Original Research Article

# Development and validation of pimavanserin tartrate by normal phase- HPTLC method

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## ARTICLE INFO

## ABSTRACT

For the determination of pimvanserin tartrate in bulk and formulation, a rapid and simple High Performance Thin Layer Chromatography at 226 nm was developed and validated. The determination was carried out on thin coated aluminum backing plates covered with 200 mm layer of silica gel G 60 F254 (10×10 cm) plate as stationary phase and using a mobile phase of methanol: chloroform: trimethylamine (4:6:0.1 v/v/v) respectively. With a correlation coefficient ($r^2$) of 0.998, the development of pimvanserin tartrate was linear in the range of 0.7 to 4.2 $\mu$g/ml. The limit of detection (LOD) was found to be 7.68 ng/spot while the limit of quantification was found to be 23.28 ng/spot. The percentage label claim of pimvanserin tartrate in bulk and formulation was found to be 99 – 101 %. The percentage found in the formulation shows that no effect of excipient on drug. The conducted procedure has the benefit of being simple and quick. As a result, it can be used to examine pimvanserin tartrate in pharmaceutical formulations.

## 1. Introduction

Parkinson's disease (PD) is a second most common neurological disease after Alzheimer's disease. The main objective of first line PD drugs were the movement dysfunction. In the L -dopa-treated PD patients psychotic systems are seen commonly as side effect. Early therapies for PD psychosis included lowering anti-PD dosages or prescribing a conventional antipsychotic, both of which have been shown to increase PD symptoms. Pimavanserin tartrate is an atypical anti-psychosis used in treatment of Parkinson's disease.[1]

Pimavanserin tartrate is a salt that is the hemitartrate salt of pimavanserin. It is chemically named as (2R, 3R)-2, 3-dihydroxybutanedioic acid; 1-[(4-fluorophenyl) methyl]-1-(1-methylpiperidin-4-yl)-3[[4(2methylpropoxy) phenyl] methyl] urea.[2]

To treat L -dopa-induced psychosis in Parkinson's disease and also for low-dose risperidone treatment in schizophrenia it act as inverse agonist at 5-HT 2A.[3]

The literature survey shows research on analytical study of PMT. The degradation study of PMT by identification and characterization of degradants,[4] bioanalytical study on UPLC/Ms method in Rat,[5] Quantification in bulk and formulation by high performance liquid chromatography.[6]

There wasn't even a publication on high performance thin layer chromatography (HPTLC) for determining PMT in bulk or in-house tablet formulation till now. So we perform this method to give precise, accurate and sensitive result by HPTLC method as per ICH guidelines.

## 2. Materials and Methods

Pure Pimavanserin tartrate provided by MSN Laboratories PVT. LTD., Hyderabad, India as a gift sample. Analytical

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reagent methanol, chloroform were of HPLC grade procured from Rankem, avantor performance materials India Ltd. Thane, India.

## 2.1. Equipment

The sample was transferred to the plate using a 100 $\mu$l sample syringe (CAMMG Hamilton, Switzerland) by a Linomate V sampler applicator with continuous nitrogen gas flow (CAMMG). The plate used for application was Aluminium plate coated with silica gel G60 $F_{254}$ plates (10×10 cm, 6mm) (E. Merck, Darmstadt, Germany; supplied by Merck India, Mumbai, India) as stationary phase. The plate was prewashed with methanol in twin through chamber and dried at 115°C for 5 min before chromatography. The applied plate was placed for development in automatic developing chamber (ADC 2 CAMMG) after mobile phase saturation.TLC scanner 3 was used to scan the developed plate, which was connected to the scanning software of the winCAT data processor software (version 1.4.10).

### 2.1.1. Stock sample preparation

To prepare 1000 $\mu$g/ml, a standard stock solution was made by accurately weighing 10 mg of PMT and transferring it into a 10 ml volumetric flask, which was then diluted with methanol up to the mark. A 0.2 $\mu$ membrane filter was used to filter solution.

### 2.1.2. Sample preparation for analysis

To prepare 10 $\mu$g/ml solution of sample by withdrawing 0.1 ml from standard stock solution in 10 ml volumetric flask and diluted with methanol.

### 2.1.3. In-house tablet formulation preparation

The in-house tablet was manufactured with a weight of 150 mg by combining 10 mg of medication with 8 mg of magnesium stearate, 8 mg of pregelatinized starch, and microcrystalline cellulose. All mixture was blended with morter and pestle. Compressed by using mini tablet compressor and make 20 tablet of 150 mg weight.

## 2.2. Validation of method

As per ICH guidelines the developed method was validated. The method validation parameter as per guidelines are linearity, precision, accuracy, ruggedness, detection of limit and quantification.

### 2.2.1. Linearity

The linearity was evaluated by taking six sets of aliquots ranging 700 to 4200 ng concentration of pimavanserin tartrate from standard stock solution. These concentration applied on plate and tested through plotting peak area versus drug concentration. The linearity of curve was observed.

### 2.2.2. Precision

The intra-day, inter-day and repeatability parameter studied as in precision. The sample application for calculation in intra-day and inter-day as 1400 ng/band, 2100 ng/band, 2800 ng/band.

### 2.2.3. Accuracy

The term accuracy refers to the degree to which the measured and true values are similar. The accuracy was measured by spiking of pre-analyzed sample with 80%, 100%, 120% extra pimavanserin standard. To study drug recovery in formulation at different level and re-analyzed the sample.

### 2.2.4. Robustness

The robustness of the HPTLC method was tested by incorporating small changes in the composition of the mobile phase, chamber saturation time, and slight change in the solvent migration distance, the results were examined.

### 2.2.5. Limit of detection and limit of quantitation

In analytical method lowest amount of sample that can be detected known as Limit of Detection (LOD) whereas the lowest amount of sample that can be quantified known as Limit of Quantification (LOQ). The detection was calculatedby (3.3 x A.S.D)/b and Quantification limit was calculated by (10 x A.S.D)/b where, b, correspond to graph slope.

## 3. Result and Discussion

The literature survey of PMT shows that the few paper on HPLC and HPTLC methods. The objective of study to accurate, precise, cost-effective study by TLC technique for the determination of PMT.

## 3.1. Optimization or development of TLC

The selection of mobile phase was performed on polarity index of solvent. The slightly tailing was observed in methanol: chloroform (4:6 v/v). For good resolution and precise result tri-ethylamine introduced. The mobile phase of methanol, chloroform, trimethylamine (4:6:0.1 v/v/v) for PMT. It shows symmetric and sharp peak with RF value of 0.69 ± 0.03 (fig. 1). The mobile phase containing chamber was saturated for 20 min at room temperature and plate activated at 5 min.

## 3.2. Study of calibration curve

The developed method of linearity was linear in range of 700 – 4200 ng/band (fig. 2). It shows the good linear relationship with applied sample concentration. The co-relation coefficient was found to be 0.998 with linear equation of 0.5682x + 1870.6 (table 1).
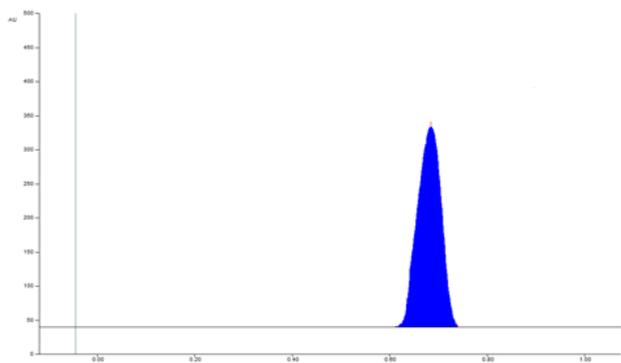
**Fig. 1:** Densitogram of PMT in optimized mobile phase

**Table 1:** Results of validation parameter (as per ICH guidelines) for PMT

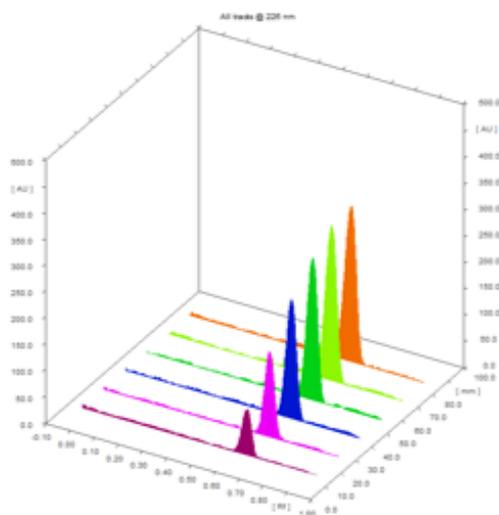| Parameters | Result |
|---|---|
| Linearity | 0.998 |
| % RSD (precision) | < 2 |
| % Recovery | 100.11 |
| Limit of detection | 7.68 ng/ml |
| Limit of quantification | 23.28 ng/ml |
| Robustness | Method was robust |



**Fig. 2:** Linearity of PMT

### 3.3. Precision

The precision was described by relative standard deviation (RSD %). The intraday and inter-day precision RSD value was found to be 0.59-1.10 and 0.80-1.7 respectively (table 2).

**Table 2:** Result of precision

| Concentration | Intra-day %RSD (should be <2) | Inter-day %RSD (should be <2) |
|---|---|---|
| 1400 | 0.96 | 1.3 |
| 2100 | 1.1 | 0.8 |
| 2800 | 0.59 | 1.71 |

### 3.4. Accuracy or recovery

The recovery studies by analytical technique of pre-analyzed sample spiked with standard PMT is known as recovery or accuracy. The results of accuracy were shown in table. The % recovery was found to be99.98 – 100.19 (table 3).

**Table 3:** Result of accuracy study

| Component | Tablet mode (mg/tablet) | % amt of standard drug added | % Drug recovery | % RSD |
|---|---|---|---|---|
| PMT | 10 | 80 | 100.19 | 0.42 |
| | | 100 | 100.11 | 0.25 |
| | | 120 | 99.98 | 0.1 |

### 3.5. Robustness

For developed HPTLC method, the standard deviation and percentage of RSD of the robustness parameter was calculated. The results of robustness given in (table 4)

### 3.6. Limit of detection and limit of quantitation

For the developed HPTLC method, the limit of detection and limit of quantification were found to be 7.6 ng/ml and 23.2 ng/ml, respectively.

## 4. Conclusion

The developed TLC/Densitometry method is a simple, accurate, and repeatable quantitative analysis for estimating Pimavanserin Tartrate in-house tablet formulation. The procedure has been validated as per ICH guidelines Q2 (R1). The method is precise and there is no intervention from any of the sample in the study. It can be concluded that the system established offers many advantages such as quick, cost-effective, simple mobile phase and simple preparation measures, more sensitive and comparative short run time.

**Table 4:** Result of robustness

| Robustness-parameter | R F | % RSD |
|---|---|---|
| **Change in Mobile Phase Composition**(Methanol: Chloroform: triethylamine) | | |
| 4:6:0.1v/v/v | 0.34 | 1.23 |
| 5: 5: 0.1v/v/v | 0.39 | 1.2 |
| 3: 7: 0.1 v/v/v | 0.31 | 1.3 |
| Mean | 0.34 | 1.24 |
| **Mobile Phase Volume** | | |
| 9 mL | 0.32 | 1.68 |
| 10 mL | 0.34 | 1.33 |
| 11 mL | 0.36 | 0.96 |
| Mean | 0.34 | 1.32 |
| **Duration of saturation** | | |
| 15 min | 0.32 | 1.56 |
| 20 min | 0.34 | 1.14 |
| 25 min | 0.38 | 0.68 |
| Mean | 0.34 | 1.12 |
| **Chromatographic Changes (Time from application to development)** | | |
| 0 min | 0.34 | 1.21 |
| 10 min | 0.34 | 0.92 |
| 15 min | 0.35 | 0.86 |
| 20 min | 0.33 | 0.72 |
| Mean | 0.34 | 0.92 |
| **Time from development to scanning** | | |
| 0 min | 0.36 | 0.98 |
| 20 min | 0.34 | 1.48 |
| 1hr | 0.35 | 1.2 |
| 1hr 30 min | 0.34 | 1.37 |
| Mean | 0.34 | 1.25 |

## 5. Source of Funding

## 6. Conflict of Interest

None.

## 7. Acknowledgement

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