ORIGINAL ARTICLE

# New stability-indicating liquid chromatographic method for determination of palbociclib (an anti-breast cancer drug)

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## Abstract

**Introduction:** A simple, sensitive stability-indicating reversed-phase high-performance liquid chromatography (RP-HPLC) method for the determination of palbociclib. Palbociclib is an anticancer drug used for the treatment of breast cancer. It is a selective inhibitor of cyclin-dependent kinases. **Materials and Methods**: Waters Model 2695 alliance HPLC system (PDA Detector) with Inertsil ODS- 3V (4.6 mm × 250 mm, 5 µm) was used for the chromatographic separation. Mobile phase consisting of ammonium acetate:acetonitrile (32:68, v/v) was delivered at a flow rate of 1.0 ml/min (detection wavelength 263 nm) on isocratic mode for the chromatographic study. **Results and Discussion**: Palbociclib obeys Beer Lambert's Law over a concentration range 5–1000 µg/ml. The limit of detection and limit of quantification are found to be 1.6378 and 4.951 µg/ml. The method was validated as per the ICH guidelines. Forced degradation studies were conducted, and the method was found to be specific. **Conclusion**: The present RP-HPLC method is simple, precise, and accurate and can be used for the routine analysis of pharmaceutical formulations.

**Key words:** Palbociclib, reversed-phase high-performance liquid chromatography, validation

## INTRODUCTION

Palbociclib [Figure 1] is a new drug used for the treatment of breast cancer. US Food and Drug Administration (FDA) has given approval for Palbociclib.[1] FDA has indicated that palbociclib has to be used along with Letrozole which is an aromatase inhibitor. Palbociclib is used in the first-line treatment for postmenopausal women with metastatic breast cancer that is estrogen receptor (ER) - positive and human epidermal growth factor receptor 2 (HER2) - negative. Palbociclib also acts as an inhibitor of cyclin-dependent kinases 4 and 6, which are involved in promoting the growth of cancer cells.[2] Addition of palbociclib to letrozole provides a novel treatment for women diagnosed with metastatic breast cancer. Dange *et al*. have established a liquid chromatographic method[3] for the simultaneous determination of palbociclib to letrozole and Song *et al*. developed a reversed-phase high-performance liquid chromatography (RP-HPLC) method for the quantification of palbociclib in capsules.[4] So far, there is not even single stability indicating liquid chromatographic method in the literature and the authors, therefore, have attempted a simple RP-HPLC method for the assay of palbociclib and validated as per the ICH guidelines.[5]

## MATERIALS AND METHODS

### Chemicals and Reagents

Palbociclib was procured from Ther Dose Pharma Pvt., Ltd., (India). Palbociclib capsules are available with brand names: IBRANCE® 125 mg; 100 mg; 75 mg; and Palbace 125 mg. All other chemicals are of AR grade, and all solvents are of HPLC grade. The analysis of palbociclib was performed using Waters 2695 alliance HPLC system with 2996PDA detector.

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### Optimized Chromatographic Conditions

Waters Model 2695 alliance HPLC system with Inertsil ODS- 3V (4.6 mm × 250 mm, 5 µm) was used for the chromatographic study. Waters 2996 Photodiode array detector was used. Chromatographic method was developed in isocratic mode with ammonium acetate with pH adjusted to 8 using TEA:acetonitrile (ACN) (38:62, v/v) was delivered with a flow rate of 1.0 ml/min and column temperature maintained at 30°C with a runtime of 30 min. The ultraviolet detection was carried out with PDA detector at 263 nm.

### Preparation of Stock Solution

25 mg of palbociclib was dissolved in 5 ml of water initially and made up to volume with ACN in a 25 ml volumetric flask and was filtered through a 0.45 µm membrane filter.

### Method Validation

The method was validated by evaluating linearity, precision, accuracy, recovery, limit of detection (LOD), limit of quantification (LOQ), system suitability, solution stability, and robustness as per the ICH Guidelines.

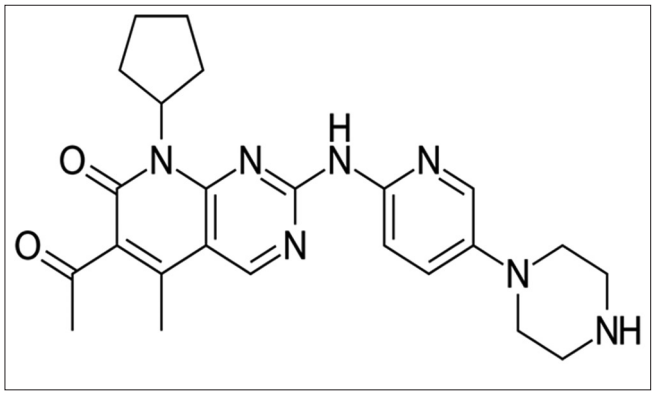


**Figure 1:** Chemical structure of Palbociclib

### Linearity, Precision, Accuracy, and Robustness Studies

Different diluted solutions of 5-1000 µg/ml were prepared from the stock solution and injected into the RP-HPLC system, and peak area of chromatogram was noted. A graph was plotted with a concentration on X-axis and mean peak area on Y-axis. Intraday and interday precision was calculated using three different concentrations on the same day and the 3 consecutive days, respectively. The accuracy of the method was proved by standard addition method, and the recovery values were determined. The robustness indicates its efficiency to remain unaffected with small and deliberate changes in the analytical procedure and provides an assurance of its analysis. The proposed method was checked for robustness by slightly changing the flow rate (± 0.1 mL), mobile phase composition (± 2%), pH (± 0.2 units), and detection wavelength (258 nm and 268 nm).

### Assay of Palbociclib in the Laboratory Prepared Formulation

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.

### Stress Degradation Studies

Forced degradation studies were performed to test the drug to withstand its properties in the applied stress conditions. Palbociclib was exposed to different stress conditions such as acidic, basic, and peroxide conditions (ICH guidelines).[6]

Acid degradation was performed by treating palbociclib drug solution with 1 ml of 5N HCl at a temperature of 80°C for 1 h in a thermostat and later the solution was neutralized with sodium hydroxide solution and diluted with mobile phase as per the requirement after filtration through 0.45 µ

**Table 1:** Highlights of present study over the previously published methods

| Mobile phase (v/v)/Detection wavelength | Column | Linearity (µg/mL) | Comments | Ref |
|---|---|---|---|---|
| Sodium dihydrogen phosphate buffer (pH 5.5):ACN: methanol (80:10:10); 254 nm | Intersil $C_8$ (4.6 mm×250 mm particle size 5 µm) | 5–50 | Simultaneous determination of palbociclib and letrozole | 3 |
| ACN: sodium acetate buffer (30:70, 0.5% TEA included); 260 nm | Kromasil C18 column (250 mm×4.6 mm, 5 µm) | 4.04–20.19 | Content determination of palbociclib | 4 |
| Ammonium acetate (pH adjusted to 8.0 with TEA: ACN (38:62); 263 nm | Inertsil ODS- 3V (4.6 mm×250 mm, 5µm) | 0.08–0.12 | Stability indicating RP-HPLC | Present method |

ACN: Acetonitrile, RP-HPLC: Reversed-phase high-performance liquid chromatography

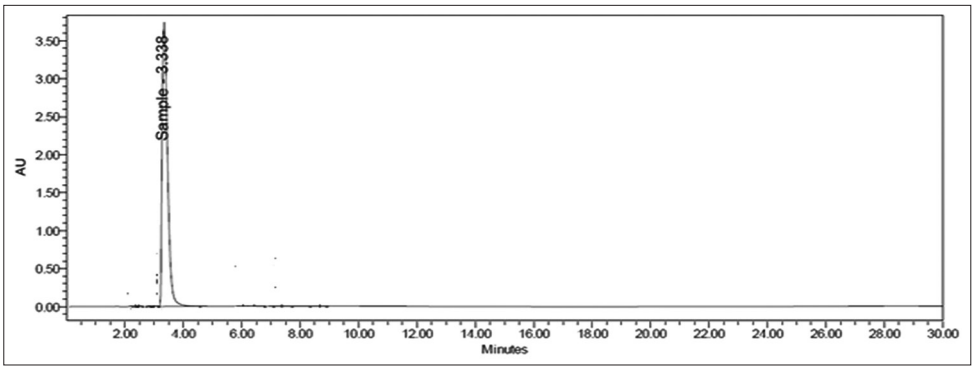


**Figure 2:** Typical chromatogram of Palbociclib (Retention time 3.338 min)

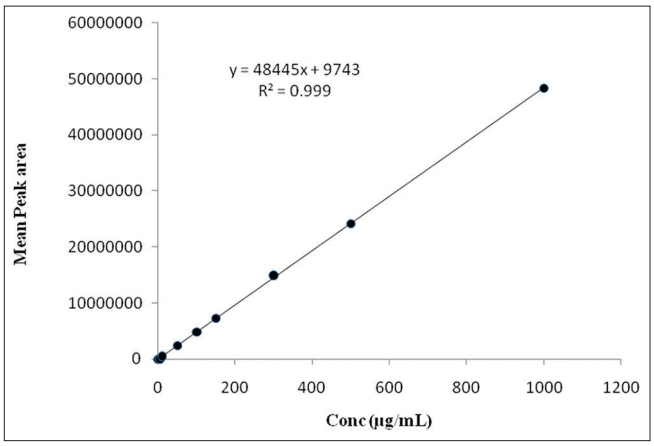


**Figure 3:** Calibration curve of Palbociclib

membrane filter. 20 µL of this solution was injected into the system, and the peak area was noted from the corresponding chromatogram.

Similarly, for base degradation, palbociclib solution was treated with 1 ml of 5N NaOH and heated on the thermostat for 80°C for 1 h and then cooled to room temperature and then neutralized with HCl and made up to volume with mobile phase and filtered through 0.45 µ membrane filter. 20 µL of this solution was injected into the system, and the peak area was noted from the corresponding chromatogram.

For peroxide degradation, 2.5 ml of drug solution was taken into a 25 ml volumetric flask and diluted with 10 ml of mobile phase. This solution was sonicated for 30 min and then treated with 5 ml of 30% hydrogen peroxide. Later, it was heated on a thermostat at 80°C for 30 min and cooled to room temperature. The resultant solution mixture was filtered through 0.45 µ membrane filter. 20 µL of this solution was injected into the system, and the peak area was noted from the corresponding chromatogram.

## RESULTS AND DISCUSSION

A simple and robust reverse-phase liquid chromatographic method has been developed, and stress studies were performed in palbociclib API in an Inertsil ODS 3V column with ammonium acetate and ACN mixture as the mobile phase. The presently proposed method was compared with the previously published methods in the literature in Table 1.

**Table 2:** Optimized conditions for determination of Palbociclib

| Parameter | Optimized chromatographic conditions |
|---|---|
| Mobile phase | Ammonium Acetate and ACN (32: 68 v/v) |
| Stationary phase | Inertsil ODS 3Vcolumn (250 mm×4.6 mm i.d., 5 µm particle size) |
| Flow rate | 1.0 mL/min |
| Detection range | 263 nm |
| Column temperature | (30°±2°C) |
| Injection volume | 20 µL |
| Detector | Waters 2996 photodiode array detector |
| Elution | Isocratic mode |
| Total runtime | 30 min |
| Retention time | 3.338±0.25 min |

ACN: Acetonitrile

### Method Development and Optimization

RP-HPLC system was initially optimized using Inertsil ODS 3V column. A mixture of with ammonium acetate and ACN (38: 62, v/v) as the mobile phase (Flow rate 1.0 mL/min) was found to be more appropriate to satisfy the system suitability parameters. The optimized chromatographic conditions were summarized in Table 2. Palbociclib was eluted as a sharp peak at 3.338 ± 0.05 min [Figure 2].

### Method Validation

The proposed method was validated by linearity, precision, accuracy, and robustness as per the ICH guidelines. The

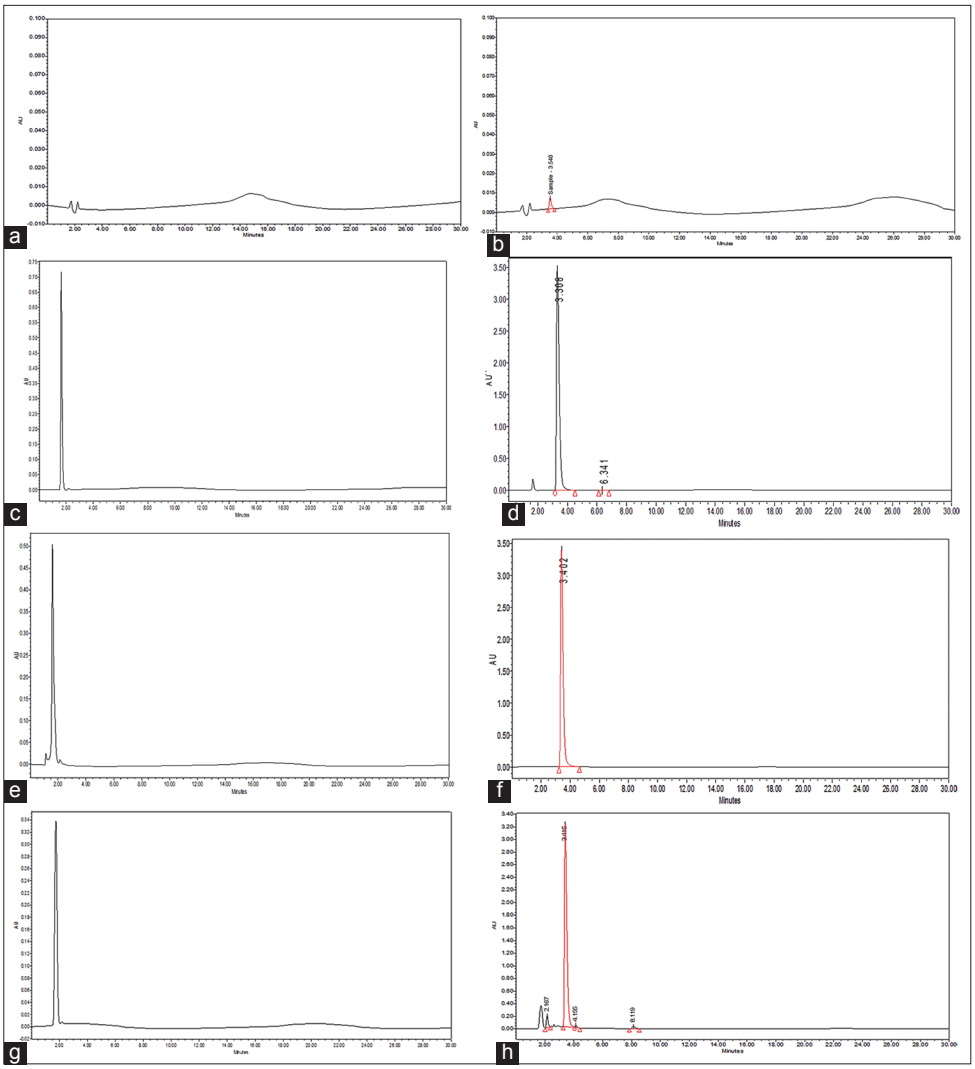


**Figure 4:** Typical chromatograms in presence and absence of palbociclib. (a) Blank, (b) palbociclib standard, (c) acid degradation blank, (d) acid degradation of palbociclib, (e) base degradation blank, (f) base degradation of Palbociclib, (h) peroxide degradation blank (Oxidation), and (i) peroxide degradation of Palbociclib

**Table 3:** Linearity of Palbociclib

| Conc.(µg/mL) | *Mean peak area | % RSD |
|---|---|---|
| 5 | 248231 | 0.32 |
| 10 | 483652 | 0.41 |
| 50 | 2419342 | 0.64 |
| 100 | 4830821 | 0.28 |
| 150 | 7261624 | 0.53 |
| 300 | 14992448 | 0.31 |
| 500 | 24187428 | 0.11 |
| 1000 | 48349042 | 0.24 |

*mean of three replicates. RSD: Relative standard deviation

calibration curve was drawn by taking a concentration of palbociclib on X-axis and the corresponding mean peak area values on the Y-axis. Palbociclib obeys Beer-Lambert's law over the concentration range 5–1000 µg/mL [Table 3] with linear regression equation y = 48445x + 9743 (correlation coefficient 0.999 [Figure 3]. The LOD and LOQ are found to be 1.6378 µg/mL and 4.951 µg/mL, respectively.

Intraday and interday precision was studied using three different concentrations of palbociclib on the same day and 3 consecutive days, respectively, and the percentage relative standard deviation (RSD) was found to be 0.21–0.42 and 0.39–0.98, respectively (<2.0%), demonstrating that the method is precise [Tables 4 and 5]. The accuracy of the method was proved by the standard addition method, and the recovery values were determined [Table 6]. The percentage RSD was found to be 0.32–0.83 (<2.0%) with a recovery of 98.51–98.72%. The percentage RSD was found to be 0.41–1.02 (<2.0%) in robustness study. The system suitability and solution stability were evaluated, and the percentage RSD was <2%.

## Stress Degradation Studies

Palbociclib was exposed to various stress conditions such as acidic, oxidative, and alkaline hydrolysis. In acidic hydrolysis,

**Table 4:** Intraday precision study of Palbociclib

| Conc. (µg/mL) | *Mean peak area | Statistical Analysis *Mean±SD (% RSD) |
|---|---|---|
| 50 | 2419342 | 2417998±7737.59 (0.32) |
| 50 | 2417354 | |
| 50 | 2417298 | |
| 100 | 4830821 | 4832965.67±10149.23 (0.21) |
| 100 | 4835624 | |
| 100 | 4832452 | |
| 150 | 7261624 | 7264438.67±30510.64 (0.42) |
| 150 | 7268938 | |
| 150 | 7262754 | |

*mean of three replicates. RSD: Relative standard deviation, SD: Standard deviation

**Table 5:** Interday precision study of Palbociclib

| Conc. (µg/mL) | *Mean peak area | | | *Mean±SD (% RSD) |
|---|---|---|---|---|
| | Day 1 | Day 2 | Day 3 | |
| 50 | 2417423 | 2414619 | 2406472 | 2412838±23645.81 (0.98) |
| 100 | 4832907 | 4836721 | 4836392 | 4835340±18857.83 (0.39) |
| 150 | 7264621 | 7266024 | 7263841 | 7264828.67±55212.70 (0.76) |

*mean of three replicates. RSD: Relative standard deviation, SD: Standard deviation

**Table 6:** Accuracy study of Palbociclib

| Laboratory prepared formulation (µg/mL) | LM-02/05 | Total conc. (µg/mL) | *Recovery (%) | % RSD* |
|---|---|---|---|---|
| 50 | 25 | 75 | 98.65 | 0.32 |
| 50 | 25 | 75 | | |
| 50 | 25 | 75 | | |
| 50 | 50 | 100 | 98.72 | 0.59 |
| 50 | 50 | 100 | | |
| 50 | 50 | 100 | | |
| 50 | 75 | 125 | 98.51 | 0.83 |
| 50 | 75 | 125 | | |
| 50 | 75 | 125 | | |

*mean of three replicates. RSD: Relative standard deviation

**Table 7:** Stress degradation studies of palbociclib

| Stress condition Medium/temp./duration | Rt (min) | Area | % Recovery | % Drug degradation | Theoretical plates | Tailing factor |
|---|---|---|---|---|---|---|
| Standard drug | 3.540 | 48349042 | 100 | - | 25192.518 | 1.363 |
| Acidic hydrolysis 5N HCl/80°C/60 min | 3.308 | 45496162 | 94.10 | 5.67 | 28758.607 | 1.281 |
| Alkaline hydrolysis 5N NaOH/80°C/60 min | 3.402 | 41496024 | 85.83 | 13.97 | 25843.469 | 1.365 |
| Oxidative 30%$H_2O_2$/70°C/1 h | 3.415<br>2.167<br>4.155<br>8.119 | 36745465 | 76.18 | 23.82 | 26795.321 | 1.342 |

palbociclib was eluted at 3.308 min. About 5.67% of degradation was observed, and while performing alkaline hydrolysis, palbociclib was eluted at 3.402 min, and 13.97% degradation was observed. During oxidation, palbociclib was eluted at 3.415 min with some other degradants observed at 2.167, 4.155, and 8.119 min (drug degradation 23.82%). In all the degradation studies, it was found that the palbociclib peak was well separated among the degradants indicating that the method is selective and specific. The system suitability parameters were well in the acceptance criteria [Table 7]. The theoretical plates are above 25000 (Acceptance criteria > 2000) and tailing factor is <1.5. Figure 4 shows the typical chromatograms obtained in absence and presence of palbociclib during stress degradation study.

### 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 indicting 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.

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## 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 cyclin-dependent 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.

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