SHORT COMMUNICATION

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Journal of Ecophysiology and Occupational Health, *Vol 25(3), DOI: 10.18311/jeoh/2025/47231, 347-353, September 2025*

ISSN (Online) : 0972-4397
ISSN (Online) : 0974-0805

Novel Method Development and Validation of Brexpiprazole in Bulk and Pharmaceutical Formulation by RP-HPLC Method

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Abstract

Objective: To develop and validate a simple, accurate, precise, and rapid method for estimation of Brexpiprazole pharmaceutical formulations using RP-HPLC. **Methods:** High-Performance liquid chromatography was used to analyse Brexpiprazole. The separation was performed on COSMICSIL, (150 X 4.6mm, 5µm) at room temperature using NaH_2PO_4 and methanol in equal volumes in isocratic condition at a flow rate of 1.0 mL/min. The detection was performed by an ultraviolet detector (UVD) at 215 nm with total run time of 5 min. Calibration curves were linear in the concentration range of 2-6 µg/mL. **Results:** Brexpiprazole retention time (Rt) was discovered to be 2.25 minutes. Linearity calibration plot was plotted in the concentration range of 2–6 µg/mL for Brexpiprazole with linear regression equation of y = 78453x - 2454.2 (R^2 = 1). The precision studies were estimated, the results were calculated as percentage RSD values, and the % RSD was calculated and obtained as 1.10 for Brexpiprazole in precision study. The %RSD was found to be with in the acceptance limit of < 2. Brexpiprazole mean Recovery was determined to be 99.14%, confirming the effectiveness of the procedure. **Conclusion:** According to ICH criteria, the designed RP-HPLC technique was verified. Brexpiprazole can be analysed using the established approach in both its bulk and commercial form.

Major Findings: A simple, rapid, and precise RP-HPLC method was developed and validated for Brexpiprazole estimation with excellent linearity (R^2 = 1), precision (%RSD = 1.10), and accuracy (99.14% recovery). The retention time was 2.25 minutes, ensuring quick analysis. This method can be used for routine quality control and stability studies of Brexpiprazole formulations.

Keywords: Antipsychotic, Dopamine Receptor, ICH, Retention Time, Schizophrenia, Validation

1. Introduction

Brexpiprazole is 7-[4-[4-(1-benzothiophen-4-yl) piperazin-1-yl] piperazin-1-yl] piperazin-1-yl] piperazin1-yl] piperazin-1-yl] piperazin-1-yl] piperazin-1 butoxy], a medical drug that belongs to antipsychotic class Figure 1. The molecular weight of Brexpiprazole is 433.6 g mol and a molecular formula of $C_{25}H_{27}N_3O_2S$. It is used treat the major depressive disorders and schizophrenia with improved efficacy and tolerability. The drug is branded as Rexulti[1].

It is a partial agonist for dopamine D_2 receptor having a "serotonin–dopamine modulating activity". The US Food and Drug Administration originally approved Brexpiprazole in July 2015 for use as a monotherapy for schizophrenia and as an adjuvant treatment for major depressive disorder in adults. Brexpiprazole metabolism is regulated by CYP2D6, contributes to its wide dosing

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Article Received on: 09.11.2024 Revised on: 17.12.2024 Accepted on: 23.12.2024

range in clinical practice. It is available in tablet form with single doses of 0.5, 1.0, 2.0, and 3.0 mg[2].

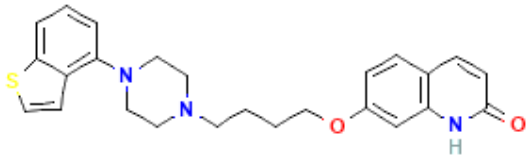


Figure 1. Molecular structure of Brexpiprazole[3]. (Source: https://pubchem.ncbi.nlm.nih.gov/compound/Brexpiprazole)

2. Materials and Methods

2.1 Instrumentation

An autosampler-equipped HPLC system Waters Alliance E2695, Model No. 2489 A UV/visible detector was utilised in the approach's development and validation. COSMICSIL was used to finish the study, and its dimensions (150 X 4.6 mm, 5µm) were obtained at room temperature. Empower 3 software was used to compile and assess the data.

2.2 Chemicals and Reagents

Otsuka Pharmaceuticals India ltd, Ahmedabad, provided the formulation dosage form (Rexulti®-4 mg) and the working standard active pharmaceutical drug Brexpiprazole. HPLC grade, Na_2HPO_4, KH_2PO_4, NaOH, HCl, and H_2O_2, alongside Acetonitrile and Methanol from Merck Specialties Ltd. All the remaining components and reagents were of an analytical grade.

2.3 Preparation of Solutions

2.3.1 Preparation of 0.1% NaH_2PO_4 Buffer

In a volumetric flask with a capacity of 1000 ml, 0.1ml of NaH_2PO_4 was added. After that, the solution was sonicated for ten minutes and filtered through a 0.45 micron membrane filter. The above standard stock solution that has been properly diluted with diluents to produce different dilutions of Brexpiprazole[4].

2.3.2 Preparation of Mobile Phase

The mixture was filtered through a 0.45 micron filter under vacuum after degassing for ten minutes in an ultrasonic water bath with 500 ml of methanol and 500 ml of NaH_2PO_4 buffer.

2.3.3 Preparation of Diluent

Mobile phase operated as the diluent.

2.3.4 Preparation of Stock Standard Solutions

4 mg of Brexpiprazole standard powder weighed and transferred into a 100 ml of clean and dry volumetric flask, add 30ml of the diluent and sonicate.

2.3.5 Procedure for Sample Solutions of Brexpiprazole

One vial of powder, containing 76 mg of Brexpiprazole, was weighed. The powder was then transferred to a 100 ml dry volumetric flask, diluent was added, and the mixture was sonicated to completely dissolve the powder, with the diluent, the volume was adjusted. The above sample solution was filtered, and then 1 ml of the filtrate was pipetted into a 10 ml volumetric flask along with 10 ml of diluent[5,6].

2.3.6 Identifying the Wavelength for Method Development

Following the production of a stock solution containing 10µg/ml of Brexpiprazole, serial dilutions in methanol were made. The wavelength was found by scanning the previously indicated average drug sample wavelength between 200 - 400 nm. The 215 nm spectrum was selected as the absorbance peak. Consequently, 215 nm is used as the wavelength of detection for the RP-HPLC analysis of Brexpiprazole[6].

2.3.7 Method Development

The tailing factor and plate count of the United States Pharmacopoeia (USP) were considered when conducting the trials that led to the development of the chromatographic technique. The kind of column and the composition of the mobile phase were the factors considered when doing the tests. Throughout the trails, the injection volume (10µl) and wavelength (flow rate -1 ml/min) remain constant. The chromatographic conditions used in experimentation for technique development have demonstrated in Table 1[7].

Table 1. Experimental conditions for developing methods

Trial no	Composition of the mobile phase	Column used	observations
1	K_2HPO_4: Acetonitrile (60:40v/v)	SUPELCO,150X4.6mm, 5µm	Theoretical plates are beyond of their limits, and the peak shape was unsatisfactory.
2	K_2HPO_4: Acetonitrile (60:40v/v)	ACE,150X4.6mm, 5µm	The peak shape is not good and the observed number of theoretical plates was quite low.
3	KH_2PO_4: Methanol (60:40v/v)	INERTSIL, C18, 250X4.6mm, 5µm	Despite Rt is more, peak form is more appropriate.
4	NaH_2PO_4: Methanol (70:30v/v)	COSMICSIL,150X4.6mm, 5µm	Rt is more
5	NaH_2PO_4: Methanol (60:40v/v)	COSMICSIL,150X4.6mm, 5µm	Less Rt but peak area has to be improved
6	NaH_2PO_4: Methanol (50:50v/v)	COSMICSIL,150X4.6mm, 5µm	Every parameter is within the suitable range, and the peak shape is good.

2.4 Construction of Calibration Curve

Mobile phase was used to dilute the standard stock solution to achieve the different concentrations 1, 2, 3, 4, 5 and 6 mg/ml. The optimum chromatographic conditions were met when the chromatograms were recorded. Using the chromatograms, the mean peak areas at various concentration levels are calculated. After that, the mean peak areas of the related concentrations were used to create the linear plot as shown in Figure 2[8].

2.5 Method Validation

In compliance with the ICH requirements, the new approach was validated for the following parameters: Detection, quantitation limits, accuracy, precision, linearity, robustness, and system suitability[9-13].

2.5.1 Selectivity (Specificity)

The specificity of the technique was assessed by adding the solution to the chromatograph. The specificity of the process was assessed by comparing the chromatograms of the tablet solutions with Brexpiprazole (standard), blank, and sample solutions. A comparison was made between the Brexpiprazole retention durations in the reference solution and the sample solution to evaluate the specificity of the procedure[14].

2.5.2 Sensitivity of the Method

The lowest concentration of analyte in a sample can be detected and measured is referred to as the "Limit of Detection" or "Limit of Quantitation". Brexpiprazole standard solutions for LOD and LOQ were prepared by dilution with an appropriate solvent. As a series of diluted solutions were examined, the LOD and LOQ were determined for each compound until the signal-to-noise (S/N) ratio yielded 3 for LOD and 10 for LOQ, respectively[15].

2.5.3 Linearity and Range

The developed method involved building a calibration curve from LOQ concentration, loading calibration curve dilutions, injecting 10µL into a column, repeating all measurements for each concentration, recording the calibration curve's area under curve versus concentration, and calculating correlation and regression values for Brexpiprazole based on the calibration curve[16].

2.5.4 Precision

For the precision investigation, the Brexpiprazole response was estimated six times at a standard concentration of 40 µg/mL (100 % solution). The data are given as a percentage of the relative standard deviation. Six estimates of the matching responses were made on the same day to carry out the precision tests. The following calculation was used to get the percentage (%RSD), which was used to express the results[17].

% RSD (Relative Standard Deviation) = (Standard deviation/Mean) × 100.

2.5.5 Accuracy/Recovery

The recovery outcomes from two placebo preparations that were precisely spiked with varying concentrations of Brexpiprazole demonstrated the accuracy of the

procedure. Using the usual addition approach, which involved adding known amounts of pure standards at three distinct levels 50%, 100%, and 150% to the formulation of the previously examined sample, recovery assessment was obtained. The formula was used to determine the amount of drug recovered, the percentage of recovery, and the amount of drug detected[18].

% RSD (Relative Standard Deviation) = (Standard deviation/Mean) × 100.

2.5.6 Robustness

Six intentional changes to a few chromatographic parameters demonstrated the robustness of the suggested approach. Changes were made to the mobile phase flow rates, pH levels, and column temperatures that were examined. Each modified condition percentage change was determined[19].

3. Results and Discussion

3.1 RP-HPLC Method Development

With a chromatogram, the placebo and blank are visible and although hrexpiprazole, whose retention period is 2.25 minutes, did not reveal any interfering peaks.

3.2 System Suitability

In the optimized conditions, peak tailing factors of was observed to be 0.92 for brexpiprazole whereas the number of theoretical plates was achieved as 6528. This method met the accepted requirements, and the relevant data were shown in Table 2.

3.3 Linearity

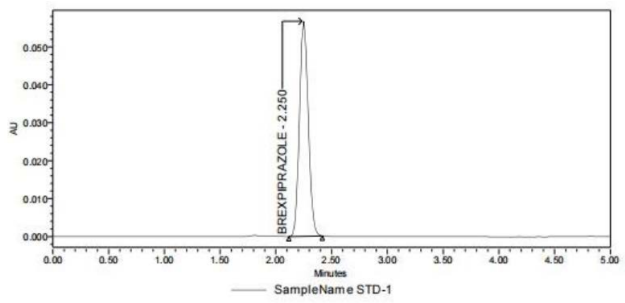
Chromatograms of solutions containing 1, 2, 3, 4, 5, and 6 mg/ml were prepared and recorded using the stock solution. After obtaining the chromatograms, the average peak areas of the corresponding concentrations were obtained, and the linearity plot was created using the mean peak areas. It was found that the correlation coefficient (R^2) had a value of 0.9996. Table 3 displayed the linearity data for Brexpiprazole, while Figure 3 displays the linearity plot.

3.4 Accuracy

To verify the accuracy at the three levels of 50%, 100%, and 150%, a recovery study was performed out. A recovery study was carried out three times for each level, and the recovery percentage and relative standard deviation are calculated[20]. Brexpiprazole mean recovery is displayed in Table 4.

3.5 Precision

The Table 5 contains the precision results. Precision was considered outstanding when it was less than 2%, expressed as a percentage Relative Standard Deviation (% RSD)[20].



	SampleName	Peak Name	RT	Area	USP Tailing	USP Plate Count	s/n
1	STD-1	BREXPIPRAZOLE	2.250	312843	1.09	3633	1255.9

Figure 2. Standard chromatogram of Brexpiprazole. Source: EMPOWER 3 Software

Table 2. System suitability analysis of Brexpiprazole

	Sample	Peak name	RT	Area	USP Tailing	USP count
1	STD-2	BREXPIPRAZOLE	2.251	312786	1.09	3620
2	STD-2	BREXPIPRAZOLE	2.253	312842	1.08	3642
3	STD-2	BREXPIPRAZOLE	2.251	313344	1.09	3638
4	STD-2	BREXPIPRAZOLE	2.257	313835	1.09	3770
5	STD-2	BREXPIPRAZOLE	2.246	313625	1.08	3722
Mean				313286.4		
%RSD				0.1		

Table 3. Linearity study results of Brexpiprazole

Sl.NO	Degree of linearity	Concentration in µg/mL	Peak Area
1	50%	2	154112
2	75%	3	232902
3	100%	4	311922
4	125%	5	390050
5	150%	6	467803

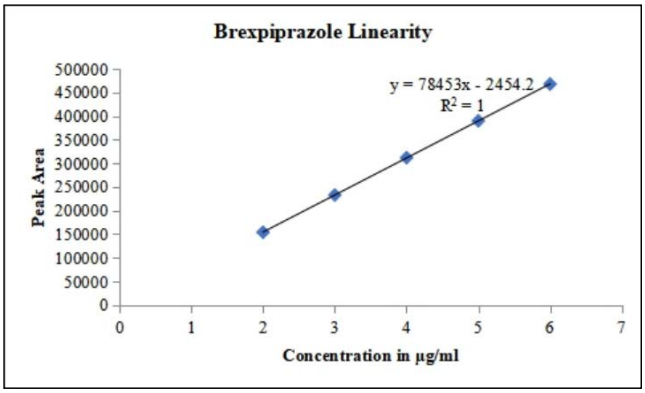


Figure 3. Linearity graph of Brexpiprazole.
SOURCE: MICROSOFT EXCEL

Table 4. Accuracy results of Brexpiprazole

Spiked Level	Sample Area	µg/mL added	µg/mL found	Percentage Recovery	Percentage
					Mean
50%	154421	1.98	1.97	99.28	99.14
50%	154101	1.98	1.96	99.07	
50%	154120	1.98	1.96	99.08	
100%	311424	3.96	3.96	100.11	100.14
100%	311451	3.96	3.96	100.12	
100%	311669	3.96	3.97	100.19	
150%	467440	5.94	5.95	100.17	100.18
150%	467402	5.94	5.95	100.17	
150%	467541	5.94	5.95	100.20	

Table 5. Precision results of Brexpiprazole

Sl.no	Peak Area	% Assay
1	311864	99.25
2	311965	99.28
3	312131	99.33
4	311273	99.06
5	312024	99.30
6	312111	99.33
%RSD	1.10	

Table 6. Robustness result of Brexpiprazole

Sl.No	Condition	Change	Area	% Change
1	Standard	No change	311922	0.00
2	Flow Rate 1	0.9 mL/min	313365	0.46
3	Flow Rate 2	1.1 mL/min	315337	1.09
4	Temperature 1	30°C	317526	1.80
5	Temperature 2	20°C	312601	0.22
6	pH 1	4.0	313864	0.62
7	pH 2	4.2	313965	0.65

3.6 Robustness

Brexpiprazole pure drug percentage change was found to be within the acceptable limit of less than 2, with a change in the level of 0.22 to 1.80. This demonstrates that a slight alteration to the analytical parameters has no effect on the outcomes and, thus, the established after a slight modification to the analytical conditions, it was concluded that the methodology was suitable for the analysis of Brexpiprazole. Robustness results were tabulated in Table 6.

3.7 LOD and LOQ

Limit of Detection and Limit of Quantification were calculated using the S/N ratio method and were 0.010 g/mL and 0.032 g/mL, respectively. Results showed that the technique was accurate and could be used for the analysis and identification of substances at the smallest amounts[21].

4. Conclusion

The developed RP-HPLC method produced results that satisfied each of the validation parameter requirements. It was discovered that the precision's RSD was less than 2% and retention period was discovered to be 2.25 minutes. Brexpiprazole mean recovery rate was discovered to be 99.14%. The sensitivity of the approach is confirmed by LOQ. It was discovered that the recently created RP-HPLC technique was simple, accurate, rapid and exact.

5. Acknowledgement

We sincerely thank the management and principal of Krupanidhi College of Pharmacy for their support.

6. Abbreviations

ICH: International Council for Harmonisation, RP-HPLC: Reverse Phase- High Performance Liquid Chromatography; LOD: Limit of Detection; LOQ: Limit of Quantification; %RSD: Percent Relative Standard Deviation; Rt: Retention Time; S/N ratio: Signal to Noise Ratio.

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