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Development and validation of stability indicating RP-HPLC method for estimation of Brexpiprazole from bulk and tablet form

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ABSTRACT

A sensitive, selective, rapid, precise, and economic stability indicating Reverse Phase High Performance Liquid Chromatographic (RP-HPLC) method were developed for the quantification of Brexpiprazole in bulk and pharmaceutical dosage form was performed on Shimadzu Model HPLC 3000 series, using a mixture methanol and water (90:10, v/v) with OPA as mobile phase with a flow rate of 0.9 mL/min. Detection was carried at 215nm. The retention time of Brexpiprazole was 5.099min. Linearity was observed over the concentration range of 10–50 µg/mL (R2 = 0.9989) with regression equation y = 71185x-482587.The Accuracy study was performed % recovery of Brexpiprazole. The % recovery was found to be 50%=100.13%,100%=99.58%,150%=99.84%.(NLT 98% and NMT 102%). The Relative standard deviation values for intraday precision and intraday precision were found to be less than 2% i.e. 0.25% and 0.40% respectively. Brexpiprazole was subjected to stress conditions (acidic, alkaline, oxidation and thermal degradation) and validated as per ICH guidelines. The validated method can be applied to perform long-term and accelerated stability studies of Brexpiprazole formulations.

Keywords: Brexpiprazole; Isocratic elution; Reversed-phase HPLC; Stability-indicating; Validation

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1. 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 C25H27N3O2S, and its molecular weight is 433.57. Brexpiprazole is a white-to-off white powder.

Literature survey revealed that Brexpiprazole was determined by UV-Visible spectroscopy and HPLC. In the present study, the authors have proposed simple validated spectrophotometric methods for the determination of Brexpiprazole in pharmaceutical dosage forms. At present, the authors have developed stability indicating RP-HPLC method for the determination of Brexpiprazole.[1,2]

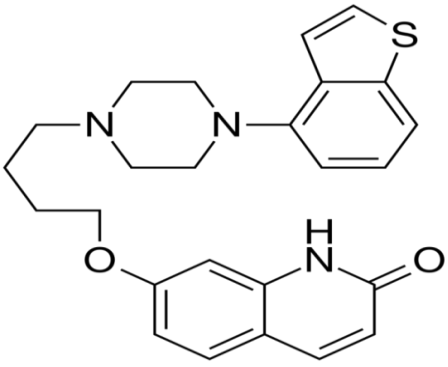

Fig No:1 Structure of Brexpiprazole.

2. MATERIAL AND METHOD:

2.1. Equipments:

HPLC System:

The method was performed on Shimadzu Model HPLC 3000 series. With colum Grace C8 (250mm x 4.6 i.d., particle size: 5 micron). UV-3000-M Detector has been used for detection

and P-3000-m Reciprocating (40MPa) pump are included in system.

Balance:

All drug and chemical were weighed on Wenser High Precision Balance (Model:PGB 100)

Sonicator:

Wenser ultra sonicator (WUC-4L)

Hot air oven: kumar laboratory oven

Photo stability chamber: make newtronic. Model IC DAC (version 1.2)

Calibrated glassware's

2.2. Materials:

Pharmaceutically pure sample of Brexpiprazole were obtained as gift samples from Macelods pharmaceutical Pvt. Ltd. Unit V GIDC, Sarigam, Gujrat State, India. HPLC grade Methanol (Merck).OPA(Rankem),was used. Brand name: Rexulti, Otsuka Pharmaceutical Co, Ltd, Tokyo, Japan

2.3.Mobile Phase:

Mobile Phase containing Methanol:water(90:10) with pH 3 adjusting by OPA .

2.4.Stock solution of Brexpiprazole:

Standard stock solution of Brexpiprazole was prepared by weighing accurately 100 mg of pure drug of Brexpiprazole transfer in 100 mL volumetric flask containing mobile Phase to get concentration 1000 µg/mL in 100mLand stock solutions was degassed by sonicated at 25 °c for 15 min. For working standard solution further dilution was made by using proper concentration of standard stock solution. the volume was made in Mobile Phase to get final concentration range of Brexpiprazole 10,20,30,40,50 µg/mL.

2.5. Preparation of Sample Solutions of Brexpiprazole:

Twenty tablets were weighed and make fine powdered; powder equivalent to 4 mg of Brexpiprazole was transferred into 10 mL volumetric flask containing Methanol and Water (90:10) to get volume up to the mark and solutions was degassed by sonicated at 25 °c for 15 min. This will be produced sample solution containing Brexipprazole 400 ug/ml.

3. RESULT AND DISCUSSION:

Selection of Analytical Wavelength:

From the standard stock solution further dilutions were done using methanol and water scanned over the range of 200 – 400 nm. The spectrum was obtained. It was observed that the drugs showed considerable absorbance at 215nm so it was selected as detection wavelength

Selection of Mobile Phase:

The standard solution of Brexpiprazole was injected into the HPLC system and run in different solvent systems. Different mobile phases like methanol and water in varying proportion of mobile phase components, varying conditions of pH were tried in order to obtain the desired system suitability parameters for the Brexpiprazole After several trials, Methanol And Water with OPA was chosen as the mobile phase, which gave good resolution and acceptable peak parameters.

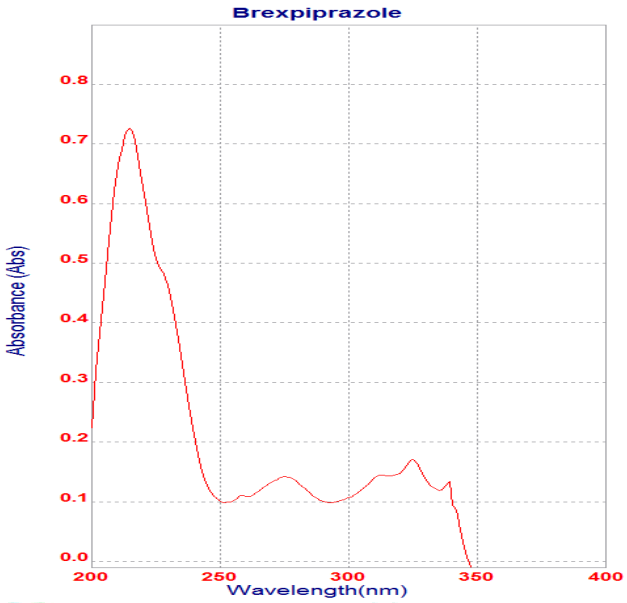

Fig No:02 UV spectrum of Brexpiprazol

Table 1: Trials of mobile phases for HPLC method of Brexpiprazole

S.N.	Mobile Phase	Observation
1.	Methanol+ Water (80:20)	RT-10.813 Poor and broad peak with another peaks.
2	Methanol+ Water (90:10)	RT- 13.713 Poor and broad peak with another peaks.
3	Methanol+ Water, OPA, PH 3 (90:10)	RT-5.099 broad peak of Brexpiprazole

System Suitability Parameter of Drug:

The column was equilibrated with the mobile phase (indicated by constant back pressure at desired flow rate). Working standard solution of drug was injected into the system. The retention time for the drug was found to be:

Brexpiprazole: 5.099ml/min System suitability parameters of Brexpiprazole are summarized in table.

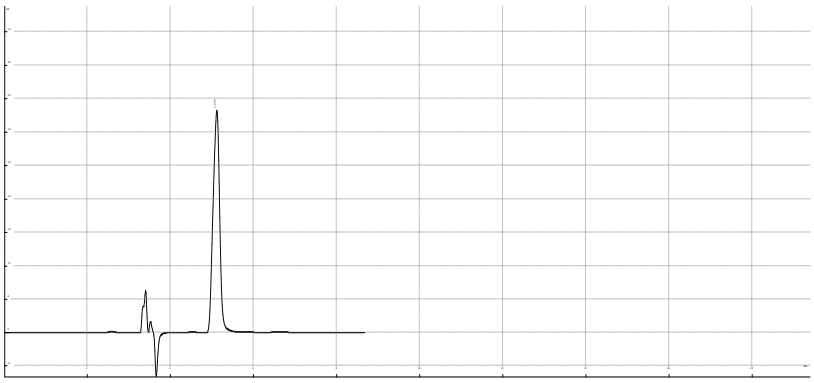

Fig.No:.03 Optimized chromatographic condition

Retention Time	Area	Resolution	T.Plate num	Asymmetry
5.099	586480	0.0	8290	1.09

Method Validation:

Linearity:

Linearity of the analytical method was performed by using five different concentration of standard stock solution (10-50ppm) The response factors were plotted against the corresponding concentrations of Brexpiprazole obtain in the calibration curve for Brexpiprazole.

Table 2: Linearity Parameter:

S.N.	Concentration µg/ml	Peak Area
1	10	237185
2	20	962099
3	30	1590373
4	40	2395273
5	50	3079829

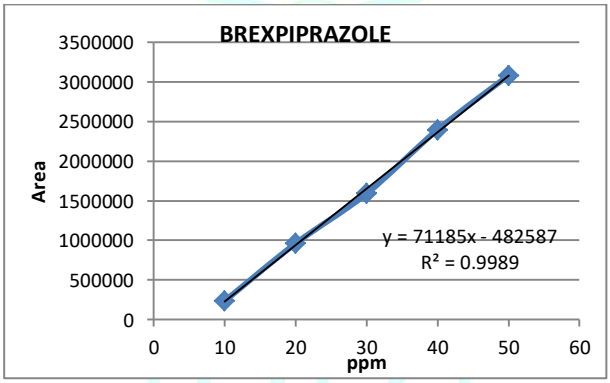

Fig No:04 Linearity Graph Of Brexpiprazole

Assay (%):

Accurately weigh and transfer 10mg of pure Brexpiprazole was transferred into 10ml clean and dry voilumteric flask. Add diluents and sonicated to dissolve it completely and made volume upto the mark with the same solvent (mobile phase). From this solution appropriate dilution of Brexpiprazole were made to get the final concentration and finally the solutions were filtered through Whatman filter paper. A 20ul sample was injected under chromatographic condition. The peak areas were measured at 215nm and the percent purity and % RSD was calculated.

Table 3: Assay Of Bulk

Drug Name	Composition in ppm	Area of Standard	Aomunt found in ppm	% Assay
Brexpiprazole	30 ppm	2080373	29.90	99.67%

Assay of Marketed formulation:

Twenty tablets were weighed and make fine powdered; powder equivalent to 4 mg of Brexpiprazole was transfred into 10 mL volumetric flask contaning Methanol and Water (90:10) to get volume up to the mark and solutions was degassed by sonicated at 25 ° c for 15 min.This will be produced sample solution containing Brexipprazole 400 ug/ml

Table 4: Assay of Marketed formulation

Drug Name	Composition in ppm	Area of Standard	Area of Sample	% Assay
Brexpiprazole	30 ppm	2080373	2073389	99.66%

Accuracy:

Recovery studies were carried out by addition of standard drug to the soln at 3 different concentration levels (50%, 100%, 150%)taking into consideration percentage purity of added bulk drug samples. These solutions were subjected to re-analysis by the proposed method and results are calculated.

Table 5: Result of Recovery studies.

Conc (%)	Sample amount (ppm)	Amount added (ppm)	Area of sample	% recovery	%mean recovery
50%	20	10	1660341	99.67%	100.13%
	20	10	1656128	99.07%	
	20	10	1665328	100.3%	
100%	20	20	2378996	100.3%	99.58%
	20	20	2381273	100.4%	
	20	20	2386276	100.08%	
150%	20	30	3065713	99.03%	99.84%
	20	30	3089741	100.01%	
	20	30	3071645	99.30%	

Precision:

Precision is expressed as the closeness of agreement between a series of measurements obtaining from multiple sampling of the same homogeneous sample. The precision method was demonstrated by inter-day and intra-day studies. Three replicate injections of a known concentration of Brexpiprazole has been injecting into HPLC and analyzed.

Inter-day study:

A standard solution containing (30ppm) of Brexpiprazole were analyzed three times on the same day and % RSD was calculated . The result are given in table

Table No: 06 Result of Inter-day studies

Brexpiprazole	
Day 1	Area
	1590373
	1593551
	1595666
Day2	1581574
	1580048
	1586199
Mean	1586199
SD	3202.97
%RSD	0.40%

Intra-day study:

A standard solution containing (30ppm) of Brexpiprazole were analyzed three times on the same day and % RSD was calculated. The result are given in table .

Table 7: Result of Intraday studies

Brexpiprazle	
Morning	Area
	1590373
	1593551
	1595666
Evening	1596366
	1589294
	1586044
Mean	1591882
SD	5277.61
%RSD	0.25 %

6 Robustness:

Robustness of the method was determined by carrying out the analysis under conditions during which flow rate, wavelength, were altered and the effects on the peak area were note.

Table 8: Result of Robustness

hanges in parameters	Values	Area	Theoretical plates	Mean	SD	%SD
Control	As per method	962099	9047			
Flow rate	0.8 mL/min	961516	7950			
(± 0.1 mL/min)	1.0 mL/min	960619	7805	961411	745.531	0.077
control	As per method	962099	9097			
Change in Wavelength	213nm	962994	7750	962677	501.345	0.052
(± 5 nm)	217 nm	962938	7564			

Ruggedness:

Ruggedness was tested for the rang of concentration 10-50ppm

Table 9: Result of Ruggedness

S.N.	Concentration µg/ml	Peak Area
1	10	236575
2	20	965495
3	30	1586542
4	40	2386276
5	50	3082830

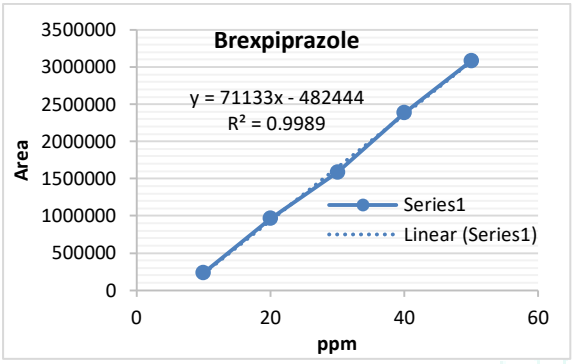

Fig No:05 Ruggedness Graph Of Brexpiprazole

Data for LOD and LOQ Limit of Detection (LOD) and Limit of Quantification (LOQ):

LOD and LOQ were calculated from the average slope and standard deviation from the calibration curve as per ICH guidelines. LOD was calculated by using this formula

$$LOD = \frac{3.3 \times \text{Std. Deviation}}{\text{Slope}}$$

$$LOQ = \frac{10 \times \text{Std. Deviation}}{\text{Slope}}$$

Where,

Std. Deviation calculated from accuracy,

And slope from linearity

S.N.	Drug	LOD	LOQ
1	Brexpiprazole	0.55 µg/ ml	1.68 µg/ ml

Stress degradation studies of bulk drug:

Stress degradation studies were carried under condition of acid, base, neutral hydrolysis, oxidation, dry heat and photolysis as per ICH Q1A R2 and Q1B Brexpiprazole were subjected to variety of stress conditions to affect degradation up to about 5-20%.

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Table 10: Summery of Forced Degradation studies of Brexpipzrazole

S.N.	Degradtion	Std area	Sample Area	Degraded up to %	Actual % Degradation
1	Acid Degradtion	3079829	2658580	86.32%	13.67%
2	Alkaline Degradtion	3079829	2701042	87.70%	12.29%
3	Peroxide Degradtion	3079829	2830779	91.91%	8.08%
4	Thermal Stress Degradtion	3079829	2874629	93.33%	6.66%
5	Photolytic Stress Degradtion	3079829	2987965	97.01%	2.98%

CONCLUSION

The proposed simultaneous estimation and validation method was found to be simple, precise, accurate and rapid for the determination of Brexpiprazole. The coefficient of correlation was obtained in acceptable range .The percentage recovery obtained in acceptable range .variation in flow rate, wavelength, does not have any effect on the % RSD of standard and assay value. The relative standard deviation of main peak area ,tailing factor and theoretical plate is well within the acceptable range . Hence the precision of given method is confirmed. Brexpiprazole shows significant degradation in acid, base, peroxide, thermal and UV Competitively, more degradation was found with acid and base degradation. Thus from the above result of the individual method is conclude that the analytical method is validated and found to be satisfactory.

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