Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2018, 10(1):55-66

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Development and Validation of Stability Indicating Assay Method and Characterization of Degradation Product for Brexpiprazole Bulk by RP-HPLC

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ABSTRACT

The stability of the drug Brexpiprazole was studied under different stress conditions like hydrolysis (acid, alkaline and neutral), oxidation, photolysis and thermal degradation as recommended by International Conference on Harmonization (ICH) guidelines. Brexpiprazole contain amine group which makes it vulnerable to oxidative degradation to give N-oxides impurity. The mass balance was found close to 95-105%. These is the only one method available for the estimation of Brexpiprazole in presence of Process and Degradant Impurity and for the Stability study of Brexpiprazole as well as characterization of its Degradation Product(N-oxide Impurity).The chromatographic separation of the drug and its impurities was achieved in a Inertsil ODS 3V (150 cm × 4.6 mm × 5 µm) column employing a isocratic elution using 20 mM Potassium hydrogen phosphate buffer at pH 6.8 in combination of Acetonitrile(50:50 v/v) at a flow rate of 1.5 mL/min and detection performed at 220 nm by RP-HPLC. Validation has been performed according to ICH guidelines. The method was validated for system suitability, linearity, precision, limits of detection and quantitation, accuracy, specificity, robustness and ruggedness. The robustness study was done for small changes in flow rate, pH of Buffer and % of ACN in mobile phase composition. Linearity range for these method was found to be 0.96 -71 µg/mL. % Recovery value of this method was 95-105%. Brexpiprazole was chemically stable in hydrolysis (acid, alkaline and neutral), photolysis and thermal degradation but unstable in oxidative degradation condition and generate N-oxide Impurity which was isolated by Preparative chromatography. Identification and structural characterization of oxidative degradation product (N-oxide Impurity) was done by LC-MS, and ^{1}H and ^{13}C NMR studies.

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

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INTRODUCTION

Brexpiprazole (7-{4-[4-(1-benzothiophen-4-yl] butoxy}quinolin-2(1H)-one) is a novel serotonin dopamine activity modulator with partial agonist activity at serotonin -1A(5-HT_{1A}) and $D_{2/3}$ receptors, combined with potent antagonist effect on 5–HT_{2A}, α1B, and α2C adrenergic receptors. Brexpiprazole is used in the treatment of agitation associated with Alzheimer's disease**,** Attention- deficit/hyperactivity disorder, Post-traumatic stress disorder**,** treatment of bipolar disorder, Adjunctive Treatment of Major Depressive Disorder, and Schizophrenia. Brexpiprazole is more potent than the other class of antipsychotic drug as well as aripiprazole, therefore lower dose can be used. Brexpiprazole has a higher affinity for serotonin $5HT_{1A}$ receptors. Aripiprazole affects the same receptors but to a lesser extent. This may give brexpiprazole advantage over aripiprazole. Brexpiprazole has a lower side effect like akathisia and extra pyramidal symptoms than aripiprazole and other class of antipsychotic drug [1]. There is no official as well as reported method available for the quantitative and qualitative estimation of

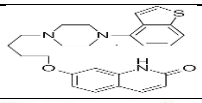
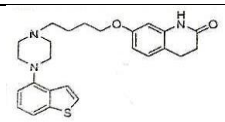
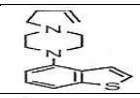
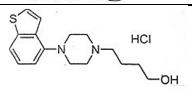
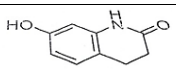
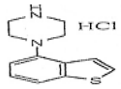
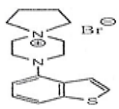
Brexpiprazole. The amount of active pharmaceutical substance is more important for activity and potency of drug. Stability indicating assay method is a quantitative analytical method based on the structural and chemical properties of each active ingredient of a drug product and that will distinguish each active ingredient from its degradation products so that the active ingredient content can be accurately measured. The chemical stability of Brexpiprazole with respect to stress conditions is presently unknown. An exhaustive study on the stability of Brexpiprazole is demanding as the current International Conference on Harmonisation (ICH) guidelines require that stability analysis should be done by using stability indicating assay methods (SIAM) [2]. After stress testing on the drug under various conditions, including hydrolysis (at various pH), oxidation, photolysis and thermal degradation and accelerated stress conditions, SIAM should be developed and validated. Forced degradation is a degradation of new drug substance at condition more severe than accelerated conditions. It is required to demonstrate specificity of stability indicating methods and also provides an insight into degradation pathways and degradation product of the drug substance and helps in elucidation of the structure of the degradation product [3]. The structural characterization of the degradation product was formed in oxidative degradation determined using Fourier transform infrared spectroscopy (FTIR), liquid chromatography–mass spectrometry (LC-MS), proton-nuclear magnetic resonance (^{1}H NMR), carbon-nuclear magnetic resonance (^{13}C NMR). 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 assay of Brexpiprazole.

EXPERIMENTAL SECTION

Reagents and Chemicals

The drug Brexpiprazole and its process related impurities (IMP) were gifted by Zydus Cadila Healthcare Ltd. (Vadodara, India). Buffer salts were purchased from Merck, 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 Fischer Scientific, India. Mobile phase was vacuum filtered through a 0.22 mm poly-tetrafluoroethylene (PTFE) filter membrane and degassed using a sonicator to remove the dissolved gases. Chemical structures for the drug, process related impurities are schematically represented in Table 1 Chemical names for the drug, and impurities are as follows:

Table 1: Chemical name and structure for brexpiprazole and its process impurities

Fig.	Name	Structure	Chemical Name
(A)	Drug:		7-(4-(4-(benzo[b]thiophen-4- yl)piperazin-1-yl)butoxy)quinolin- 2(1H)-one,
(B)	Dihydro Imp:		7-(4-(4-(benzo[b]thiophen-4- yl)piperazin-1-yl)butoxy)-3,4- dihydroquinolin-2(1H)-one,
(C)	Butene Imp:		1-(benzo[b]thiophen-4-yl)-4-(butoxy-3-en-1-yl)piperazine
(D)	Hydroxy Imp.:		4-(4-(benzo[b]thiophen-4- yl)piperazin-1-yl)butan-1-ol hydrochloride,
(E)	KSM-I Imp:		7-hydroxyquinolin-2(1H)-one
(F)	KSM-II Imp:		1-(benzo[b] thiophen-4- yl)piperazine hydrochloride
(G)	Spirocyclic Imp:		8-(benzo[b]thiophen-4-yl)-5,8- diazaspiro[4.5]decan-5-ium bromide

Instrumentation and Chromatographic Conditions

High performance liquid chromatography (HPLC):

Dionex Ultimate 3000 equipped with an Autosampler, quaternary pump a thermostat column compartment and Photo Diode Array detector (Dionex Technologies) was used for method development, force degradation and validation. The data were evaluated by Chromeleon software 6.8. Solutions were degassed by Ultra Sonication (Power sonic 420, Labtech, Korea). The pH of the mobile phase was adjusted as required by a pH meter (Thermo Orion model 420a, USA). The analyses were carried out on Inertsil ODS 3 V (150 cm × 4.6 mm × 5 µm). The measurements were carried out at a wavelength of 220 nm for the analytes. The mobile phase was prepared by combination of 20 mM potassium di hydrogen phosphate in 1000 ml Mili Q water (pH 6.2 with diluted (10% w/v) Potassium Hydroxide solution) and Acetonitrile taken in an equal ratio (50:50% v/v). Flow rate used was 1.5 mL/min.

Sample Preparation for Analysis

Impurity stock solution preparation:

Weigh accurately and Transfer about 7.5 mg of each KSM-I, KSM-II, Spirocyclic compound, Hydroxy impurity, Butene Impurity, and Dihydro impurity standards into a 50 ml volumetric flask. Dilute up to volume with diluent, sonicate for 5 min to dissolve and mix. Transfer 10 ml of this solution in to a 100 ml volumetric flask. Dilute to volume with Diluent and Mix. (15 ppm).

System suitability solution preparation:

Weigh accurately and transfer about 50.0 mg of Brexpiprazole API standard in to a 100 ml of volumetric flask. Add 80 ml of diluent, sonicate to dissolve, and further dilute 5.0 ml of above solution in to 50 ml volumetric flask, make up volume to mark with diluent and mixed well having concentration of 50.0 µg/ml of Brexpiprazpole API.

Standard solution:

Weigh accurately and transfer about 50 mg of Brexpiprazole Standard into 100 ml volumetric flask, add 70 ml diluent, sonicate to dissolve and make up the volume with diluent. Further dilute 5 ml of above solution in to 50 ml volumetric flask add 70 ml diluent, sonicate to dissolve make up the volume with diluent (50 ppm Brexpiprazole).

Method Validation

Developed method was validated as per ICH guideline Q2 (R1) [4].

Specificity:

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. These studies were performed in two parts, Specificity part A and Specificity part B.

In specificity part A: Specific specificity generally refers to a method that produces a response for a single analyte only. Spiked sample of Brexpiprazole API was used and observed the separation and resolution between Brexpiprazole API and all process impurities.

In specificity part-B: Forced degradation study was carried out on Brexpiprazole API under various stress conditions like acidic hydrolysis, basic hydrolysis, oxidative degradation, thermal degradation, photolytic degradation.

Acid hydrolysis: Forced degradation studies in acidic media were performed by adding 10 mL of 2 N HCl in 50 mg Brexpiprazole bulk solid sample in 100 ml volumetric flask. The mixture was then keep it at Room Temperature for 24 HR, after this it was neutralized with 2 N NaOH. The resultant mixture was diluted up to 100 ml with diluent. Further dilution was done by taking 5 ml from above mixture and make up the volume up to 50 ml in 50 ml volumetric flask with diluent.

Base hydrolysis: Forced degradation studies in basic media were performed by adding 10 mL of 2 N NaOH in 50 mg Brexpiprazole bulk solid sample in 100 ml volumetric flask. The mixture was then kept at Room Temperature for 24 hr, after this it was neutralized with 2 N HCl. The resultant mixture was diluted up to 100 ml with diluent. Further dilution was done by taking 5 ml from above mixture and make up the volume up to 50 ml in 50 ml volumetric flask with diluent.

Oxidative degradation: Forced degradation studies in oxidative media were performed by adding 10 mL of 10% H_2O_2 in 50 mg Brexpiprazole bulk solid sample in 100 ml volumetric flask. The mixture was then kept it at Room

Temperature for 24 hr then, the resultant mixture was diluted up to 100 ml with diluent. Further dilution was done by taking 5 ml from above mixture and make up the volume up to 50 ml in 50 ml volumetric flask with diluent.

Thermal degradation: In thermal degradation was done by put the Brexpiprazole Bulk solid sample at 105ºC for 7 days in the oven, after which it was cooled at room temperature, transferred 50 mg of above treated sample in to 100 mL volumetric flask and diluted it up to 100 mL with diluent. Further dilution was done by taking 5ml from above mixture and make up the volume up to 50ml in 50ml volumetric flask with Diluent.

Photolytic degradation: In photolytic degradation was done by put the Brexpiprazole Bulk solid sample for 1 cycle in the photolytic chamber (U.V/Not less than 200 watt hour's square meters^{-1}, VIS/ not less than 1.2 million Lux), transferred 50 mg of above treated sample into 100 mL volumetric flask and diluted it up to 100 ml with diluent. Further dilution was done by taking 5ml from above mixture and make up the volume up to 50 ml in 50 ml volumetric flask with Diluent. Peak purity was carried out for Brexpiprazole Bulk peak by using PDA detector in stress condition. The mass balance studies were calculated for each type of stress study.

Limit of detection (LOD) and Limit of Quantification (LOQ):
The LOD is defined as the lowest amount of analyte in a sample which can be detected, but not necessarily quantify as an exact value and the LOQ was defined as the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy [4]. 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.

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 [4]. Linearity test solution for the assay method was prepared by diluting the stock solution to the required concentrations. Six different concentration levels of the solutions were prepared in this range from 20% to 140% of the assay analyte concentration (10.25, 30.74, 40.99, 51.23, 61.19 and 71.73 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 of an analytical procedure expresses 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 assay method was evaluated in triplicates at three different concentration levels, 80%, 100%, and 120% i.e. 40, 50, 60 µg/mL in the bulk 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 95–105%.

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 (Interday-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.

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.

i) Change in % organic phase of mobile phase by ± 5.0%
ii) Change in pH of buffer of water by ± 10% of set pH
iii) Change in the flow rate of the mobile phase by ± 10% of the original flow rate.

Ruggedness:
Standard solution of the drug substance should be analyzed while systematically varying in operating condition. The measured value of the drug substance level and effect on precision, retention, and separation factor should be noted. The condition examined should include the following:

a) Different operator in same Lab.
b) Changing to a new column (same type and manufacture).

Solution stability:
The solution stability was also carried out to check the stability of both the solutions (standard and sample) till 48 h when stored at ambient temperature in laboratory. It was performed by doing the analysis of both the solutions at 0, 12, 24, and at 48 hr intervals and comparing the results with the freshly prepared standard solutions analyzed simultaneously.

Characterization of Oxidative Degradation Product of Brexpiprazole
The degradation product obtained in Oxidative stress degradation studies (designated as N-Oxide) was targeted for its structural characterization. The Oxidative degradation product was analyzed on LC-MS instrument. The mass spectrometer was run in positive ionization mode and negative ionization mode with turbo ion spray interface and mass to charge (*m*/*z*) ratio was recorded. Initially the parent ion (m+1) and (m-1) values for N-Oxide was obtained using LC-MS. Further structure elucidation of oxidative impurity was done by ^{1}H NMR and ^{13}C NMR studies.

RESULT AND DISCUSSION

Optimization of Chromatographic Condition
In the initial stage of method development of the drug involved selection of Buffer and screening of column, which have given good resolution with appropriate system suitability peak. Brexpiprazole is an ion forming substance and it is very sensitive to pH, therefore it was necessary to use buffer as mobile phase to maintain its buffer capacity and better separation. Preliminary studies were done and based on that select mobile phase, in the preliminary studies the use of methanol along with the buffer. During forced degradation studies, chromatogram of the stressed mixtures showed co-eluting peaks with longer retention time. The problem was resolved when acetonitrile was used alone with the buffer. The addition of acetonitrile along with buffer led to the resolution of the degradation products and the drug. Analytes (process related impurities, degradation products, and the drug) were found to be a mixture of acidic, basic and neutral components. Hence, a modification in pH altered the separation selectivity for ionized or unionized solutes. Several trials were made with the mobile phase by varying the pH of the buffer and also changing the buffer component.

- 20 mM Potassium hydrogen phosphate use in combination of Acetonitrile (50:50). The method had a total run time of 15 min using the Isocratic elution method. In this combination, buffer pH was checked with 3 and further trials were done with pH values of 5–7. Better resolution was obtained by adjusting the pH of the buffer to 6.8, which illustrates the sensitivity of the polar compounds and the non-polar compounds in a less acidic environment (Figure 1).
- Peak purity for analyte peak was found to be greater than 0.99.

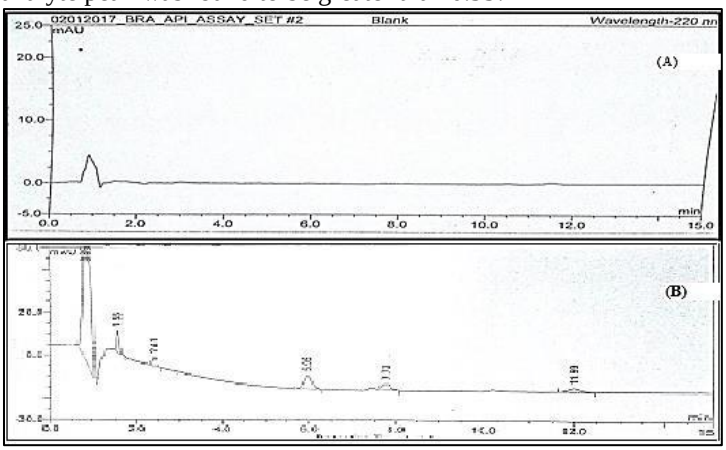


Figure 1: Typical chromatograms, (A) blank chromatogram, (B) sample spiked chromatogram

Method Validation

Isocratic elution method which was developed to separate the analyte from its related substance (process related impurities and degradation products) and quantification of analyte was checked for its efficacy and was validated by the following parameters.

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 five standard replicates (Table 2). The values are within the acceptable range indicating that the system is suitable for the intended analysis.

Table 2: System suitability parameters for brexpiprazole

Parameters	Observation[a]	RSD
Rt (min)	5.95	0.42
Peak area	2808.329	0.22
Theoretical plates	5369	1.01
Asymmetry	1.14	0.53

[a]Mean of five replicates

Specificity:

Specific specificity generally refers to a method that produces a response for accurately measuring a single analyte only and evaluated by measuring the response for compound of interest only and by purity of analyte peak. The peak of Brexpiprazole obtain at Rt of 5.94 min and well separated from process Impurity which were KSM-II, KSM-I, Hydroxy Impurity, Spirocyclic Impurity ,Butene Impurity , Dihydro Impurity obtain at Rt of 0.86 min, 0.95 min, 1.55 min, 2.41 min,7.79 min, 11.99 (Figure 1) and Oxidative Degradant Product peak obtain Rt of 2.0 min. (Figure 2). Upslope similarity, down-slope similarity and 3-point peak purity for analyte were found to be greater than 0.99. This confirms that the method has the ability to unambiguously determine the drug even in the presence of process related impurities and degradation products.

Limit of detection (LOD) and limit of quantification (LOQ):

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.

Linearity:

The linearity range was found to be 0.96 -71 µg/mL. The correlation coefficient was found to be >0.999 (Figure 3). The result showed that an excellent correlation existed between the peak area and concentration of the analyte (Table 3).

Table 3: Linearity data of calibration curve for brexpiprazole

Linearity Level	Conc. of Brexpirazole (µg/mL)	Response observed (AUC) a
LOQ	0.9688	9.918
20%	10.2478	567.669
60%	30.7433	1693.75
80%	40.991	2259.388
100%	51.2388	2791.325
120%	61.4866	3344.081
140%	71.7343	3900.266

Accuracy:

Accuracy was determined by spiking the analyte (Brexpiprazole API) at 80%, 100%, and 120% level of their specified limits in dosage forms. The % Recoveries of analyte were found to be in the range of 95-105% (Table 4).

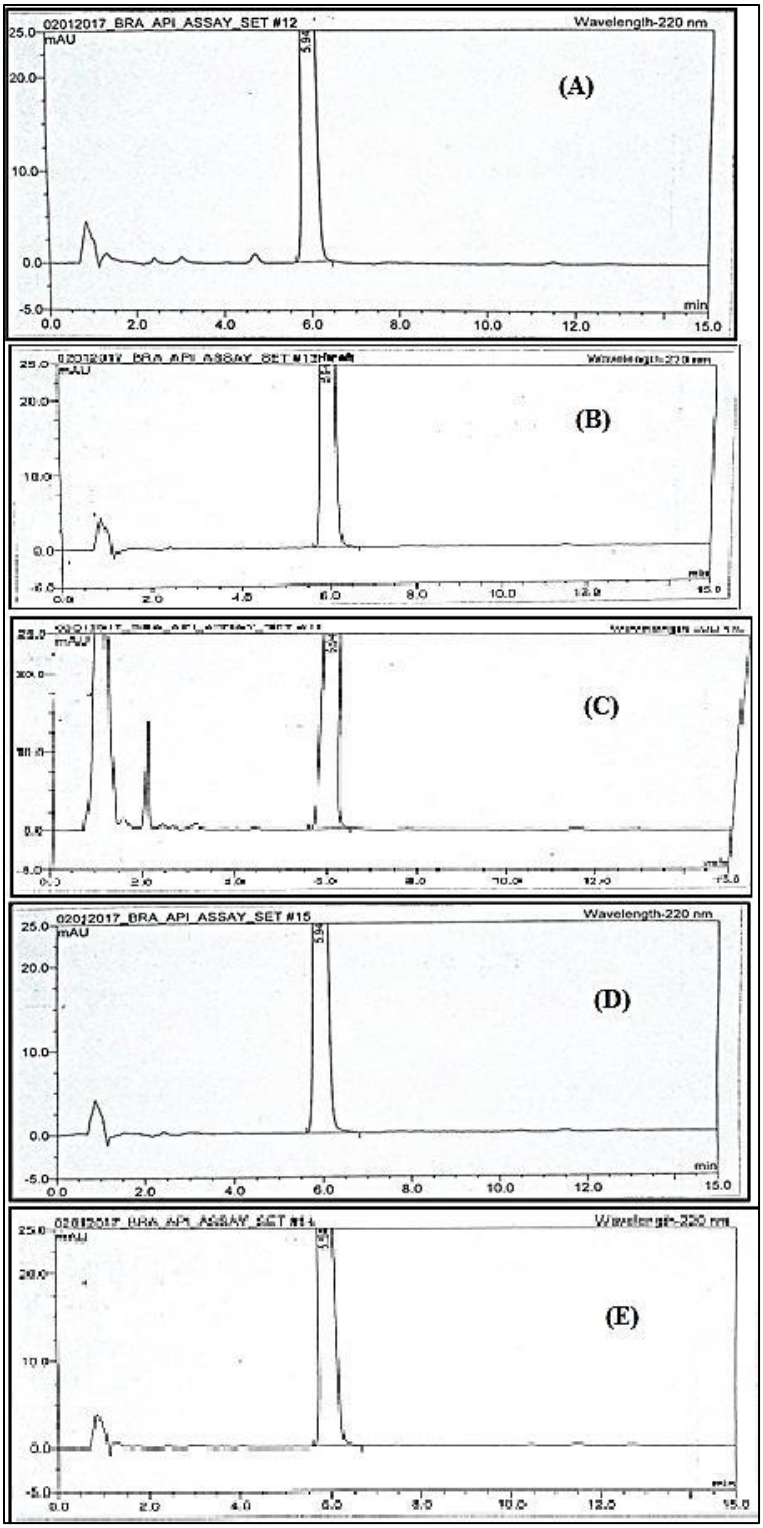


Figure 2: Forced degradation chromatograms, (A) sample in 2 N HCl room temperature for 24 hr, (B) sample in 2 N NaOH room temperature for 24 hr, (C) sample in 10% H_2O_2 room temperature for 24 hr, (D) sample in thermal degradation 7 day at 105°C, (E) sample in photolytic degradation

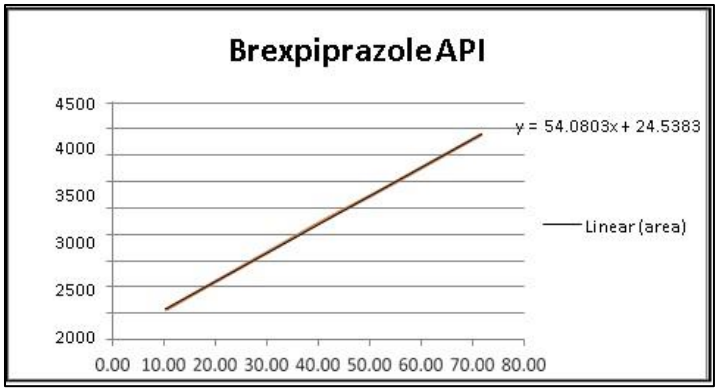


Figure 3: Calibration curve of developed RP-HPLC method for brexpiprazole

Table 4: Accuracy data for brexpiprazole

Level	Weight in mg	Area	%Recovery	Mean Recovery	SD	%RSD
I (80% Wrt to target concentration)	39.79	2094.053	96.073	95.85	0.5586	0.58
	40.4	2130.203	96.256			
	40.05	2088.802	95.21			
II (100% Wrt to target concentration)	51.49	2731.804	96.801	96.72	0.4019	0.42
	50.42	2682.514	97.071			
	50.08	2642.72	96.28			

Precision:

The results (Table 5) of all the precision studies (Repeatability, intraday, interday and different analysts), shows that the mean assay values and RSD values are within the acceptance criteria which proves the good precision of developed method.

Table 5: Precision data for brexpiprazole

Precision study	Observation	
	Mean Assaya	RSD
Repeatability a	96.61	0.58
Intraday b	96.72	0.89
Interday c	95.68	0.9
Different analyst d	97.14	0.85

[a]n= 6 , [b]Mean value of initial, 3 h, 6 h interval observations; [c]Mean value of day I and day II observations; [d]Mean value of analyst I and analyst II observations

Robustness

The results of robustness studies are summarized in Table 6. In any condition RSD value of sample is not deviating more than 2.0% indicating that the method is robust in nature.

Table 6: Robustness data for brexpiprazole

Robustness Condition	Rt	T	A	RSDa
- 5% Acetonitrile (Buffer: Acetonitrile; 55:45 v/v)	6.25	12009	1.11	0.16
+ 5% Acetonitrile (Buffer: Acetonitrile; 45:55 v/v)	5.67	10936	1.19	0.12
- 10% Changed pH of Buffer	5.27	10398	1.07	0.22
+ 10% Changed pH of Buffer	6.52	10999	1.06	0.11
- 10% Change in flow rate - 1.4 mL/min	6.27	11236	1.09	0.15
+ 10% Change in flow rate - 1.6 mL/min	5.78	11895	1.13	0.42

[a]From five values of standard area; T= Theoretical plates; A = Asymmetry

Ruggedness

The method should be rugged enough with respect to all critical parameters so as to allow routine laboratory use **(**Table 7).

Table 7: Ruggedness data for brexpiprazole

Parameter	Area	Mean	SD	RSD
Analyst change	2803.676	2805.093	2.0039	0.07
	2806.51			
Column lot change	2761.784	2763.885	2.972	0.11
	2765.987			

Solution stability:

From the results of the solution stability study (Table 8), it was found that both the solutions are stable over the specified period of analysis by the developed method at ambient temperature for 48 h.

Table 8: Solution stability study of brexpiprazole by SIAM

Interval	Observation			
	% Assay		% Difference	
	STD*	Sample*	STD	Sample
Initial	99.98	99.58	-	-
12 h	99.93	99.36	0.05	0.22
24 h	99.54	99.42	0.44	0.16
48 h	99.36	99.12	0.62	0.46

* Results are from duplicate injection of same solution

Stress Degradation Behavior of Brexpiprazole:

Brexpiprazole was found to be stable in stress degradation conditions like acid, alkali, neutral, thermal, photolytic, and accelerated stress studies as there was no much decrease in area of Brexpiprazole and no additional peaks was observed compared to their respective initial samples, But one degradation product peak (designated as N-Oxide) was observed in chromatogram of Peroxide treated Brexpiprazole (Figure 3) and the peak area was decreased by approximately 12.5% compared to initial sample. The separation of Oxidative degradation Impurity was done by Preparative Chromatography. The results of forced degradation studies, with approximate % degradation are given in Table 9. The mass balance was calculated, from the responses obtained Brexpiprazole and all the degradation products obtained after stress studies.

Table 9: Stress degradation studies for brexpiprazole

Degradation stage	Condition	%Known	Major Degradant	%	Mass
Acidic Degradation	2 N HCl Room Temperature for 24	3.2743	-	95.11	98.4
Basic Degradation	2 N NaOH Room Temperature for	2.8174	-	96.84	99.7
Oxidative Degradation	10% H2O2 Room Temperature for	3.3097	9.2433	90.08	102.6
Thermal Degradation	7 Day at105° C	3.4098	-	97.28	100.6 9
Photolytic Degradation	1 cycle (not less than1.2 million lux	3.2877	-	96.48	99.8

LC-MS Studies on Oxidative Degradation Product of Brexpiprazole

During the stress degradation studies of Brexpiprazole by developed and optimized RP-HPLC method, only one degradation product (N-Oxide) was obtained in oxidative stress condition which was further subjected to LC-MS study for characterization and structural elucidation. Figure 4 shows LC-MS spectra of N-Oxide Impurity with obtained m+1 peak at 450.05 m/z, and m-1 peak at 448.05 m/z which indicate proposed molecular weight of N-Oxide Impurity as 449.05 m/z (Table 10).

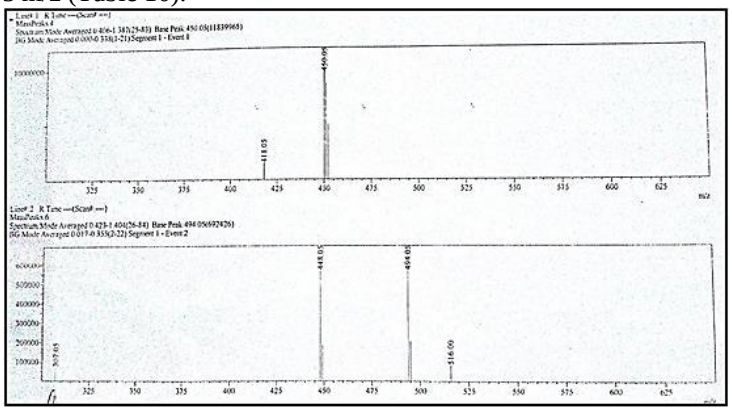


Figure 4: Representative LC-MS spectra of oxidative degradation product of brexpiprazole

Table 10: Molecular mass studies for oxidative degradation product of brexpiprazole

Compound Name	m+1 peak	m-1 peak	Molecular mass
N-Oxide Impurity	450.05	448.05	449.05

NMR Studies on Oxidative Degradation Product of Brexpiprazole

For characterization and structural elucidation ^{1}H NMR and ^{13}C NMR studies done on Brexpiprazole oxidative degradant product (N-Oxide Impurity) (Figures 5-7) (Tables 11 and 12).

^{1}H NMR study Oxidative Degradation Product of Brexpiprazole

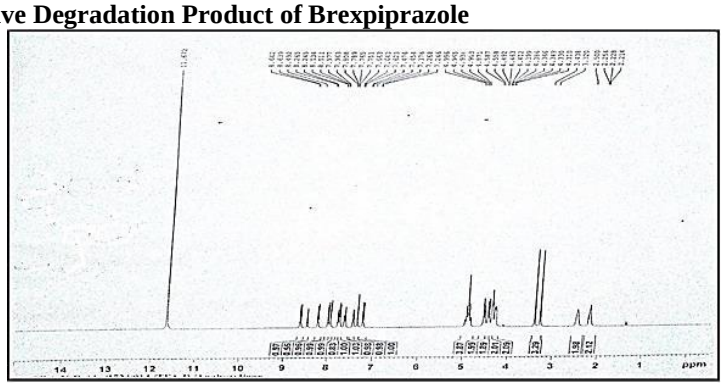


Figure 5: Representative ^{1}H NMR spectra of oxidative degradation product of brexpiprazole

Table 11: ^{1}H NMR interpretation of oxidative degradation product of brexpiprazole

1H NMR in DMSO d6 at 400.13 MHZ			
Chemical shift in ppm	**Multiplicity**	**Assignment of Group**	**Number of Proton**
11.59	S	-NH	1
8.7	d(J=9.6HZ)	4	1
8.2	d (J=5.6HZ)	23	1
8	d(J=8.0HZ)	22	1
7.9	d(J=9.6HZ)	3	1
7.6	t(J=8HZ)	21	1
7.5	d(J=5.6HZ)	24	1
7.2	d(J=7.6HZ)	5	1
5	M	8,20	2
4.5	d(J=9.6HZ)	6	1
4.4	t(J=6.4HZ)	11	2
4.3	t(J=6.8HZ)	14	2
3.5	S	16,17	4
3.3	S	15,18	4
2.43	M	13	2
2.12	M	12	2

^{13}C NMR Oxidative Degradation Product of Brexpiprazole

From the ^{13}C NMR Spectra interpretation it can be suggested that the molecule contain 25 carbon atom in the structural formula.

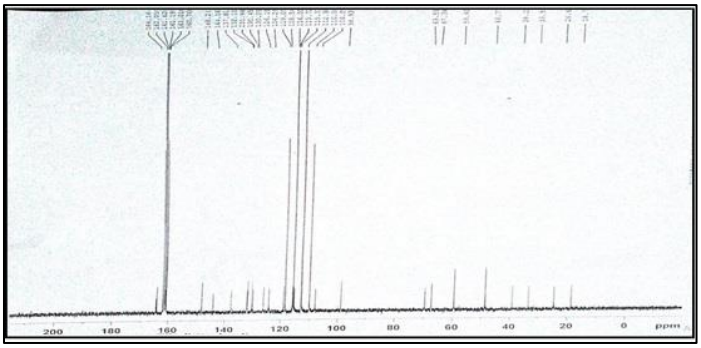


Figure 6: Representative ^{13}C NMR spectra of oxidative degradation product of brexpiprazole

Table 12: ^{13}C NMR interpretation of oxidative degradation product of brexpiprazole

^{13}C NMR in DMSO d6 at 100.61 MHZ		
Chemical shift in ppm	**Chemical shift in ppm**	**Chemical shift in ppm**
162.69	1	2
160.91	1	7
148.7	1	9,19,26
141.12	1	
140.85	1	
140.43	1	4
133.82	1	25
129.66	1	3,5,8,21,23,24
126.21	1	
125.52	1	
122.33	1	
118.92	1	
117.04	1	
113.72	1	10
112.44	1	6,20,22
111.28	1	
99.07	1	
68.07	1	11
57.81	1	14
53.41	2	16,17
52.17	2	15,18
27.01	1	12
23.16	1	13

Prediction of Structure of N-Oxide Impurity

Proposed molecular weight of Degradation product (N-Oxide Impurity) was 449.05 m/z and NMR studies indicate that nitrogen moiety of Brexpiprazole is retained in molecule of Degradant product. Degradation product has 16 Da more than Brexpiprazole molecular mass, proposed addition of one oxygen atom in Brexpiprazole moiety. The structure of Degradation product (N-Oxide Impurity) is proposed in Figure 7.

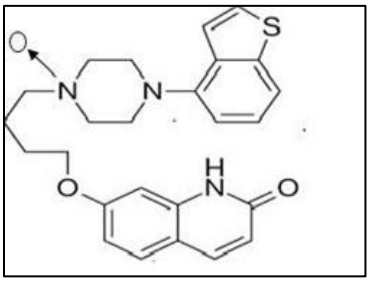


Figure 7: Proposed structure of oxidative degradation product (N-Oxide impurity)

CONCLUSION

The developed and validated stability indicating RP-HPLC assay method is specific, sensitive and robust. The method found to be advantageous and simple by using isocratic elution mode. The results from the stress degradation studies show that Brexpiprazole is susceptible to Oxidative stress condition and only one degradation product was found as N-oxide Impurity. The LC-MS, ^{1}H NMR and ^{13}C NMR studies were used for structural elucidation and characterization of N-oxide Impurity.

ACKNOWLEDGMENT

The authors are grateful to Zydus Cadila Healthcare Ltd., Dabhasa, Vadodara for providing the facilities to carry out this research work.

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**.