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Characterization of Pimavanserin Stress Degradation Products by LCMS/MS and Optimization of Green Analytical HPLC Method for Quantification of Pimavanserin and its Impurities

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Green HPLC analytical procedures can quantify contaminants since they employ few hazardous chemicals and waste, reducing environmental impact and improving laboratory safety. In literature, no green analytical method reported for quantification of impurities in pimavanserin and hence this study aimed to establish green analytical method. The method comprises XBridge BEH C18 3.0 µm (100 mm × 4.6 mm) C18 column along with ethanol and 0.01 M aqueous orthophosphoric acid in 60:40 (v/v) at 0.75 mL/min flow and 249 nm wavelength. These conditions proved to be appropriate for asymmetric peak shape along with good resolution and permissible tailing. This method produces well correlated linearity in 3-18 µg/mL for pimavanserin and 0.03-0.18 µg/mL for impurities. This method exhibit a very sensitive detection limit of 0.01 µg/mL that enables precise and accurate impurity quantification at very low concentrations. All validation parameters performed and permissible results observed for both pimavanserin and impurities. Different stress conditions like acid, base, oxidative, thermal and photolytic was analyzed for pimavanserin to evaluate method effectiveness to resolve stress degradation products (DPs). The stress study identifies two distinct DPs in acid and base degradation chromatograms and was named as DP 1 and DP 2. The applicability of MS^n studies and mass fragmentation confirms DP 1 as (4-fluorobenzyl)(1-methylpiperidin-4-yl)carbamic acid and DP 2 as [4-(2-methylpropoxy)benzyl]carbamic acid. The GAPI (Green Analytical Procedure Index) and AGREE (Analytical GREEnness) metric tools were employed to assess the method greenness. This proposed green method can significantly reduce the usage of hazardous solvents without losing chromatographic performance and method efficiency. This study concluded that the method is suitable for quantifying pimavanserin and its impurities along with identification of degradation products.

Keywords: Pimavanserin, Green analytical method, GAPI, AGREE, Degradation products.

INTRODUCTION

Pimavanserin is an antipsychotic medication utilized to treat and manage hallucinations and delusions associated with Parkinson's disease psychosis [1] and belongs to antipsychotic class of drug used to treat depression orders but it not a dopamine receptor [2]. Pimavanserin, IUPAC name is N-(4-fluorophenylmethyl)-N-(1-methylpiperidin-4-yl)-N′-(4-(2-methylpropyloxy)phenylmethyl)carbamide and appears to be round and offwhite powder with film coating. Side effects of pimavanserin are slow heart rate, trouble in breathing, sometimes fainting and discomfort in chest along with hive like swellings on eyelids, hands, leg and on face also [3]. Pimavanserin, marketed as Nuplazid, developed by Acadia Pharmaceuticals and was granted FDA approval in 2014. Pimavanserin is directly works on brain to control the hallucinations and delusions, but it is not used to treat behavioural problems in adults [4]. The action of mechanism of pimavanserin was distinct from other anti-psychotics and it does not exert its effects through dopaminergic pathways. Instead, pimavanserin acts as an inverse agonist and antagonist at serotonin 5-HT2A receptors and to a lesser extent at 5-HT2C receptors [5].

In literature, one HPLC [6] and one UPLC [7] procedure was available for the quantification of pimavanserin impurities. Few methods [8-10] reported for the quantification of pimavanserin in formulations. The literature survey revealed one

HPTLC [11] and UHPLC-MS/MS [12] reported for quantification of pimavanserin in formulations and human plasma samples, respectively. Three impurities are found in pimavanserin *viz*. impurity 1 is N-(4-fluoro-benzyl)-1-methylpiperidin-4-amine with 222.3017 g/mol of mass and formula of $C_{13}H_{19}N_2F$. Impurity 2 have molecular weight of 179.2587 g/mol with formula of $C_{11}H_{17}NO$ and name of 1-[4-(2-methylpropoxy)-phenyl]methanamine. Whereas impurity 3 chemically called as 1,3-*bis*(4-isobutoxybenzyl)urea with molecular formula is $C_{23}H_{32}N_2O_3$ and molecular mass of 384.5117 g/mol. Structure of pimavanserin and impurities is given in Fig. 1. In literature, no green analytical method reported for quantification of pimavanserin impurities. Green analytical methods align with green chemistry principles that minimize hazardous chemical usage, minimize the waste generation and conserve resources. Furthermore, these methods can lead to cost savings and increased efficiency, making them an attractive option for the accurate and reliable quantification of impurities in pharmaceutical compounds. Keep this in consideration, this research work intended to propose green analytical method for quantification of pimavanserin impurities by HPLC and its evaluation of its degradation behaviour using LC-MS/MS technique.

EXPERIMENTAL

A gift sample of pimavanserin (purity >98%) along with impurity 1, 2 and 3 was obtained from MSN Laboratories Ltd. Hyderabad, India. All the analytical grade chemicals utilized in this study were purchased from Merck Chemicals, India. HPLC grade solvents like ethanol, methanol, acetonitrile, water are purchased from SD Fine Chemicals, India. Commercially available pimavanserin tablets with brand name NUPLAZID® (10 mg) were procured from MSN Laboratories Ltd., New Delhi, India.

Instrumentation: Analytical experiments were performed on Agilent HPLC-UV series with 2695 equipped with quaternary pump along with degasser. The instrument also equipped with autosampler and column temperature-controlled compartment. Analyzed data was exported Empower 3 software for accurate interpretation of reports. LC-MS/MS (1290 series, Agilent, USA) system was employed for characterization of degradation products (DPs) and the system coupled with Q-TOF mass detector. Ionization was performed on ESI positive mode for analytes including DPs. Analyzed raw data was exported using software Mass Hunter Workstation for interpretation of mass spectral information. Mettler Toledo (Switzerland) pH meter was utilized for pH measurements and complete dissolution of samples were performed with the assistance of ultrasonic bath (Oscar Ultrasonic Pvt Ltd.) sonicator.

Standard solution preparation: In a cleaned 100 mL flask, 100 mg of standard was accurately weighed and transferred to the flask and then dissolved in 50 mL of ethanol. This solution was sonicated for complete solubility and filtered through nylon membrane having the pore size of 0.22 µm. Filtered solution was further makeup with ethanol and stored. This solution was used a standard solution. From these, the working solutions were prepared for the analysis.

Forced degradation study: Stress study was studied by following ICH Q1 A (R2) guidelines [13] and reported literature [14-16]. Accurately weighed 100 mg of drug was transferred to 100 mL volumetric flask with 50 mL of diluent. Then 5 mL of 2 N HCl was added and refluxed for 8 h at 80 ºC. Solution was neutralized with 2 N NaOH solution after 3 days and make-up the volume up to the mark. A 5 mL of solution was further diluted to 20 mL of diluent, this solution was filtered through 0.22 µm. Filtered solution was diluted to 100% level concentration and used for analysis. For base degradation study, 100

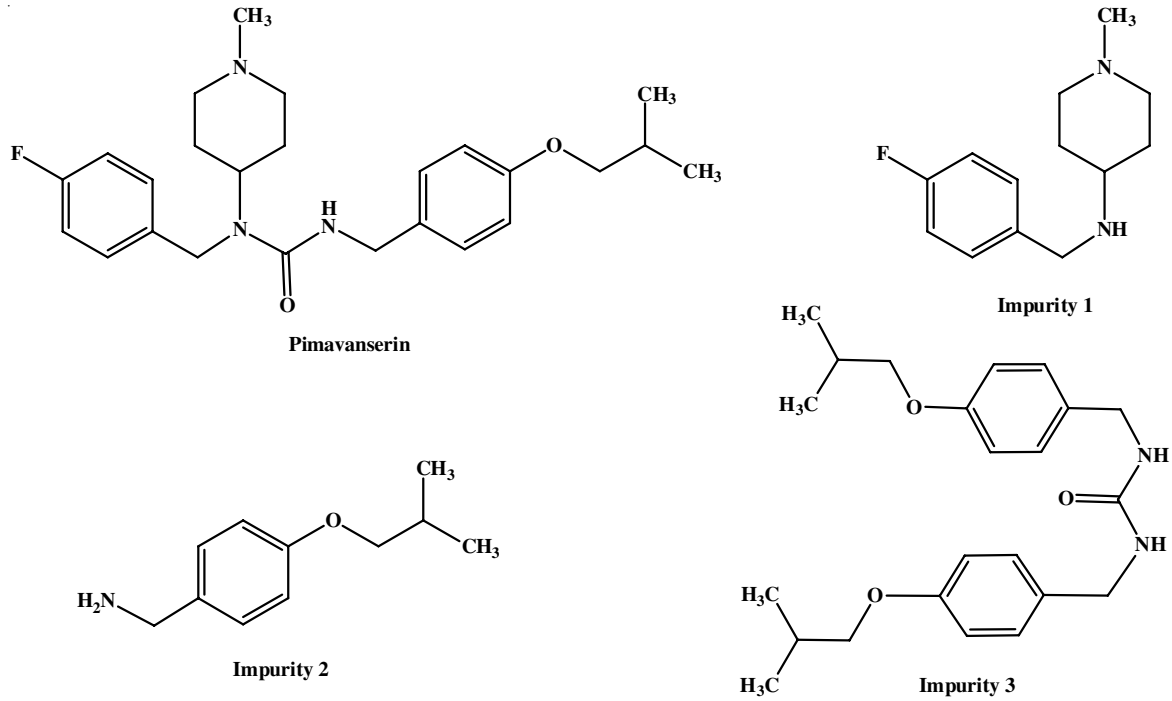


Fig. 1. Molecular structure of pimavanserin

mg of drug was transferred to 100 mL flask containing 5 mL of 3 N NaOH solution and 45 µL diluent. This solution was refluxed at 80 ºC for 8 h, after that 3 N HCl solution added to neutralize and then makes up the solution till mark. Then 5 mL of this solution was transferred to flask having 20 mL diluent. This solution was filtered through 0.22 µm and filtered solution was diluted to 100% level concentration before analysis.

In oxidative degradation, 100 mg of drug was weighed and transferred to 50 mL of flask, which has already filled with diluent and 5 mL of 30% H_2O_2. This solution was kept in dark for 3 days at room temperature. This solution was filtered through 0.22 µm and filtered solution was diluted to 100% level concentration before analysis. The standard drug was kept separately in an air oven at 80 ºC for 2 days and UV chamber (200 W h/m^2) at room temperature for 3 days for thermal and UV light stress study respectively. These stress exposed pure drug was diluted to 100% concentration level and was analyzed in the proposed method.

Analytical method validation: Method validation was performed in accordance with Q2 (R1) guidelines prescribed by ICH [17] and literature available [18-22].

System suitability: System suitability was assessed by repeatedly injecting pimavanserin solution with 1% impurities and method efficiency were proved by summarizing parameters with acceptable limits set as resolution (>2), tail factor (<2), plate count (>3000) and% RSD of area results (<2).

Linearity: An analytical method is linear if there's a significant relationship between response and tested concentration, with an R^2 value of 0.999 or higher. The linear equation, Y = aX ± b, where Y = area response, X = analyte concentration and a and b are the slope and intercept, respectively, was used. Six different concentrations of pimavanserin solution mixed with 1% of each impurity were analyzed and the area responses were recorded to evaluate the method's range. A linear curve was plotted for pimavanserin and each impurity, showing the concentration against area response.

Accuracy: It was assessed in triplicate by spiking pimavanserin and impurities at 50% to 150% of target concentration. The % recovery and system suitability for each analyte were calculated to evaluate method accuracy using the formula:

$$\text{Accuracy (\%)} = \frac{\text{Experimental concentration}}{\text{Prepared concentration}} \times 100$$

Precision and ruggedness: The 100% spiked level solution was prepared and analyzed within the calibration range in two settings: one within a single day (n = 6) and another over two different days (n = 3/day). This helped to assess the repeatability and reproducibility of method. The ruggedness was tested by deliberately varying conditions like analyst, column and day, while keeping the method parameters constant. The % RSD of peak area responses for pimavanserin and impurities in each study was calculated, accepting the results below 2% as satisfactory.

Robustness: Method robustness was assessed by making small, planned changes to parameters like temperature, pH of mobile phase and others. These adjustments included varying detector wavelength by ±5 nm, flow rate by ±0.05 mL/min and mobile phase ratio ±5. Changes in % area response and system suitability were recorded to confirm method robustness.

Sensitivity: The method's sensitivity was assessed by determining the LOD and LOQ for impurities. LOD is the lowest detectable concentration and LOQ is the lowest quantifiable concentration of the analyte in the matrix using this instrument. These concentrations should remain consistent across accuracy, precision and linearity ranges for each injection. LOD and LOQ were calculated based on the slope (S) and standard error (σ) derived from the calibration linearity as follows:

$$\text{LOD} = \frac{3.3\sigma}{S} \text{ and } \text{LOQ} = \frac{10\sigma}{S}$$

Characterization of degradation products: The stress-induced solutions were assessed through HPLC-MS/MS system using specified parameters proposed in this study. The peaks corresponding to pimavanserin, impurities and DPs were detected using ESI in both positive and negative ionization modes across a mass range of 10 to 500 *m/z*. To ensure that the LCMS system received 25% of the column eluents, certain precautions had to be taken during the experiment. This was facilitating with the assistance of splitter arranged in between column and MS detector.

Evaluation of method greenness: For the green evolution of quantitative proposed method GAPI and AGREE metric tools were employed. By using green evolution different parameters were evaluated to check the drug safety, health and environmental impact. The evolution is done in several aspects like collection of samples, employed method, solvents and reagents used, consumption of energy, procedure for waste disposal and other relevant factors. Present study was conducted to develop AGREE, GAPI and environmental sustainability to reach safe and best qualitative and quantitative analysis of pimavanserin.

RESULTS AND DISCUSSION

Method optimization was initiated by using Waters X bridge C18 column with 50 mm × 4.6 mm particle size along with different types of organic modifiers to result asymmetric peak shape, resolution and tailing. But the conditions found to be not suitable for the asymmetric peak shape the mobile phase is altered and changed pH for the better result. But unfortunately, the changed mobile phase was also found to be not suitable. Then the column was shifted to XBridge BEH C18 3.0 µm (100 mm × 4.6 mm) C18 column along with the ethanol and 0.01 M orthophosphoric acid in 60:40 (v/v) was found to be suitable for asymmetric peak shape along with good resolution and tailing. So, the method was optimized these conditions for the development of pimavanserin along with impurities. In this conditions wavelength maximum was set to 249 nm based on iso-absorption wavelength of pimavanserin and impurities.

Degradation study of pimavanserin: Pimavanserin was investigated for the degradation behaviour under different stress conditions using HPLC. The stress conditions were conducted and findings are summarized in Table-1, indicating that the nominal degradation observed for pimavanserin under oxidative, thermal and photolytic conditions suggesting its stability in these environments. The drug degradation was observed in both acid (Fig. 2a) and base (Fig. 2b) degradation studies. These

TABLE-1
FORCED DEGRADATION CONDITIONS FOR PIMAVANSERIN

Stress condition	Concentration of stress study	Conditions	Time	Degradation (%)	Identified DPs
Acid	HCl (2 N)	80 °C	8 days	16.51	DP 1 & DP 2
Base	NaOH (3 N)	80 °C	8 h	14.26	DP 1 & DP 2
Oxidative	H_2O_2 (30%)	Room temperature	3 days	3.13	–
Thermal	–	80 °C	2 days	3.95	–
UV	200 W h/m^2	Room temperature	3 days	4.26	–

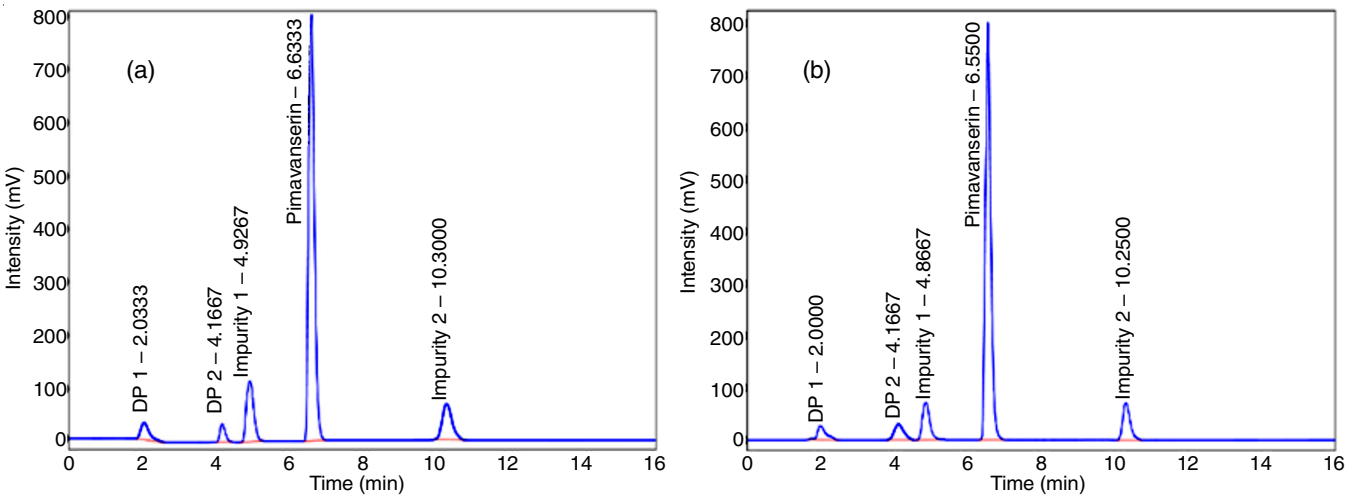


Fig. 2. Acid (a) and base (b) degradation chromatogram that clearly resolve DP 1 and 2 along with known impurities and standard pimavanserin in the proposed method

chromatograms in these conditions show two new peaks corresponds to DPs at 1.2 min and 2.1 min, respectively along with known impurity 1 and 2. These impurities were designated as DP 1 and DP 2 respectively based on time of detection and were further characterized using LCMS/MS.

LC–MS/MS studies of pimavanserin, its impurities and DPs: The exact mass determination was depends on investigation of high-resolution mass fragmentation patterns. The exact mass forecast of pimavanserin and DP structure was gained by using RDB measurements and adhering to the nitrogen rule. The protonated DPs elemental composition and their product ions are shown in Table-2 and Figs. 3-6 displays fragmentation pathway of pimavanserin, impurity 1, 2 and 3 respectively.

DP 1: Protonated DP 1 is formed under both acid and base degradation studies which is shown in Fig. 2. Total molecular weight of DP 1 is 266.3112 with $C_{14}H_{19}FN_2O_2$ of molecular formula. DP 1 is eluted at 2.0333 in acid degradation study and 2.0000 min in base degradation study. Form DP 1 total molecular formula loss of $C_4H_8NO_2$ resulted to from $C_{10}H_{11}FN$ product ion with 164.8 molecular weight. From this loss of C_2H_2 forms another product ion with molecular formula of C_8H_9FN with 138.161 molar mass. From *N*-[(*Z*)-(4-fluorocyclohexa-2,4-dien-1-ylidene)methyl]methaniminium loss of CH_2 forms another distinct product ion with formula of C_7H_7FN formed with 124.135 molar mass. This product is again loss HN group and resulted to form another product ion with 109.120 molar mass and name of the formed product is (4-fluorocyclohexa-2,4-dien-1-ylidene)methylium. The degradation pathway of DP 1 is elucidated in Fig. 7 and formed DP molecular name is as (4-fluorobenzyl)(1-methylpiperidin-4-yl)carbamic acid.

DP 2: DP 2 is formed in both acid and base degradation studies with $C_{12}H_{17}NO_3$ molecular formula and 223.268 is the molar mass of the formed compound. In this stress condition peak was eluted at 4.1667 min in both acid and base stress conditions. Loss of OH group from DP 2 forms the fragmented product ion of $C_{12}H_{16}NO_2$ with mass of 206. 260 *m/z* known as (E)-N-formyl{4-[(2-methylprop-2-en-1-yl)oxy]cyclohexa-1,3-dien-1-yl}methaniminium. From this formed fragmented ion again losses CHN group and formed another product ion with molecular formula of $C_{11}H_{15}O_2$ with 163.235 *m/z* of molar

TABLE-2
ELEMENTAL COMPOSITION STUDY RESULTS OF PIMAVANSERIN, IMPURITIES AND ITS DPs

Compound	m.f.	*m/z* calculated	*m/z* observed	Error (ppm)	Fragmentation (m+1)
Pimavanserin	$C_{25}H_{34}FN_3O_2$	427.5545	427.5547	0.468	372.4667, 274.2817, 167.2147, 110.1203, 97.1023
Impurity 1	$C_{13}H_{19}FN_2$	222.3016	222.3017	0.450	198.2718, 164.2389, 154.2441, 105.1286
Impurity 2	$C_{11}H_{17}NO$	179.2584	179.2587	1.670	137.1705, 124.1524, 108.1293, 92.1299
Impurity 3	$C_{23}H_{32}N_2O_3$	384.5113	384.5117	1.040	343.4320, 272.2906, 148.1931, 110. 1452, 92.1299
DP 1	$C_{14}H_{19}FN_2O_2$	266.3110	266.3112	0.750	165.1988, 139.1616, 125.1350, 110.1203
DP 2	$C_{12}H_{17}NO_3$	223.2684	223.2682	0.900	207.2603, 164.2356, 108.1293, 92.1299

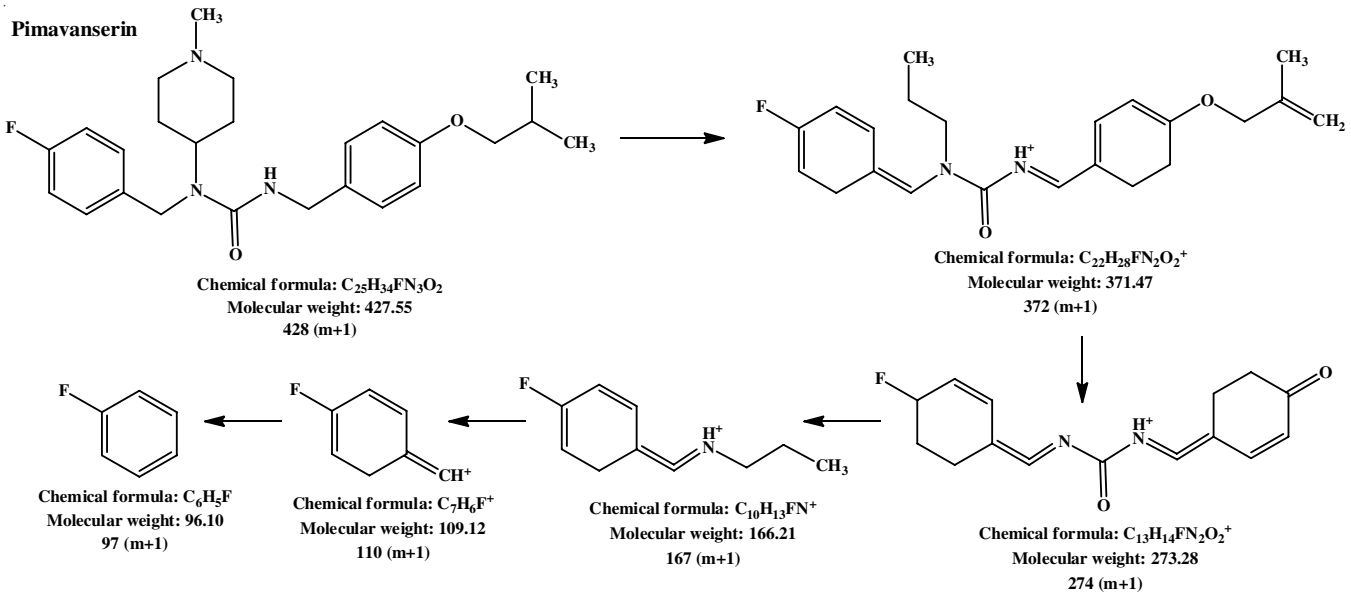


Fig. 3. Degradation pathway for pimavanserin

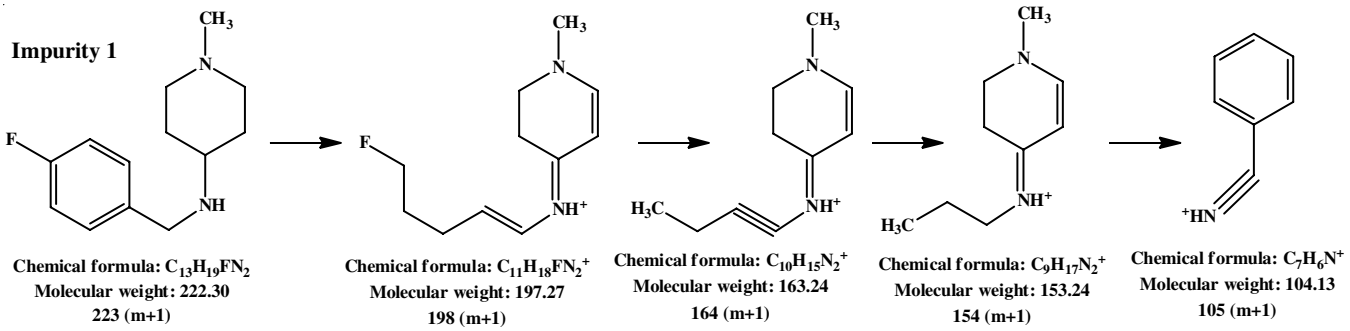


Fig. 4. Degradation pathway for impurity 1

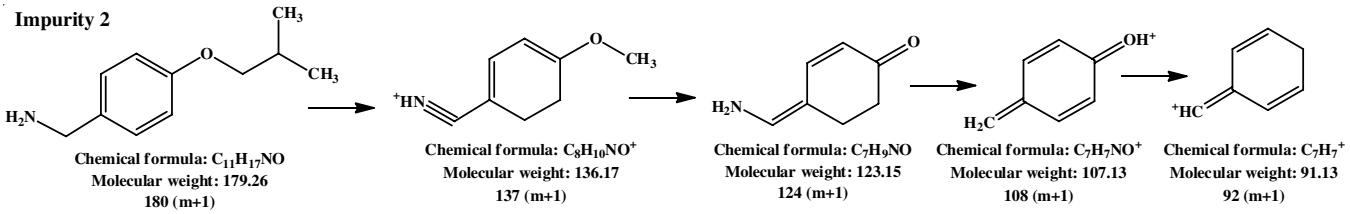


Fig. 5. Degradation pathway for impurity 2

mass. This formed product ion name is (4-methylphenyl)-(2-methylprop-2-en-1-yl)oxonium. From the formed compound C_4H_8 *i.e.* unsaturated olefinic hydrocarbons was eliminated to form another product ion with 107.129 *m/z*, with C_7H_7O molecular formula. This formed compound name is (4-methylidene-cyclohexa-2,5-dien-1-ylidene)oxonium. Only oxygen is eliminated and formed another fragmented ion C_7H_7 with 91.129 *m/z*. This formed compound name is cyclohexa-2,5-dien-1-ylidenemethylium. The proposed whole degradation pathway of DP 2 is shown in Fig. 8. By all these patterns, the proposed DP 2 name is [4-(2-methylpropoxy)benzyl]carbamic acid.

Method validation

Linearity: The relationship between concentration and area response of pimavanserin and impurities in the proposed method was evaluated in linearity study. Linear concentration range was found to be 3-18 µg/mL for pimavanserin and 0.03-0.18 µg/mL for studied impurities. Regression equation was observed for pimavanserin is $y = 59098x + 84751$ ($R^2 = 0.9998$) whereas $y = 560118x + 684.63$ ($R^2 = 0.9991$) for impurity 1, $y = 897491x + 361.49$ ($R^2 = 0.9986$) for impurity 2 and $y = 463543x + 939.53$ ($R^2 = 0.9985$) for impurity 3. These results confirmed that the proposed method is reliable and accurate quantification of pimavanserin and its impurities within the specified concentration ranges.

Precision and ruggedness: Table-3 indicates that the assay percentages and% RSD for precision and ruggedness for pimavanserin and its impurities demonstrate high accuracy and precision. Pimavanserin showed an assay value of 98.25% with % RSD of 0.57% in intraday precision, 0.43 in day 1 precision,

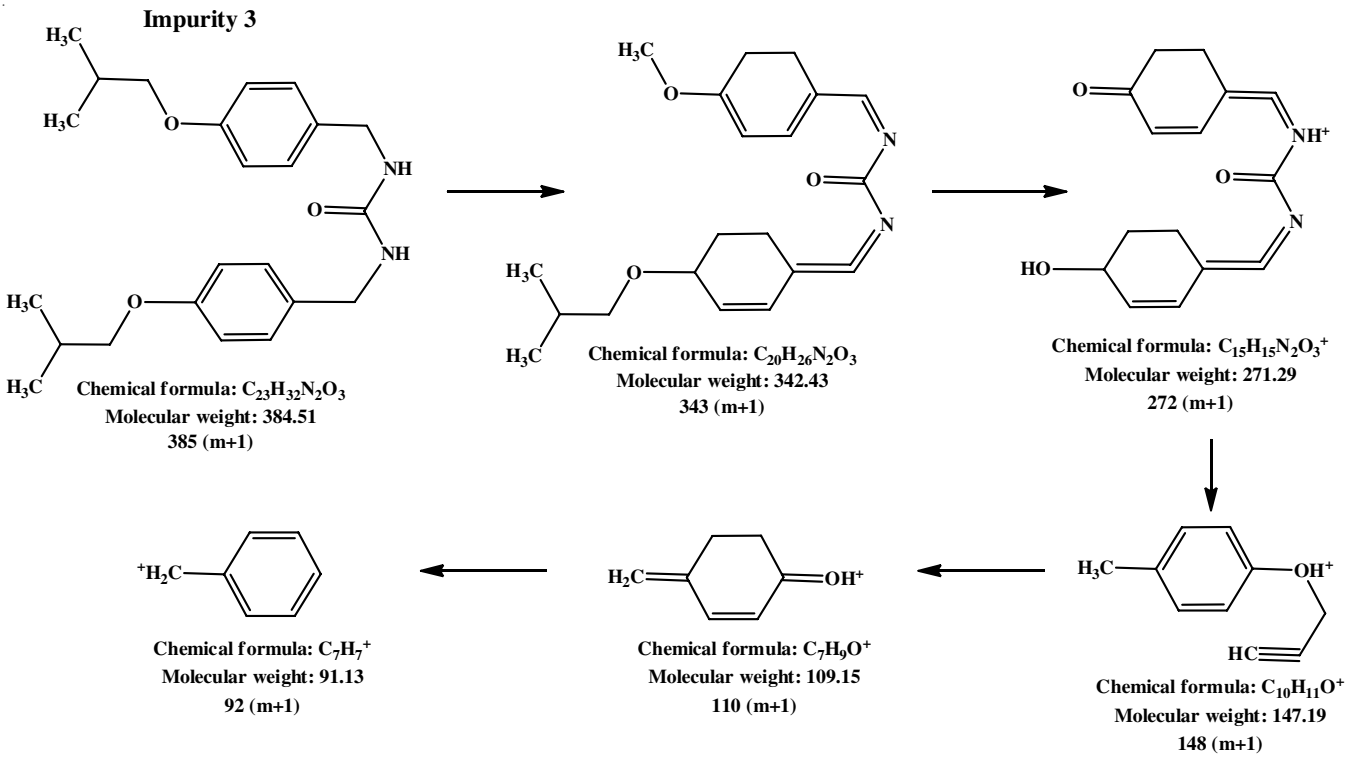


Fig. 6. Degradation pathway for impurity 3

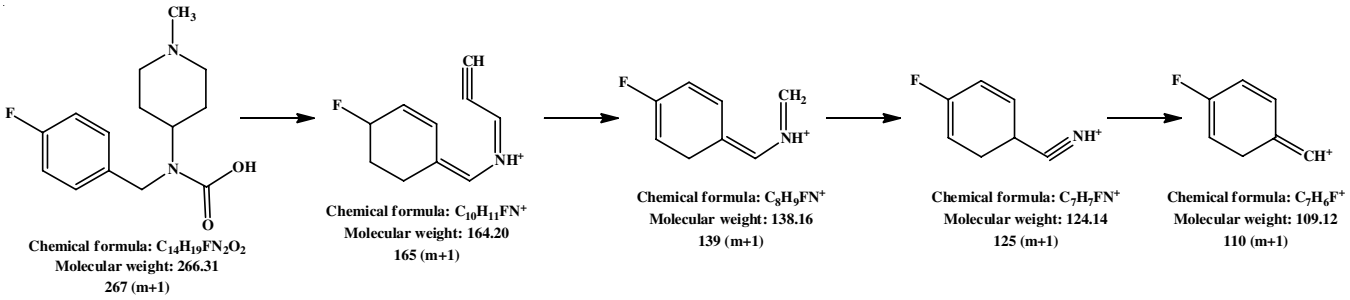


Fig. 7. Degradation pathway for DP 1

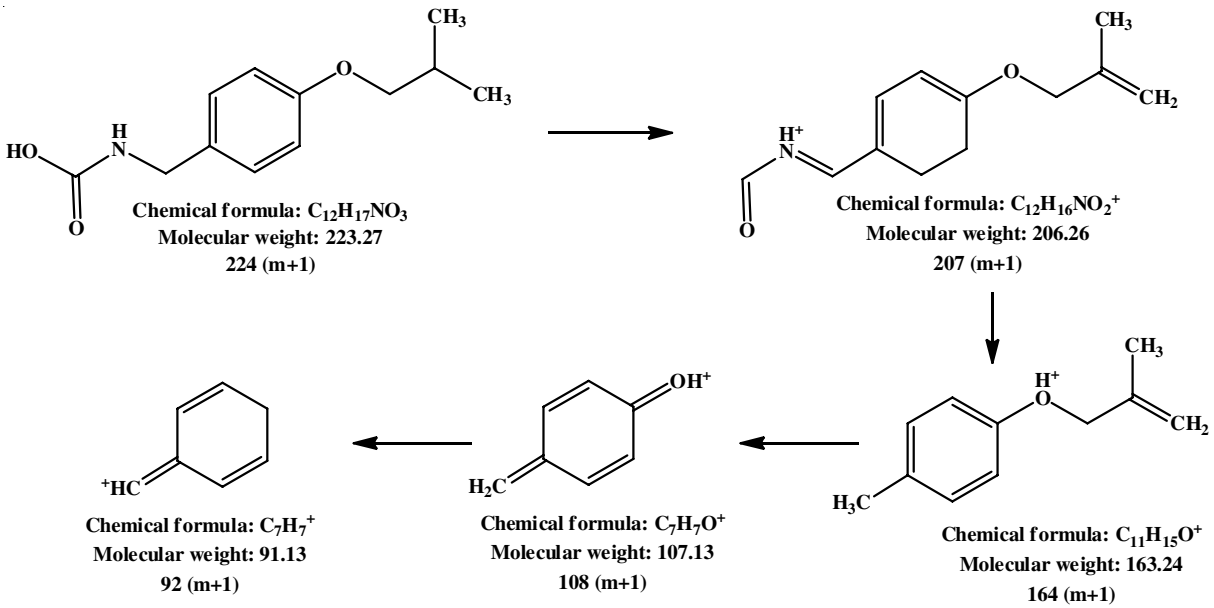


Fig. 8. Degradation pathway for DP 2

TABLE-3
PRECISION AND RUGGEDNESS RESULTS OF PIMAVANSERIN AND ITS IMPURITIES

Analyte	Assay (%)	% RSD in intra-day precision	% RSD in inter-day precision		% RSD in ruggedness
			Day 1	Day 2	
Pimavanserin	98.25	0.57	0.43	0.91	0.35
Impurity 1	99.36	0.81	0.59	0.96	0.57
Impurity 2	99.04	0.36	0.89	0.74	0.49
Impurity 3	98.73	0.58	0.43	0.67	0.82

TABLE-4
ROBUSTNESS RESULTS OF PIMAVANSERIN

Condition changed	Resolution between pimavanserin and impurity 1	Peak asymmetry	Peak plate count	Assay (%)
Ethanol and orthophosphoric acid in 65:35 (v/v)	16.38	1.08	11876	98.94
Ethanol and orthophosphoric acid in 55:45 (v/v)	16.31	1.07	10976	99.63
0.80 mL/min flow rate	16.40	0.93	11543	99.04
0.70 mL/min flow rate	16.35	1.09	11932	101.76
254 nm wavelength	16.47	0.95	10878	99.15
244 nm wavelength	16.44	1.02	10483	99.18

0.91 in day 2 precision and 0.35 in ruggedness. Impurity 1 had an assay value of 99.36% with % RSD of 0.81% in intraday precision and 0.57 in ruggedness reflecting reliable quantification. Impurity 2 exhibited an assay value of 99.04% with the lowest% RSD of 0.36% in intraday precision suggesting exceptional precision in the intraday measurements. Impurity 3 recorded an assay value of 98.73% with % RSD of 0.58% in intraday precision and 0.82 in ruggedness. The results validated that assay values near 100% for all analytes indicate accurate quantification, while the low % RSD values illustrate the high precision and reproducibility of method, thus affirming its reliability for the accurate and precise determination of pimavanserin and its impurities.

Robustness: The robustness results in Table-4 indicate that the method used for the analysis of pimavanserin and its impurities is reliable under various conditions. When the mobile phase composition was changed to ethanol and orthophosphoric acid in 65:35 (v/v), the resolution between pimavanserin and impurity 1 was 16.38 with % assay of 98.94%. Altering the mobile phase composition to 55:45 (v/v) resulted in the resolution of 16.31 and % assay of 99.63%. Similarly, acceptable level results observed while changes made in mobile phase flow as well as detector wavelength. These results demonstrated that the proposed method remains robust and showing consistent performance in terms of resolution, peak asymmetry, peak plate count and assay percentage across a range of conditions.

Accuracy: Accuracy was performed in three different stages *i.e.* one level at 50% concentration level, second level at 100% concentration and third one at 150% concentration level. For 50% concentration level 9 µg/mL was used of pimavanserin and 0.09 µg/mL was used for its impurities. For 100% concentration level 12 µg/mL was used of pimavanserin and 0.12 µg/mL was used for its impurities. For 150% concentration level 15 µg/mL was used of pimavanserin and 0.15 µg/mL was used for its impurities. The results of recovery and accuracy shows with the range of limit (Table-5).

LOD and LOQ: The method sensitivity for analyzing pimavanserin and its impurities was demonstrated by LOD and LOQ results achieved. The LOD for impurities was observed to be 0.01 µg/mL, indicates the method capability to detect even minute quantities of impurities with high precision. The LOQ for the impurities was achieved to be 0.03 µg/mL signifies the method ability to not only detect but also accurately quantify low levels of impurities. These lower LOD and LOQ values proved high sensitivity of proposed method ensures reliable detection and quantification of impurities in pimavanserin formulations.

TABLE-5
ACCURACY RESULTS OF
PIMAVANSERIN AND ITS IMPURITIES

Target amount (µg/mL)	Spiked amount (µg/mL)	Final amount (µg/mL)	Recovery (%)	%RSD
Pimavanserin				
6	3	9	99.497	0.347
6	6	12	99.717	0.188
6	9	15	99.443	0.375
Impurity 1				
0.06	0.03	0.09	100.512	0.430
0.06	0.06	0.12	100.203	0.711
0.06	0.09	0.15	100.098	0.238
Impurity 3				
0.06	0.03	0.09	100.014	0.300
0.06	0.06	0.12	100.074	0.143
0.06	0.09	0.15	99.544	0.905
Impurity 2				
0.06	0.03	0.09	100.030	0.078
0.06	0.06	0.12	99.859	0.831
0.06	0.09	0.15	100.199	0.338

Solution stability: In the evaluation of solution stability, 100% level standard solution was stored in room temperature for various time intervals. At regular time intervals sample and standard is injected to evaluate the stability of pimavanserin. The obtained result was calculated in percentage for relative difference of the injected samples and the % difference was found for sample is 0.27 and 0.33 for standard was found. These results demonstrated that both sample and standard solutions

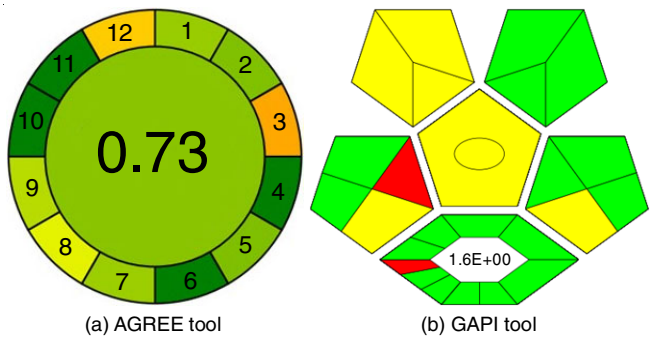
of pimavanserin exhibit excellent stability over 48 h with negligible degradation in assay value. The results of the solution stability are given in Table-6.

TABLE-6
SOLUTION STABILITY OF PIMAVANSERIN

Time interval	Sample	Standard
0 h (Initial)	99.45	99.59
24 h (after)	99.13	99.32
48 h (after)	99.18	99.26
% difference	0.27	0.33

Assessment of green analytical chemistry: Analytical green method was developed for pimavanserin and impurities for the assessment of greener by using the eco-friendly solvents. More common and hazardous solvents like acetonitrile and methanol was not used in this study. Specifically eco-friendly solvents like ethanol and water for the assessment of greenness evaluation. For the preparation of mobile phase and for the preparation of solution only these two solvents used. Along with column was used 100 mm and less runtime was used for the study to make it less consumption of solvents and energy.

To measure the penalty points, the analytical method of pimavanserin eco-scale metric tool was used. Proposed method of pimavanserin shows total 23 penalty points were achieved, hence pimavanserin analytical method of analysis gained total 76 points that indicates the excellent performance of the reported method. AGREE software was used for evaluation of pimavanserin which has 12 green analytical principles to check the criteria will be tuneable or not. From these 12 principles, each principle is assigned to check the score which is ranged from 0.1-1.0. After analysis of AGREE tools for the analysis of pimavanserin total score was achieved is 0.73, which indicates that the proposed method is eco-friendly which don't effects nature. The results of AGREE pictogram is represented in Fig. 9a. The GAPI tool, which utilizes a set of pictograms and pentagrams to visually represent the environmental impact of the proposed method, provided a detailed analysis method's greenness. Significantly, the proposed method did not have any red pictograms, indicating no major environmental concerns, *i.e.* it shows 1.6E+00 value for GAPI tool. Instead, aspects related to sample handling and preparation were marked with yellow pictograms (Fig. 9b). This highlighted the method's compliance with eco-friendly practices and its reduced environmental risks.



(a) AGREE tool (b) GAPI tool

Fig. 9. GAPI and AGREE pictogram noticed for the proposed method

In literature, Radic *et al*., 2021 [7] reported UPLC based method for quantification of impurity A to D of pimavanserin. Whereas the method reported by Navaneeswari *et al*. [6] was applicable for quantification of impurity A to H of pimavanserin. Both these methods are not applicable for resolution and quantification of impurity 1, 2 and 3 of pimavanserin. These approaches do not incorporate the principles of green chemistry and fail to assess the environmental sustainability of the methodology. Unlike previous methods, the proposed technique allows for the resolution and quantification of impurity 1, 2 and 3 of pimavanserin. This method offers a significant advancement by incorporating green chemistry principles, ensuring a more environmentally friendly approach. Additionally, this method includes the characterization of degradation products enhancing the comprehensive analysis of substance.

Conclusion

An accurate HPLC method was developed for quantitative evaluation of pimavanserin and impurities in formulations and stability samples. Different stress conditions were performed and these stress conditions produce two degradation products (DPs) in acid and base stress conditions. In remining studies like oxidative, thermal and photolytic degradation studies shows no degraded compounds for the pimavanserin and impurities. DPs were analyzed by using LC-MS/MS study and fragmentation pathway clearly explained. From this study, DP1 is identified as (4-fluorobenzyl)(1-methylpiperidin-4-yl)carbamic acid whereas DP 2 is named as [4-(2-methylpropoxy)-benzyl]carbamic acid. Apart from this HPLC method, the development and validation parameters like linearity, precision, accuracy and robustness was studied. The proposed analytical method was further assessed for the greenness evaluation by using green assessing tools like GAPI and AGREE. The method is suitable for quantifying pimavanserin and its DPs in both active pharmaceutical ingredients and formulations. Thus, it can be used effectively in quality control and stability studies for routine applications.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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