VALIDATED METHOD DEVELOPMENT FOR THE ASSAY OF BREXPIPRAZOLE IN PHARMACEUTICAL FORMULATIONS

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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 Inertsil ODS C8 150*4.6, 3um at ambient temperature. The separation achieved employing a mobile phase consisting of 0.1%v/v Trifluoro acetic acid in water: Acetonitrile (30:70). The flow rate was 0.7 ml/ minute and ultra violet detector at 315nm. The average retention time for Brexpiprazole was found to be 2.16 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.

Key words: Brexpiprazole, Isocratic, HPLC, TFA, C8, Acetonitrile, Methanol and validation.

Introduction

Brexpiprazole, sold under the brand name **Rexulti**, is an atypical antipsychotic. It is a dopamine D_2 receptor partial agonist and has been described as a "serotonin–dopamine activity modulator" (SDAM). The drug received FDA approval on July 13, 2015 for the treatment of schizophrenia, and as an adjunctive treatment for depression.[2] Although it failed Phase II clinical trials for attention-deficit hyperactivity disorder (ADHD), it has been designed to provide improved efficacy and tolerability (e.g., less akathisia, restlessness and/or insomnia) over established adjunctive treatments for major depressive disorder (MDD).

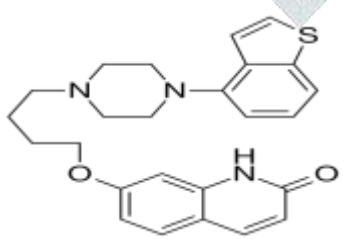


Figure 1 Structure of Brexpiprazole

Mechanism of action: Although the mechanism of action of brexpiprazole in the treatment of MDD and schizophrenia is unclear, the efficacy of brexpiprazole may be attributed to partial agonist activity at serotonin 1A and dopamine D2 receptors, and antagonist activity at serotonin 2A receptors.

Instruments

WATERS HPLC, Model: 2695 with 2487detector, with an automated sample injector. The output signal was monitored and integrated using Empower 2 software.

Table 1: List of Equipments

S.NO	Equipment's	Model	Company
1	Electronic Balance	SAB224CL	SCALETEC
2	Ultra-Sonicator	SE60US	ENERTECH
4	Thermal oven	---------	YAMTO
5	pH Meter	PH-7000	SMIS
6	Filter Paper 0.45 microns	---------	MILLI PORE

Chromatographic conditions

The separation was achieved by using Inertsil ODS C8 150*4.6mm, 3um column and a mixture of buffer (0.1% TFA in water) and Acetonitrile in the ratio of 300: 700 has been used as mobile phase. The flow rate of the mobile phase was 1.0 ml/min and the UV detector was set 315 nm. The total run time of the analysis is 4 minutes and the chromatogram is shown in Figure 2.

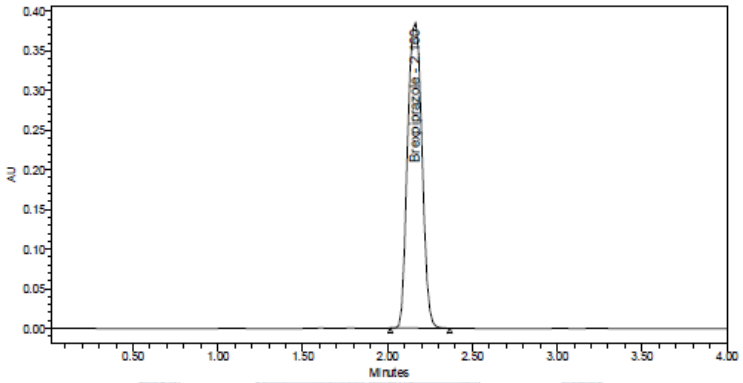


Fig 2: Chromatogram for optimized method

Materials

Preparation of mobile phase : Transfer 500ml of HPLC water into 1000ml of beaker add Trifluoro acetic acid. Transfer the above solution 300ml of Trifluoro acetic acid, 700ml of Acetonitrile is used as mobile phase. They are mixed and sonicated for 20min.

Preparation of the brexpiprazole standard and sample solution:

Preparation of standard stock solution: A 20mg of pure Brexpiprazole were weighed and transferred to 25 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 containing 800µg/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 Diluent to give a solution containing 80µg/ml of Brexpiprazole.

Method Validation

The developed method was validated as per ICH guidelines[20,21,23] for linearity, specificity, precision, accuracy and robustness.

Results and Discussions

System suitability

After achieving optimized chromatographic conditions, the system suitability was assessed for five replicate injections of API working standard solution. The percentage relative standard deviations (% RSD) for the peak responses were determined. The % RSD value is within the acceptable criteria as per validation parameters. The analytical system complies with the requirements specified by the system suitability. The Results are tabulated in the Table 2 and Table 3.

Table 2 : System suitability data of Brexpiprazole

Parameter	Brexpiprazole	Acceptance criteria
Retention time	2.160	+-10
Theoretical plates	3062	>2000
Tailing factor	1.07	<1.50
% RSD	0.24	<2.00

Table 3 Standard Results of Brexpiprazole

S.NO	Sample name	RT	Area	USP plate count	USP tailing
1.	Injection1	2.160	2266149	3090	1.07
2.	Injection 2	2.16	2280505	3045	1.07
3.	Injection 3	2.16	2268912	3048	1.07
4.	Injection 4	2.159	2271941	3058	1.06
5.	Injection 5	2.159	2272966	3069	1.06

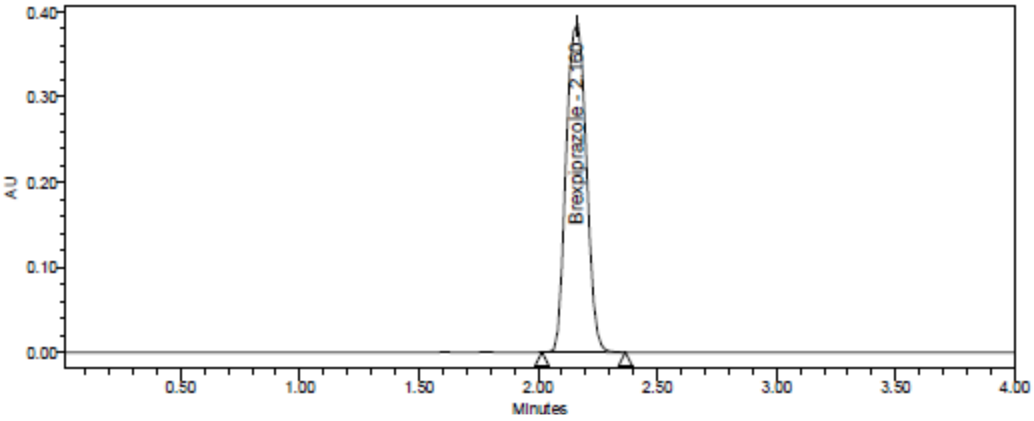


Fig-7: Typical Chromatogram of Standard; Injection-1

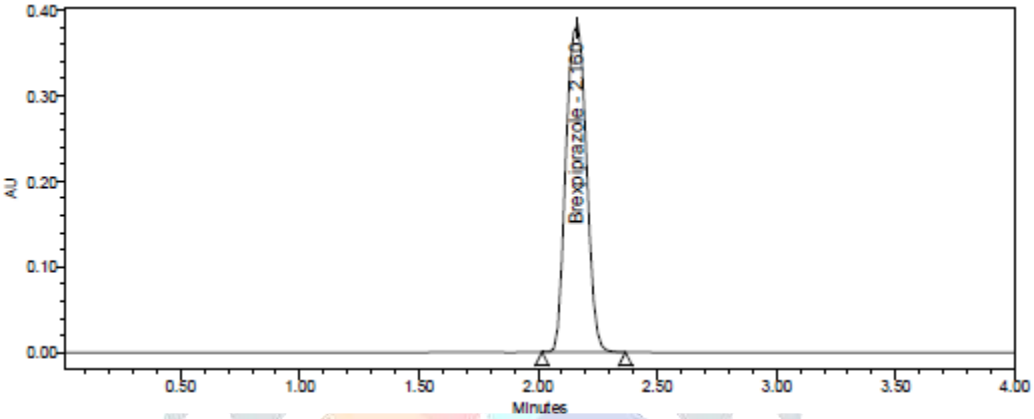


Fig-8: Typical Chromatogram of Standard; Injection-2

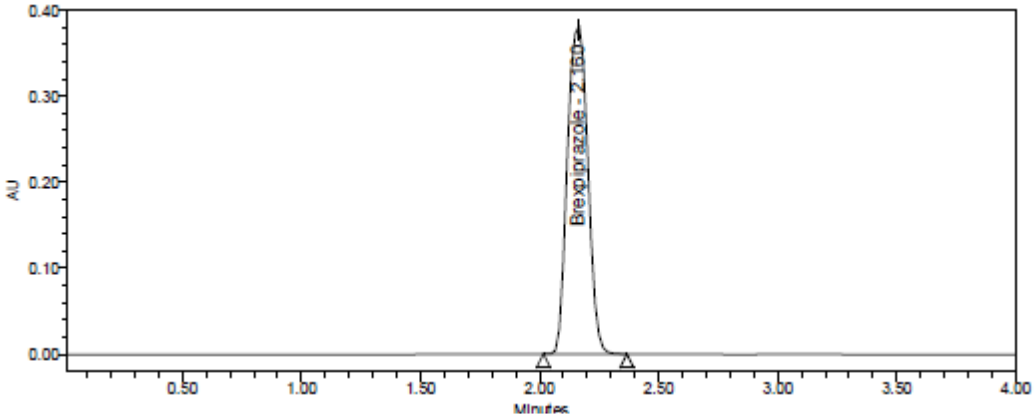


Fig-9: Typical Chromatogram of Standard; Injection-3

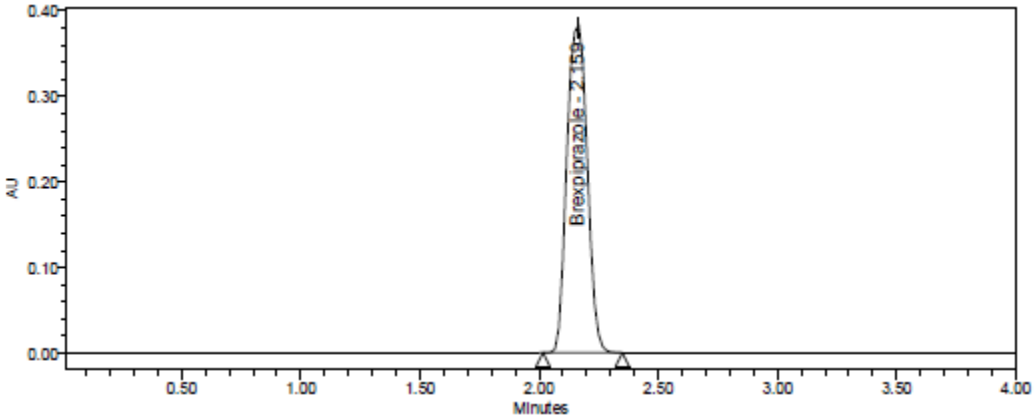


Fig-10: Typical Chromatogram of Standard; Injection-4

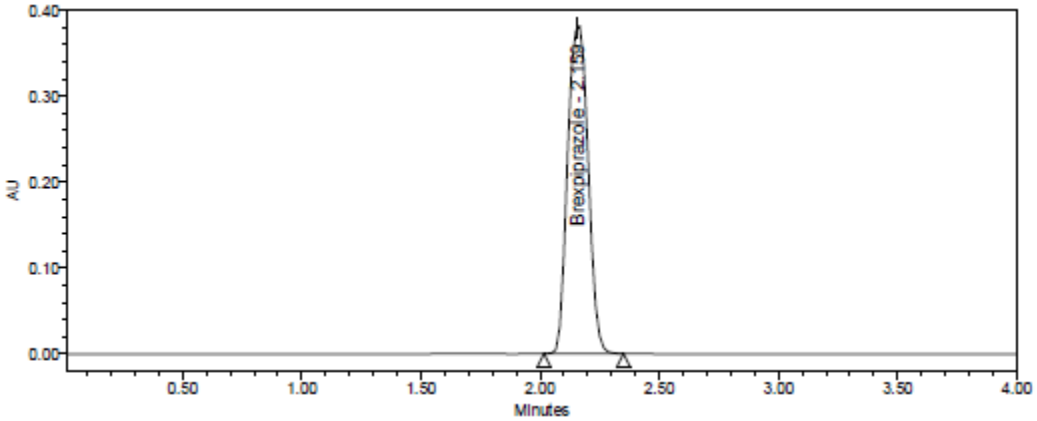


Fig-11: Typical Chromatogram of Standard; Injection 5

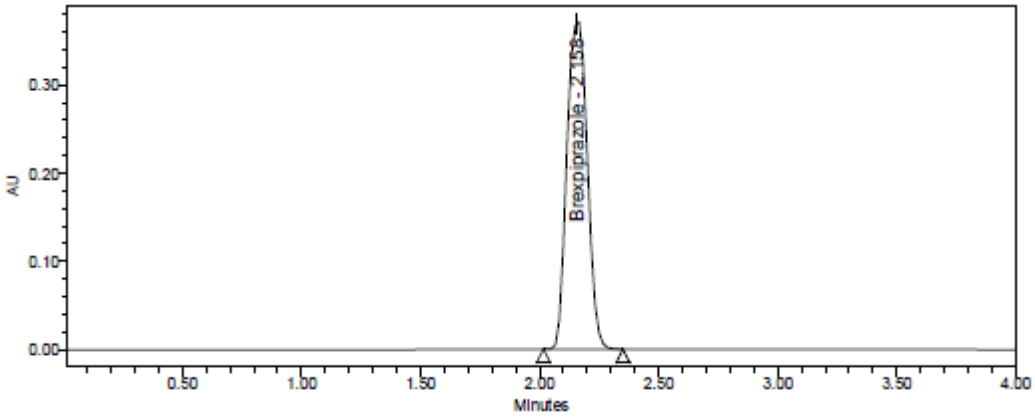


Fig-12: Typical Chromatogram of Sample-1

SPECIFICITY

The developed method was found to be specific for Brexpiprazole as no peaks were observed for blank and placebo. The results of specificity were tabulated in table 4 and the corresponding chromatogram is shown in figure 3.

Table 4: Specificity data for Brexpiprazole

S.NO	Sample name	Rt Brexpiprazole
1	Standard	2.160
2	Sample	2.155
3	Blank	-

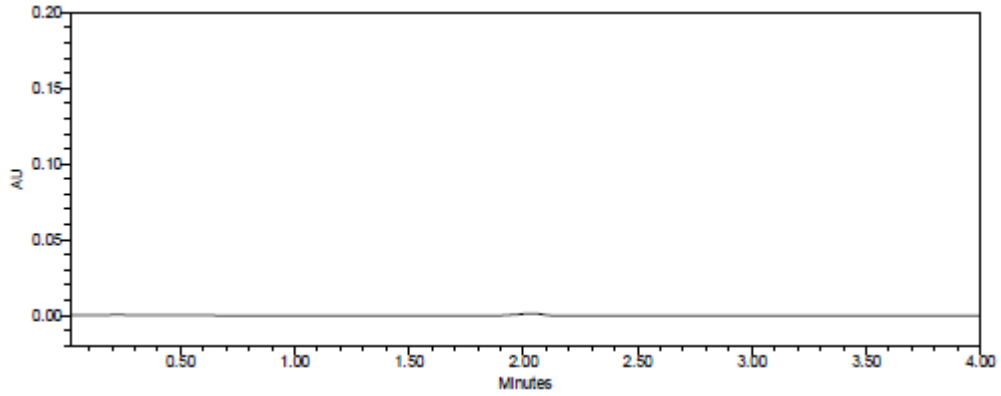


Fig-13: Typical chromatogram of the Blank

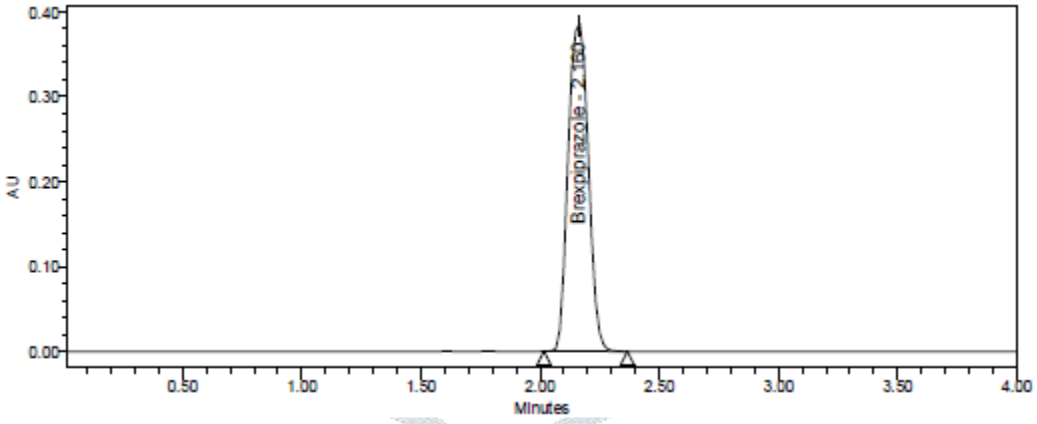


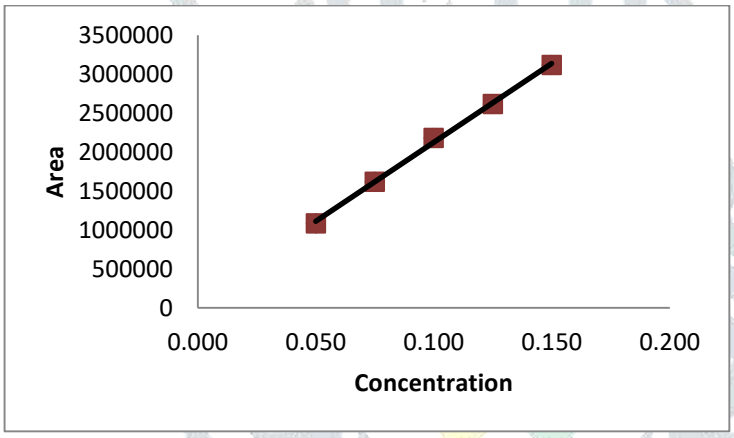
Fig-14: Chromatogram representing specificity of standard

3. LINEARITY

In the concentration range of 50.0 – 150.0 µg/ml for Brexpiprazole standard curve was obtained. A statistical method known as linear regression analysis was used to evaluate the linearity of the curve. To assess the linearity of the proposed method slope, intercept and correlation coefficient [r2] of standard curve was calculated and was given in Figure-4. The results were given in the Table- 5 and the results of LOD and LOQ were given in table 6. From the data obtained (For Brexpiprazole), the method was found to be linear within the proposed range.

Table 5: Linearity data for Brexpiprazole

S.NO	level	Area
1.	50	1084377
2.	75	1619406
3.	100	2180449
4.	125	2614171
5.	150	3117855
Correlation coefficient		**0.9991**



Linearity plot of Brexpiprazole

Table 6: LOD and LOQ values Calculated from calibration curve:

	Brexpiprazole
LOD	**0.0063** mg
LOQ	**0.0192** mg

ACCURACY

Accuracy is defined as the closeness of results obtained by that method to the true value for the sample. Accuracy is expressed in terms of percentage recovery. Recovery % is determined by the standard addition method. In the present study recovery studies were carried out at 50%, 100% and 150% spiked levels. The results of Recovery % were given in Table – 7.

Table- 7: Accuracy data for Brexpiprazole

S.NO	Accuracy level	injection	%Recovery
1	50%	1	99.8
		2	98.8
		3	99.3
2	100%	1	98.2
		2	99.2
		3	99.2
		4	99.3
		5	98.6
		6	98.5
3	150%	1	100.0
		2	98.3
		3	98.5