

Comparison of UV-Spectrophotometric and RP-HPLC Methods for Quantification of Abemaciclib in Solid Dosage Form

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Abstract: Abemaciclib is an anticancer drug that inhibits specific enzymes called CDKs, particularly CDK4 and CDK6. This medication is not included in any official pharmacopoeia. The objective of this study was to develop, validate, and compare spectrophotometric [zero-order UV spectrophotometric, first-order derivative spectroscopy method, and second-order derivative UV spectrophotometric method] and high-performance liquid chromatography (HPLC) methods for the determination of Abemaciclib in the pharmaceutical dosage form (tablets). Method A is a simple zero-order UV spectrophotometric method established for the determination of Abemaciclib in methanol at 235 nm. Method B is a first-order derivative spectrophotometric method, and method C is a second-order derivative spectrophotometric method that involves measurement of amplitude at 225 nm and 220 nm, respectively. Method D is by HPLC, which was carried out using a C₁₈ column, mobile phase consisting of acetonitrile: 0.1 % ortho phosphoric acid (70:30 v/v/v) with a flow rate of 1 ml/minute and detection done at 343 nm, which provide a sharp peak with a short retention time of 6.641 minutes. The developed analytic methods were statistically validated. As a result of analyses performed using spectrophotometric and liquid chromatographic methods, it was determined that both methods were precise, accurate, and robust, with RSD < 1%. Recovery values were within the normal range (98-100%). A statistical comparison of both analytical methods was performed, and no statistical significance was observed. These methods have been found to be reliable, fast, accurate, and simple for the quantitative analysis of Abemaciclib and can be used for quality control testing.

Keywords: abemaciclib; UV spectrophotometric; high-performance liquid chromatography; anticancer drug.

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1. Introduction

Abemaciclib, chemically N-[5-[(4-ethylpiperazin-1-yl) methyl]pyridine-2-yl]-5-fluoro-4-(7-fluoro-2-methyl-3-propan-2-benzimidazole-5-yl)pyrimidine-2-amine, (Fig. 1), and its molecular formula is C₂₇H₃₂F₂N₈, is a pharmaceutical compound that belongs to a class of drugs known as cyclin-dependent kinase (CDK) inhibitors [1]. It is used primarily in the treatment of certain types of cancer, with a focus on breast cancer. Abemaciclib inhibits specific

enzymes called CDKs, particularly CDK4 and CDK6. These enzymes play a crucial role in cell cycle regulation [2]. By inhibiting CDKs, abemaciclib helps slow down or halt the uncontrolled growth of cancer cells. Common side effects of abemaciclib may include diarrhea, fatigue, nausea, abdominal pain, and decreased appetite. Diarrhea is a notable side effect and is often managed with dose adjustments or anti-diarrheal medications [3]. Various analytical techniques have been documented in the literature for quantifying abemaciclib in samples. One method was reported for resolving abemaciclib impurities [4]. One bio-analytical HPLC method was reported for the quantification of abemaciclib in rat plasma in combination with letrozole [5]. Few HPLC-MS/MS bioanalytical methods have been reported for the quantification of abemaciclib in biological samples, either alone [6,7] or in combination with its metabolites [8,9] or other drugs [10-12]. UPLC/MS bioanalytical method reported for quantification of abemaciclib in biological samples [13-15]. A literature survey revealed that zero-, first-, and second-order UV spectrophotometric methods, as well as HPLC methods, have been reported for other drugs [16-31]. Hence, drawing on existing studies, an effort is made to develop both UV and HPLC methods for determining Abemaciclib in pharmaceutical tablet formulations that exhibit enhanced precision, simplicity, accuracy, reproducibility, resilience, and cost-effectiveness. The established analytical method was validated in accordance with the International Council for Harmonisation (ICH) requirements.

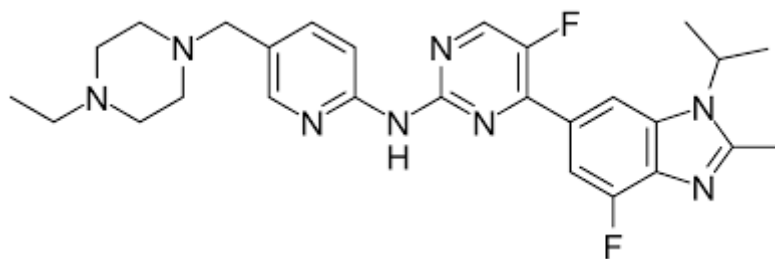


Figure 1. Abemaciclib structure (Obtained from open source Drug Bank).

2. Materials and Methods

2.1. Materials.

Abemaciclib bulk powder was obtained as a generous gift from Dr. Reddy's Lab, Hyderabad, India. The commercially available tablets of Abemaciclib (150 mg content) were purchased from the pharmacy. Chemical reagents employed in this study (methanol, acetonitrile, orthophosphoric acid, HPLC-grade water, etc.) were of analytical grade and procured from E. Merck India Ltd., Mumbai, India.

2.2. Instruments.

Spectrophotometric studies were performed on a double-beam Lab India 3000 + UV-Visible spectrophotometer (LAB INDIA INSTRUMENTS) equipped with a 10 mm matched quartz cell and UV WIN software. The solvent used was methanol to prepare the standard stock and for the serial dilutions of the Abemaciclib bulk form. The samples were placed in 1 cm quartz cells, and absorbance was recorded. The chromatographic method was performed using a Shimadzu HPLC system comprising an LC-20AD pump, a binary gradient pump, an SCL-20A system controller, and a variable-wavelength programmable PDA detector. A Rheodyne injector 7725i was fitted with a 20 μ L loop, which was used, and the data were recorded and evaluated using LC solutions software version 5.0.

2.3. Chromatographic conditions.

Chromatographic analysis was performed on an Enable C₁₈ G column (250 × 4.6 mm i.d, 5 µL). The mobile phase consisted of acetonitrile and 0.1 % orthophosphoric acid at a 70:30 % v/v ratio. The flow rate was 1 mL/min, the injection volume was 20 µL, and detection was carried out at 343 nm using a PDA detector.

2.4. Preparation of stock solutions.

2.4.1. UV spectrophotometry method.

Precisely 100 mg of Abemaciclib was weighed and transferred to a 100 ml volumetric flask for UV spectrophotometry. The solute was dissolved in methanol to create the solution, thereafter sonicated, and diluted to the specified volume to obtain a standard stock solution with an Abemaciclib concentration of 1000 µg/ml. A 100 µg/mL working standard solution of Abemaciclib was prepared by appropriately diluting the previously formulated stock solution in methanol. In the method development for A, B, and C, the wavelengths were documented within the 200–400 nm scanning range after preparing a standard solution of Abemaciclib at 10 µg/ml from a working standard solution of 100 µg/ml in methanol. The basic zero-order UV, first-derivative, and second-derivative spectra of the solution were documented.

2.4.2. HPLC method.

For HPLC, acetonitrile: 0.1 % orthophosphoric acid was used as the mobile phase. For the preparation of the standard stock solution, 25 mg of Abemaciclib was accurately weighed and transferred to a 25-ml volumetric flask. The content was dissolved in acetonitrile, further sonicated, and diluted to the desired concentration to obtain a standard stock solution with a final Abemaciclib concentration of 1000 µg/ml. By appropriate dilution of the stock solution, a 100 µg/ml working standard solution was prepared with the mobile phase. For the development of the method in the selection of wavelength(s), 10 µg/ml of Abemaciclib standard solution was prepared from the working standard solution (100 µg/ml) in the mobile phase. The 20 µl solution (10 µg/ml) was injected at a flow rate of 1 ml/minute in a C₁₈ column at room temperature. The chromatogram was obtained, and the peak areas were recorded.

2.5. Proposed methods.

Method A is a zero-order UV spectrophotometric method in which the simple UV spectrum of Abemaciclib was acquired, showing an absorption maximum (λ_{max}) at 235 nm. Aliquots of the working standard solution were transferred into a series of 100 ml volumetric flasks and diluted to the mark with methanol. The absorbance of the resulting solutions was measured at 235 nm against methanol as a blank. The calibration curve was plotted by measuring absorbance at various concentrations.

Method B is a derivative spectrophotometric method in which the simple UV spectrum of Abemaciclib (zero-order spectra) was first converted to first-order derivative spectra. Maxima occur at 225 nm and minima at 250 nm. Aliquots of the working standard solution were transferred into a series of 100 mL volumetric flasks and diluted up to the mark with methanol. First-derivative spectra were obtained, which showed an absorbance maximum at 225 nm and a minimum at 250 nm. A calibration curve was plotted at an absorbance maximum of 225 nm versus concentration of 5–25 µg/mL.

Method C is second-order; the simple UV spectrum of Abemaciclib was obtained (zero-order spectra) and then derivatized to first-order derivative spectra. Maxima occur at 220 nm and minima at 228.2 nm. Aliquots of the working standard solution were transferred into a series of 100 mL volumetric flasks and diluted up to the mark with water. First-derivative spectra were obtained, which showed an absorbance maximum at 220 nm and a minimum at 228.2 nm. A calibration curve was plotted at an absorbance maximum of 220 nm versus concentration of 5–25 µg/mL.

Method D is the HPLC method in which the working standard solution is diluted with the mobile phase to obtain a concentration of 10 µg/mL. The calibration curve was plotted between peak area and a concentration range of 10–50 µg/mL.

2.6. Marketed formulation.

Twenty tablets (marketed product) were weighed accurately, and the average weight was estimated. The tablets were crushed uniformly to obtain a fine powder. The amount of powder corresponding to 100 mg of Abemaciclib was transferred into a 100 ml volumetric flask, sonicated for 15 minutes with sufficient ethanol to dissolve the drug, and the volume was adjusted to the mark with ethanol. The solution was filtered through Whatman filter paper No. 41. The filtrate was diluted to 100 ml with methanol to produce a 15 µg/ml solution. This solution was analyzed using the above methods (A, B, and C), and the % estimation was calculated from the respective calibration curves.

For HPLC, a tablet powder equivalent to 25 mg was accurately weighed and diluted up to 25 ml with acetonitrile, sonicated for 15 minutes. The solution obtained was filtered through Whatman filter paper No. 41 and further diluted with mobile phase to obtain a concentration of 30 µg/mL. The solution was analyzed by the above method (Method D), and the % estimation was done using the calibration curve.

2.7. Method validation.

Analytical methods have been validated in accordance with the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use. Validation parameters (Linearity, selectivity, accuracy, precision, limit of detection and quantification, and robustness) have been investigated. System suitability testing was performed for injection repeatability (relative standard deviation of retention time and peak area response), tailing factor, peak asymmetry, and theoretical plate number using a standard solution (Abemaciclib 30 µg/ml).

Standard calibration curves were obtained in both methods by analyzing a series of standard solutions. These standard solutions were prepared in triplicate, and linearity was assessed by linear regression. The selectivity of both methods was assessed by comparing the spectra and chromatograms obtained from standard and sample preparations used in the pharmaceutical preparations.

The limits of detection and quantification have been determined using the slope of calibration curve (s) and standard error (σ) as displayed in the following equations 1 and 2.

$$\text{LOD} = 3.3\sigma/s \quad (1)$$

$$\text{LOQ} = 10\sigma/s \quad (2)$$

Precision of all methods was analyzed in terms of both repeatability (intraday precision) and intermediate precision (interday precision). The repeatability was determined from five replicate injections of a freshly prepared Abemaciclib solution (assay concentration, 30 µg/ml)

using the same equipment on the same day. In order to determine intermediate precision, the experiment was also replicated by analysing the newly prepared solutions at the same concentrations on three consecutive days. Precision was expressed as R.S.D.% % of a series of measurements.

The percentage recovery was determined using three preparations at three different levels of the reference drug Abemaciclib to ensure accuracy. The findings were expressed as the percentage of Abemaciclib recovered in the sample and R.S.D.% %. The robustness of analytical methods was evaluated by making small changes in method conditions. For the HPLC method, samples have been analyzed under different conditions, including changes in the mobile-phase flow rate (± 0.1 mL/min) and acetonitrile content ($\pm 2\%$), and the effects on system suitability parameters have been observed. For the UV method, samples have been analyzed under different conditions, such as using different brands of methanol.

2.8. Statistical comparison of methods.

Based on the validation results, the above-mentioned methods were suitable for routine quality control analysis of Abemaciclib in commercial formulations. The recovery percentages were statistically compared when both methods were applied to a commercial drug formulation. For this purpose, the F-test and t-test were applied.

3. Results and Discussion

The choice of analytical method depends on factors such as the complexity of the sample, the nature of the drug, and the intended use of the method. For quality control in drug analysis, the fastest and simplest method is the most wanted. However, spectrophotometric methods are also widely used because they are inexpensive and easy to perform.

Method A is a simple UV spectrophotometric method. In this method, the simple UV spectrum of Abemaciclib in ethanol and water was obtained, showing an absorption maximum (λ_{max}) at 235 nm. The overlay spectrum of the Abemaciclib standard solutions and the spectrum of the sample solution are given in Figure 2. Good linearity was achieved over the concentration range of 5–30 $\mu\text{g/mL}$ for standard solutions of Abemaciclib. The linear regression equation was found to be (equation 3):

$$Y=0.0602X+0.0036 \quad (3)$$

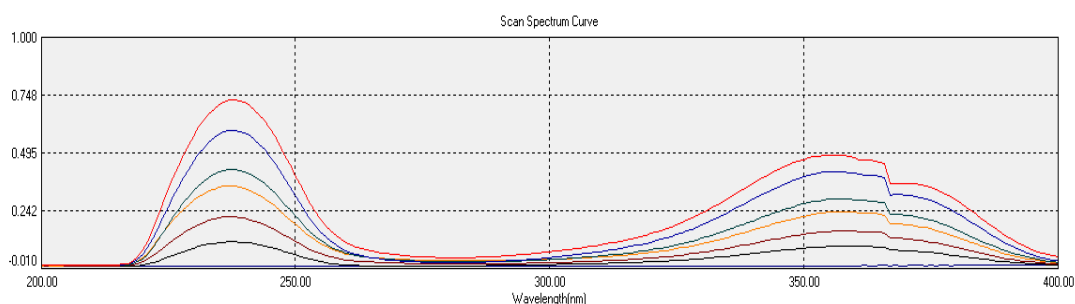


Figure 2. Zero-order UV Spectrum from method A.

With r^2 value of 0.9997. The exact data obtained for the evaluated methods are presented in Table 5.

Method B is the derivative spectrophotometric method. In this method, the simple UV spectrum of Abemaciclib is first-order-derivative, exhibiting an absorption maximum (λ_{max}) at 225 nm. The overlay spectrum of the Abemaciclib standard solutions and the spectrum of

the sample solution are given in Figure 3. Good linearity was achieved over the concentration range of 5–30 µg/mL for standard solutions of Abemaciclib. The linear regression equation was found to be (equation 4), with r^2 value of 0.9972.

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$$Y=0.005X+0.002 \quad (4)$$

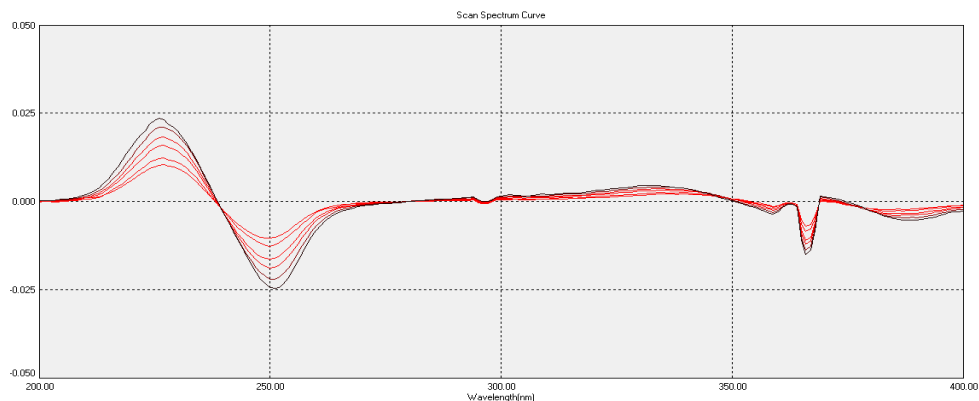


Figure 3. First-order UV Spectrum from method B.

Method C is the derivative spectrophotometric method. In this method, the simple UV spectrum of Abemaciclib is derivatized to a second-order spectrum, which exhibits absorption maxima (λ_{max}) at 220 nm. The overlay spectrum of the Abemaciclib standard solutions and the spectrum of the sample solution are given in Figure 4. Good linearity was achieved over the concentration range of 5–30 µg/mL for standard solutions of Abemaciclib. The linear regression equation was found to be (equation 5), with r^2 value of 0.9983.

$$Y=0.002X+0.005 \quad (5)$$

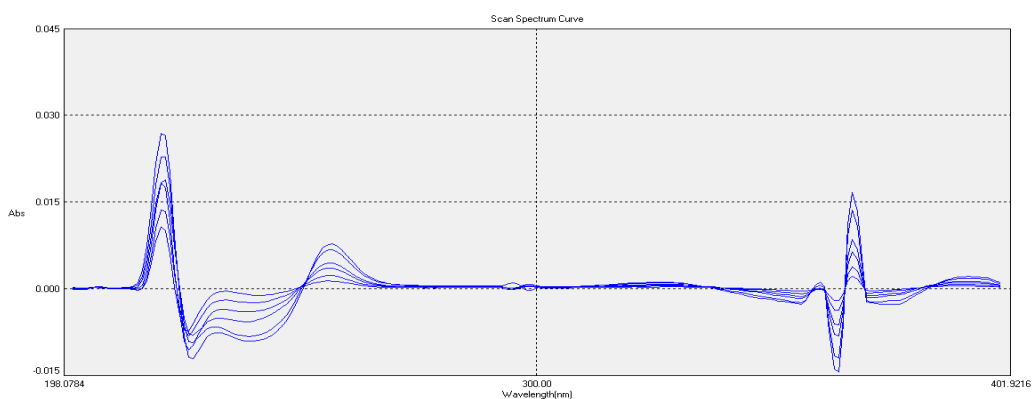


Figure 4. Second-order UV Spectrum from method C.

Method D is a HPLC method in which acceptable separations, with a retention time of 3.9 minutes for Abemaciclib, were obtained by use of a C_{18} column and acetonitrile: 0.1 % ortho phosphoric acid (40:60v/v), at 1.0 ml/minute, as a mobile phase [29,30]. A sharp, symmetrical peak was obtained for Abemaciclib when analyzed under these conditions (Figure 5). This retention time enables the quick estimation of the drug molecule, which is significant for routine analysis. The detection wavelength was fixed at 343 nm from the UV spectra. No interference from diluents, impurities, or excipients present in the pharmaceutical formulations was observed at this detected wavelength. The linear regression equation was found to be (equation 6), with r^2 value of 0.9998.

$$Y=43482X+16095 \quad (6)$$

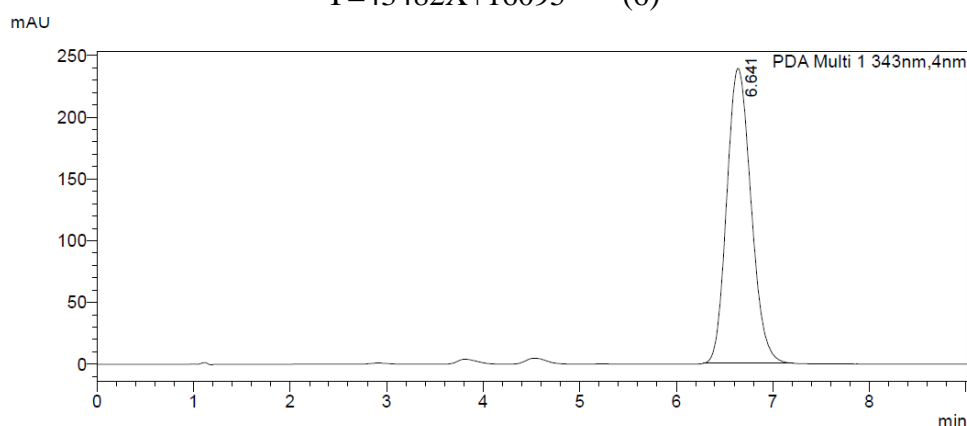


Figure 5. Typical chromatogram of Abemaciclib from method D.

A linear correlation was observed in both spectrophotometric and chromatographic methods over a concentration range of 5-30 µg/ml for UV and 10-60 µg/ml for HPLC. Beer’s law was well fitted to the developed linear concentrations in this analysis. Accuracy was determined by calculating recovery, and the mean was calculated (Table 1), which ranged from 98%–100% as prescribed by USP, indicating that the method is free of excipient interference. The method’s precision, according to the ICH guideline, was assessed by replicate analyses of the marketed formulation. It was expressed as ±SD and % RSD. This method was found to be rugged, with a number of significant changes in the analytical conditions, such as different times (intra-day) (Table 2), different days (inter-day), etc (Table 3). The method’s robustness was evaluated by testing the effects of minor variations in experimental variables, such as changes in solvents and detection wavelengths, on analytical performance. The minor differences in each of the factors didn't affect the findings dramatically. This indicates that the method developed for routine analysis is reliable; the situation is highlighted in Table 4.

Table 1. Accuracy of spectrophotometric and RP-HPLC methods.

Method	Level	Amount taken (µg/ml)	Amount added (%)	% Recovery	± SD	%RSD
A	80%	16	80	100.31	1.509	1.49
	100%	20	100	101.9		
	120%	24	120	98.9		
B	80%	16	80	99.32	0.46	0.46
	100%	20	100	100.23		
	120%	24	120	99.65		
C	80%	16	80	99.01	0.36	0.36
	100%	20	100	98.89		
	120%	24	120	98.32		
D	80%	24	80	100.03	0.31	0.31
	100%	30	100	99.89		
	120%	36	120	99.43		

Table 2. Inter-day precision of the UV and RP-HPLC method.

Method	% Estimation			± SD	%RSD
	Day 1	Day 2	Day 3		
A	99.12	99.43	99.02	0.21	0.22
B	98.35	99.12	98.31	0.45	0.46
C	98.12	98.77	99.42	0.65	0.65
D	99.43	99.97	100.11	0.35	0.35

The methods were successfully used to determine the amount of Abemaciclib present in tablets, and the results were reported as \pm SD and % RSD, which are within the limit, i.e., less than 2, prescribed by USP for finished products (Table 4). All the validation parameters [31] are summarized in Table 5.

Table 3. Inter-day precision of the UV and RP-HPLC method.

Method	% Estimation			\pm SD	%RSD
	0 hr	3 hr	6 hr		
A	99.34	99.21	99.12	0.11	0.11
B	98.56	99.02	98.89	0.67	0.68
C	98.14	98.17	99.23	0.65	0.66
D	99.76	99.62	99.91	0.14	0.14

Table 4. Drug assay and statistical comparison of UV and HPLC methods.

Analysis method	Name of the formulation	Labelled claim	Amount found (mg)	% RSD	F test	t test
Method A	Marketed product	150 mg	148.31 \pm 2.61	1.32	2.886	0.982
Method B	Marketed product	150 mg	147.94 \pm 2.48	1.25	2.914	1.971
Method C	Marketed product	150 mg	147.82 \pm 2.21	1.24	3.674	1.892
Method D	Marketed product	150 mg	148.92 \pm 2.01	1.01	1.926	0.911

* Average of five determinations; ** The tabulated values at 95% confidence limit are 2.78 (t-value) and 6.39 (F-value) at four degrees of freedom.

Table 5. Summary of the validation parameters of UV and HPLC analysis.

Parameters	Method A	Method B	Method C	Method D
Analytical wavelength (nm)	235	225	220	343
Beer's law range (μ g/ml)	5-30	5-30	5-30	10-60
Correlation coefficient	0.9991	0.9912	0.9931	0.9998
Standard regression equation	Y = 0.0602X + 0.0036	Y = 0.005X + 0.002	Y = 0.002X + 0.005	Y = 43482x + 16905
Slope	0.0602	0.005	0.002	4382
LOD (μ g/ml)	0.232	0.082	0.076	0.03
LOQ (μ g/ml)	0.646	0.241	0.232	0.12
Accuracy (average % recovery)	99.66-100.31	98.71-99.2	98.43-99.12	99.12-99.89
Precision (average% RSD)				
Intraday	0.22	0.46	0.65	0.35
Interday	0.11	0.68	0.66	0.14
Robustness (average% RSD)	0.23	0.54	0.62	0.12
Ruggedness (average% RSD)	0.25	0.35	0.43	0.21
% Assay	98.87	98.62	98.54	99.28

Acetonitrile:water in the ratios 80:20 v/v and 50:50 were employed; however, a noisy baseline and less intense peaks were observed. A more intense peak was observed when the mobile phase composition, acetonitrile:0.1 % ortho-phosphoric acid (70:30 v/v), was employed. Employing acetonitrile:0.1 % ortho-phosphoric acid (40:60 v/v) instead of water resulted in linear elution with less tailing. Asymmetry is a crucial factor in RP-HPLC, and a value between 1 and 1.5, and NMT 2.0, is seldom required per ICH guidelines, as observed in the case of the acetonitrile:0.1 % ortho-phosphoric acid (40:60 v/v) mobile phase composition. The following observed characteristics, asymmetry (1.1732) and column efficiency (8433.6),

indicated that this employed mobile phase has better eluting characteristics than the previously developed method. However, methanol is not a green solvent; when acetonitrile:water was used, a less intense peak was observed. When methanol: water was utilized, signal splitting and baseline noise were observed. However, the use of acetonitrile:0.1 % ortho-phosphoric acid (70:30 v/v) resulted in less tailing and proper system suitability parameters.

3.1. Application to pharmaceutical preparations.

Chromatographic and spectroscopic methods have been applied in pharmaceutical formulations. Test results for the tablet containing Abemaciclib sold in pharmacies were presented in Table 5. These results are very close to the amounts indicated on the tablet label. The UV and LC methods recommended in this report can be applied appropriately to analyze Abemaciclib in pharmaceutical preparations.

3.2. Statistical comparison of methods.

F-test and t-test were applied to statistically compare both methods. Statistical tests revealed that there was no significant difference between the experimental values. Obtained during the analysis with both methods. The calculated t-value and F-value were found to be lower than the table values for both methods at the 95% confidence level. It is clear from this report that both of the recommended UV and LC methods are appropriate for the determination of Abemaciclib in drug formulations. Data for statistical comparisons of the LC and UV methods are shown in Table 5.

4. Conclusions

UV spectrophotometric methods (Methods A, B, and C) generally do not require complex operations and procedures. It takes less time and is economical. These cases show the advantages of the UV method over the LC method. Statistically compared, the LC method (method D) is more precise and accurate than the UV method. Statistical tests revealed no significant difference between the experimental values obtained with both methods. It is clear from this report that both of the recommended UV and LC methods are appropriate for the determination of Abemaciclib in drug formulations. Excipients in pharmaceutical preparations have not interfered with the mobile phase, which can be prepared very easily. Both suggested analytical methods are reproducible, precise, and linear, and can be used for routine analysis of Abemaciclib in different pharmaceutical forms.

Author Contributions

Conceptualization, S.R.K. and K.G.M.; methodology, D.V. and Y.S.R.; writing – original draft preparation, D.V. and N.V.R.R.; supervision, D.V. and Y.S.R.

Institutional Review Board Statement

Not applicable.

Informed Consent Statement

Not applicable.

Data Availability Statement

Data supporting the findings of this study are available upon reasonable request from the corresponding author.

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Conflicts of Interest

The authors declare no conflict of interest.

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