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Degradation Behaviour of Brexpiprazole: Isolation, Characterization and Structural Elucidation of New Degradants

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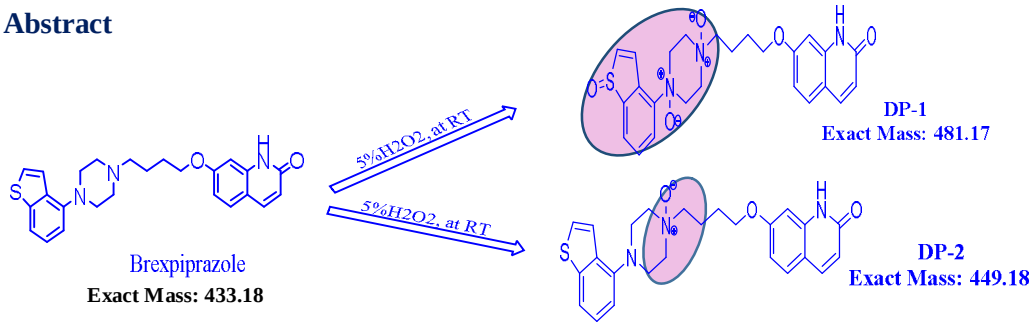
ABSTRACT

The aim of the present work is to study the stability of the Brexpiprazole (BREX) drug which is carried out under various stress conditions like acid, alkaline and oxidation according to International Conference on Harmonization (ICH) guidelines. BREX drug is stable under acidic and alkaline conditions where degradation is not observed, but when exposed to oxidation condition degradation occurs. Two degradation products are observed out of which DP-2 already reported in literature a DP-1is not reported anywhere in literature.LC-QTOF analysis is performed to separate the drug and its degradation impurities which were accomplished on C18 BEH UPLC column (50 mm X 2.1mm, 1.7 µm) using 0.05% Formic Acid in water and 0.05%Formic Acid in Acetonitrile as mobile phase. The flow rate is 0.6mL/min and detection is monitored at 215nm.The degradation product obtained is isolated by preparative HPLC. Characterization and structural elucidation of degradation product was studied by NMR, LCMS and HRMS.

Graphical Abstract



Keywords: Preparative HPLC, NMR, LCMS, HRMS, Brexpiprazole, Stability indicating method.

INTRODUCTION

Brexpiprazole (BREX) is a novel D2 dopamine and serotonin 1A partial agonist, called Serotonin Dopamine Activity Modulator (SDAM) and a potent antagonist of serotonin 2A receptors,

noradrenergic alpha 1B and 2C receptors. Partial agonists have both clogging properties and exhilarating properties at the receptor they bind to. The ratio of clogging activity to stimulating activity regulates a portion of its clinical effects. BREX has more blocking and less stimulating activity than its predecessor, aripiprazole, which may decrease its risk for agitation and restlessness [**1-3**]. Brexpiprazole is approved for the treatment of schizophrenia and depression, but also for agitation and other behavioural symptoms in patients with Alzheimer's disease [**4**]. Even though it failed Phase II clinical trials for Attention Deficit Hyperactivity Disorder (ADHD), it has been considered toprovide improved efficacy and tolerability (e.g., less akathisia, restlessness and/or insomnia) over established adjunctive treatments for Major Depressive Disorder (MDD) [**5, 6**]. Brexpiprazole has a greater affinity for the 5HT1A receptors. Brexpiprazole has a side effect like akathisia, weight gain and nasopharyngitis [**7**]. The determination of stability testing is to deliver evidence on how the quality of a drug substance or drug product varies with time under the influence of diverse environmental factors and to establish a re-test period for the drug substance or a shelf life for the drug product which is necessary for regulatory documentation [**8-10**]. The studies of stress testing of drug substance helps in identification of degraded products which further help to establish the degradation pathways and the intrinsic stability of the molecule and validate the steadiness indicating power of the analytical methods used. The Structural elucidation and characterization of degradation product(DP-1) which formed in oxidative degradation was not reported anywhere in literature which is determined by liquid chromatography mass spectrometry (LCMS), High resolution mass spectrometry (HRMS), Two-dimensional nuclear magnetic resonance studies(2DNMR).

MATERIALS AND METHODS

Reagents and Chemicals: The drug Brexpiprazole was purchased from Hyderabad local market. Formic acid, Ammonium bicarbonate, hydrochloric acid, sodium hydroxide, methanol and acetonitrile of (LC-MS grade), Milli Q water, Millipore were procured from Merck, India. Hydrogen peroxide was acquired from Sigma Aldrich (Germany).

Instrumentation: Liquid chromatography Mass Spectrometry (LCMS): ACQUITY BEH C18, 2.1mm×50mm, 1.7µcolumn was chosen as stationary phase. Mobile phase comprised of 0.05% Formic acid in aqueous and 0.05% formic acid in acetonitrile with flow rate 0.6 mL min^{-1} was used. The column temperature was maintained at 30°C and UV wavelength was monitored at 215nm.The mixture of tetrahydrofuran (THF), acetonitrile and water was used as diluent.

High Resolution Mass Spectrometry: Samples were analysed on the Thermo Q Exactive Version: Orbitrap MS 2.8 Build 2688 with ESI ion source. Sample was analysed in positive mode and negative mode. Caffeine (*m/z*: 193 Da) was used as internal standard to calibrate the mass range and accuracy. Mass data was acquired in positive mode and negative mode using Thermo Excalibur Version: 3.0.63 software.

Preparative High- Performance Liquid Chromatography (Prep-HPLC): Preparative HPLC is ever gaining in importance as purification method in the pharmaceutical industry. Prep-HPLC is used for purification and isolation of impurities present in the mixture. Preparative HPLC equipped with water pump module 2545, Waters PDA detector module 2998 monitored at 215 nm, column: X-Bridge-C18 (250 X19 mm) 5µ, mobile phase A: 0.01% formic acid; mobile phase B: Acetonitrile: T% of B:0.0/10,8.0/60,10/60,13/98,13.1/10,16/10.

Nuclear Magnetic Resonance Spectroscopy: NMR analysis of API and Isolated impurities of Brexpiprazole taken on Bruker AVANCE NEO 400MHz NMR instrument equipped with 5mm Smart probe TM(PA BBO BBF-H-D-05 Z SP) with Z- gradient shim system which has the sensitivities as 500:1 and 225:1 for ^{1}H and ^{13}C nuclei respectively and also equipped with sample Xpress auto sampler with 60 samples hold capacity. All the NMR analysis has been performed at 298K probe temperature with fine automatic tuning and matching for the frequency of respective nuclei. ^{1}H NMR

spectra were referenced to tetra methyl Silane (TMS) singlet at zero (0) ppm and referenced DMSO-D6 septet at 39.5 ppm in carbon NMR.

Stress degradation studies: According to ICH guidelines stress studies are done by exposing the drug substances under several stress conditions like acidic, basic and oxidation. Brexpiprazole drug was forcibly degraded under different stress conditions like acidic hydrolysis, basic hydrolysis and oxidative degradation.

Acid stress studies: Acid stress studies were performed in acidic conditions by exposing to 1N HCl in 100 mg Brexpiprazole drug in 100 mL volumetric flask. The solution was set aside at room temperature for 24 h.

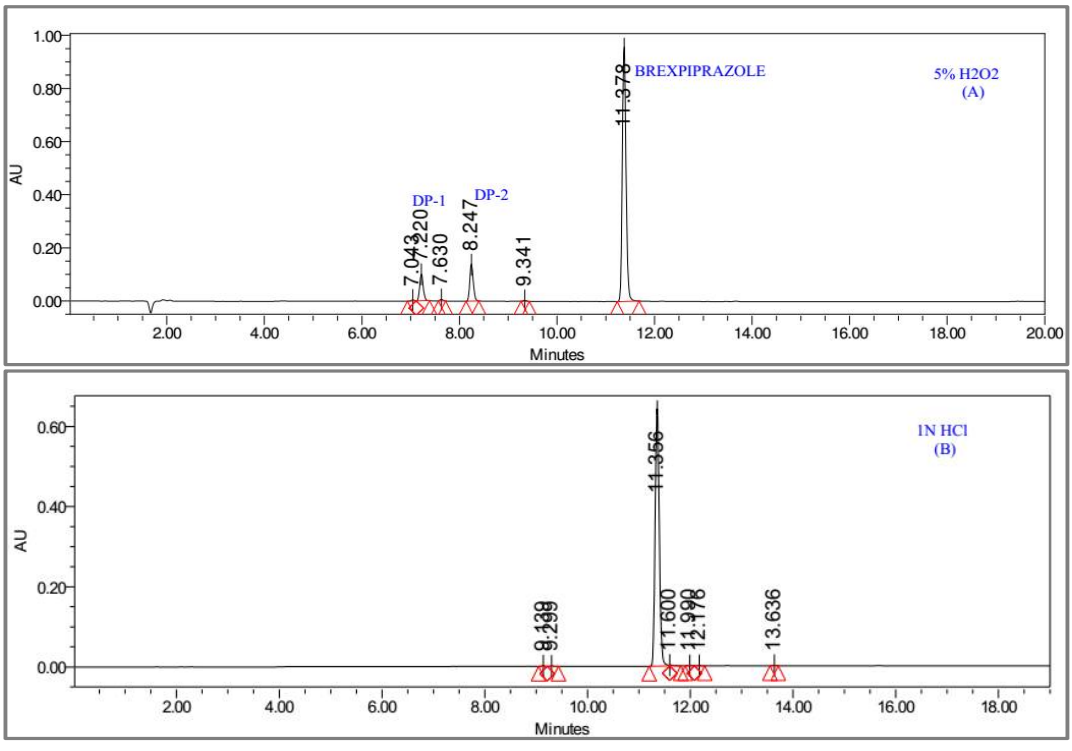
Base stress studies: Base stress studies were performed in basic conditions by adding 10 mL of 1N NaOH in 100 mg Brexpiprazole drug in 100 mL volumetric flask. The solution was set aside at room temperature for 24 h.

Oxidative stress studies: Oxidative stress studies in oxidative media were performed by adding 10 mL of 5% H2O2 in 100 mg Brexpiprazole drug in 100 ml volumetric flask. The solution was set aside at room temperature for 24 h.

Characterization and Structural elucidation of degradation Product: The degradation product was observed under Oxidative stress degradation studies which are scrutinized on LC-MS instrument was isolated by Prep-HPLC and characterized by NMR studies (1D, 2D experiments).

RESULTS AND DISCUSSION

BREX was stable in stress degradation conditions like acid and alkali hydrolysis where it is labile to the oxidation conditions. When it is exposed to the 5% hydrogen peroxide two degradant products were observed at room temperature after 24h and continued till 48h to enhance the impurity percentages (Figure 1).



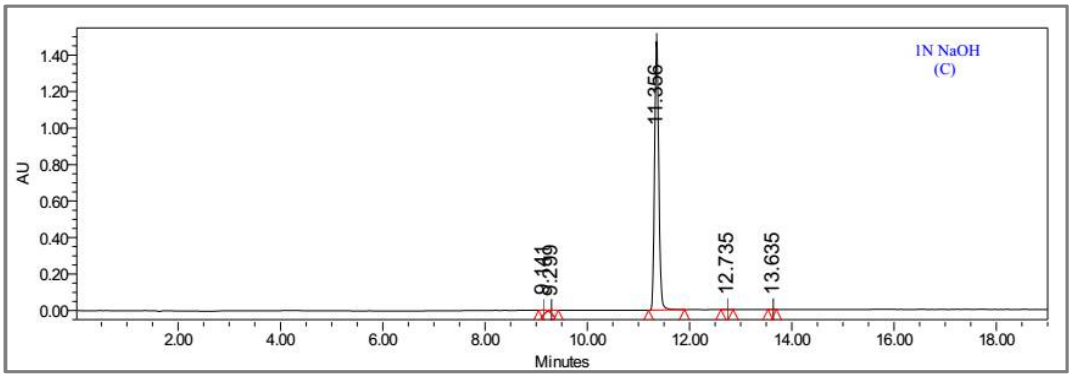
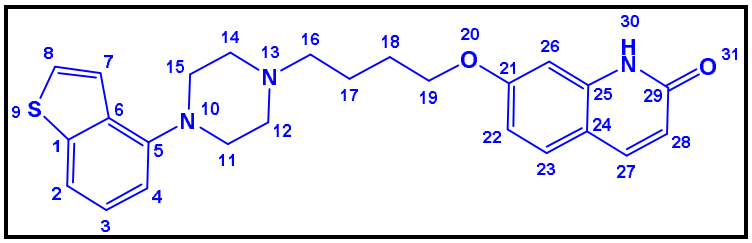
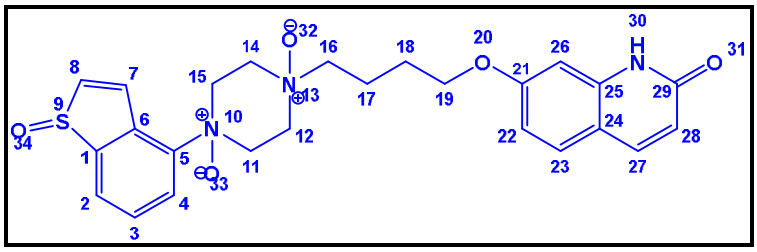


Figure 1. (A) Hydrogen peroxide, (B) Acid, (C) Base degradation chromatograms of Brexpiprazole.

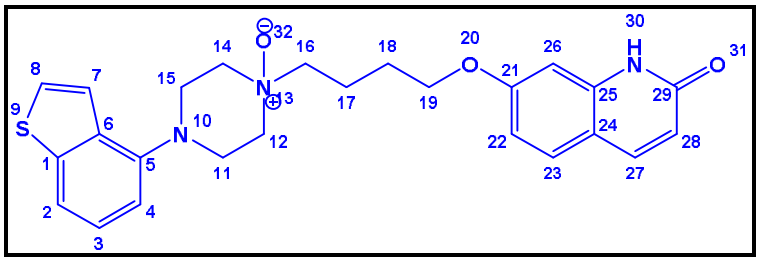
Isolation of oxidative degradation products: Preparative HPLC was used for isolation of oxidative degradant products as per the method described in the section. Characterization was carried out by HR-MS, NMR (1D and 2D) Studies.DP-2 structure was already reported in the literature [**11**] which is a metabolite [**12**]. DP-1 is new degradant product (Figure 2) shows the degradant impurity.



Brexpiprazole
Chemical Formula $C_{25}H_{27}N_3OS$, Molecular Weight: 433.57



DP-1
Chemical Formula $C_{25}H_{27}N_3O_5S$, Molecular Weight: 481.57



DP-2
Chemical Formula $C_{25}H_{27}N_3O_3S$, Molecular Weight: 449.57

Figure 2. Chemical structures of Brexpiprazole and its oxidative degradation products.

Structure elucidation of DP-1: The experimental HRMS m/z 482.1739 (M+H) shows there is a 48 m/z difference from the BREX API m/z 434.1890 (M+H) (Fig 3). From the fragmentation pattern of HR-MS data reveals that three oxygen atoms are attached to the molecule. There was no change in the

RBDE (13.5) for both BREX API and DP-1 (Figure 4). It was further confirmed by 1HNMR, 13C NMR, HSQC, HMBC and COSY. The difference in chemical shifts of proton and carbon NMR data has been revealed that where exactly changes has occurred in the molecule on treatment with 5% hydrogen peroxide (Figure 5). Carbon and proton chemical shifts of CH14, CH12, CH15, CH11, CH16, CH7 and CH8 are significantly changed. The chemical shifts of the two protons (H7 and H8) of thiophene ring of BREX API (Table 1) were observed 7.40 ppm and7.69 ppm with coupling constant J=5.6Hz but in DP-1 it was changed to 7.67 and 7.34 with J=7.10Hz.Variance in coupling constant observed is 1.5Hz. It has been observed that there was a drastic shift in PPM of C6 and C4 carbons as well as C11 and C15 shifted up field to nearly ~5PPM. C12, C14 and C16 are shifted down field to ~10 ppm whereas rest of the molecule chemical shifts does not change much as shown in table 2.

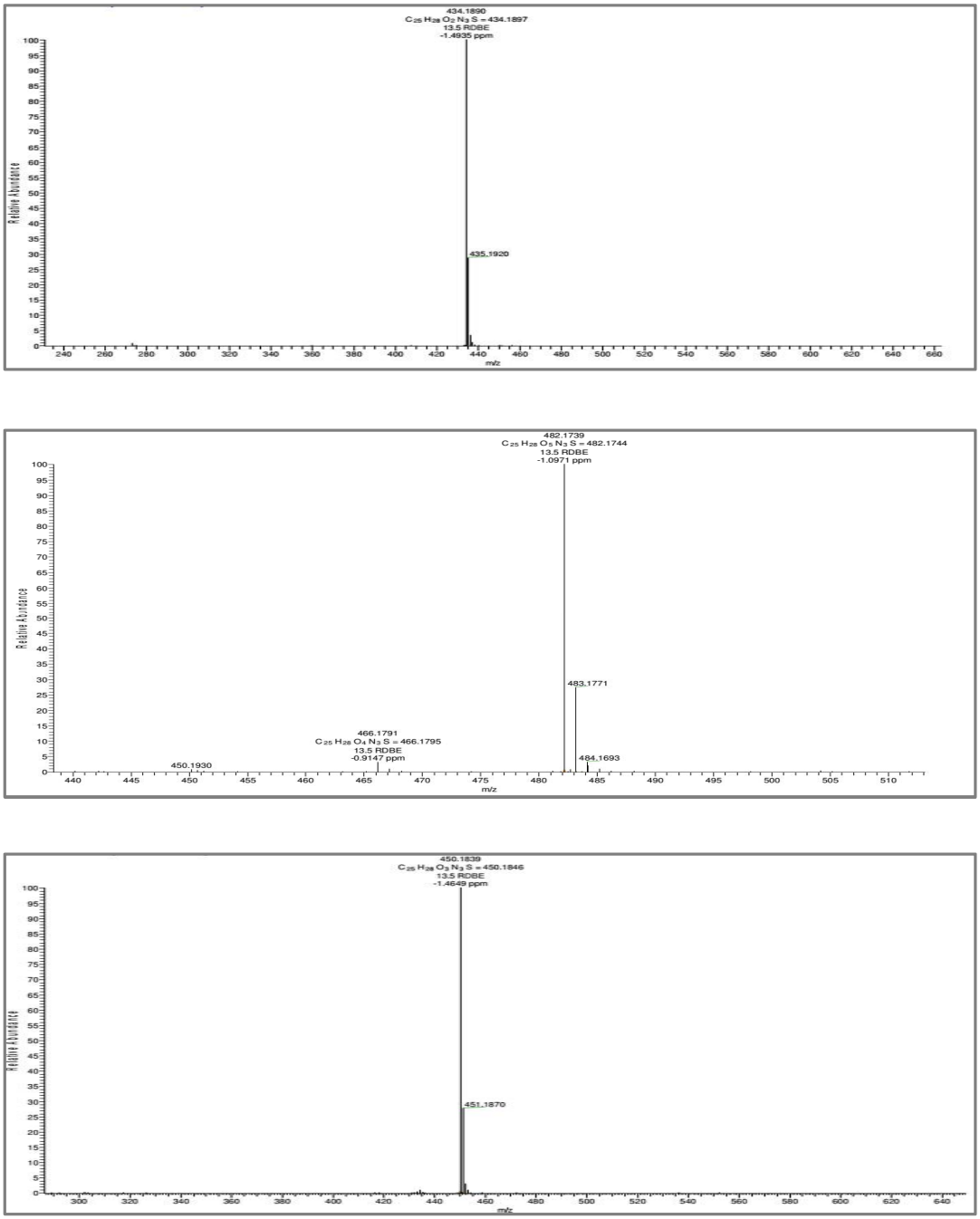


Figure 3. A) HRMS data of**API, B)** HRMS data of **DP-1** and **C)** HRMS data **of DP-2**

Table-1. ^{1}H ,^{13}CNMR data of BREX-API

Atom No	Type of Atom	^{1}H Chemical Shift (PPM) Coupling Const(J)	^{13}C Chemical Shifts(ppm)
1	C	-	140.37
2	CH	7.62(d, 8.0Hz, 1H)	116.6
3	CH	7.26(t, 8.0Hz, 1H)	125.06
4	CH	6.88(d, 8.0Hz, 1H)	112.00
5	C	-	148.24
6	C	-	133.35
7	CH	7.40(d, 5.6Hz, 1H)	121.86
8	CH	7.69(d, 5.6Hz, 1H)	125.79
9	S	-	-
10	N	-	-
11	CH2	3.05(broad s, 2H)	51.71
12	CH2	2.61(broad s, 2H)	52.96
13	N	-	-

S-singlet, d-doublet, t-triplet, dd-doublet of doublet

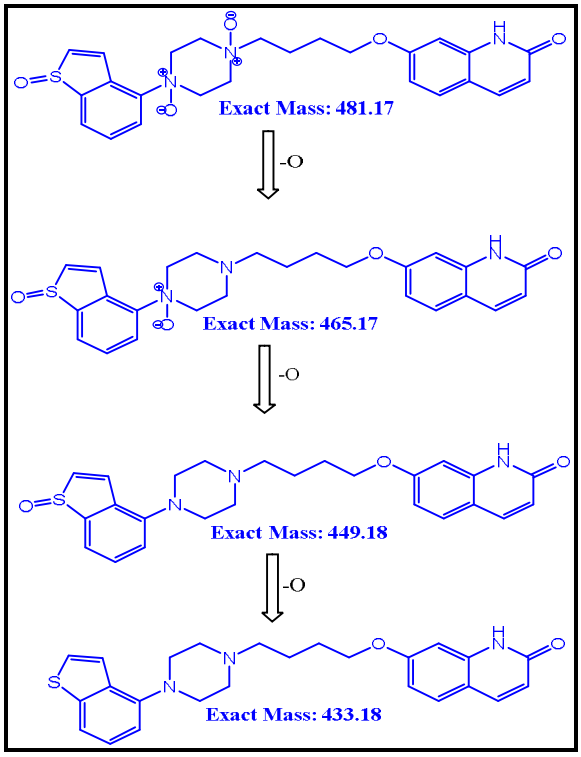


Figure 4. HRMS fragmentation pathway of DP-1.

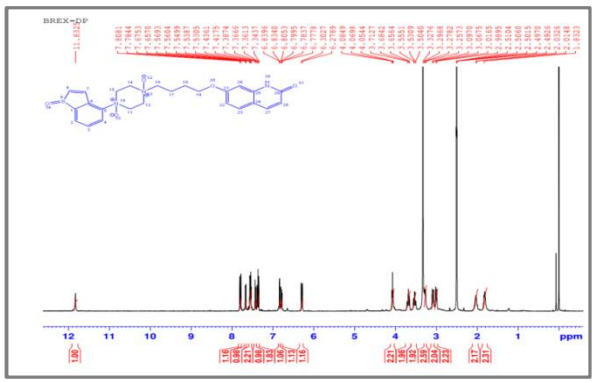


Figure 5. 1H-NMR Data of DP-1

Table 2. ^{1}H , ^{13}C, NMR data of DP-1

Atom No	Type of Atom	1H Chemical Shift (PPM) Coupling Const(J)	13C Chemical Shifts(ppm)
1	C	-	137.78
2	CH	7.43(d, 8.4Hz, 1H)	114.58
3	CH	7.54(t, 8.4Hz, 1H)	132.14
4	CH	7.38(d, 8.4Hz, 1H)	123.91
5	C	-	148.26
6	C	-	122.58
7	CH	7.67(d, 5.6Hz, 1H)	130.29
8	CH	7.34(d, 5.6Hz, 1H)	123.91
9	S	-	-
10	N	-	-
11	CH2	3.09(d, 12.0Hz, 2H) 3.68(t, 11.2Hz, 2H)	46.56
12	CH2	2.98(d, 10.8Hz, 2H) 3.53(t, 10.7Hz, 2H)	63.30
13	N	-	-
14	CH2	2.98(d, 10.8Hz, 2H) 3.53(t, 10.7Hz, 2H)	63.30
15	CH2	3.09(d, 12.0Hz, 2H) 3.68(t, 11.2Hz, 2H)	46.56
16	CH2	3.27(t, 8.4Hz, 2H)	69.92
17	CH2	2.03(m, 2H)	18.4
18	CH2	1.81(m, 2H)	26.17
19	CH2	4.06(t, 6.0Hz, 2H)	67.45
20	O	-	-
21	C	-	160.31
22	CH	6.78(dd, 8.8, 2.4Hz, 1H)	110.77
23	CH	7.53(d, 9.2Hz, 1H)	129.20
24	C	-	113.3
25	C	-	140.7
26	CH	6.83(d, 2.4Hz, 1H)	98.65
27	CH	7.80(d, 9.6Hz, 1H)	139.94
28	CH	6.30(d, 9.6Hz, 1H)	118.53
29	C	-	162.28
30	NH	11.83(s, 1H)	-
31	O	-	-
32	O	-	-
33	O	-	-
34	O	-	-

S-singlet, d-doublet, t-triplet, dd-doublet of doublet

APPLICATION

The stress degradation studies indicate that Brexpiprazole is liable to Oxidative stress condition where two degradation products DP 1 and DP 2 were found.

CONCLUSION

The outcome from the stress degradation studies indicates that Brexpiprazole is liable to Oxidative stress condition where two degradation products were obtained. Out of which one is N-oxide impurity already reported in the literature and the other one is DP-1 chemically named as 1-(1-oxidobenzo[b] thiophen-4-yl)-4-(4-((2-oxo-1,2-dihydroquinolin-7-l)oxy)butyl)piperazine 1,4-dioxide not reported earlier in the literature. This compound was characterized by LC-MS, HRMS and NMR (1D, 2D) studies.

Conflicts of Interest: There are no conflicts to declare.

Ethical approval: This article does not contain any studies with human participants or animals performed by any of the authors.

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