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New method develment for the estimation of neuroactive drug (pimavanserin) by RP-HPLC method

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ABSTRACT

RP-HPLC method has been developed & validated for the analysis of Pimavanserin API. The HPLC is the method of choice in the field of analytical chemistry since this method is specific, robust, linear, precise and accurate and the limit of detection is low and also it offers: Speed (many analysis can be accomplished in 20 min or less), Greater sensitivity (various detectors can be employed) Improved resolution (wide variety of stationary phases) Reusable columns (expensive columns but can be used for many analysis) Ideal for the substances of low viscosity Easy sample recovery, handling and maintenance. To develop a precise, linear, specific & suitable stability indicating RP-HPLC method for analysis of Pimavanserin, different chromatographic conditions were applied & the results observed in chapters. Isocratic elution is simple, requires only one pump & flat baseline separation for easy and reproducible results. So, it was preferred for the current study over gradient elution. In case of RP-HPLC various columns are available, but here Symmetry ODS RP C_{18}, 5µm, 15mm x 4.6mm i.d. column was preferred because using this column peak shape, resolution and absorbance were good. Mobile phase & diluent for preparation of various samples were finalized after studying the solubility of API in different solvents of our disposal (methanol, ethanol, water, DMF, DMSO).

Keywords: RP-HPLC, Pimavanserin, Stability studies, method development, method validation

INTRODUCTION[1 2 3 4 5]

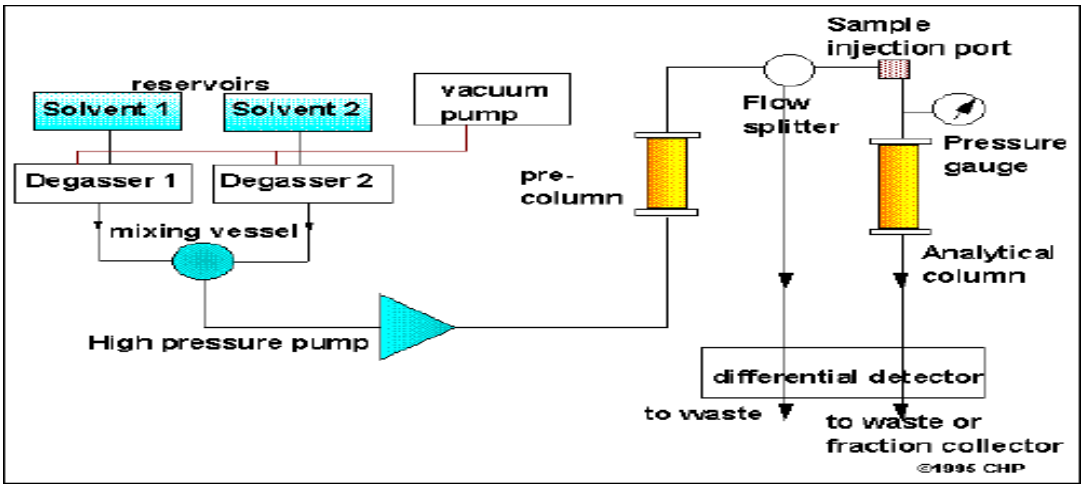
Different pharmaceutical analytical techniques are available that are extremely sensitive, provide precise, accurate and detailed information regarding the sample. The various analytical techniques used are categorized as follows: Spectral methods (UV, IR spectroscopy), Chromatographic methods (TLC, HPLC, GC),Electro analytical methods (potentiometer, conductometry, amperometry) Biological and microbiological methods (assay of vitamins and antibiotics),Radioactive methods (RIA),Physical methods (DTA, DSC, TGA),Miscellaneous methods (Titrimetric methods). In some instrumental procedures the sample is destroyed (destructive methods), where as in others it remains unchanged (non –destructive) and may be used in subsequent studies. The choice of an instrumental procedure for the determination of a specific element or compound really involves two choices: 1) the instrument to be used.2) the chemical system.

High-Performance Liquid Chromatography (Hplc)

The HPLC is the method of choice in the field of analytical chemistry since this method is specific, robust, linear, precise and accurate and the limit of detection is low and also it offers: Speed (many analysis can be accomplished in 20 min or less), Greater sensitivity (various detectors can be employed) Improved resolution (wide variety of stationary phases) Reusable columns (expensive columns but can be used for many analysis) Ideal for the substances of low viscosity Easy sample recovery, handling and maintenance. Instrumentation leads itself to automation and quantification

(less time and less labour) Precise and reproducible Integrator itself does calculations.

Instrumentation[4]



System Suitability Parameters [10]: The parameters that are affected by the changes in chromatographic conditions are: Retention time (t_R), Resolution (R_S), Capacity factor ($k`$), Selectivity (α), Number of Theoretical plates (N), Height equivalent to a Theoretical plate **(**HETP), Asymmetry factor Tailing factor.

Statistical Parameters: Linear regression [13], Standard deviation [14], Percentage relative standard deviation (% RSD), Standard error of mean (S.E)[15]

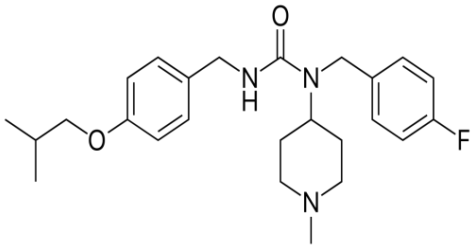
Hplc Method Development is a good method development strategy should require only as many experimental runs as are necessary to achieve the desired final result. Finally method development should be as simple as possible, and it should allow the use of sophisticated tools such as computer modeling. During initial method development, a set of initial conditions.

DRUG PROFILE

Name [33] **:** Pimavanserin

Description : Pimavanserin (ACP-103), trade name Nuplazid, is a drug developed by Acadia Pharmaceuticals which acts as an inverse agonist on the serotonin receptor subtype 5-HT2A, with 40x selectivity over 5-HT2C

Structure :



Molecular Weight : 427.564

Chemical Formula : $C_{25}H_{34}FN_3O_2$

Indication [34] **:** Investigated for use/treatment in neurologic disorders, parkinson's disease, psychosis, schizophrenia and schizoaffective disorders, and sleep disorders.

Mechanism of action : ACP-103 reduces haloperidol-induced akathisia in patients with schizophrenia. ACP-103 is a 5-HT2A inverse agonist, to reduce the side effects associated with antipsychotic drug treatment with haloperidol.

METHOD DEVELOPMENT

Table 1: List of Instrument used

S. No.	Instruments/Equipments/Apparatus
1.	HPLC with Empower2 Software with Isocratic with UV-Visible Detector (Waters).
2.	ELICO SL-159, INDIA UV – Vis spectrophotometer
3.	Electronic Balance (SHIMADZU ATY224)
4.	Ultra Sonicator (Wensar wuc-2L)
5.	Thermal Oven
6.	Symmetry ODS RP C_{18},5µm, 15mm x 4.6mm i.d.
7.	P^H Analyzer (ELICO)

8.	Vacuum filtration kit (BOROSIL)

CHEMICALS / REAGENTS USED

Table 2: List of Chemicals used

S.N.	Name	Specifications		Manufacturer/Supplier
		Purity	Grade	
1.	Doubled distilled water	99.9%	HPLC	Sd fine-Chem ltd; Mumbai
2.	HPLC Grade Water	99.9%	HPLC	Sd fine-Chem ltd; Mumbai
3.	Methanol	99.9%	HPLC	Loba Chem; Mumbai.
4.	Ethanol	99.9	L.R.	Sd fine-Chem ltd; Mumbai
5.	DMSO	99.9%	HPLC	Loba Chem; Mumbai.
6.	DMF	99.9	L.R.	Sd fine-Chem ltd; Mumbai

SOLUBILITY STUDY

Table 3: Lists of Solvents

SOLVENT	SOLUBILITY
Methanol	Soluble
Ethanol	Soluble
DMF	Soluble
DMSO	Soluble
Acetonitrile	Insoluble
Water	Insoluble

Method Development and its Validation for Pimavanserin By RP-HPLC

Selection of Wavelength

The standard & sample stock solutions: were prepared separately by dissolving standard & sample in a solvent in mobile phase diluting with the same solvent. (After optimization of all conditions) for UV analysis. It scanned in the UV spectrum in the range of 200 to 400nm. This has been performed to know the maxima of Pimavanserin, so that the same wave number can be utilized in HPLC UV detector for estimating the Pimavanserin. The scanned UV spectrum is attached in the following page.

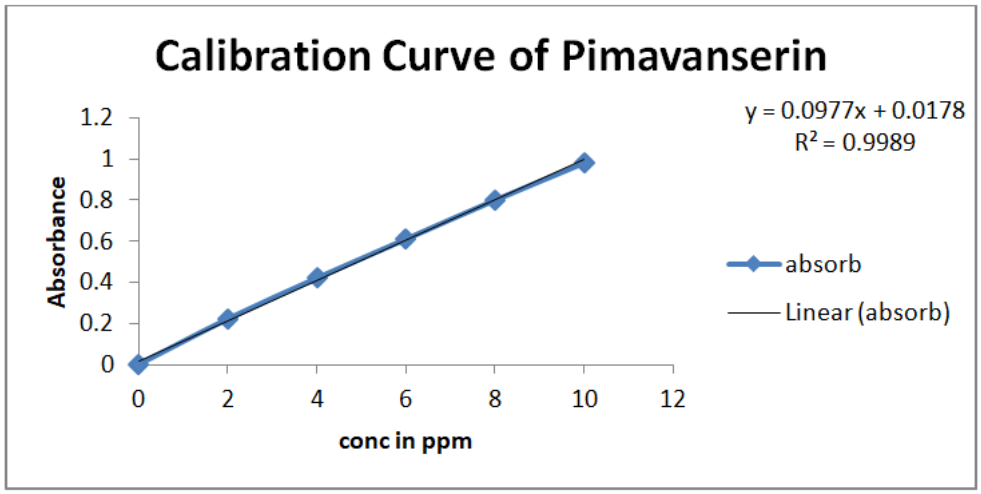


Fig 1: UV Calibration Curve for Pimavanserin

Table 4: Results of Calibration Curve for Pimavanserin

Concentration	Absorbance
0	0
2	0.222
4	0.421
6	0.611
8	0.801
10	0.982

While scanning the Pimavanserin solution we observed the maxima at 240nm. The UV spectrum has been recorded on T60-LAB INDIA make UV–Vis Spectrophotometer model UV-2450.

Optimization of Chromatographic Conditions

The chromatographic conditions were optimized by different means. (Using different column, different mobile phase, different flow rate, different detection wavelength & different diluents for sample preparation etc.

Table 5: Summary of Process Optimization

Column Used	Mobile Phase	Flow	Wave	Observation	Result
Symmetry ODS RP C_{18},5µm, 15mm x	Water : Acetonitrile	0.8	240nm	Low	Method
Symmetry ODS RP C_{18},5µm, 15mm x	Water : Methanol	1.0	240nm	Very low	Method
Symmetry ODS RP C_{18},5µm, 15mm x	Methanol : Acetonitrile	1.0	240nm	Tailing peak	Method
Symmetry ODS RP C_{18},5µm, 15mm x	Phosphate buffer (pH=4.20) :	1.0	240nm	Broad Peak	Method
Symmetry ODS RP C_{18},5µm, 15mm x	Phosphate buffer (pH=3.80) :	1.0	240nm	Tailing peak	Method
Symmetry ODS RP C_{18},5µm, 15mm x	Methanol : ACN = 70:30	1.0	240nm	Good Peak	Method

Table 6: Summary of Optimized Chromatographic Conditions

Mobile phase	Methanol : ACN = 70:30
Column	Symmetry ODS RP C_{18},5µm, 15mm x 4.6mm i.d.
Flow rate	1.0 ml/ min.
Wavelength	240nm
Sampling System	Automatic
Temp. of Auto sampler	Ambient
Volume of injection	10µl
Run time	07 mins
Mode of Separation	Isocratic

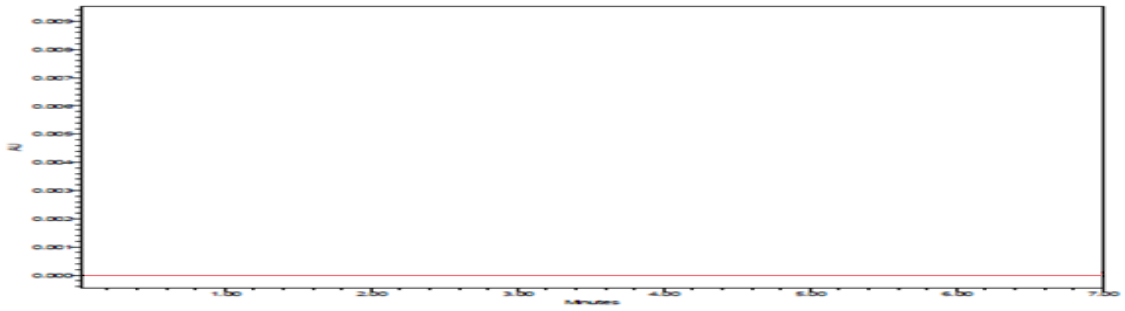


Fig 2: Chromatogram for Blank Solution

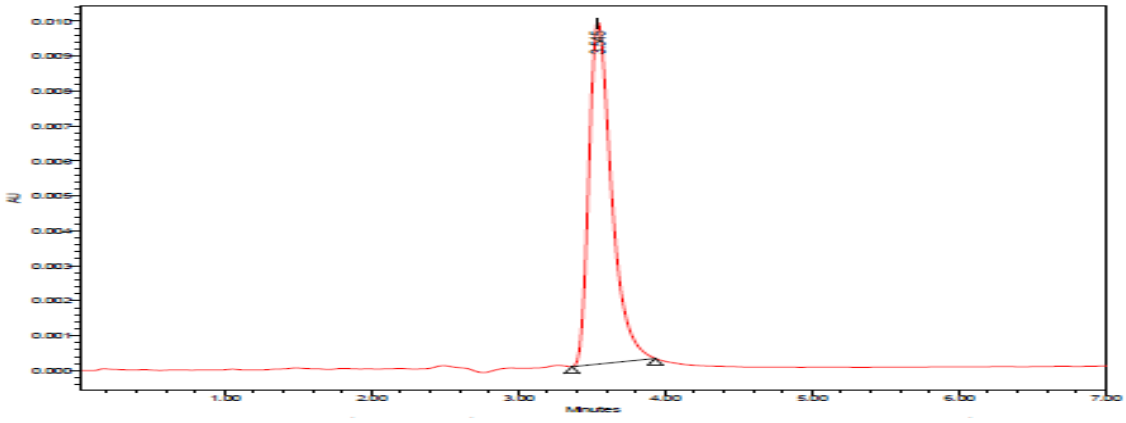


Fig 3: Chromatogram of Pimavanserin in Optimized Condition

Table 7: Peak results of Optimized Condition

Drug Name	RT	Peak Area	Tailing Factor	Theoretical Plates
Pimavanserin	3.545	255415	1.09	2612

Preparation of mobile phase

700ml of Methanol and 300ml of Acetonitrile were mixed well and degassed in ultrasonic water bath for 15 minutes. The solution was filtered through 0.45 µm filter under vacuum filtration.

Final Result & Discussion

The selected and optimized mobile phase was Methanol : ACN (70:30) and conditions optimized were flow rate (1.0 ml/minute), wavelength (240nm), Run time was 07 mins. Here the peaks were separated and showed better resolution, theoretical plate count and symmetry. The proposed chromatographic conditions were found appropriate for the quantitative determination of the drug.

METHOD VALIDATION

Accuracy

Recovery study: To determine the accuracy of the proposed method, recovery studies were carried out by adding different amounts (80%, 100%, and 120%) of pure drug of PIMAVANSERIN were taken and added to the pre-analyzed formulation of concentration 10µg/ml. From that percentage recovery values were calculated. The results were shown in table 8.

Table 8: Accuracy Readings

Sample ID	Concentration (µg/ml)		Peak Area	% Recovery of Pure drug	Statistical Analysis
	Amount	Amount Found			
S_1 : 80 %	8	7.9	569989	98.77763	Mean= 99.1357%
S_2 : 80 %	8	8.0	578751	100.3245	S.D. = 1.056284
S_3 : 80 %	8	7.8	567312	98.30503	% R.S.D.= 1.06
S_4 : 100 %	10	9.8	709788	98.76633	Mean= 100.1784%
S_5 : 100 %	10	10.0	723395	100.6881	S.D. = 1.238596
S_6 : 100 %	10	10.1	726176	101.0809	% R.S.D.= 1.2385
S_7 : 120 %	12	11.8	846927	98.44573	Mean= 99.57136%
S_8 : 120 %	12	11.9	853840	99.25935	S.D. = 1.30981
S_9 : 120 %	12	12.1	868706	101.009	% R.S.D. = 1.315

From the Accuracy Method, we observed that the mean %Recovery of the drug are 99.1357%, 100.1784% and 99.57136% which is within the range of 98-102% and %RSD is within the range <2 i.e. 1.06%, 1.2385% and 1.315% respectively.

Precision: Repeatability

The precision of each method was ascertained separately from the peak areas & retention times obtained by actual determination of six replicates of a fixed amount of drug. Pimavanserin (API). The percent relative standard deviation was calculated for Pimavanserin are presented in the table-9.

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Table 9: Repeatability readings

HPLC injection Replicates of	Retention Time	Peak Area
Replicate – 1	3.538	704122
Replicate – 2	3.540	704232
Replicate – 3	3.537	702658
Replicate – 4	3.545	702541
Replicate – 5	3.547	702863
Replicate – 6	3.545	702754
Average	**3.542**	**703195**
Standard Deviation	**0.004195**	**768.8287**
% RSD	**0.118443**	**0.10933**

From the Precision method, we observed that the %RSD of the AUC is 0.10933 and Rt is 0.118443 which are within the acceptable range as per ICH guidelines.

Intermediate Precision

The Intermediate Precision consists of two method :-Intra Day: In Intra Day process, The 80%, 100% and 120% concentration are injected at different intervals of time in same day. Inter Day: In Inter Day process, The 80%, 100% and 120% concentration are injected at same intervals of time in different days.

Table 10: Results of intra-assay & inter-assay

Conc. Of Pimavanserin (API) (µg/ml)	Observed Conc. Of Pimavanserin (µg/ml) by the proposed method			
	Intra-Day		Inter-Day	
	Mean (n=6)	% RSD	Mean (n=6)	% RSD
8	7.79	0.61	8.10	0.95
10	9.52	0.50	10.11	0.92
12	12.01	0.65	11.92	0.72

The intra & inter day variation of the method was carried out & the high values of mean assay & low values of standard deviation & % RSD (% RSD < 2%) within a day & day to day variations for Pimavanserin revealed that the proposed method is precise.

Linearity & Range

0µl injections of each concentration were injected into the HPLC system and chromatographed under the optimized conditions. Calibration curve was constructed by plotting the mean peak area (Y-axis) against the concentration (X-axis).

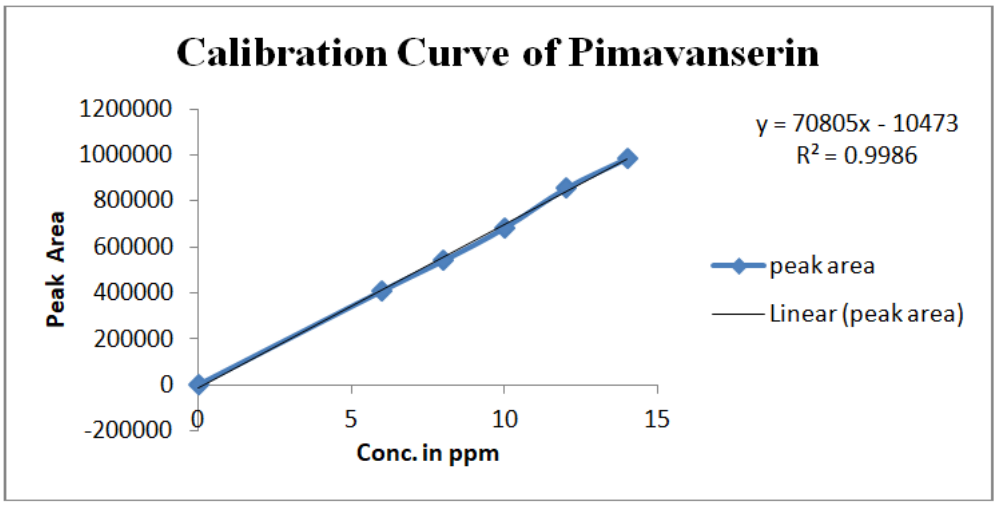

Fig 4: Calibration curve of Pimavanserin (API)

Table 11: Linearity Results

CONC.(µg/ml)	MEAN AUC (n=6)
0	0
6	409852
8	540864
10	684126
12	856125
14	986458

We observed that the calibration curve showed good linearity in the range of 6-14 µg/ml, for Pimavanserin (API) with correlation coefficient (R^2) of 0.998. A typical calibration curve has the regression equation of y = 70805x + 10473 for Pimavanserin.

Method Robustness

Robustness of the method are also in favour of (Table-12, % RSD < 2%) the developed RP-HPLC method for the analysis of Pimavanserin (API).

Table 12: Result of method Robustness test

Change in parameter	% RSD
Flow (1.1 ml/min)	0.64
Flow (0.9 ml/min)	0.68
More Organic	0.71
Less Organic	0.68
Wavelength of Detection (246 nm)	0.94
Wavelength of detection (242 nm)	0.92

LOD & LOQ

The Minimum concentration level at which the analyte can be reliable detected (LOD) & quantified (LOQ) were found to be 0.09 & 0.27µg/ml respectively.

System Suitability Parameter

System suitability testing is an integral part of many analytical procedures. The tests are based on the concept that the equipment, electronics, analytical operations and samples to be analyzed constitute an integral system that can be evaluated as such. Following system suitability test parameters were established. The data are shown in Table-13.

Table 13: Data of System Suitability Parameter

S.No.	Parameter	Limit	Result
1	Resolution	Rs > 2	8.4
2	Asymmetry	T ≤ 2	Pimavanserin =0.19
3	Theoretical plate	N > 2000	Pimavanserin =3245

4	Tailing Factor	T<2	Pimavanserin =1.09

Estimation of Pimavanserin in Pharmaceutical Dosage Form

Label claim:Each tablet contains: 17 mg Twenty Tablets were taken as per I.P. method, and The solution prepared was injected in five replicates into the HPLC system and the observations were recorded. Recorded data are shown in Table-14

Table 14: Recovery Data for estimation Pimavanserin in Nuplazid

	Labelled amount of	Mean (± SD) amount	Assay % (± SD)
Nuplazid (17 mg) (New Life	17mg	16.89 (± 0.456)	99.35 (± 0.393)

The amount of drug in Nuplazid was found to be 16.89 (± 0.456) mg/tab for Pimavanserin & % assay was 99.35 %.

Table 15: Results of Force Degradation Studies of Pimavanserin API.

Stress condition	Time	Assay of active	Assay of degraded	Mass Balance
Acid Hydrolysis (0.1 M HCl)	24Hrs.	96.64	3.36	100.0
Basic Hydrolysis (0.I M	24Hrs.	97.08	2.92	100.0
Thermal Degradation (60 ^{0}C)	24Hrs.	98.42	1.58	100.0
UV (254nm)	24Hrs.	95.75	4.25	100.0
3 % Hydrogen peroxide	24Hrs.	98.72	1.58	100.0

RESULTS AND DISCUSSION

To develop a precise, linear, specific & suitable stability indicating RP-HPLC method for analysis of Pimavanserin, different chromatographic conditions were applied & the results observed are presented in previous chapters.

In case of RP-HPLC various columns are available, but here Symmetry ODS RP C_{18}, 5µm, 15mm x 4.6mm i.d. column was preferred because using this column peak shape, resolution and absorbance were good. Mobile phase & diluent for preparation of various samples were finalized after studying the solubility of API in different solvents of our disposal (methanol, ethanol, water, DMF, DMSO). The drug was found to be soluble in Methanol, Ethanol, DMF and DMSO. Insoluble in Acetonitrile and Water. Using these solvents with appropriate composition newer methods can be developed and validated.

Detection wavelength was selected after scanning the standard solution of drug over 200 to 400nm. From the U.V spectrum of Pimavanserin it is evident that most of the HPLC work can be accomplished in the wavelength range of 240 nm conveniently. Further, a flow rate of 1 ml/min & an injection volume of 10µl were found to be the best analysis.

The result shows the developed method is yet another suitable method for assay which can help in the analysis of Pimavanserin in different formulations.

CONCLUSION

A sensitive & selective RP-HPLC method has been developed & validated for the analysis of Pimavanserin API. Further the proposed RP-HPLC method has excellent sensitivity, precision and reproducibility. The result shows the developed method is yet another suitable method for assay, purity which can help in the analysis of Pimavanserin in different formulations.

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