Chinese Journal of Health Management
Volume 20 Issue 3, Year of Publication 2026, Page 308-315 | DoI-10.564220/1674-0815.2026.57

ISSN: 1674-0815

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DoI-10.564220/1674-0815

Chinese Journal of Health Management

Chinese Medical Association

Development and Validation of a Stability-Indicating HPLC Method for Pimavanserin hemitartrate: A Green Analytical Approach

Santosh Vilas Gandhi[1], Atul Arjun Baravkar[2], Vipul Bhamare[1], Jayashri Randhir Jagtap[3], Rajashri Sunil Chvan[3], Deepali Arvind Nanaware[3]

[1]AISSMS College of Pharmacy, Pune, India

[2*]Agricultural Development Trust's School of Pharmacy & Research Centre, Baramati, Pune, India

[3]Pune District Education Association's Seth Gavind Raghunath Sable College of Pharmacy, Saswad, Pune, India

Corresponding Author Email id: **atul200678@gmail.com**

Article Information
Received: 14-12-2025
Revised: 18-01-2026
Accepted: 11-02-2026
Published: 22-03-2026

Keywords
Pimavanserin hemitartrate, Stability-Indicating Method, HPLC, Forced Degradation, Method Validation, Green Analytical Chemistry.

ABSTRACT:

This study presents the creation, validation, and implementation of a stability-indicating high-performance liquid chromatography (HPLC) technique for the quantitative analysis of Pimavanserin hemitartrate, alongside the forced degradation and separation of degradation products. An environmentally friendly and robust HPLC method was fine-tuned utilizing a BDS Hypersil C8 column (250 × 4.6 mm, 5 µm), with a mobile phase consisting of methanol and 1% formic acid in water (85:15 v/v), operating at a flow rate of 2.0 mL/min and detection at 225 nm. The method demonstrated remarkable linearity (10–60 µg/mL) with a correlation coefficient (R^2) of 0.9978, along with satisfactory precision, accuracy, robustness, and specificity in compliance with ICH guidelines. To verify the stability-indicating capability, forced degradation studies were conducted under acidic, alkaline, oxidative, thermal, and photolytic conditions. Notably, degradation was significant in acidic and alkaline environments, with % recovery decreasing to 59.43% and 46.47%, respectively. Under thermal and photolytic conditions, minimal degradation was observed, indicating the drug's stability in those scenarios. The method's environmental friendliness was improved by employing methanol and aqueous formic acid as the mobile phase, avoiding buffers or hazardous solvents, thus aligning with sustainable analytical methodologies.

INTRODUCTION:

Parkinson's disease (PD) ranks as the second most prevalent neurological disorder following Alzheimer's disease. The primary aim of first-line PD medications was to address movement dysfunction. In patients with PD who are treated with L-dopa, psychotic symptoms frequently manifest as a side effect. Initial treatments for PD-related psychosis involved reducing anti-PD medication dosages or administering a conventional antipsychotic, both of which have been demonstrated to exacerbate PD symptoms. Pimavanserin tartrate is classified as an atypical

antipsychotic utilized in the management of Parkinson's disease. Pimavanserin tartrate is a salt, specifically the hemitartrate salt of Pimavanserin (Figure 1). Its chemical designation is (2R, 3R)-2,3-dihydroxybutanedioic acid; 1-[(4-fluorophenyl) methyl]-1-(1-methylpiperidin-4-yl)-3[[4(2-methylpropoxy) phenyl] methyl] urea. This compound functions as an inverse agonist at the 5-HT_{2A} receptor, aimed at treating L-dopa-induced psychosis in Parkinson's disease, as well as for low-dose risperidone therapy in schizophrenia .[1-3] Various analytical methods have been reported for Pimavanserin hemitartrate estimation. A stability-indicating HPLC and HPTLC method showed degradation under stress but no distinct degradation products reported.[4-9] However, most lack use of green solvents. Thus, there is a need for a comprehensive method combining green HPLC analysis for degradation product separation.

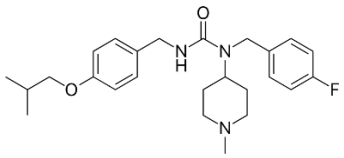

Figure 1: Pimavanserin hemitartrate

EXPERIMENTAL:

Reagents and Chemicals:

The chemicals use for analysis are Methanol (HPLC Grade), HPLC grade water, Formic acid, NaOH, HCl, 30% H_2O_2. All chemicals, including methanol and buffer solvents were purchasedt from Loba Chemicals Laboratories Pvt. Ltd., Mumbai.

Instruments and Chromatographic Condition:

The HPLC analysis was carried out using a JASCO system equipped with a PU-2080 pump, PU-2075 UV detector, and Borwin software (v1.5) for data acquisition. Chromatographic separation was achieved on a BDS Hypersil C8 column (250 × 4.6 mm, 5 µm particle size) using an isocratic mobile phase consisting of methanol and 1% formic acid in HPLC-grade water in the ratio of 85:15 (v/v). The flow rate was maintained at 2.0 mL/min with an injection volume of 20 µL, and detection was carried out at 225 nm over a 10-minute run time. In addition to the HPLC system, several other instruments were employed during the analysis. A Shimadzu electronic weighing balance (model ATX-224R) was used for accurate weighing of samples. A Pharma Solutions sonicator was utilized for solution preparation, and an Extra Pure Lab Link water purification system provided high-purity water for analytical use. For stability studies, a hot air oven (BOMEDICA) was used for thermal degradation testing, and a photo stability chamber (Newtronic NEC103RSPI) was used for photolytic degradation assessment.

Preparation of Solution:

Pimavanserin hemitartrate was soluble in methanol, therefore, methanol was chosen for preparing various sample solutions. 10 mg of Pimavanserin hemitartrate was weighed and transferred to 10 mL volumetric flask. It was then dissolved in methanol and volume was made up to get standard stock solution of 1000 µg/mL. From the stock solution further dilutions were made to get solutions having concentration of 10-60 µg/mL.

Selection of Analytical Wavelength:

A solution of 20 µg/mL in methanol solvent was prepared from standard stock solution(1000µg/mL) and scanned over 200 - 400nm in UV Spectrophotometer. The maximum absorbance was shown at 225 nm. Hence it was selected as analytical wavelength; The UV spectrum is given in Figure 2.

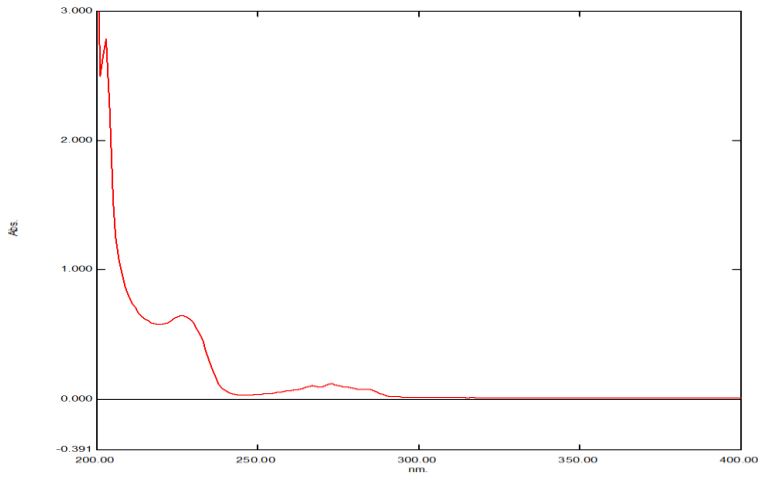

Figure 2: UV-Spectra of Pimavanserin Hemitartrate

METHOD VALIDATION:

Linearity:

Working standard solutions were prepared from standard stock solution of Pimavanserin hemitartrate (1000 µg/mL). Linearity was tested for the range set in a concentration of 10-60 µg/mL. The calibration curve was made using six standard solutions of different concentrations (10, 20, 30, 40, 50 and 60 µg/mL). The working solutions were prepared by diluting an appropriate volume of the stock solution with methanol.

Precision:

The precision of the analytical method was evaluated by assessing both Intraday and Inter-day variability at three concentration levels (20, 40, and 60 µg/mL) of Pimavanserin hemitartrate.

Assay:

Assay of Pimavanserin hemitartrate was performed on the marketed formulation of Pimavanserin hemitartrate brand name PIMAVERSE, marketed by Sun Pharma Laboratories LTD. One capsule was taken, and it's contents were weighed accurately. An amount of powder equivalent to 10 mg Pimavanserin hemitartrate was accurately weighed and dissolved in methanol to prepare a stock solution of 1000 µg/mL. The volumetric flask was sonicated for 10 minutes to enable complete dissolution of the drug and solution was filtered using Whatman filter paper. A 0.2 mL aliquot of the filtrate was further diluted using methanol in a 10 mL volumetric flask to obtain a working solution of 20 µg/mL.

Accuracy:

The accuracy of the method was determined using the standard addition method. Marketed formulation to which known amount of the API to be analyzed was added. The blend prepared for the assay was spiked with pure drug substance at 50%, 100% and 150% levels. Accuracy was evaluated at 3 concentration levels covering the predefined range with 3 replicates each.

Limit of detection (LOD) and limit of quantitation (LOQ):

The detection and quantitation limits of the drug were calculated from the calibration curve. The calculations were based on the standard deviation of the y-intercept and the slope of the calibration curve.

Robustness:

The developed method was tested for robustness by making small but deliberate changes in the mobile phase ratio, flow rate and detection wavelength.

Forced Degradation Study

The stress testing was performed in accordance with ICH Q1A (R2) guidelines, involving the exposure of the drug to a range of stress conditions over different time periods. Optimizations were made regarding the strength of the reagents and the duration of exposure with or without heat. The refined stress conditions were subsequently applied in the HPLC method.

Acid Degradation:
1 mL was taken from Pimavanserin hemitartrate working solution (1000 µg/mL), and combined with 5 mL of 5 N HCl and 5 mL methanol in a 10 ml volumetric flask. The solution was kept at room temperature for about 2 hours. After the reaction, the resulting solution, with a concentration of 100 µg/mL was injected into the system.

Alkaline Degradation:
1 ml was taken from Pimavanserin hemitartrate working solution (1000 µg/mL), and combined with 5 mL of 5 N NaOH and 5 mL of methanol in a 10 mL volumetric flask. The solution was kept at room temperature for about 2 hours. After the reaction, the resulting solution of 100 µg/mL was injected into the system.

Oxidation Degradation:
1 mL was taken from Pimavanserin hemitartrate working solution (1000 µg/mL), and combined with 5 mL of 30% H_2O_2 v/v in a 10 mL volumetric flask. The solution was placed at room temperature for about 2 hours. The resulting solution of 100 µg/mL was injected into the system.

Thermal Degradation:
For thermal degradation, the drug in solid state was placed in an oven at 80ºC for 4 hours. The sample was removed from the oven, cooled to room temperature, weighed and dissolved in methanol to obtain a final concentration of 100 µg/mL of Pimavanserin hemitartrate. The resulting solution was then injected into the HPLC system.

UV degradation:
For Sunlight exposure, the drug in solid state was placed in sunlight for 4 hours. The sample was then weighed and dissolved in methanol to obtain a final concentration of 100 µg/L of Pimavanserin hemitartrate. The resulting solution was then injected into the HPLC system.

RESULTS AND DISCUSSION:
Linearity:

Six replicates of these working solutions were analysed and the results are summarized in the table 1. The overlay of Pimavanserin hemitartrate linearity (10-60 µg/mL) shown in Figure 3. The values were plotted as concentration versus peak area to obtain the calibration curve shown in Figure 4.

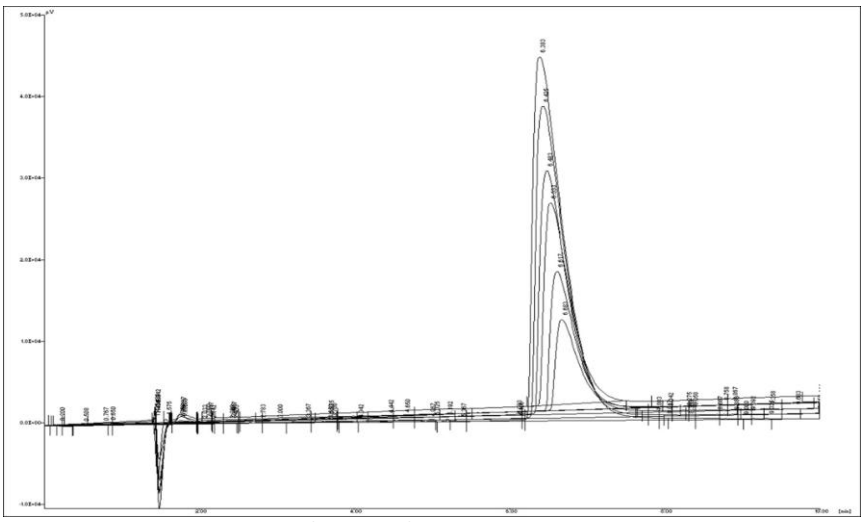

Figure 3: Chromatogram of overlay of Pimavanserin Hemitartrate (10-60 µg/mL)

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Volume 20 Issue 3, Year of Publication 2026, Page 308-315 | DoI-10.564220/1674-0815.2026.57

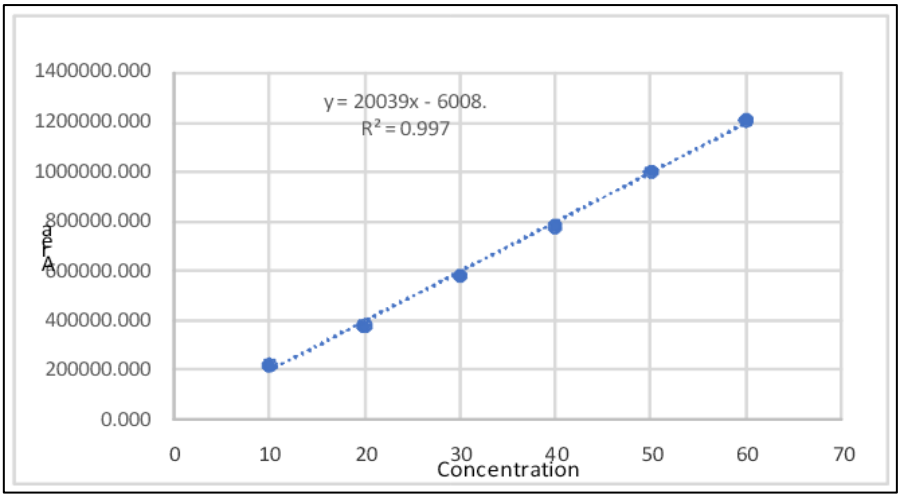

Figure 4: Calibration Curve of Pimavanserin Hemitartrate (10-60 µg/mL)

Table 1: Results for Linearity.

	Conc. (µg/mL)					
Sr. No.	**10**	**20**	**30**	**40**	**50**	**60**
1	220615	388066	569094	780317	990551	1211475
2	222481	380149	587763	782504	1033363	1231968
3	219123	373138	593608	762504	992827	1208909
4	216176	380594	575063	781144	984487	1205879
5	221582	371105	568041	783340	1006993	1194563
6	222481	381084	589650	797650	1000163	1204784
AVG	220409.667	379022.667	580536.500	781243.167	1001397.333	1209596.333
SD	2432.152	6116.983	11163.958	11199.952	17502.525	12389.282
%RSD	1.103	1.614	1.923	1.434	1.748	1.024

LOD and LOQ:

The limit of detection (LOD) and limit of quantitation (LOQ) were calculated using the equations LOD (3.3* σ/S) and LOQ (10* σ/S), where, σ = the standard deviation of response at the y-intercept or at the lowest concentration, and S = slope of the calibration curve. The LOD and LOQ were found to be 0.829 µg/mL and 2.513 µg/mL, respectively

Precision:

Intraday precision (n=3) was evaluated by analysing each concentration on the same day. Interday precision (n=3) was assessed by analysing each concentration on three consecutive days. The relative standard deviation (% RSD) for both intraday and interday analyses was found to be less than 2%. The results of intraday and interday precision are summarized in Tables 2 and 3, respectively.

Table 2: Results for Intraday Precision.

Intraday (n=3)						
Conc (µg/mL)	**0 hr.**	**2 hr.**	**4 hr.**	**Average**	**SD**	**% RSD**
20	379495	3844105	379723	381107	2598.269	0.681
40	740308	750056	764273	751545	12051.748	1.603
60	1210974	1231968	1198336	1213759	16988.126	1.399

Table 3: Results for Interday Precision

Interday (n=3)						
Conc (µg/mL)	**Day 1**	**Day 2**	**Day 3**	**Average**	**SD**	**% RSD**
20	380149	373138	380594	377960	4182.186	1.106
40	782504	762504	781144	775384	11175.115	1.441
60	1231968	1208909	1205879	1215585	14268.463	1.173

Assay:

Sample solution was injected into the HPLC system and the percent recovery was calculated. The results are shown in Table 4

Table 4: Results of Assay study

Sr. no.	Peak Area	Amount recovered (µg/mL)	% Recovery	AVG %	SD	% RSD
1	383598.0	19.442	99.707	99.587	1.061	1.066
2	389493.0	19.737	98.683			
3	397791.0	20.151	100.753			
4	388471.0	19.686	98.428			
5	380878.0	19.307	99.029			
6	398468.8	20.185	100.923			

Accuracy: Accuracy of the method was tested at 50, 100 and 150 % level. The results are presented in Table 5

Table 5: Accuracy results

Level	Peak Area	Amount recovered (µg/mL)	% Recovery	AVG %	SD	% RSD
50%	598955	30.189	100.631	99.459	1.018	1.024
	587898	29.638	98.792			
	588878	29.686	98.955			
100%	786989	39.573	98.932	99.465	0.636	0.639
	796900	40.067	100.168			
	789890	39.717	99.294			
150%	987698	49.589	99.177	99.613	0.662	0.665
	999699	50.188	100.375			
	988798	49.644	99.287			

Robustness:
The robustness of the method was evaluated by making changes to the analytical conditions deliberately. The ratio of mobile phase components was adjusted to methanol and 1% formic acid in water at 87:13 v/v and 83:17 v/v. The flow rate was varied by ±0.2 mL/min, and the detection wavelength was varied by ±1 nm. The results indicated that the method remained robust under these conditions.

FORCE DEGRADTION STUDY:
To check stability of the drug under various stress conditions and to assess the stability-indicating capability of the method, forced degradation studies were conducted under acidic, alkaline, oxidative, thermal, and photolytic conditions. Significant degradation was observed in acidic and alkaline environments, with % recovery decreasing to 59.43% and 46.47%, respectively. Under thermal and photolytic conditions, minimal degradation was observed, indicating the drug's stability under these conditions. The results are summarised in Table 6 and chromatograms of forced degradation are presented in Figures 5 to 9.

Table 6: Summary of degradation parameters for Pimavanserin Hemitartrate

Sr. No.	Stress Condition	Concentration and time	% Recovery
1	Acidic Hydrolysis	2 N HCl for 2 hrs	59.434
2	Alkaline hydrolysis	2 N NaOH for 2 hrs	46.468
3	Oxidation Condition	30% H_2O_2 for 2 hrs	90.110
4	Thermal degradation	80 ºC for 4 hrs	89.004
5	Photodegradation	200 watt hours/m^2	85.822

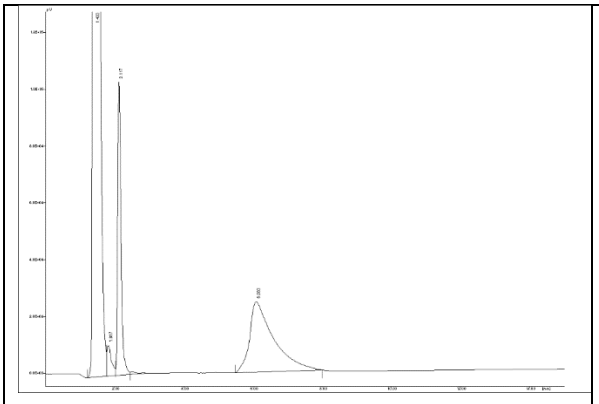

Figure 5: Chromatogram of Acidic hydrolysis condition (100 µg/mL)

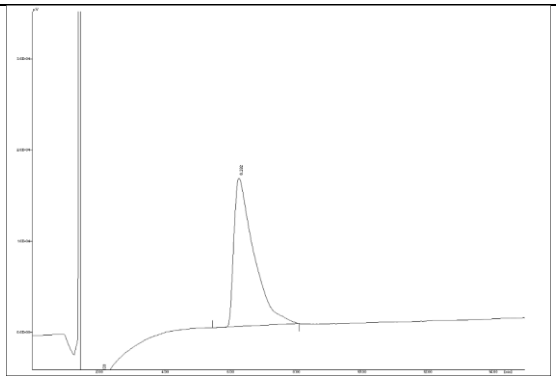

Figure 6: Chromatogram of Alkaline Degradation condition (100 µg/mL)

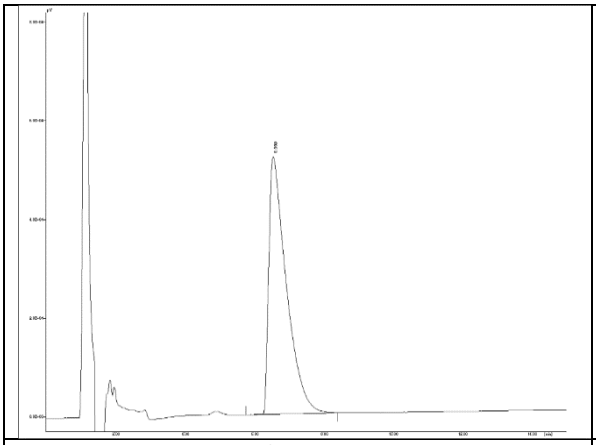

Figure 7: Chromatogram of Oxidation Degradation condition (100 µg/mL)

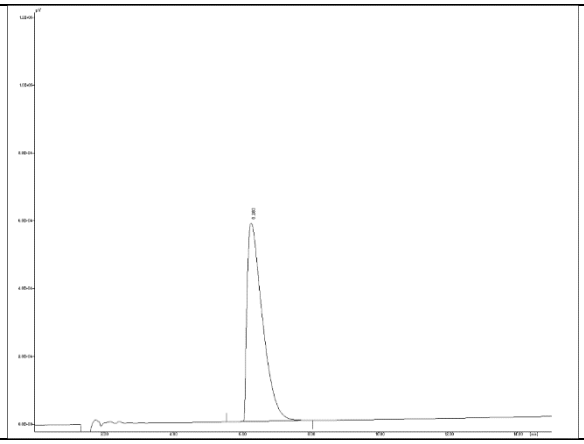

Figure 8: Chromatogram of Thermal Degradation condition (100 µg/mL)

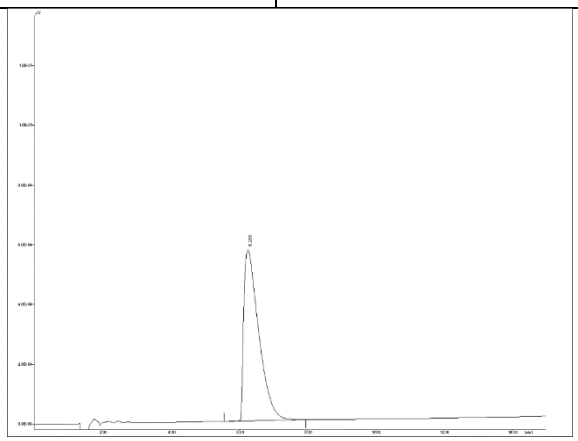

Figure 9: Chromatogram of drug under sunlight exposure (100 µg/mL)

GREEN ASSESSMENT: [10, 11]

The HPLC method developed achieved a higher greenness score compared to the reported method[7] due to optimized chromatographic conditions that effectively minimize solvent consumption and energy use, in addition to reducing analysis time.

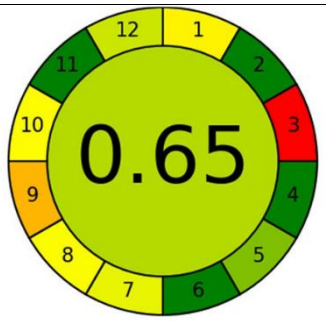

Figure 10: Reported HPLC method

Figure 11: Developed HPLC method

CONCLUSION:

An efficient, accurate, and precise stability-indicating HPLC method was successfully developed and validated for estimating Pimavanserin hemitartrate. The validation process was conducted in accordance with ICH Q2 (R1) guidelines, confirming the method's reliability for routine analysis. The method demonstrated its effectiveness under various stress conditions, including acid and base hydrolysis, oxidative degradation, dry heat, and photolytic stress studies. Significant degradation was observed under acidic, basic, and oxidative conditions. Overall, the

developed HPLC method provides a straightforward, accurate, and precise approach for the quantification of Pimavanserin hemitartrate.

STATEMENT AND DECLARATIONS

Ethical Approval: Not applicable. No human or animal subjects were involved.
Conflict of Interest: The authors declare no conflicts of interest.
Funding: No external funding was received.

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