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Original Article

# QbD Driven Method Development and Validation of Palbociclib by LC-MS/MS

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## ABSTRACT

**Background:** Palbociclib, an oral active cyclin dependent kinase inhibitor, is used in the treatment of metastatic breast cancer with narrow index and variable pharmacokinetics, hence the need of sensitive optimized analytical approach with LC-MS/MS offering superior detection with QbD approach is innovative strategy. **Aim:** The present study investigation involves the development and validate a method for quantification of Palbociclib in bulk and solid dosage form using a QbD-driven approach. **Materials and Methods:** The method was optimized according to ICH guidelines and performed on a quadrapole triple mass spectrometer with positive reaction mode. **Results:** The UPLC-hyphenated MS method was simple, reproducible, and accurate, with inter and intra-day precision results less than 15% and analyte accuracy (96-102%). The extraction recovery rate was 85-102%, and the linearity over the concentration range was 0.0106 µg/mL and 0.0321 µg/mL, respectively compared to earlier methods. Also, there was less matrix interference with simple sample extraction. **Conclusion:** The results reveal the method developed and validated revealed sensitivity, linearity, accuracy, precision, selectivity and rapid analysis in less run time (<10 min) which can be applied in Quality control labs as per ICH guidelines as done by the QbD method.

**Keywords:** Analytical method development, ICH, LC-MS/MS, Metastatic Breast Cancer, NSCLC, Palbociclib, Quality control.

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## INTRODUCTION

Oral Cyclin-Dependent Kinase 4 and 6 (CDK4/6) reversible inhibitors like Palbociclib (PCB) are frequently used to treat breast cancer approved by US-FDA in 2015 under the brand name of Ibrance. Through phosphorylating the Retinoblastoma Protein (Rb), it inhibits the tumor-suppressor action of Rb. CDK4/6 and cyclin D are necessary for the cell cycle to transition from the G1 to the S phase.[1,2] This oral CDK4/6 inhibitor is quite potent and shows promise as an anticancer agent, particularly against the ERα+ subtype of breast cancer. The medication took a median of 4-6 hr to achieve $C_{max}$, and research backs up the liver's metabolism.[2] Oxidation and sulfonation were the main principal metabolic routes, with acylation and glucuronidation playing a minor role. The term "Quality by design" (QbD) describes the attainment of a specific, predictable quality with intended and specified parameters.[3] Achieving sensitivity and specificity, minimizing matrix effects through sample preparation, and guaranteeing precise quantification-particularly in intricate biological matrices like plasma-are some of the difficulties that the LC-MS analysis of PCB, a CDK4/6 inhibitor, faces. For accurate detection and quantification, very sensitive LC-MS techniques are needed for biological samples containing low quantities of PCB. The sensitivity and accuracy of the analysis may be impacted by matrix effects. Accurate quantification depends on specificity. Protein precipitation, Solid Phase Extraction (SPE), and internal standards are all part of sample preparation. Although stable isotope-labeled internal standards can increase precision and accuracy, they can be costly and challenging to acquire. Reliable therapeutic drug monitoring and other clinical uses depend on quantification. Palbociclib concentrations in the samples should be covered by the LC-MS method's adequate linear range. Palbociclib must be stable in the sample matrix and throughout storage, therefore stability is also essential. Separating the parent drug from its metabolites, identifying drug interactions, and getting around financial and schedule limitations are other factors to take into account. Advanced LC-MS approaches,

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such multi-omics analysis, are necessary to discover changed metabolic pathways and drug targets in order to comprehend the processes of PCB resistance. Because a quality-by-design approach places more of an emphasis on risk assessment and management than does a standard or conventional approach help to develop that results in a method that is more robust or tough. Understanding dependent variables, different factors, and their interaction effects through a desired series of tests on the responses to be examined is a crucial part of QbD.[4] Information about PLB metabolite investigations can be found in the literature. According to literature reports, the most commonly used equipment is LC-MS with a triple quadrupole mass spectrometer; MS/MS is usually used for increased specificity.[5-13] Use of LC-MS/MS for PCB measurement, utilizing protein precipitation, C18/biphenyl columns, and triple-quadrupole detection are reported. These methods have achieved high sensitivity, accuracy, and precision, which have utility in preclinical and clinical settings. Innovations include solid-phase extraction cleaning and multi-drug panels for therapeutic medication monitoring. Tissue stability is crucial for technique robustness.[8] Because it is believed to be a potential alternative to animal testing, the use of very high sensitivity MS for micro-dosing research is currently generating a lot of interest.[9,10] The goal of the current study is to create a straightforward, sensitive, specific, selective, quick, and validated UPLC-ESI-MS/MS method for estimating the dosage of PCB in tablets and bulk.[11]

## MATERIALS AND METHODS

### Instruments and Software Used

ELICO SL 210 UV-vis spectrophotometer with two sample holders was employed. The matched quartz cells were used for all absorbance measurements. Analytical balance is used to weigh the sample accurately. Waters Acquity SDS Ultra Performance LC system (Waters, India) coupled with MASS spectrometer Mass Lynx version 4.1 SCN805 was used. Design expert 13.0 version was applied for optimization of method development trials.

### Standards and Reagents

Palbociclib reference standard was obtained as a gift sample from Hetero labs Hyderabad. The capsules of standard drug Palbociclib (Brand-Palbace) of 125 mg were procured from Hetero drugs, Hyderabad, Telangana. The drug belongs to BCS class I with extreme low water solubility instead methanol was used to dissolve the drug throughout the experiment. Water, Acetonitrile, formic acid was of HPLC grade. Chemicals used in the study were of highest analytical grade.[12,13]

### UV-vis spectrometric analysis

#### *Standard stock solution of Palbociclib*

10 mg of the PCB Figure 1A was accurately weighed and transferred to 10 mL volumetric flask. Small amount of methanol was added to dissolve and made upto the volume with the same (1000 µg/mL). 1 mL sample solution is pipette out from above and made for transfer to a 10 mL volumetric flask,[7,8] volume was adjusted to the mark with same solvent to obtain strength100 µg/mL. The dilutions were further made from the stock solutions to make 10 µg/mL solution and was scanned by a UV-vis spectrophotometer in range of 180-380 nm to establish the $\lambda_{max}$ and thereby determining the linearity as shown in Figure 1B.[14,15]

### Method Validation

The method was validated based on the protocol furnished in ICH $Q^2$ ($R^1$) to determine range, precision, accuracy, robustness, linearity, ruggedness, Detection Limit (LOD) and Limit of Quantification (LOQ).

### Precision

The drug Palbociclib (10 µg/mL) was analyzed six times for intra-day precision in order to examine intra-day and inter-day precision. The results are shown in Table 3. As a general rule, inter-day precision is measured using the same concentration solution while simultaneously altering the temperature, analyst, and equipment. The precision data in Table 3 shows the intra- and inter-day variation in the parameters.

### Study of Linearity and Range

Appropriate aliquots of samples from the stock solution were used to provide a range of values between 1 and 12 µg/mL Figure 1C. The absorbance of these solutions was measured at 220 nm after the volume was adjusted with diluent, and a calibration curve showing absorbance vs. concentration was plotted Figure 1.[11] According to reports, the linearity-optimized technique was obtained between 1 and 12 µg/mL. Concentrations in the range of 1-12 µg/mL Figure 1C were prepared taking appropriate aliquots of samples from the stock solution. Adjusted the volume to the mark with diluent and the absorbance of these solutions were recorded at 220 nm and calibration curve was plotted, absorbance vs. concentration Figure 1C. The method optimized for linearity was reported to be within the range limits of 1-12 µg/mL.[12]

### Detection and quantification limits

By applying the slope technique and standard deviation of response as specified by ICH recommendations, the LOD and LOQ of palbociclib were ascertained to be 0.8977 and 0.2925, respectively.[10]

### Accuracy

Accuracy was calculated three different levels i.e.50%, 100%and150% as triplicate experiment Table 4. Recovery studies were carried out by adding known amount of standard solution to the pre-analyzed sample solution as seen in Table 6.[9] The% recovery was then calculated by using Equation 1:

$$\% \text{ Recovery} = \frac{\text{Total amount of drug found} - \text{Amount of drug found on pre} - \text{analyzed basis}}{\text{Amount of pure drug added}} \quad ..(1)$$

## Robustness

Three aliquots of 10ppm test solution were prepared and scanned at a wavelength of ±1 nm of $\lambda_{max}$. The absorbance values were tabulated in Table 4.

## Ruggedness

The degree of reproducibility of test results obtained was given in the Table 5. It was carried out by two different analysts on different days to check the reproducibility which indicates the method developed is rugged.[16]

## Analytical Quality by Design

For the successful application of analytical QbD principles, a suitable, step-by-step methodology has been developed. In the first step all the requirements for the method like dilution process, sonication time, slit width, scan speed, mobile phase ratio, pH of the mobile phase, runtime, flow rate of mobile phase were considered. The variables that were taken into consideration that directly affect the peak's retention duration were primarily the mobile phase ratio, the mobile phase's pH, and the flow rate. The experiment was created to establish x and y correlations in the presence of noise z in order to mitigate the risk involved. Of the above discussed requirements pH of mobile phase along with its flow rate were chosen as variables that influence retention time and the responses were recorded in the Table 1. The experiments were performed as 3,7. 11 runs using full factorial design of the

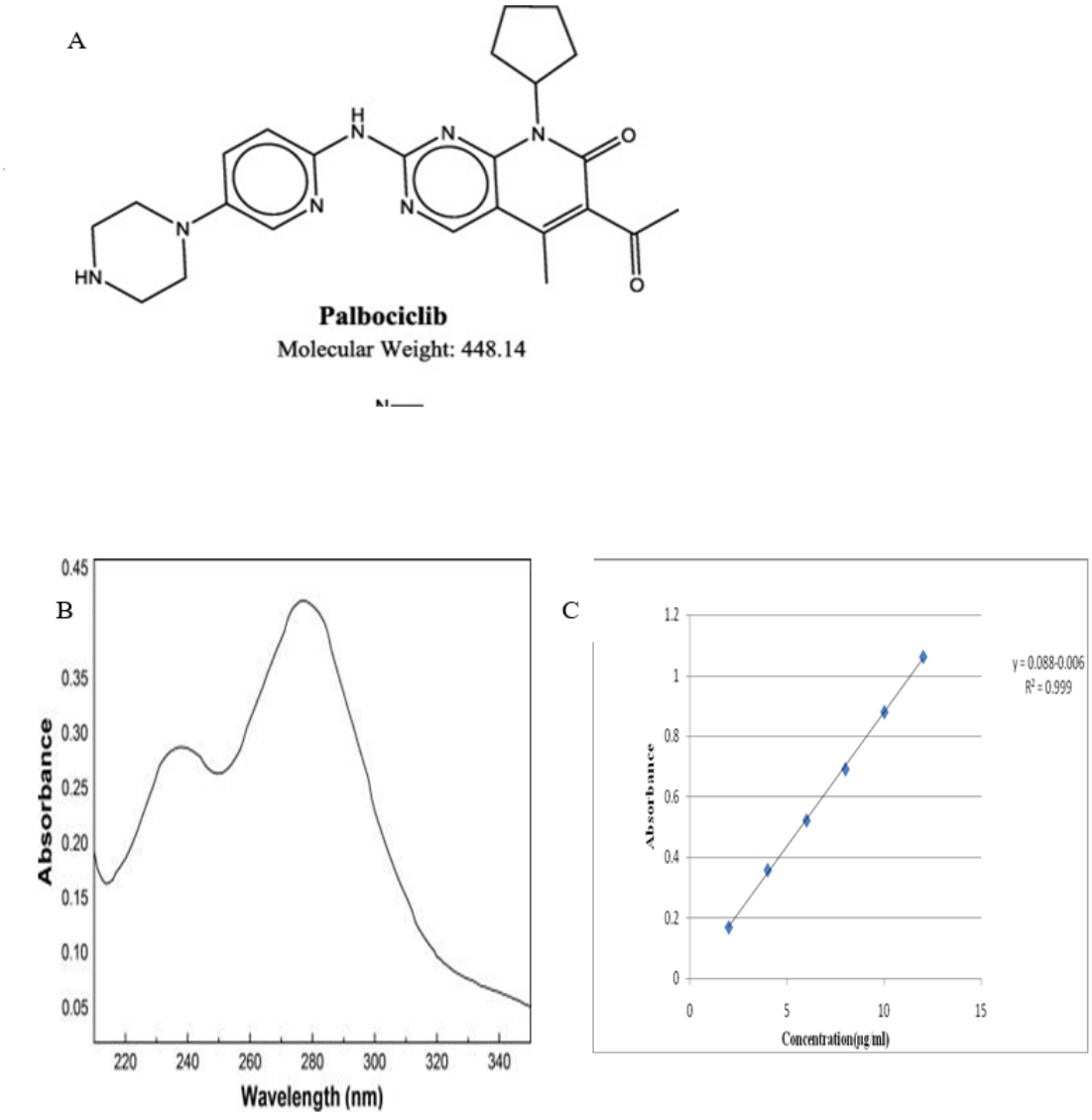



**Figure 1:** A) Structure of Palbociclib B) UV-vis Spectrum showing Palbociclib in methanol C) Calibration Curve of Palbociclib at 220 nm.

**Table 1:** Illustrating impact of factor x and y on the retention time response.

| Std | Run | Factor 1 A:pH 4.5 | Factor 2 B:Flow rate (mL/min) | Response Retention time (Min) |
|---|---|---|---|---|
| 3 | 1 | 55 | 1 | 3.5 |
| 11 | 2 | 60 | 0.75 | 4 |
| 6 | 3 | 67.0711 | 0.75 | 3.56 |
| 8 | 4 | 60 | 1.10355 | 4.5 |
| 5 | 5 | 52.9289 | 0.75 | 3.5 |
| 2 | 6 | 65 | 0.5 | 3.5 |
| 9 | 7 | 60 | 0.75 | 3.3 |
| 10 | 8 | 60 | 0.75 | 3.1 |
| 7 | 9 | 60 | 0.396447 | 3.4 |
| 1 | 10 | 55 | 0.5 | 3.2 |
| 4 | 11 | 65 | 1 | 4 |

**Table 2:** Application of the developed LC-MS/MS method for estimation in capsule.

| Sl. No. of each capsule | Weight of the capsule (mg) |
|---|---|
| 1. | 120.1 |
| 2. | 120.5 |
| 3. | 120.6 |
| 4. | 120.2 |
| 5. | 120.4 |
| 6. | 120.0 |
| 7. | 120.9 |
| 8. | 120.6 |
| 9. | 120.9 |
| 10. | 120.8 |
| Average | 120.5 |

standard designs, and ANOVA for Linear model was calculated for response of Retention time. An F-value attributable to high range has an 8.18% possibility of being the result of noise, according to the Model F-value of 3.48. P-values indicating significance was found to be less than 0.05, indicating optimized and accuracy of method with the model terms.[3,7]

Furthermore, case B is a noteworthy approach having these attributes. Insignificant qualities are indicated by values greater than 0.1. Therefore, the report of model reduction may improve the model as illustrated in Figures 2A and 2B, when the approach is seen with more minor model words (not justified as per hierarchy). In comparison to the pure mistake, the Lack of Fit is not substantial, according to the Lack of Fit F-value of 0.35. A significant Lack of Fit F-value has an 86.80% probability of being caused by noise. We want the model to fit, thus a non-significant lack of fit is ideal. The difference observed is more than 0.2, meaning that the predicted value of $R^2$ (-0.0869) is far from the adjusted value of $R^2$ (0.3315), which is close to the usual expected value. This could point to a significant block effect or a potential issue with your data and/or model. Factors like the reduction model, response, response transformation, response outliers, etc. that are pertinent to the model optimization parameters. Confirmation runs should most likely be used for the analysis of tested empirical models. The signal/noise ratio should have sufficient precision measurements. It is determined that a signal/noise ratio greater than 4 is ideal. It is suggested that the 4.729 significant ratio indicates a sufficient signal. This important model is a simple approximation that can be used to navigate the space design. It was discovered that the retention times were +3.60, +0.1106 A, and +0.2945 B. It is possible to estimate the equation in terms of coded factors for responses to suggested levels related to each factor. By default, the components' high and low levels are denoted by the numbers +1 and -1, respectively. By comparing the factor coefficients, the coded equation can be used to determine the elements' respective impacts.[13] The model fits statistics show moderate accuracy and precision in predicting the response variable. The standard deviation is 0.3373, with a mean response value of 3.60. The coefficient of variation is 9.38%, indicating acceptable variability. The coefficient of determination is 0.4652, explaining 46.5% of the response volatility. The adjusted $R^2$ score is 0.3315, indicating a moderate decline in model efficacy as seen in Figures 2A and 2B. The anticipated $R^2$ value is significantly lower (0.0879), indicating restricted predictive capability on novel data. Despite this, the precision ratio is 4.7286, exceeding the acceptable threshold, indicating the model's suitability for design space.

## LC-MS/MS Analysis

### *Sample preparation for Method Development*

A 100 mL volumetric flask, methanol, and 10 mg of palbociclib API were used in the first solution. 1000 µL of the first solution was pipetted into a 100 mL volumetric flask to create the second solution. Pipetting 50 µL, 25 µL, 20 µL, 15 µL, 10 µL, 5 µL, and 2.5 µL into a 100 mL volumetric flask allowed for the preparation of a range of concentrations.

## Application of developed method

The developed UPLC-MS/MS method Table 1 was applied to determine PCB concentrations at steady state in API and formulation were accurately recorded the sampling time points.[6,19]

## Preparation of Solution

Initially 120.5 mg of powdered granules was transferred into a 100 mL volumetric flask, added suitable quantity of methanol to dissolve, and made up to the volume with the same to give a solution having a concentration of 1000 µg/mL as seen in Table 2. From the above solution 1000 µL was pipetted and transferred into a 100 mL volumetric flask to give a solution having a concentration of 1000 ng/mL. Then series of solutions of 500 ng/mL, 250 ng/mL, 200 ng/mL, 150 ng/mL, 100 ng/mL and 50 ng/mL were prepared by pipetting 50 µL, 25 µL, 20 µL, 15 µL, 10 µL and 5 µL into a 100 mL volumetric flask respectively.

## Validation of Mass Spectrophotometric Analysis Method

### *Specificity and recovery*

A single blank sample was injected to make sure no interfering chemicals were present. Selectivity was assessed by contrasting the chromatograms from the spiked peaks and blank. PCB had a retention time of almost 1 min. The retention periods of the analytes and the blank samples showed no discernible

**Table 3: Accuracy Data of UV-vis spectroscopic analysis and Precision data of Palbociclib at Lower as well as optimum concentrations.**

| Measured absorbance in triplicate of Accuracy level | Sample(bulk +formulation) | Observed Absorbance | % of spiked API that has been Recovered | %RSD |
|---|---|---|---|---|
| 50% | 2.50+5.0 | 0.5577 | 99.36 | 1.06% |
| | 2.50+5.0 | 0.5497 | 98.76 | |
| | 2.50+5.0 | 0.5612 | 99.65 | |
| 100% | 5.0+5.0 | 0.7734 | 98.05 | 0.903% |
| | 5.0+5.0 | 0.7963 | 98.42 | |
| | 5.0+5.0 | 0.7862 | 98.53 | |
| 150% | 7.50+5.0 | 1.0288 | 98.66 | 0.914% |
| | 7.50+5.0 | 1.0269 | 98.28 | |
| | 7.50+5.0 | 1.0193 | 96.86 | |
| Method Precision | | | Intermediate Precision | |
| | Area | RT | Area | RT |
| At LOQ level | 168 | 1.01 | 167 | 1.01 |
| | 167 | 1.01 | 168 | 1.01 |
| | 167 | 1.00 | 167 | 1.01 |
| | 168 | 1.01 | 168 | 1.00 |
| | 167 | 1.00 | 168 | 1.00 |
| | 168 | 1.00 | 168 | 1.00 |
| Average | 167.5 | 1.005 | 167.6 | 1.005 |
| Standard Deviation | 0.5477 | 0.0054 | 0.5163 | 0.0054 |
| Percentage Relative Standard Deviation | 0.33 | 0.54 | 0.31 | 0.54 |
| At 100% level | 1749 | 1.00 | 1748 | 1.01 |
| | 1748 | 1.01 | 1747 | 1 |
| | 1750 | 1.01 | 1749 | 1.01 |
| | 1748 | 1.01 | 1748 | 1.01 |
| | 1749 | 1.00 | 1747 | 1 |
| | 1748 | 1.01 | 1748 | 1 |
| Average | 1748.6 | 1.006 | 1747.833 | 1 |
| Standard Deviation | 0.8164 | 0.0051 | 0.7527 | 0.0054 |
| Percentage Relative Standard Deviation | 0.05 | 0.51 | 0.04 | 0.55 |

**Table 4: Precision and Robustness Data of UV-vis spectroscopic analysis.**

| Sl. No. | Concentration(µg/mL) | Absorbance Values | |
|---|---|---|---|
| 1. | 10.0 | 0.8842 | |
| 2. | 10.0 | 0.8785 | |
| 3. | 10.0 | 0.8835 | |
| 4. | 10.0 | 0.8617 | |
| 5. | 10.0 | 0.8822 | |
| 6. | 10.0 | 0.8775 | |
| %RSD | | 0.89% | |
| **Robustness** | | | |
| **Sl. No.** | **Concentration(µg/mL)** | **219 nm** | **221 nm** |
| 1. | 10 | 0.8512 | 0.8825 |
| 2. | 10 | 0.8493 | 0.8798 |
| 3. | 10 | 0.8515 | 0.8826 |
| %RSD | | 0.12% | 0.16% |

**Table 5: Ruggedness Data of UV-vis spectroscopic analysis and Linearity Data of LC Method.**

| Ruggedness | | | | |
|---|---|---|---|---|
| Day1 | | | Day2 | |
| **Analyst1** | | **Analyst2** | **Analyst1** | **Analyst2** |
| Concentration(µg/mL) | Absorbance | Absorbance | Absorbance | Absorbance |
| 10 | 0.8832 | 0.8568 | 0.8793 | 0.8642 |
| 10 | 0.8795 | 0.8493 | 0.8548 | 0.8756 |
| 10 | 0.8815 | 0.8536 | 0.8675 | 0.8845 |
| %RSD | 0.17% | 0.13% | 0.14% | 0.15% |
| **Linearity** | | | | |
| **Name of the API** | **Sample name** | **Conc(ng/mL)** | **Retention time(min)** | **Peak Area** |
| Palbociclib | CC-1 | 1.0 | 1.00 | 168 |
| | CC-2 | 2.5 | 1.01 | 364 |
| | CC-3 | 5.0 | 1.00 | 875 |
| | CC-4 | 7.5 | 1.00 | 1300 |
| | CC-5 | 10.0 | 1.00 | 1749 |
| | CC-6 | 12.5 | 1.00 | 2245 |
| | CC-7 | 15.0 | 1.00 | 2347 |

interference. Every stage of recovery involved an assay of the test samples ($n$=6) at different concentration levels.

## Precision, Linearity, LLOQ and Accuracy

A range of concentrations, from 1.0 to 500 ng/mL, were created. 100% concentration and precision at the LOQ level were prepared for injection into the HPLC. To verify the method's linearity, a series of concentrations were made and injected (Table 3). Three medication product concentration levels-50%, 100%, and 150%-are used to assess accuracy. 1 mL of 25 ng/mL PCB API solution and 1 mL of 50 ng/mL drug product solution were mixed evenly to test the method's accuracy at 50%. For 100% level 1 mL from 50 ng/mL of PCB API solution and 1 mL of 50 ng/mL of drug product solution were mixed uniformly. To check the recovery at 150% level, 1 mL from 75 ng/mL of palbociclib API solution and 1 mL of 50 ng/mL of PCB solution were mixed well and injected into the HPLC system after the same has been properly equilibrated with the mobile phase.[17,18]

Robustness Slight variation in the mobile phase B composition (±5%) was checked by injecting six times of palbociclib API solution having a concentration of 100 ng/mL.

**Table 6: Accuracy level of Spiking at various levels.**

| %Level of spiking | Drug substance (ng/mL) | Drug Product (µg/mL) | Obtained Peak Area | %Recovery | Average<br>Standard deviation<br>%RSD |
|---|---|---|---|---|---|
| 50 | 25 | 50 | 1231 | 101.4 | 99.7 |
| | 25 | 50 | 1235 | 98.6 | 1.5 |
| | 25 | 50 | 1237 | 99.2 | 1.46 |
| 100 | 50 | 50 | 1747 | 99.7 | 100.0 |
| | 50 | 50 | 1751 | 100.1 | 0.3 |
| | 50 | 50 | 1752 | 100.3 | 0.35 |
| 150 | 75 | 50 | 2152 | 98.2 | 99.0 |
| | 75 | 50 | 2164 | 99.2 | 0.7 |
| | 75 | 50 | 2168 | 99.6 | 0.71 |

## RESULTS

### UV-vis Spectroscopic analysis

Palbociclib in its bulk and oral dose form was determined using the UV-vis Spectrophotometric method Figure 3C, which was verified and shown to be linear Table 3, fast, sensitive, accurate, exact, and repeatable with a satisfactory correlation coefficient. Palbociclib in bulk and dosage form can be successfully estimated using the developed spectrophotometric method.[19,20] It is discovered that the relative standard deviation is substantially within the bounds. With an RSD between 0.9 and 1.03% for 50 to 150%, the accuracy was determined to be statistically significant. With an RSD of 0.89%, the precision was confirmed in the concentration range of 10-50 µg/mL Table 5. At 219 and 221 nm, the robustness Table 4 was confirmed with an RSD of 0.12-0.16%. After two days of calculations using two analytes, the ruggedness Table 5 was determined to have an RSD between 0.13 and 0.17% (Table 6).

At a starting range of 55%, a difference between the composition of mobile phase B (0.1%) formic acid and its matching mobile phase A (45%) was found. Since $p$ (<0.05) is regarded as significant, it was not discovered throughout the experimental runs Table 7. A diagonal line in the factorial design indicates that a flow rate of less than 0.2 mL/min can be used to optimize the process. The red-colored design point in Figure 2B below denotes ideal circumstances for method development.

### Optimized UPLC chromatographic conditions

The analysis was conducted using the Waters Acquity Ultra Performance LC system (Waters, India). Using an X-terra column (2.1 mm × 100 mm, 3.5 µm, Sigma Aldrich, India), the chromatographic separation was achieved. With a gradient mode of elution, binary gradient separation consists of A (acetonitrile as organic phase) and B (0.1% formic acid as aqueous phase). During the 3-min run, the mobile phase flow was kept at 0.2 mL/min. The auto-sampler temperature was set to 10°C, while the column temperature was set to not exceed 30°C with a fluctuation of ±3.0°C. The mass spectrometer Mass Lynxversion4.1SCN805 (Waters, India) was connected to the UPLC system. The Electrospray Ionization (ESI-positive ion ES+) source was used to run the mass spectrometer in MRM mode. By injecting a 1 ng/mL concentration under gradient elution in the appropriate mobile phase ratio, the mass spectrum of the standards was produced. To comprehend the precursor and product ions, the Palbociclib sample was examined utilizing the Mass Scan and Daughter Scan functions as in Figure 3A.

The PCB peaks, which formed a transition in MRM mode, were discovered at 448.20 m/z and 380.08 m/z. Over the course of several injections, the Collision Energy (CE) was gradually optimized. With a 5 V gap between injections, the mode was run between 10 and 30 V, as in Figures 3B and 3C. The strongest signal for the channel occurred at 30 V after the maximum point was attained. The ideal cone voltages were at 30 V, while the ion source and desolvation temperatures were kept at 150°C and 500°C, respectively. Mass Lynx software was utilized for the acquisition and processing of the data Figures 4A-4D.

### UPLC coupled Mass spectrometric analysis

#### *Specificity*

The UPLC system was stabilized for 10 min for smooth injections, and no peak was observed when a blank sample was injected.

### Limit of Detection along with Limit of Quantification

The analytical method's sensitivity was evaluated using the Limit of Detection (LOD) and Limit of Quantification (LOQ).[15-20] The LOD was determined using calibration curve parameters and a specific formula, indicating excellent sensitivity for detecting and quantifying extremely low analyte concentrations as seen in Equations 2 and 3.

$$LOD = 3.3 \times \frac{\text{Standard Deviation}}{\text{Slope}} = (3.3 * 0.5477)/170.415 = 0.0106 \text{ ng/mL} \ldots (2)$$

$$LOQ = 10 \times \frac{\text{Standard Deviation}}{\text{Slope}} = (10 * 0.5477)/170.415 = 0.0321 \text{ ng/mL} \ldots (3)$$

## Range and Accuracy

From the obtained Linearity data Table 5 the current method detects and quantifies palbociclib within the linearity range of 0.0321 ng/mL-150 ng/mL, as validated according to ICH $Q^2$ RH guidelines and within the limits.

## Robustness

The difference in the composition of the mobile phase was seen in six replicate injections of the standard (25, 75, 100% level), as in Figures 5A-5E. The gradient program's mobile phase composition was changed at the 3rd and 13th min, and the sample was run with no discernible changes in peak area or retention duration Table 3.

## DISCUSSION

The LC- MS/MS method developed for Palbociclib has demonstrated a significant sensitivity over the reported analytical technique. Few studies have employed either HPLC or LCMS individually often reporting long run time such as 15- 25 min, less sensitivity with solvent effects. The present method achieved a prominent chromatographic separation in less than 10 min thereby enhanced separation with decreased operational costs. Earlier reported methods have LLOQ values generally in the range of 2-10 ng/mL compared to present method with marked LLOQ of 1 ng/mL and lower LQC valued 0.0321 ng/mL with a high detection in compliance with ICH and EMA requirements

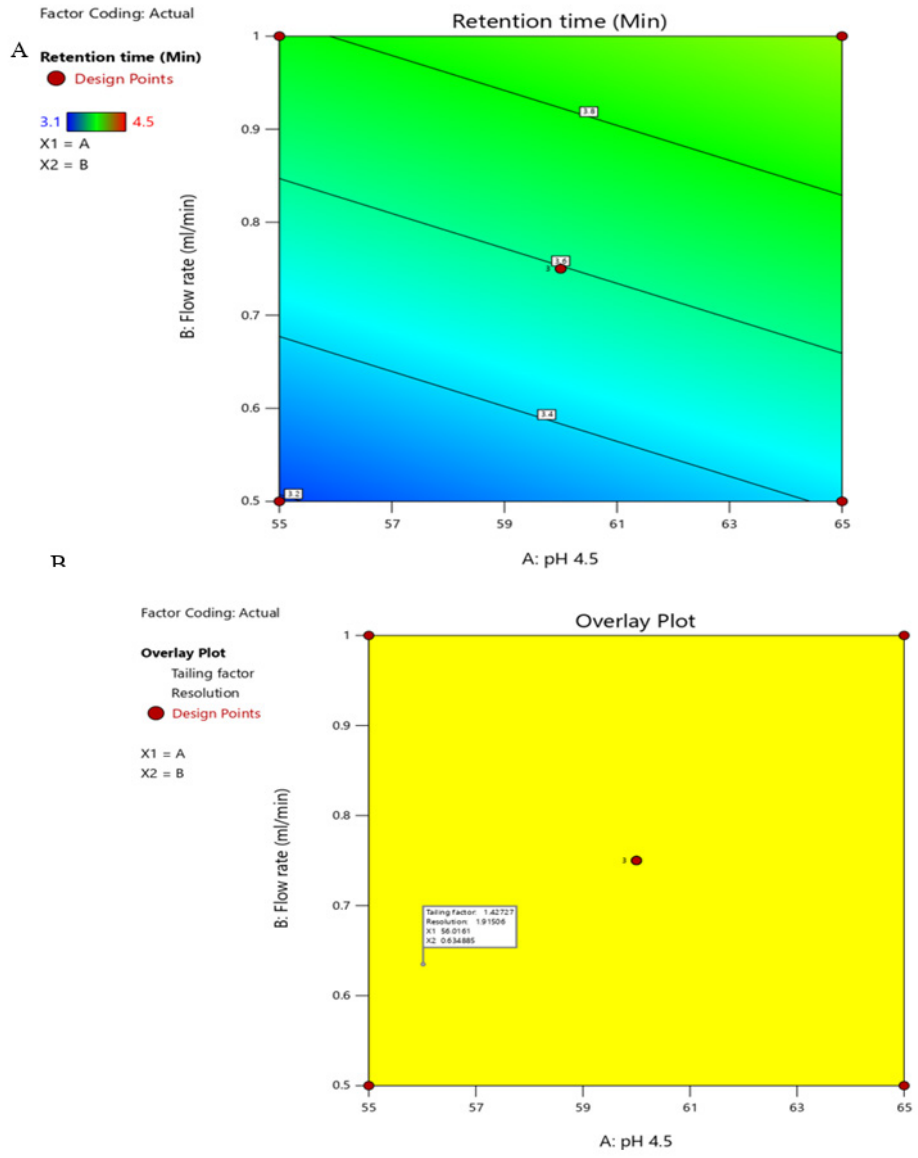



**Figure 2:** Graph representing A) Effect of variables x1 and x2 on retention time. B) overlay of x1 and x2 on tailing factor along with resolution.

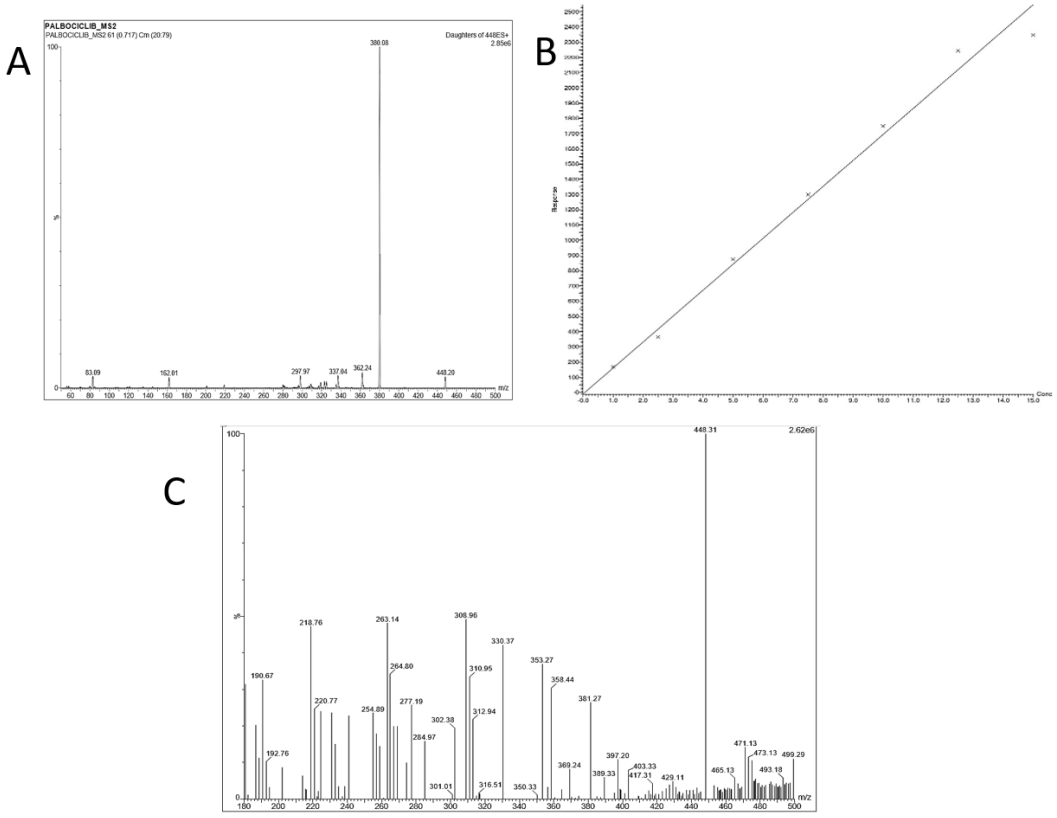



**Figure 3:** A) Mass spectra depicting Palbocilib API with m/z value of 448.20 with a base peak at m/z 380.08. B) Mass spectra depicting PCB in capsule formulation with m/z value of 448.31.C) Graph illustrating linearity results of Palbociclib in bulk drug.

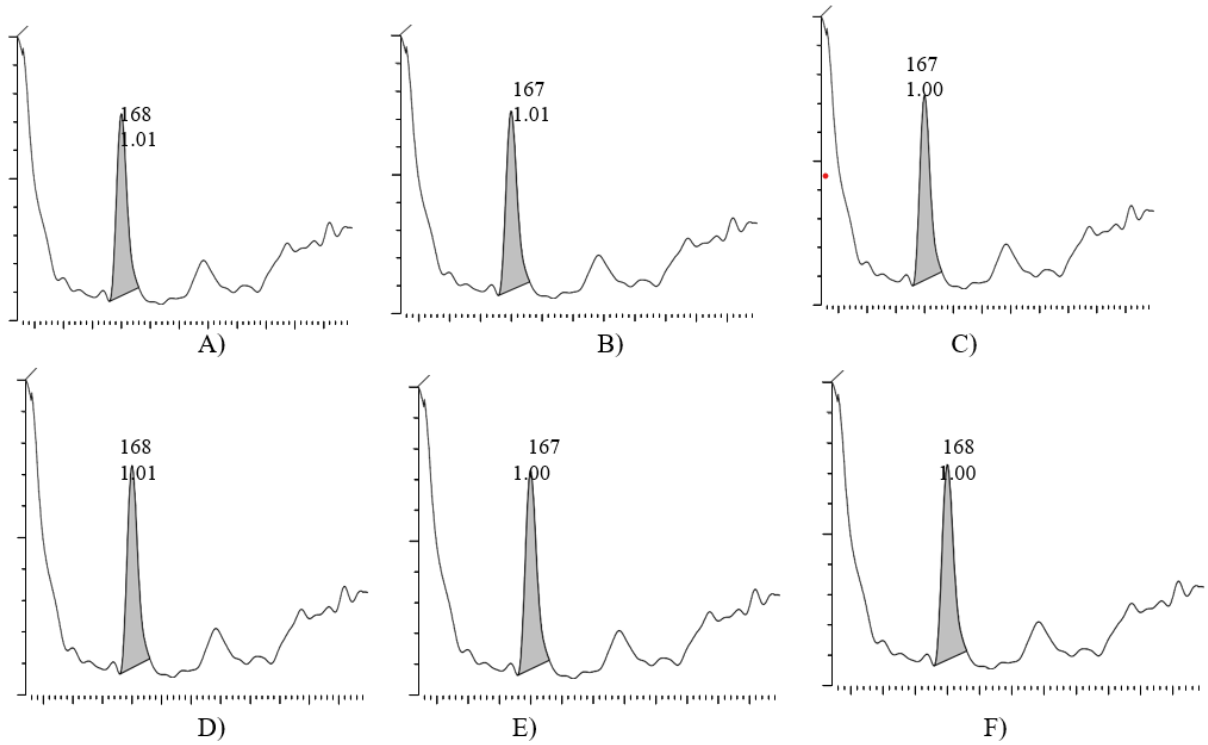



**Figure 4:** A) LC obtained Chromatograms showing Intraday precision at LOQ Level A-F: Injections (1-6) B) LC obtained chromatograms(A-C) showing injections (1-3) in triplicate of PCB at spiked 50% of Drug product concentration. C) LC obtained chromatograms showing injections in triplicate of PCB at spiked 100% of Drug product concentration. D) LC obtained chromatograms (A-C) showing injections in triplicate of PCB at spiked 150% of Drug product concentration.

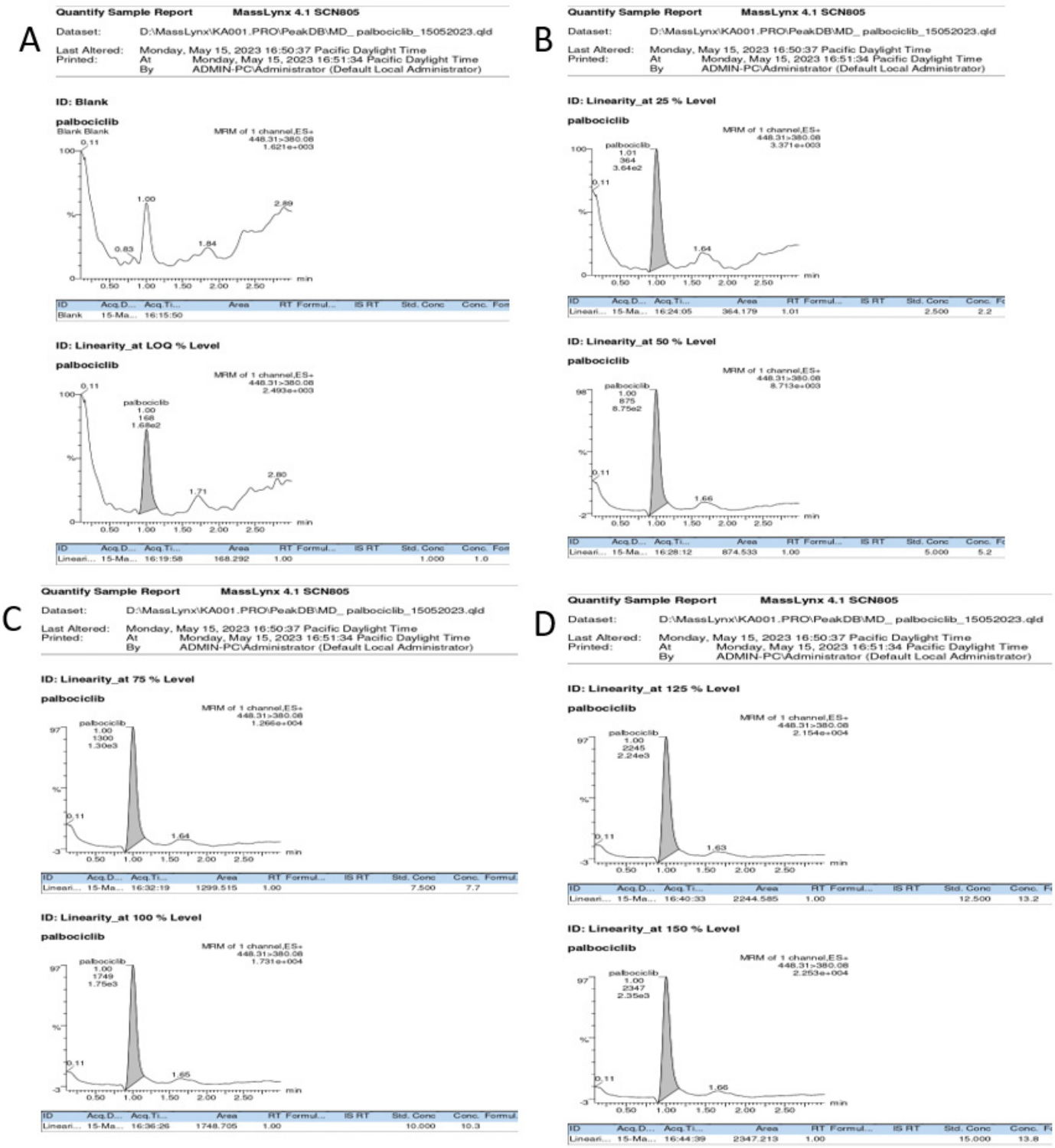



**Figure 5:** Typical LC chromatogram of A) blank (upper chromatogram) and 25% (lower chromatogram) level. B) PCB at 25%(upper chromatogram) and 50% (lower chromatogram) levels. C) PCB at 75% (upper chromatogram) and 100% (lower chromatogram) levels. D) PCB at 125%(upper chromatogram) and 150%(lower chromatogram) level.

proving its application I meeting regulatory expectations for robustness, matrix effect and reproducibility.[1,2] Thus, this sensitivity is operative in pharmacokinetic study along with trace metal quantification in biomatrices. Sample preparation involved no complex extraction and clean up decreasing the source of error.[3,4] As a matter of sensitivity interest the linearity has been critical with improved correlation coefficient $r^2$ of 0.9939 proving improved performance in selected range of concentration compared to earlier studies reporting in the range of 0.98-0.995 including the cancellation of ion suppression and less matrix

**Table 7: Rows and columns depicting Peak area at -5% in the left column and +5% in the right along with retention time of the varied mobile phase B composition, respectively.**

| Injection number | Peak Area | | Retention time of the peak (min) | |
|---|---|---|---|---|
| Injection 1 | 2152 | 2166 | 1.00 | 1.00 |
| Injection 2 | 2168 | 2169 | 1.01 | 1.01 |
| Injection 3 | 2164 | 2162 | 1.01 | 1.00 |
| Injection 4 | 2162 | 2167 | 1.00 | 1.00 |
| Injection 5 | 2165 | 2164 | 1.01 | 1.01 |
| Injection 6 | 2167 | 2163 | 1.00 | 1.00 |
| Average | 2163 | 2165 | 1.005 | 1.00 |
| Standard Deviation | 5.7965 | 2.639 | 0.0054 | 0.005 |
| % Relative standard deviation | 0.267 | 0.121 | 0.544 | 0.514 |

effects nullifying the variability.[5-14] Precision and accuracy within the % bias range of 0.3-0.5% and 95-103% accuracy (99.7% mean) demonstrated high reliability compared to earlier data reported in range of 1-5% in precision.[15-17] Thus, this improved method is more reliable and practical for routine QC analysis with high speed, sensitive operation efficacy.[19,20]

## CONCLUSION

A novel, rapid, selective and highly sensitive UPLC MS/MS assay was developed and validated for determination of PCB in bulk and its capsule formulation. The MS detection settings were optimized to reach linear ranges as per previously reported clinical trials. The majority of the ion suppression was consistent across the matrices and analyte concentrations in terms of matrix effects eluting in 10 min. The inter-day and intra day precision (%bias) was between 0.3-0.5%. The accuracy ranged from 98-103% with 99.7% which was found to be within the limits. These results are in compliance with the analytical technique validation recommendations published by the ICH and EMA. The method is advantageous as it was found to be economical, simple in sample preparation and peak has been eluted within less run time. The calibration curve was found to be linear whereas the least square linear Regression ($R^2$) was found to be 0.9939. The LLOQ is 1 ng/mL and LQC is found to be 0.0321 ng/mL. The results conclude that this method was more sensitive than the alternative methods which were previously published. The intraday and inter-day accuracy and the precision were determined at three levels of concentrations. Thus, the drug Palbociclib which is an essential tyrosine inhibitor in the cancer treatment could be analyzed easily by novel UPLC-MS/MS method developed in marketed samples with maximum recovery of the drug. The method application can be extended to pilot study for understanding the *in vivo* kinetics. The novel UPLC hyphenated with mass spectrophotometric analysis method which has been developed could analyze the nature of drug, stability and compared to previous reported methods has considerably favored to improve the linearity. Thus, the validated method has scope to be applicable in the quality control laboratory for estimation of Palbociclib drug in tablet and bulk dosage forms.

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## ABBREVIATIONS

**LOD:** Limit of Detection; **LOQ:** Limit of Quantification; **PCB:** Palbociclib; **ICH:** International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use; **MS:** MASS Spectrophotometer; **UV-vis:** Ultraviolet-visible Spectrophotometry.

## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

## SUMMARY

Palbociclib can be determined in bulk and oral dosage form using the UV-vis spectrophotometric method, which has demonstrated notable efficacy and dependability. The linearity and sensitivity of the method are confirmed, and an adequate correlation coefficient shows accurate quantification in accordance with ICH recommendations. The accuracy and precision of the procedure have been confirmed, and the RSD values stay within reasonable bounds. Robustness evaluations verify the method's stability under slight changes in analytical conditions. To increase efficiency, the composition and flow velocity of the mobile phase had to be optimized. Palbociclib detection has good specificity thanks to the UPLC system and mass spectrometry, whereas precursor and product ions were identified using the electrospray ionization approach. With computed Limit of Detection and Limit of Quantification values of 0.0106 ng/mL and 0.0321 ng/mL, respectively, the method showed outstanding specificity. Multiple injections and changes in the composition of the mobile phase were used to verify the method's consistency and robustness.

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