RESEARCH PAPER

A Novel Stability-Indicating RP-HPLC Method for the Simultaneous Determination of Palbociclib and Its Impurities in API and Pharmaceutical Dosage Forms

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Received: 17th Dec, 2025; Revised: 10th Feb 2026; Accepted: 15th Feb, 2026; Available Online: 30th March, 2026

ABSTRACT
In this present study, it is developed and validated a novel RP-HPLC method has been developed and validated for quantifying Palbociclib and its associated impurities in both active pharmaceutical ingredient (API) and finished dosage forms. The method enables the simultaneous determination of assay and related substances and is distinguished by its accuracy, specificity, selectivity, simplicity, and precision. With six specified impurities present in Palbociclib, the method offers a reliable and efficient approach for their determination. Using a YMC Triart C18 column, Palbociclib and its impurities were effectively separated. During the gradient phase, acetonitrile and orthophosphate buffer were used as the mobile phase. With a total run time of 60 minutes and a column temperature of 30°C, detection was carried out at 230 nm. Over the concentration range of 0.5–200 g/mL, the method demonstrated linearity. The method was validated according to ICH guidelines, and forced degradation studies confirmed its stability-indicating characteristics. According to the developed method, it is specific, selective, precise, accurate, and robust, so it can be used in routine quality control analysis.

Keywords: *Assay, Related substance, Palbociclib, Validation, ICH guidelines.*
How to cite this article: Kshatriya AG and Andal P, A Novel Stability-Indicating RP-HPLC Method for the Simultaneous Determination of Palbociclib and Its Impurities in API and Pharmaceutical Dosage Forms. Int J Drug Deliv Technol. 2026;16(3): 275-280. DOI: 10.25258/ijddt.16.3.33
Source of support: Nil.
Conflict of interest: None

INTRODUCTION

Palbociclib is a drug used to treat specific kinds of breast cancer in both women and men. It is hormone-receptor positive and HER2-negative, particularly if it has spread to other parts of the body. Researchers are also looking into its use for other cancer types[1]. Palbociclib prevents cancer cells from growing by blocking specific proteins. Cyclin-dependent kinase inhibitors are a class of drugs that affect cyclin-dependent kinase. It belongs to a group of drugs known as cyclin-dependent kinase inhibitors[2].

Palbociclib is a type of medication that affects how cells divide and grow. It is part of a category of medications called cyclin-dependent kinase inhibitors. Pfizer Inc developed Palbociclib after researchers found that cyclin-dependent kinases play a crucial role in controlling cell growth[3]. The drug received FDA approval in March 2015 for treating advanced or metastatic breast cancer that is HR-positive and HER2-negative. In April 2019, its use was expanded to include male patients, following reports that confirmed its safety and effectiveness based on real-world data[4,5]. The number of methods available for Palbociclib[6]. Few methods are available for the determination of impurities, but these method does not include the below impurities and no method available which determine the assay and related substance of Palbociclib within a single run method[7,8]. This method gives an advantage to determine the four impurities and Palbociclib content as well as its impurities[9]. This method is stability-indicating and to prove that forced degradation study has been performed for the same. All observations found satisfactory[10].

MATERIALS

Water HPLC 2695 model and Shimadzu with PDA detector, YMC Triart-C18, 250 mm x 4.6 mm, 5 µm is found suitable, flow rate is variable along with gradient. Sartorius balance and Venus make a pH meter used for the above study. Mobile phase buffers are prepared using potassium phosphate, triethylamine, and orthophosphoric acid. In the mobile phase, acetonitrile is used as a solvent.

METHOD

Chromatographic Conditions

This experiment was conducted on a YMC Triart C18 column (4.6 x 250 mm, 5µm). A UV detection was carried out using 230 nm with a column oven maintained at 30 °C. Flow rate 0.8 ml /minute. The injection volume was 20µL and the run time was 60 minutes for each analysis. Mentioned in Table 1.

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Table 1: Chromatographic conditions

Time (Minute)	0	10	22	40	50	55	60
Mobile phase-A (%)	82	72	65	25	25	82	82
Mobile phase-B (%)	18	28	35	75	75	18	18

PREPARATION OF THE MOBILE PHASE

Mobile phase-A: A solution of 1.36g potassium phosphate and 1 ml triethylamine must be dissolved in 1000 ml Milli Q water, and the pH should be adjusted to 2.10 with OPA.

Mobile Phase-B: Acetonitrile

Diluent: 0.1% OPA in water and Acetonitrile in a ratio of 50:50 v/v.

Standard Working Solution preparation

Prepared a 50 ppm solution of Palbociclib

Prepared a 500 ppm solution of Palbociclib by dissolving in diluent. Sonication and shaking method used during preparation[11].

Enhancement of the RP-HPLC method

A reliable reverse-phase high-performance liquid chromatography method was designed for the simultaneous quantification of the drug and its related impurities. Various mobile phase combinations & column types were used to investigate the superior resolution and peak clarity. Individual standard impurities were first analyzed to determine their retention characteristics, followed by spiked injections of Palbociclib samples containing known impurities. Through systematic trials, the chromatographic conditions were optimized. Each impurity was determined based on its retention time and relative retention time during spiking experiments. The finalized method is capable of determining both the assay and related substances within a single analytical run[12].

Validation study

Validation of this method was done using ICH Q2 (R2) guidelines.

System suitability

This step is essential in chromatographic method development, as it verifies that the system provides adequate resolution and reproducible performance for reliable analysis. The process involves examining the entire system, which includes the equipment, electronics, analytical procedures, and samples. System suitability parameters were evaluated to ensure the performance of the HPLC system for the analysis of Palbociclib. The USP theoretical plate count was found to be 8825, indicating good column efficiency. It was found that peak area and retention time had a %RSD of 0.45% and 0.23%, respectively, demonstrating excellent precision and consistency. These results confirm that the system meets the suitability criteria for routine analysis. Mentioned in Table 2.

Specificity

Injected blank, placebo, and Palbociclib, spiked sample, and sample solution. The chromatogram of the sample solution was examined alongside the individual impurities and the placebo solution. Further, the comparison of the chromatogram made with forced degradation samples. All the specified impurities are found separately from each other. The blank interference was observed at any retention time for Palbociclib or any impurity[13]. The chromatogram of blank, spiked sample, and structures shown in Figure 3.

Linearity

Linearity established for impurities and Palbociclib individually. For Palbociclib, the linearity covered from 0.5 ppm to 200 ppm and for impurities, 0.2 ppm to 2 ppm. As the method is usable for an assay method, the higher range for Palbociclib is covered up to 200 ppm. Calculated the Relative response factor (RRF) for the active drug and its impurities, for all impurities, RRT found within 0.8 to 1.2. The correlation coefficient, y-intercept, and slope has been tabulated and given in Table 3.

Precision

Prepared spiked impurity sample solution and assay sample solution six preparation and injected under the chromatography. Followed by Precision, Intermediate precision also on another system, another column, another analyst, and on another day. Calculated and compared the results of the precision and intermediate study. The result is satisfactory and based on it is concluded that the method is Precise. The results are captured in Table 4.

Accuracy

Two methods were used to check accuracy: one compared result to an impurity standard, and the other relied solely on the Palbociclib standard with Response Factor (RF). Impurities were added to a sample solution, and an exact amount of Palbociclib was added to a placebo solution. Prepared three different levels of solutions, ranging from the Limit of Quantification (LOQ) to 150%, following the established procedure. The accuracy was confirmed for both the impurities and Palbociclib, and the recovery rates fell within acceptable limits. Additionally, the accuracy was verified using only the Palbociclib standard with RF. Thus, impurities can be quantified using just the Palbociclib standard through this method. The results are detailed in Table 5.

Robustness

The robustness of the developed HPLC method was evaluated by introducing small but deliberate changes to critical method parameters, including detection wavelength, column temperature, and mobile phase pH. The assay of Palbociclib was not significantly affected by

minor changes in wavelength (±2 nm), with a 0.0% difference observed at both 228 nm and 232 nm, confirming the method's insensitivity to small variations in detection wavelength. Similarly, changes in column temperature (25°C and 35°C) resulted in minimal assay variation, with a maximum difference of only 0.6%, indicating thermal stability of the method. Adjustments in mobile phase pH from 2.00 to 2.20 had no impact on the assay results, with a 0.0% difference observed. These findings confirm that the method is robust and reliable under slight variations in analytical conditions, ensuring consistent performance during routine quality control analysis[14].

Forced degradation study

Different stress conditions, such as base, Acid, thermal, peroxide, photolytical, and water hydrolysis, were forced degradation samples prepared and injected under the system. Prepared API and stressed the sample individually. Verified the peak purity of individual Palbociclib and all its each impurity. All peak found well resolved and peak purity passes. The peak purity determined using empower software[15]. Results are summarized in Table 6.

Filter Validation

A filter compatibility study was conducted to assess the potential impact of filtration on the quantification of impurities in the spiked sample of Palbociclib. Two methods were used to process the spiked sample: centrifugation and filtration through a 0.45 m PVDF membrane filter. The impurity content obtained from the centrifuged sample was 100.1%, while the filtered sample showed an impurity level of 99.9%, resulting in a negligible difference of 0.2%. This minimal variation confirms that the PVDF filter does not adsorb or interfere with the analyte or its impurities. Therefore, filtration through a 0.45 µm PVDF membrane filter is suitable for sample preparation in the developed HPLC method[16,17].

Solution stability

The stability of the assay test solution was evaluated over a period of 36 hours at room temperature to ensure the reliability of analytical results during routine analysis. The initial assay value of Palbociclib was found to be 99.9%. After 12 hours, the assay was 99.3%, corresponding to a 0.6% difference from the initial value. At 36 hours, the assay slightly decreased to 99.0%, showing a total difference of 0.9%. which is less than 2.0%, which is the general specified limit. These results indicate that the assay test solution of Palbociclib remains stable for at least 36 hours under ambient laboratory conditions, with no significant degradation or loss of potency. Therefore, the prepared solutions can be considered stable within this timeframe for analytical use[18].

RESULTS AND DISCUSSION

System suitability

Table 2: RF and RRT of Palbociclib impurities in Spiked sample

Peak	~RT	~RRT
Palbociclib	16.12	1.0
Imp-A	10.00	0.62
Imp-B	21.20	1.32
Imp-D	24.05	1.49
Imp-F	26.06	1.62
Imp-E	29.90	1.85
Imp-C	40.01	2.48

Specificity

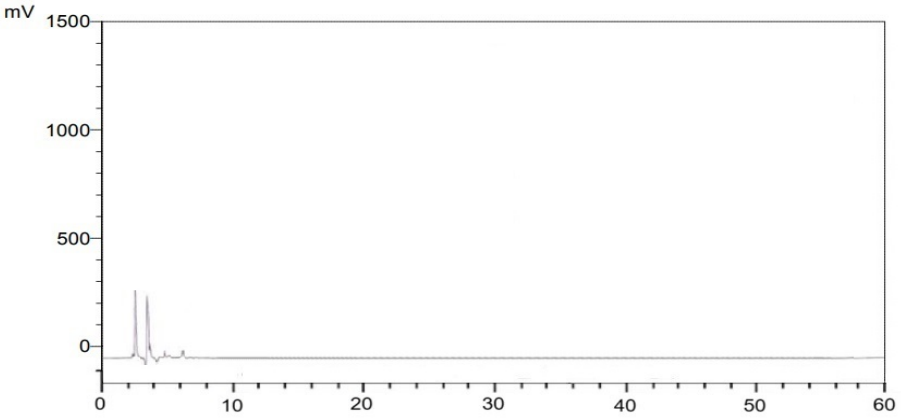

Fig. 1: Chromatogram of Blank

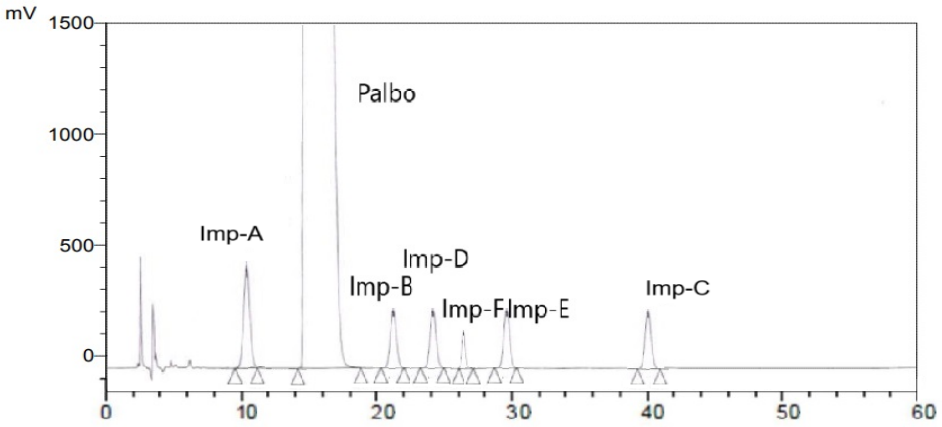

Fig. 2: Structure and name of impurities

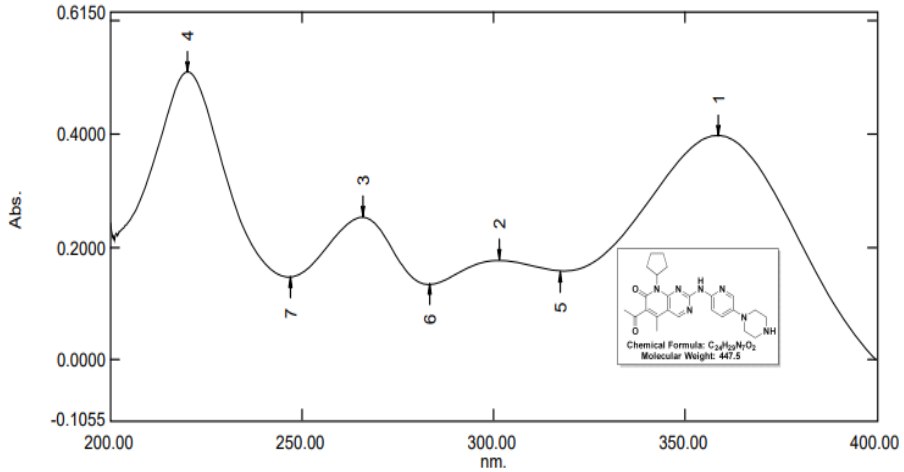

Fig. 3: Spectra (UV) of Palbociclib

Linearity:

Table 3: Linearity results for Palbociclib

Parameters	Palbociclib	Impurity-A	Impurity-B	Impurity-C	Impurity-D	Impurity-E	Impurity-F
Slope	37887	36693	35763	38878	36565	36815	33905
Intercept	224.9	217.8	898.7	-1640.7	918.9	323.4	201.2
%y-Intercept	0.24	0.24	1.01	-1.71	1.01	0.35	0.24
Correlation (R)	0.999	1.000	1.000	0.999	1.000	1.000	1.000
R^2	1.000	1.000	1.000	0.999	1.000	1.000	1.000

Precision:

Table 4: Result of Precision study

Sl.No.	Sample -1	Sample -2	Sample - 3	Sample -4	Sample - 5	Sample-6	%Average	% RSD
Impurity-A	97.6	96.8	98.2	101.5	97.5	99.1	98.5	1.7
Impurity-B	100.4	101.0	103.7	103.9	102.4	102.6	102.3	1.4
Impurity-C	96.6	97.3	96.7	97.6	99.5	99.6	97.9	1.4
Impurity-D	99.4	98.6	100.1	100.2	99.3	100.9	99.8	0.8
Impurity-E	96.7	95.4	95.8	97.2	97.5	96.6	96.5	0.9
Impurity-F	98.4	98.1	98.1	98.2	100.1	97.9	98.5	0.8

Accuracy:

Table 5: Result for Accuracy of Impurities

	% Level*	LOQ Level	100% Level	150% Level
Impurity-A	**Average %Recovery**	97.0	98.5	98.3
	% RSD	2.5	1.5	1.3
Impurity -B	**Average %Recovery**	95.1	102.3	102.7
	% RSD	3.0	2.5	1.3
Impurity -C	**Average %Recovery**	102.7	97.9	95.6
	% RSD	5.3	4.0	2.0
Impurity -D	**Average %Recovery**	96.3	98.8	96.9
	% RSD	1.1	1.5	1
Impurity -E	**Average %Recovery**	95.5	96.2	95.7
	% RSD	0.7	1.3	1.3
Impurity -F	**Average %Recovery**	95.5	98.5	95.7
	% RSD	1.7	2.0	3.0

Forced degradation:

Table 6: Forced degradation Results for Palbociclib

Name of the Solution	Stress testing		
	Total impurities (%)	% Assay	Mass Balance
Control Sample	0.28	99.5	99.8
Thermal Stress Sample (80ºC/48 hours)	0.35	100.6	101.2
Photolytic Stress Sample (1.2m Lux hrs)	0.31	99.9	100.4
Water Hydrolysis (H2O/2mL)	0.32	98.9	99.4
Base Stress Sample the (5N NaOH/2mL)	6.2	94.2	100.6
Acid Stress Sample (5N HCl/2mL)	1.5	98.9	100.6
Oxidation Stress Sample (3% H2O2/2 mL)	0.8	97.5	98.5

Exposed the sample to above condition for 5 hours * Purity Angle should be less than Purity threshold as per empower software for peak purity.

The evaluation was conducted on a range of trials involving various types of buffers, mobile phases, organic solvents, and pH levels. After careful consideration, the YMC Triart C18 column 250mm x 4.6mm with a particle size of 5µ, was found to deliver excellent results, such as better resolution and symmetrical peaks. By utilizing a phosphate buffer in combination with Acetonitrile, the sensitivity was optimized. Furthermore, the gradient played a pivotal role in the successful separation of impurities. The wavelength selection for the UV spectra of Palbociclib proved to be crucial. During validation, the developed method was found to be not only specific/selective, accurate, and precise, but also robust.

To ensure the reliability of the results, a filter study was performed using PVDF filters, and the solution stability was assessed. Both the standard solution and the sample solution of Palbociclib are stable for up to 36 hours when diluted in an acidic mixture of water and acetonitrile. Various pH mobile phases were investigated, and pH 2.1 was ultimately deemed the most suitable for the aforementioned method. The choice of wavelength was based on the UV spectra of Palbociclib. Moreover, the quantification of impurities was achieved by calculating the RF (Response Factor), necessitating the use of only Palbociclib as the standard. This approach proves highly effective for the quantification of impurities. Overall, this method offers cost-effectiveness, simplicity, and reliability.

CONCLUSION

For the Validation study, the guidelines set by the International Conference on Harmonization. The results show that the method is simple, specific, precise, accurate, and reliable. This method allows for easy analysis of Palbociclib, whether in its active pharmaceutical ingredient or dosage form. Both the assay and the test for related substances can be determined using this one method. The proposed method met ICH guidelines and showed great accuracy, linearity, precision, specificity,

limit of detection, and limit of quantification. This validated HPLC method can be suitable for regular quality control tests of Palbociclib and its impurities in pharmaceuticals.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge Scientia Qualitek® in Navi Mumbai for generously arranging the drug Palbociclib and related items. The authors also appreciate the tremendous support received from Scientia Qualitek and the opportunity to use their facilities to conduct this study.

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