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Structural Elucidation of Novel Degradation Products of Pioglitazone by Nuclear Magnetic Resonance Spectroscopy and High Resolution Mass Spectrometry

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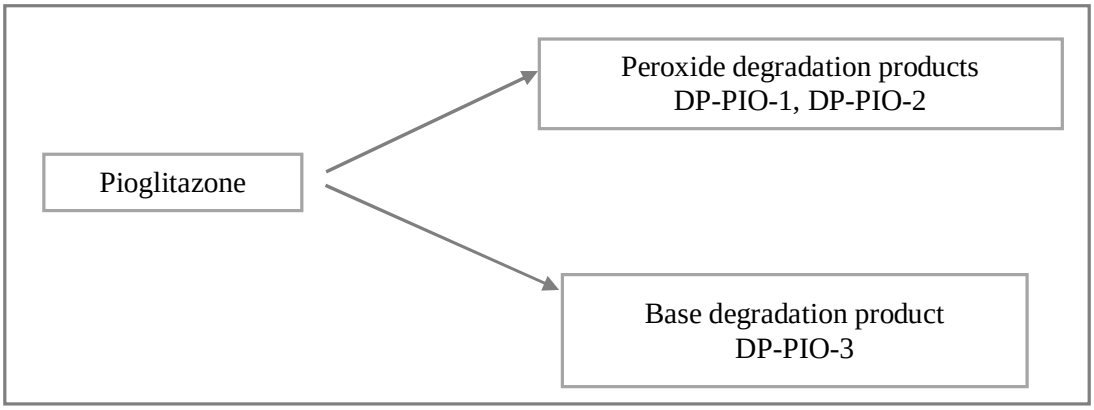
ABSTRACT

Pioglitazone hydrochloride is an anti-diabetic agent and it is used to treat type 2 diabetes. It is a class of thiazolidinediones and it was subjected to stress degradation under acidic basic and peroxide hydrolysis according to ICH guidelines-stability testing of new drug substances and products Q1A(R2). The thiazolidinediones group was cleaved and rearranged under base and peroxide mediated hydrolysis and stable in acidic hydrolysis conditions. Two major degradants referred as DP-PIO-1, DP-PIO-2 were formed during peroxide hydrolysis and one degradant which is referred as DP-PIO-3 was formed in base catalyzed hydrolysis. These impurities were isolated by using preparative HPLC, DP-PIO-1 and DP-PIO-2 are novel degradants and structures were elucidated by Nuclear Magnetic Resonance Spectroscopy (1D,2D NMR) along with High Resolution Mass Spectrometry.

Graphical Abstract



Keywords: Pioglitazone degradation products, Structure elucidation, HRMS, NMR.

INTRODUCTION

Pioglitazone hydrochloride is an anti-diabetic agent and it is thiazolidinedione class of drug [**1**], used to treat type 2 diabetes mellitusand it activates the nuclear peroxisome proliferator activated receptor which increases insulin sensitivity in liver and skeletal muscle cells [**2-4**]. Pioglitazone possess one chiral centre and IUPAC nomenclature is 5-(4-(2-(5-ethylpyridin-2-yl)ethoxy)benzyl)thiazolidine-2,4-dione. The drugs can undergo physicochemical degradation during manufacturing and storage and sometimes loss of activity. Complete knowledge of API's stability profile is essential to prevent those risks during manufacturing and storage condition. The object of the present study is to know the degradation behaviour of pioglitazone under the stress conditions and the stability study was performed as per ICH guidelines and other regulatory authorities [**5-8**].

There are few reports available on characterisation and degradation of pioglitazone [**9, 11**] and the present study two novel degradants were identified. The structures have been confirmed on the basis of Nuclear Magnetic Resonance spectroscopy(1D,2D-NMR),High Resolution Mass Spectroscopy and the degradants were identified asDP-PIO-1 (3-(4-(2-(5-ethylpyridin-2-yl)ethoxy)phenyl)propanoic acid), DP-PIO-2 (2-(2-(4-(3-amino-3-oxo-2-sulfinopropyl)phenoxy)ethyl)-5-ethylpyridine 1-oxide), DP-PIO-3(3-(4-(2-(5-ethylpyridin-2-yl)ethoxy)phenyl)-2-mercaptopropanoicacid).

MATERIALS AND METHODS

Chemicals and Reagents: Pioglitazone was a gifted sample from an API unit in Hyderabad. Solvents and chemicals used for the experiment were HPLC grade water filtered with 0.25µ filter, Acetonitrile (Rankem),Methanol(Merck),Formicacid(Merck),Trifluoroaceticacid(Merck),Ammoniumbicarbonate(Fisher scientific),DMSO-d6(Cambridge isotope limited).

Liquid Chromatography-High Resolution Mass Spectrometry (HRMS): Mass accuracy was measured with Q-TOF micro mass instrument equipped with MCP detector and Electrospray ionization source. The optimum conditions are desolvation gas flow 650 L h^{-1}, capillary voltage 3000v, conevoltage 30v, MCP voltage 2800v, resolution 5000. Leucine Enkephalin (556.2771Da $[M+H]^+$) was used to lock mass correction, mass accuracy (in ppm) was calculated with Masslynx software.

Column: Kinetex C-18 2.1 x 50 mm 1.7µm

Mobile Phase: A-0.1% formic acid in water, B-0.1% formic acid in acetonitrile.

Gradient: (Time/%of A) 0/97,5/5,5.5/5,6/97.

Flow rate: 0.6mL min^{-1}, Column Temp 35°C.

Automated Mass mediated Purification System: Purification was performed with Waters Preparative HPLC system and it was coupled with 2545 binary gradient module, 2767 sample manager, 515 make up pump, automated fraction collector and QDA mass detector.

Column: X select CSH C-18 19 x 250 mm 5 µm.

Mobile phase: A-10mM Ammonium bicarbonate in water, B-Acetonitrile

Gradient: (Time/% of B) 0/40, 12/90, 15/98, 17/98,20/40

Flow rate: 19 mL min^{-1}, wavelength at 230nm, Ionization mode ES+.

Nuclear Magnetic Resonance Spectroscopy: Pioglitazone degradation impurities were recorded on Bruker 400 MHz resolution NMR instrument and it was coupled with Broad Band Observe Probe, auto sampler. The data was processed with Bruker top spin software, solvent was used for the experiment DMSO-d6 and tetra methyl silent (TMS) used as internal standard.

Stress methods: The stress conditions base catalyzed hydrolysis and oxidation were carried out as per ICH guideline Q1A (R2), 5% hydrogen peroxide solution was used for peroxide mediated hydrolysis and refluxed for 12h at 60°C, the formation of degradant percentage was very low and the reflux continued to 48 h at 60°C, 1N sodium hydroxide solution was used for base hydrolysis and refluxed for 24 h.

RESULTS AND DISCUSSION

The formation of Pioglitazone degradation impurities was monitored with LC-MS (method details as mentioned in 2.2), 1 mL of the pioglitazone reaction mass was diluted with mobile phase initial composition and 5 µL was injected in to the LC-MS system. Two major degradants were identified in peroxide hydrolysis, one degradant was identified from base hydrolysis (Figure 1) and identified degradants were taken for separation.

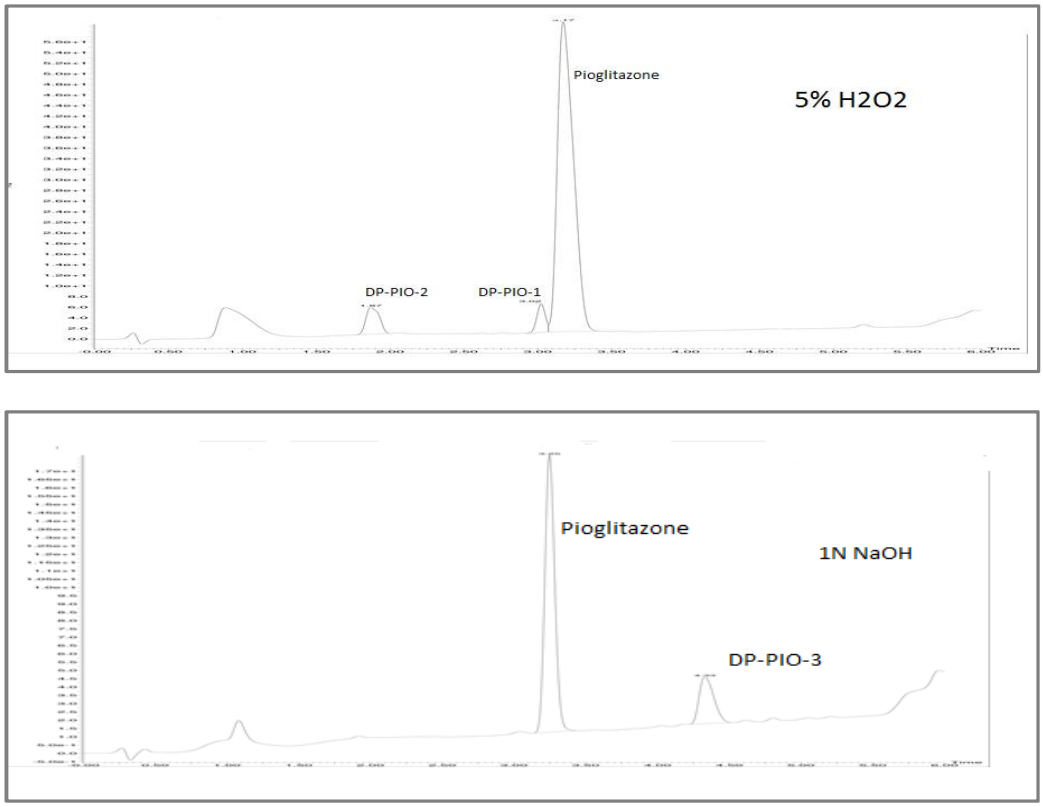


Figure 1. Chromatograms of Peroxide,base mediated degradation.

Isolation of peroxide, base degradation products: The degradation products were isolated through automated purification system as mentioned in the method details in section 2.3and the resulting fractions were collected separately. Individual fractions were lyophilized to remove the mobile phase. Degradation products resulting from the fractions were assigned as DP-PIO-1, DP-PIO-2, DP-PIO-3 and characterized by high resolution mass spectroscopy, NMR of the above degradants only DP-PIO-3 was the known degradant whereas DP-PIO-1 and DP-PIO-2 are completely novel and to our knowledge they have not been reported in the literature. The proposed structures were shown in figure 2.

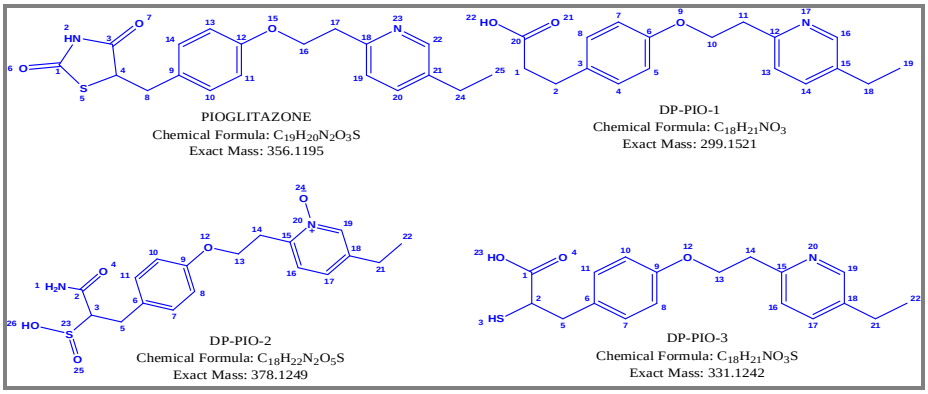


Figure 2. Chemical structures of Pioglitazone and it's degradation products.

Reaction mechanism: The probable reaction mechanism for formation of degradation products was shown in figure 3, 4, 5.

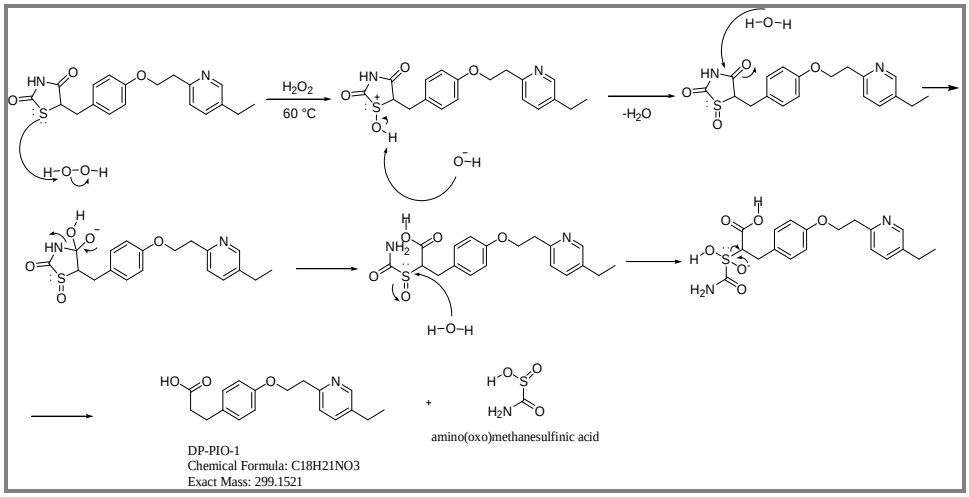


Figure 3. Probable Reaction mechanism for DP-PIO-1.

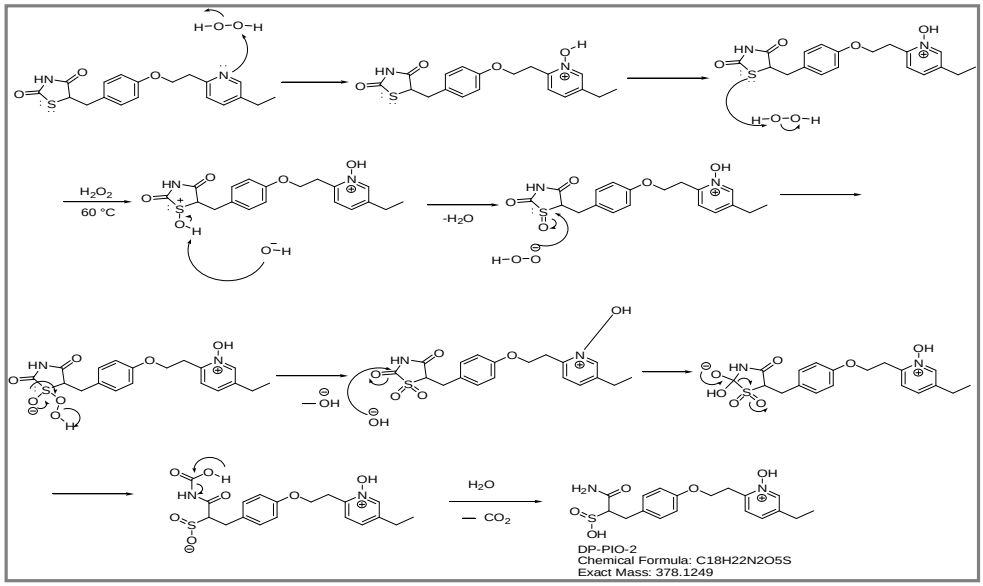


Figure 4. Probable Reaction mechanism for DP-PIO-2.

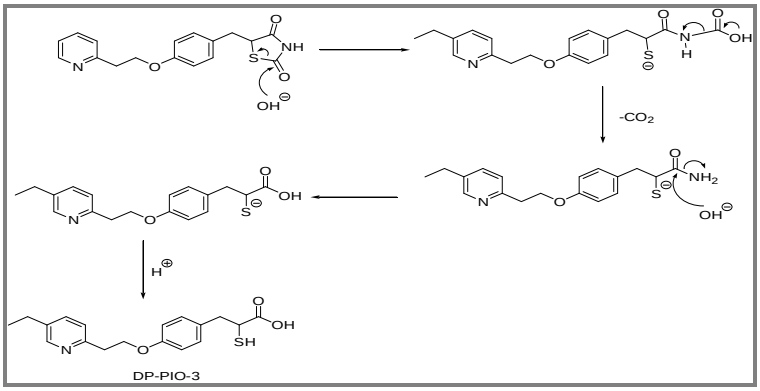


Figure 5. Probable Reaction mechanism for DP-PIO-3.

Structure elucidation of DP-PIO-1: The mass spectrum of DP-PIO-1 showed a protonated molecular ion peak 300.1605 $[M+H]^+$ and protonated molecular formula $C_{18}H_{22}NO_3$ was obtained from HRMS with below 2 ppm error. The HRMS spectrum of DP-PIO-1 was shown in figure 6.

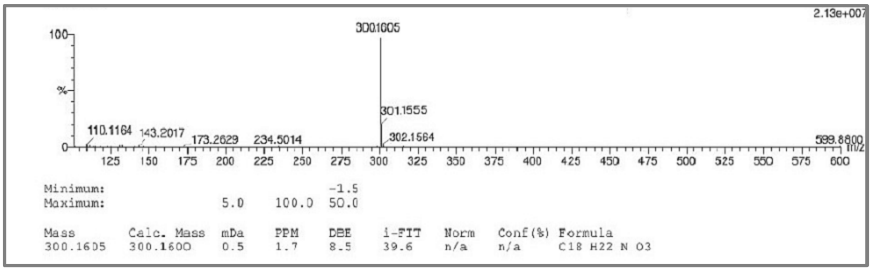


Figure 6. HRMS spectrum of DP-PIO-1.

DP-PIO-1 possesses 7 aromatic protons and 13 aliphatic protons. Thiazolidine-2,4-dione ring protons of Pioglitazone were absent in DP-PIO-1. ^{13}C NMR data revealed that DP-PIO-1 had 12 aromatic carbons and 6 aliphatic carbons. HSQC experiment was provided the information that DP-PIO-1 had one methyl group, 5 methylene groups and 7 methynes. In COSY Experiment, H-2proton (2.72 ppm) correlated with H-1 proton (2.42 ppm). This correlation indicated that H-2 and H-1 protons are coupled to each other and both H-1 and H-2 protons are adjacent to each other. In HMBC Experiment,

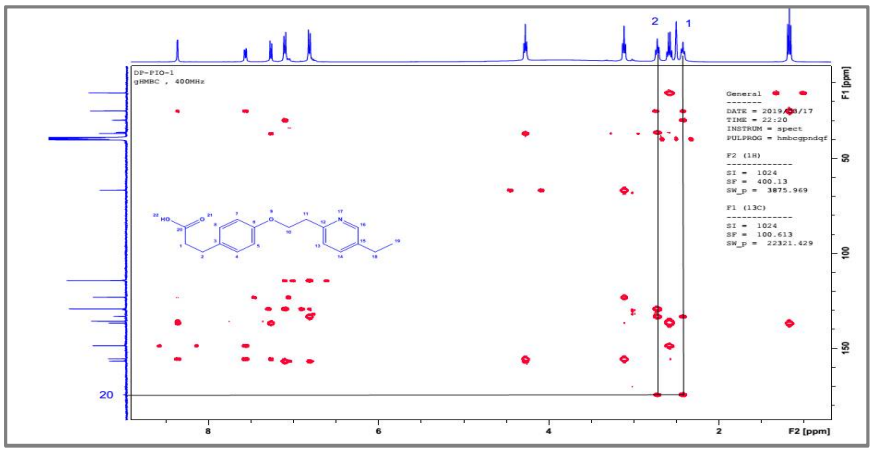


Figure 7. HMBC Spectrum of DP-PIO-1.

H-1 proton (2.42 ppm) and H-2 proton (2.72 ppm) correlated with 20th position acid carbonyl carbon at 174.3 ppm as shown in figure 7. This main key proton versus carbon correlation in HMBC supports structure of DP-PIO-1 as shown in figure 2.

Structure elucidation of DP-PIO-2: The mass spectrum of DP-PIO-2 showed a protonated molecular ion peak 379.1325[M+H]$^+$ and protonated molecular formula $C_{18}H_{23}N_2O_5S$ was confirmed by HRMS. The HRMS spectrum of DP-PIO-2 was shown in figure 8.

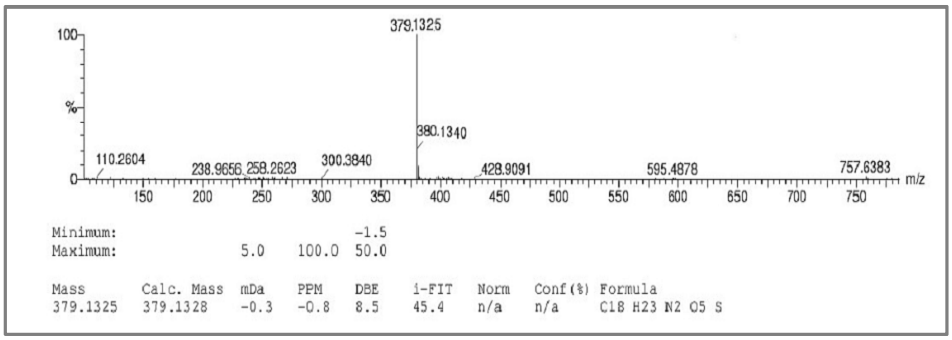


Figure 8. HRMS spectrum of DP-PIO-2.

DP-PIO-2 had 7 aromatic protons, 12 aliphatic protons and two amide protons. Amide protons were observed at 6.75 ppm and 7.07 ppm. Thiazolidine-2,4-dione ring protons were found to be absent in DP-PIO-2. DP-PIO-2 was N-oxide compound and oxygen attached on 20th position. It was confirmed by ^{1}H NMR and ^{13}C NMR. In 1H NMR experiment pyridine ring protons of DP-PIO-2 was moved to up field when compared to pioglitazone hydrochloride pyridine ring protons and in similar way in ^{13}C NMR, pyridine ring carbon chemical shift values drastically changed when compared to pioglitazone hydrochloride pyridine ring carbons. These changes in the chemical shift values of pyridine ring in 1H NMR and ^{13}C NMR of DP-PIO-2 compared to drug supported that oxygen was attacked on 20th position nitrogen as shown in figure 2. In COSY experiment H-3proton (3.36 ppm) correlated well with H-5(3.02 ppm). In HMBC Experiment, H-3proton (3.36 ppm) and H-5 proton (3.02 ppm) correlated with both C-2(170.1 ppm) and C-6(131.8 ppm). The HMBC spectrum of DP-PIO-2 was shown in figure 9.

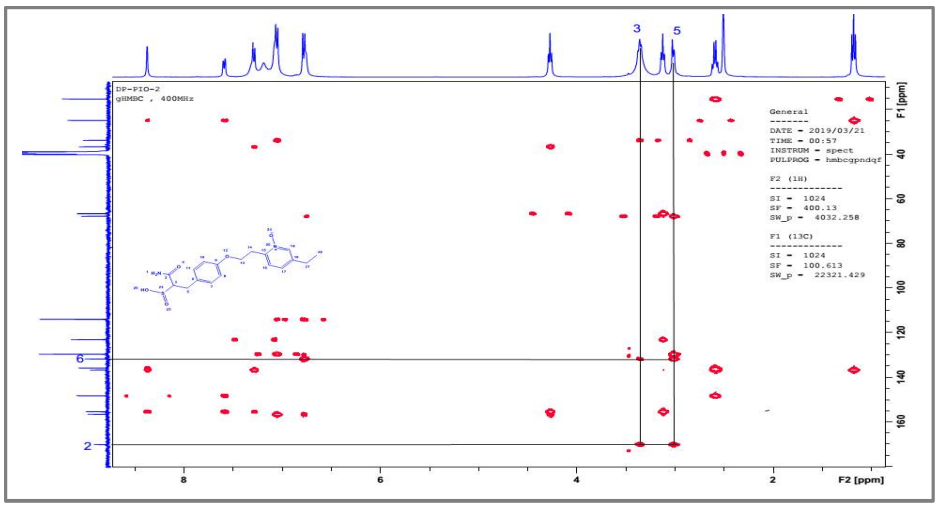


Figure 9. HMBC spectrum of DP-PIO-2.

These main key proton versus carbon correlation in HMBC supporting to structure of DP-PIO-2 as shown in figure 2.

Structure elucidation of DP-PIO-3: The mass spectrum of DP-PIO-3 showed a protonated molecular ion peak at 332.1323[M+H]$^+$ and protonated molecular formula $C_{18}H_{22}NO_3S$ was obtained from HRMS with below 1 ppm error. The HRMS spectrum of DP-PIO-3 was shown in figure10.

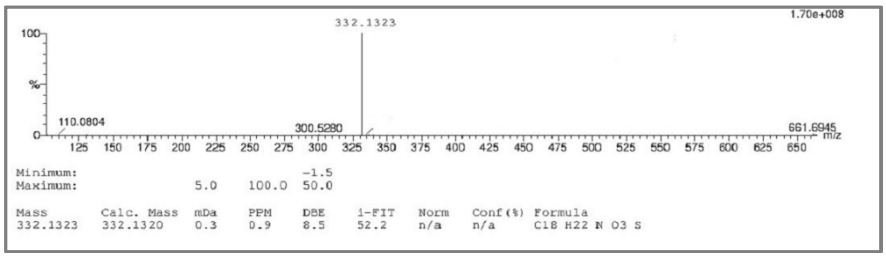


Figure 10. HRMS spectrum of DP-PIO-3.

The key correlation in HMBC supporting to structure of DP-PIO-3 as shown in figure 11.

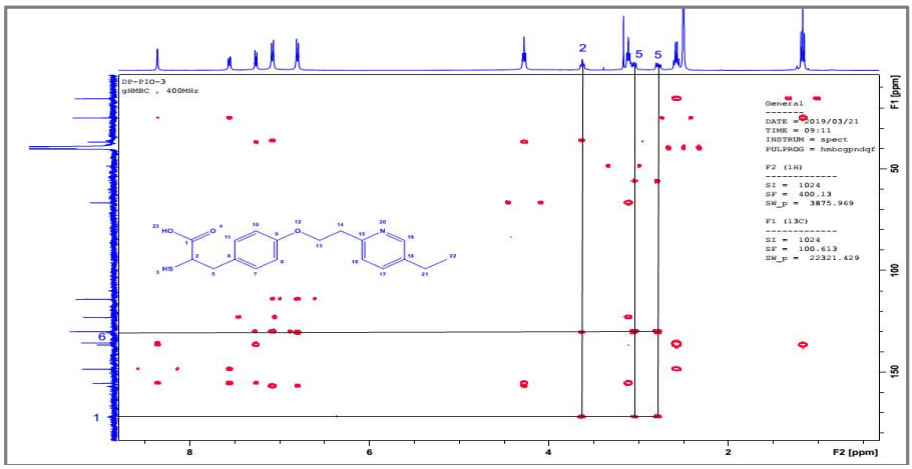


Figure 11. HMBC spectrum of DP-PIO-3.

^{1}H NMR, ^{13}C NMR Spectral data of Pioglitazone and its degradation products were shown in table 1.

Table 1. ^{1}H NMR, ^{13}C NMR Spectral data of Pioglitazone and its degradation products in ppm.

Assignment	Pioglitazone		DP-PIO-1		DP-PIO-2		DP-PIO-3	
	1H	13C	1H	13C	1H	13C	1H	13C
1	--	171.6	2.42	36.2	6.75,7.07	--	--	172.1
2	12	--	2.72	29.8	--	170.1	3.63	55.6
3	--	175.6	--	133.2	3.36	67.8	--	--
4	4.87	52.9	7.1	129.2	--	--	--	--
5	--	--	6.81	114.2	3.02	33.8	2.78,3.01	36
6	--	--	--	156.7	--	131.8		130.4
7	--	--	6.81	114.2	7.1	129.6	7.08	130
8	3.06,3.29	36.2	7.1	129.2	6.78	114	6.81	114.1
9	--	129.1	--	--	--	156.6		156.9
10	7.15	130.4	4.27	66.7	6.78	114	6.81	114.1
11	6.87	114.4	3.12	36.8	7.1	129.6	7.08	130
12	--	156.9	--	155.6	--	--	--	--
13	6.87	114.4	7.27	123	4.27	66.6	4.3	66.7
14	7.15	130.4	7.56	135.7	3.12	36.7	3.1	36.8
15	--			136.6		155.4		155.5
16	4.38	65.4	8.36	148.5	7.28	123.1	7.27	123
17	3.45	32.4	--	--	7.6	136	7.56	135.6
18	--	151.3	2.58	24.9	--	136.7		136.6
19	7.93	127	1.17	15.4	8.37	148.3	8.4	148.5
20	8.37	145	--	174.3	--	--	--	--
21	--	141.2	--	--	2.6	24.9	2.58	24.9
22	--	140.3	--	--	1.17	15.4	1.17	15.4
23	--	--	--	--	--	--	--	--
24	2.77	24.6	--	--	--	--	--	---
25	1.23	14.6	--	--	--	--	--	--

APPLICATION

The Pioglitazone stress degradation studies provides degradation pathway ,degradation products and stress degradation studies show the chemical behavior of the molecule which in turn helps in the development of formulation and package.

CONCLUSION

Three major degradation products were identified in pioglitazone during peroxide and base hydrolysis, two impurities are unknown (DP-PIO-1, DP-PIO-2) and one known impurity (DP-PIO-3) were isolated. These impurities were separated with automated purification technique and characterized by using NMR, HRMS.

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Conflict of interest: All authors declare that they have no conflict of interest.

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

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