IJPSR (2019), Volume 10, Issue 8 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 15 November 2018; received in revised form, 11 February 2019; accepted, 28 February 2019; published 01 August 2019

ISOLATION AND STRUCTURAL ELUCIDATION OF DEGRADATION PRODUCTS OF RANOLAZINE

Santhosh Guduru [1, 2], V. V. S. R. N. Anji Karun Mutha [1, 2], B. Vijayabhaskar [1], Jagadeesh Narkedimilli [3], Muralidharan Kaliyaperumal [1], Raghu Babu Korupolu [2], Kishore Babu Bonige [2] and Chidananda Swamy Rumalla [*1]

Department of Medicinal Chemistry [1], GVK Biosciences Pvt. Ltd., IDA Mallapur, Hyderabad - 500076, Telangana, India.
Department of Engineering Chemistry [2], Andhra University, Visakhapatnam - 530003, Andhra Pradesh, India.
Centre for Chemical Sciences and Technology [3], Institute of Science and Technology, Jawaharlal Nehru Technological University Hyderabad, Hyderabad - 500085, Telangana, India.

Keywords:

Ranolazine, Degradation products, HRMS, NMR

Correspondence to Author:
Dr. Chidanandaswamy Rumalla

Principle Scientific Manager, GVK Biosceinces Pvt. Ltd., Plot No. 125, 126, Mallapur, Hyderabad - 500076, Telangana, India.

E-mail: chidanand_swamy@yahoo.co.in

ABSTRACT: Degradation studies are important to know the potentials degradation products and to develop a stability indicating method. Ranolazine active pharmaceutical ingredients subjected to in detailed forced degradation study using several stressing agents (HCl, NaOH, H_2O_2). Degradation products of Ranolazine under hydrolytic and oxidative stress conditions were identified, and their stabilities were assessed. Three degradation products were formed when the drug was subjected to acid stress and two products were formed in oxidative stress condition. Ranolazine was stable to base hydrolysis. The degradants were separated on a C-8 column employing preparative HPLC using gradient elution. The structures of all the five degradation products (DP-1, DP-2, DP-3, DP-OX1, and DP-OX2) were established by extensive 1D (^1H, ^{13}C) and 2D (COSY, HSQC and HMBC) NMR spectroscopic studies and mass spectra. The products were identified as 2-(4-(2-hydroxy-3-(2-hydroxyphenoxy) propyl) piperazine-1-yl) acetic acid (DP-1), 2-(4-(2-hydroxy-3-(2-methoxy phenoxy) propyl) piperazine-1-yl) acetic acid (DP-2), N-(2,6-dimethylphenyl)-2 -(4-(2-hydroxy-3 (2-hydroxyphenoxy) propyl) piperazine-1-yl) acetamide (DP-3), 1-(2-((2,6-dimethylphenyl)amino)-2oxoethyl)-4-(2-hydroxy-3-(2-methoxy phenoxy) propyl) piperazine 1, 4-dioxide (DP-OX1), 4-(2-((2, 6 dimethyl phenyl) amino)-2-oxoethyl)-1-(2-hydroxy-3-(2-methoxy phenoxy) propyl) piperazine 1-oxide (DP-OX2). All the degradants reported here are novel except DP-OX1.

INTRODUCTION: Ranolazine is a racemic mixture, chemically it is described as N-(2, 6-dimethylphenyl) -2 -(4-(2-hydroxy -3 -(2-methoxy phenoxy) propyl) piperazine-1-yl) acetamide and the FDA approves it in January 2006 as a second-line treatment of chronic angina.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.10(8).3763-69

The article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.10(8).3763-69

The pharmacological action of Ranolazine is believed to lie in its ability to alter the trans-cellular sodium current by altering the intracellular sodium level. Ranolazine affects the sodium-dependent calcium channels during myocardial ischemia [1, 2, 3].

During the drug developmental stage, the identification and characterization of degradation products are required to know its influence on the safety and efficacy of the pharmaceutical products. The safety of the final products not only depending on toxicological properties of the active pharmaceutical ingredients but also the presence of unwanted impurities in the products.

Like organic impurities, carrying over from starting materials, reagents, intermediates or by-products are considered as potential genotoxic impurities (PGIs) which may cause cancer in humans [4, 5]. To control the drug chemical impurities is a huge challenge for the pharmaceutical industry. The toxic effect of minor impurities results in the adverse drug reactions knowing that identification and structure elucidation and assessment of the toxicity of the degradation products is crucial from quality and safety perspective of the drug products [6]. Considering safety issues, identification and characterization of degradation products are suggested by all regulatory authorities and agencies including the International Conference on Harmonization (ICH) and other regulatory authorities [7, 8, 9]. As per ICH guideline Q3A and Q3B, assessment of toxicity of degradation products is essential as the guidelines include stringent reporting, identification, characterization of degradation products [10].

Ranolazine is a well-known drug manufactured commonly as a bulk drug. There are few reports published on the stability studies on Ranolazine in bulk drug and tablet dosage form by HPLC and HPTLC [11]. UV spectrophotometric and LC methods are developed for routine analysis (Assay, Uniformity) of Ranolazine tablets [12]. A systematic study to isolate and identify the degradation products has never been attempted. Effect of Ranolazine on myocardial metabolic ischemia observed by ^{31}P NMR [13]. Effect of Ranolazine on renal functions in the patients [14]. Examine the usage of Ranolazine in various cardiovascular conditions [15]. HPLC method developed for researcher involved in the formulation and quality control of Ranolazine [16]. We have taken up the present work to investigate the Ranolazine behavior in the acid, base and peroxide media and fully characterize the degradants using spectroscopic techniques like HRMS and NMR.

MATERIALS AND METHODS:

Chemicals and Reagents: Ranolazine was a kind gift sample from a manufacturing unit in Hyderabad. Solvents and buffers used for analysis were HPLC grade acetonitrile (Merck), formic acid (Alfa Aesar), dimethyl sulfoxide$_6$ containing 0.03% (v/v) TMS (Cambridge isotope limited) and water used was Milli-Q grade.

Liquid Chromatography - Mass Spectrometry: Column: ACQUITY BEH C18, 2.1 mm × 50 mm, 1.7µ; mobile phase A: 0.1% formic acid (Aq); mobile phase B: acetonitrile; T/% of B: 0.0/2.0, 0.3/2.0, 5.0/98, 6.0/98, 6.1/2.0, 6.5/2.0; diluent: mobile phase; detection: 215 nm.

Preparative HPLC: Preparative HPLC equipped with water pump module 2545, Waters PDA detector module 2998 at 215 nm, column: Symmetry C8 (300 × 19 mm) 7µ, mobile phase A: 0.1% formic acid (Aq); mobile phase B: acetonitrile: T% of B: 0.0/20, 8.0/50, 11/50, 11.1/20, 14/20.

High-Resolution Mass Spectrometry: Samples were analyzed on the waters micro mass Q-TOF equipped with ESI ion source. The sample was analyzed in positive mode and negative mode. Leucine encephalin (m/z: 555.62268 Da) was used as internal standard to calibrate the mass range and accuracy. Mass data were acquired in positive mode and negative mode using Mass lynx software.

Nuclear Magnetic Resonance Spectroscopy: The ^{1}H, ^{13}CNMR and 2D NMR spectra of base degradation impurities were recorded in DMSO-d$_6$ solvent on Bruker 400 MHz Avance -III HD NMR spectrometer equipped with broadband observe (BBO) probe. The ^{1}H and ^{13}C chemical shifts are reported on the δ scale in ppm, relative to tetramethylsilane (TMS) as an internal standard. The spectra were set to δ 0.00 ppm in ^{1}H NMR (TMS) and δ 39.50 ppm in ^{13}C NMR (DMSO-d$_6$).

RESULTS AND DISCUSSION: The degradants were formed after 12 h of stirring in the media. However, it was continued until 24 h to enrich their yields. For the analytical study, 1 ml of the resultant degradation solution was dissolved in acetonitrile and diluted with mobile phase, and 10 µl was injected into the LC-MS system. Drug solution treated with peroxide showed two degradants and drug solution treated with acid showed three degradants **Fig. 1**. However, no degradation products were formed in base treated drug solutions. Acid and peroxide treated solution was taken up for isolation.

Isolation of Acid and Oxidation Degradation Products: The acid and oxidation degradation products were isolated at room temperature by the

method described in the experimental section. The fractions corresponding to the five peaks were collected and lyophilized. Degradation products were labeled as DP-1, DP-2, DP-3, DP-OX1, and DP-OX2. The chemical structures of these degradation products were deduced from the analysis of HRMS and 1D, 2D NMR data. Literature survey revealed that DP-OX1 was already reported [14], but H and ^{13}C NMR values were not reported. The DP-1, DP-2, DP-3 and DP-OX2 were found to be novel. **Fig. 2** shows structures of Ranolazine and degradation products DP-1, DP-2 and DP-3, DP-OX1 and DP-OX2. Mechanism of formed degradation products during acidic hydrolysis and oxidation with H_2O_2 were explained as shown in **Fig. 3**.

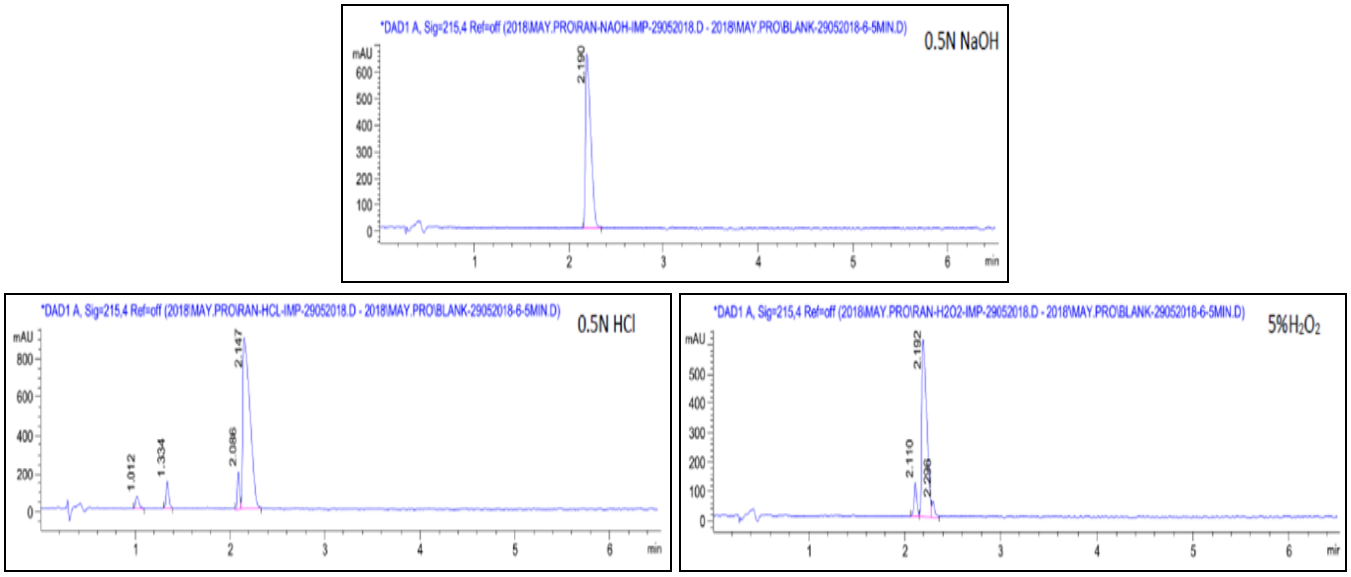

FIG. 1: BASE, ACID AND HYDROGEN PEROXIDE CHROMATOGRAMS OF RANOLAZINE DRUG SUBSTANCE

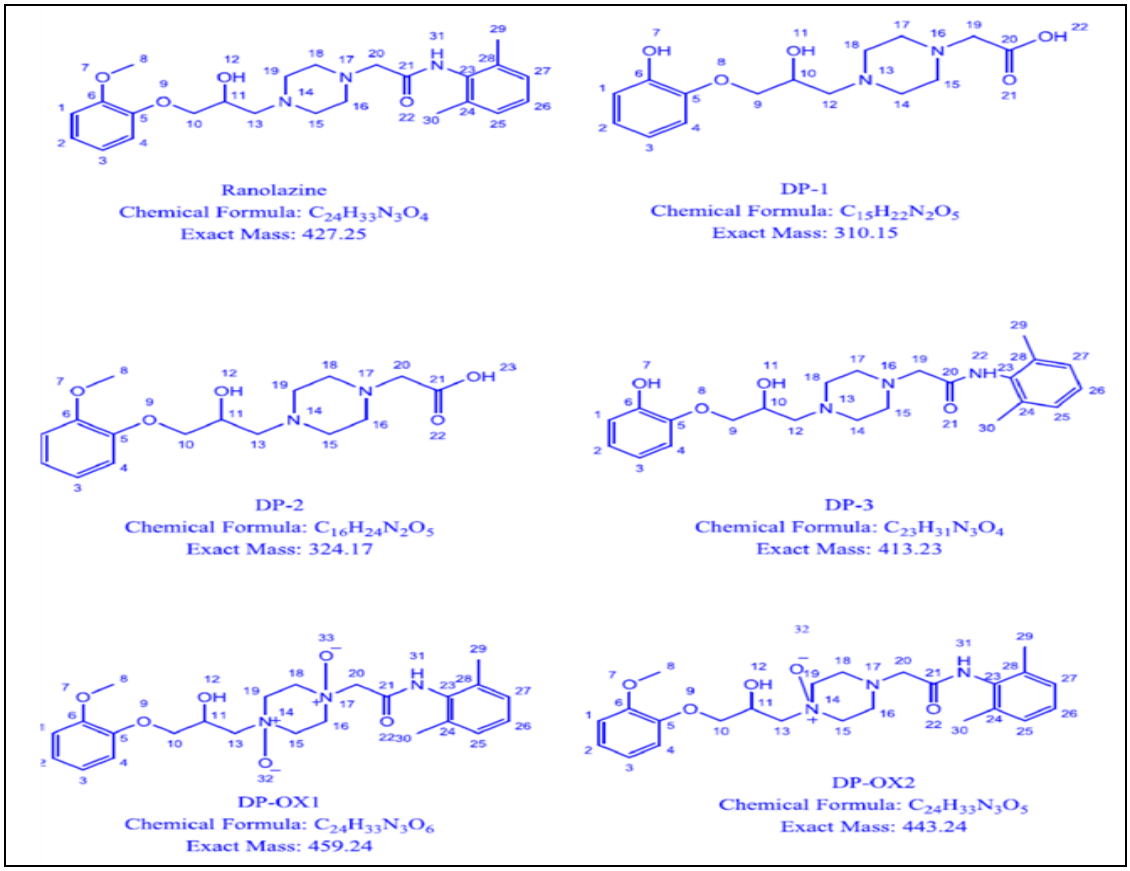

FIG. 2: STRUCTURES OF RANOLAZINE DRUG SUBSTANCE AND ITS DEGRADATION PRODUCTS

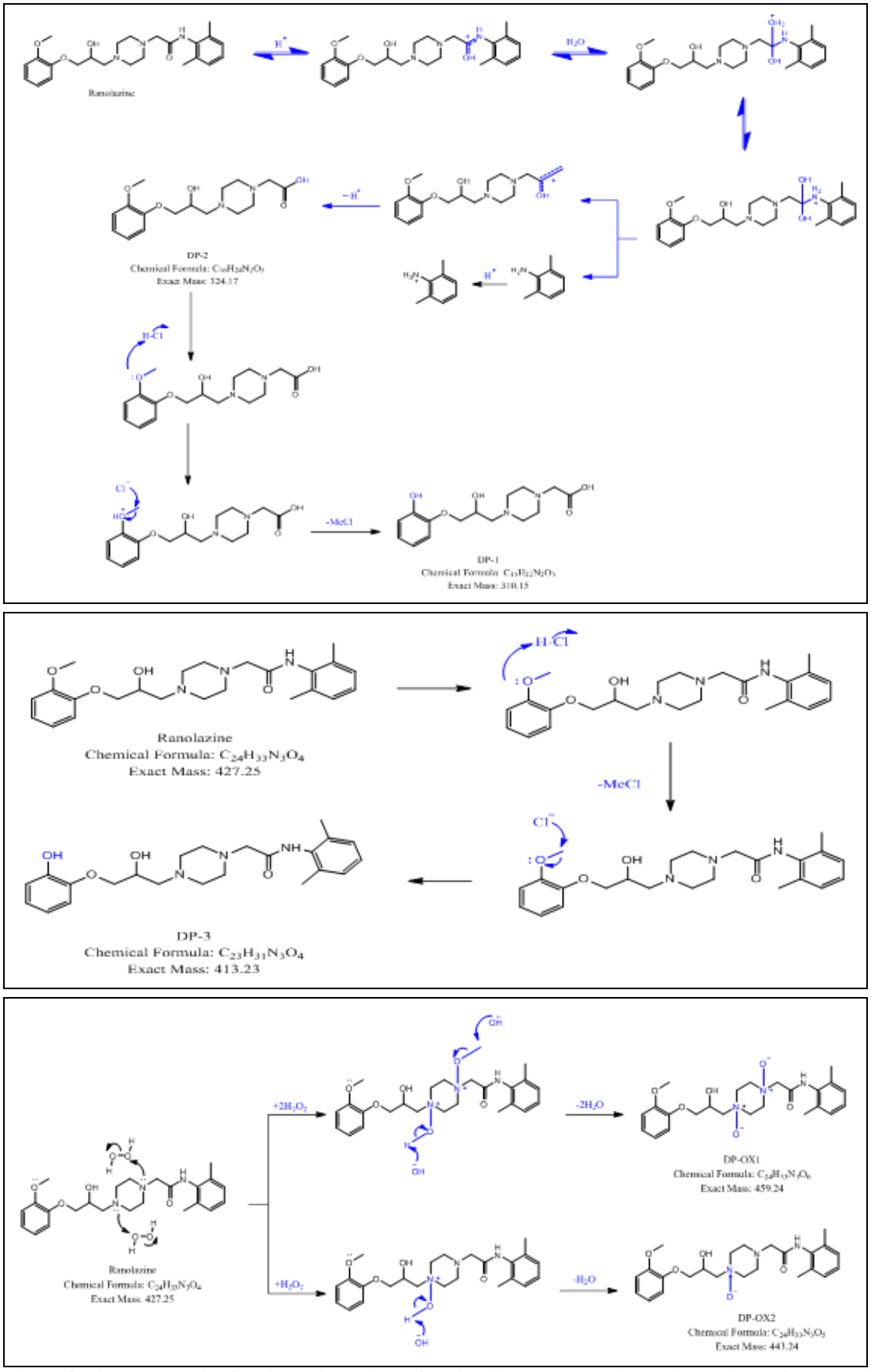

FIG. 3: PLAUSIBLE MECHANISM FOR FORMATION OF DP-1, DP-2, DP-3, DP-OX1, AND DP-OX2

Structure Elucidation of DP-1: The HRMS showed a protonated molecular ion peak at m/z 311.1613 $[M+H]^+$ corresponding to molecular formula $C_{15}H_{22}N_2O_5$. The ^1H NMR spectrum

revealed that the DP-1 had 4 aromatic protons and 15 aliphatic protons. The ^{13}C NMR spectrum showed 7 aromatic carbons and 8 aliphatic carbons. HSQC analysis revealed that there are 5 methines and 7 methylenes in the compound. ^{1}H, ^{13}C chemical shift values were assigned for DP1 as shown in **Table 1** and **Table 2** by correlating COSY, HSQC, and HMBC experimental data. In this compound dimethyl benzene group of drug substance was absent. It was due to the conversion of the amide group to carboxylic acid during acidic hydrolysis. Methoxy group protons of ranolazine drug substance were also absent in DP-1 because methoxy group is demethylated during acidic hydrolysis.

Structure Elucidation of DP-2: The HRMS showed a protonated molecular ion peak at m/z 325.1768 $[M+H]^+$ corresponding to molecular formula $C_{16}H_{24}N_2O_5$. The ^{1}H NMR spectrum revealed that the DP-2 had 4 aromatic protons and 18 aliphatic protons. Exchangeable protons were not observed in ^{1}H NMR due to the presence of moisture. The ^{13}C NMR spectrum showed 7 aromatic carbons and 9 aliphatic carbons. HSQC analysis revealed that there are 5 methines, 1 methyl and 7 methylene in the compound. Proton and carbon chemical shift values of this compound were assigned from the interpretation of COSY, HSQC and HMBC data as shown in **Table 1** and **Table 2**. In this compound dimethyl benzene group and NH proton of Ranolazine drug substance were absent, indicating hydrolytic cleavage of amide group to form carboxylic acid during acidic hydrolysis.

Structure Elucidation of DP-3: The HRMS showed a protonated molecular ion peak at m/z 414.2380 $[M+H]^+$ corresponding to molecular formula $C_{23}H_{31}N_3O_4$. The ^{1}H NMR spectrum revealed that the DP-3 had 7 aromatic protons and 21 aliphatic protons. Exchangeable protons were not observed in ^{1}H NMR due to the presence of moisture. The ^{13}C NMR spectrum showed 13 aromatic carbons and 10 aliphatic carbons.

HSQC analysis revealed that there are 8 methines, 2 methyls and 7 methylenes in the compound. Proton and carbon chemical shift values of this compound were assigned using COSY, HSQC and HMBC data as shown in **Table 1** and **Table 2**. In this compound only methoxy group protons of Ranolazine drug substance were absent, indicating demethylation of a methoxy group to hydroxy group during acidic hydrolysis.

Structure Elucidation Of DP-OX1: The HRMS showed a protonated molecular ion peak at m/z 460.2458 $[M+H]^+$ corresponding to molecular formula $C_{24}H_{33}N_8O_6$ which can be attributed to the reported impurity N- Di-Oxide [14] which mass is 32 mass units higher than drug (Ranolazine) mass 427.25. The ^{1}H NMR spectrum revealed that the DP-OX1 had 24 aliphatic protons and 7 aromatic protons. The ^{13}C NMR spectrum showed 11 aliphatics carbon and 13 aromatic carbons. ^{1}H, ^{13}C chemical shift values for DP-OX1 were assigned by ^{1}H, ^{13}C, COSY, HSQC, and HMBC experiments. The assignments of DP-OX1 are shown in **Table 1** and **Table 2**.

The number of protons and carbons observed were the same as the drug, but significant changes were observed concerning chemical shift values when compared with the drug. 15, 19 position proton chemical shift values went downfield to 3.27, 3.41, 4.19, 4.26 ppm from 2.56 ppm (drug). 16, 18 position proton chemical shift values also went downfield 3.35, 3.5, 4.04, 4.26 ppm from 2.56 ppm (drug). 13, 20 position proton chemical shift values also moved to downfield as shown in **Table 1**. These substantial shifts in proton chemical shift values indicated the presence of oxygen on both 14 and 17 position nitrogens, resulting in deshielding of adjacent protons. 15, 19 and 16, 18 position carbon chemical shift values also moved downfield compared to drug substance as shown in **Table 2**. These carbon chemical shift value changes also indicated the deshielding of carbons from the oxygen present on both 14, 17 position nitrogens as shown in **Fig. 2**.

Structure Elucidation of DP-OX2: The HRMS showed a protonated molecular ion peak at m/z 444.2504 $[M+H]^+$ corresponding to molecular formula $C_{24}H_{33}N_3O_5$ which can be attributed to mono oxygenated compound with 16 mass units higher than drug (Ranolazine) mass 427.25. The ^{1}H NMR spectrum revealed that the DP-OX2 had 24 aliphatic protons and 7 aromatic protons. The ^{13}C NMR spectrum showed 11 aliphatics carbon and 13 aromatic carbons.

TABLE 1: ^{1}H NMR SPECTRAL DATA OF RANOLAZINE AND ITS DEGRADATION PRODUCTS IN DMSO-D_6 AT 25 °C, δ IN PPM, J IN HZ, TMS AT 0 PPM

Proton Assignment Number	Ranolazine	DP-1	DP-2	DP-3	DP-OX1	DP-OX2
1	6.95	6.78	6.96	6.81	7.0	6.99
2	6.88	6.78	6.88	6.81	6.92	6.91
3	6.88	6.72	6.88	6.74	6.87	6.88
4	6.97	6.9	6.96	6.93	6.97	6.97
7				8.22		
8	3.75		3.75		3.77	3.75
9		3.79, 3.94		3.86, 3.96		
10	3.86, 3.95	3.96	3.89	4.09	3.92	3.9
11	3.96		4.0		4.58	4.55
12	4.81	2.41, 2.48		2.64,2.71		
13	2.37, 2.48		2.54, 2.63		3.39,3.73	3.28,3.52
14		2.68		2.77		
15	2.56	2.52	2.89	2.68	3.41,4.26	3.4,3.51
16	2.56		2.72		3.5,4.04	2.75,3.07
17		2.52		2.68		
18	2.56	2.68	2.72	2.77	3.35,4.26	2.82,3.04
19	2.56	3.14	2.89	3.17	3.27,4.19	3.18,3.59
20	3.11		3.31		4.24	3.23
22				9.02		
23						
25, 27	7.06			7.07	7.07	7.08
26	7.06			7.07	7.07	7.08
29, 30	2.13			2.14	2.18	2.14
31	9.15				12.7	9.33

TABLE 2: ^{13}C NMR SPECTRAL DATA OF RANOLAZINE AND ITS DEGRADATION PRODUCTS IN DMSO-D_6 AT 25°C, δ IN PPM REFERENCED TO DMSO-D_6 AT 39.5 PPM

Proton Assignment Number	Ranolazine	DP-1	DP-2	DP-3	DP-OX1	DP-OX2
1	112.4	115.6	112.3	115.7	114.2	114.2
2	120.7	121.5	120.8	121.6	121.4	121.7
3	120.9	119.2	121.1	119.2	120.9	120.9
4	113.6	113.9	113.7	114.1	112.4	112.5
5	148.4	146.7	148.2	146.6	148.1	148.1
6	149.2	146.9	149.7	147	149.3	149.3
8	55.5		55.5		55.6	55.8
9		72.2		72		
10	71.9	66.6	71.7	66	71.3	71.5
11	66.6		66.3		65.1	65.1
12		60.5		60.2		
13	61.2		60.3		68.5	69.3
14		52.4		52.9		
15	53.3	51.9	51.5	52.1	59.4	62.2
16	53.2		51.3		57.4	46.8
17		51.9		52.1		
18	53.2	52.4	51.3	52.9	58.8	47.3
19	53.3	58.9	51.5	61	60.9	65.9
20	61.5	169.7	58.3	167.8	68.2	60.1
21	168		169.3		162.7	168.1
22						
23	135.0			135	134.6	135
24, 28	135.1			135.2	134	135.4
25, 27	127.7			127.7	127.9	127.9
26	126.4			126.5	126.2	126.8
29, 30	18.2			18.3	18.5	18.4

^{1}H, ^{13}C chemical shift values for DP-OX2 were assigned using ^{1}H, ^{13}C, COSY, HSQC, and HMBC experiments. The assignments of DP-OX2 are shown in **Table 1** and **Table 2**. A number of protons and carbons observed were the same as drug molecules except significant changes were pronounced concerning the chemical shift values of few protons and few carbons when compared with the drug. 15, 19 position proton chemical shift values went downfield to 3.18 ppm, 3.4 ppm, 3.51 ppm and 3.59 ppm from 2.56 ppm (drug). 13th position proton chemical shift value went

downfield to 3.28 ppm and 3.52 ppm from 2.37 ppm and 2.48 ppm (drug). These substantial shifts in the proton chemical shift values indicated the presence of oxygen on 14th position nitrogen. 15, 19 position carbon chemical shift values also went downfield to 62.2 ppm, 65.9 ppm from 53.3 ppm (drug) and in a similar fashion 13th position carbon resonance also went downfield compared with the drug as shown in **Table 2**. These changes in the carbon chemical shift values also indicated the presence of oxygen on 14th position nitrogen. HRMS, 1D, 2D NMR data matched with the structure shown in **Fig. 2** for DP-OX2.

CONCLUSION: Three novel degradant products DP-1, DP-2, and DP-3 were formed during acid hydrolysis of Ranolazine and characterized by ^{1}H and ^{13}C NMR data including COSY, HSQC and HMBC experiments. Two degradation products DP-OX1 and DP-OX2 were identified during the oxidative degradation of Ranolazine. DP-OX2 degradant product so far has not been published in the literature. Here, we have deduced the structure of DP-OX2 for the first time. The structures of all the degradants were unambiguously elucidated by HRMS and NMR techniques.

ACKNOWLEDGEMENT: The authors wish to thank the management of GVK Biosciences Pvt. Ltd., for supporting this work.

CONFLICT OF INTEREST: All the authors do not have any conflict of interest.

SUPPLEMENTARY DATA: All the HRMS and NMR spectra are provided as supplementary data.

REFERENCES:

1. Mohammed A, David SF and Amadou KSC: Safety and efficacy of Ranolazine for the treatment of chronic angina pectoris. Clin Med Insights Ther 2013; 5: 1-4.
2. Banon D, Filion KB, Budlovsky T, Franck C and Eisenberg MJ: The usefulness of Ranolazine for the treatment of refractory chronic stable angina pectoris as determined from a systematic review of randomized controlled trails. Am J Cardiol 2014, 113: 1075-82.
3. /http://www.en.wikipedia.org/wiki/RanolazineSAndNeurolex.org/wiki/Category:Ranolazine.
4. ICH, Stability testing of new drug substances and products Q1A(R2), in: International Conference on Harmonisation, IFPMA, Geneva, 2003.
5. WHO, Draft Stability Testing of Active Pharmaceutical Ingredients and Pharmaceutical Products, World Health Organization, Geneva, 2007.
6. CPMP, Note for Guidance on Stability Testing: Stability Testing of Existing Active Substances and Related Finished Products, Committee for Proprietary Medicinal Products, EMEA, London, 2002.
7. Bakshi M and Singh S: Development of validated stability indicating assy method critical review. J Pharm Biomed Anal 2002, 28: 1011-40.
8. Sharma T, Moitra SK, Sudam C SI and Sankar DG: Stability indicating LC Method for the determination of Ranolazine HCl in the bulk drug and in pharma-ceutical dosage form. Int J Pharm Pharm Sci 2011; 3: 327-32.
9. Khedkar AN, Veer SU, Rakh MS and Rao JR: Stability indicating method development and validation of ranolazine hydrochloride in bulk and tablet dosage form by HPTLC. Int J Pharm Clin Res 2015; 7: 77-83
10. Tapas LK, Dibya PR and Subrata S: A validated stability indicating Reversed Phase High Performance Liquid Chromatographic method of Ranolazine dihydrochloride and characterization of ITS degradation products. Int J Pharm Pharm Sci 2013; 5: 61-66.
11. Vakamulla M, Anumala RR, Mukkanti K and Suryanarayana MV: A novel reverse phase stability indicating RP-UPLC method for the quantitative determination of fifteen related substances in Ranolazine drug substance and drug product. Talanta 2012; 97: 563-73.
12. Virpul P and Saurabh P: Spectrophotometric and stability indicating RP-HPLC method for estimation of Ranolazine in tablet dosage form. Int J Pharmaceutical Sciences and Research 2018; 9: 1218-25.
13. Gerald MP, Heewon K, Gabriel V, Laurn C, Hooman M, Jeffrey H and Norman L: Impact of Ranolazine on myocardial metabolic ischemia detected by phosphorus-31 Magnetic Resonance Spectroscopy. Journal of Cardiovascular Magnetic Resonance 2016; 18: 97.
14. Saminder SK, Zubin A and Craig N: Ranolazine and hallucinations. Journal of Medical Case 2017; 8: 90-92.
15. Mezincescu A, Karthikeyan VJ and Sunil KN: Ranolazine: A true pluripotent cardiovascular drug or jack of all trades, master of none? Sultan Qaboos University Medical Journal 2018; 18: 1.
16. Patel VD and Raj H: Ranolazine: A review on analytical method and its determination in the synthetic mixture. International Journal of Pharmaceutical Research & Allied Sciences 2016; 5: 1-7.

How to cite this article:
Guduru S, Mutha VVSRNAK, Vijayabhaskar B, Narkedimilli J, Kaliyaperumal M, Korupolu RB, Bonige KB and Rumalla CS: Isolation and structural elucidation of degradation products of Ranolazine. Int J Pharm Sci & Res 2019; 10(8): 3763-69. doi: 10.13040/IJPSR.0975-8232.10(8).3763-69.