for selectivity, precision, linearity and accuracy. All validation parameters were within the acceptable range. The assay methods were found to be linear from 51.5 - 154.5µg/ml for Brexpiprazole and 48.5 - 145.5µg/ml of Fluoxetine.

KEYWORDS: Brexpiprazole and Fluoxetine, Isocratic, HPLC, Inertsil ODS 3V, Formic acid, Methanol and validation.

BREXPIPRAZOLE

Brexpiprazole is an atypical antipsychotic drug. It is a D2 dopamine partial agonist called serotonin-dopamine activity modulator. The drug received FDA approval on July 13, 2015 for the treatment of schizophrenia and as an adjunctive treatment for depression. Although it failed Phase II clinical trials for ADHD, it has been designed to provide improved efficacy and tolerability over established adjunctive treatments for major depressive disorder (MDD).

Brexpiprazole is chemically designated as 7-{4-[4-(1-benzothiophen-4-yl) piperazin-1-yl] butoxy} quinolin-2(1H)-one. Its molecular formula is C25H27N3O2S and its molecular weight is 433.6 g/mol.
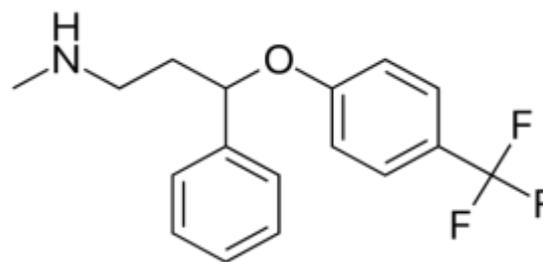
FLUOXETINE

Fluoxetine also known by trade names **Prozac** and **Sarafem** among others is an antidepressant of the selective serotonin reuptake inhibitor class. It is used for the treatment of major depressive disorder, obsessive–compulsive disorder, bulimia nervosa, panic disorder and premenstrual dysphoric disorder. It may decrease the risk of suicide in those over the age of 65.[2] Fluoxetine has also been used to treat premature ejaculation. It is taken by mouth.

Fluoxetine is chemically designated as *N*-methyl-3-phenyl-3-[4-(trifluoromethyl) phenoxy]propan-1-amine. Its molecular formula is $C_{17}H_{18}F_3NO$, and its molecular weight is 309.33 g·mol^{-1}.

Structure of Brexpiprazole

Structure of Fluoxetine

EXPERIMENTAL

Equipments: The chromatographic technique performed on a waters 2695 with 2487 detector and Empower2 software, reversed phase C18 column (Inertsil ODS 3V 250*4.6,5μ) as stationary phase, Ultrasonic cleaner, Scaletech analytical balance, Vaccum micro filtration unit with 0.45μ membrane filter was used in the study.

Materials: Pharmaceutically pure sample of Brexpiprazole/Fluoxetine were obtained as gift samples from Fortune pharma training institute, Sri Sai nagar colony, KPHB, Hyderabad, India.

HPLC-grade Methanol was from qualigens reagents pvt ltd. Formic acid (AR grade) was from sd fine chem.

Chromatographic conditions

The sample separation was achieved on a (5 μ, 250 cm X 4.6 mm i.d.) Inertsil ODS 3V C18 column, aided by mobile phase mixture of 0.1%v/v Formic acid in water: Methanol (35:65). The flow rate was 0.8 ml/ minute and ultra violet detector at 263nm that was filtered and degassed prior to use, Injection volume is 10 μl and ambient temperatures.

Preparation of mobile phase

Buffer Preparation: Taken accurately 1ml of Formic acid in 1000mL of water.

Mobile phase: Then added 35 volumes of buffer and 65 volumes of Methanol mixed well and sonicated for 5 min.

Diluents: Water: Methanol 50:50 v\v.

Preparation of standard stock solution

A 10 mg of pure Brexpiprazole and 4 mg of Fluoxetine were weighed and transferred to 10 ml of volumetric flask and dissolved in Methanol. The flask was shaken and volume was made up to mark with diluent to give a primary stock solution. From the above solution 0.2ml of solution is pipette out into a 10 ml volumetric flask and volume was made up to mark with water: methanol to give a solution containing 20μg/ml of Brexpiprazole and 20 μg/ml Fluoxetine.

Preparation of sample solution

Accurately weighed twenty tablets were ground to obtain fine powder equivalent to 10mg of Brexpiprazole and 4mg of Fluoxetine sample were weighed and transferred to 10 ml of volumetric flask and dissolved in diluent. The flask was shaken and volume was made up to mark with diluent to give a primary stock solution. From the above solution 0.2 ml of solution is pipette out into a 10 ml volumetric flask and volume was made up to mark with diluents(Water: Methanol) to give a solution containing 20 μg/ml of Brexpiprazole and 8.0 μg/ml Fluoxetine.

RESULTS AND DISCUSSIONS

Determination Of Working Wavelength (λ max): 10 mg of the Brexpiprazole and Fluoxetine standard drug is taken in a 10 ml volumetric flask and dissolved in Diluent and volume made up to the mark, from this solution 0.1ml is pipette into 10 ml volumetric flask and made upto the mark with the Water to give a concentration of 10 μg/ml. The above prepared solution is scanned in uv between 200-400 nm using Water as blank. The λmax was found to be 263nm After several initial trails with mixtures of methanol, water, ACN and buffer in various combinations and proportions, a trail with a mobile phase mixture of 0.1%v/v Formic acid in water: Methanol (35:65). The flow rate was 0.8 ml/ minute brought sharp peaks. The chromatogram was shown in Figure-1**.**
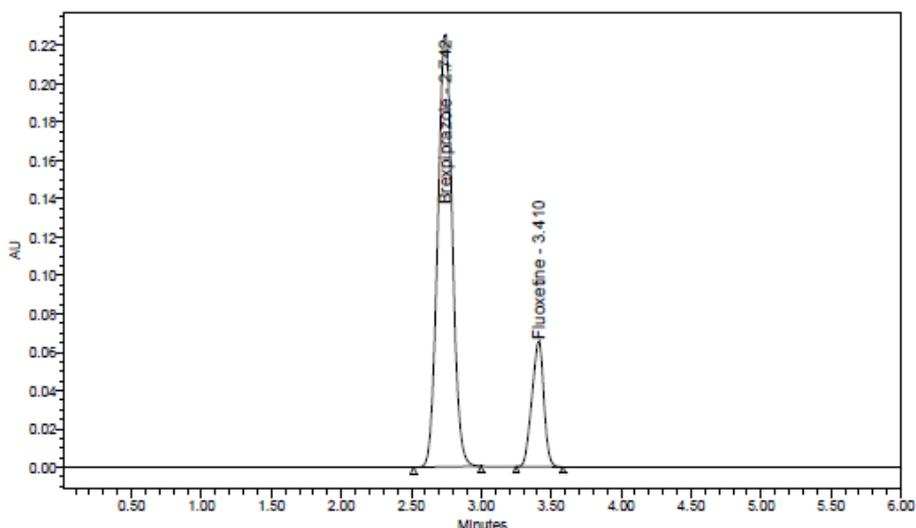

Figure 1: Chromatogram of Brexpiprazole / Fluoxetine.

METHOD VALIDATION
Linearity
Linearity was studied by analyzing five standard solutions covering the range of 10.0 -30.0µg/ml for Brexpiprazole and and 4.0 - 12.0µg/ml Fluoxetine. From the primary stock solution 0.1ml,0.15ml,0.2ml, 0.25ml,0.3 ml of aliquots are pipette into 10 ml volumetric flasks and made up to the mark with the water to give a concentrations of 10.0 µg /mL,

15.0µg/mL, 20.0µg/mL, 25.0µg/mL and 30.0 µg/mL of Brexpiprazole and 4.0g/mL,6.0µg/mL, 8.0µg/mL, 10.0µg/mL and 12.0 µg/mL Fluoxetine.

Calibration curve 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.

Table No: 1: Linearity data of Brexpiprazole.

Level	Concentration (mg/mL)	Peak area
50%	0.010	805292
75%	0.015	1230869
100%	0.020	1650263
125%	0.025	2003936
150%	0.030	2476964
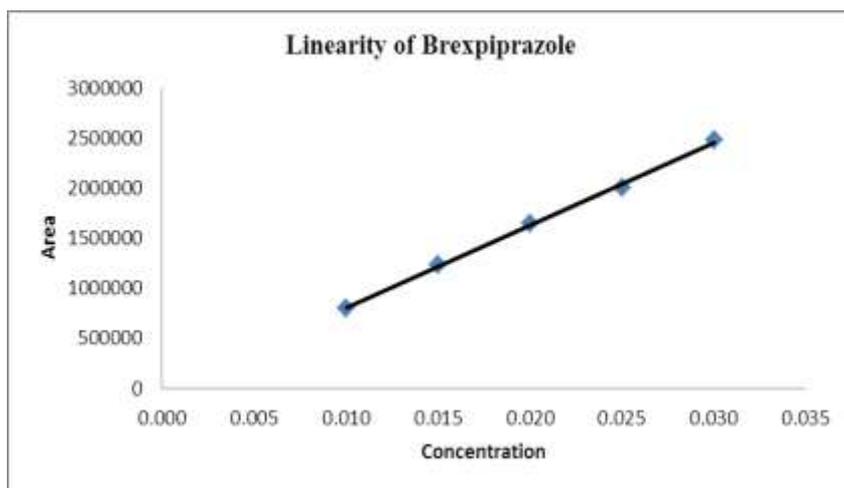
Fig. 2: Linearity (calibration) curve of Brexpiprazole.

Table No: 2: Linearity data of Fluoxetine.

Level	Concentration (mg/mL)	Peak area
50%	0.004	191845
75%	0.006	293607
100%	0.008	391482
125%	0.010	482719
150%	0.012	587779
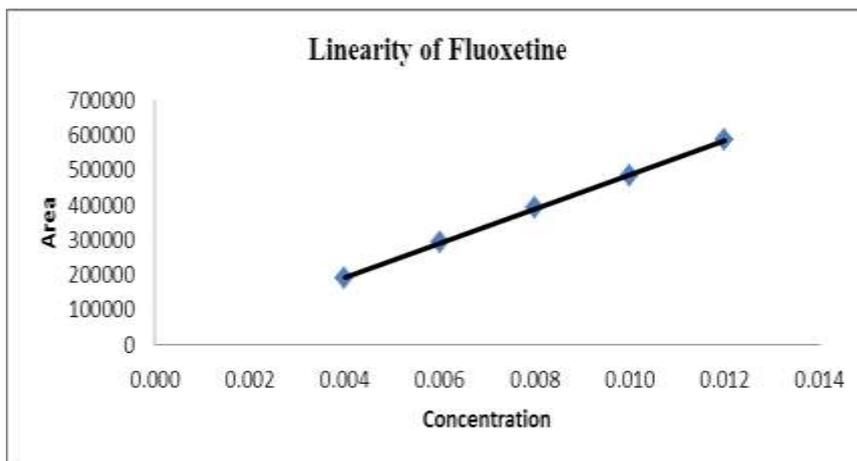
Fig. 3: Linearity (calibration) curve of Fluoxetine.

RESULT

A linear relationship between peak areas versus concentrations was observed for Brexpiprazole and Fluoxetine in the range of 50% to 150% of nominal concentration. Correlation coefficient was 0.9993 and 0.9998 for both Brexpiprazole and Fluoxetine which prove that the method is linear in the range of 50% to 150%.

Limit of detection and limit of quantification

The limit of detection (LOD) and limit of quantification (LOQ) were separately determined based on standard

deviation of the y-intercept and the slope of the calibration curve by using the equations (1) and (2), respectively.

LOD = 3.3 σ /S ………. (1)
LOQ =10 σ /S ……….. (2)

Where,
σ = the standard deviation of the response (STEYX)
S = the slope of the calibration curve
The slope S may be estimated from the calibration curve of the analyte.

Table no. 3: LOD and LOQ values Calculated from calibration curve.

Parameter	Brexpiprazole mg	Fluoxetine mg
LOD	0.001	0.0002
LOQ	0.004	0.001

Method precision (repeatability)

The precision of the method was checked by repeated preparation(n=6) of 20.0μg/ml of Brexpiprazole and

8.0μg/ml Fluoxetine without changing the parameter of the proposed chromatographic method. And measure the peak areas and retention times.

Table 4: Summary of peak areas for method precision of Brexpiprazole.

Sample No	Retention time	Peak area	% Assay
1	2.736	1632978	99.3
2	2.737	1652064	100.4
3	2.735	1634393	99.0
4	2.733	1627717	98.6
5	2.737	1641561	99.6
6	2.734	1624831	98.6
Mean	2.735	1635591	99.2
%RSD	0.06	0.61	0.72

Table 5: Summary of peak areas for method precision of Fluoxetine.

Sample No	Retention time	Peak area	% Assay
1	3.409	386524	98.6
2	3.409	394240	99.0
3	3.403	387392	99.3
4	3.407	387707	99.1
5	3.408	390900	99.6
6	3.407	387473	99.3
Mean	3.407	389039	99.2
%RSD	0.07	0.76	0.31

RESULT

Results of variability were summarized in the above table. Percentage relative standard deviation (%RSD) was found to be less than 2.0% which proves that method is precise.

Accuracy (recovery study)

The accuracy of the method was determined by calculating the recoveries of Brexpiprazole and Fluoxetine by analyzing solutions containing approximately 50%, 100% and 150% of the working strength of Brexpiprazole and Fluoxetine. The percentage recovery results obtained are listed in Table 6 &7.

Table No. 6: Recovery data of Brexpiprazole.

LEVEL	S.NO	%Recovery of Brexpiprazole	Average
50	1	99.6	99.5%
	2	99.8	
	3	99.2	
100	1	99.3	99.6%
	2	100.4	
	3	99.0	
150	1	100.5	99.9%
	2	99.5	
	3	99.6	

Table No. 7: Recovery data of Fluoxetine.

LEVEL	S.NO	%Recovery of Fluoxetine	Average
50	1	99.7	99.6%
	2	99.4	
	3	99.6	
100	1	98.6	99.0%
	2	99.0	
	3	99.3	
150	1	98.8	99.0%
	2	98.7	
	3	99.4	

RESULT
Results of accuracy study are presented in the above table. All the results indicate that the method is highly accurate.

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. 8&9).

Table No. 8: Results of of Brexpiprazole.

Parameter	Rt of Brexpiprazole	Theoretical plates	Asymmetry
Decreased flow rate (0.7ml/min)	3.109	3622	1.03
Increased flow rate (0.9ml/min)	2.438	3129	1.03
Wave Length 261nm	2.742	3326	1.03
265nm	2.741	3339	1.04

Table No. 9: Results of of Fluoxetine.

Parameter	Rt of Fluoxetine	Theoretical plates	Asymmetry
Decreased flow rate (0.7ml/min)	3.878	9497	0.93
Increased flow rate (0.9ml/min)	3.032	6399	0.94
Wave Length 261nm	3.410	7093	0.93
265nm	3.411	7459	0.93

RESULT
The results of Robustness of the present method had shown that changes are not significant we can say that the method is Robust.

Ruggedness
The ruggedness of the method was studied by analyzing the sample and standard preparations by two analysts. The results were shown in Table no.10&11.

Table No. 10: Results of Brexpiprazole.

		%Assay	%RSD
Analyst-1	**BREXPIPRAZOLE**	99.3	0.78%
Analyst-2		100.4	

Table No. 11: Results of Fluoxetine.

		%Assay	%RSD
Analyst-1	**FLUOXETINE**	98.6	0.29%
Analyst-2		99.0	

RESULT

The %RSD assay values between two analysts was calculated, this indicates the method was rugged.

Table No. 12: Summary of Brexpiprazole.

S.No	Parameter	Result	Acceptence criteria
1	System suitability Theoretical plates Asymmetry Retention time %RSD	2.740 3322 1.04 0.31	Not less than 2000 Not more than 2.0 Not more than 2.0
2	Specificity	Specific	Specific
3	Method precision(%RSD)	0.61	Not more than 2.0%
4	Linearity Range(mcg/ml) Correlation coefficient(r^2)	10.0 -30.0 0.9993	Not less than 0.990
5	Accuracy (Mean % recovery) 50% 100% 150%	99.5 99.6 99.9	97 - 103%
6	Robustness	All the system suitability parameters are within the limits.	

*RSD = Relative standard deviation.

Table No. 13: Summary of Fluoxetine.

S.No	Parameter	Result	Acceptance criteria
1	System suitability Theoretical plates Asymmetry Retention time %RSD	3.410 7305 0.93 0.41	Not less than 2000 Not more than 2.0 Not more than 2.0
2	Specificity	Specific	Specific
3	Method precision(%RSD)	0.76	Not more than 2.0%
4	Linearity Range(mcg/ml) Correlation coefficient(r^2)	4.0 – 12.0 0.9998	Not less than 0.990
5	Accuracy (Mean % recovery) 50% 100% 150%	99.6 99.0 99.0	97 - 103%
6	Robustness	All the system suitability parameters are within the limits.	

CONCLUSION

From the above experimental results it was concluded that, this newly developed method for the simultaneous estimation of BREXPIPRAZOLE and FLUOXETINE was found to be simple, precise, accurate and high resolution and shorter retention time makes this method more acceptable and cost effective and it can be effectively applied for routine analysis in research institutions, quality control department in meant in industries, approved testing laboratories.

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