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Gradient High Performance Liquid Chromatography method for determination of related substances in (7-{4-[4-(1-Benzothiophen-4-YL] Butoxy} Quinolin-2(1H)-one) dosage form

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ABSTRACT

A sensitive HPLC method was developed and validated for the estimation of related substances in Brexpiprazole in drug Product. The developed method is found to be specific, reproducible, and stability indicating. Kromasil100-5 C18 (150x4.6mm), 5µ column was used and mobile phase consisted of mixture of phosphate buffer of pH5.2 and ACN in gradient program is used at a flow rate of 1.0mL/min at a wave length of 215 nm. The detector linearity was established from concentrations ranging from LOQ-150% of specification level with a correlation co-efficient of 0.999. The method was also validated for specificity, LOD, LOQ, accuracy, robustness, precision. The method is proved to be robust with respect to change in flow rate, pH, organic phase composition and column temperature. The proposed method is found to be sensitive, precise, rapid, reproducible, and offers good column life.

Keywords: RP-HPLC; Stability indicating method; Brexpiprazole; validation.

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INTRODUCTION [1-3]

Brexpiprazole (7-{4-[4-(1-benzothiophen-4-yl] butoxy} quinolin-2(1H)-one) is serotonin dopamine activity modulator with partial agonist activityat serotonin -1A -(5-HT1A) and D2 and D3 receptors, combined with potent antagonist effect on 5–HT2A, α1B, and α2C adrenergic receptors. Brexpiprazole is used in the treatment of Alzheimer's disease, hyperactivity disorder, Post-traumatic stress disorder, treatment of bipolar disorder, Adjunctive Treatment of Major Depressive Disorder, and Schizophrenia. Brexpiprazole is more potent drug than the other class of antipsychotic drugs like aripiprazole, therefore lower dose used. Brexpiprazole is higher affinity for serotonin 5HT1A receptors. Aripiprazole also higher affinity for serotonin 5HT1A receptors but lesser extent compare to Brexpiprazole. The amount of active pharmaceutical substance is more important for activity and potency of drug. Stability indicating related substances method is a quantitative analytical method based on the structural and chemical properties of each active ingredient of a drug product. In the present work, intrinsic stability of the drug Brexpiprazole was found and a selective, precise and accurate RP-HPLC method was developed for Stability indicating Related Substances of Brexpiprazole.

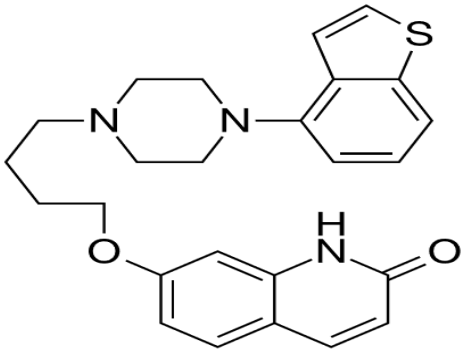


Figure 1: Chemical structure of Brexpiprazole

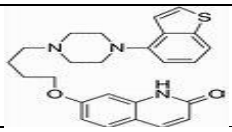
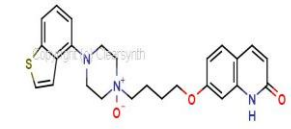
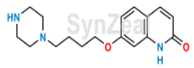
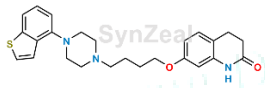
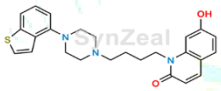
In the present work, a simple, rapid, precise and sensitive reverse phase HPLC method was developed and validated for the estimation of Brexpiprazolein pharmaceutical tablet dosage form.

MATERIALS AND METHODS [4-9]

Instrumental and analytical conditions

Reagents and chemicals

The drug Brexpiprazole and its process related impurities were gifted by Vimta Labs Ltd. (Hyderabad, India). Buffer salts were purchased from Merck and Sigma Aldrich, India. Highly purified water for HPLC was obtained from Milli Q plus water purifying system, Millipore. Methanol and acetonitrile of HPLC grade were obtained from RANKEM, India. Mobile phase was vacuum filtered through a 0.45µm Polyvinyl Dene Fluoride (PVDF) filter membrane and degassed using a sonicator to remove the dissolved gases.

Name of Impurity	Structure	Classification	Chemical Name
Brexpiprazole		NA	(7-{4-[4-(1-benzothiophen-4-yl] but oxy} quinolin-2(1H)-one
Dihydro Brexpiprazole		Process	7-(4-(4-(benzo[b]thiophen-4-yl)piperazin-1-yl)butoxy)-3,4-dihydroquinolin-2(1H)-one
Brexpiprazole N-Oxide		Degradent	7-(4-piperazin-1-yl)butoxy) quinnolin-2(1H)-one
N-Alkylated Brexpiprazole		Process	7-(4-(4-(benzothiophen-4-yl)piperazin-1-yl)butoxy)-N-Methyl quinnolin-2-one
O-Alkylated Brexpiprazole		Process	7-(4-(4-(benzothiophen-4-yl)piperazin-1-yl)butoxy)-8Methyl quinnolin-2-one

Equipment:

Waters e2695 gradient system with Empower-2 software and 2996 module Photo Diode Array detectors equipped with a quaternary solvent delivery pump, automatic sample injector and column thermostat was used for the analysis.

Chromatographic conditions:

Column : Kromasil 100 5 C-18 (150×46mm,5µ)

Pump mode : Gradient

Flow rate : As per Gradient

Detection : UV, 215nm

Column oven temperature : 35°C

Injection Volume : 50µL

Run time : 50 minutes

Table 1: Gradient Programme

Time (min)	Flow (ml/min))	Mobile Phase-A (%v/v)	Mobile Phase-B (%v/v)
0.01	1.0	75	25
20.00	1.0	50	40
30.00	1.0	45	55
35.00	1.5	25	75
42.00	1.5	25	75
43.00	1.0	80	20
50.00	1.0	80	20

Rinsing solvent: 100% Methanol (To avoid the carryover)

Preparation of analytical solutions

Preparation of diluent-1 (0.1% Orth phosphoric acid)- Prepare a required volume by diluting 1 ml of Orth phosphoric acid to 1000 ml with mill-Q water and mix well.

Preparation of diluent-2 Prepare a required volume of degassed mixture of 0.1% Orth phosphoric acid and Methanol in the Ratio of 30:70 v/v.

Preparation of mobile phase

Preparation of mobile phase-A Prepare a required volume of degassed mixture of pH 5.2 buffer and Acetonitrile in the ratio of 95:5v/v

Preparation of mobile phase-B

Use degassed Acetonitrile as Mobile Phase-B

Diluent preparation: Mixture of Water and acetonitrile taken in the ratio 50:50 v/v was used as diluent.

Preparation of the Brexpiprazolestandard solution

Weigh and transfer accurately about 42 mg of Brexpiprazole standard into a 200 ml clean and dry volumetric flask. Add about 120 ml of methanol and sonicate to dissolve completely for about 15-20 minutes. Dilute to volume with diluent-1 and mix well. Transfer 4 ml of above solution into 100 ml clean, dry volumetric flask, dilute to volume with diluent-2 and mix well. Further dilute 3ml above solution to 100 ml clean, dry volumetric with diluent-2 and mix well. Filter through PVDF Millipore or suitable by discarding about 5ml of initial solution.

Preparation of sample solution

Transfer 10 tablets into a 100 ml clean and dry volumetric flask, add about 10ml of diluent-1 and sonicate for about 10 minutes until all tablets completely disperse, add about 70 mL methanol and sonicate at room temperature for about 45 minutes with every 5 minutes intermittent shaking. Make up to the volume with diluent-1 and mix. Centrifuge the solutions at 5000rpm for 10 minutes. Filter the Supernatant solution through finer porosity membrane filter (PVDF-0.45µ) by discarding about 5 ml initial solution

Preparation of placebo solution

Weigh and Transfer the placebo powder equivalent to about 2.5mg of Brexpiprazole into a 100 ml clean and dry volumetric flask, add about 10ml of diluent-1 and sonicate for about 10 minutes, add about 70 mL methanol and sonicate at room temperature for about 45 minutes with every 5 minutes intermittent shaking. Make up to the volume with diluent-1 and mix. Centrifuge the solutions at 5000rpm for 10 minutes. Filter the Supernatant solution through finer porosity membrane filter (PVDF-0.45µ) by discarding about 5 ml initial solution.

Method Development and Validation of HPLC

The proposed analytical method was validated according to ICH guidelines (Q2B) with respect to certain parameters such as system suitability, specificity, linearity, accuracy, precision, robustness and ruggedness.

Specificity

The specificity was carried out to determine whether there is any interference of any impurities in retention time of analytical peak[9]. The specificity of the method was determined by observing the interference of any of the possible impurities and excipients.

Linearity

The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in sample. Linearity test solution for the Related Substances method was prepared by diluting the stock solution to the required concentrations. Five different concentration levels of the solutions were prepared in this range from 10% to 150% of the RS analyte concentration (0.025, 0.050, 0.125, 0.2520 and 0.376 µg/mL). RSD value for the slope and Y-intercept of the calibration curve was calculated. Peak area under the curve (average peak area of five observations) was plotted against the respective concentration level. Straight lines were obtained and the calibration equation obtained from regression analysis was used to calculate the corresponding predicted responses. Y intercepts obtained for the drug and other analytes were insignificant.

Accuracy

Accuracy is the closeness of agreement between value which is accepted either as a conventional true value or an accepted reference value and the value found The accuracy of the Related Substances method was evaluated in triplicates at three different concentration levels, 50%, 100%, and 150% i.e. 0.495,0.990, 1.485 µg/mL in the drug sample. Percentage recoveries were calculated from the slope and Y-intercept of the calibration curve developed for the drug. Percentage recoveries for the drug and impurities were within the range 90–110%. .

Precision

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision may be considered at three levels: repeatability (same day), intermediate precision (Intraday-Three different day, Intraday- different time interval on the same day), and reproducibility (different lab). Precision was carried out by injecting six replicates at the 100% level. The RSD of the peak areas of each impurity was calculated.

Limit of Detection and quantification

The LOD is defined as the lowest amount of analyte in a sample, but not necessarily quantify as an exact value and the LOQ was defined as the lowest amount of analyte in a sample determined with suitable precision and accuracy . The LOD and LOQ for Brexpiprazole was determined as a S/N ratio of 3:1 and 10:1 respectively by injecting a series of dilute solution with known concentration.

Robustness

To determine the robustness, three parameters were varied: flow rate, pH and percent composition of the organic modifier. Deliberate changes in the following parameters which affect area of Brexpiprazole and system suitability parameters were studied.

1) Change in % organic phase of mobile phase by ± 5.0%
2) Change in pH of buffer of water by ± 10% of set pH
3) Change in the flow rate of the mobile phase by ± 10% of the original flow rate

System Suitability

System suitability test was used to verify whether the system was adequate for the analysis to be performed; it was an integral part of chromatographic method development. The system suitability parameters were evaluated for the developed method by calculating the RSD values of retention

time, peak area, asymmetry, and theoretical plates of Six standard replicates

RESULTS AND DISCUSSIONS

The present investigation reported is a new RP-HPLC method development and validation of estimation of Brexpiprazole. In order to get the optimized RP-HPLC method, various mobile phases and columns were used. From several trials final method is optimized with the following conditions:

Linearity

A series of solutions were prepared using Brexpiprazole standard at concentration levels from 1% to 150% of standard concentration level and each solution was injected into HPLC. Hence the results were obtained within the limit.

Table 2: Linearity values for Brexpiprazolesolution

%Level	Concentration(µg/ml)	Area	Statistical Analysis	
10	0.025	10749	Slope	446259
20	0.050	21863	Intercept	-777
50	0.125	54732	Correlation Coefficient	0.999
100	0.250	110828		
150	0.376	168162		

Table 3: Linearity values for Brexpiprazole N-Oxide

%Level	Concentration(µg/ml)	Area	Statistical Analysis	
10	0.025	9665	Slope	390208
20	0.051	19167	Intercept	-476
50	0.126	48783	Correlation Coefficient	0.999
100	0.253	98388		
150	0.379	148700		

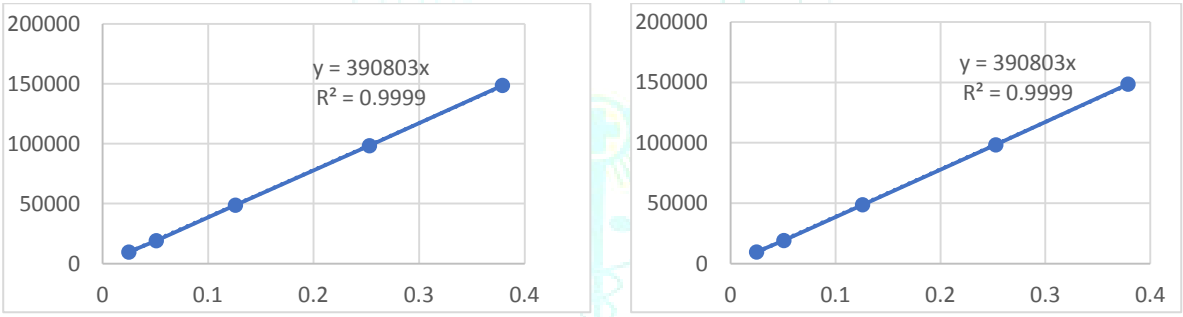


Figure 2: linearity curve for Brexpiprazole and Brexpiprazole N-Oxide.

System suitability

System suitability test was used to verify whether the system was adequate for the analysis to be performed; it was an integral part of chromatographic method development. The system suitability parameters were evaluated for the developed method by calculating the RSD values of retention time, peak area, asymmetry, and theoretical plates of Six standard replicates.

Table 4: System suitability parameters

Parameter	Observation
Retention time	13.61
USP Tailing	1.0
Plate Count	35790
%RSD	0.6

Accuracy of the proposed HPLC method

Sample Solutions were prepared in triplicate using equivalent amount of placebo present in Brexpiprazole Tablets Spiked with Known amount of Brexpiprazole drug substance at levels LOQ, 50%,100% and 150% of specification as per test method and injected each solution into HPLC.

Table 5: Accuracyvalues for Brexpiprazole N-Oxide (for LOQ Level)

Level	Amount added(%w/w)	Amount Found(%w/w)	% Recovery
LOQ Level Sample-1	0.0594	0.0543	91.4
LOQ Level Sample-2	0.0594	0.0602	101.3
LOQ Level Sample-3	0.0594	0.0575	96.8

Statistical Analysis							
Mean	96.5	SD	5.0	%RSD	5.2	95%Confidence Interval(±)	12.4

%Recovery should be between 85.0 and 115.0 for LOQ level and up to 0.2% specification

Table 6: Accuracyvalues for Brexpiprazole N-Oxide (50% to 150% level)

Level/Sample ID	Amount Added(%w/w)	Amount Found(%w/w)	%Recovery	Statistical Analysis	
50%Level Sample-1	0.495	0.464	93.7	Mean	94.0
50%Level Sample-2	0.495	0.468	94.5	SD	0.5
50%Level Sample-3	0.495	0.464	93.7	%RSD	0.5
100%Level Sample-1	0.990	0.941	95.1	Mean	95.0
100%Level Sample-2	0.990	0.944	95.4	SD	0.5
100%Level Sample-3	0.990	0.936	94.5	%RSD	0.5
150%Level Sample-1	1.485	1.399	94.2	Mean	93.8
150%Level Sample-2	1.485	1.391	93.7	SD	0.3
150%Level Sample-3	1.485	1.390	93.6	%RSD	0.3

Overall Statistical Analysis							
Mean	94.3	SD	0.7	%RSD	0.7	95% Confidence(±)	0.5

%Recovery should be between 85.0 and 115.0. %RSD should not be more than 10.0 for each level. The recovery results indicated that the test method has an acceptable level of accuracy for the determination of Related Substance in Brexpiprazole tablets is form LOQ to 150% of specification level

Precision

The precision of the method was ascertained from determinations of peak areas of six replicates of sample solution and one Standard.

System Precision

Standard Solutions was prepared as per test method and injected six times into HPLC as per methodology.

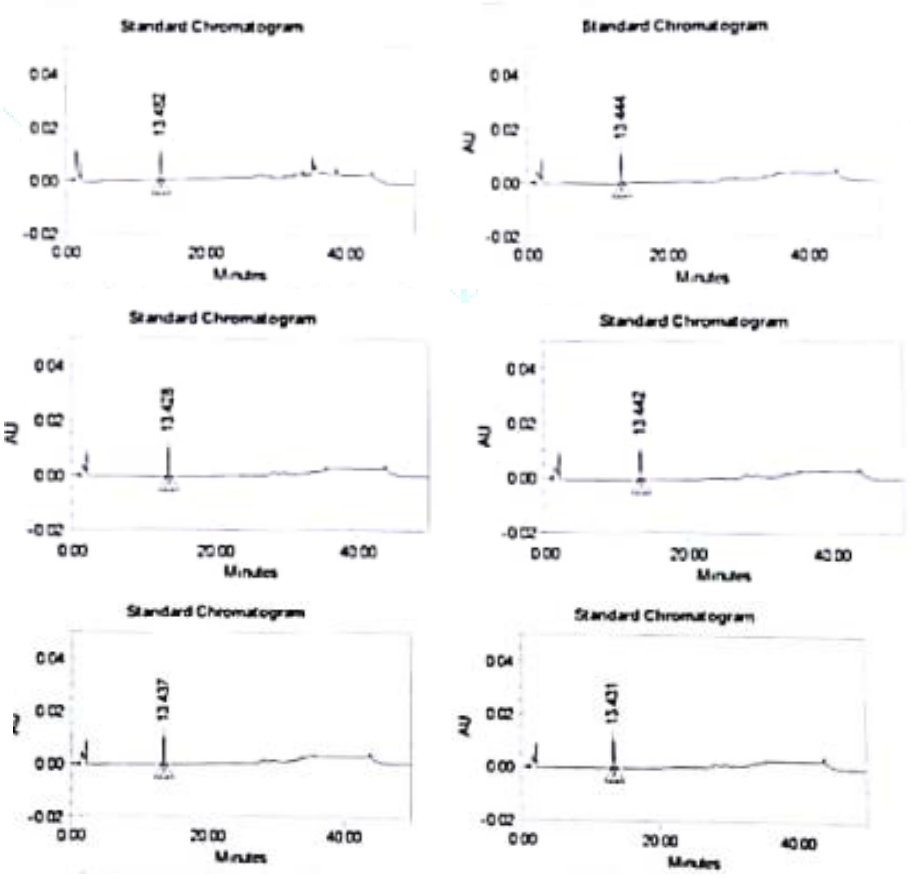


Figure 3: System Precision chromatograms

Table 7: System precision values for Brexpiprazole

RESULTS	
Injection ID	Area of Brexpiprazole
1	113368
2	113058
3	112972
4	112257
5	112618
6	112942
Statistical Analysis	
Mean	112869
SD	384.4
%RSD	0.3

%RSD for the peak areas of Brexpiprazole obtained from six replicate injection of standard solution should not more than 5.0

Method Precision

Six Sample Solutions was prepared as per test method and injected into HPLC as per methodology.

Table 8: System precision values for Brexpiprazole

RESULTS				
Injection ID	Brexpiprazole		Brexpiprazole N-Oxide	
	%w/w	%Recovery	%w/w	%Recovery
Sample 1	0.501	100	0.941	95.1
Sample 2	0.487	97.2	0.943	95.3
Sample 3	0.493	98.4	0.936	94.5
Sample 4	0.494	98.6	0.933	94.2
Sample 5	0.493	98.4	0.924	93.3
Sample 6	0.495	98.8	0.933	94.2
Statistical Analysis				
Mean	0.494	98.6	0.935	94.4
SD	0.004	0.9	0.007	0.7
%RSD	0.8	0.9	0.7	0.7
95% Confidence Interval	0.004	0.9	0.007	0.7

%RSD should not be more than 10.0 for the results of Brexpiprazole obtained from the six determinations. % Recovery should Between85.0 to 115.0.

Robustness

Standard and Sample Solution spiked with known Related Substance at Specification level were prepared as per test method and injected into HPLC at different deliberately varied conditions to evaluate method ability to remain unaffected.

The varied conditions include change in Flow Rate by ±10%, Column Oven Temperature by ±5%°C, Gradient Composition by ±1%Absolute with respect to Mobile Phase-B, Wavelength by±5nm, Organic Composition in Mobile Phase-A by±1 and pH of the Buffer ±0.1 Units.

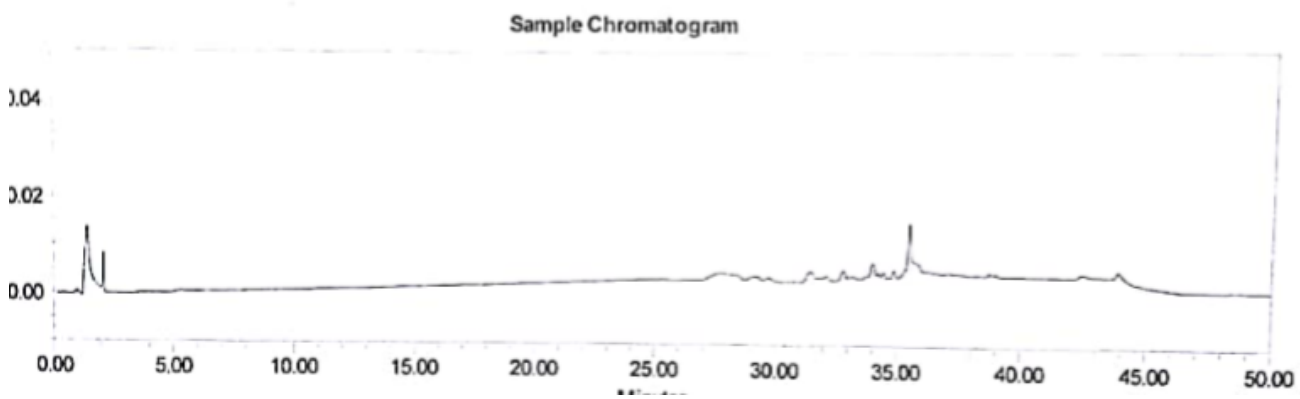


Figure 4: Diluent chromatograms

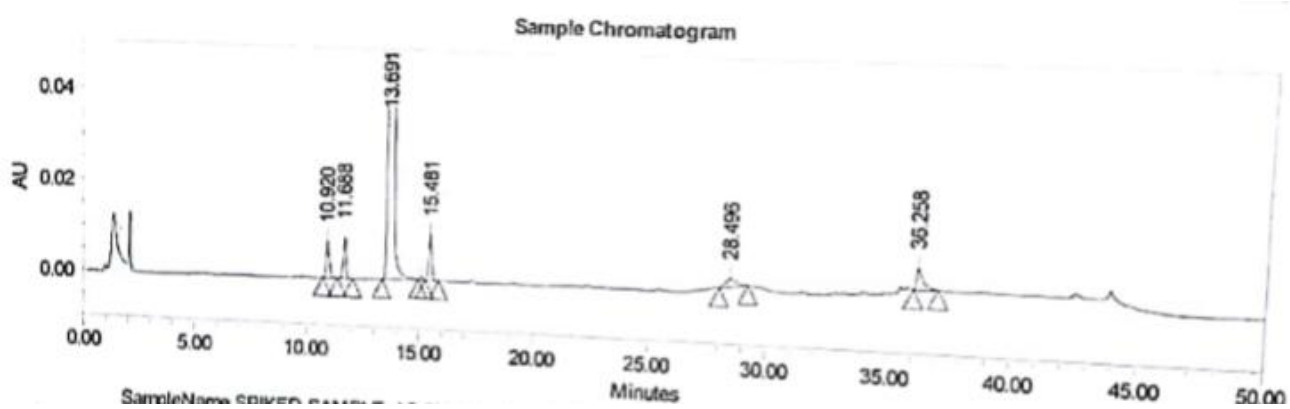


Figure 5: Sample chromatograms (As such)

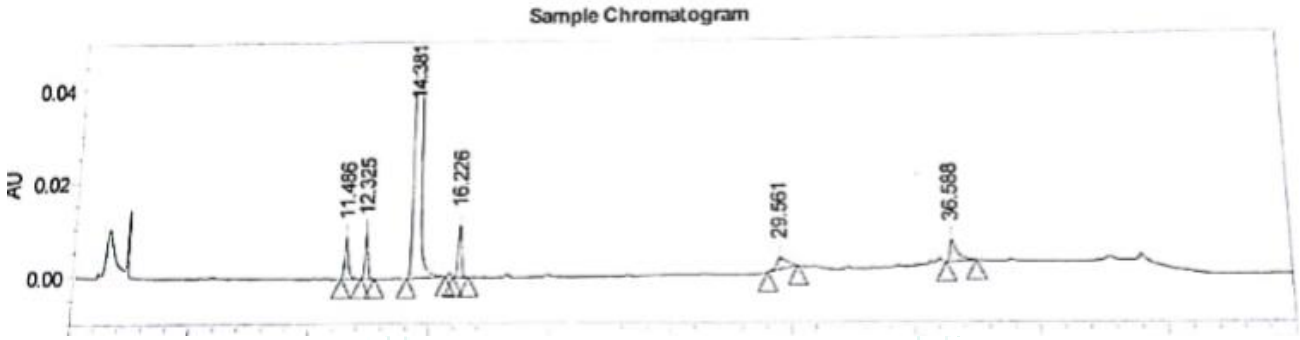


Figure 6: Sample chromatograms (0.9ml/min)

Figure 7: Standardchromatogram(0.9ml/min

Table 6: Robustness study of BrexpiprazoleStandard Solution

Parameter	Variation	System Suitability	
		USP Plate Count	USP Tailing
Flow Rate	-10%	35366	1.2
	+10%	30882	1.2
Column oven	-5°C	32504	1.2
	+5°C	32256	1.1
Wave length	-5 nm	32740	1.2
	-5 nm	32465	1.1
Gradient Composition Mobile Phase-A	-1% Absolute	36684	1.2
	+1% Absolute	30220	1.2
Gradient Composition Mobile +Phase-A	-1% Absolute	36104	1.2
	+1% Absolute	30556	1.2
pH of Buffer	-0.1 Units	32644	1.2
	+0.1 Units	33874	1.2

Table 6: Robustness study of Brexpiprazole Spiked Sample Solution

Parameter	Variation	Brexpiprazole N-Oxide	N-Alkylated-7-Hydroxy Carbostyril	Brexpiprazole	Di hydro Brexpiprazole
Flow Rate	-10%	0.79	0.87	1.00	1.13
	+10%	0.78	0.86	1.00	1.13
Column Oven Temperature	-5°C	0.80	0.88	1.00	1.14
	+5°C	0.77	0.84	1.00	1.14
Wavelength	-5nm	0.79	0.86	1.00	1.13
	+5nm	0.79	0.86	1.00	1.13
Gradient Composition Mobile Phase-A	-1% Absolute	0.80	0.87	1.00	1.13
	+1% Absolute	0.78	0.86	1.00	1.14
Gradient Composition Mobile Phase-B	-1% Absolute	0.79	0.87	1.00	1.13
	+1% Absolute	0.78	0.86	1.00	1.14
pH of Buffer	-0.1 units	0.81	0.87	1.00	1.13
	+0.1units	0.84	0.84	1.00	1.13

From spiked sample with known related substances obtained from different robustness conditions, there Related Substances at each varied conditions.

CONCLUSION

The developed and validated stability indicating RP-HPLC Related Substances method is specific, Sensitive and robust. The method found to be advantageous and simple by using Gradient elution mode. All the analytical data for all method validation parameters tested and found out to be satisfactory. The developed method can suitably use by quality control department to determine the Related substances in commercial and stability test samples of Brexpiprazole Tablets.

REFERENCES

1. National drug monograph Brexpiprazole (REXULTI®), VA Pharmacy Benefits Management Services Medical Advisory Panel and VISN Pharmacist Executives, 2016.
2. ICH- Harmonized Tripartite Guideline, Q1B, Stability Testing: Photo Stability Testing of New Drug Substances and products, 1996.
3. ICH- Harmonized Tripartite Guideline, Q3A (R1), Impurities in New Drug Substance, 2006.
4. ICH-Harmonized Tripartite Guideline, Q2 (R1), Validation of Analytical Procedures: Text and Methodology, 2005.
5. National Alliance on Mental Illness Mental health by the numbers. Available at: www.nami.org/Learn-More/Mental-Health-By-the-Numbers [accessed on August 16, 2015].
6. Das S., Barnwal P., Blessed W.A., Mondal S., Saha I. Ther Adv Psychopharmacol. 2016: 6(1):3954.
7. Wikipedia [internet], [reviewed 2017 Sep 2; cited 2017 Sep 27]. Avai lable from:htt ps:// en.wi kipedia. org/wik i/Brexpiprazole, oct. 4th, 2017.
8. Sravani A., & Durga C.H.N., Divya U., Suneetha C.H., Suresh P., Rao B.T., Sudheer C., Indo Am J Pharm Res.2017: 7: 8560-8565.
9. ICH Guideline Q2(R1) (1996) on Validation of Analytical Procedures: Text an d Methodology, InternationalConference on Harmonization, Geneva, 6 Nov 1996.