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Research Article

Quantification of Pimavanserin in Bulk and Tablet Dosage Form Using A Stability Indicating High Performance Liquid Chromatographic Method

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ABSTRACT

Background: Pimavanserin, an antipsychotic agent, is used to treat patients suffering with Parkinson's disease. Till now no stability indicating reverse phase HPLC method was reported for the quantification of pimavanserin in bulk and tablet dosage form. Hence in the present study, a new sensitive, precise and accurate stability indicating reverse phase HPLC method with photodiode array detector has been developed for the quantification of pimavanserin in bulk and tablet dosage form.

Methods: Separation and analysis of pimavanserin was achieved on Kromasil C18 (5 µm, 250 mm × 4.6 mm) column using 0.1M NaH_2PO_4, methanol and acetonitrile in ratio of 55:30:15 (v/v/v) as mobile phase at 25°C. The flow rate was 1.0 ml/min. The effluents were monitored with detector set at 239 nm. The method validation was done with regard to the guidelines by the International Conference on Harmonization. Pimavanserin was subjected to acid, alkali and neutral hydrolysis, hydrogen peroxide oxidation, thermal degradation, and photo (sunlight) degradation.

Results: Linear relationship was obtained between the concentration of drug and peak area response in the range of 4.25-34.0 µg/ml. The limits of detection and quantitation were found to be 0.027 µg/ml and 0.089 µg/ml, respectively. All the validation characteristics were within the acceptance criteria. The peaks of degradation products were well resolved from the pimavanserin peak.

Conclusion: The developed and validated method is able to quantify the pimavanserin in the presence of degradation products.

Introduction

Pimavanserin is an antipsychotic drug that acts as a selective antagonist on the serotonin subtype 5-HT2A receptor subtype and 40 fold increased selectivity towards 5-HT2C receptor subtype.[1-4]

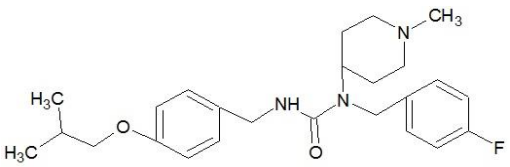

Figure 1. Chemical structure of pimavanserin

Pimavanserin has no considerable affinity or activity towards 5-HT2B 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). United States Food and Drug Administration approved pimavanserin in 2016 for the treatment of delusions and hallucinations allied with psychosis experienced by a few patients suffering with Parkinson's disease.[5-8]

Up to date only one method based on ultra performance liquid chromatography tandem mass spectrometry has been developed for the quantification of pimavanserin and applied to routine pharmacokinetic study of pimavanserin in rats.[9]

Knowledge about the molecule's stability helps in choosing correct formulation and package plus providing appropriate storage conditions, which is vital for regulatory documentation.[10-12] 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.

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Materials and Methods

Solvents and chemicals

Pimavanserin reference standard was provided kindly by Lara drugs Pvt Ltd., (Hyderabad, India). Nuplzid (Acadia Pharmaceuticals Inc., San Diego) tablets labeled to contain 17 mg of pimavanserin were obtained from a local pharmacy market. HPLC grade acetonitrile and methanol were purchased from Merck India Ltd (Mumbai, India). Analytical reagent sodium dihydrogen orthophosphate, orthophosphoric acid, hydrogen peroxide, hydrochloric acid and sodium hydroxide were supplied by Sd. Fine Chemicals Ltd (Mumbai, India). HPLC grade water was prepared using Milli-Q system (Millipore, USA).

Instrumentation

Waters Alliance HPLC system 2695 Module with a 2998 PDA detector, degasser, auto sample injector and column oven was used in the present analysis. Data acquisition and processing was done with Empower 2 software. Method development and validation was done using Kromasil, C18, 5 µm, 250 mm × 4.6 mm analytical column.

Optimized HPLC conditions

Isocratic elution was performed with a mobile phase comprised of filtered (using a 0.45 µm membrane filter) and degassed 0.1M NaH_2PO_4: methanol: acetonitrile (55:30:15, v/v/v) adjusted to pH 5 with orthophosphoric acid and pumped at a flow rate of 1.0 ml/min. The column temperature was set at 25 °C. The samples were injected at 10 µl injection volume and eluted samples were analyzed at a wavelength of 239 nm. The total runtime was 6 min.

Standard stock and working solutions

The standard stock solution (170 µg/ml) of pimavanserin was prepared by dissolving an accurately weighed 17 mg of pimavanserin reference standard in 100 ml of mobile phase. The pimavanserin working standard solutions (4.25 µg/ml, 8.5 µg/ml, 12.75 µg/ml, 17.0 µg/ml, 21.25 µg/ml, 25.5 µg/ml, 29.75 µg/ml and 34.0 µg/ml) were obtained by appropriately diluting the standard stock solution with mobile phase.

Preparation of placebo blank solution

Accurately weighed common excipients, starch (40 mg), hydroxyl cellulose (35 mg), gum acacia (35 mg), lactose (20 mg), sodium citrate (35 mg), talc (40 mg), sodium alginate (35 mg) and magnesium stearate (35 mg), were made into a homogeneous mixture. An accurately weighed (100 mg) homogeneous mixture was transferred to a 100 mL volumetric flask containing 30 mL of mobile phase. The contents of the flask were sonicated (20 min) and filtered (0.45 µm membrane filter). The filtrate was made upto to 100 mL with mobile phase.

Construction of calibration curve

Aliquots (10 µl) 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

Ten tablets were crushed into powder. The tablet powder weight equivalent to 17 mg of pimavanserin was transferred to 100 ml volumetric flask and sonicated with 30 ml of mobile phase for 20 min. The volume was made to 100 ml with mobile phase and filtered through 0.45 µm membrane filter. The stock tablet sample solution was then diluted aptly with mobile phase to get the final concentration of 17 µg/ml of pimavanserin. 10 µl of pimavanserin tablet sample solution (17 µg/ml) prepared was injected into the HPLC system and analyzed the developed method. The nominal content of pimavanserin in the tablet was calculated either using the calibration graph or regression equation.

Forced degradation studies

The forced degradation studies were performed through the analysis of pimavanserin tablet sample solution (17 µg/ml), which was exposed to accelerated degradation conditions as per the International Conference on Harmonization (ICH) guidelines.[13] All results were compared to a reference solution, prepared in the same day.

Acid and alkaline hydrolysis

10 ml of pimavanserin tablet sample solution was mixed with 10 ml of 0.1 N HCl (for acidic degradation acid) or 10 ml of 0.1 N NaOH (for alkaline degradation) in 100 ml volumetric flasks. The solutions were sonicated at room temperature for 30 min. After this period, the acid and alkali degraded solutions were neutralized with apt volume of 0.1 N NaOH and 0.1N HCl, respectively. The resulting solutions were diluted with mobile phase to get a concentration of 17 µg/ml, filtered and injected.

Thermal and photo degradation

10 ml of pimavanserin tablet sample solution was transferred to volumetric flask (100 ml) and exposed to 105°C for 30 min in oven (for thermal degradation) or exposed to sun light for 24 hr (for photo degradation). After the specified period of degradation, the resulting solution was diluted with mobile phase for a concentration of 17 µg/ml, filtered and injected.

Oxidative and neutral degradation

10 ml of 30% hydrogen peroxide solution (for oxidative degradation) or 10 ml of deionised water (for neutral degradation) was added into a 100 ml volumetric flask containing 10 ml pimavanserin tablet sample solution. After sonication for 30 min at room temperature, the solutions were diluted to 100 ml with mobile phase until 17 µg/ml. These solutions were filtered and injected.

Results and Discussion

Method development

Several experimental trials were performed to develop a stability indicating HPLC method for the quantification of pimavanserin in tablets. ODS C18 column (150 mm × 4.6 mm i.d., 5 µm particle size) and Kromasil C18 column (250 mm × 4.6 mm i.d., 5 µm particle size) with different temperatures were tried. Kromasil C18 column (250 mm × 4.6 mm i.d., 5 µm particle size) with 25 ºC temperature was found as most suitable column, as it produced symmetrical peaks with high sensitivity within a reasonable analysis time. As a result, the same column was used. Several variations in the mobile phase composition, flow rate and pH were performed to study the possibilities for getting better system performance. 0.1M NaH_2PO_4, methanol and acetonitrile in the ratio of 55:30:15 (v/v/v) with 5.0 pH at a flow rate of 1.0 ml/min was optimal for good peak shape and fine separation in a reasonable time with good sensitivity. The detector response of pimavanserin was studied. At a wavelength of 239 nm, highest sensitivity was obtained. Hence, the same wavelength was used for the detection and analysis. The result of method optimization led to the elution of pimavanserin from the column at 3.897 min (Figure 2).

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.[14]

System suitability

System suitability parameters like peak tailing, plate count, and percent relative standard deviation for retention time and peak area response were calculated to demonstrate that the HPLC system performed well. For this study, pimavanserin standard solution (17 µg/ml) was injected into the HPLC system in five replicates. The obtained values were in the acceptable limits as given in Table 1.

Selectivity

The selectivity of the method was evaluated by comparison of chromatograms of mobile phase blank, placebo blank, pimavanserin tablet sample solution (17 µg/ml) with pimavanserin standard solution (17 µg/ml). The representative chromatograms of the four samples are shown in Figure 3. No extra peak or interference peak was observed in the chromatograms of mobile phase blank, placebo blank and tablet sample solution. The retention time of pimavanserin in tablet sample solution and standard solution was same. Hence, the method is selective.

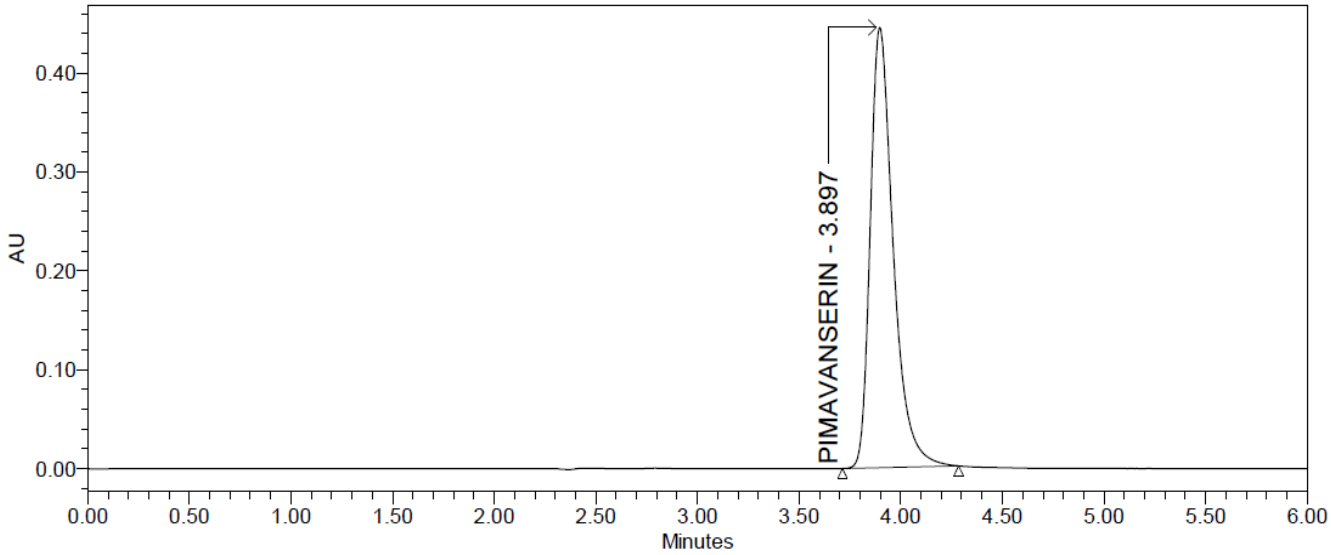

Figure 2. Chromatogram of pimavanserin after method optimization.

Table 1. System suitability parameters of the developed method for pimavanserin.

Injection No.	RT (min)	Peak area (mAU)	Plate Count	Tailing
1	3.896	4134605	5990	1.42
2	3.893	4144584	6035	1.43
3	3.891	4065286	6145	1.45
4	3.889	4165002	6111	1.44
5	3.887	4091773	6113	1.43
Mean	**3.891**	**4120250**	**6078.800**	**1.434**
%RSD	**0.090**	**0.988**	**1.053**	**0.795**
Recommended limit[15]	**RSD ≤ 2**	**RSD ≤ 2**	**> 2000**	**≤ 2**

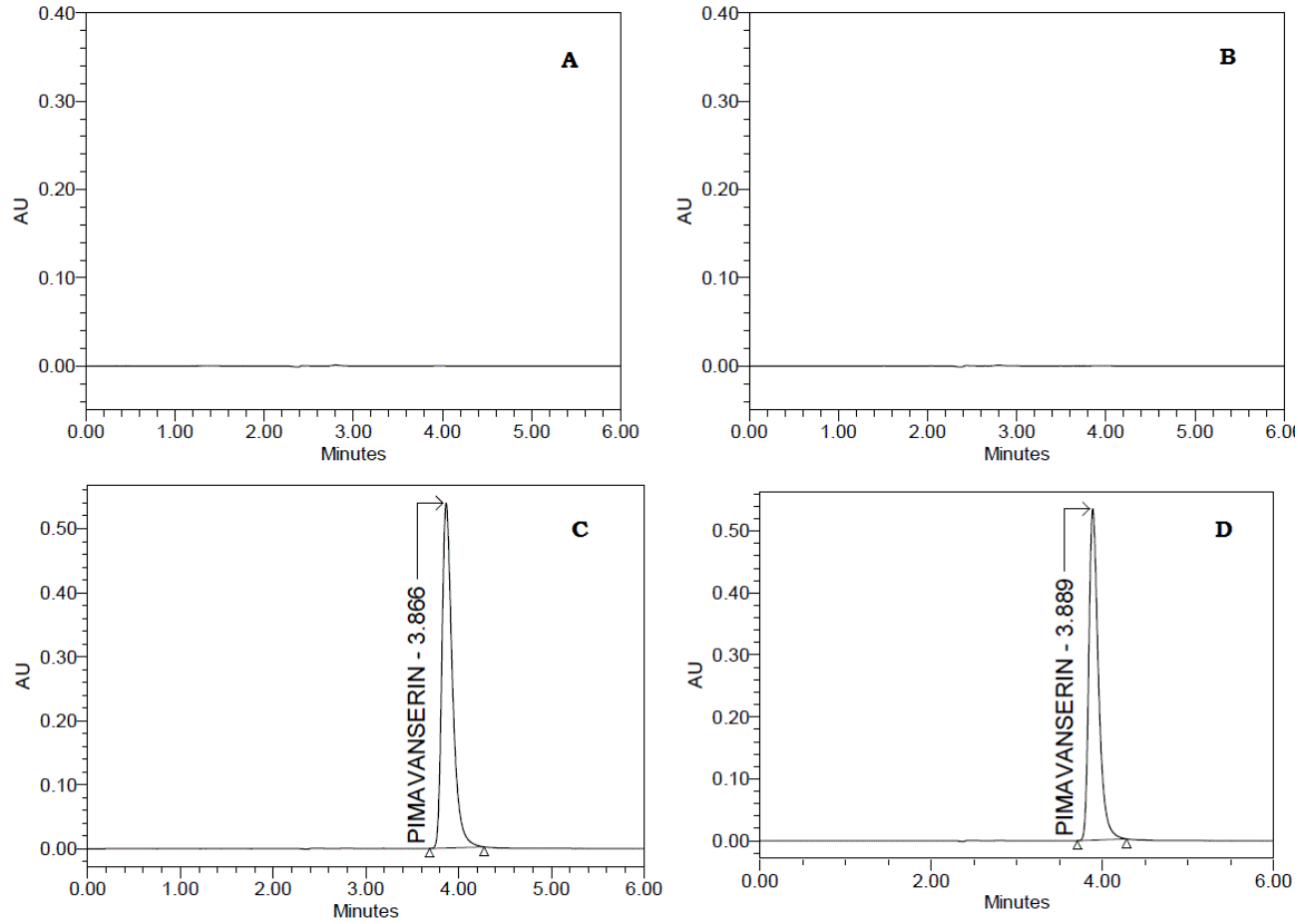

Figure 3. HPLC chromatograms of solutions of (A) mobile phase blank (B) Placebo blank (C) Pimavanserin tablet sample (D) Pimavanserin standard.

Specificity

To prove the specificity and stability indicating nature of the proposed method, forced degradation studies were done. The pimavanserin tablet sample solution was subjected to acid, base, oxidative, thermal, neutral and photo degradation. The chromatograms of pimavanserin treated with acid, base, hydrogen peroxide, heat, sun light and water are shown in Figure 4. Results of the forced degradation studies are summarized in Table 2. Peak of pimavanserin was well resolved from the degradation products produced. The obtained results indicating that the developed method could evaluate pimavanserin in presence of their degradants.

Specificity was also assessed using photodiode array detection to make sure the peak homogeneity and to evaluate peak purity in applied stress conditions for pimavanserin. For this purpose, the peak threshold value and peak purity angles were calculated using Waters empower 2 software. The peak threshold means the minimum purity value which means the peak is pure. These values are based on noise statistical analysis in spectra. If the peak purity angle value is lesser than the peak threshold value, within the noise of the system the peak is considered spectrally homogeneous. The results were shown in Table 2. The peak threshold value was greater than peak purity angle (Table 2), which confirmed the purity and homogeneity of pimavanserin peak in all the degradation conditions applied.

Table 2. Forced degradation results of pimavanserin in tablet sample.

Degradation condition	Peak area (mAU)	Percent of drug		Peak purity		Retention time of degradant
		Recovered (%)	Degraded (%)	Purity angle	Purity threshold	
Undegraded	4120250	100.00	-	-	-	-
Acidic	3597561	87.05	12.95	0.319	0.839	2.412, 2.752
Basic	3764973	91.10	8.9	0.255	0.756	2.408, 2.753
Oxidative	3592725	86.94	13.06	0.311	0.799	2.403, 2.754
Thermal	3464638	83.84	16.16	0.245	0.842	2.417, 2.758
Photo	3694840	89.41	10.59	0.305	0.801	2.407, 2.757
Neutral	3719610	90.01	9.99	0.294	0.766	2.413, 2.754

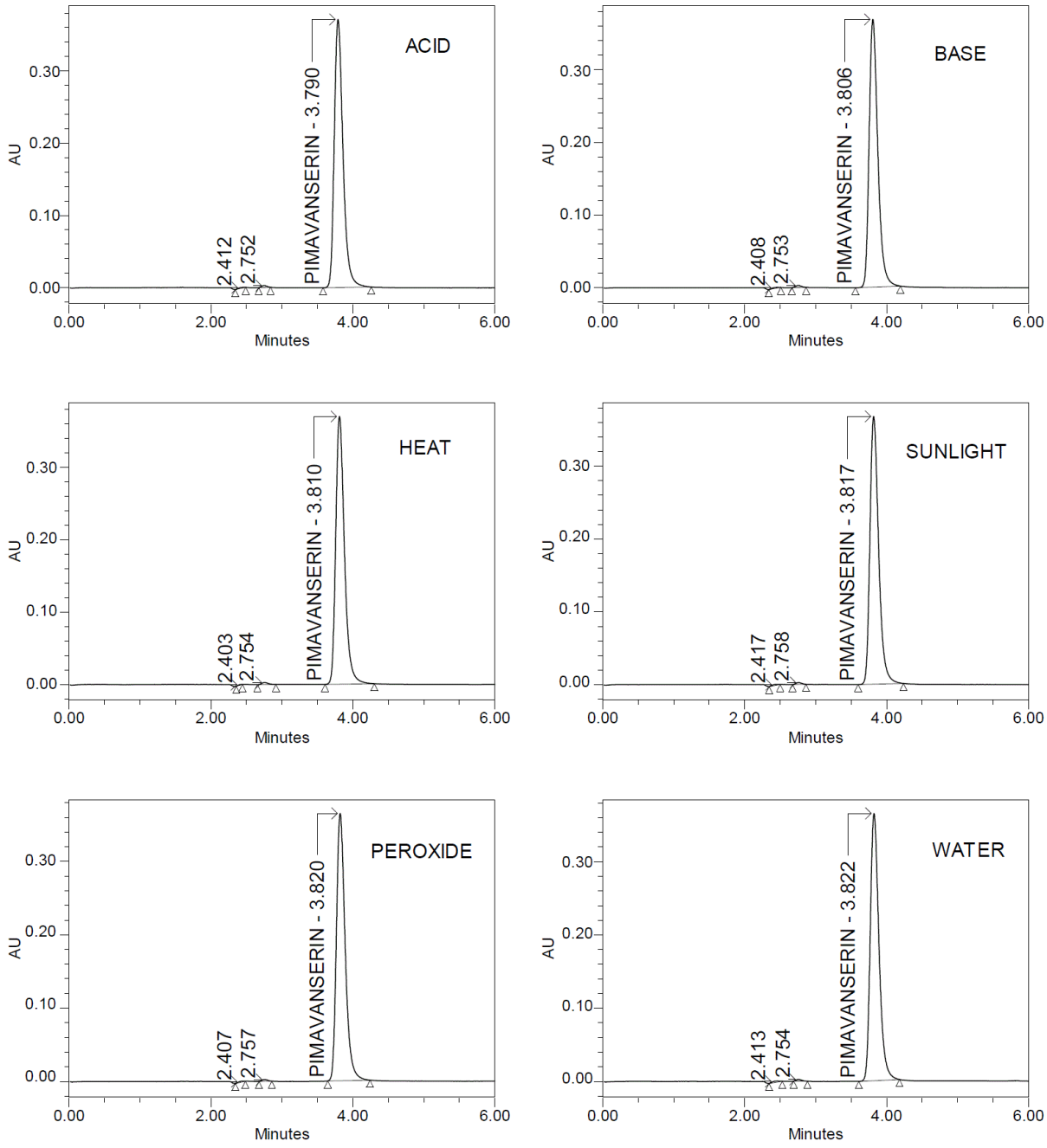

Figure 4. HPLC chromatograms of pimavanserin tablet sample solution after exposed to different forced degradation conditions.

Linearity

The calibration curve was constructed by plotting the peak area response of pimavanserin against the concentration (µg/ml). Calibration curve was linear over a range of concentration 4.25-34.0 µg/ml. Linear regression equation and regression coefficient (R^2) were y = 24239 x + 3715 and 0.9997, respectively for pimavanserin. The results showed excellent correlation exists between the peak area response and concentration.

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 0.027 µg/ml and 0.089 µg/ml, respectively. The values showed that the sensitivity of the method was good.

Precision

The precision of the HPLC method for pimavanserin was evaluated by analyzing standard solution (17 µg/ml) six times. Percentage relative standard deviation (%RSD) of peak area response of pimavanserin was used to assess the precision. The results of precision exhibited %RSD below 2.0% (Table 3), indicating the adequate precision of the method.

Table 3. Precision and accuracy of the method for pimavanserin.

Precision		Accuracy		
Injection No.	Peak area (mAU)	Concentration of pimavanserin (µg/ml)		Recovery (%)
		Taken	Found	
1	4122502	17	16.96	99.75
2	4129745	17	16.99	99.93
3	4123040	17	16.96	99.77
4	4127866	17	16.98	99.88
5	4127814	17	16.98	99.88
6	4124015	17	16.96	99.79
Mean	**4125830**	**-**	**16.97**	**99.83**
RSD (%)	**0.073**	**-**	**0.073**	**0.073**

Table 4. Recovery of pimavanserin by the proposed method.

Spiked level (%)	Concentration of pimavanserin (µg/ml)		Recovery (%)	Mean recovery (%)
	Spiked	Found		
50	8.5	8.48	99.81	
	8.5	8.47	99.71	99.77
	8.5	8.48	99.78	
100	17.0	16.98	99.86	
	17.0	16.97	99.81	99.86
	17.0	16.98	99.90	
150	25.5	25.46	99.83	
	25.5	25.44	99.75	99.80
	25.5	25.45	99.81	

Table 5. Robustness of the method.

Parameter varied	Plate count	Peak tailing
Column temperature - 23 ºC	4458	1.30
Column temperature - 27 ºC	5173	1.34
Flow rate – 0.9 ml/min	4539	1.32
Flow rate – 1.1 ml/min	5566	1.33
Mobile phase ratio (0.1 M NaH_2PO_4: methanol: acetonitrile) - 55:35:10 *v/v/v*	6010	1.41
Mobile phase ratio (0.1 M NaH_2PO_4: methanol: acetonitrile) - 55:25:20 *v/v/v*	5954	1.34
Mobile phase pH – 4.8	5973	1.48
Mobile phase pH – 5.2	5982	1.31
Detection wavelength – 237 nm	6008	1.39
Detection wavelength – 241 nm	6011	1.41
Recommended limit	> 2000	≤ 2

Accuracy

The method accuracy for pimavanserin was determined by analyzing standard solution (17 µg/ml) six times. The accuracy of the results was demonstrated by calculating the percent recovery. The results showed adequate accuracy performance for the determination of pimavanserin (Table 3).

Recovery

The newly developed HPLC method was further evaluated for its accuracy by the analysis of the placebo spiked with pure pimavanserin at three different concentration levels. Recovery of the spiked pimavanserin was determined by the proposed method thrice. The recovery values (Table 4), indicating that the developed method ensure the acquisition of reliable accurate data for pimavanserin at different concentrations.

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 (Table 5). Therefore the method is considered as robust.

Conclusion

For the first time, a stability indicating HPLC with photodiode 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.

Conflict of interests

The authors claim that there is no conflict of interest.

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