World Journal of Pharmaceutical and Life Sciences
WJPLS
www.wjpls.org
SJIF Impact Factor: 6.129

DEVELOPMENT AND VALIDATION OF ANALYTICAL METHOD FOR THE ESTIMATION OF PIMAVANSERIN IN BULK AND ITS DOSAGE FORM BY RP-HPLC

Nagaraju Pappula* and Venkata Lavanya Kantu

Department of Pharmaceutical Analysis, Hindu College of Pharmacy, Guntur – 522002, Andhra Pradesh, India.

Corresponding Author: Dr. Nagaraju Pappula
Department of Pharmaceutical Analysis, Hindu College of Pharmacy, Guntur - 522002, Andhra Pradesh, India.

Article Received on 03/01/2022 **Article Revised on 24/01/2022** **Article Accepted on 14/02/2022**

ABSTRACT

Background: To Develop and validate RP-HPLC method for the estimation of Pimavanserin in bulk and its pharmaceutical dosage form. The objective of the present work is to develop and validate a HPLC method with PDA detector. In the method development of Pimavanserin, we have decided to carry out our project work by incorporating the Reverse phase High performance Liquid chromatography (RP-HPLC). Then the developed method will be validated according to ICH guidelines for its various parameters. ***Method***: In the process of HPLC method development, the optimization was done by changing the mobile phase ratio, column and flowrate. Method development focuses on identifying buffer type, strength and P^H of organic solvent. Implementing small changes to optimize selectivity and enchanced resolution. Different trails were performed and finally the optimized method was found to be suitable. The mobile composition of ACN: water: Potassium Di hydrogen orthophosphate (0.02M) = 40:10:50 v/v, buffer pH was adjusted to 6.0 with orthophosphoric acid, agilent column of C_{18} (250 X 4.6 X 5µm) and flow rate 1.2 ml/min. The efficient and reproducible method was developed for determination of pimavanserin and chromatogram is obtained with good resolution. ***Results:*** Initial assay parameters were based on physical-chemical properties of Pimavanserin. Due to the octanol-water partition coefficient (Log P) -2.8 of the parent compound, a C_{18} column was selected for development. A UV scan of Pimavanserin showed a maximal absorbance at 290 nm. The process of method development was conducted and optimized on a agilent column of C18 (250 X 4.6 X 5µm), operated at ambient temperature. This column provides efficient and reproducible separations of non-polar compounds while minimizing solvent usage. Isocratic mobile phase was optimized to resolve Pimavanserin, on the C_{18} column, with ACN: water: Potassium Di hydrogen orthophosphate (0.02M) = 40:10:50 v/v, buffer pH was adjusted to 6.0 with Ortho phosphoric acid. This proportion of mobile phase was found to provide a reproducible and well resolved peak with an average peak tailing factor of 1.59. Based on the pressure limitations of the column and the HPLC system, flow rates were maximized to decrease assay time without adversely affecting the system or the column. Typical pressure throughout the method ranges from 3550 to 3750 psi. The length of time in each portion of the assay was varied to accommodate a 5-min run time. ***Conclusion:*** The study was focused to develop and validate HPLC method for estimation of Pimavanserin in tablet dosage form. For routine analytical purpose it is desirable to establish methods capable of analyzing huge number of samples in a short time period with good robustness, accuracy and precision without any prior separation steps. HPLC method generates large amount of quality data, which serve as highly powerful and convenient analytical tool. The method shows good reproducibility and good recovery. From the specificity studies, it was found that the developed methods were specific for Pimavanserin.

KEYWORDS: Pimavanserin, Isocratic, RP-HPLC and anti-psychotic.

INTRODUCTION

Pimavanserin **(Fig 1)** is an antipsychotic drug that acts as a selective antagonist on the serotonin subtype 5-HT_2A receptor subtype and four folds increased selectivity towards 5-HT_2C receptor subtype.[1]

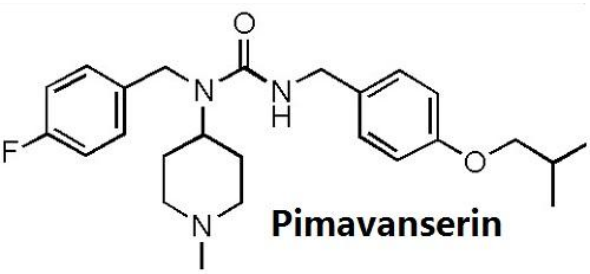

Figure 1: Chemical structure of Pimavanserin.

Pimavanserin has no considerable affinity or activity towards 5-HT_2B or dopamine receptors. Chemically Pimavanserin is described as 1-[(4-fluorophenyl) methyl]-1-(1-methylpiperidin-4-yl)-3-[[4-(2-methylprop-oxy) phenyl] methyl] urea (Figure 1). It is used for the treatment of delusions and hallucinations allied with psychosis experienced by a few patients suffering with Parkinson's disease. Up to date only one method based on ultra performance liquid chromatography tandem mass spectrometry[5] has been developed for the quantification of pimavanserin and applied to routine pharmacokinetic study of pimavanserin in rats. Knowledge about the molecule's stability helps in choosing correct formulation and package plus providing appropriate storage conditions, which is vital for regulatory documentation for this purpose a stability indicating analytical method is necessary. The determination of pimavanserin in pharmaceutical dosage form by stability indicating high performance liquid chromatography coupled with photodiode array detector has not been reported. Hence, the current study was proposed to determine the content of pimavanserin in tablet dosage form using stability indicating HPLC method.

MATERIALS AND METHODS

Instrumentation

- WATERS HPLC, Model: Aliance 2695, Photo diode array detector (PDA), with an automated sample injector.
- The output signal was monitored and integrated using Empower 2 software.
- Agilent C18 (4.6 x 250mm, 5 µm, Make: Waters) column was used for separations.

Analytical method development and Optimization

The present study was carried out to develop and validate RP-HPLC method for the estimation of Pimavanserin in bulk and application of the developed method for the determination of assay (% purity) of the marketed formulation.

Standard stock and working solutions

Standard preparation

100 mg of Pimavanserin was accurately weighed, transferred to a 100 ml volumetric flask, dissolved in the diluent and final volume was made up to the mark with the same to get a standard stock solution of 1 mg/ml.

Construction of calibration curve

Aliquots (10µl) of pimavanserin working standard solutions were injected into the HPLC system and eluted by the mobile phase under the optimum HPLC conditions. The peak area response versus the final concentration of pimavanserin in µg/ml was plotted. On the other hand, the regression equations were derived

Analysis of pimavanserin in tablet sample solution

Twenty tablets were weighed and average weight was calculated. The tablets were ground to fine powder and a quantity of powder equivalent of 25 mg was accurately weighed and transferred to a volumetric flask of 25 ml capacity. 15 ml of mobile phase was transferred to volumetric flask and sonicated for 10 min. The final volume was made upto the mark with the same to get the sample stock solution of 1000 µg/ml. The solution was filtered through 0.45 µ membrane filter. An aliquot of 2 ml was transferred to a 25 ml volumetric flask and diluted upto the mark with mobile phase to get a final concentration is 80µg/ml. 10µl of this solution was injected into the chromatograph and the chromatogram was recorded. The peak area was determined and the amount of Pimavanserin was calculated.

RESULTS AND DISCUSSION

Method validation

The method validation was done according to the ICH guidelines in terms of system suitability, selectivity, specificity, linearity, sensitivity, precision, accuracy and robustness.[2-4]

System suitability

Prior to the validation study, system suitability tests were performed by measurement of general characteristics such as peak symmetry, number of theoretical plates, retention time, tailing factor etc. The results obtained were satisfactory and in accordance with guidelines and shown in Table 1.

Table 1: System suitability parameters of Pimavanserin.

S. No.	Parameter	Description/Value
1	Stationary Phase	Agilent C_{18} Column (150 mm × 4.6 mm and 5µm)
2	Mobile Phase	ACN: water: Potassium Di hydrogen orthophosphate (0.02M) 40:10:50 v/v, Buffer pH was adjusted to 6 with Ortho phosphoric acid
3	Flow rate	1.2 ml/min
4	Detection Wavelength	290.0 nm
5	Detector	Photo diode array
6	Injection	Auto sampler –Waters, model 717 plus
7	Injection volume	10µl
8	Column Temperature	Ambient
9	Run time	5 min
10	Elution Technique	Isocratic
11	Diluent	Mobile Phase

Specificity

Specificity of an analytical method is its capability to measure the analyte precisely and particularly in presence of parts that may be likely to be present in the sample matrix. Chromatograms of standard and sample prove that the method was specific.

Linearity

The linearity plot (Figure 2) was constructed with five concentration at the level of 40-120% (40, 60, 80, 100, 120 µg/ml of Pimavanserin). The response of the drug was found to be linear in the studied concentration range and the linear regression equation was y = 95602x - 9465.4. The correlation coefficient was found to be 0.9997.

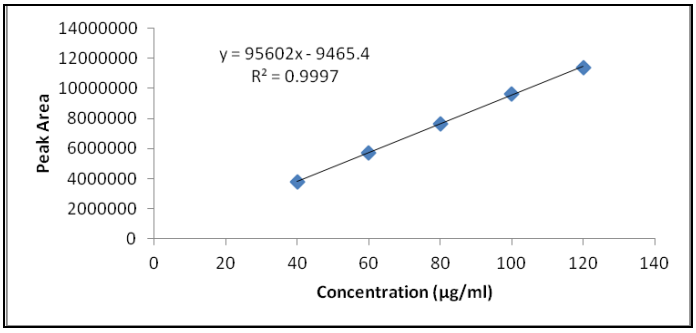

Figure 2: Linearity curve for Pimavanserin.

Sensitivity

The method sensitivity was determined with respect to limit of detection (LOD) and limit of quantitation (LOQ). The LOD and LOQ for pimavanserin were assessed at a signal-to-noise ratio of 3:1 and 10:1 respectively. The determined LOD and LOQ values are 0.0006µg/ml and 0.002µg/ml, respectively. The values showed that the sensitivity of the method was good.

Accuracy

The method accuracy for pimavanserin was determined by analyzing standard solution at 50, 100, 150% level.

The accuracy of the results was demonstrated by calculating the percent recovery. The results showed adequate accuracy performance for the determination of pimavanserin in figure 3-5.

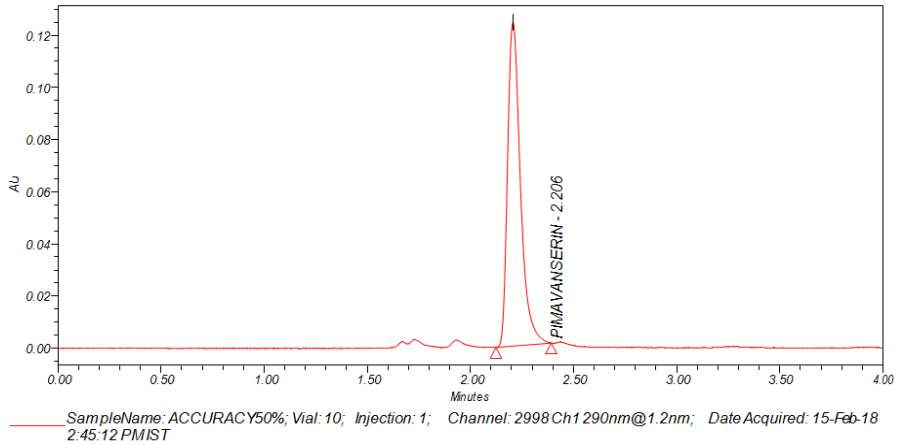

Figure 3: Accuracy Chromatogram of Pimavanserin at 50%.

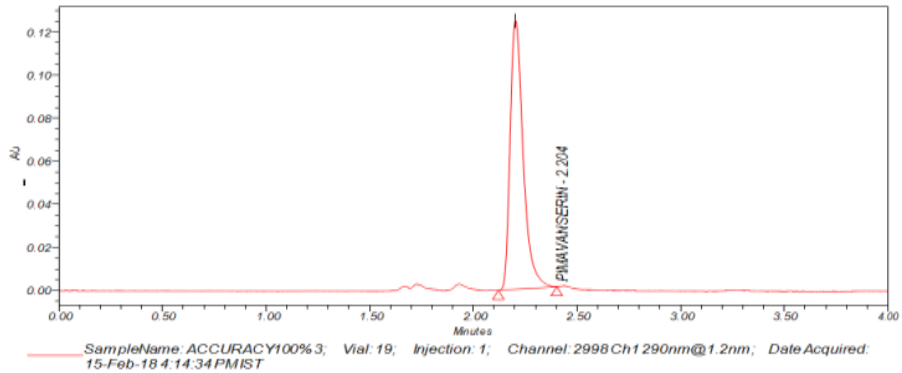

Figure 4: Accuracy Chromatogram of Pimavanserin at 100%.

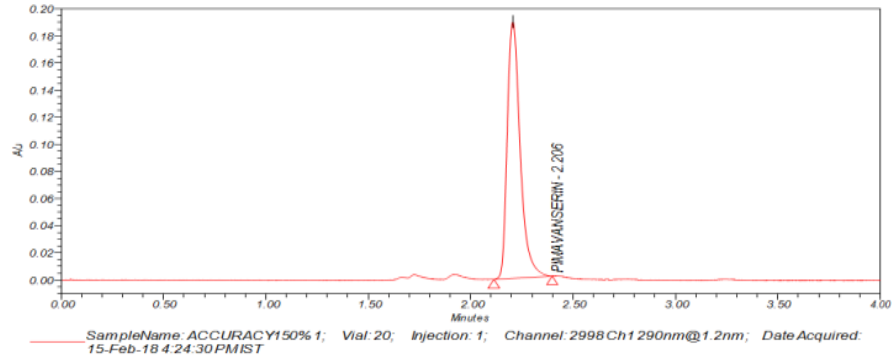

Figure 5: Accuracy Chromatogram of Pimavanserin at 150%.

Precision

Intra and inter-day precision of the analytical method was determined by performing method precision for three times in same day and followed by three consequent days. %RSD was calculated and found to be within the specified limits (<2 %). Table 2 shows the results of precision and figure 6 & 7 shows precision chromatograms.

Table 2: Summary of precision study for Pimavanserin.

S. No	Intraday precision		Inter day precision	
	Peak Area	% Assay	Peak Area	% Assay
1.	7522699	99.05	7622699	100.37
2.	7642606	100.63	7642606	100.63
3.	7618974	100.32	7618974	100.32
4.	7676367	101.07	7676367	101.07
5.	7765881	102.25	7665881	100.94
6.	7532006	99.17	7632006	100.49
Average	7626422	**100.42**	7643089	**100.63**
STDEV	91578.98	**1.21**	23440.36	**0.31**
% RSD	**1.20**	**1.20**	**0.31**	**0.31**

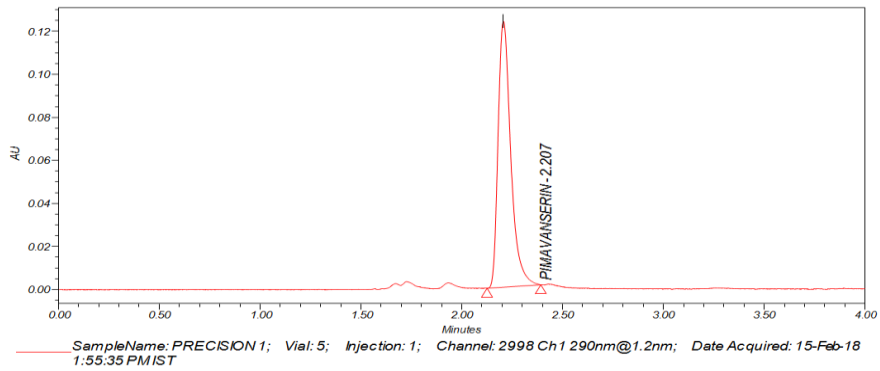

Figure 6: Chromatogram of Inter precision study of Pimavanserin.

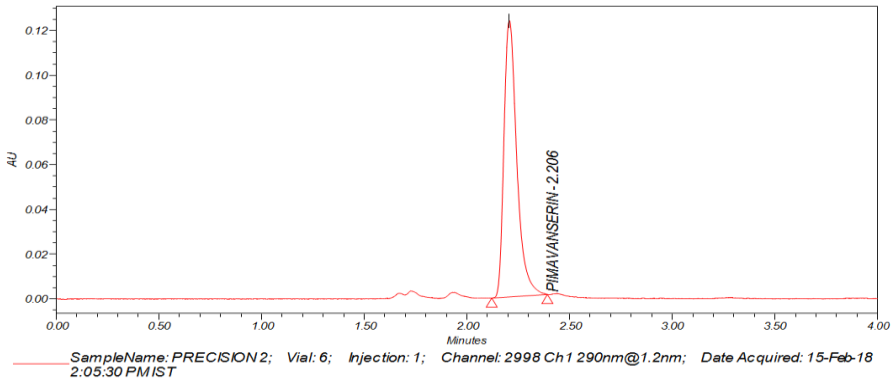

Figure 7: Chromatogram of Intraday precision study of Pimavanserin.

Robustness

Method robustness was established by deliberately varying the experimental conditions such as flow rate (±0.1 ml/min),column oven temperature(±2°C),mobile phase components ratio (±5%), pH of mobile phase(±0.2 units) and detection wavelength(±2 nm).The study was carried out on the same day with pimavanserin standard solution of concentration 17 µg/ml. In each case, plate count and peak tailing were calculated. The calculated values were within the acceptance limits. Therefore the method is considered as robust and shown in table 3.

Table 3: Evaluation data of robustness study for Pimavanserin.

Robust conditions	Rt (min)	Peak area	% Assay
1.3 ml/min flow rate	3.283	7592252	99.97
Column temp at 28°C	3.278	7642606	100.63
Column temp at 32°C	3.275	7418974	97.68
Wave length 268 nm	3.278	7676367	101.07
Wave length 292 nm	3.277	7665881	100.94

Degradation studies (stress testing)

Forced degradation study was performed to evaluate the stability of the developed method using the stress conditions like exposure of sample solution to acid (0.1 N HCl), base (0.1 N NaOH), peroxide (H_2O_2), UV light, and water. Investigation was done for the degradation products. For acid treatment 5 ml of stock solution of Pimavanserin was taken in a 25 ml volumetric flask; to it 5 ml of 0.1 N HCl was added and kept aside at room temperature for 24 hrs. This solution was neutralized with alkali 0.1 N NaOH and diluted suitably to a final concentration of 400 µg/ml. Same procedure was followed for alkali (0.1 N NaOH) treated sample and neutralized with acid (0.1 N HCl) and sample was treated with 10% solution of H_2O_2 for peroxide treated samples. 20µl all the sample solutions were injected into the chromatograph and chromatograms were recorded. The results of forced degradation studies were shown in figure 8.

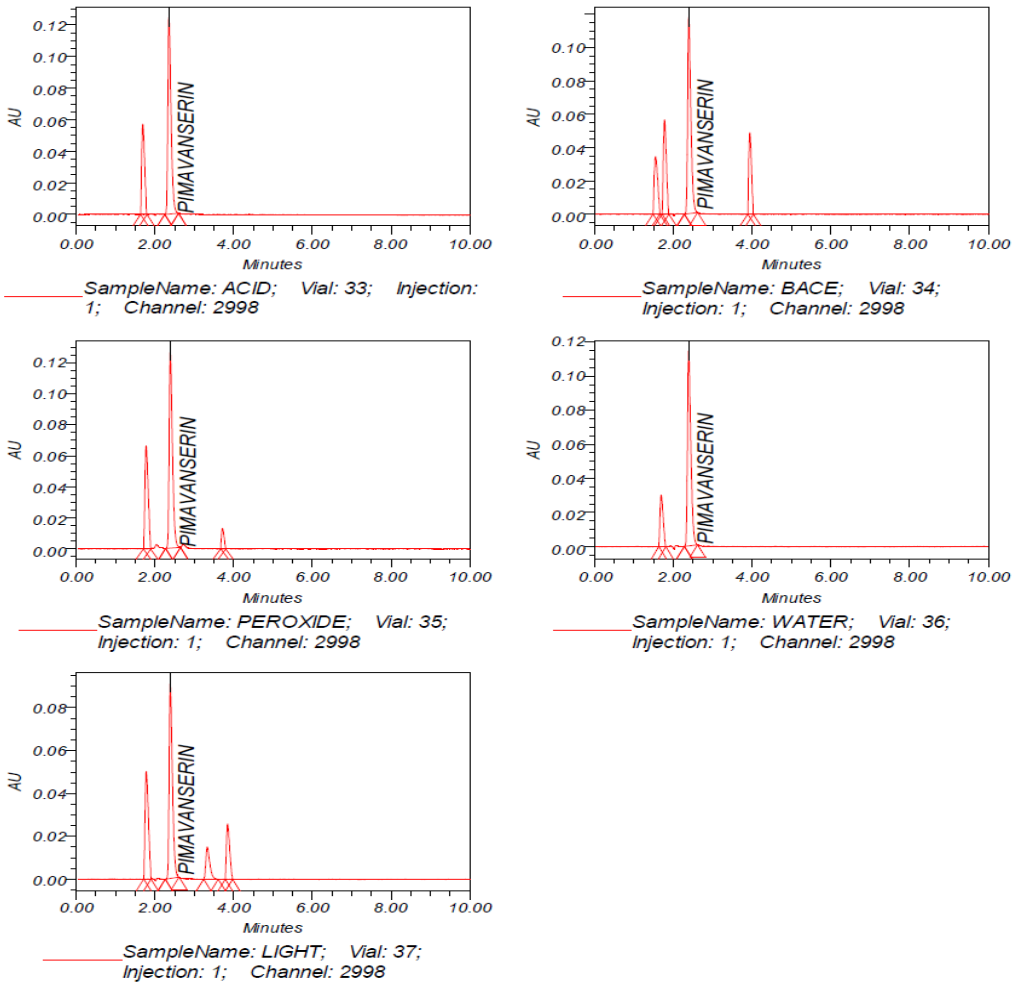

Figure 8: Forced degradation study of Pimavanserin.

CONCLUSION

For the first time, a stability indicating HPLC with photo diode array detector method has been developed and validated for the assay of pimavanserin in bulk and tablet dosage form. Analysis of pimavanserin in vitro by the proposed method was valid. All parameters satisfied the acceptance criteria of the ICH guidelines. The stability indicating nature of the developed method indicated that the pimavanserin could be assayed in the presence of their degradation products. Therefore the developed and validated stability indicating method can be employed for the routine estimation of pimavanserin in quality control laboratories.

REFERENCES

1. https://www.accessdata.fda.gov/drugsatfda_docs/label/2016/207318lbl.pdf
2. ICH: Q2B, Analytical Validation-Methodology, 1996.
3. ICH: Q2A, Text on validation of Analytical procedure, 1994.
4. ICH: Q2(R1),Validation of Analytical procedures. Text and Methodology, 2005.
5. Shixiao Wangaet al., Development of a UPLC–MS/MS method for determination of pimavanserin tartrate in rat plasma: Application to a pharmacokinetic study. Journal of Pharmaceutical Analysis, December 2017; 7(6): 406-410.