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R E S E A R C H A R T I C L E

RP-HPLC Method Development and Validation of Pimavanserin

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A B S T R A C T

New method was established for method development and validation of Pimavanserin by RP-HPLC method. The chromatographic conditions were successfully developed for the separation of Pimavanserin by using Inertsil ODS C18 column (4.6×150mm) 5µ. The wavelength for detection was selected by scanning standard Pimavanserin over a wide range of wavelength 200nm to 400nm. UV spectra at 290nm was selected as the detection wavelength in different mobile phase were tried but satisfactory separation, well resolved and good symmetrical peaks were obtained on a Waters C18 column with the mobile phase ACN: water: Potassium Di hydrogen ortho phosphate (0.02M) 40:10:50 v/v Buffer pH 6.0 adjusted with ortho phosphoric acid.

Keywords: Pimavanserin, Inertsil ODS C18 column, RP-HPLC method

A R T I C L E I N F O

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C O N T E N T S

1. Introduction

In High-performance liquid chromatography, mobile as well as the stationary phase compete for the distribution of the sample components. In case of HPLC, separation is based on adsorption and partition. Adsorption

chromatography employs high-surface area particles that adsorb the solute molecules. Usually a polar solid such as silica gel, alumina or porous glass beads and a non-polar mobile phase such as heptanes, octane or chloroform are used in adsorption chromatography. In partition chromatography, the solid support is coated with a liquid stationary phase. The relative distribution of solutes between the two liquid phases determines the separation. The stationary phase can either polar or non-polar. If the stationary phase is non-polar, it is called normal phase partition chromatography. If the opposite case holds, it is called reversed-phase partition chromatography. In normal phase mode, the polar molecule partition preferentially in to the stationary phase and are retained longer than non-polar compounds. In reverse phase partition chromatography, the opposite behaviour is observed.

Drug profile:

Molecular Formula: $C_{40}H_{50}N_8O_6$

IUPAC Name: 1-(4-Fluorobenzyl)-3-(4-isobutoxybenzyl)-1-(1-methylpiperidin-4-yl)urea

Structure:

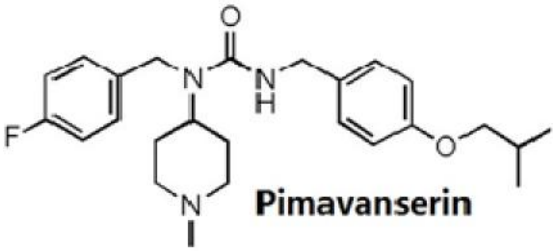

Fig 1: Structure of Pimavanserin

Mechanism of action:

Pimavanserin acts as an inverse agonist and antagonist at serotonin 5-HT2A receptors with high binding affinity (Ki 0.087 nM) and at serotonin 5-HT2C receptors with lower binding affinity (Ki 0.44 nM). Pimavanserin shows low binding to σ1 receptors (Ki 120 nM) and has no appreciable affinity (Ki >300 nM) to serotonin 5-HT2B, dopamine (including D2), muscarinic acetycholine, histamine, or adrenergic receptors, or to calcium channels. Pimavanserin has a unique mechanism of action relative to other antipsychotics, behaving as a selective inverse agonist of the serotonin 5-HT2A receptor, with 40-fold selectivity for this site over the 5-HT2C receptor and no significant affinity or activity at the 5-HT2B receptor or dopamine receptors.

2. Materials and Methods

Instrument used:

Table 1: List of Instruments

Equipment	Model	Company
Electronic Balance	ER200A	ASCOSET
Ultra-Sonicator	SE60US	ENERTECH
Heating Mantle	BTI	BIO TECHNICS INDIA
Thermal oven	---------	NARANG
pH Meter	AD102U	ADWA
Filter Paper 0.45 µ	---------	MILLI PORE

Chemicals used:

Table 2: List of Chemicals

Chemicals/standards and reagents	Grade	Make
Ortho-Phosphoric Acid	AR	Finar
Methanol	HPLC	Merck
Water	HPLC	Loba Chemi
Pimavanserin	API	Dr. Reddy's Lab

Mobile phase preparation:

400 mL (40%) of Acetonitrile, 100 mL (10%) of HPLC grade water and 500 mL (50%) Potassium Dihydrogen orthophosphate buffer pH 6.0 were mixed in a 1000 ml volumetric flask and kept for sonication in an ultrasonic water bath for 5 minutes. The solution was filter through 0.45 µ filter under vacuum filtration. Mobile phase was used as diluent.

Standard preparation:

100mg 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.

Preparation of working standard solution:

2 ml of the standard stock solution was diluted to, in a 25 ml volumetric flask to get 80 µg/ml working standard solution.

Determination of wave length:

The working standard solution of Pimavanserin was scanned in the range of 200-400 nm using mobile phase as blank. The drug showed maximum absorbance at 290 nm, which was selected for the determination.

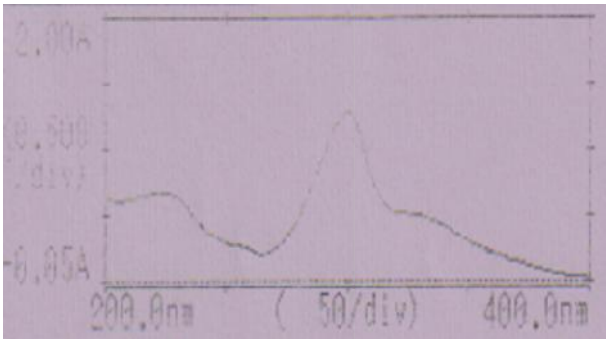

Fig 2: UV-VIS Spectrum of Pimavanserin

3. Results and Discussion

Table 3: Optimized Chromatographic conditions

Parameter	Description/Value
Stationary Phase	Waters C_{18} Column with 150 mm × 4.6 mm i.d and 5 µm Particle size
Mobile Phase	ACN : water : Potassium Di hydrogen ortho phosphate (0.02M) 40:10:50 v/v Buffer pH 6.0 adjusted with Ortho phosphoric acid
Flow rate	1.2 ml/min
Wavelength	290.0 nm
Detector	Photo diode array

Injection	Autosampler –Waters, model 717 plus
Injection volume	10 µl
Column Temperature	Ambient
Run time	5 mins
Elution Technique	Isocratic
Diluent	Mobile Phase

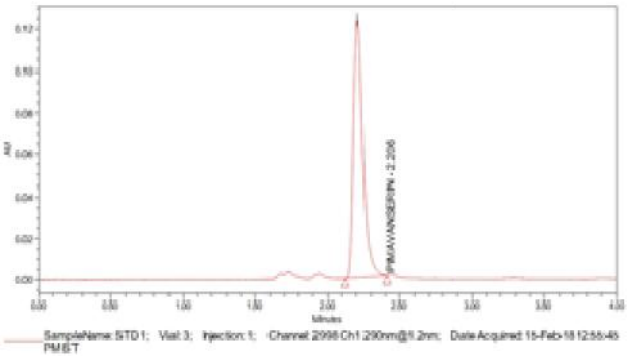

Fig 3: Chromatogram of Optimized trial

Results for Optimized Trial:

Good peak with more than 5200 theoretical plate count. And Rt was brought down to 2.206 mins. The Theoretical plates & tailing factor ware found to be within limits. So this trail was considered and validated according to ICH guidelines.

Linearity:

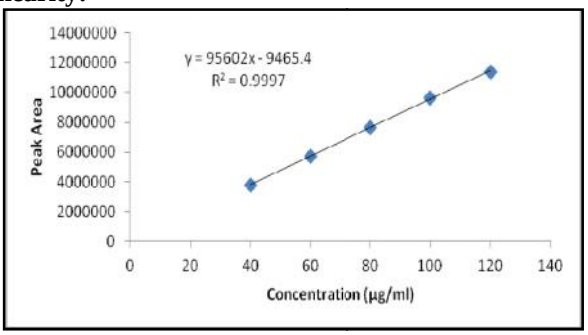

Fig 4: Linearity curve for Pimavanserin

Accuracy:

The accuracy of the method was assessed by standard addition method. % Recovery for three concentrations (corresponding to 50, 100 and 150 % of test solution concentration) were determined. For each concentration three replicates were prepared. The mean recovery of Pimavanserin was found to be 100 %.

Limit of detection and Limit of quantitation:

The limit of detection (LOD) and limit of quantitation (LOQ) were determined by using standard deviation of response and slope of the calibration curve. The LOD and LOQ of the proposed method were found to be 0.0006 and 0.002 µg/ml respectively.

Assay: The proposed method was applied for the tablet of Pimavanserin and the mean % assay was found to be 100 %. The chromatogram shows that no interference from excipients.

Table 4: System Suitability results

S. No	Parameter	Result	Acceptance Limit
1.	Retention time (Rt)*	2.206 min	--
2.	Resolution factor*	--	--
3.	Number of theoretical plates (N)*	6755	More than 4000
4.	Tailing factor (T)*	1.00	Less than 2
5.	Capacity factor (K)*	-	0.5<K<20
* Number of injections: 6 replicates			

Table 5: Linearity results

Linearity Level	Concentration (µg/ml)	Peak Area
50%	40	3793458
75%	60	5716681
100%	80	7661150
125%	100	9620341
150%	120	11401823
Slope		95602
Intercept		9465.4
Regression coefficient		0.9997

Table 6: Evaluation data of accuracy study for Pimavanserin

Sample No.	Spiked Level	Sample Weight (mg)	Sample Area	µg/ml added	µg/ml found	% Recovery	% Mean Recovery
1	**50%**	197.50	3831749	40.00	40.36	100.90	100
2	**50%**	197.50	3811606	40.0000	40.1493	100.37	

3	**50%**	197.50	3773683	40.0000	39.7499	99.37	
4	**50%**	197.50	3730428	40.0000	39.2942	98.24	
5	**50%**	197.50	3813458	40.0000	40.1688	100.42	
6	**50%**	197.50	3752716	40.0000	39.5290	98.82	
1	**100%**	395.00	7594479	80.0000	79.9960	100	
2	**100%**	395.00	7499061	80.0000	78.9909	99	100
3	**100%**	395.00	7761150	80.0000	81.7516	102	
1	**150%**	592.50	11362905	120.0000	119.6904	99.74	
2	**150%**	592.50	11401823	120.0000	120.1004	100.08	
3	**150%**	592.50	11382080	120.0000	119.8924	99.91	100
4	**150%**	592.50	11392046	120.0000	119.9974	100.00	
5	**150%**	592.50	11400193	120.0000	120.0832	100.07	
6	**150%**	592.50	11390759	120.0000	119.9838	99.99	

Table 7: Robustness data

Robust condition	Rt (Min)	Peak area	% Assay
1.1 ml/min flow rate	3.282	7554002	99.46
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
Average	3.28	7591680.33	99.96
SD	0.00	96455.64	1.27
%RSD	0.09	1.27	1.27

4. 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.

5. Acknowledgement

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