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Original Research Article

Dissolution method development and validation of brexpiprazole

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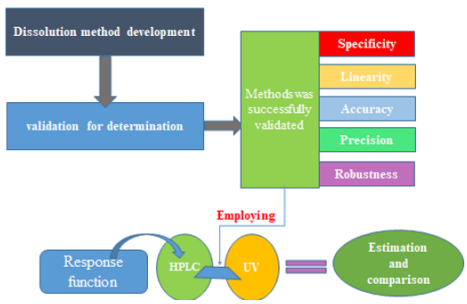
ABSTRACT

Background : A stability-indicating RP-HPLC method was established and validated for the determination of Brexpiprazole in bulk drug-using 1260 Infinity HPLC having column Hemochrom C-8, 25 cm X 4.6 mm x 5 μ, USP Apparatus Type II (Paddle), and sonicator. The prepared mobile phase was filtered as per the standard procedure. Analysis was carried out at wavelength 227 nm, flow rate 1ml per minute, 18-22 °C temperature, with an injection volume of 50 μl with time 21 minutes.
Results: It was found that interference of diluent and placebo is not more than 0.5% of Brexpiprazole. The individual % recovery was found to be between 97.0% to 100.0%, the linearity was within the range of 10- 60 μg mL with a Correlation Coefficient of 0.999, Slope of the regression line was found to be 1299178.9578 which means all parameters were found to be within range. In precision % RSD for % released dissolution values were found to be 99.16, in robustness, all the parameters like change in the flow rate, wavelength, and in RPM shows that the developed method was robust.
Conclusion : The proposed approach performed well in terms of sensitivity, precision, accuracy, linearity and range, robustness. The well-known RP-HPLC method for the study of Brexpiprazole was shown to be trustworthy, as well as easy, consistent, cost-effective, and exact. For quality control or routine quantification, this method is applicable to determine Brexpiprazole in a bulk and pharmaceutical dosage form. This developed method required less time.

1. Introduction



Brexpiprazole is a BCS class II type called (7-{4-[4-(1-benzothiophen-4-yl) piperazin-1-yl] butoxy} quinoline-2(1H)-one is a new medication in psychiatric [1,2] and also in the treatment of depression which has a high affinity for monoamine neurotransmitters like serotonin, dopamine, and noradrenaline receptors, For other major depressive disorder like Alzheimer's disease, schizophrenia[3,4], neurobehavioral disorders[5], combat disorder, bipolar disorder treatment, adjunctive treatment it is mostly used. It has more potency than other antipsychotic drugs with little aqueous solubility and substantial intestinal permeability.[6,7]

Brexpiprazole was originally approved in the United States in July 2015 for use as an adjunctive action for

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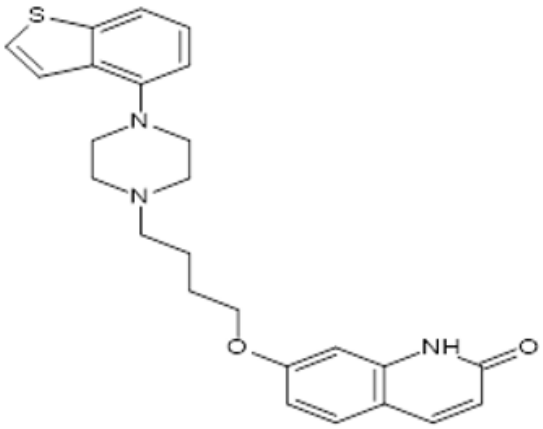

Figure 1: Structure of Brexpiprazole[8]

major depressive disorder (MDD) and schizophrenia.[9] Antipsychotics are first and second-generation drugs that are mainly active as D2 receptor antagonists.[10,11] Brexpiprazole acts as a partial agonist of the serotonin 5-HT1A receptor and the dopamine D2 and D3 receptors. Partial agonists have both blocking properties and stimulating properties at the receptor they bind to. The ratio of blocking activity to stimulating activity determines a portion of its clinical effects. .[12] brexpiprazole is a substrate of CYP2D6 and CYP3A4, like its predecessor aripiprazole. Participants in the clinical trials are advised to avoid grapefruit, Seville oranges and related citruses. The literature survey discovered that by UV-visible spectroscopy and HPLC.[13–17] method Brexpiprazole was determined. In the current work, the authors have proposed dissolution method development and validation of Brexpiprazole which is a simple, precise, robust, and valid RP-HPLC method for the estimation of Brexpiprazole in the pharmaceutical active dosage form.

2. Materials and Methods

2.1. Chemicals and solvents

Brexpiprazole standard (Purity ≥ 99.7), triethylamine, phosphoric acid, Methanol, HPLC grade water (Millipore).

2.2. Instrumentation

The instrument employed in the present work were analytical microbalance (Make: Mettler Toledo, Model: XP56), 1260 Infinity HPLC having column Hemochrom C-8, 25 cm X 4.6 mm x 5 μ, USP Apparatus Type II (Paddle), sonicator (Make: Elma, Model: S300H),

2.3. Method development

2.3.1. Optimization of the chromatographic conditions

The system used was Agilent 1260 Infinity HPLC having column Hemochrom, mobile phase was prepared by a combination of 20 ml of triethylamine with 1000 ml of HPLC grade water and 680 ml of HPLC Grade Methanol. pH was adjusted to 4.0 ± 0.05 with phosphoric acid, degassed by sonicator, and mix well. The diluent used was water. Analysis was carried out at wavelength 227 nm, flow rate 1ml per minute, 18-22 °C temperature, with an injection volume of 50 μl with time 21 minutes.

2.3.2. Preparation of working standards

The standard solution of Brexpiprazole prepared as 27.5 mg of pure drug was dissolved in a 250 mL volumetric flask and diluted to volume with water. Further 10 mL of stock was diluted to 50 mL with diluent, further, dilute 5 ml of the above-diluted solution to 10 ml with diluent.

2.3.3. Method validation

By analyzing linearity, precision (method and intermediate), accuracy, LOD, LOQ, deterioration, and robustness, the present created technique was verified according to ICH and FDA requirements.

2.3.3.1. Specificity. Specificity is the ability to access unequivocally the analyte in the presence of components that may be expected to be present. Separate vials were prepared and injected to check the interference. The standard solution was prepared to have a percentage purity of 99.61%. The analysis was done using various methods such as Blank solution, Control standard solution, Test solution and Placebo solution respectively.

2.3.3.2. Accuracy. Accuracy was conducted in the range of 20 % to 150 % of working concentration of 10 mg strength. Solutions of each accuracy level were prepared in triplicate. The study was performed by using placebo tablets of 10 mg strength. Determination of percent recovery was carried out in each study.

2.3.3.3. Linearity and range. The linearity parameter of an analytical procedure is its ability to obtain test results that are directly proportionally to the concentration of analyte in the sample. Linearity was conducted for the Brexpiprazole concentration between 20% to 150% level of limit concentration. Graphically region was plotted. The range was evaluated based on linearity.

2.3.3.4. Precision. Precision was conducted by using tablets of 10.0 mg strength. This study was performed on 6 different jars. Standard solutions were prepared by purity 99.61%, Weight 27.69mg, and Concentration 11.03 μg/ml. In the precision determination method, the blank and standard solution method was used, also the intermediate

precision determination method was done by using standard and blank solution.[18]

2.3.3.5. Robustness. The robustness was performed by using different samples with chromatogram injection and check the parameter to carrying out deliberate variations like Flow rate (±10%), Wavelength (+10 % at 230 nm and -10 % at 224 nm), RPM (±10%) and the volume of the dissolution medium (changed by ±10%)

Table 1: Method parameters of HPLC for robustness

Sr. No.	Parameter	Actual Parameter	Parameter to be changed	
1	HPLC (Flow Rate)	1.000 ml	0.900 ml	1.100 ml
2	HPLC (Wavelength)	227 nm	224 nm	230 nm
3	Dissolution apparatus: RPM	75	68	82
4	Dissolution apparatus: Volume of Dissolution Medium	900 ml	890 ml	910 ml

3. Results

3.1. Method development

The developed chromatographic conditions were optimized to determine Brexpiprazole in drug substance and dosage form. The symmetrical peak was found with Hemochrom C-8, 25 cm X 4.6 mm x 5 μ or equivalent column at 18-22 °C, and the mobile phase consisted of 20 ml of Triethylamine with 1000 ml of HPLC grade water and 680 ml of HPLC Grade Methanol. Adjust the pH of this solution to 4.0 ± 0.05 with phosphoric acid. Detector performed at 227 nm and the flow rate was 1.0 mL/min. The injection volume was 50 μl and the run time was 25 min.

3.1.1. Specificity

The developed chromatographic method passed specificity criteria and the results of specificity are given the table 2-3.

3.1.2. Accuracy

The accuracy was done by analyzing samples at six different levels of concentrations the recovery of the method was determined by spiking Brexpiprazole active substances. The % recovery results are shown in table 4-5.

3.1.3. Linearity

Linearity was conducted for the Brexpiprazole concentration between 20% to 150% level of limit concentration. Range was evaluated based on linearity.

Table 2: Summary of results for specificity study

Sr. No.	Solution Name	Area	% Interference	Remark
1	Blank	ND	NA	NA
2	Control standard	13785046	99.4	Due to Brexpiprazole
3	Calibration standard	13874561	100.0	Due to Brexpiprazole
4	Placebo solution	ND	NA	NA
5	Drug substance	13963158	100.6	Due to Brexpiprazole
6	Drug substance + Placebo	13930289	100.4	Due to Brexpiprazole
7	Test solution	13877334	100.0	Due to Brexpiprazole

ND-

Table 3: Result of specificity

Sr. No.	Evaluation parameter	Results	Acceptance Criteria
1.	Specificity	Retention time of Brexpiprazole peak in the test solution is comparable to that in standard solution.	Retention time of Brexpiprazole peak in test solution should be comparable to that in standard solution.
		Interference of diluent and placebo is not more than 0.5% of Brexpiprazole	Interference of diluent and placebo should not be more than 0.5% of Brexpiprazole

Linearity summary is given in the graph is in figure 2 and in table from 6.

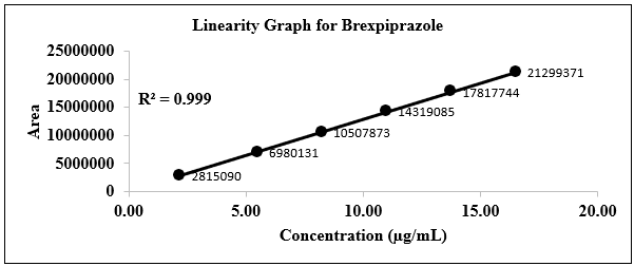

Figure 2: Linearity graph ofBrexpiprazol

Table 4: Accuracy levels area response

Levels	% Level w.r.t. working concentration	The concentration of levels in μg/mL	Area	Added Concentration in μg/mL	Recovered Concentration in μg/mL	% Recovery (Rounded)
		2.20	2819899	2.20	2.16	98.0
1	20	2.20	2804934	2.20	2.14	97.0
		2.20	2820437	2.20	2.16	98.0
		5.50	6964933	5.50	5.32	97.0
2	50	5.50	6989944	5.50	5.34	97.0
		5.50	6985517	5.50	5.34	97.0
		8.25	10499850	8.25	8.03	97.0
3	75	8.25	10509541	8.25	8.03	97.0
		8.25	10514228	8.25	8.04	98.0
		11.00	14260907	11.00	10.90	99.0
4	100	11.00	14336655	11.00	10.96	100.0
		11.00	14359693	11.00	10.98	100.0
		13.76	17818661	13.76	13.62	99.0
5	125	13.76	17820402	13.76	13.62	99.0
		13.76	17814170	13.76	13.62	99.0
		16.51	21328801	16.51	16.31	99.0
6	150	16.51	21329407	16.51	16.31	99.0
		16.51	21239906	16.51	16.24	98.0
Average % Recovery				98.2		
Standard Deviation				1.0603		
Relative standard Deviation				1.1		
Minimum % Recovery				97.0		
Maximum % Recovery				100.0		
Sample size				18		
Confidence Coefficient				1.96		
Margin of error				0.4898		
95% Confidence interval upper limit				98.7		
95% Confidence interval lower limit				97.7		
95% Confidence interval				97.7-98.7		

Table 5: Accuracy results

Sr. No.	Evaluation Parameter	Results	Acceptance Criteria
1	Individual % Recovery	Between 97.0% to 100.0%	Between 95.0% and 105.0%
2	Mean % recovery (n=18)	98.2	Between 97.0% and 103.0%
3	95 % Confidence Interval	97.7 - 98.7	To be reported

Table 6: Linearity and range results

Sr. No.	Evaluation Parameter	Results	Acceptance criteria
1	Correlation Coefficient	0.999	≥ 0.99
2	Y – Intercept	-99954.1606	To be reported
3	% Y – Intercept	-0.7	$\leq \pm 5$
4	Slope of regression line	1299178.9578	To be reported
6	Residual sum of square	40291650086	To be reported
7	Range	2.20 to 16.51	To be reported

3.1.4. Precision

The Method precision and Intermediate precision (ruggedness) were prepared and evaluated six samples at 100% of the target sample concentration as per the method. The results of % assay and % RSD are presented in Table 7-9 and in figure 3.

4. Method Prescision

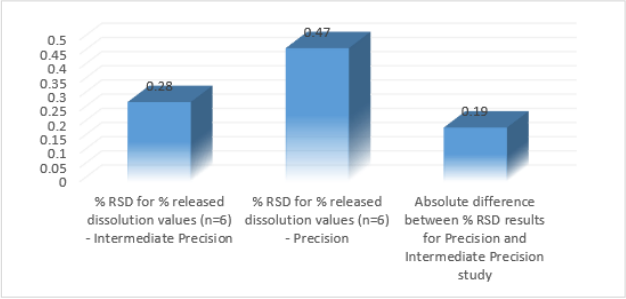

Figure 3: Intermediate precision results

4.1.

4.1.1. Robustness

The robustness of the method was studied by small variation of the chromatographic conditions such as flow (1.0 ± 0.2 mL/min), column temperature (30 ± 5°C), and mobile phase composition (±10% absolute). The results are given in Table 10-16.

Change in Rpm – Dissolution Apparatus

5. Discussion

In this RP-HPLC method, the Retention time of the Brexpiprazole peak in the test solution is comparable to that in the standard solution. It was found that interference of diluent and placebo is not more than 0.5% of Brexpiprazole. The individual % recovery was found to be between 97.0% to 100.0%, the linearity was within the range of 10- 60 μg mL with a Correlation Coefficient of 0.999, Slope of the regression line was found to be 1299178.9578 which means all parameters were found to be within range. In precision % RSD for % released dissolution values were found to be 99.16, in robustness, all the parameters like change in the flow rate, wavelength, and in RPM shows that the developed method was robust.

6. Conclusion

This study is a stability-indicating analysis that was done in accordance with ICH/FDA criteria. The proposed approach performed well in terms of sensitivity, precision, accuracy, linearity and range, robustness. The well-known RP-HPLC method for the study of Brexpiprazole was shown to be trustworthy, as well as easy, consistent, cost-effective, and exact. For quality control or routine quantification, this method is applicable to determine Brexpiprazole in a bulk and pharmaceutical dosage form. This developed method required less time.

7. Abbreviations

RP-HPLC- Reversed-phase high-performance liquid chromatography, USP- United States Pharmacopeia**,** LOD- Limit of detection LOQ- Limit of quantification, D2- Dopamine receptor, ICH**-** International Council on Harmonisation, FDA- Food drug administration, RPM- Rotation per minute, ND- Not detected, NA- Not applicable, RSD- Relative standard deviation.

8. Source of Funding

None.

9. Conflict of Interest

None.

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Table 7: Observation summary of test solution

Test	Retention time	Area	%Drug released
1	17.82	14132702	99.10
2	17.83	14135590	99.12
3	17.82	14167535	99.35
4	17.79	14062104	98.61
5	17.81	14090551	98.81
6	17.83	14254142	99.96
Average	17.817	14140437.333	99.158
SD	0.0151	66889.6467	0.4691
% RSD	0.08	0.47	0.47

Table 8: System suitability test for precision study

Sr. No.	Evaluation Parameter	Results	Acceptance Criteria
1	% Recovery of control standard against average Calibration standard.	101.2	Between 98.0% and 102.0%
2	% Recovery of bracketing / closing standard against average calibration standard.	99.7	Between 98.0% and 102.0%
3	% RSD of minimum 5 replicate injections of the calibration standard.	0.09	NMT 2.0 %

Table 9: Observation summary of test solution

Test	Retention time	Area	% Drug released
1	17.81	13881294	98.44
2	17.82	13907429	98.63
3	17.8	13799749	97.86
4	17.81	13896722	98.55
5	17.81	13854417	98.25
6	17.81	13871104	98.37
Average	17.810	13868452.500	98.352
SD	0.0063	38490.0843	0.2730
% RSD	0.04	0.28	0.28

Table 10:

Sr. No.	Parameter	Actual Parameter	Parameter to be changed	
1	HPLC (Flow Rate)	1.000 ml	0.900 ml	1.100 ml
2	HPLC (Wavelength)	227 nm	224 nm	230 nm
3	Dissolution apparatus: RPM	75	68	82
4	Dissolution apparatus Volume of Dissolution Medium	900 ml	890 ml	910 ml

Table 11: Parameters ofrobustness

Test	Retention time	Area	% Drug released
1	16.96	13302656	99.13
2	16.95	13219849	98.51
3	16.95	13250240	98.74
4	16.95	13266822	98.86
5	16.95	13243665	98.69
6	16.96	13229258	98.58
Average	16.953	13252081.667	98.752
SD	0.0052	29695.5794	0.2221
% RSD	0.03	0.22	0.22

Table 12: Observation summary of test solution for change in flow rate (+10%)

Test	Retention time	Area	% Drug released
1	18.82	15179637	98.93
2	18.83	15161521	98.81
3	18.82	15182428	98.95
4	18.82	15183974	98.96
5	18.81	15163972	98.83
6	18.82	15189733	98.99
Average	18.820	15176877.500	98.911
SD	0.0063	11457.9327	0.0747
% RSD	0.03	0.08	0.08

Table 13: Observation summary of test solution for change in flow rate (-10%)

Test	Retention time	Area	% Drug released
1	17.72	10558518	98.94
2	17.72	10548074	98.84
3	17.72	10566387	99.01
4	17.73	10553938	98.89
5	17.72	10561257	98.96
6	17.72	10563592	98.98
Average	17.722	10558627.667	98.937
SD	0.0041	6707.4416	0.0622
% RSD	0.02	0.06	0.06

Table 14: Observation summary of test solution for change in wavelength (+10%)

Test	Retention time	Area	% Drug released
1	17.72	14654726	98.55
2	17.72	14635423	98.42
3	17.72	14642595	98.47
4	17.73	14665109	98.62
5	17.73	14655124	98.55
6	17.73	14654220	98.54
Average	17.725	14651199.500	98.525
SD	0.0055	10520.1795	0.0701
% RSD	0.03	0.07	0.07

Table 15: Observation summary of test solution for change in flow rate (-10%)

Test	Retention time	Area	% Drug released
1	18.02	14318703	99.69
2	17.97	14248589	99.20
3	17.98	14234946	99.11
4	17.97	14195429	98.83
5	17.97	14161434	98.60
6	17.98	14220280	99.01
Average	17.982	14229896.833	99.073
SD	0.0194	53346.2295	0.3701
% RSD	0.11	0.37	0.37

Table 16: Observation summary of test solution for change in RPM (+10%)

Test	Retention time	Area	% Drug released
1	17.97	14254419	99.25
2	17.97	14208519	98.93
3	17.98	14256434	99.26
4	17.97	14217491	98.99
5	17.98	14234364	99.11
6	17.95	14216445	98.98
Average	17.970	14231278.667	99.087
SD	0.0110	20522.7920	0.1432
% RSD	0.06	0.14	0.14

Table 17: Observation summary of test solution for change in volume of dissolution medium (+10%)

Test	Retention time	Area	% Drug released
1	17.97	14174713	98.62
2	17.97	14217690	98.92
3	17.98	14193228	98.75
4	17.98	14183120	98.68
5	17.99	14118944	98.23
6	17.99	14141478	98.39
Average	17.980	14171528.833	98.598
SD	0.0089	35817.3659	0.2501
% RSD	0.05	0.25	0.25

Table 18: Observation summary of test solution for change in volume of dissolution medium (-10%)

Test	Retention time	Area	% Drug released
1	17.96	14154612	98.48
2	17.97	14166845	98.57
3	17.97	14158635	98.51
4	17.97	14152137	98.46
5	17.97	14124843	98.27
6	17.97	14161002	98.52
Average	17.968	14153012.333	98.468
SD	0.0041	14721.6052	0.1042
% RSD	0.02	0.10	0.11

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