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Original Article

DEVELOPMENT AND VALIDATION OF STABILITY INDICATING HPTLC METHOD FOR PIMAVANSERIN TARTRATE

MRINALINI C. DAMLE[1*], ROSHANI R. PARDESHI[2], SHUBHANGI R. BIDKAR[2]

[1]Department of Quality Assurance, AISSMS College of Pharmacy, Kennedy Road, Near RTO Pune, Pune-411001, Maharashtra, India. [2]Master of Pharmacy, Department of Pharmaceutical Quality Assurance, AISSMS College of Pharmacy, Kennedy Road, Near RTO Pune-411001, Maharashtra, India

*Corresponding author: Mrinalini C. Damle; *Email: damle_mc@aissmscop.com

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ABSTRACT

Objective: The aim of current research work was to investigate degradation behavior of Pimavanserin tartrate upon exposure to stress conditions recommended by ICH Q1A (R2) and Q1B guidelines.

Methods: Chromatographic separation was achieved on Merck's TLC aluminum plates pre-coated with silica gel G 60 F_{254} as stationary phase and Methanol: Chloroform (2:8 v/v) as mobile phase. Densitometry scanning was carried out at 224 nm.

Results: The retardation factor (Rf) was observed to be 0.56±0.02. Pimavanserin tartrate showed degradation in all stress conditions, but no degradation product was found in any stress condition. Peak purity was found to be 0.999 indicating no interference by degradation products to drug peak. The developed HPTLC method was successfully validated as per ICH Q2 (R1) guideline. Method was found to be linear within the range of 400-2000 ng/band with correlation coefficient R^2= 0.9982. % RSD for intra-day and inter-day precision were found to be 1.35 and 1.78 % and % recovery was found to be in range 98-102 %. LOD and LOQ were found to be 17.58 ng/band and 53.27 ng/band respectively.

Conclusion: A simple, economic stability indicating high performance thin layer chromatography method has been developed and validated for Pimavanserin tartrate. It is used for the treatment of delusions and hallucinations in Parkinson's disease.

Keywords: Pimavanserin tartrate, HPTLC, Forced degradation, Validation, Antiparkinson

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INTRODUCTION

Pimavanserin tartrate is an atypical anti-psychotic and anti-Parkinson drug approved in April 2016 by the USFDA, for the treatment of delusions and hallucinations in Parkinson's disease [1-4]. Chemically it is (2*R*, 3*R*)-2, 3-dihydroxybutanedioic acid; 1-[(4-fluorophenyl) methyl]-1-(1-methylpiperidin-4-yl)-3-[[4-(2-methylpropoxy) phenyl] methyl] urea. Twenty to forty percent of Parkinson's disease patients experience psychotic symptoms. Parkinson disease psychosis (PDP) is characterised by sensory abnormalities, paranoid delusions, and primarily visual hallucinations. The precise origin of PDP is unknown; however, research suggests that structural and neurochemical alterations, irregularities in sleep, and abnormalities in visual processing may all play a role. Parkinson disease is thought to cause psychosis when acetylcholine is blocked. The development of psychosis is hypothesised to be influenced by excessive dopaminergic activity in the limbic system and cerebral cortex, which can be brought on by dopaminergic medications used to treat the motor symptoms of Parkinson disease. PDP is treated in part by a dopaminergic drug dose decrease.

An antipsychotic effect of Pimavanserin occurs via selective inverse agonist activity at serotonin 5-HT2A receptors, less potent inverse agonist and antagonist activity at serotonin 5-HT2C receptors. This decreases the elevated serotonin levels at these receptors, which help in reducing hallucinations and delusions [5-7]. Pimavanserin tartrate is freely soluble in water, soluble in methanol, ethanol, DMSO, dimethylformamide, dichloromethane and practically insoluble in cyclohexane.

Extensive literature survey reveals that few analytical and bioanalytical methods, including the UV-spectrophotometric method, HPLC, UPLC-MSMS has been reported in literature [8-14] But no stability indicating HPTLC method has been reported yet. HPTLC is a simple, economic, time-saving and labour-saving method compared to sophisticated methods like UPLC, HPLC-MS.

The current research was to develop and validate stability indicating high-performance thin layer chromatography (HPTLC) method for Pimavanserin tartrate.

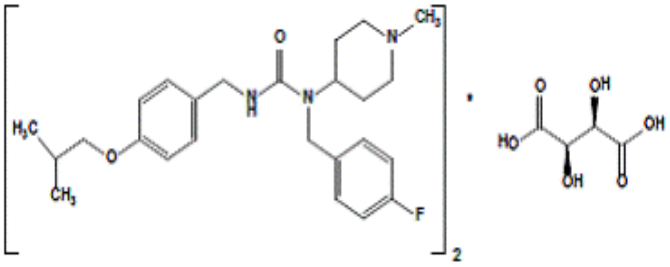

Fig. 1: Structure of pimavanserin tartrate

MATERIALS AND METHODS

Chemicals and reagents

Pimavanserin tartrate working standard was received as a gift sample from Zyduscadila healthcare Ltd, Thane, India. Chloroform AR grade, Methanol AR grade, Sodium hydroxide (NaOH), Hydrochloric acid (HCl), Hydrogen peroxide solution 30% v/v (H_2O_2) were purchased from LobaChemie Pvt. Ltd. Mumbai, India. Merck Pvt. Ltd. sold TLC plates that were pre-coated with silica gel G60 F_{254}.

Instrument

The sample application was carried out using a Hamilton microliter syringe (100 µl) and CAMAG HPTLC equipment with a Linomat 5 sample applicator operating under a moderate stream of nitrogen. For densitometry scanning and detection, a CAMAG TLC SCANNER 3 was employed. Win CATS software, version 1.4.3, was used to collect the data. Using a UV-visible spectrophotometer (Make: JASCO, Model: V730), UV-spectral analysis was carried out. Photostability study was performed in the photostability chamber (Make-NEWTRONIC, Model-NEC103RSPI).

Preparation of standard stock solution

By precisely weighing 10 mg of pimavanserin tartrate and adding it to a 10 ml volumetric flask, which was then filled with methanol to make it 10 ml, a standard stock of pimavanserin tartrate (1000 µg/ml) was created. To create a standard solution containing 100 µg/ml Pimavanserin tartrate, 1 ml of this standard stock solution was obtained and diluted to 10 ml using methanol as the solvent.

Selection of analytical wavelength

Using methanol as a solvent, a solution containing 10 µg/ml of pimavanserin tartrate was made from the standard solution (100 µg/ml). This solution was then scanned in a UV spectrophotometer between 200-400 nm.

Optimization of chromatographic conditions

After several trials using different mobile phases in varying solvent ratios to produce acceptable peak parameters, methanol: chloroform (2:8 v/v) was chosen as the mobile phase, producing a satisfactory peak shape with Rf value of 0.56. Chromatographic separation was performed using a 100 µg/ml standard solution of pimavanserin tartrate, and a twin trough TLC chamber was utilized for development on Merck's TLC aluminum plates that had been pre-coated with silica gel G60 F_{254}. By setting the developing chamber aside at room temperature (RT), chamber saturation was accomplished for 30 min. At RT, the plate underwent development and air drying. Detection and densitometry scanning was performed at wavelength 224 nm.

Forced degradation studies

Forced degradation studies were carried out according to ICH Q1A (R2) [15] and Q1B [16] guidelines, to prove stability indicating the ability of the developed method. A literature for optimization of stress conditions was preferred [17, 18]. Standard solution of Pimavanserin tartrate (1000 µg/ml) was subjected to hydrolysis under different pH (Acidic, Basic and Neutral), thermal, oxidation and photolysis. Stress conditions were optimized to achieve degradation of about 10-30 %. The optimized condition is as follows.

Hydrolysis under acidic, basic and neutral condition

The degradation samples for hydrolysis under acid, basic and neutral condition were prepared by treating 1 ml of standard solution of Pimavanserin tartrate (1000 µg/ml) with 1 ml of 0.5 N HCl, 1 ml of 0.5 N NaOH, and 1 ml distilled water respectively. The volume was made up to 10 ml with methanol; this solution was kept for 24 h at RT and then applied on a TLC plate. The plate development was done with mobile phase and scanned at 224 nm.

Hydrogen-peroxide-induced oxidation degradation

The degradation sample was prepared by treating 1 ml Pimavanserin tartrate (1000 µg/ml) with 1 ml of 30% v/v H_2O_2 solution and volume was made up to 10 ml with methanol. The solution was kept for 24 h in a dark place at RT and then applied on a TLC plate. The plate development was done with a mobile phase and scanned at 224 nm.

Thermal degradation

Solid state Pimavanserin tartrate was exposed to 60°C in a hot air oven for 6 h. A solution of this exposed drug in methanol was prepared and appropriately diluted to get a 100 µg/ml solution that was applied on the TLC plate. The plate development was done with a mobile phase and scanned at 224 nm.

Photolytic degradation

In a photostability chamber, pimavanserin tartrate was separately subjected to fluorescence light up to an illumination of not less than 1.2 million lux h and UV light up to an illumination of not less than 200-watt h/m2. 100 µg/ml solution of this exposed drug was applied on TLC plate. The plate development was done with mobile phase and scanned at 224 nm.

Validation of analytical method

Validation of the developed HPTLC method was carried out as per the ICH Q2 (R1) [19] guideline. Literature methods were studied for procedural details of validation parameters [20, 21].

Specificity

Peak purity profiling studies and assay was carried out for evaluating the specificity of the method. Peak purity for the peaks of all degradation conditions were carried out with the help of spectral detection by win CAT software.

Assay

For the assay, a synthetic combination of pimavanserin tartrate and excipients that are often used was utilized. From a 1000 µg/ml stock solution, 6 replicates of the sample solution (100 µg/ml) were created. After sonication and filtration, 8 µl volume of each sample solution was applied on the TLC plate. The plate development was done with a mobile phase and scanned at 224 nm. Peak area was recorded and % recovery was calculated.

Linearity

Standard solution of Pimavanserin tartrate (100 µg/ml) was spotted on TLC plate with spotting volume 4, 8, 12, 16, 20 µl to achieve the range 400-2000 ng/band. The development of the plates was completed using an optimized process. By graphing the amount of spots versus the peak area, the calibration curve was obtained.

Precision

Application of 6 replicates of the 800 ng/band concentration of pimavanserin tartrate on TLC plates on the same day after a brief interval of time and on three successive days, respectively, was used to test intra-day precision and inter-day precision. Calculated was the % RSD.

Accuracy

Accuracy of the method was determined by standard addition method. Assay solution, a synthetic mixture of the product's components, was examined by adding a known quantity of pure drug at levels of 50, 100, and 150 %. 3 replicates of 3 concentrations (1200 ng/band, 1600 ng/band, and 2000 ng/band) were evaluated and % recovery was calculated.

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

Calculated from the calibration curve was the Pimavanserin tartrate detection and quantitation limit. LOD and LOQ calculations were performed using the ensuing formulae.

$$\mathrm{LOD} = \frac{3.3 \times \sigma}{S}$$

$$\mathrm{LOQ} = \frac{10 \times \sigma}{S}$$

Where σ = the standard deviation of y-intercept or standard deviation of responses at lowest concentration. S = slope of the calibration curve.

Robustness

Robustness of the developed method was determined by making small, deliberate changes in optimized conditions like mobile phase composition, saturation time and time from application to development, time from development to scanning, change in scanning wavelength to determine its effect on result (peak area). Calculated was the % RSD.

RESULTS AND DISCUSSION

Maximum absorbance for pimavanserin tartrate was reported at 224 nm. It was chosen as an analytical wavelength as a result; the UV spectrum is shown in fig. 2.

Optimized chromatographic conditions included mobile phase of methanol: chloroform (2:8 v/v) saturated for 30 min and the detection wavelength is 224 nm. Rf value of Pimavanserin tartrate was found to be 0.56±0.02. Densitogram is shown in fig. 3 and optimized chromatographic conditions are given in table 1.

Pimavanserin tartrate showed degradation in all stress conditions, but no degradation product was found in any stress condition. Noninterference by any degradant was confirmed by peak purity using spectral detection by software and multiwavelength scanning. The peak purity was found to be 0.999, indicating the non-interference of degradation product or impurity and method is specific. Summary of forced degradation studies is given in table 2 and 3D Densitogram of multiwavelength scanning of optimized forced degradation condition of Pimavanserin tartrate shown in fig. 4.

Table 1: Optimized chromatographic parameter

Parameter	Condition used for analysis
Stationary Phase	Merck's TLC aluminum plates pre-coated with silica gel G 60 F_{254}
Mobile Phase	Methanol: Chloroform (2:8 v/v)
Band Length	6 mm
Saturation time	30 min
Detection wavelength	224 nm
Rf Value	0.56±0.02

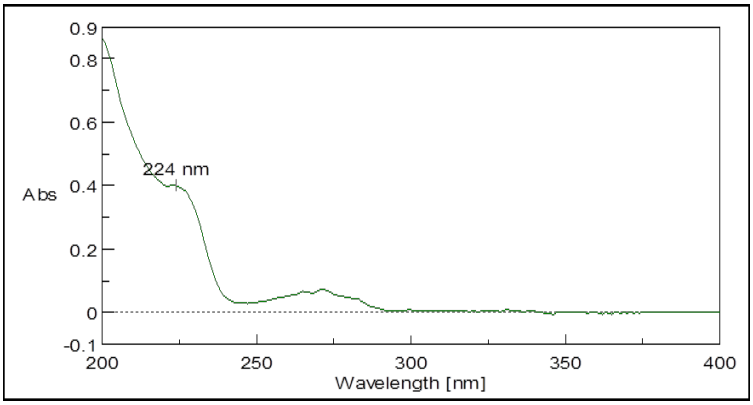

Fig. 2: UV-spectrum of pimavanserin tartrate (10 µg/ml)

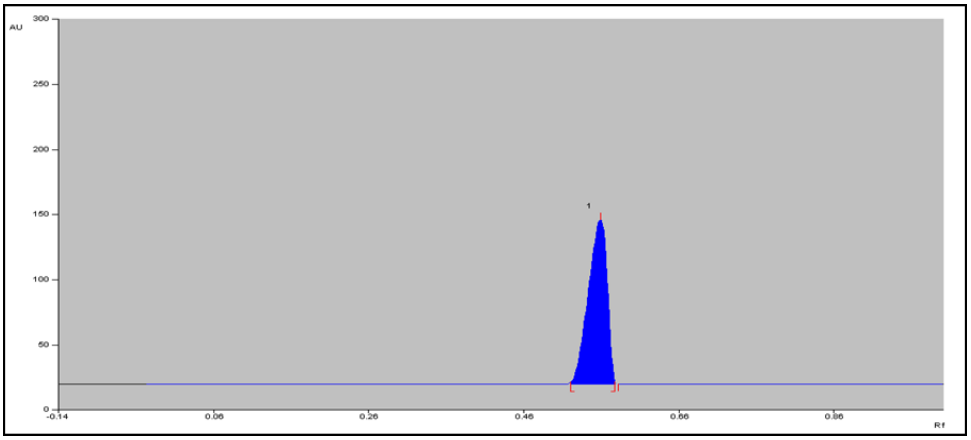

Fig. 3: Densitogram of pimavanserin tartrate (1000 ng/band, Rf = 0.56±0.02)

Table 2: Summary of forced degradation of pimavanserin tartrate

S. No.	Parameter	Condition	% Degradation	Peak purity	
				r (s, m)	r (m, e)
1	Acid	1 ml of 0.5 N HClfor 24 h at RT	21.14	0.9998	0.9987
2	Base	1 ml 0.5 N NaOH for 24 h at RT	24.87	0.9997	0.9989
3	Neutral	1 ml water for 24 h at RT	15.62	0.9995	0.9997
4	Oxidation	1 ml 30 % v/v H_2O_2 for 24 h at RT	18.86	0.9990	0.9987
5	Thermal	60 °C, 6 h	22.27	0.9985	0.9996
6	UV	200-watt h/m2	11.48	0.9991	0.9987
7	Fluorescence	1.2 million lux h	13.05	0.9999	0.9995

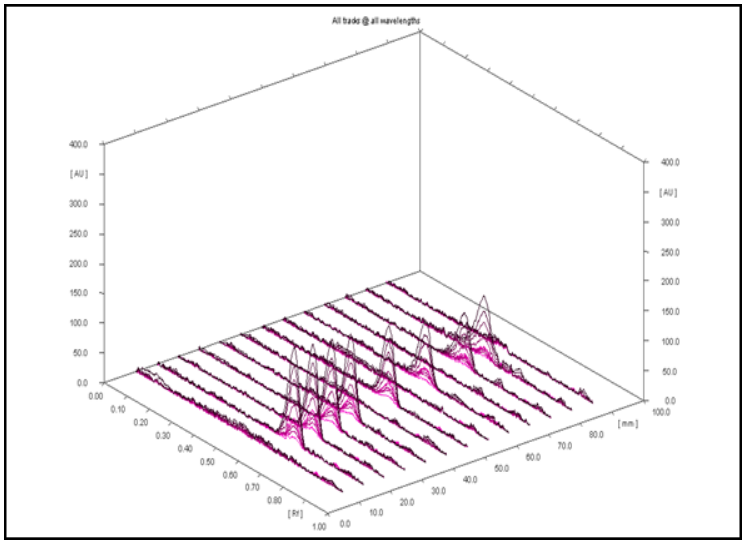

Fig. 4: 3D densitogram of multiwavelength scanning of optimized forced degradation condition of pimavanserin tartrate (track1, 5, 7, 9, 12 Blank, track1–Standard, track 2-UV, track 3-Fluoro, track 4-Thermal, track 6-Oxidation, track 8-Basic, track 10-Acidic, track 11-Neutral)

Developed HPTLC method was successfully validated as per ICH Q2 (R1) guideline. The results are shown in table 3 to table 7.

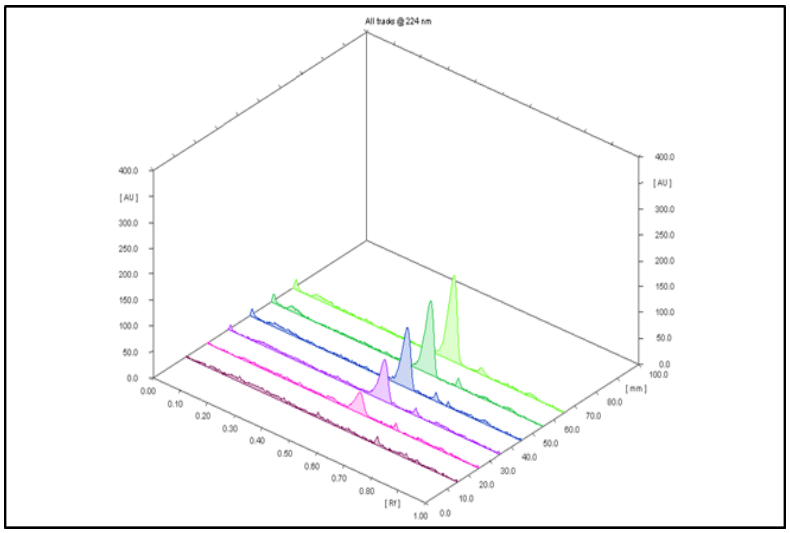

Fig. 5: 3D densitogram of linearity of pimavanserin tartrate (Rf = 0.56±0.02)

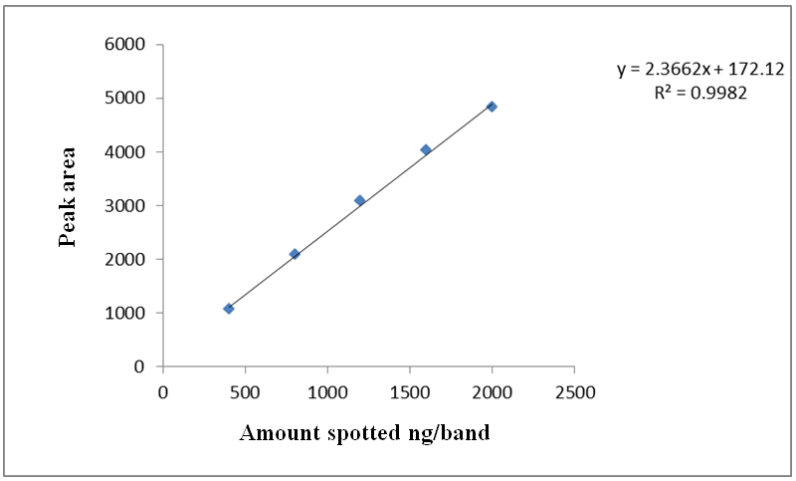

Fig. 6: Calibration curve of pimavanserin tartrate

Table 3: Linearity of pimavanserin tartrate

	Amount spotted of pimavanserin tartrate (ng/band)				
Replicate	**400**	**800**	**1200**	**1600**	**2000**
1	1069.4	2062.8	3147.8	4068.8	4902.4
2	1054.0	2080.5	3015.2	4000.7	4717.7
3	1062.0	2023.0	3043.6	3934.8	4734.5
4	1078.8	2075.8	3065.4	3985.4	4865.5
5	1074.4	2104.1	3109.3	4021.4	4896.5
6	1056.6	2075.3	3082.0	4076.2	4845.0
AVG	1067.367	2070.25	3077.217	4015.883	4826.883
SD	12.60566	26.81513	47.22289	52.96045	80.97247

For Linearity, 5 different concentrations (400, 800, 1200, 1600 and 2000 ng/band) and for each 5 different concentrations numbers of experiments are 6.

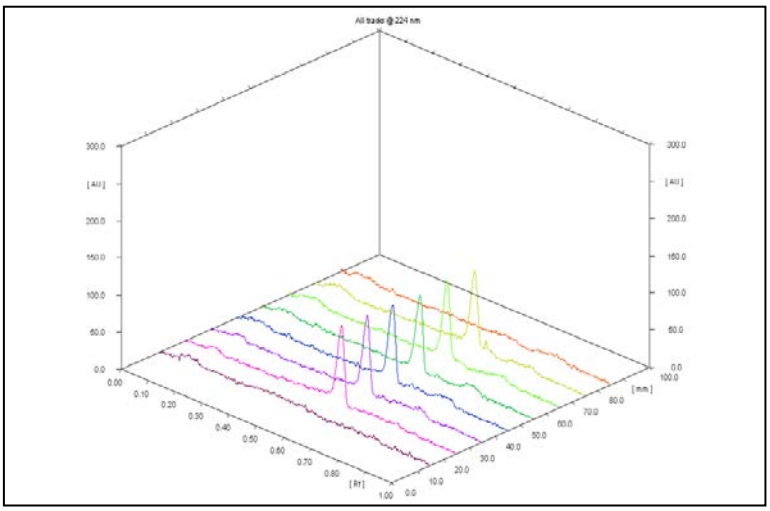

Fig. 7: 3D Densitogram of precision of pimavanserin tartrate (6 replicate of 800 ng/band)

Table 4: Intra-day and inter-day precision details of pimavanserin tartrate

Precision	**Mean peak area (n=12)**	**Standard deviation (SD)**	**%RSD**
Intra-day precision (800ng/band)	2169.567	29.32223	1.35
Inter-day precision (800 ng/band)	2121.508	37.8149	1.78

For Intra-day Precision, numbers of experiments are 12 and the average is 2169.567 and Standard Deviation (SD) is 29.32223 and % Relative Standard deviation %RSD is 1.35.

For Inter-day Precision, numbers of experiments are 12 and the average is 2121.508 and Standard Deviation (SD) is 37.8149 and % Relative Standard deviation % RSD is 1.78.

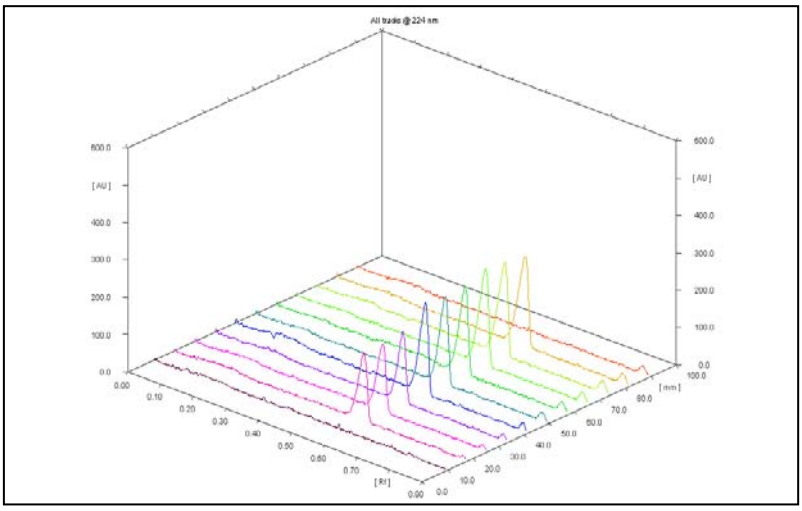

Fig. 8: 3D densitogram of accuracy study (track1-blank, track 2,3,4–50% level, track 5,6,7-100% level, track 8,9,10–150% level)

Table 5: Recovery study

S. No.	% Level	Initial amount (ng/band)	Amount added (ng/band)	% Recovery	Mean % recovery	% RSD
1	50	800	400	99.78 100.49 101.98	100.75	1.11
2	100	800	800	101.02 100.72 99.7	100.48	0.68
3	150	800	1200	100.67 99.17 99.68	99.84	0.76

For recovery study, numbers of experiments are 3 for each 3 different concentrations.

Table 6: Robustness study

Parameter	Robust condition	% RSD
Mobile phase composition	2.2:7.8	1.88
(Methanol: Chloroform 2:8v/v,±0.2 ml)	1.8:8.2	1.61
Saturation time (30±5 min)	25 min	1.61
	35 min	1.84
Time from application to development (10 min, 20 min)	After 10 min	1.74
	After 20 min	1.86
Time from development to scanning (30 min, 60 min)	After 30 min	1.73
	After 60 min	1.75
Change in wavelength (224±1 nm)	223 nm	1.34
	225 nm	1.52

Table 7: Summary of validation parameters

S. No.	Validation parameters		Result
1	Linearity		y = 2.3662x+172.12, R^2= 0.9982
2	Range		400-2000 ng/band
3	Intra-day Precision (%RSD)		1.35
	Inter-day precision (%RSD)		1.78
4	Assay		101.95
5	Accuracy	50 % level	100.75
		100 % level	100.48
		150 % level	99.84
6	LOD	Using SD of responses at lowest concentration	17.58 ng/band
		Using SD of y-intercept	23.58 ng/band
7	LOQ	Using SD of responses at lowest concentration	53.27 ng/band
		Using SD of y-intercept	71.47 ng/band
8	Robustness		Robust
9	Specificity		Specific

The earlier research work used a sophisticated method like HPLC UPLC-MSMS for the degradation analysis of Pimavanserin. The developed stability indicating HPTLC method for Pimavanserin tartrate is simple, economic and robust. We have carried out a degradation study as per ICH Q1A (R2) [15] and Q1B [16] guidelines and the developed method was validated as per ICH Q2 (R1) [19] guideline. we have carried out the thermal degradation in a photo stability chamber as specified in ICH Q1B guideline, while Panda sagar *et al.*[9] carried out photolytic degradation by exposing drug sample to UV light (365 nm) for 3 h and KoduriGeeta *et al.*[10] performed photolytic degradation by exposing drug sample to direct sunlight and we found comparable degradation (11.48% under UV Light and 13.05% under Fluorescence Light). In another study by Shaik Saida *et al.* [11] it was observed that drug is prone to hydrolytic and oxidation degradation with 24.02% degradation under acidic, 14.63% under basic and 11.84% under oxidation conditions, respectively. Our observations fairly match with this study results (21.14%, 24.87% and 15.62% for Hydrolytic degradation under acidic, basic and neutral, respectively and 18.86% for oxidative degradation). Thermal degradation of pimavanserin was reported in earlier research work we have observed 22.27% degradation under thermal condition (60°Cin a hot air oven for 6 h). We found degradation of Pimvanserin tartrate under all stress conditions (Hydrolysisunder Acidic, Basic and Neutral Condition, Hydrogen-Peroxide induced Oxidation Degradation, Thermal Degradation, Photolytic Degradation) but we do not observed any degradation product's peaks. There was a decrease in peak area of pimavanserin tartrate degradation sample and purity of drug peak (No interference by any degradant at drug peak) was confirmed by spectral detection and multiwavelength scanning (Range of 204-294 nm with wavelength increment of 10 nm). Peak purity found to be 0.999, indicating that there is no interference of any other peak of degradation product. The developed method can be used in routine analysis of Pimavanserin tartrate in Quality control laboratories and need further optimization to be used for quantitative analysis of degradation products.

CONCLUSION

In the current work, a simple, economic stability-indicating high-performance thin-layer chromatography method has been developed and validated for Pimavanserin tartrate. Pimavanserin tartrate was found to be sensitive to all stress conditions, but no degradation product was found in any stress condition. Non-interference by any degradant was confirmed by peak purity using spectral detection by software and multiwavelength scanning. The peak purity value was found within the limit confirming stability indicating nature of the developed method. Thus, this method can conveniently be used for quantitative analysis of Pimavanserin tartrate on routine basis.

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Nil

AUTHORS CONTRIBUTIONS

MCD designed the work. RRP and SRB contributed for the analysis and data collection parts of the work. MCD, RRP and SRB contributed to the interpretation of the results.

CONFLICT OF INTERESTS

Declared none

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