WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
Coden USA: WJPRAP Impact Factor 8.63
Volume 15, Issue 11, 2511-2520. Research Article ISSN 2277–7105

A NOVEL ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF PRALSETINIB BY UV SPECTROSCOPIC METHOD

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Article Received on 05 May 2026,
Article Revised on 25 May 2026,
Article Published on 03 June 2026,

https://doi.org/10.5281/zenodo.20536954

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How to cite this Article: Dr. Santhosh Illendula*[1], Chinta Gowtham[2], Kodigela Sreeja[2], Nallabothula Sandhya[2], Singireddy Akanksha[2], Kurthi Manasa[2], Sunnapu Shiva[2], Dr. Suthakaran Raj[3] (2026). A Novel Analytical Method Development And Validation For The Estimation Of Pralsetinib By Uv Spectroscopic Method. World Journal of Pharmaceutical Research, 15(11), 2511-2520.

ABSTRACT

A new simple, accurate, rapid, precise and reproducible spectrophotometric method for the quantitative estimation Pralsetinib in bulk form. The developed visible spectrophotometric method for the quantitative estimation of Pralsetinib is based on measurement of absorption at maximum wavelength 260 nm using with Orthophosphoric acid**:** Acetonitrile (60**:**40 v/v) as a solvent. The stock solution of Pralsetinib was prepared, and subsequent suitable dilution was prepared in diluent to obtained standard curve. The standard solution of Pralsetinib shows absorption maxima at 260 nm. The drug obeyed beer lambert's law in the concentration range of 5 - 25 µg/ml with regression 0.9999 at 260 nm. The overall % recovery was found to be 99.64% which reflects that the method was free from the interference of the impurities and other excipients used in the bulk form. The low value of % RSD was indicative of accuracy and reproducibility of the method. The % RSD for inter-day and intra-day precision was found to be 0.4499 and 0. 7497, respectively which is<2%

hence proved that method is precise. The results of analysis have been validated as per International Conference on Harmonization (ICH) guidelines. The developed method can be adopted in routine analysis of Pralsetinib in bulk form.

KEYWORDS: Pralsetinib, UV Visible Spectrophotometry, Method development, Validation, ICH guidelines, Acetonitrile**,** Orthophosphoric acid, Accuracy, Precision.

INTRODUCTION

Pralsetinib N-[(1S)-1-[6-(4-fluoropyrazol-1-yl) pyridin-3-yl] ethyl]-1-methoxy-4-[4-methyl-6-[(5-methyl-1H-pyrazol-3-yl) amino] pyrimidin-2-yl] cyclohexane-1-carboxamide.

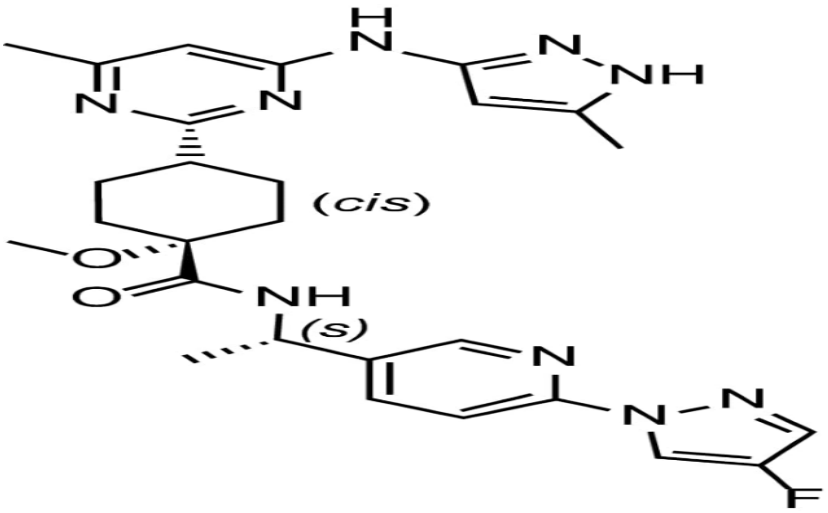

Figure -1: Structure of Pralsetinib.

Mechanism of action : Rearranged during transfection (RET) is a transmembrane receptor tyrosine kinase containing extracellular, transmembrane, and intracellular domains whose activity is required for normal kidney and nervous system development. Constitutive RET activation is achieved through chromosomal rearrangements producing 5' fusions of dimerizable domains to the 3' RET tyrosine kinase domain leading to constitutive dimerization and subsequent autophosphorylation; the most common fusions are KIF5B-RET and CCDC6-RET, although more than 35 genes have been reported to fuse with RET Constitutive activation leads to increased downstream signalling and is associated with tumor invasion, migration, and proliferation.

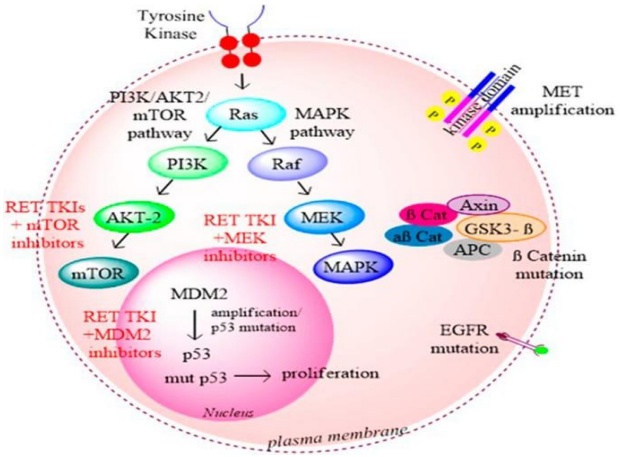

Figure 2: Mechanism of Pralsetinib.

MATERIALS AND METHODS

Chemicals and Reagents: Orthophosphoric acid**:** Acetonitrile.

Instruments: SHIMADZU UV-1800 UV–Vis spectrophotometer, Electronic Balance **(CITIZEN BALANCE CTG302-300),** Ultra Sonicator **(SOLTEC),** and P^H Analyzer **(INFRA DIGI IR 501),** Distillation unit **(BOROSIL),** Vaccum filtration unit **(BOROSIL).**

Reagents and Solutions

Diluent preparation: In a 100ml volumetric flask take 60:40 Orthophosphoric acid**:** Acetonitrile.

Preparation of Standard Solutions

Accurately weighed 100mg of Pralsetinib was weighed accurately and transferred into 100ml volumetric flask. About 10 ml of diluent was added and sonicated to dissolve. The volume was made up to the mark with same solvent. The final solution contained about 100µg/ml of Pralsetinib Working standard solution of Pralsetinib containing 10µg/ml for method. Finally add those above solutions and prepare the final solution is about 10µg/ml.

Determination of wavelength of maximum absorbance for Pralsetinib

The absorbance of the final solution scanned in the UV spectrum in the range of 200 to 400nm against solvent mixture as blank.

Optimization of selection of Solvent

It is well known that the solvents do exerts a profound effect on the quality and the shape of the peak. The choices of solvents for UV method development are: Methanol, Ethanol, Acetonitrile, Isopropyl alcohol, DMF, OPA & KH_2PO_4 etc. First optimize the different

solvents. From those solvents Orthophosphoric Acid and Acetonitrile combination satisfied the all the optimized conditions.

Wavelength Selection

The standard solutions are preparing by transferring the standard drug in a selected solvent or mobile phase and finally diluting with the same solvent or diluent. That prepared solution is scanned in the visible wavelength range of 200-400nm. This has been performed to know the maxima of Pralsetinib. While scanning the Pralsetinib solution we observed the maxima at 260 nm. The UV spectrum has been recorded on (SHIMADZU UV-1800) make UV – Vis spectrophotometer model UV-1800.The scanned UV spectrum is attached in the following page. The λ_{max} of the Pralsetinib.

METHOD VALIDATION

1. 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 Pralsetinib 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-1.

2. 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. Pralsetinib (API) the percent relative standard deviations were calculated for Pralsetinib is presented in the Table-2.

Intermediate Precision

Intra-assay & inter-assay

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 Pralsetinib revealed that the proposed method is precise. The results were shown in Table-3.

3. Linearity & Range

The calibration curve showed good linearity in the range of 5-25µg/ml, for Pralsetinib (API) with correlation coefficient (r^2) of 0.999976 (Fig-3). A typical calibration curve has the regression equation of y = 0.0459x + 0.0009 for Pralsetinib.

Standard solutions of Pralsetinib in the concentration range of 5 µg/ml to 25 µg/ml were obtained by transferring (5,10,15,20 and 25 ml) of Pralsetinib stock solution (100ppm) to the series of clean & dry 10 ml volumetric flasks. The volumes in each volumetric flask were made up with the solvent system and mixed.

The absorbances of the solutions were measured at 274 nm against the solvent system as blank and calibration curve is plotted. The Lambert-Beer's Law is linear in concentration range of 5 to 25 µg/ml at 260 nm for Pralsetinib. The results were shown in Table-4.

4. Method Robustness

Robustness of the method was determined by carrying out the analysis under different Wavelength i.e., at 258 nm, and 262 nm. The respective absorbances of 10µg/ml were noted SD < 2%) the developed UV-Spectroscopic method for the analysis of Pralsetinib (API). The results were shown in Table-5.

5. LOD & LOQ

The LOD and LOQ were calculated by the use of the equations LOD = 3.3 × σ / S and LOQ = 10 × σ / S where σ is the standard deviation of intercept of Calibration plot and S is the average of the slope of the corresponding Calibration plot.

The Minimum concentration level at which the analyte can be reliable detected (LOD) & quantified (LOQ) were found to be 0.2261 & 0.6854 µg/ml respectively.

RESULTS AND DISCUSSION

The standard solutions of Pralsetinib with Orthophosphoric acid and Acetonitrile subjected to a scan individually at the series of wavelengths of 200 nm to 400 nm. Absorption maximum of Pralsetinib was found to be at 260 nm. Therefore, 260 nm was selected as λ_{max} of Pralsetinib for the present study. The calibration curve of Pralsetinib was found to be linear in the range of 5-25 µg/ml at 260 nm. Therefore, it was clear that Pralsetinib can be determined without interference of any irrelevant substance in single component pharmaceutical

products. The used technique was initially attempted on bulk drugs in their synthetic sample and concentrations were estimated.

The % recovery was carried out at 3 levels, 80%, 100% and 120% of Pralsetinib standard concentration. Three samples were prepared for each recovery level. The solutions were then analyzed, and the percentage recoveries were found to be satisfactory within the acceptable limits as per the content of the label claim for marketed tablet dosage form. The newly developed method was validated according to the ICH guidelines and the method validation parameters.

The developed method was subjected to do the various method validation parameters such as specificity, accuracy, precision, linearity and range, limit of detection and limit of quantification, robustness and ruggedness.

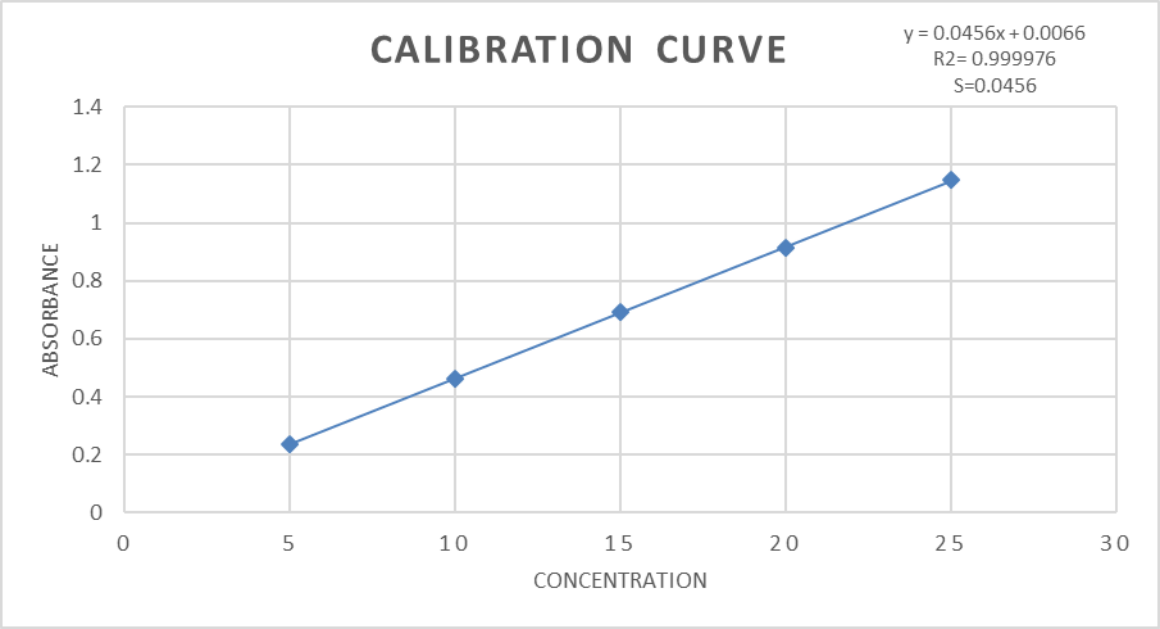

Fig- 3: Calibration curve of Pralsetinib (API).

Linearity range was found to be 5-25 µg/ml for Pralsetinib at 260 nm. The correlation coefficient was found to be 0.9999, which shows good linearity between above range. The slope was found to be 0.0459 and intercept was found to be 0.0009 which was close to zero intercept.

Table-1: Results of accuracy.

Level of Recovery	Sample Conc. (µg/ml)	Recorded conc. (µg/ml)	Absorbance	% Recovery	Mean % Recovery
80%	8	7.926	0.365	99.07	99.11%
80%	8	7.945	0.362	99.31	
80%	8	7.918	0.366	98.97	
100%	10	9.981	0.461	99.81	98.89%
100%	10	9.815	0.454	98.15	
100%	10	9.871	0.458	98.71	
120%	12	11.971	0.552	99.75	99.55%
120%	12	11.954	0.549	99.61	
120%	12	11.917	0.545	99.30	

The results obtained for the accuracy study (recovery method) from three sample studies (n 3) for each level indicated that the mean of the % recovery was 99.11% and 98.89% and 99.55% for Pralsetinib in mixture (10 g/ml). Here the mean % recovery is in between 98-102 % thus showing that the analytical technique has a good recovery study.

Repeatability

Table-2: Results of Repeatability.

Sr. No.	Conc. (µg /ml)	Wavelength (nm)	Absorbance
1	10	260	0.456
2	10	260	0.459
3	10	260	0.461
4	10	260	0.458
5	10	260	0.465
6	10	260	0.458
Mean + S.D.			0.4595
Standard Deviation			0.003146
% RSD			0.6846

Repeatability study showed a R.S.D of 0.6846% for Pralsetinib. Thus, it is concluded that the analytical technique has a good repeatability precision as R.S.D for the drug were less than 2%.

Table-3: Results of intra-Day & inter-Day.

Conc. taken (µg/mL)	Observed Conc. Of Pralsetinib (µg/ml) by the proposed method			
	Intra-Day		Inter-Day	
	Absorbance	Statistical Analysis	Con. found (µg/mL)	Statistical Analysis
10	0.461	Mean = 0.462667	0.461	Mean = 0.462
10	0.465	SD = 0.002082	0.464	SD = 0.003464
10	0.462	%RSD = 0.4499%	0.466	%RSD =0.7497%

Table-4: Results of Linearity.

Concentration (µg /ml)	Absorbance(n=6)
5	0.233
10	0.456
15	0.692
20	0.914
25	1.149

Table-5: Result of Method Robustness Test Wavelength (258nm -262nm)

Concentration(µg/ml)	Wavelength	Absorbance	Statistical Analysis
10	258	0.451	Mean = 0.454833
10		0.451	
10		0.453	SD = 0.004215
10	262	0.459	
10		0.461	% RSD = 0.9267
10		0.454	

Acceptance criteria: %RSD value below 2 obtained.

CONCLUSION

Unlike GC and HPLC techniques, UV Spectrophotometry is simple and inexpensive. The spectrophotometric method requires simple reagents, which an ordinary analytical laboratory can afford and the procedures do not involve any critical reactions. Moreover, the proposed method is found to be simple, sensitive, accurate and with good precision, this approach could be considered for the analysis of Pralsetinib drug in the quality control laboratories.

ACKNOWLEDGEMENT

The authors are grateful to the management of Vijaya College of Pharmacy, Hyderabad for providing the facilities to carry out the present research work.

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