

Development and Validation of HPLC Method For Quantifying Anticancer Agent in Pharmaceutical Formulation

Chanchal Wankhede*, Dr.Santosh Payghan, Prof. Amol Wadhawe, Prof. Ashwini Pundkar

1. Research scholar, Dept. of Pharmaceutical Quality Assurance, Rajesh Bhiayya Tope College of Pharmacy, Chh.Sambhajinagar.
2. Principal. Dept. of Pharmaceutical Sciences, Rajesh Bhiayya Tope College of Pharmacy, Chh.Sambhajinagar.
3. Professor, Dept. of Pharmaceutical Sciences, Rajesh Bhiayya Tope College of Pharmacy, Chh.Sambhajinagar.
4. Professor, Dept. of Pharmaceutical Sciences, Rajesh Bhiayya Tope College of Pharmacy, Chh.Sambhajinagar.

Email id: uionyou65@gmail.com

ABSTRACT

Palbociclib is a selective cyclin-dependent kinase (CDK) 4/6 inhibitor which is widely used in the treatment of hormone receptor-positive and human epidermal growth factor receptor 2 (HER2) negative breast cancer. In recent years, targeted anticancer therapies such as CDK inhibitors have gained significant importance due to their improved effectiveness and reduced side effects compared to conventional chemotherapy. Therefore, the development of reliable and accurate analytical methods for the estimation of such drugs in pharmaceutical dosage forms is essential to ensure their quality, safety, and efficacy. High performance liquid chromatography (HPLC) is one of the most commonly used analytical techniques in pharmaceutical analysis because of its high sensitivity, precision, and reproducibility. The present study was undertaken to develop and validate a simple, rapid, precise, accurate, and economical reverse phase HPLC (RP-HPLC) method for the quantitative estimation of Palbociclib in pharmaceutical capsule dosage form. Chromatographic separation of Palbociclib was achieved using a C18 column with an optimized mobile phase consisting of suitable organic solvent and buffer in appropriate proportion. The flow rate and detection wavelength were optimized to obtain a sharp and symmetrical peak with good resolution. The detection of Palbociclib was carried out using a UV detector.

Keywords: Palbociclib; Cyclin-dependent kinase 4/6 inhibitor; Anticancer agent; RP-HPLC; Analytical method development; Method validation;

INTRODUCTION

The ongoing and interconnected activities related to research and development, quality control, and quality assurance in the pharmaceutical industry involve the creation and validation of analytical methods. Analytical method development and validation play a vital role in ensuring the identity, purity, strength, and quality of pharmaceutical products. The assessment and management of equivalence, accuracy, and risk in analytical procedures are crucial to obtain reliable and reproducible results. Validation of an analytical method demonstrates that the method is suitable for its intended purpose and produces consistent and accurate results. An effective analytical method should be simple, precise, sensitive, and economical. It should be easy for analytical professionals to perform and capable of providing trustworthy data. The development and validation of a robust analytical method can significantly improve precision, reduce systematic errors, and save time and resources by avoiding repeated experimental trials.^[1,2] Analytical chemistry employs advanced techniques to determine the qualitative and quantitative composition of pharmaceutical substances. Both qualitative and quantitative analysis are essential in pharmaceutical research, development, and quality control. The reliability of analytical data largely depends on the performance of analytical instruments and methods. Therefore, proper selection and validation of analytical techniques are essential in pharmaceutical laboratories.

RESULTS AND DISCUSSION

The present study was carried out for the development and validation of RP-HPLC method by QbD approach for the quantitative estimation of Palbociclib in pharmaceutical tablet dosage form.

7.1.1 UV Absorption Spectrum of Palbociclib

The UV absorption spectrum of Palbociclib was recorded by scanning a solution of 10 µg/mL in methanol over the wavelength range of 200–400 nm using Shimadzu UV-1800 spectrophotometer. Following is the UV absorption spectrum of palbociclib showing maximum absorbance at 220 nm :

Table 7.1: UV Absorption Data of Palbociclib in Methanol

Wavelength	Absorbance (standard, 10 µg/mL)
210	1.25
220	1.45
230	1.20
240	0.85
254	0.35
270	0.15
300	0.02

Method validation table:

Table 7.2: UV Method Validation Parameters

Parameter	UV method results
Linearity Range	5-25µg/mL
Correlation coeff. (r^2)	0.999
LOD	0.125µg/mL
LOQ	0.380µg/mL
Precision (%RSD)	≤2%
Accuracy (%Recovery)	98.53%
%RSD	<2%

Above palbociclib's characteristic broad absorption peak at $\lambda_{\text{max}} = 220$ nm (scanned 200-350 nm range in methanol-phosphate buffer).The calibration curve was found to be linear in the concentration range of 2–12 µg/mL with a correlation coefficient (R^2) of 0.999, indicating good linearity of the developed method.

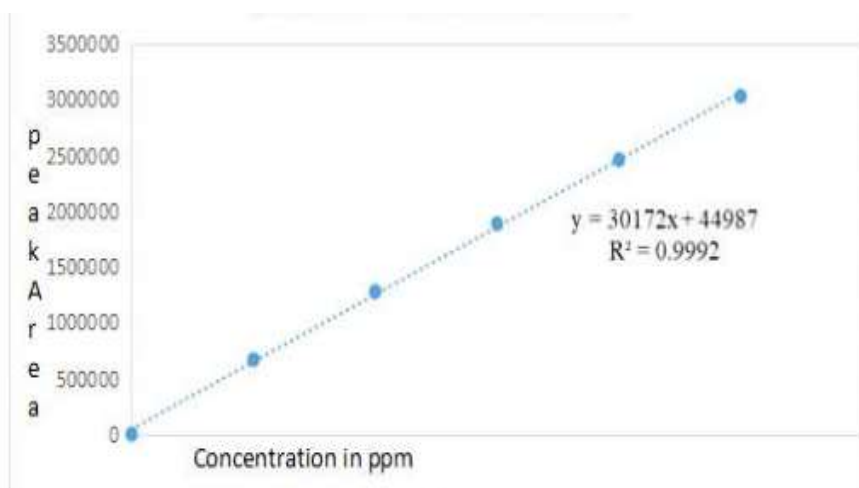


Fig 7.2: The calibration curve of Palbociclib

Table 7.3: Calibration curve data of Palbociclib by RP-HPLC

concentration µg/mL	mean peak area	Mean ± SD
5	42,256	42256±145
10	84,489	84489±1410
15	1,26,734	126734±1415
20	1,68,945	168945±1420
25	2,11,123	211123±1425
30	2,53,412	253412±1430

The calibration curve was found to be linear over the concentration range of 5–1000 µg/mL with regression equation $y = 48445x + 9743$ and correlation coefficient $R^2 = 0.999$, indicating excellent linearity of the developed RP-HPLC method.

7.1.3 Typical Chromatogram of Palbociclib

The optimized chromatographic conditions were finalized after systematic optimization of mobile phase composition, flow rate, and detection wavelength using QbD approach. The optimized chromatographic conditions are summarized in Table 5.2.

Table 7.4: Optimized Chromatographic Conditions for RP-HPLC Analysis of Palbociclib

Sr.No	Parameters	Optimized Condition
1	Column	Inertsil ODS – 3V (250 x 4.6mm, 5µg)
2	Mobile Phase	Ammonium Acetate : Acetonitrile (38:62 v/v)
3	Flow Rate	1.0 mL/min
4	Detection Wavelength	263nm
5	Injection Volume	20µL
6	Column Temperature	30°C ±2°C
7	Elution Mode	Isocratic
8	Total Runtime	30 min
9	Retention time	3.338± 0.25min
10	Detector	PDA Detector

Palbociclib showed a sharp and well-defined peak at retention time of 3.338 min under the optimized chromatographic conditions, confirming suitability of the developed RP-HPLC method for routine analysis.

7.2 Method Validation

The developed RP-HPLC method was validated as per ICH Q2(R1) guidelines. The following validation parameters were evaluated:

7.2.1 System Suitability

System suitability test was performed before validation by injecting standard solution of Palbociclib six times into the RP-HPLC system under optimized chromatographic conditions. Parameters such as theoretical plates, tailing factor, retention time and %RSD of peak area were evaluated.

Table 7.5: System Suitability Parameters of Palbociclib

Sr. No	Parameter	Results	Acceptance Criteria
1	Retention Time(min)	3.338±0.25	Consistent
2	Theoretical Plates (N)	25192	≥ 2000
3	Tailing Factor	1.363	≤ 2.0
4	%RSD of Peak Area	0.32	≤ 2.0%

System suitability parameters were found to be within the acceptance criteria, confirming that the RP-HPLC system was suitable for the analysis of Palbociclib."

5.2.2 Specificity

Specificity of the developed RP-HPLC method was determined by injecting blank solution (methanol), placebo solution and standard solution of Palbociclib separately into the HPLC system under optimized chromatographic conditions.

Table No. 7.6 : Specificity Data of Palbociclib

Sr. No.	Solution Injected	Retention Time(min)	Peak area	Interference
1	Blank (Methanol)	-	-	None
2	Placebo solution	-	-	None
3	Palbociclib Standard	3.338	4830821	-

No interference was observed at the retention time of Palbociclib in blank and placebo solutions, confirming the specificity of the developed RP-HPLC method

7.2.2 Accuracy

Accuracy of the method was determined by recovery experiment. Recovery studies were carried out by adding known amount of standard drug solution to the laboratory prepared formulation at three concentration levels i.e. 80%, 100% and 120%. The %recovery was calculated and reported in tables below.

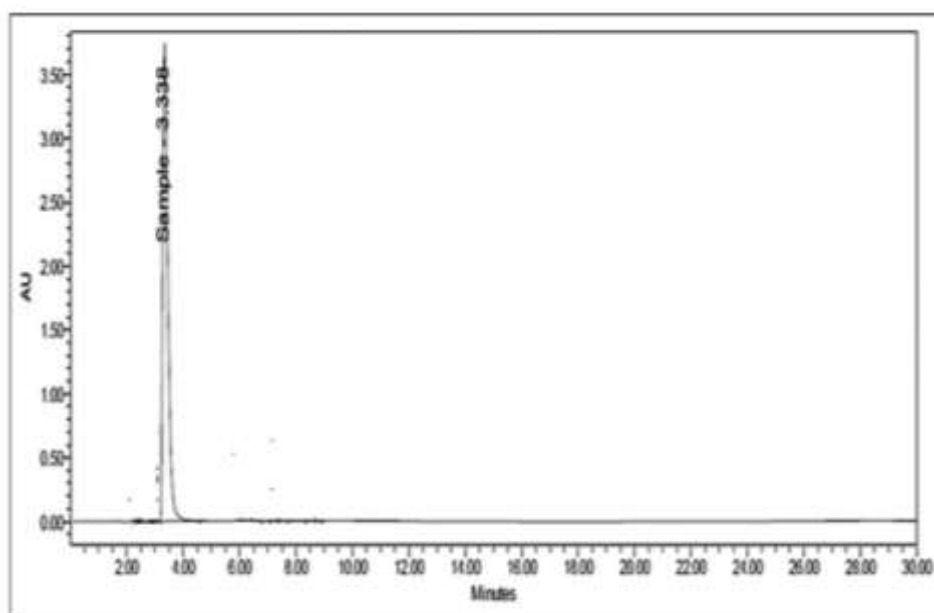


Fig 7.5: Chromatogram for Accuracy 80% of Palbociclib

Table No. 7.7: Accuracy Study of 80%

Retention Time	Area	Theoretical Plates
3.308	45,496,162	28758

Table No. 7.8: Accuracy Study of 100%

Retention Time	Area	Theoretical Plates
3.402	48349042	25843

Table No. 7.9 : Accuracy Study of Palbociclib

Sr. No.	%Level	Conc. (µg/mL)	Amount Added (µg/mL)	Total Conc. (µg/mL)	%Recovery	%RSD
1	80%	50	40	90	98.65	0.32
2	100%	50	50	100	98.72	0.59
3	120%	50	60	110	98.51	0.83

7.2.4 Precision

The precision of the analytical method was studied by analysis of multiple sampling of homogeneous sample. The precision was expressed as standard deviation or relative standard deviation. The experiment was repeated as an intraday and interday precision. The average %RSD values for determination of Palbociclib were calculated. The interday and intraday studies are presented in Tables below.

A) Intraday Precision (Repeatability)

Table No. 7.10: Intraday Precision Results of Palbociclib

Sr. No	Conc. (µg/mL)	Area 1	Area 2	Area 3	Mean ± SD	%RSD
1	50	2419342	2417354	2417298	2417998±7737.59	0.32
2	100	4830821	4835624	4832452	4832965± 10149.23	0.21
3	150	7261624	7268938	7262754	7264438± 30510.64	0.42

7.2.5 Limit of Detection (LOD) and Limit of Quantification (LOQ)

The LOD and LOQ were calculated based on standard deviation of the response and the slope of the calibration curve using the following formulas as per ICH Q2(R1) guidelines:

$$\text{LOD} = 3.3 \times \sigma / S$$

$$\text{LOQ} = 10 \times \sigma / S$$

Where:

σ = Standard deviation of the response

S = Slope of the calibration curve

Table No. 7.11: LOD and LOQ Values of Palbociclib

Sr. No.	Parameter	Value
1	Slope (S)	48445
2	Standard Deviation (σ)	24085
3	LOD	1.6378($\mu\text{g/mL}$)
4	LOQ	1.951($\mu\text{g/mL}$)

The LOD and LOQ were found to be 1.6378 $\mu\text{g/mL}$ and 4.951 $\mu\text{g/mL}$ respectively, indicating high sensitivity of the developed RP-HPLC method for quantification of Palbociclib."

7.2.6 Robustness

Robustness of the method was determined by slightly changing the chromatographic conditions deliberately one at a time while keeping all other parameters constant. The following parameters were varied: flow rate (± 0.1 mL/min), mobile phase composition ($\pm 2\%$), and detection wavelength (± 5 nm).

Effect of Less Flow Rate (0.9 mL/min)

Table No. 7.12: Robustness — Less Flow Rate

Retention Time	Area	Theoretical Plates
3.450	4572361	24895

Table No.7.13: Robustness Results of Palbociclib

Sr. No.	Parameter Changed	Condition	Retention Time(min)	%RSD	Theoretical Plates
1	Flow Rate	0.9ml/min	3.450	0.45	24895
2	Flow Rate	1.1mL/min	3.210	0.38	26432
3	Mobile phase	+2%ACN	3.298	0.52	25341
4	Mobile phase	-2%ACN	3.389	0.61	24987
5	Wavelength	258nm	3.3338	0.41	25192
6	Wavelength	268nm	3.338	0.55	25912

Assay of Palbociclib

Palbociclib is still under development, and therefore, Palbociclib capsules were prepared in our laboratory with the available excipients and then extracted with mobile phase. This solution was filtered and diluted as per the requirement with mobile phase. The percentage recovery of palbociclib was found to be 98.93–99.21%. No interference of excipients was observed.

Conclusions

The validated stability indicating method developed for the determination of Palpociclib is specific and selective and more economical. Palpociclib is found to be more sensitive towards oxidation than alkaline environment. This method can be excellently applied for the determination of Palpociclib in pharmaceutical formulations.

References

1. Novel Drug Approved for Breast Cancer: Palbociclib (Ibrance). Medscape; 2015. Available from: <https://www.medscape.com>. [Last accessed on 2015 Feb 03].
2. Tamura K, Mukai H, Naito Y, Yonemori K, Kodaira M, Tanabe Y, et al. Phase I study of palbociclib, a cyclindependent kinase 4/6 inhibitor, in japanese patients. Cancer Sci 2016;107:755-63.

3. Dange Y, Bhinge S, Salunkhe V. Optimization and validation of RP-HPLC method for simultaneous estimation of palbociclib and letrozole. *Toxicol Mech Methods* 2018;28:187-94.
4. Song WJ, Wang Z, Qiu F, Li W. Content determination of Palbociclib capsules. *Chin J New Drugs* 2017;26:2468-71.
5. ICH Validation of Analytical Procedures: Text and Methodology Q2 (R1). International Conference on Harmonization; 2005
6. ICH Stability Testing of New Drug Substances and Products Q1A (R2). International Conference on Harmonization; 2003.