DEGRADATION STUDY OF PIMAVANSERIN: IDENTIFICATION, ISOLATION AND STRUCTURAL CHARACTERIZATION OF DEGRADANTS

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ABSTRACT

Pimavanserin (PMV) is a novel antipsychotic drug that is used for the treatment of Parkinson's disease and psychosis. PMV stability is characterized by subjecting it to systematic forced degradation in various stress conditions such as acid, base, peroxide, UV-light and heat as per ICH guidelines. Three PMV degradants were identified, one each from acid, base and peroxide catalysed degradation. All the three PMV degradants were isolated on C18 column by using preparative HPLC with gradient elution method and labelled as DP-1, DP-2 and DP-3, and their structures were elucidated by mass and 2D NMR spectral analysis. DP-1 was identified as bis (4-isobutoxyphenyl) methane, DP-2 was identified as 1,3-bis(4-isobutoxybenzyl)urea and DP-3 was identified as N-oxide product of PMV. Interestingly, both acid and base degradation products of PMV (DP-1 and DP-2) contain Iso butoxy benzyl amine portion of the drug which is stable, and the remaining fragment of PMV is sensitive to stress. PMV degradation products, DP-1 and DP-2 were confirmed as dimers, and DP-3 as N-oxide product resulting from the peroxide mediated oxidation. Furthermore, PMV was found to be stable for 48 hrs when exposed to UV-light and subjected to 70 °C.

Keywords: Pimavanserin, Degradation Products, UPLC-MS, NMR.

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INTRODUCTION

Pimavanserin (PMV) is an atypical antipsychotic drug, approved in the year 2014 by FDA for the treatment of delusions and hallucinations with psychosis, affected with Parkinson's disease¹. PMV, unlike other atypical antipsychotics, does not act through dopamine receptor antagonism but acts as an inverse agonist and antagonist of serotonin (5HT_{2A}) receptors^{2,4}. PMV is also being studied for the treatment of schizophrenia, depression and agitation. During PMV synthesis, two process impurities were identified and reported as 1, 3-bis (4-isobutoxybenzyl) urea and N-oxide (4-(1-(4-fluorobenzyl)-3-(4-isobutoxybenzyl) ureido)-1-methylpiperidine 1-oxide)^{5,6}. Koduri *et.al.* studied and reported the stability of PMV in bulk and tablet dosage form using HPLC without much attention to the structural characterization of degradation products⁷. Shixiao Wang *et.al* has developed UPLC-MS/MS Method for Determination of Pimavanserin Tartrate in Rat Plasma⁸. To the best of our knowledge, a detailed PMV stability study was not reported in the literature. Hence, in the present research, we conducted a systematic PMV stability study for the first time as per the ICH guidelines⁹⁻¹⁰, and identified the degradation products by UPLC-MS, isolated by preparative HPLC and further degradant structures were

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elucidated by 2D NMR. PMV acid and base degradation products contain Iso butoxy benzyl amine portion of the drug which is stable, and the remaining fragment of PMV is sensitive to stress.

EXPERIMENTAL

Materials and Methods

Pimavanserin (PMV) drug substance (API) was a kind gift from SMS Pharmaceuticals Limited, R&D center, Hyderabad, Telangana state, India. Acetonitrile and Formic acid were procured from Merck, USA. Dimethyl sulfoxide-d₆ containing 0.03 % (v/v) of TMS was purchased from Cambridge Isotope Ltd., UK. Milli-Q water is collected from the in-house Millipore instrument. All reagents used are of the highest purity.

Pimavanserin Degradation Study

PMV degradation study was conducted as per ICH guidelines⁹⁻¹⁰ and details of stress conditions are summarized in Table-1. In brief, for acid hydrolysis, 500 mg of PMV was solubilized in 5.0 mL of 0.5 N Hydrochloric acid solution and refluxed for 12 h at RT. For base degradation, 500 mg of PMV was solubilized in 5.0 mL of 0.5 N Sodium hydroxide solution and refluxed for 12 hr at RT. For oxidative degradation, 500 mg of PMV was solubilized in 5.0 mL of 30% H₂O₂ and refluxed for 48 hr at RT. For heat stress, 500 mg of PMV was kept in an oven at 70 °C for 48 hr. The resultant degradation solutions were diluted in 100 mL of water:acetonitrile::50:50. For photolytic stress, 500 mg of PMV was kept under UV light for 48 hr. The resultant degradation solution was diluted in 100 mL of water:acetonitrile::50:50.

Liquid Chromatography-Mass Spectrometry (UPLC-MS)

PMV acid degradation study samples were analyzed by using Thermo Ion trap mass analyzer (LCQ FLEET) and alkali, oxidative, UV and heat degradation study samples were analyzed on Waters UPLC-MS instrument with a single quadrupole mass analyzer.

PMV acid degradation study samples along with the freshly prepared sample with identical treatment were diluted with water:acetonitrile:: 50:50 and injected on to the Acquity column; BEH C18 (2.1 mm x 50 mm x 1.7μ) and the peaks were resolved using gradient flow method with mobile phase A: 0.1% Formic acid in water and mobile phase B: 0.1% Formic acid in acetonitrile, % B: 0/3, 0.3/3, 2.8/98, 4.5/98, 4.51/3, 5.5/3. And mass parameters are with ion source ESI mode, and source parameters capillary voltage: 15V, cone voltage: 3.5KV, source temp: 380°C, sheath gas: 40 L/hr, aux gas: 8L/hr. the sample was analyzed in a negative mode.

PMV alkali, oxidative, UV and heat degradation study samples along with freshly prepared samples with identical treatment were diluted with water: acetonitrile::50:50 and injected on to the X-Bridge C18 column (3.0 mm x 50 mm x 2.5μ) and the peaks were resolved using gradient flow method with mobile phase A: 10 mM ammonium acetate in water and mobile phase B: acetonitrile, %B: 0/5, 3/100, 4.5/100, 4.7/5, 5.5/5. And mass parameters, Waters SQD with ion source ESI mode, and various source parameters capillary voltage: 3.5V, cone voltage: 50V source temp: 359°C, desolvation gas: 750 L/hr, cone gas: 50L/hr samples were analyzed in positive mode.

Preparative HPLC

PMV acid, base, and oxidative degradation crude substance was diluted in a mixture of water and acetonitrile (50:50), degradant peaks were collected by Gilson preparative HPLC equipped with module 331&332, diode array detector at 215 & 254 nm, Kromasil C18 column (19mm x150 mm x 5.0 μ), mobile phase A: 10 mM ammonium bicarbonate in water and mobile phase B: acetonitrile with gradient elution method, %B: 0/10, 12/85, 17/85, 18/10, 20/10 at a flow of 16 mL/min.

Nuclear Magnetic Resonance (NMR) Spectroscopy

PMV acid, base, and oxidative degradation products, 5mg of each were solubilized in 0.6 mL of Dimethyl sulfoxide-d₆ and NMR data was recorded with a 500 MHz instrument equipped with Broadband observe probe (BBO). ¹H, ¹³CNMR chemical shifts were recorded on δ scale in ppm with respect to tetramethylsilane (TMS) as an internal reference.

RESULTS AND DISCUSSION

In the present study, PMV is subjected to acid, base, oxidative, UV and heat stress as per ICH guidelines⁹⁻¹⁰. PMV is stable in heat and photolytic conditions. PMV acid, base and peroxide mediated degradation peaks were resolved and analyzed by UPLC-MS. PMV acid degradation product eluted at retention time 4.26 min and it is labeled as DP-1, (Fig.-1 and Table-2). PMV base and oxidative degradation products eluted at RT 3.48 min and 2.60 min and labeled as DP-2 and DP-3, respectively (Fig.-1 and Table-2). The mass of DP-1, DP-2 and DP-3 were found to be m/z 311.31 in negative mode, m/z 385.56 in positive mode and m/z 444.58 in positive mode (Fig.-2), respectively. For structural characterization, PMV is subjected to acid, base and oxidative stress conditions, and degradation peaks were isolated by preparative HPLC and lyophilized to obtain solid compound. All the three degradation products (DP-1, DP-2 and DP-3) are characterized by LC-MS, 1D and 2D NMR analysis and their structures are illustrated in (Fig.-3)

Fig.-1: Acid, Base, and Hydrogen Peroxide Degradation Chromatogram of Pimavanserin

Fig-2: Acid, Base, and Hydrogen Peroxide Degradation Mass Spectrum of Pimavanserin

Fig-3: Chemical Structures of Pimavanserin and its Degradation Products

Table-1: Acid, Base, Oxidative, Heat and Photolytic Stress Degradation Conditions

Stress Methods	Degradation Conditions	Degradation Study at	Time
Hydrolysis	Acid- 0.5 N HCl	Room Temperature /stirring	16 hours
Hydrolysis	Base- 0.5 N NaOH	Room Temperature /stirring	16 hours
Oxidation	30% Hydrogen Peroxide	Room Temperature /stirring	24 hours
Dry Heat	Exposed to	70 °C	2 days
Photolytic	Exposed to	Near UV lamp	2 days

Table-2: Percent Pimavanserine and its Acid, Base and Oxidative Degradation Products

Degradation method	% Pimavanserine Recovered	Degradation product DP-1 (%) at RT 4.26 min	Degradation product DP-2 (%) at RT 3.48 min	Degradation product DP-3 (%) at RT 2.60 min
Acid	75.98	24.02		
Base	85.33		14.63	
Oxidative	87.99			11.84
Thermal	-	-	-	-
Photolysis	-	-	-	-

DP-1 Structural Confirmation

PMV acid degradation product, DP-1 mass spectrum revealed a deprotonated molecular ion peak with m/z 311.31 in negative mode, corresponding to molecular formula $C_{21}H_{28}O_2$. 1H NMR spectra of DP-1 revealed six proton signals, two aromatic and 4 aliphatic. Proton NMR data of DP-1 showed 1-isobutoxy-4-methylene benzene ring protons, similar to PMV with the exclusion of (1-(4-fluorobenzyl)-1-(1-methylpiperidin-4-yl) urea). This suggests that during acid hydrolysis of PMV, C-12 and NH-13 bond is cleaved (Fig.-3). ^{13}C NMR spectrum of DP-1 showed 8 carbon signals, 4 aromatic and 4 aliphatic, in contrast to 28 signals in PMV. The mass spectrum of DP-1 confirms the presence of 21 carbon atoms, suggesting dimerization, which is confirmed by 2D NMR. 1H , ^{13}C ppm values of DP-1 are presented in Table-3. Further, carbon-hydrogen connectivity's in DP-1 is confirmed by HMBC (Fig.-4).

Fig.4: HMBC spectrum of DP-1

DP-2 Structural Confirmation

PMV base degradation product, DP-2 mass spectral data revealed a molecular ion peak with m/z 385.56 $[M+H]^+$ in positive mode, confirms to chemical formula $C_{23}H_{32}N_2O_3$. 1H NMR spectra of DP-2 revealed 7 proton signals, 3 aromatic and 4 aliphatic. A comparison of 1H NMR data of DP-2 with PMV revealed the presence of only 1-(4-isobutoxybenzyl) urea protons. This suggests bond cleavage between N-15, C-17

and N-15, C-19 positions of PMV (Fig.-3). DP-2 ^{13}C NMR shows 9 carbon signals, 5 aromatic and 4 aliphatic in contrast to 28 signals in PMV. The mass spectrum of DP-2 confirms the presence of 23 carbon atoms, suggesting dimerization, which is confirmed by 2D NMR. ^1H , ^{13}C ppm values of DP-2 are presented in Table-3. Further carbon-hydrogen connectivity's in DP-2 is confirmed by HMBC (Fig.5).

Fig.5: HMBC spectrum of DP-2

DP-3 Structural Confirmation

PMV oxidative degradation product, DP-3 mass spectrum revealed a protonated molecular ion peak with m/z 444.58 $[\text{M}+\text{H}]^+$ confirms to molecular formula $\text{C}_{25}\text{H}_{34}\text{FN}_3\text{O}_3$. DP-3 mass (m/z 444.58) is 16units higher than PMV mass (m/z 427.26), suggesting peroxide mediated oxidation. DP-3 ^1H NMR spectrum revealed 25 aliphatic hydrogens, 8 aromatic and one exchangeable amide hydrogen. ^{13}C NMR spectrum revealed 12 aliphatic carbons and 13 aromatic carbons. ^1H , ^{13}C chemical shift values for DP3 were shown in Table-3. The number of proton and carbon signals in DP-3 and PMV are similar, with comparative drastic chemical shifts observed for few protons and carbons in DP-3 with respect to PMV. C-25 N-methyl protons in PMV shifted from 2.29 ppm to 2.99 ppm in DP-3. Further, H-21 and H-23 protons in PMV shifted from 2.30 ppm and 2.96 ppm, to 2.89 ppm and 3.33ppm, respectively in DP-3. Similarly, H-20, H-24 protons in DP-3 moved to the downfield comparedto PMV as represented in Table-3. C-25 N-methyl carbon resonance 44.3 ppm in PMV shifted to downfield 60.5 ppm in DP-3. C-21 and C-23 carbon chemical shifts in PMV shifted from 53.8 ppm to 64.8 ppm in DP-3. These changes in proton and carbon chemical shift values suggest oxidation on N-22 position in PMV (Fig.-3).

Table-3: ^1H and ^{13}C NMR Data of Pimavanserin and its Degradation Products DP-1, DP-2 and DP-3.

Assignment	$^1\text{H}(\text{PPM})$ API- Drug	^{13}C (PPM) API- Drug	$^1\text{H}(\text{PPM})$ DP-1	^{13}C (PPM) DP-1	$^1\text{H}(\text{PPM})$ DP-2	^{13}C (PPM) DP-2	$^1\text{H}(\text{PPM})$ DP-3	^{13}C (PPM) DP-3
1	6.83	114.1	6.82	114.3	6.85	114.7	6.83	114
2	-	157.4	-	156.9	-	157.6	-	157.4
3	6.83	114.1	6.82	114.3	6.85	114.7	6.83	114
4	7.1	128.1	7.08	129.5	7.14	128.8	7.09	128.1
5	-	133	-	133.6	-	132.7	-	133
6	7.1	128.1	7.08	129.5	7.14	128.8	7.09	128.1
7	-	-	-	-	-	-	-	-
8	3.70	74	3.68	73.7	3.7	73.7	3.70	73.7

9	1.99	27.7	1.97	27.7	1.99	27.7	1.99	27.6
10	0.96	19	0.95	19.0	0.86	19.05	0.96	19.1
11	0.96	19	0.95	19.0	0.96	19.05	0.96	19.1
12	4.18	43.1	3.78	39.5	4.03	42.4	4.18	43.1
13	6.89	-			6.35	-	6.93	-
14	-	157.4			-	158.0	-	157.4
15	-	-					-	-
16	-	-					-	-
17	4.41	44.03					4.44	44.1
18	-	136.7					-	136.6
19	4.02	51.1					4.15	49.8
20,24	1.50,1.69	28.66					2.27,1.33	24.8
21,23	2.30,2.96	53.8					3.33,2.89	64.8
22	-	-					-	-
25	2.29	44.3					2.99	60.5
26,30	7.24	128.3					7.25	128.3
27,29	7.12	114.8					7.12	114.8
28	-	160.8					-	160.8

Koduri *et.al*⁷, for the first time studied the stability of PMV in API and formulations as per ICH guidelines using HPLC-UV and reported as photo and heat-labile and it is further unstable in acid, base and oxidative stress conditions. The reported degradants peaks in HPLC-UV chromatogram is identical and formed to the same extent in all the stress conditions. In the present study, we conducted the systematic forced degradation studies of PMV as per ICH guidelines and characterized the structure of degradation products for the first time. In our studies, PMV was found to be stable upon exposure to UV light (for 24 hrs) and heat (70 °C for 24 hrs), which is contradictory to Koduri *et.al*⁷. PMV in acid, base and oxidative stress conditions gives rise to degradation products, DP-1, DP-2 and DP-3, respectively. DP-1 is characterized as bis(4-isobutoxyphenyl)methane, DP-2 is characterized as 1,3-bis(4-isobutoxybenzyl)urea and DP-3 is characterized as 4-(1-(4-fluorobenzyl)-3-(4-isobutoxybenzyl)ureido)-1-methylpiperidine 1-oxide). DP-2 and DP-3 are reported as process related impurity⁵⁻⁶. However, we report for the first time, PMV degradation products: DP-2 and DP-3 are formed in the basic and oxidative degradation conditions, which is helpful in monitoring the PMV manufacturing process to reduce the potential impurities. PMV acid degradation product (DP-1), bis(4-isobutoxyphenyl)methane is a novel degradant formed by C12 and N13 bond cleavage, and further dimerization of 4-isobutoxyphenyl methane fragment.

CONCLUSION

PMV is stable upon exposure to UV light and heat. PMV forms three degradation products, one each in acid (DP-1), base (DP-2) and oxidative (DP-3) stress conditions. DP-1, DP-2, and DP-3 were isolated by preparative HPLC, mass was identified by UPLC-MS and structures were elucidated by 2D NMR. DP-2 and DP-3 were reported earlier as a PMV process impurity and a metabolite in *in vivo* clinical plasma samples⁵⁻⁶, respectively. We have identified the formation of DP-2 in basic and DP-3 in oxidative conditions for the first time. DP-1 is the novel PMV degradant product formed by the dimerization of 4-isobutoxyphenyl methane fragment. To the best of our knowledge, the present study is the most comprehensive PMV degradation study with structural elucidation of degradants.

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