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

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ABSTRACT

A sensitive, selective, rapid, convenient, and economic stability indicating Reverse Phase High Performance Liquid Chromatographic (RP-HPLC) method were developed for the Validation and development for the Estimation of Brexpiprazole in bulk and pharmaceutical dosage form was performed on EZ- Chrome Software, Column: Kromasil C18,Column Dimension: (250 mm X 4.6 mm i.d.) 5µm ,Column Oven temperature: 40°C .using Methanol : 0.1%TFA (40:60%, v/v) as mobile phase with a flow rate of 1ml/min. Detection was carried at 216nm. Linearity was observed over the concentration range of 80-120 µg / ml .(R2=0.99983) with regression equation Y=mx=c,Y=1179203.12x+7782378.40. From the Accuracy study was performed % recovery of Brexpiprazole. The % recovery was found to be 80%=100.16, 100%=99.91%, 120%=98.86%. The overall recovery is 99.64% while %RSD for overall recovery is found to be 0.842. The relative standard deviation values for interday and intraday precision was found to be less than 2% i.e. 0.434 and 0.549 respectively. The LOD AND LOQ values were found to be LOD = 0.96 µg/ml, LOQ=2.91 µg/ml respectively. Brexpiprazole was subjected to stress conditions (acidic, alkaline, oxidation and thermal degradation) and validated as per ICH guidelines. The validated method can be applied to perform long-term and accelerated stability studies of Brexpiprazole formulations.

Keywords: Brexpiprazole: Reversed-phase HPLC; Stability-indicating; Validation

INTRODUCTION

Brexpiprazole functions as a serotonergic, noradrenergic, and dopaminergic agent. Chemically known as 7-{4-[4-(1-Benzothiophen-4-yl) piperazin-1-yl] butoxy} quinoline-2(iH)-one, it is a tiny molecule with the molecular formula $C_{25}H_{27}N_3O_2S$ and molecular weight 433.57 g/mol. Brexpiprazole has a melting point of 183°C (decomposition), is non-hygroscopic, and crystallizes into a white to off-white powder. With a pKa of 7.8, this medication is classified as weakly basic and is insoluble in water. Brexpiprazole (Figure 1) is used as an adjuvant treatment for Major Depressive Disorder (MDD) and as a treatment for schizophrenia. [1]

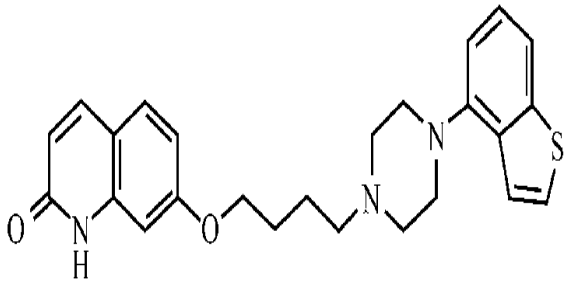

Figure No 1: Structure of Brexpiprazole

Based on a review of the literature, Brexpiprazole was identified using HPLC and UV-Visible spectroscopy. The authors of this work have suggested straightforward, proven spectrophotometric techniques for figuring out how much brexpiprazole is in prescription dose forms. As of right now, the authors have created a stability-indicating RP-HPLC method to measure brexpiprazole. [2]

MATERIALS AND METHODS

Chemicals and Reagents:

HPLC grade Methanol (Merk), Acetonitrile (Merk), Analytical grade Ortho-phosphoric acid (Thermofisher Scientific), Trifluro acetic acid (Thermofisher Scientific) was used. Brexpiprazole was obtained from Vidisha Analytical Lab.Siddhi lab provided the HPLC grade water.

Instrumentation and Software:

An Agilent HPLC system with 1260 InfinityII model and for data collection and processing ,the chromatograms were registered using Openlab EZ chrome on Windows based computer system .Brexpiprazole concentrations were determined using Kromasil C18 column. [3]

Selection of solvent:

Methanol was selected as the solvent for dissolving Brexpiprazole.

Preparation of standard solutions for UV scans:

In order to prepare stock solution, weighed accurately 10 mg Brexpiprazole and transferred into 20 ml volumetric flask, added 15 ml of methanol and sonicated to dissolve the standard completely and diluted up to the mark with methanol (500 PPM). Further diluted 0.8 ml to 20 ml with methanol. (20 PPM)

Selection of analytical wavelength:

Methanol as a blank and Brexpiprazole standard solution (20 PPM) was scanned from 400 nm to 200 nm. Absorption maxima were determined for drug. Brexpiprazole showed maximum absorbance at 216 nm shown in results.

Preparation of standard stock solution for Chromatographic development:

Brexpiprazole Standard stock solution was prepared by dissolving 10 mg Brexpiprazole into a 20 ml clean and dried volumetric flask added about 15 ml of methanol to dissolve it completely and made volume up to the mark with methanol (500 PPM). Further diluted 2 ml of stock solution to 10 ml with mobile phase (100 PPM). It was prepared in mobile phase of each trial and injected in development trials.

Selection of analytical wavelength for HPLC method development:

Analytical wavelength for the examination was selected from the wavelength of maximum absorption from the spectrophotometric analysis and it was 216 nm. [4, 5]

METHOD VALIDATION

The method was validated for system suitability, linearity and limit of quantitation (LOQ) and limit of detection (LOD), Precision, accuracy, selectivity and robustness.

Linearity:

Preparation of linearity solution 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 the sample. 5 levels of Linearity were performed from 80% to 120% of working concentration [6]. Linearity Brexpiprazole stock solution: Weighed 25 mg of Brexpiprazole and dissolved in 25 ml with methanol. (1000 ppm)

Precision and accuracy:

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 tablet test sample Precision of an analytical method is the degree of agreement among individual test results when the procedure is applied repeatedly to multiple samplings of a homogenous sample. Precision of an analytical method is usually expressed as standard deviation or relative standard deviation. Precision was performed on Test sample. % Assay value for each sample (Individual sample) and mean assay value for precision (6 samples), mean assay value intermediate precision (6 samples), mean assay value for precision plus intermediate precision sample (12 samples): 90- 110% % RSD: % RSD for precision study samples (6 samples), Intermediate precision study samples (6 samples) and precision plus intermediate precision sample (12 samples)

The accuracy of an analytical method is the closeness of test results obtained by that method to the true value. The accuracy of an analytical method is determined by applying the method to analyzed samples to which known amounts of analyte have been added [7, 8].

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 Determination. Standard solution was injected under different chromatographic conditions. a) Changes in flow rate by ±10%. (±0.1ml/min), b) Change in column oven temperature. (± 2°C), c) Change in wavelength (± 3 nm) Following changes made under Robustness ,Change in Wavelength , Change in flow rate ,Change in column oven temperature Chromatography (System suitability) acceptance criteria should not get failed [9].

Assay of marketed Formulations (Tablets)

Rexulti 1 mg test sample is available in market. But it was not available in Indian market. Hence physical lab mixture prepared at lab level by using following formula Tablet Preparation: Physical lab mixture of Tablet prepared at lab level by formula, 120 mg as average weight considered for preparing tablet mixture.

Sr. No.	Ingredients	Role	Qty (mg)
1	Brexpiprazole API	Active ingredient	1 mg
2	Placebo	NA	119 mg
Total			120 mg

Table No 1: Physical lab mixture of Tablet prepared at lab level by following formula

Sr. No.	Ingredients	Role	Qty (mg)
1	Lactose	Filler	80
2	Starch	Binder	5
3	Magnesium stearate	Lubricant	5
4	Talc	Glidant	5
5	crospovidone	Disintegrants	5
Total			100 mg

Total 10 gm of placebo prepared:

Table No 2: Placebo prepared at lab level by using formula

Force degradation studies:

Forced degradation studies have been performed for stability indicating properties and to evaluate degradation of drug product [10, 11].

For sample preparation, weighed accurately 20 mg of Brexpiprazole and transferred it in a clean and dried 20 ml of volumetric flask, added 12 ml of Methanol, sonicated it for 15 minutes with intermittent shaking. Made the volume up to the mark with Methanol. Filter the solution through 0.45 µ PVDF syringe filter discarding 3-5 ml of filtrate. Further dilute 1 ml of filtrate to 10 ml with mobile phase. (Exact wt of Brexpiprazole API is 20.2 mg).

Placed sufficient amount of Brexpiprazole API in petri dish and kept in hot air oven at 105º C for 48 hrs. After 48 hours sample was taken out and kept in desiccator to reach at room temperature for thermal degradation studies.Similarly, acid degradation, alkaline degradation and peroxide degradation studies performed [12].

RESULTS AND DISCUSSION

Using Methanol: 0.1%TFA (40:60%, v/v) as mobile phase with a flow rate of 1ml/min. Detection was carried at 216nm. Linearity was observed over the concentration range of 80-120 µg/ml. (R2=0.99983) with regression equation Y=mx+c, Y=1179203.12x+7782378.40. From the Accuracy study was performed % recovery of Brexpiprazole. The % recovery was found to be 80%=100.16, 100%=99.91%, 120%=98.86%. The overall recovery is 99.64% while %RSD for overall recovery is found to be 0.842. The relative standard deviation values for interday and intraday precision was found to be less than 2% i.e. 0.434 and 0.549 respectively. The LOD AND LOQ values were found to be LOD = 0.96 µg/ml, LOQ=2.91 µg/ml respectively. Brexpiprazole was subjected to stress conditions (acidic, alkaline, oxidation and thermal degradation) and validated as per ICH guidelines. The validated method can be applied to perform long-term and accelerated stability studies of Brexpiprazole formulations.

Linearity:

From the calibration curve it was concluded that the Brexpiprazole shows linear response in the range of 80.0-120.0 µg/ml. The Regression value was found well within the limit.

Level	Conc (µg/mL)	Area	Mean	% RSD
80%	80.00	101900847	101910525	0.050
		101865315		
		101965413		
90%	90.00	113819208	113864980	0.062
		113946251		
		113829481		
100%	100.00	126287501	126302423	0.019
		126329634		
		126290135		
110%	110.00	137231807	137264706	0.027
		137305421		
		137256891		
120%	120.00	149157183	149170818	0.073
		149068749		
		149286521		

Table No 3: Linearity Data for Brexpiprazole

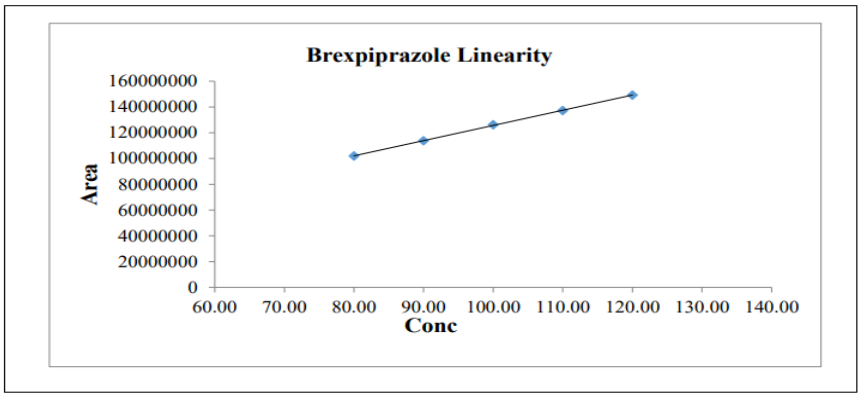

Figure No 2: Calibration curve of Brexpiprazole

Precision and Accuracy:

Precision: % Assay value for each sample (Individual sample) and mean assay value for precision (6 samples), mean assay value intermediate precision (6 samples), and mean assay value for precision plus intermediate precision sample (12 samples): 90- 110% % RSD for precision study samples (6 samples), Intermediate precision study samples (6 samples) and precision plus intermediate precision sample (12 samples): NMT 2.0 here,% Assay and % RSD was found well within acceptance limit and hence method is precise (Reproducible) Accuracy: % Recovery for each level and overall recovery: 98.0 to 102.0% ; % RSD for each level and overall recovery is NMT 2.0 Data interpretation: Recovery of analytical procedure was found well within acceptance criteria at all 3 levels. % Recovery not get hampered by changed in analyte concentration.

	Sample	Test Sample (mg)	Area	% Assay
Repeatability	Sample 1	480.2	123485639	97.69
	Sample 2	480.6	125686934	99.35
	Sample 3	479.8	125029864	99.00
	Sample 4	480.3	124855967	98.76
	Sample 5	480.5	124836051	98.70
	Sample 6	479.6	125645639	99.53
	Mean			98.84
	STD DEV			0.6497
	% RSD			0.657
Intermediate precision (Inter-Day)	Sample 1	480.9	125026413	98.77
	Sample 2	479.2	125694832	99.65
	Sample 3	480.6	124796128	98.65
	Sample 4	480.7	125596826	99.26
	Sample 5	479.5	124663521	98.77
	Sample 6	479.8	125685423	99.52
	Mean			99.10
	STD DEV			0.4301
	% RSD			0.434
Repeatability Plus Inter-day	Mean			98.971
	STD DEV			0.5432
	% RSD			0.549

Table No 4: Result of Intra- day and Inter- Day Precision for Brexpiprazole test sample assay

Level (%)	Area	Recovered conc (µg/mL)	Added conc (µg/mL)	% Recovery	Mean Recovery	% RSD
80	101250631	80.14	80.00	100.18	100.16	0.6642
	101896527	80.65	80.00	100.81		
	100542931	79.58	80.00	99.48		
100	126350429	100.00	100.00	100.00	99.91	0.9491
	127369851	100.81	100.00	100.81		
	124978862	98.92	100.00	98.92		
120	149623973	118.42	120.00	98.68	98.86	0.2260
	149785426	118.55	120.00	98.79		
	150263989	118.93	120.00	99.11		

Table No 5: Result and statistical data of Accuracy of Brexpiprazole

Robustness:
And from the above results, it was concluded that the system suitability test result was found well within the limits and analytical method was robust

Change in Parameter	R.T.	Standard area	Asymmetry	Theoretical plates
Wavelength by +3 NM (219 NM)	3.54	151155692	1.19	12110
Wavelength by -3 NM (213 NM)	3.53	106686694	1.19	12515
Flow rate by +10% (1.1mL/min)	3.21	114531086	1.19	11653
Flow rate by -10% (0.9mL/min)	3.92	140301943	1.22	12606
Column oven temp by +2ºC (42 ºC)	2.53	126353961	1.19	12359
Column oven temp by -2ºC (38 ºC)	2.56	125973864	1.21	12072

Table No 6: Result of Robustness study

Assay of marketed formulations:
a) Physical lab mixture: Average weight of tablet = 120 mg

Sample	Area	% Assay	Mean Assay
Sample 1	125956004	99.67	99.71
Sample 2	126102536	99.74	

Table No 7: Assay results of Marketed Test samples

% Assay found should be in the range of 90-110%. And from the above results, it can be concluded that the assay result is within the limit for Marketed Test samples and sample can be used for validation

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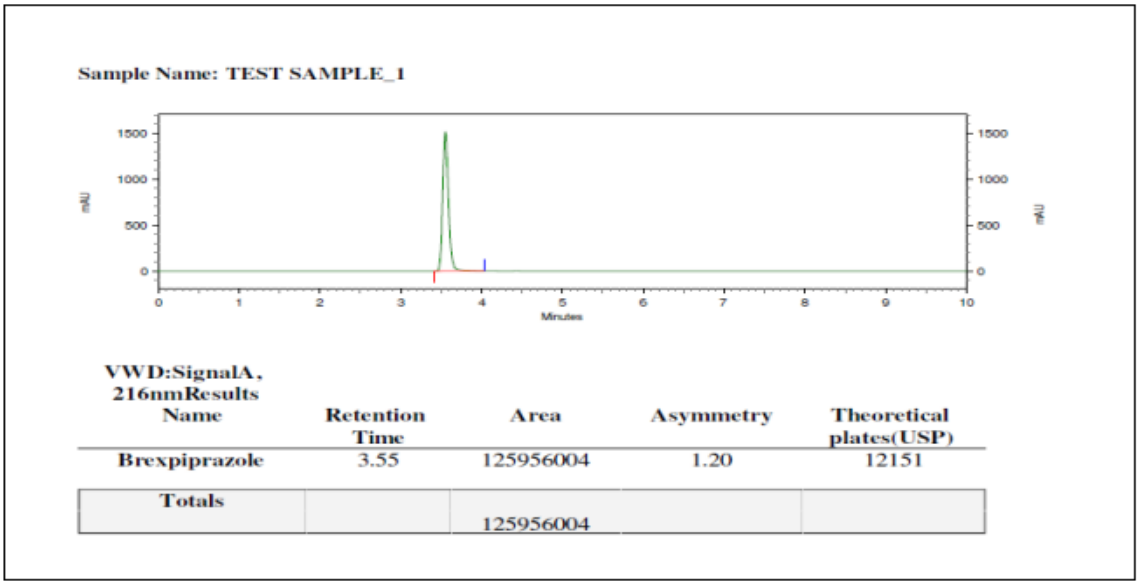
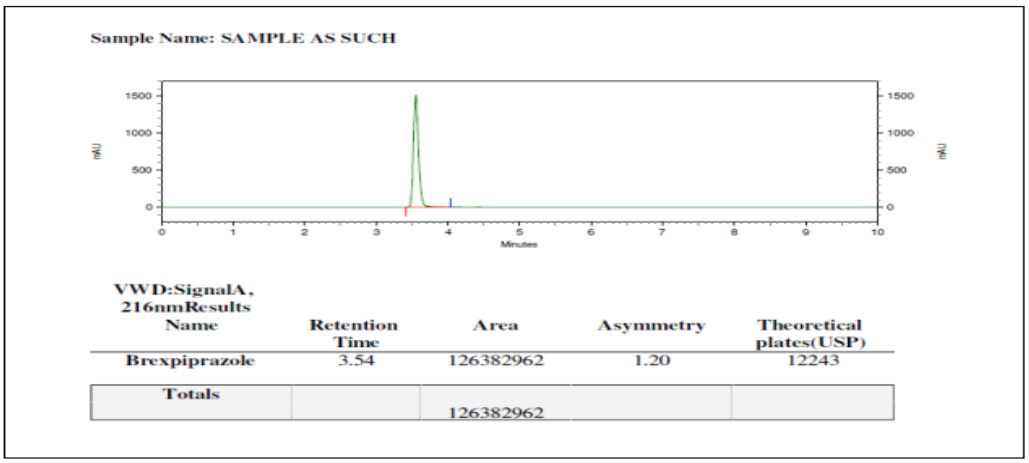


VWD:SignalA, 216nmResults

Name	Retention Time	Area	Asymmetry	Theoretical plates(USP)
Brexpiprazole	3.55	125956004	1.20	12151
Totals		125956004		

Figure No 3: Typical chromatogram of test sample 1 of physical lab mixture Acceptance criteria

Force Degradation Studies:



VWD:SignalA, 216nmResults

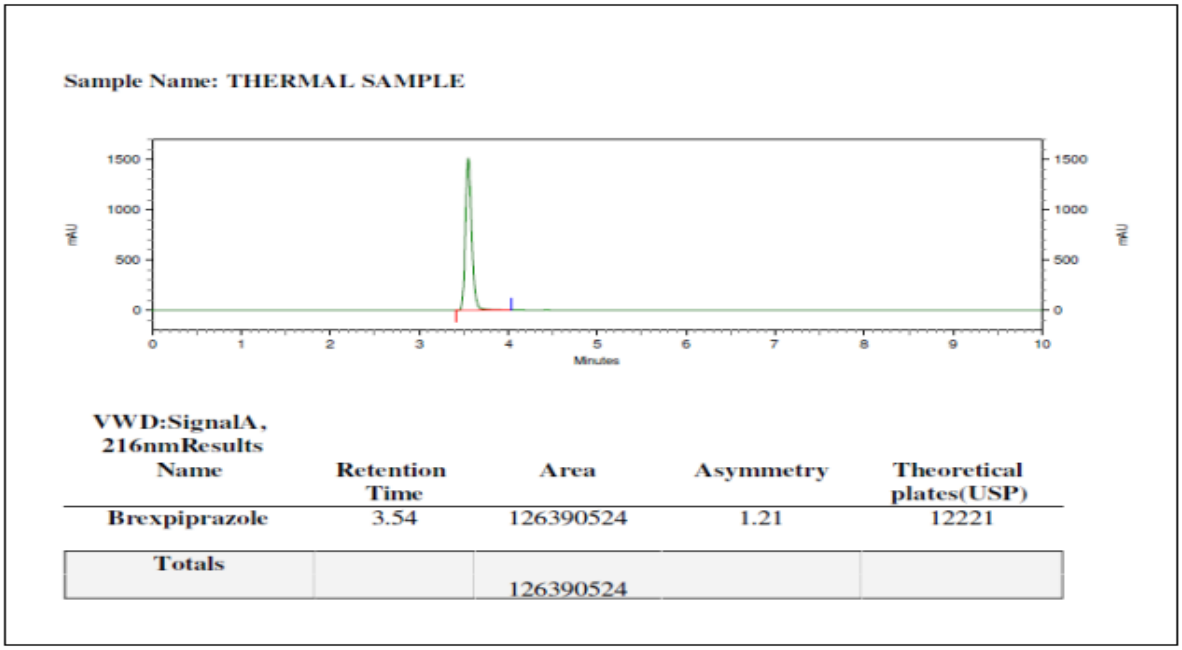
Name	Retention Time	Area	Asymmetry	Theoretical plates(USP)
Brexpiprazole	3.54	126382962	1.20	12243
Totals		126382962		

Figure No 4: Typical chromatogram of Control sample

Result:

Control sample (as such sample) assay found to be 99.04%

Thermal Degradation:



VWD:SignalA, 216nmResults

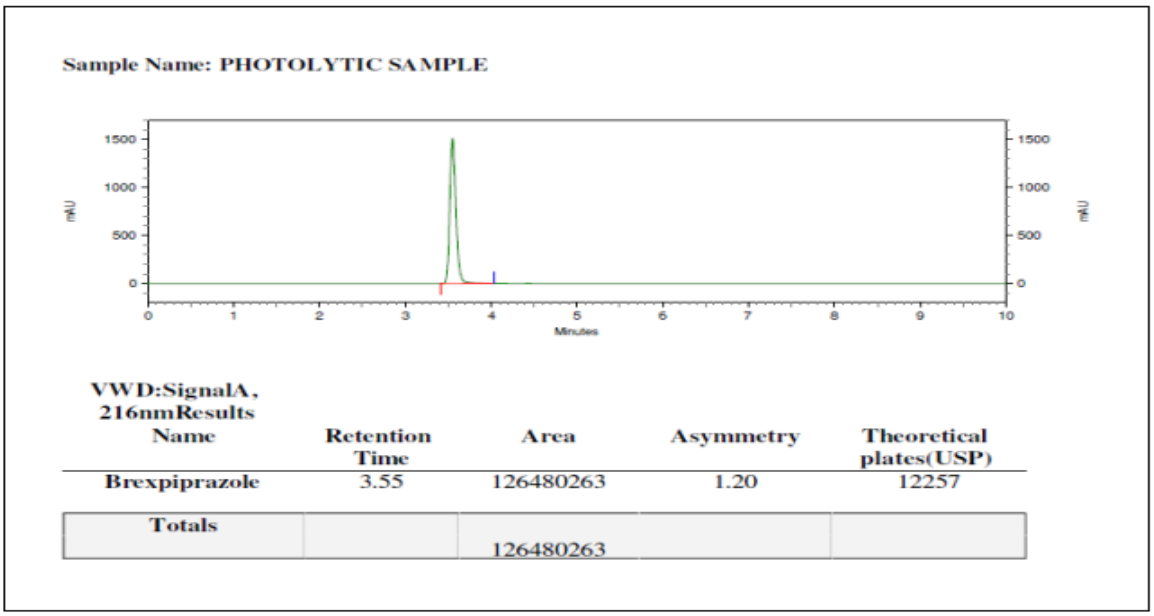
Name	Retention Time	Area	Asymmetry	Theoretical plates(USP)
Brexpiprazole	3.54	126390524	1.21	12221
Totals		126390524		

Figure No 5: Typical chromatogram of Thermal sample

Result:

After exposing the sample at 105°C for 48 hours, No degradation found.

Photolytic degradation:



VWD:SignalA, 216nmResults

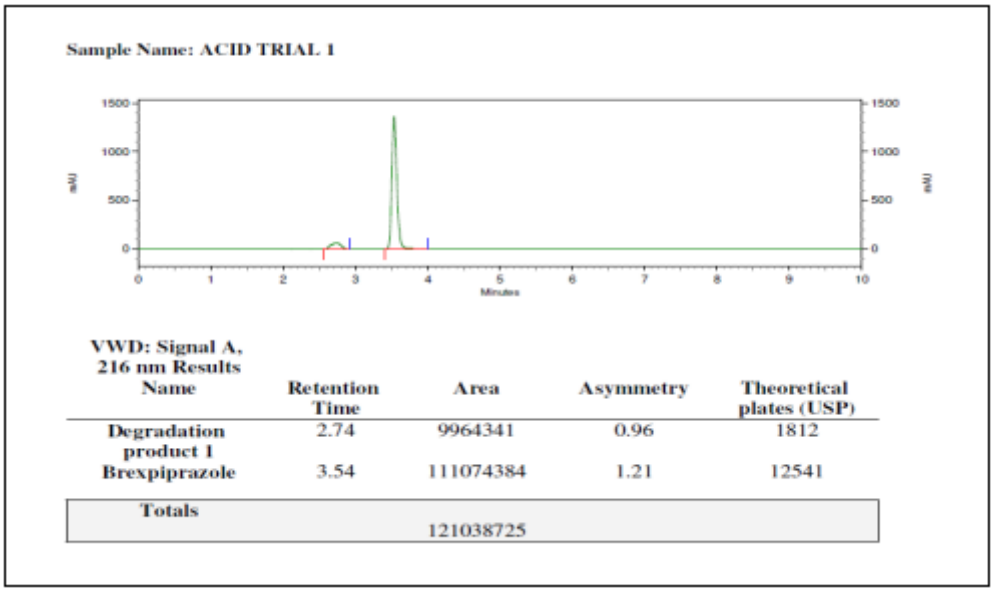
Name	Retention Time	Area	Asymmetry	Theoretical plates(USP)
Brexpiprazole	3.55	126480263	1.20	12257
Totals		126480263		

Figure No 6: Typical chromatogram of Photolytic sample

Result:

After exposing the sample at direct sun light for 72 hours, No degradation found

Acid Degradation:



VWD: Signal A, 216 nm Results

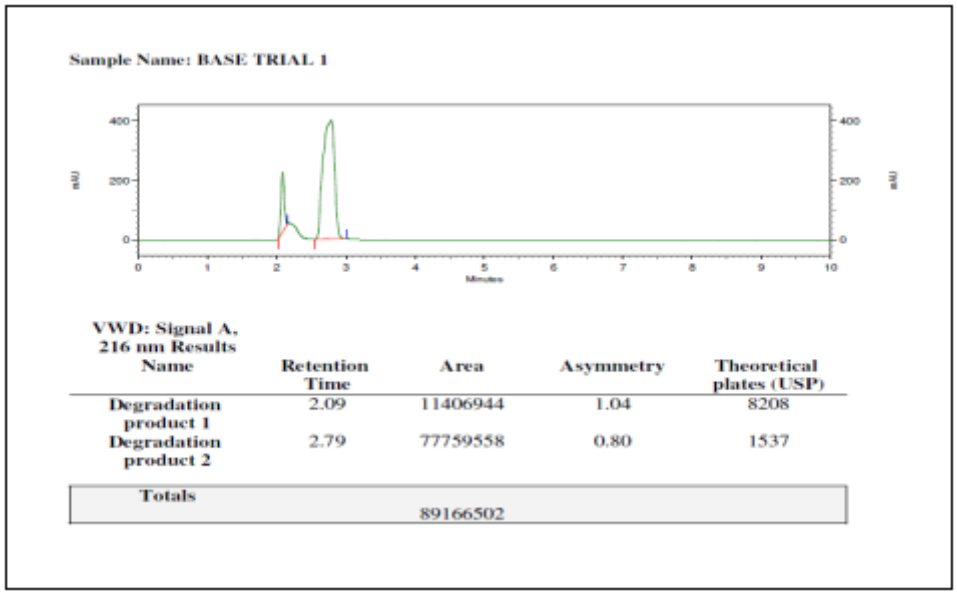
Name	Retention Time	Area	Asymmetry	Theoretical plates (USP)
Degradation product 1	2.74	9964341	0.96	1812
Brexpiprazole	3.54	111074384	1.21	12541
Totals		121038725		

Figure No .7: Typical chromatogram of sample exposed at Acid condition under trial 1

Result:

After exposing the sample for acidic condition under trial 1, 10.34% degradation found.

Base Degradation:



VWD: Signal A, 216 nm Results

Name	Retention Time	Area	Asymmetry	Theoretical plates (USP)
Degradation product 1	2.09	11406944	1.04	8208
Degradation product 2	2.79	77759558	0.80	1537
Totals		89166502		

Figure No 8: Typical chromatogram of sample exposed at Base condition under trial 1

Result:

After exposing the sample for base condition under trial 1, 100% degradation found.

Peroxide degradation:

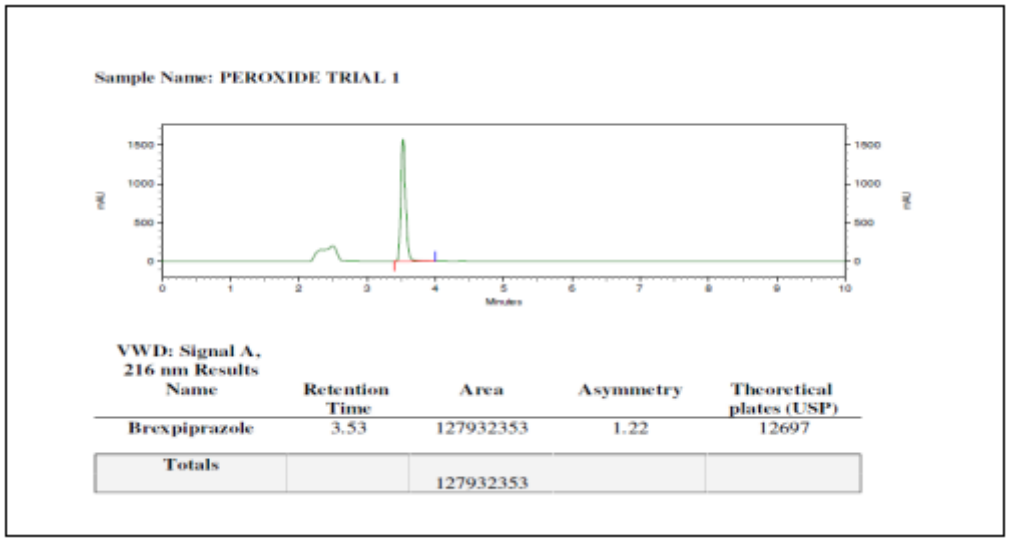


Figure No. 9: Typical chromatogram of sample exposed at Peroxide condition under trial 1

Result:

After exposing the sample for peroxide condition under trial 1, no degradation found.

CONCLUSION

The present work involved the development of simple, accurate, precise and suitable RP-HPLC method. Literature survey revealed that several methods have been reported for determination of Brexpiprazole in bulk drug or in pharmaceutical dosage forms. 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.

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