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

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ABSTRACT

Analytical method was developed for the estimation of Brexpiprazole drug substance by liquid chromatography. The chromatographic separation was achieved on C18 column (Inertsil ODS 3V 150*4.6, 5um) at ambient temperature .the separation achieved employing a mobile phase consists 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 315nm. The average retention time for Brexpiprazole found to be 2.27 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 50-150µg/ml for Brexpiprazole.

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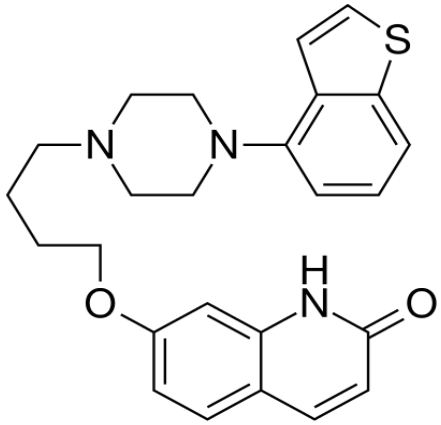
INTRODUCTION

Brexpiprazole is an antipsychotic medication. It works by changing the actions of chemicals in the brain. Brexpiprazole is used to treat the symptoms of schizophrenia. It is also used together with other medications to treat major depressive disorder in adults. Brexpiprazole is a novel D2 dopamine and serotonin 1A partial agonist, called serotonin-dopamine activity modulator (SDAM), and a potent antagonist of serotonin 2A receptors, noradrenergic alpha 1B and 2C receptors.

Brexpiprazole is chemically designated as 7-{4-[4-(1-benzothiophen-4-yl) piperazin-1-yl]butoxy}-1,2-dihydroquinolin-2-one. Its molecular formula is $C_{25}H_{27}N_3O_2S$, and its molecular weight is 433.57. Brexpiprazole is a white-to-off white powder. It is freely soluble in methanol and practically insoluble in water.



Structure of Brexpiprazole.

EXPERIMENTAL:

Equipments:

The chromatographic technique performed on a waters 2695 with 2487 detector and Empower2 software, reversed phase C18 column (Inertsil 5µ, 150 mm × 4.6 mm) 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 were obtained as gift samples from Fortune pharma training institute, sri sai nagar, KPHB and 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 C18 (5 µ, 15 cm X 4.6 mm i.d.) INERTSIL 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 315nm that was filtered and degassed prior to use, Injection volume is 10 µl and ambient temperatures.

Preparation of mobile phase:

Buffer Preparation:

Take accurately 1ml of formic acid in 1000mL of water

Mobile phase:

Then add 35 volumes of buffer and 65 volumes of Methanol mixed well and sonicated for 5 min.

Preparation of standard stock solution:

A 50mg of pure Brexpiprazole were weighed and transferred to 50 ml of volumetric flask and dissolved in methanol. The flask was shaken and volume was made up to mark with methanol to give a primary stock solution containing 1000µg/ml. From the above solution 1ml of solution is pipette out into a 10 ml volumetric flask and volume was made up to mark with methanol to give a solution containing 100µg/ml of Brexpiprazole.

Preparation of sample solution:

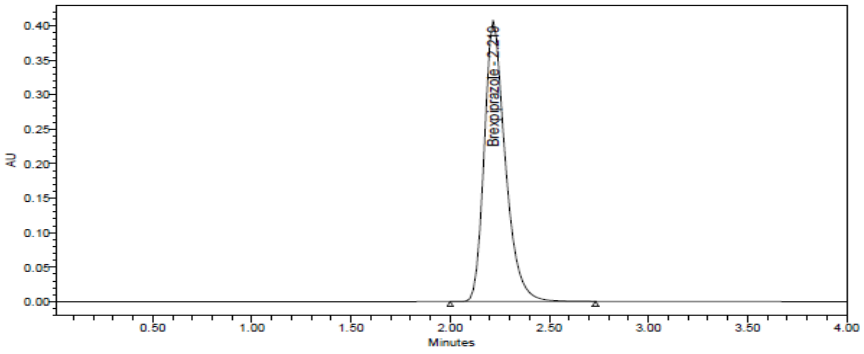
A 50mg of Brexpiprazole sample were weighed and transferred to 50 ml of volumetric flask and dissolved in methanol. The flask was shaken and volume was made up to mark with methanol to give a primary stock solution containing 1000µg/ml. From the above solution 1ml of solution is pipette out into a 10 ml volumetric flask and volume was made up to mark with methanol to give a solution containing 100µg/ml of Brexpiprazole.

RESULTS AND DISCUSSIONS:

Determination Of Working Wavelength (λ max):

10 mg of the Brexpiprazole 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 315nm

After several initial trails with mixtures of methanol, water, ACN and buffers 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.



	Peak Name	RT	Area	% Area	USP Tailing	USP Plate Count
1	Brexpiprazole	2.219	2996965	100.00	1.30	2107

Figure: 1 Chromatogram of Brexpiprazole.

METHOD VALIDATION

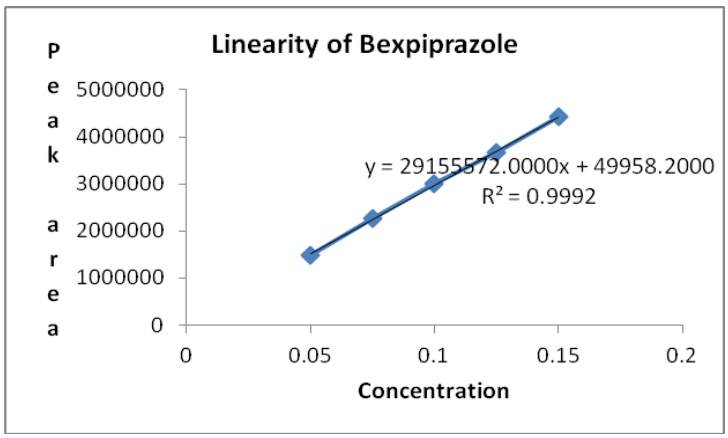
Linearity:

Linearity was studied by analyzing five standard solutions covering the range of 50-150 µg/ml of Brexpiprazole. From the primary stock solution 0.5ml,0.75ml,1.0ml,1.25ml,1.5 ml of solution pipette into 10 ml volumetric flasks individually and made up to the mark with methanol to give a concentrations of 50µg/mL , 75µg/mL ,100µg/mL ,125µg/mL and 150 µg/mL of Brexpiprazole .

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.

Level	Concentration (mg/mL)	Peak area
50%	0.05	1477294
75%	0.075	2265508
100%	0.10	3000811
125%	0.125	3658939
150%	0.150	4425025



FigureNo.2: Linearity (calibration) curve of Brexpiprazole.

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 (3) and (4), respectively.

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

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

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.

Table no.2: LOD and LOQ values Calculated from calibration curve:

	mg
LOQ	0.013
LOD	0.004

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, 100 µg/ml of BREXPIPRAZOLE without changing the parameter of the proposed chromatographic method.

Table.3: Summary of peak areas for method precision.

Sample No	Retention time	Peak area	% Assay
1	2.219	2982351	100.6
2	2.220	2988289	100.8
3	2.220	2939752	99.1
4	2.218	2995127	101.0
5	2.219	2984599	100.6
6	2.220	2996062	101.0
Mean	2.22	2981030	100.5
%RSD	0.04	0.70	0.71

Accuracy (recovery study):

The accuracy of the method was determined by calculating the recoveries of BREXPIPRAZOLE by analyzing solutions containing approximately 50%, 100% and 150% of the working strength of Brexpiprazole. The percentage recovery results obtained are listed in Table 4

Table No.4: Accuracy data.

LEVEL	S.No	%Recovery of BREXPIPRAZOLE	Average
50	1	98.8	99.2%
	2	99.6	
	3	99.2	
100	1	100.6	100.2%
	2	100.8	
	3	99.1	
150	1	99.1	99.2%
	2	100.2	
	3	99.6	

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 ±0.1 ml/min. The results were shown in (Table no.5).

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.6.

Table No.5: Results of Robustness study.

Parameter	Rt of BREXPIPRAZOLE	Theoretical plates	Asymmetry
Decreased flow rate (0.7ml/min)	2.653	2260	1.31
Increased flow rate (0.9ml/min)	1.907	1884	1.29
Wave Length 313nm	2.219	2107	1.30
317	2.219	2126	1.31

Table No.6: Results of Ruggedness.

		%Assay	%RSD
Analyst-1	BREXPIPRAZOLE	100.6	0.14%
Analyst-2		100.8	

Table No.7: Validation parameters of evaluated method:

S. No	Parameter	Limit	Value Obtained
1	Linearity concentrations Range (mg/mL) Correlation coefficient	NLT 0.990	0.05 to 0.15 mg\ml 0.9996
2	Method precision (Repeatability) (%RSD, n = 6)	98.0 to 102.0 %	99.1 to 101.0 %
3	ACCURACY(%Recovery)	98-102%	98.8 to 100.8%
4.	Robustness Flow Variation(0.7mL to 0.9 mL/min) Wavelength Variation (313nm to 317nm)	It should be meet System suitability criteria	Complies
5.	Ruggedness (Intermediate Precision) (%RSD analyst to analyst variation)	NMT 2%	0.14%

*RSD = Relative standard deviation.

CONCLUSION

From the above experimental results and parameters it was concluded that, this newly developed method for the estimation of BREXPIPRAZOLE 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 and approved testing laboratories.

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