WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 9, Issue 15, 1473-1491. Research Article ISSN 2277– 7105

RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR ESTIMATION OF BREXPIPRAZOLE IN BULK DRUG AND DOSAGE FORM

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Article Received on
21 October 2020,

Revised on 11 Nov. 2020,
Accepted on 01 Dec. 2020

DOI: 10.20959/wjpr202015-19380

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ABSTRACT

One spectrophotometric and several HPLC methods have been reported for determination of Brexpiprazole in drugs and in pharmaceutical dosage forms. Hence, in the present study, a new, sensitive, suitable and robust reversed-phase high performance liquid chromatography method was developed and validated for the determination of Brexpiprazole in bulk drug and in tablet formulation. In RP-HPLC method, Acetonitrile: Methanol and 10 mM phosphate buffer adjusted to a pH2.0±0.05 by diluted OPA. (45:35:20 %v/v) was used as mobile phase, at a flow rate of 1.0ml/min, on HPLC system containing UV- detector with Openlab EZchrome software and Water sperisorb C18 column (100 mm x 4.6; 5µm). The detection was carried out at 216 nm. The method gave suitable retention time i.e. 4.7 min for Brexpiprazole. The results of analysis in the method were validated in terms of Filter study, Solution stability, specificity, linearity, accuracy, precision (Repeatability and intermediate precision), limit of detection, limit of quantification and robustness. A simple and precise method was developed for the assay of Brexpiprazole in bulk drug and in tablet formulation. method need regular reagents for doing analysis and also less time consuming, it can be performed routinely in industry for routine analysis of bulk drug and marketed product of Brexpiprazole.

KEYWORDS: RP-HPLC, Brexpiprazole, Acetonitrile, Validation.

INTRODUCTION

The analytical technique of high performance liquid chromatography is used extensively throughout the pharmaceutical industry. It is used to provide information on the composition of drug related samples. The information obtained may be qualitative, indicating what compound present in the sample or quantitative are providing the actual amounts of compound in the sample. Reverse phase chromatography refer to the use of a polar mobile phase with a nonpolar stationary phase in contrast, to normal phase being employed with a nonpolar mobile phase. Liquid chromatography is based upon the phenomenon that, under the same condition, each component in a mixture interacts with its environment differently from other components. Since HPLC is basically a separating technique, it is always used in conjunction with another analytical tool for quantitative and qualitative analysis. Reverse phase chromatography refers to the use of a polar eluents contrast to normal phase chromatography, where a polar stationary phase is employed with a phase chromatography is widely in use.[1-5]

Brexpiprazole drug is sold under the name Rexulti among others and it is typical antipsychotic drug. As an adjunctive treatment of major depressive disorder (MDD) and for treatment of schizophrenia. Brexpiprazole is approved for the treatment of schizophrenia, and as an adjunctive treatment for major depressive disorder (MDD). Brexpiprazole is soluble in organic solvents such as ethanol, DMSO, and dimethyl formamide (DMF), sparingly soluble in aqueous buffers.[13-18]

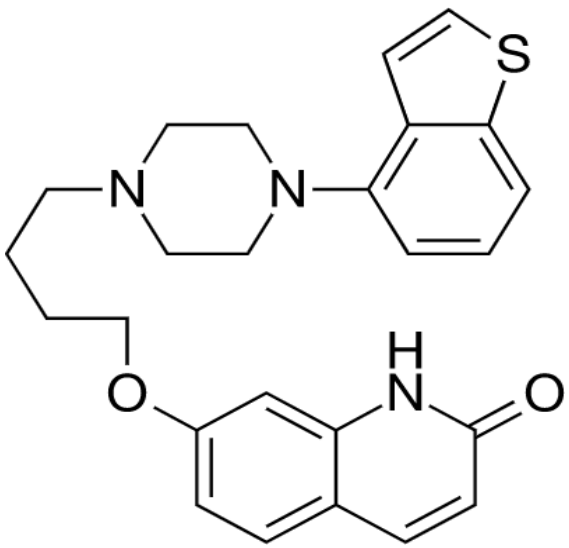


Figure 01: Structure of 7-[4-[4-(1-benzothiophen-4-yl) piperazin-1-yl] butoxy] quinolin-2(1H)-one.

MATERIAL AND METHOD

Instrumentation used

HPLC system consist of Analytical Technologies Ltd. having model 1260 Infinity II and other instruments used such as Electronic Weighing balance (Azcet High Precision Balance), UV-Visible spectrophotometer, Digital PH meter, Vacuum pump and ultra Sonicator were used.[5-7]

Chemicals and Reagent

Brexpiprazole was procured from Alkem Laboratories Ltd., HPLC grade methanol, water and all other chemicals which are analytical grade such as ortho-phosphoric acid was used for analysis.

Preparation of standard stock solutions

In order to prepare stock solution, weigh accurately 10 mg Brexpiprazole and transferred into100 ml volumetric flask, dissolve with 70 ml of methanol and diluted up to the mark with Methanol (100ppm). Further diluted 1 mL to 10 mL with methanol.

Selection of analytical wavelength: Brexpiprazole showed maximum absorbance at 216 nm shown in results.

Method development by RP – HPLC

Preparation of standard stock solution

Standard solution was prepared by dissolving 10 mg of Brexpiprazole into a 100 mL clean anddry volumetric flask, added about 70 mL of methanol to dissolve it completely and made volumeup to the mark with methanol (100ppm).[8-9]

Optimization of HPLC method

Following trials are taken for estimation of Brexpiprazole.

Principle: Reversed Phase Liquid Chromatography with gradient elution and UV detection.

Instrumentation and Finalized chromatographic conditions

The following chromatographic conditions were established by trial and error and were kept constant throughout method development.

Standard solution: Brexpiprazole 100 PPM

Column: Water spherisorb C18.

Column Dimension: (100 mm X 4.6 mm i.d.) 5µm

Detector: U.V. Detector

Wavelength: 216 nm

Flow Rate: 1.0 ml/min

Mobile phase: Acetonitrile: Methanol: 10 mM Phosphate buffer adjusted pH 2.0 by OPA.(45:35:20)

Injection Volume: 20 µl

Run Time: 10 min

Preparation of system suitability test (working standard solution)

Weighed about 10 mg of Brexpiprazole standard and transferred in 100 mL volumetric flask, added 70 mL methanol, sonicated to dissolve it. Made volume up to the mark with methanol. Pipette out 5 ml from standard stock solution and transferred into 10 ml volumetric flask and made volume up to the mark with Methanol (50 mcg), chromatograms were recorded. System suitability is a Pharmacopoeial requirement and is used to verify, whether the chromatographic system is adequate for analysis to be done. The tests were performed by Collecting data from five replicate injection of standard drug solution and the results are recorded.

1. **Brexpiprazole Drug (API)**

Drug sample solution was prepared by dissolving 10 mg of Brexpiprazole API into a 100 mL clean and dry volumetric flask, added about 70 mL of methanol to dissolve it completely by sonication and made volume up to the mark with methanol (100ppm). Filtered through suitable filter discarding suitable volume of sample solution. Further diluted 5 mL of filtrate solution to 10 mL with methanol (50ppm).

2. **Tablet formulation**

Weighed 20 tablets and calculated the average weight. Crushed tablets to fine powder and weighed powder material equivalent to 10 mg of Brexpiprazole and transferred to 100 mL clean and dry volumetric flask, added about 70 mL of methanol to dissolve it completely by sonication and made volume up to the mark with methanol (100ppm). Filtered through suitable filter discarding suitable volume of sample solution. Further diluted 5 mL of filtrate solution to 10 mL with methanol (50ppm).

VALIDATION OF RP-HPLC METHOD[10-12]

The developed method for estimation of Brexpiprazole was validated as per ICH guidelines for following parameters.

1. Filtration study

Filtration study of an analytical procedure checks the interference of extraneous components from filter, deposition on filter bed and compatibility of filter with sample. This study will be conducted with sample of 1.0 mg strength of Rexulti tablet. Filtration study carried out with unfiltered and filtered test solution. During filtration activity 0.45 µm PVDF 0.45 and 0.45 µm Nylon syringe filters used by discarding 5 mL of aliquot sample.

2. Stability of analytical solution

Stability study will be conducted for standard solution and test solution. Test solution stability will be performed using test sample of 1 mg of Rexulti tablet. Stability study will be performed at normal laboratory conditions.

3. Specificity

Specificity is the ability to access unequivocally the analyte in the presence of components which may be expected to be present.

4. Linearity and Range

Linearity was performed by diluting standard stock solution. From standard stock solution aliquots of 0.5, 3.0, 5.0, 6.0, 7.5 ml was taken and diluted to 10 ml with diluent such that the final concentration of Brexpiprazole in the range of 5 to 75 µg.

5. Accuracy (% Recovery)

The accuracy of the analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value of the value found, Accuracy will be conducted in the range from 50 % to 150 % of working concentration of 1 mg strength. Solution of each accuracy level will be prepared in triplicate. The study will be performed by using placebo. % Recovery calculated for each sample.

6. Precision

Precision of an analytical procedure expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogeneous test under the prescribed conditions. Precision is of two types, Repeatability and Intermediate precision. It is performed on API sample.

7. Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

8. Limit of detection and limit of quantitation

Detection limit and quantitation limit were determined based on the standard deviation y-intercept of five calibration curve and average slope of six calibration curve as mentioned.

RESULT AND DISCUSSION

Preliminary analysis

Description

The sample of Brexpiprazole was observed for its colour and texture. It is off-white, odourless and amorphous powder. The melting point was found to be 271-173^{0}C.

Solubility

The solubility in methanol is slightly soluble and it insoluble in water, Acetonitrile, Phosphate buffer and 0.1N HCl.

Selection of solvent

Methanol was selected as the solvent for dissolving Brexpiprazole.

Selection of analytical wavelength

Observation

The standard solution was scanned between 200 nm to 400nm. Wavelength of maximum absorption was determined for drug. Brexpiprazole showed maximum absorbance at 216 nm. It is shown in Figure No.2. Therefore 216 nm considered as an analytical wavelength for further determination.

Method development by RP – HPLC

Results of optimization of HPLC method

The optimization of HPLC method were done for the selection of proper mobile phase for method development. Pure drug products were injected and run in different solvent systems. In this, different trials are taken with different ratio of mobile phase. For trials methanol and water at different flow rate and PH were used. Different combinations of mobile phases were tried for selection of proper mobile phase are given below

System suitability test

Table no. 1: Results for system suitability test of brexpiprazole.

Sr. No.	Conc. (µg/ml)	R.T. (Min)	Area	Mean	S.D	RSD	Plate Count	Asymmetry
1	50	4.78	81565783	81476252	54336.12	0.07	4504	1.53
2	50	4.78	81490153				4508	1.52
3	50	4.78	81437004				4514	1.51
4	50	4.77	81443737				4516	1.51
5	50	4.78	81444582				4024	1.52

System suitability acceptance criteria

1. Relative standard deviation of the area of analyte peaks in standard chromatograms should not be more than 2.0 %.
2. Theoretical plates of analyte peak in standard chromatograms should not be less than 2000.
3. Tailing Factor (Asymmetry) of analyte peaks in Standard Chromatograms should be less than 2.0

Data interpretation: It was observed from the data tabulated above; the method complies with system suitability parameters. Hence, it can be concluded that the system suitability parameter meets the requirement of method validation.

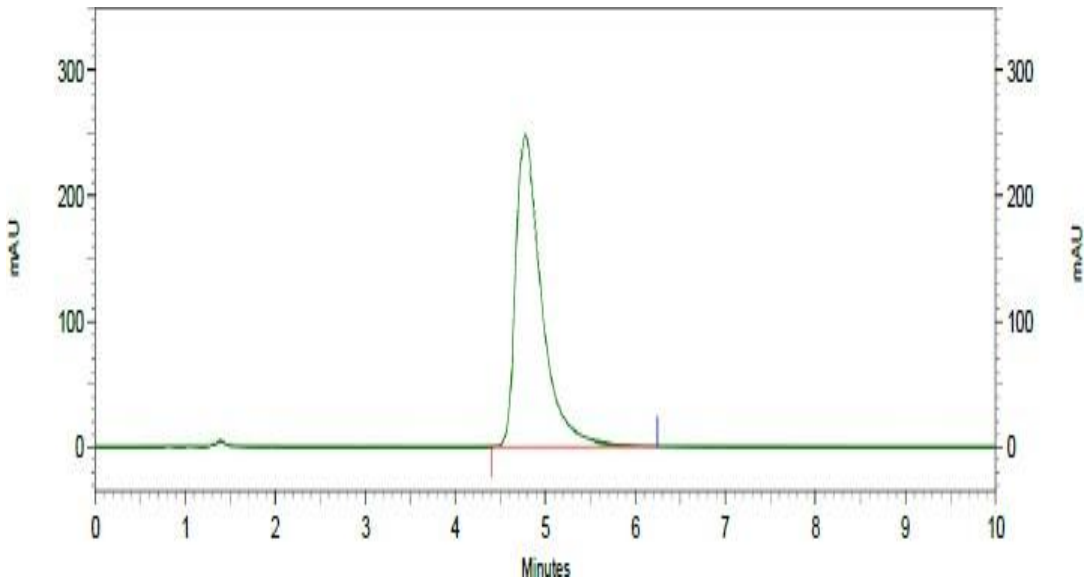


Figure no. 02: Typical chromatogram Standard solution 1 of system suitability solution.

Analysis of Test samples (Assay)

a) Brexpiprazole API

Table no. 2: Assay results of brexpiprazole API.

Area of standard	Area of sample	% Assay
81476252	81504965	100.04

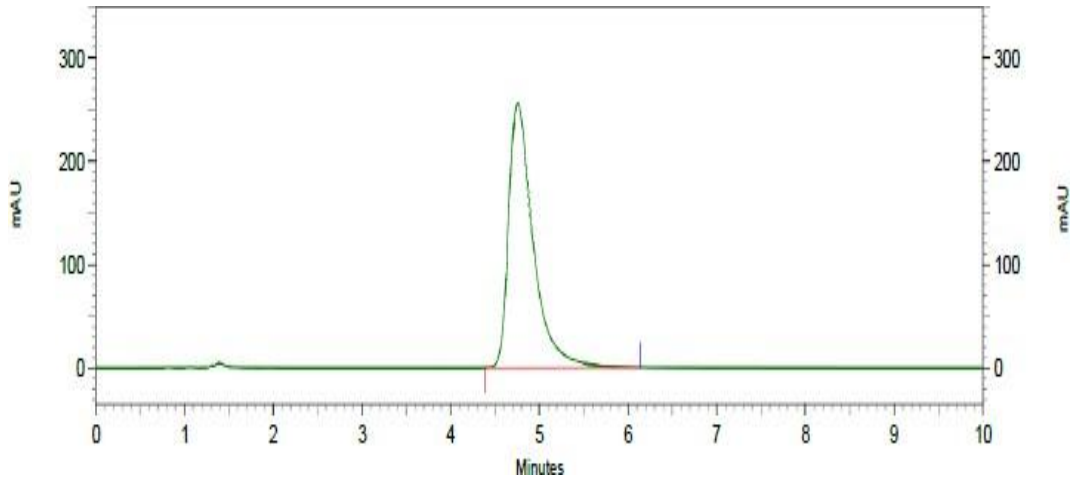


Figure no. 03: Typical chromatogram brexpiprazole API test sample.

b) Brexpiprazole tablet (Rexulti tablet 1mg)

Table no. 3: Assay results of brexpiprazole tablet 1mg.

Label Claim (mg/tab)	Area of Standard	Area of sample	% Assay
1 mg	81476252	**8**1482473	99.03

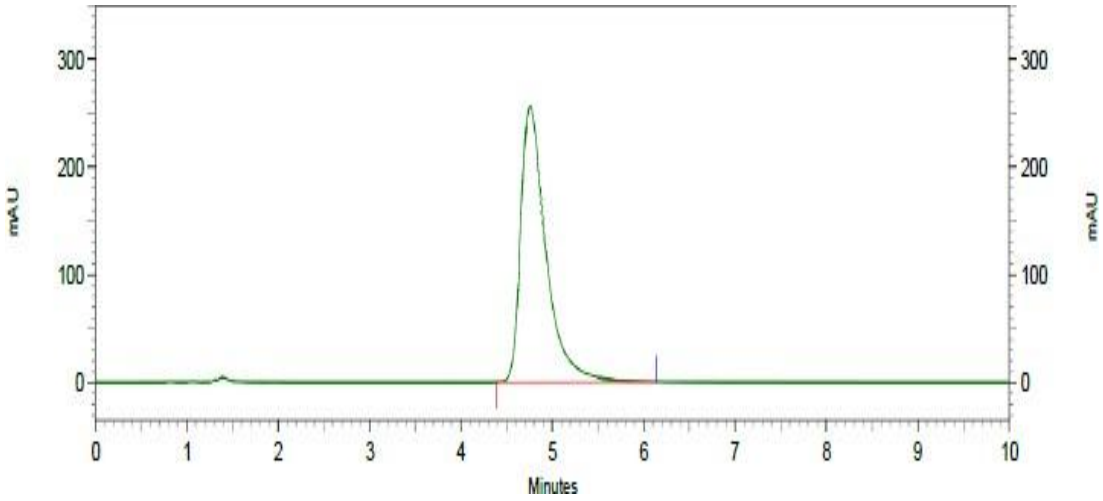


Figure no. 04: Typical chromatogram Brexpiprazole tablet (Rexulti tablet 1 mg).

VALIDATION OF RP-HPLC METHOD

1) Filtrationstudy

Filtrationstudyofananalyticalprocedurecheckstheinterferenceofextraneouscomponents

from filter, deposition on filter bed and compatibility of filter with sample. Performed on Tablet test samples.

Table no. 04: Results of filter study.

Sample description	Area	% Absolute difference
Unfiltered	81636364	NA
0.45 µ PVDF filter	81714271	0.10
0.45 µ Nylon filter	81726883	0.11

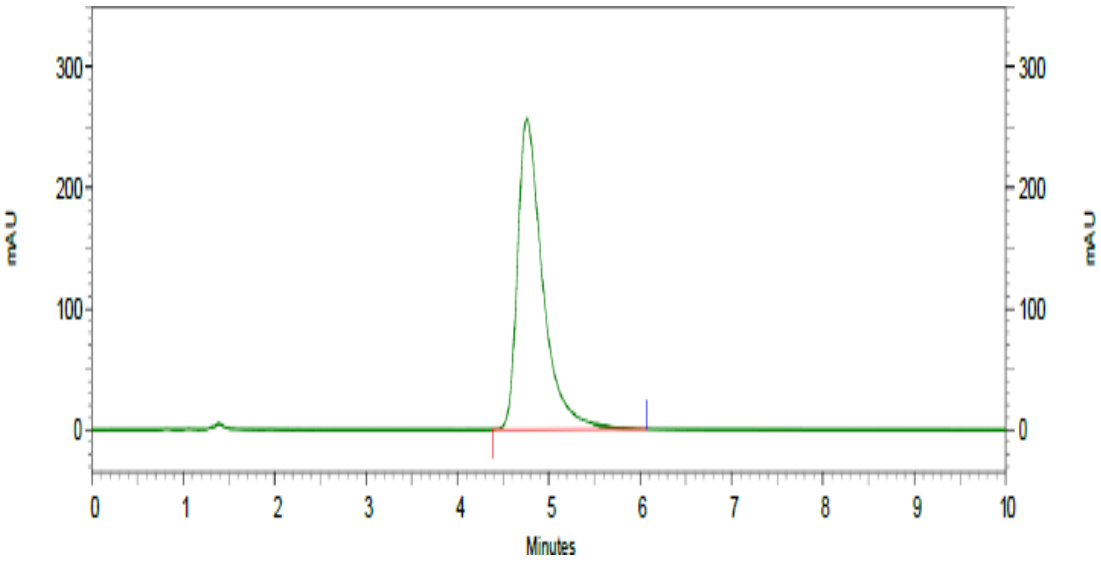


Figure no. 05: Typical chromatogram of unfiltered sampl.

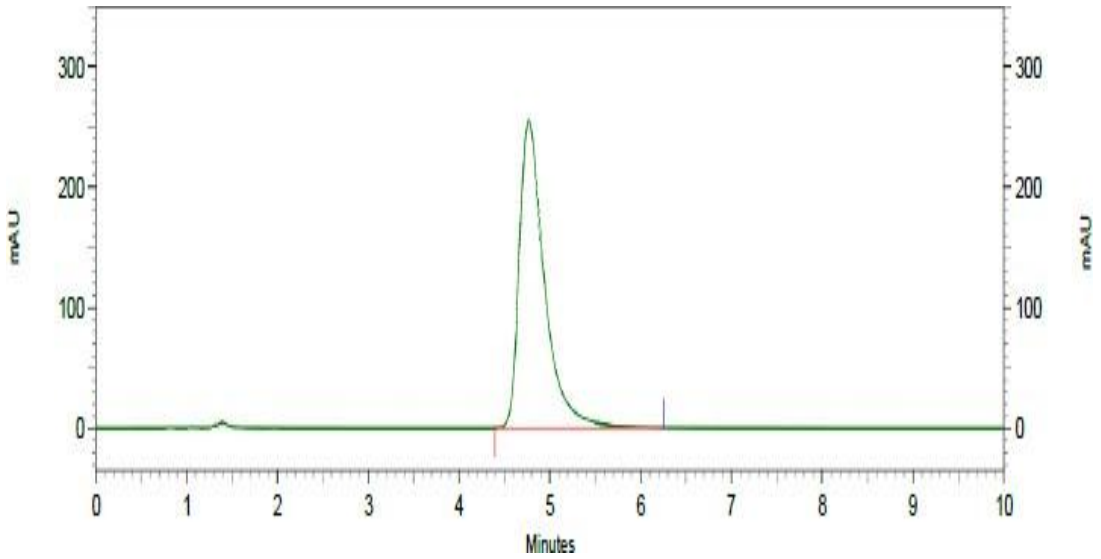


Figure no. 06: Typical chromatogram of sample filtered through 0.45µ PVDF filter.

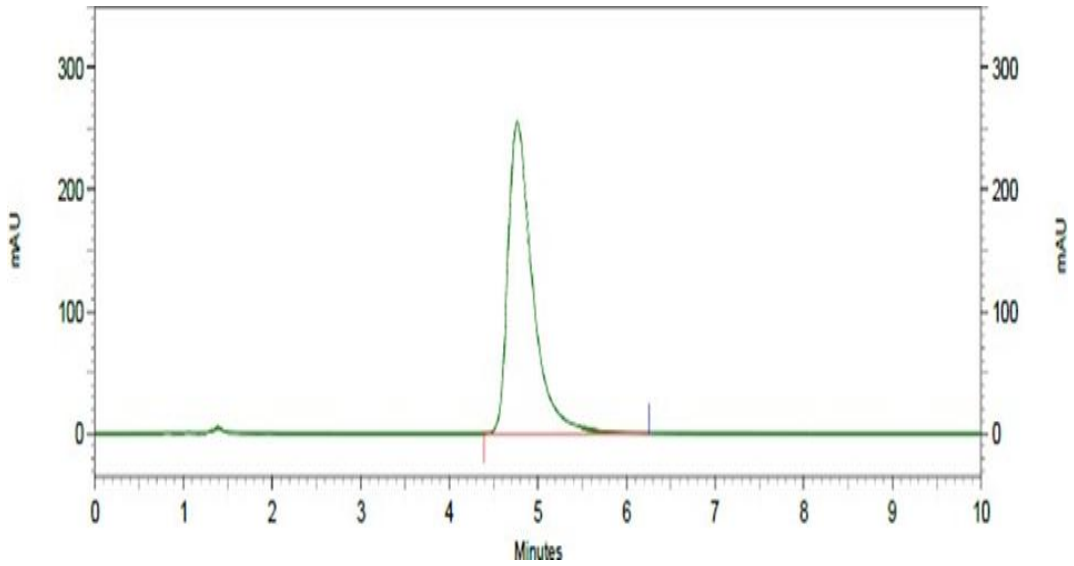


Figure no. 07: Typical chromatogram of sample filtered through 0.45µ Nylon filter.

Solution Stability: Stability study was conducted for standard solution and test solution. Test solution stability was performed using test sample of 1 mg of Rexulti tablet. Stability study was performed at normal laboratory conditions. The solution was stored at normalilluminatedlaboratoryconditionsandanalysedafter12hoursand24hours.[14-18]

Table no. 5: Results of solution stability.

Sample solution			Standard solution		
Time point	**Area**	**% Absolute difference**	**Time point**	**Area**	**% Absolute difference**
Initial	81844723	NA	Initial	81796341	NA
12 Hours	82059683	0.26	12 Hours	81754126	0.05
24 Hours	82061450	0.26	24 Hours	81812853	0.02

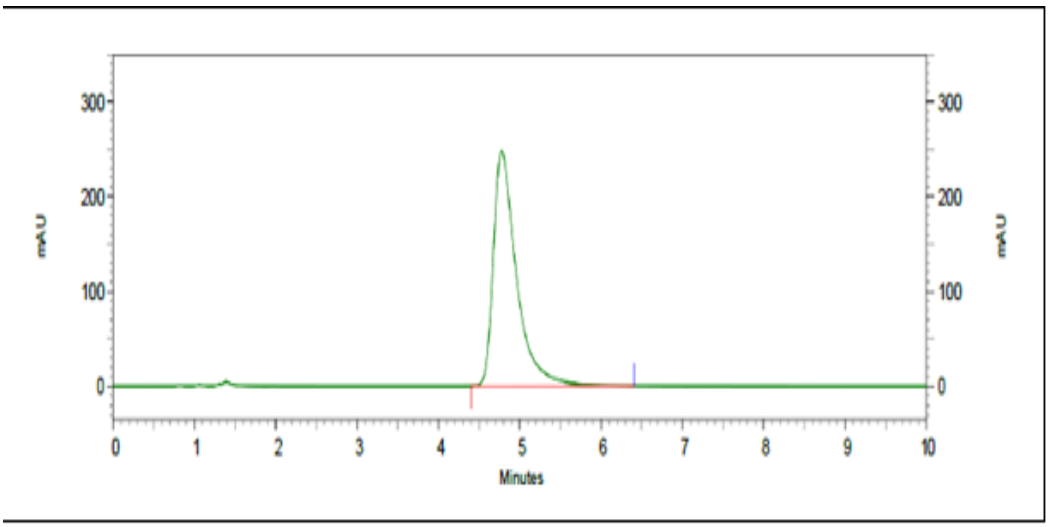


Figure no. 08: Typical chromatogram of standard solution Initial.

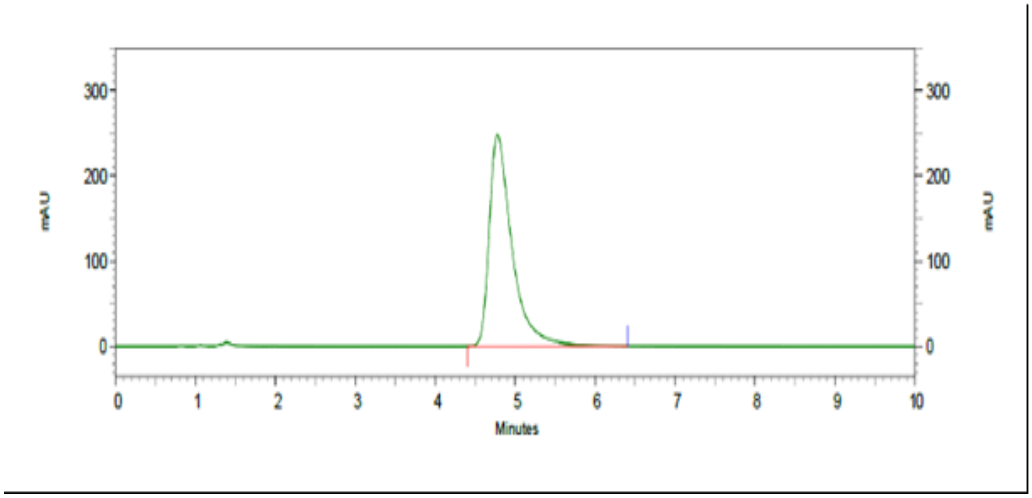


Figure no. 09: Typical chromatogram of Standard solution After 24 Hrs.

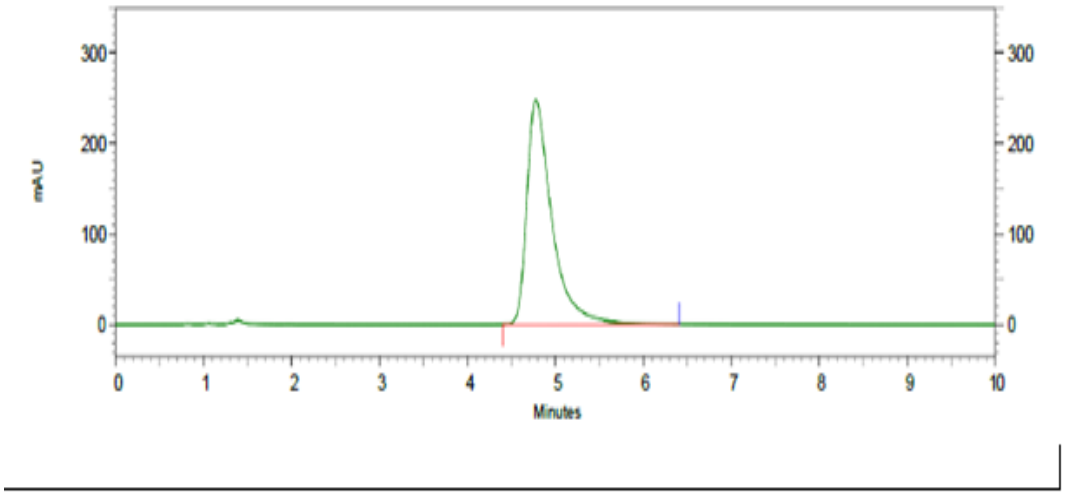


Figure no. 10: Typical chromatogram of Test sample solution Initial.

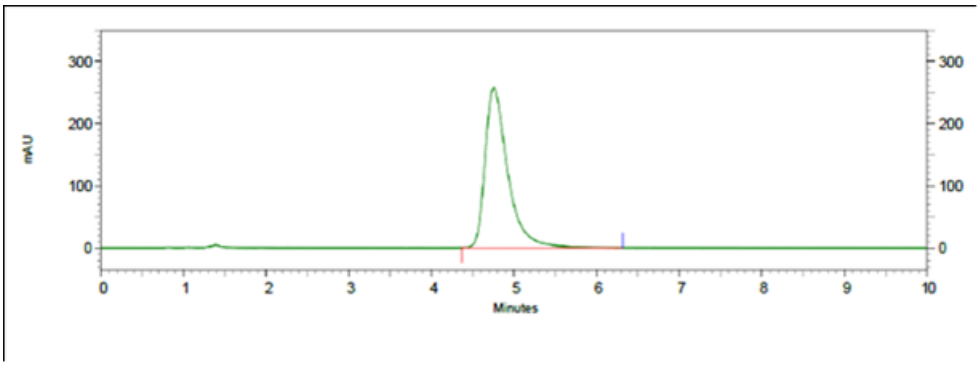


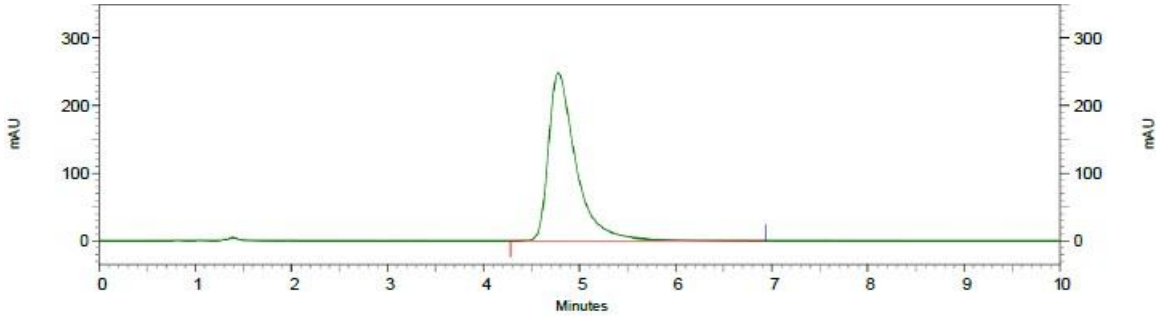
Figure No. 11: Typical chromatogram of Test sample solution After 24 Hrs.

Specificity: Specificity is the ability to access unequivocally the analyte in the presence of components which may be expected to be present.

Blank, standard solution and test sample prepared and injected to check peak purity.

Table no. 6: Results of specificity.

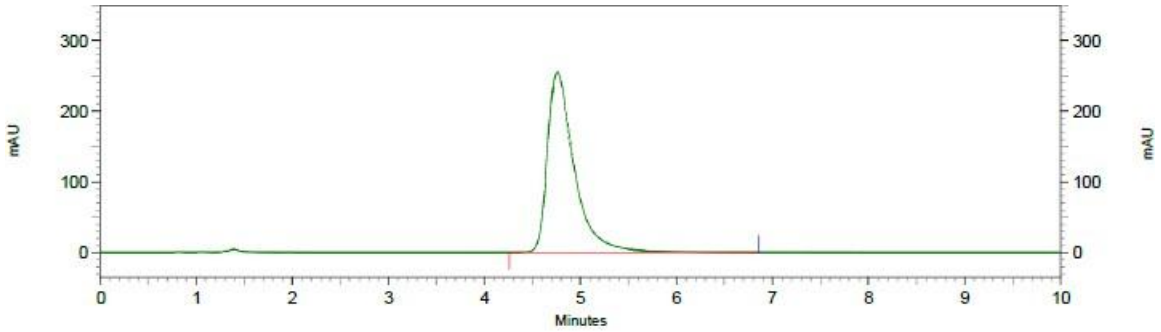
Description	Observation
Blank	No interference at R.T. of Brexpiprazole in blank
Standard solution	Peak purity was 0.998
Test sample	Peak purity was 0.998



VWD: Signal A, 216 nm Results

Name	Retention Time	Area	Asymmetry	Theoretical plates (USP)	Peak purity
Brexpiprazole	4.78	82394529	1.54	4500	0.998
Totals		82394529			

Figure no. 14: Typical chromatogram of standard solution.



VWD: Signal A, 216 nm Results

Name	Retention Time	Area	Asymmetry	Theoretical plates (USP)	Peak purity
Brexpiprazole	4.76	82384263	1.57	4555	0.998
Totals		82384263			

Figure no. 15: Typical chromatogram of Test solution.

Linearity and Range

Linearity of an analytical method is its ability to elicit test results that are proportional to the concentration of analyte in samples within a given range.

Table no. 7: Linearity data for brexpiprazole.

Level	Actual Conc (µg/mL)	Area	Mean	% RSD
10%	5.05	8110823	8112125	0.141
		8101424		
		8124127		
60%	30.3	48451517	48495690	0.140
		48461426		
		48574128		
100%	50.5	81487397	81490000	0.003
		81492385		
		81490217		
120%	60.6	97933800	97932212	0.002
		97932853		
		97929984		
150%	75.75	122572592	122557241	0.017
		122534278		
		122564852		
Straight line equation		Y = 1620101.119x - 279840.1481		
Correlation Coefficient		0.9999		

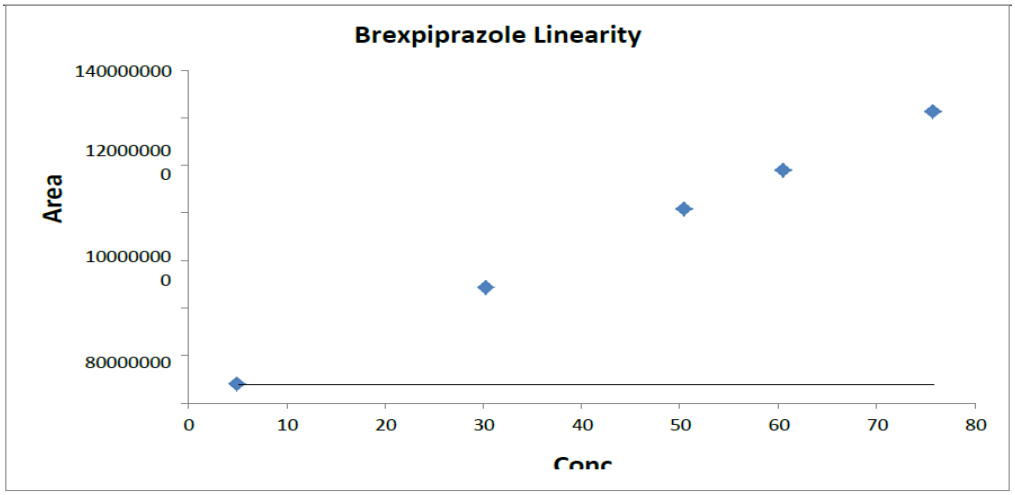


Figure no. 16: Calibration curve of brexpiprazole.

Table no. 8: Data for calibration curve of brexpiprazole.

Parameters	Result
Detection Wavelength	216 nm
Beer's law limit	5-75 µg/ml
Slope	1620101.119
Intercept	-279840.1481
Correlation coefficient (R^2)	0.9999

Accuracy (Recovery)

Table no. 09: Result and Statistical data of accuracy of brexpiprazole.

Level (50 %)	API wt (mg)	Area	Recovered conc. (µg/mL)	Added conc. (µg/mL)	% Recovery
50	5.1	40511076	25.36	25.50	99.45
	5.1	40502418	25.35	25.50	99.41
	5.1	40488276	25.34	25.50	99.37
100	10.2	81468534	51.00	51.00	100.00
	10.3	81482473	51.00	51.50	99.03
	10.2	81474283	51.00	51.00	100.00
150	15.2	122471536	76.66	76.00	100.87
	15.3	122484271	76.67	76.50	100.22
	15.2	122451724	76.65	76.00	100.86

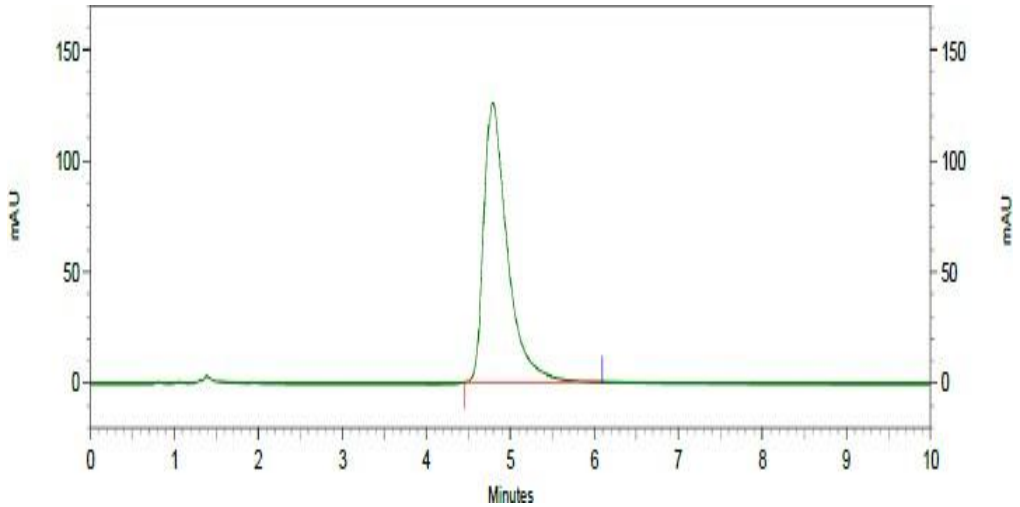


Figure no. 17: Typical chromatogram of accuracy 50%.

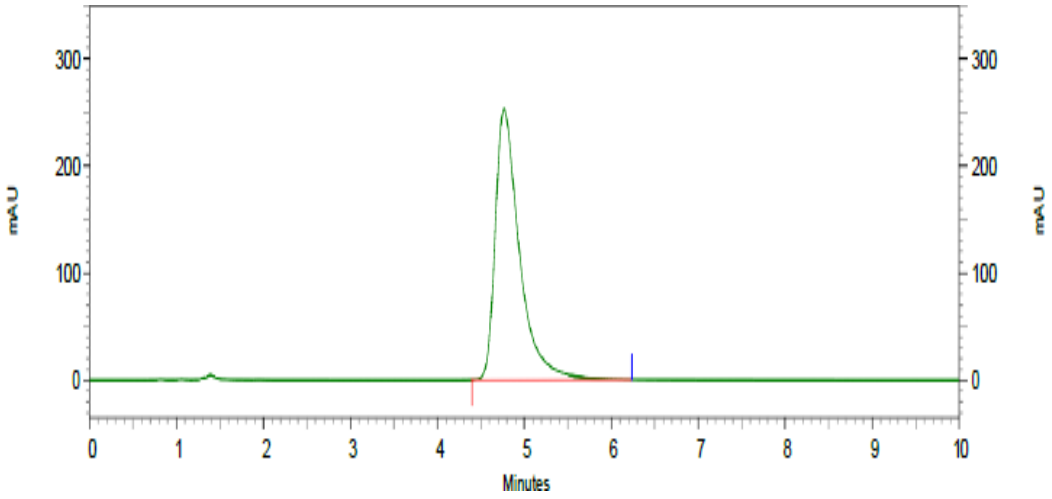


Figure no. 18: Typical chromatogram of accuracy100%.

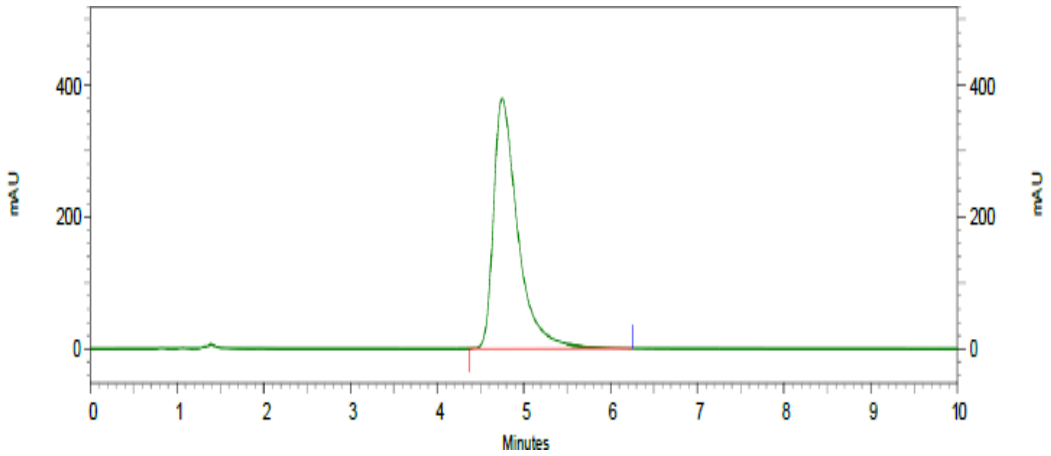


Figure no. 19: Typical chromatogram of accuracy 150%.

Precision

Table no. 10: Result of Intra- day and Inter- Day Precision for Brexpiprazole.

	Sample	API (mg)	Area	% Assay
Intra-Day	Sample 1	10.2	81583287	100.13
	Sample 2	10.3	81379405	98.91
	Sample 3	10.2	81412771	99.92
	Sample 4	10.2	81504965	100.04
	Sample 5	10.3	81752571	99.36
	Sample 6	10.2	81886359	100.50
	Mean			**99.81**
	STD DEV			**0.575500**
	% RSD			**0.577**
Inter-Day	Sample 1	10.1	81435214	101.85
	Sample 2	10.2	81723641	101.20
	Sample 3	10.1	81264789	101.63
	Sample 4	10.3	81538691	99.99
	Sample 5	10.2	81426638	100.84
	Sample 6	10.1	81642384	102.10
	Mean			**101.27**
	STD DEV			**0.772410**
	% RSD			**0.763**
Intra-day Plus Inter-day	**Mean**			**100.539**
	STD DEV			**1.00088**
	% RSD			**0.996**

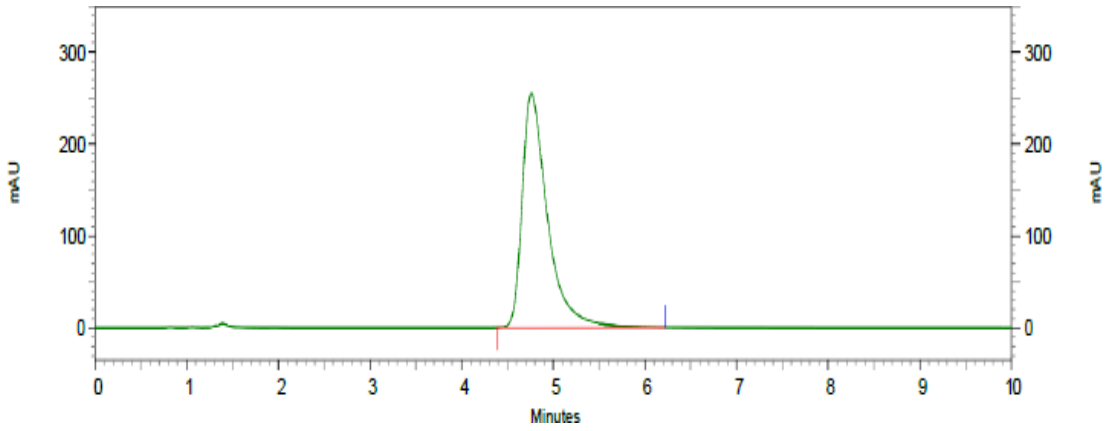


Figure no. 20: Typical chromatogram of Intra-day precision.

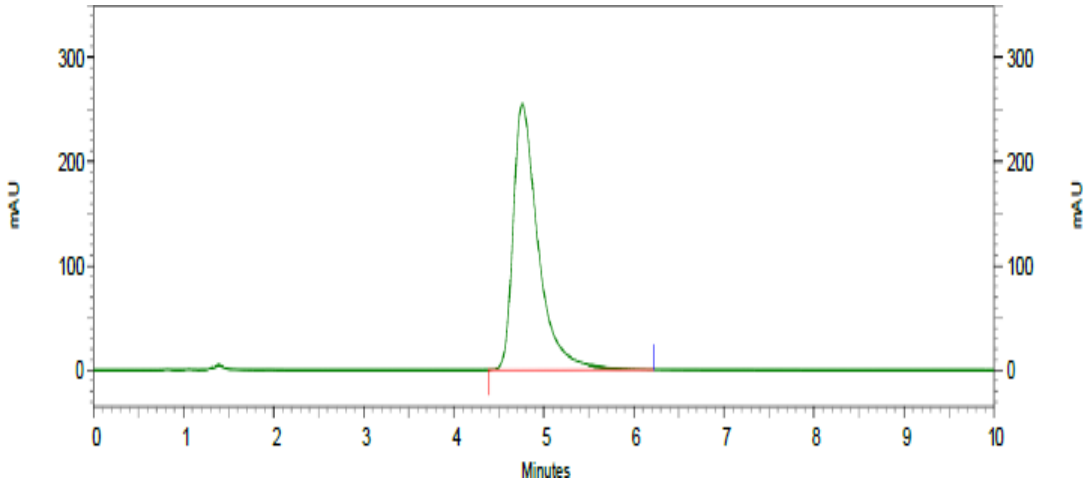


Figure no. 21: Typical chromatogram of Inter-day precision.

Robustness

Following changes made under Robustness:

- Change in Wavelength
- Change in flow rate
- Change in column oven temperature

Table no. 11: Result of robustness study of brexpiprazole.

Change in Parameter	Standard area	Sample Area	% Assay	Abs Diff w.r.t. Precision
Wavelength by +3 NM	77846476	77787485	99.92	0.11
Wavelength by -3 NM	81943133	83018511	100.09	0.28
Flow rate by +10% (1.1mL/min)	74318886	74384865	100.09	0.28
Flow rate by -10% (0.9mL/min)	91156286	91999115	99.83	0.02
Column oven temp by +2ºC	81552147	81585261	100.04	0.23
Column oven temp by -2ºC	81325871	81324173	100.00	0.19

Limit of detection

The LOD is the lowest limit that can be detected. Based on the S.D. deviation of the response and the slope. The limit of detection (LOD) may be expressed as:

LOD = 3.3 (SD)/S

Where, SD= Standard deviation of the Y intercept

S = Slope

LOD = 3.3 x 199302.9539/ 1620101.119 = 0.41 µg/ml

The LOD of Brexpiprazole was found to be 0.41 µg/ml

Limit of quantitation

The LOQ is the lowest concentration that can be quantitatively measured. Based on the S.D. deviation of the response and the slope.

The quantitation limit (LOQ) may be expressed as LOQ = 10 (SD)/S

Where, SD = standard deviation of Y intercept S = Slope

LOQ = 10 x 199302.9539/ 1620101.119 = 1.23 µg/ml

The LOQ of Brexpiprazole was found to be 1.23 µg/ml

CONCLUSION

The present work involved the development of simple, accurate, precise and suitable RP-HPLC method. Hence, in the present study, a new, sensitive and suitable reversed-phase high performance liquid chromatography method was developed and validated for the determination of Brexpiprazole in bulk drug and pharmaceutical dosage form. The detection was carried out at 216nm.The results of analysis in the developed method were validated in terms of linearity, accuracy, precision, robustness, limit of detection and limit of quantification. The developed method has several advantages, including reproducibility of results, rapid analysis, simple sample preparation and improved selectivity as well as sensitivity. The regression coefficient (r2) for each analyte is not less than 0.999 which shows good linearity. The % recovery was in the acceptable range in tablet dosage form. The %RSD was also less than 2% showing high degree of precision of the proposed method. Since the developed method is robust and reproducible and also less time consuming, it can be performed for routine analysis in pharmaceutical industry for bulk drug of Brexpiprazole and also in pharmaceutical dosage form.

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