IDENTIFICATION, SYNTHESIS AND STRUCTURAL ELUCIDATION OF IMPURITIESOBSERVED IN FORCED DEGRADATION STUDY OFRANOLAZINE ACTIVE PHARMACEUTICAL INGREDIENT

SantoshKumar and Swati Goyal *

Department of Chemistry, Dr. A. P. J. Abdul Kalam University, Indore, Madhya Pradesh

*Corresponding Author Email: swatipc2011@gmail.com

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Abstract:

Impurities are the unwanted substance that present in active pharmaceutical ingredient(API) and finished products has no therapeutic properties even they may have some adverse and toxic effects too.Hence for the safety of patients the potential unknown impurities must be identified and quantify by the specific methods. During forced degradation study of ranolazine API, two major unknown impurities observed in oxidative degradation through chromatographic purity test on HPLC. With help of LC-MS the molecular weight of impurities found.Precise synthesis method developed for the synthesis of these unknown impurities. C18 column with mobile phase ammonia and acetic acid in water and acetonitrile used for chromatographic purity test with gradient elution. As these impurities formed majorly in oxidative stress condition of API. Based on molecular weight of the impurities it is propose that one impurity is an oxide of the parent molecule and the second one is bis- oxide of the parent Molecule. With the help of HPLCchromatogram, MS spectra and NMR data the structure of impurities elucidated.

Keywords: Impurities, force degradation, structural elucidation, LC-MS, NMR.

1. Introduction

Ranolazine used for the treatment of chronic angina (chest pain). Ranolazine should not be used during an acute (emergency) episode of angina[1].The FDA authorized ranolazine for the treatment of persistent angina in 2006. Ischemia causes angina, which characterized by chest discomfort [2].The goal of treating stable angina is to diminish symptoms and the development of ischemia, as well as to prevent myocardial infarction and mortality. Aspirin, P2Y12 inhibitors,

angiotensin-converting enzyme inhibitors (ACEI), and angiotensin receptor blockers are all common treatmentsm [3,4].Off-label uses include the treatment of ventricular tachycardia and other arrhythmias [5].However, there is quite rare evidence observed to back up this claim. As part of the interprofessional team, this activity covers the indications, mechanism of action, routes of administration, key adverse effects, contraindications, and monitoring of ranolazine so that physicians can direct patient care in treating illnesses for which it is appropriate [6,7].Symptoms of chronic stable angina pectoris (CSAP) remain in 25% of patients despite the administration of standard antianginal drugs (blockers, calcium channel blockers, and nitrates) and revascularization procedures[8].Ranolazine, sold under the trade name Ranexa is a piperazine derivative, a well-tolerated medication that selectively inhibits the late sodium current. Additionally, ranolazine has beneficial metabolic properties and does not affect heart rate or blood pressure [9].Ranolazine patented in 1986 is an active piperazine derivative and is available in an intravenous oral and an oral form. Ranolazine (Ranexa; CV Therapeutics Inc, Palo Alto, Calif) in the form of sustained-release is manufactured and it has maximal plasma concentrations (Cmax) typically seen 4 to 6 hours after administrationwith a prolonged absorption phase [10].The use of ranolazine as an adjunct therapeutic option in patients with chronic stable angina is well established in the armamentarium of guideline-directed medical therapy [11].

2. Method and Materials

2.1 Reagents and samples

Acetonitrile was purchased from Thermo Fisher Scientific (Fair Lawn, NJ) was HPLC grade. A Milli-Q water purification system (Millipore, Billerica, MA) was used to further purify glass-distilled water.Triethylaminewas purchased from Merck, phosphoric acidwas purchased from Merck.Ranolazine(API) of Covalent laboratories private limited, India.

2.2 Reference standard and sample solutions preparation

Preparation of system suitability solution:

Weigh and transfer about 25mg of Ranolazine working standard/reference standard in 250mL volumetric flask add about 120mL of diluent and sonicate with intermittent shaking to clear solution, dilute to the volume with diluent.

Further pipette 1 mL of this solution into 100-mL vol. flask, dilute to volume with diluent.

Test Solution Preparation: Weigh and transfer powdered API equivalent to 100 mg of Ranolazine to a 100mL volumetric flask, add 70mL of diluent and sonicate for about 5 minutes with intermittent shaking. Dilute with diluent to volume and mix. Filter the solution through 0.45µ PVDF filter.

2.3 Instrumentation

The MS/MS system API 2000 of (Applied Biosystem Inc., California), controlled by Analyst® software (version 1.5.1) used for fragmentation of ranolazine and Impurities. HPLC consist of a Agilent 1200 series quartnary pump, a column compartment, an autosampler, a UV detector (Agilent 1200series).

2.4 Forced degradation study

Oxidative stress Condition: During forced degradation of API by hydrogen peroxide two impurities generates majorly. To identify the impurities by LC-MS a LC-MS compatible method has been develop.

2.5 Chromatographic conditions

The analysis was carried out on a Xterra RP 18 (4.6 mm×150 mm, 5 µm-particle diameter). Mobile phase A contains 2.0 mL of Ammonia Solution(25 %)into 2000ml of water, adjusted the pH to 6.0 with dilute acetic acid solution and acetonitrile 950:50 v/v. Mobile phase B contains2.0 mL of Ammonia Solution(25 %)into 2000ml of water, adjusted the pH to 6.0 with dilute acetic acid solution and acetonitrile 100:900 v/v100%.UV detection was at 225 nm, the flow rate kept at 1.0 mL/min. Column oven temperature was 40°C, and the data acquisition time was 95min. The pump mode was gradient and the program was as follows, time (min)/A (*v/v*):B (*v/v*); T0.00/95:5, T15/08:20, T50/65:35, T55/65:35, T65.0/25:75 and T80.0/25:75, T82.0/95:5, T95/95:5

2.6 Mass spectrometry

LC-MS analysis of ranolazine and impurities carried out using the optimized MS parameters. The parameters are electrospray ionization (EPI) positive ionization mode, declustering potential (DP) 10 V, entrance potential (EP) 10 V, collision energy, curtain gas: 20.0 L/h, ion source gas 1: 50.0 L/h, ion source gas 2: 50.0 L/h, ion spray voltage (IS): 5500 V, temperature (TEM): 450.0 °C, and Interface heater. Mass range acquired from *m*/*z* 75to *m*/*z* 1250 in 0.1 amu steps with dwell time of 2.0 s. For data acquisition and processing Analyst software (version 1.5.1) was used . Molecular weights of all components were determined by use of protonated molecular ions ($[M+H]^+$) and were confirmed using minor adduct ions of $[M+Na]^+$ and [M+K]+ peaks.

3. Results and Discussion

3.1 Impurity analysis by LC-MS

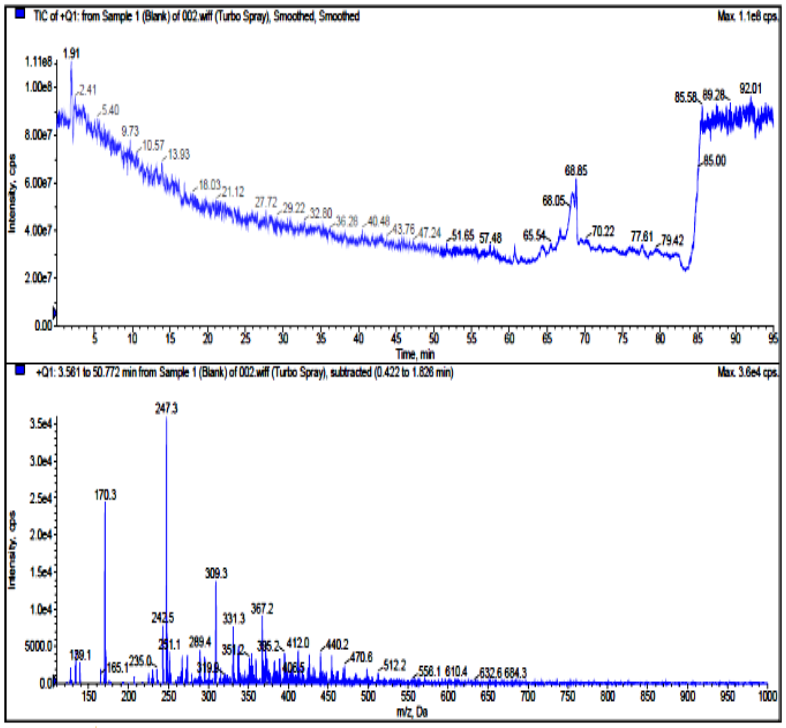


Figure 1: LC-MS chromatogram of Blank

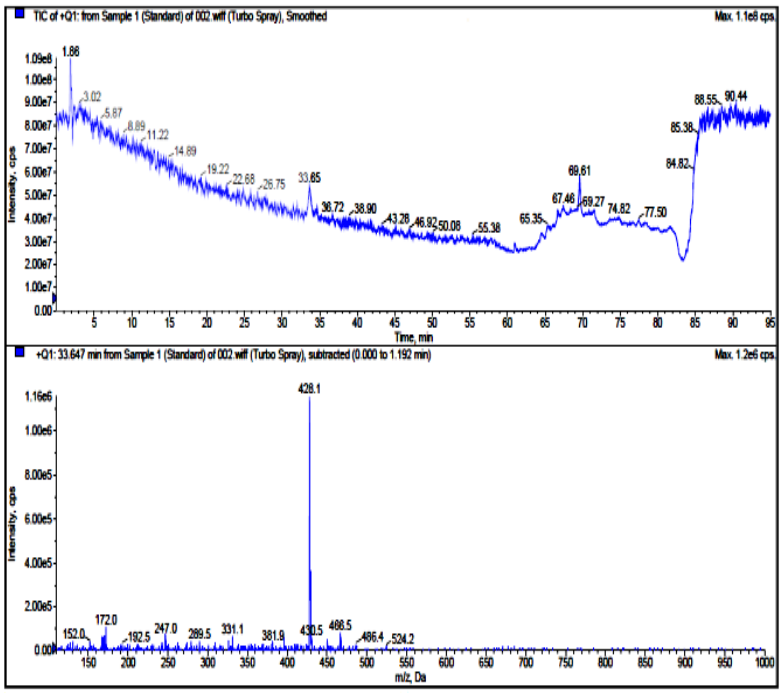


Figure 2: LC-MS chromatogram of Standard (Ranolazine)

As these impurities formed majorly in oxidative stress condition of API. Based on the molecular weight of the impurities it indicates that these impurities might be formed by addition of oxygen molecule in the parent molecule. Impurities are result of oxidation reaction. Based on molecular weight of the impurities it is also proposed that one impurity might be an oxide of the parent molecule and the second one might be Bis- Oxide of the parent Molecule.

Further, it is decided to synthesize the impurities and characterize the impurities later on. Several trials had been taken to synthesize the impurities in pure form.

Followings are the finalized method of the synthesis for the impurities observed in forced degradation of Ranolazine by hydrogen peroxide treatment,

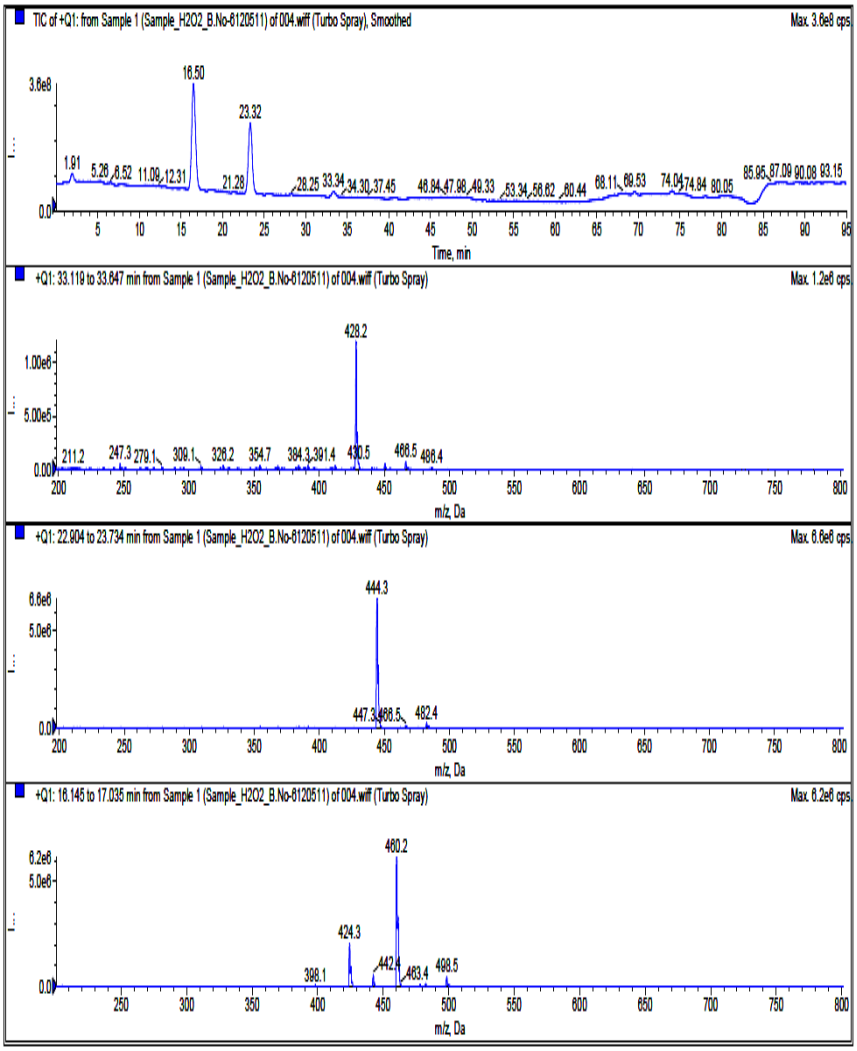


Figure 3: LC-MS chromatogram of peroxide degradedAPI (Sample)

Synthesis of Ranolazine N-Oxide:1 g of ranolazine API dissolved in 50ml methanol, added 5 mL 30% Hydrogenperoxidekept at room temperature for 90 minutes. Transfer the reaction mixture in separating funnel. Added 50 ml water and 50 ml Dichloromethane.Shake it for 15 minutes to extract.Collect the lower layer. Again added 50 ml of Dichloromethane.Shake it for 15 minutes to extract.Collect the lower layer. Dried on rotatory evaporator. After complete drying collect the dried powder which is Ranolazine N-Oxide impurity.

Synthesis of Ranolazine Bis (N-Oxide):1 g of ranolazine API dissolved in 50ml methanol, added 0.77 g of sodium tungustate and 5 mL 30% Hydrogenperoxide. Stirred the reaction mixture at magnetic stirrer for 2 hours at room temperature. Added 5 ml 30% Hydrogenperoxide. Transfer the

reaction mixture in separating funnel. Added 50 ml water and 50 ml Dichloromethane.Shake it for 15 minutes to extract.Collect the lower layer. Again added 50 ml of Dichloromethane.Shake it for 15 minutes to extract.Collect the lower layer. Dried on rotatory evaporator. After complete drying collect the dried powder which isRanolazine Bis (N-Oxide).

After completion of synthesis, impurities were analyze on HPLC, NMR and Mass spectrometry. Followings are the chromatograms:

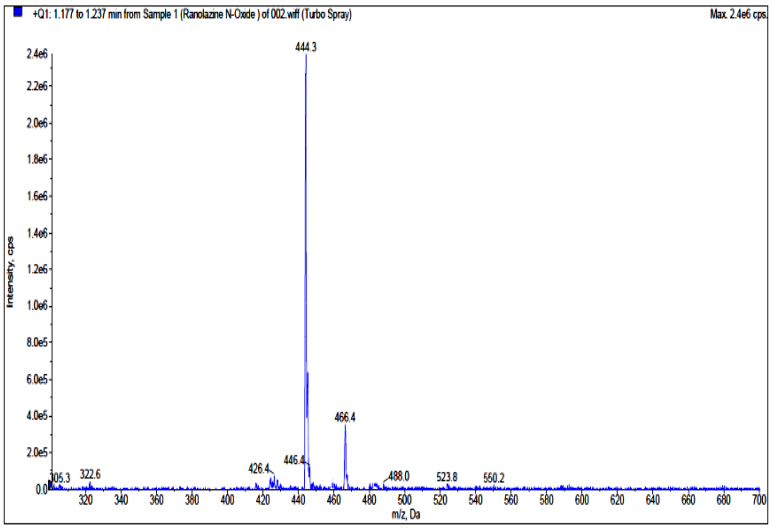


Figure 4: Mass chromatogram of Ranolazine N-Oxide

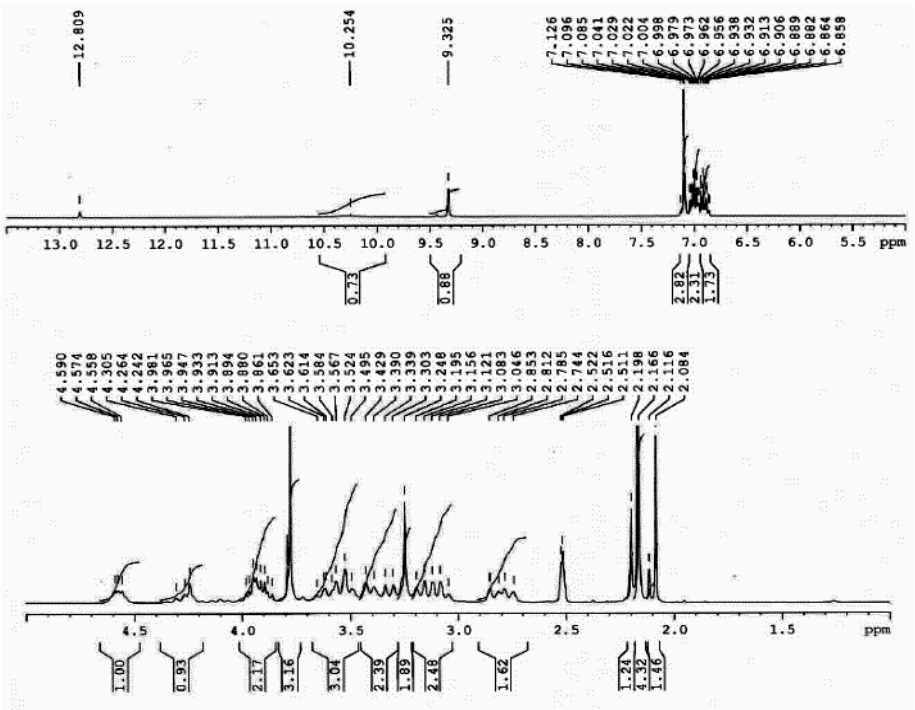


Figure 5: 1 H NMR spectra of Ranolazine N-Oxide

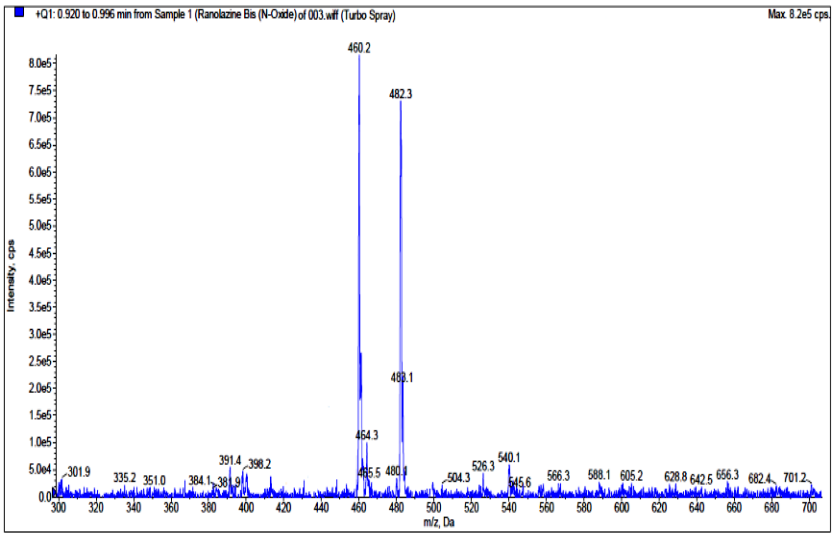


Figure 6: Mass chromatogram of Ranolazine Bis (N-Oxide)

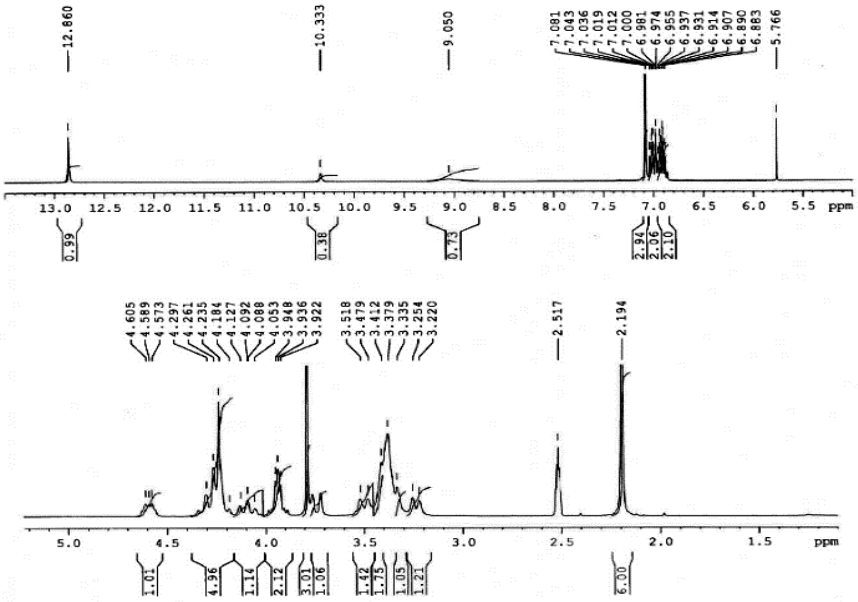


Figure 7: 1 H NMR spectra of Ranolazine Bis (N-Oxide)

Compound Information:

Name of Compound: Ranolazine Bis (N-Oxide)

IUPAC Name: 1-(2-((2,6-dimethylphenyl)amino)-2-oxoethyl)-4-(2-hydroxy-3-

(2methoxyphenoxy)propyl)piperazine 1,4-dioxide

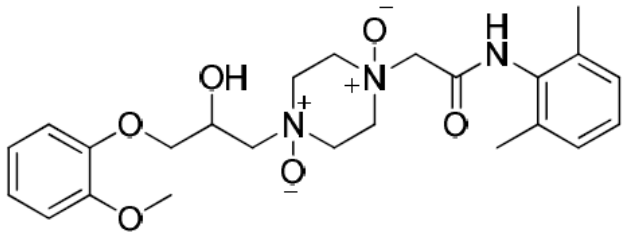
Molecular Formula: $C_{24}H_{33}N_3O_6$ Molecular Weight:459.55 g/mol

Description: Off-white Solid

Purity: HPLC Purity 86.85 %

Storage Condition: At 2-8 °C under inert atmosphere

Structure:



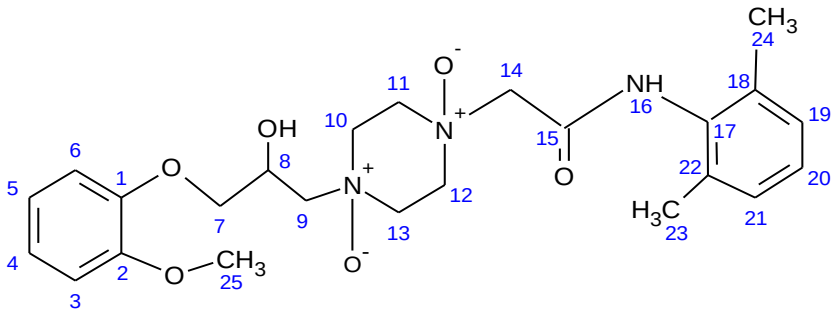
Characterization Technique:

A) Mass Spectroscopy: Mass spectra of Ranolazine Bis (N-Oxide) shows the molecular ion peak at m/z 460.3 in positive mode scan, which is in consistent with the molecular weight of Ranolazine Bis (N-Oxide) i.e. 459.55

Molecular weight	Molecular Ion $(M+H)^+$
459.55	460.3

B) NMR Spectroscopy:

Structure:



1)^{1}H NMR: The Proton magnetic resonance spectrum of Ranolazine Bis (N-Oxide) in DMSO obtained at 300 MHz NMR spectrometer. The following chemical shift observed.

Table1:

Sr.No.	Carbon No.	Number of proton	Multiplicity	Chemical Shift (δ)
01	23,24	6	m	2.194
02	12a	1	s	3.220
03	12b	1	s	3.254
04	11	2	d	3.335-3.412
05	13a	1	s	3.479-3.518
06	13b	1	s	3.700
07	10, 25a	3	t	3.800
08	25b,25c	2	d	3.922-3.948
09	9a	1	t	3.776-3.790
10	9b, 7, 14	5	p	4.235-4.297
11	8	1	s	4.573-4.605
12	3,6	2	d	6.883-6.955
13	4,5	2	d	6.974-7.043
14	19,20,21	3	t	7.081
15	16	1	s	9.050
16	OH	1	s	10.333

2) ^{13}C NMR:

Table 2:

Sr.No.	Carbon No.	Chemical Shift
1	C-23/ C-24	19.159
2	C-25	55.378
3	C-13	56.093
4	C-10	57.751
5	C-12	59.276 & 59.972
6	C-11	61.608
7	C-7	65.589
8	C-9	68.837 & 68.837

9	C-14	71.818
10	C-3/C-6	112.965 &114.706
11	C-4/C-5	121.260 & 121.904
12	C-20	126.516
13	C-19/C21	128.277
14	C-18/C-22	134.349 & 135.067
15	C-8/C-17	148.561
16	C-1/ C-2	149.776
17	C-15	163.010

3) In D2O Exchange experiment two H atom disappeared at Chemical Shift (δ) 9.325 and 10.254 respectively, which were NH and OH respectively.

Compound Information:

Project: Ranolazine ER Tablet

Name of Compound: Ranolazine N-Oxide

IUPAC Name:1-(2-((2,6-dimethylphenyl)amino)-2-oxoethyl)-4-(2-hydroxy-3-(2-methoxyphenoxy)propyl)piperazine 1-oxide

Molecular Formula: $C_{24}H_{33}N_3O_5$ Molecular Weight:443.55 g/mol

Batch No.:RMD900/10/2017-01 Pharmacopeia Status: Inhouse

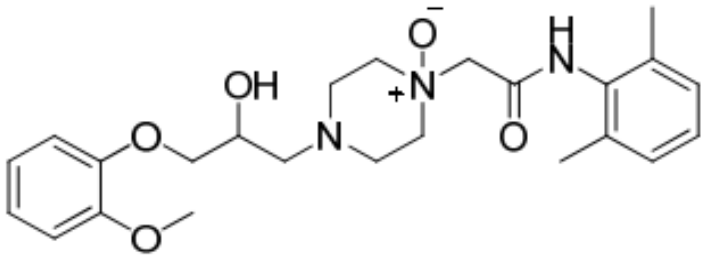
Source: Alkem Laboratories Ltd.

Description: Off-white Solid

Purity: HPLC Purity 90.84 %

Storage Condition: At 2-8 °C under inert atmosphere

Structure:



Characterization Technique:

A) UV Spectroscopy: The UV spectra of Ranolazine N-Oxide in methanol is scanned from 190.0 to 400.0nm on UV Spectrophotometer Model No. UV-1800 (Instrument ID:AD-UV-07) and shown maximum absorption 1.8042 at 196.2 nm.

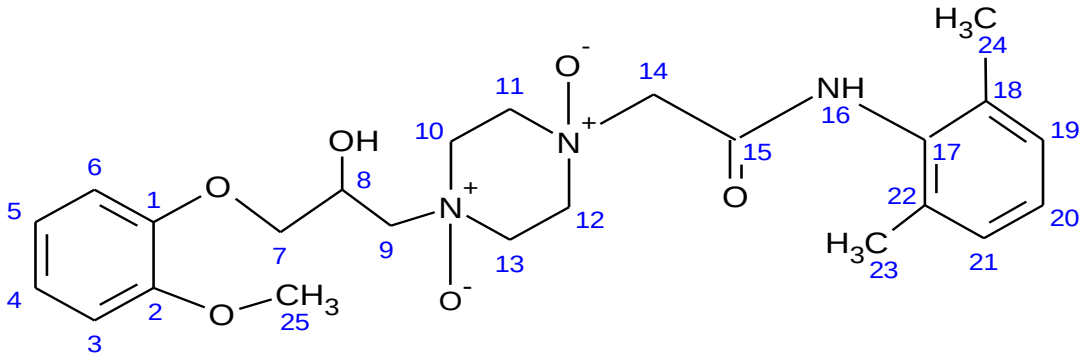
Wavelength (λ) nm	Absorbance
196.2	1.8042

B) Mass Spectroscopy: Mass spectra of Ranolazine N-Oxide shows the molecular ion peak at m/z 444.1 in positive mode scan, which is in consistent with the molecular weight of Ranolazine N-Oxide i.e. 443.53

Molecular weight	Molecular Ion (M+H)+
443.55	444.1

C) NMR Spectroscopy:

Structure:



1) ^{1}H NMR: The Proton magnetic resonance spectrum of Ranolazine N-Oxide in DMSO obtained at 300 MHz NMR spectrometer. The following chemical shift observed.

Table 3:

Sr.No.	Carbon No.	Number of proton	Multiplicity	Chemical Shift (δ)
01	23,24	6	m	2.084-2.198
02	12	2	d	2.744-2.853
03	11	2	d	3.046-3.195
04	13	2	d	3.248
05	10	2	d	3.303-3.429
06	25	3	t	3.495-3.653
07	9a, 14	3	t	3.861
08	7a, 9b	2	d	3.880-3.981
09	7b	1	s	4.242-4.305
10	8	1	s	4.558-4.590
11	4,5	2	d	6.858-6.956
12	3,6	2	d	6.962-7.096
13	19,20	3	t	7.126
14	16	1	s	9.325
15	OH	1	s	10.254

2) ^{13}C NMR:

Table 4:

Sr.No.	Carbon No.	Chemical Shift
1	C-24	16.597
2	C-23	17.042
3	C-25	53.952
4	C-13	58.392
5	C-10	60.422
6	C-12	63.261
7	C-11	64.191
8	C-7	69.826
9	C-9	110.810
10	C-14	112.591
11	C-3/C-6	119.150

12	C-4/C-5	119.835
13	C-20	124.840
14	C-19/C21	126.017
15	C-18/C-22	133.340
16	C-17	133.595
17	C-8	146.368
18	C-1/ C-2	147.649
19	C-15	166.201

3) In D2O Exchange experiment two H atom disappeared at Chemical Shift (δ) 9.325 and 10.254 respectively, which were NH and OH respectively,

4. Conclusion

The structure of majorly generated impurities during oxidative forced degradation of Ranolazine active pharmaceutical ingredient are confirmed asRanolazine N-Oxide and Ranolazine Bis (N-Oxide)respectively based on above analyticalevidences.The structures of all the degradants were elucidated by HPLC, MS and NMR techniques.

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