# Analytical studies on zero order and first order derivative and area under curve UV-spectrophotometric methods for estimation of pimavanserin tartrate in bulk and In-house tablet formulation

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## Abstract

The proposed of work to establish simple, rapid, sensitive, economical and accurate UV spectrophotometric methods for the quantificationof Pimavanserin tartrate in bulk material and *in-house* tablet formulation. This study is designed to validate the developed methods as per ICH guidelines. Pimavanserin tartrate an atypical anti-psychotic drug also used for the treatment of Parkinson Disease. Four simple UV Spectroscopy methods developed and validated for the estimation of Pimavanserin tartrate, by using double beam spectrophotometer (UV-2450, Shimadzu, Japan). Maximum absorbance (λmax) of pimavanserin tartrate was observed at 226 nm used methanol as a solvent. The calibration curve of concentration range 5-30 µg/ml obeyed Beer lambert law. The % recovery was found to be in the range of 98-101%. Precision values observed less than 2 in the terms of % RSD that shows precise nature of developed methods. It was concluded that statistical analysis and the result amongst all four methods, AUC method is most simple, specific, accurate and precise. All four methods can be used as routine analysis of Pimavanserin tartrate in bulk and pharmaceutical formulations.

**Keywords:** Area under curve, Derivative-spectrophotometry, Pimavanserin tartrate, UV Spectroscopy.

## Introduction

Pimavanserin tartrate (PMT) is chemically (2*R*, 3*R*) - 2,3 - dihydroxybutanedioic acid; 1-[(4-fluorophenyl) methyl] -1- (1-methylpiperidin-4-yl) -3- [[4-(2-methylpropoxy) phenyl] methyl]urea (Fig. 1). Pimavanserin tartrate (PMT) is one of the atypical anti-psychotic which is also used for the treatment of Parkinson.[1] When it synthesized Pimavanserin has been administered as its tartrate salt. [2]PMT is not a dopamine receptor antagonistbutis having inverse agonist on 5-HT2A[3-7] subtype receptor for the treatment of psychosis in Parkinson's disease (PD) and used in the treatment of schizophrenia. It's also having significant effect on insomnia, it's selective serotonin 5- HT2A receptor inverse agonist, to the slow wave sleep.[8]

Literature survey gives details about analytical methods for determination of PMT. In High Performance Liquid Chromatographic used to Quantification of Pimavanserin in Bulk and Tablet Dosage Form Using A Stability Indicating method,[9] ultrafast LC for estimation pimavanserin in pharmaceuticals.[10] and UPLC-MS for estimation of Pimavanserin tartrate in rat plasma [11] but still no UV spectrophotometry method has been developed for the determination of PMT in bulk and tablet formulation. At the same time, our goal was use to AUC and amplitude technique to established zero order and first order derivative.

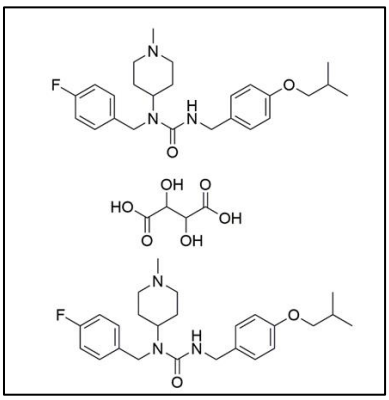

**Fig. 1:** Chemical structure of pimavanserin tartrate

## Materials and Methods

### Chemicals

Pure PMT was received from MSN Laboratories PVT. LTD, Hyderabad, India as a gift sample. All chemicals and reagents used were of analytical grade (Rankem India Ltd. Thane, India)

### Instrumentation

Use of a double beam UV-VIS spectrophotometer (UV-2450, Shimadzu, Japan), consist of 10 mm quartz cell, connected to a computer loaded with a UV Probe spectra software version 2.21. The instrumental obtained the spectra as follows various procedure of parameters, wavelength range in between 200–400 nm; scanning speed: medium; sampling interval 1.0 nm. All weights were taken on an electronic balance. (Model- AUX 120, Shimadzu, Japan)

**Preparation of standard stock solution and selection of determination of lambda (λmax):** A stock standard solution was prepared by dissolving 10mg of PMT in a100mL of methanol to get concentration of 100µg/mL. From it, further dilution concentration of 10µg/mL was prepared and scanne din the UV-visiblerange 400–200nm. Maximum absorbance observed at 226 nm. (Fig. 2)

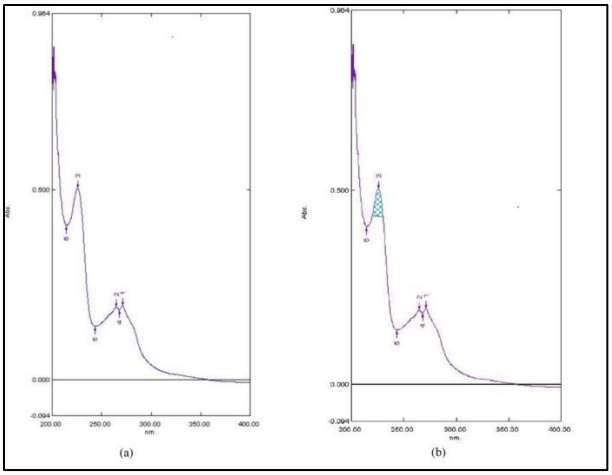


**Fig. 2:** Zero order spectrum (a) and Area under curve AUC; (b) selected wavelength

**Method A (Zero order) and Method B (Zero order AUC):** For method A and method B, prepared different standard solutions sets of PMT range from (5-30 µg/mL) from the standard stock solution of PMT (100 µg/mL).For this aliquots of 0.5 – 3.0 mL of standard stock solution are separately pipetted out and transferred to volumetric flask series with capacity up to 10 mL, then volume was made up to the methanol. For method A absorbance was observed at 226 nm shown (Fig. 2(a)). While method B area under curve (AUC) of the zero-order spectrum was recorded between 219.20 and 230.40 nm shown in (Fig. 2(b)). Method A and Method B calibration curves are constructed by plotting zero order spectrum concentration against absorbance and AUC.

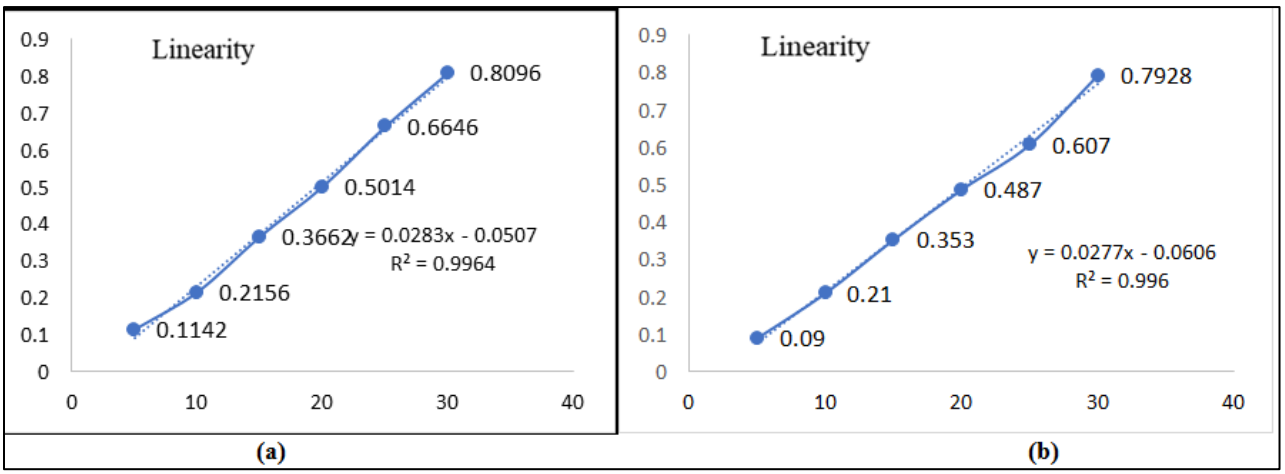


**Fig. 3:** Calibration Curve of PMT for Method A (a); and Method B (b)

**Method C (First order derivative) and Method D (First order derivative AUC):** Using UV probe 2.21 version software with delta 4 and scaling factor 10 for method C and method D spectra of previous solution derivatized into first-order spectra. The amplitude of Method C was shown at 232 nm in (Fig. 4(a)). While spectrum selected for method D first order derivative AUC between 228.20 and 238.40 nm shown in (Fig. 4(b)).Method C and D calibration curves are developed by plotting concentration versus amplitude and first-order spectrum AUC respectively, given in (Fig. 5).

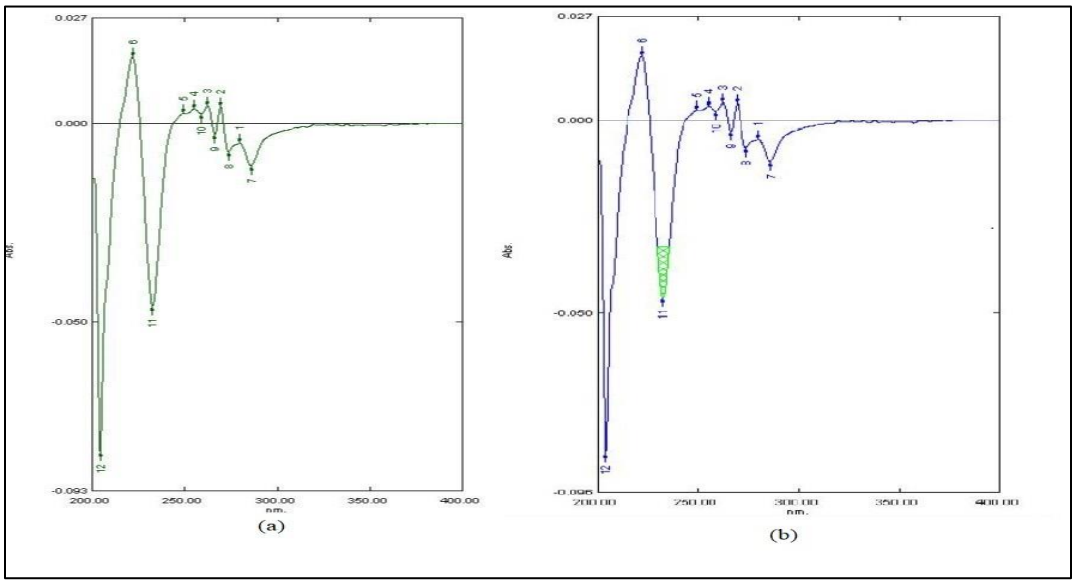


**Fig. 4:** First order derivative spectrum (a) and area under curve; (b) Between selected wavelengths of pimavanserin Tartrate.

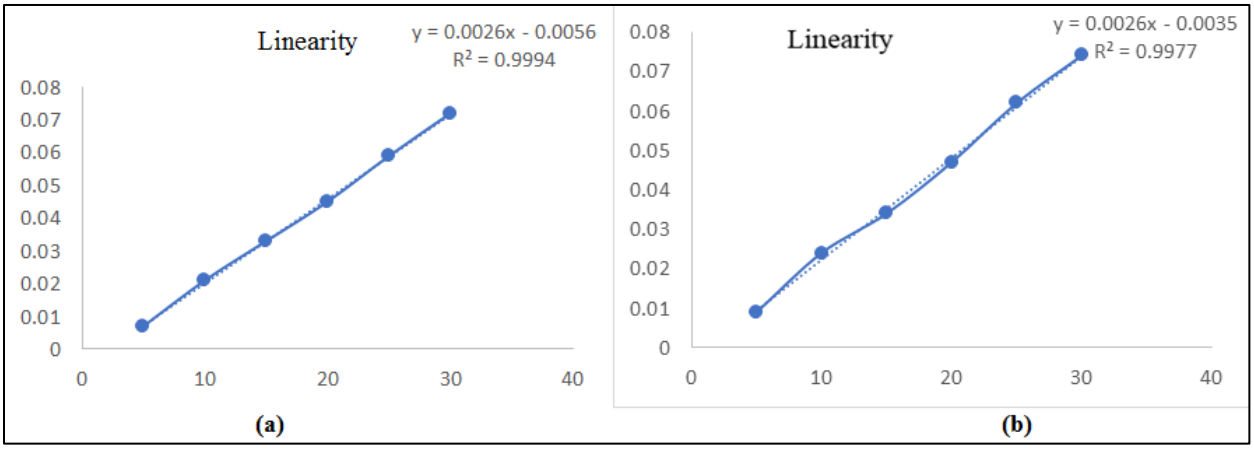


**Fig. 5:** Calibration Curve of Pimavanserin Tartrate for Method C (a); and Method D (b)

**Study of marketed formulation**

Formulation of *In-house* tablet preceded taking 10 mg drug with filler or binding agents like Magnesium stearate 8 mg, pregelatinized starch 8mg and micro crystalline cellulose as quantity sufficient for 150 mg. Mixed altogether through mortar and pestle. Repress in the mini tablet compressor and gets 150 mg of tablet containing about 10 mg of PMT. Formulation of 20 tablet takes place and weigh and average about 149.95 mg, brings a tablets, weigh and triturate to makes fine powders with the mortar and pestle. Quantity of powder equivalents to tablet. Place into 100 volumetric flask, add 50 ml of methanol, sonicated for

15 min and volume adjust to mark. Solvent were filtered through whatman filter paper no.41. 2 ml of filter solution pour in a 10 ml volumetric flask and make up with methanol gets 20 µg/mL of solution. The absorbance measured and the sample concentrations are calculated from the equation of linearity.

**Validation of the method**

The aim of method was validated with regards to various parameters i.e. linearity, accuracy, precision, repeatability, limit of detection, limit of quantification and ruggedness as per ICH guidelines.[12]

**Linearity studies**

Analysis of six standard PMT solutions of concentrations 05, 10, 15, 20, 25, and 30 µg / mL evaluated the linearity of the "methods A, B, C, and D".In the concentration range 5-30 µg / mL, the calibration curve was obeyed The Beers and lambert law and the chart was plotted between concentrations versus absorption, amplitude and AUC.

**Accuracy**

A defined quantity of stock standard solution has been applied at different levels to the pre-analyzed sample solutions (20 µg/mL) known amounts of solutions were added from standard stock solution at different levels, i.e. 80%, 100%, and 120%. After these formations re-analyzed through the different methods.

**Precision**

Precision was studied as intraday and interday variations. In this method takes 3 different concentration 15, 20, 25 µg/mL for 3 different times within a day for intraday precision, while interday precision above same concentration and times, performed in three days for all methods were developed.

**Sensitivity**

The Sensitivity used for the estimation of limit of detection (LOD) and limit of quantification (LOQ) of PMT. The LOQ and LOD were calculated using equation LOD=3.3 × Avg. S.D/Sand LOQ=10 × Avg. S.D/S, where'Avg. S.D' is the average standard deviation of the absorbance amplitude, and AUC and 'S' is the slope of the corresponding calibration curve.

**Repeatability**

Repeatability was determined by analyzing 20 µg/mL concentration of pimavanserin tartrate solution for six times for all methods.

**Ruggedness**

Ruggedness of the proposed methods was determined by analyzing aliquots for 20 µg/mL concentrationsof PMT from standard stock solution by two analysts using the same operational and environmental conditions for all methods.

## Results and Discussion

**System validation**

PMT has been tested for linearity, accuracy, precision, sensitivity, LOD, LOQ, repeatability and ruggednessof the following parameters. As per the guidelines, the results were found to be acceptable. The regression analysis data shown in Table 1.

**Table 1:** Optical characteristics and linearity data of Pimavanserin tartrate

| Parameters | Method A | Method B | Method C | Method D |
|---|---|---|---|---|
| Beer-Lambert's range(µg/mL) | 05-30 | 05-30 | 05-30 | 05-30 |
| Lambda Max (nm) | 226 | 219.20-230.40 | 232 | 228.20-238.40 |
| Slope | 0.0283 | 0.0277 | 0.0026 | 0.0026 |
| Intercept | 0.0507 | 0.0606 | 0.0056 | 0.0035 |
| Correlation coefficient | 0.9964 | 0.996 | 0.9994 | 0.9977 |

**Linearity:** From the linear regression data, the calibration curves shown in (Fig. 3 & 5) linear relationship over the 5-30 µg / mL concentration range for PMT were cleared for "methods A, B, C, and D".

**Accuracy:** Accuracy determined from the pretested sample solution at three different concentrations i.e. 80%, 100%, 120%. The % Recovery values showed that the accuracy of the methods was found to be satisfactory.

**Table 2:** Accuracy

| Drug | Methods | Initial amount [µg/mL] | Amount added [µg/mL] | Amount recovered [µg/mL, n = 3] | % Recovered | % RSD |
|---|---|---|---|---|---|---|
| Pimavanserin Tartrate | A | 20 | 16 | 36.10 | 100.64 | 0.29 |
| | | 20 | 20 | 40.06 | 100.30 | 0.35 |
| | | 20 | 24 | 44.08 | 100.36 | 0.21 |
| | B | 20 | 16 | 35.94 | 99.63 | 0.2 |
| | | 20 | 20 | 39.99 | 99.98 | 0.18 |
| | | 20 | 24 | 44.00 | 100.02 | 0.17 |
| | C | 20 | 16 | 35.98 | 99.98 | 0.32 |
| | | 20 | 20 | 40.03 | 100.04 | 0.26 |
| | | 20 | 24 | 44.08 | 100.09 | 0.22 |
| | D | 20 | 16 | 35.99 | 99.99 | 0.51 |
| | | 20 | 20 | 40.04 | 100.40 | 0.70 |
| | | 20 | 24 | 44.01 | 100.03 | 0.42 |

n- number of determinations Precision

**Intra-day:** For intraday precision studies three replicates of three different concentration 15, 20, 25 µg/mL was analyzed at different times in same day. The % RSD and data disclosein (Table 3).

**Inter-day:** For inter-day precision three replicates of three different concentrations 15, 20, 25 µg/mL was analyzed in different days subsequently. The% RSD and data disclose in (Table 3).

**Table 3:** Precision studies

| Drug | Methods | Concentration [µg/mL] | Intra-day [n = 3] | % RSD | Inter-day [n = 3] | % RSD |
|---|---|---|---|---|---|---|
| Pimavanserin Tartrate | | 15 | 14.91 | 0.57 | 14.84 | 0.43 |
| | A | 20 | 19.95 | 0.41 | 19.92 | 0.59 |
| | | 25 | 25.05 | 0.34 | 20.03 | 0.31 |
| | | 15 | 14.87 | 0.34 | 14.93 | 0.22 |
| | B | 20 | 19.97 | 0.42 | 19.97 | 0.18 |
| | | 25 | 25.11 | 0.30 | 25.07 | 0.28 |
| | | 15 | 14.98 | 0.15 | 14.88 | 0.13 |
| | C | 20 | 20.01 | 0.55 | 20.01 | 0.52 |
| | | 25 | 25.13 | 0.19 | 25.20 | 0.21 |
| | | 15 | 15.08 | 0.19 | 10.10 | 0.35 |
| | D | 20 | 19.88 | 0.14 | 20.08 | 0.12 |
| | | 25 | 25.07 | 0.21 | 25.13 | 0.19 |

n- number of determinations

**Sensitivity:** The LOD and LOQ for PMT in "method A" were found to be 0.13 µg and 0.39 µg, in "method B" 0.106 µg and 0.321 µg, "method C" 0.143 µg and 0.434 µg, while "method D" 0.147 µg and 0.445 µg.

**Repeatability:** The results of repeatability in terms of % RSD for "methods A, B, C, and D," were observed less than 2 that shows precise nature of developed methods. Results are shown in (Table 4).

**Table 4:** Repeatability studies

| Drug | Methods | Amount taken [µg/mL] | Amount found [µg/mL] | % Amount found [n =6] | Mean ± SD | % RSD |
|---|---|---|---|---|---|---|
| Pimavanserin Tartrate | A | 20 | 19.96 | 99.82 | 99.82 ± 0.51 | 0.51 |
| | B | 20 | 20.04 | 100.21 | 100.21 ± 0.49 | 0.49 |
| | C | 20 | 19.88 | 99.44 | 99.44± 0.50 | 0.50 |
| | D | 20 | 19.94 | 99.32 | 99.32 ± 1.10 | 0.74 |

n- number of determinations

**Ruggedness:** The results of ruggedness were in acceptable range that is % RSD values less than 2 for all the developed methods as shown in (Table 5). The results proved no statistical differences between different analyst using same operational and environmental condition. That's why it signifies the developed methods are rugged in nature.

**Table 5:** Ruggedness studies

| | | Analyst I | | Analyst II | |
|---|---|---|---|---|---|
| Drug | Methods | % Amount found ± SD [n = 3] | % RSD | % Amount found ± SD [n = 3] | % RSD |
| Pimavanserin Tartrate | A | 100.41 ± 0.52 | 0.52 | 100.20 ± 0.36 | 0.36 |
| | B | 100.07 ± 0.58 | 0.58 | 100.37 ± 0.57 | 0.57 |
| | C | 99.80 ± 0.76 | 0.76 | 99.87 ± 0.78 | 0.78 |
| | D | 99.61 ± 0.79 | 1.23 | 99.87 ± 1.12 | 1.12 |

n- number of determinations

**Analysis of *in-house* Tablet Formulation:** From PMT T *in-house*tablet formulation amount of PMT estimated by using methods A, B, C and D were founded about 100.68%, 100.77%, 100.38%, and 100.44%, respectively. The % amount found from tablet formulation indicates that there was not too much interruption from excipients present *in-house* tablet formulation.

## Conclusion

All the methods developed are economical, simple, reliable, accurate and robust and can be used for the analysis of Pimavanserin tartrate. These methods are developed for the quantification of pimavanserin tartrate. It can be also used in routine quality control of the formulations containing pimavanserin tartrate.

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**None.**

## Conflict of Interest

None.

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