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Development and Validation of a QbD-Based Stability indicating UV Spectrophotometric Method for Simultaneous Estimation of Lenvatinib and Palbociclib in Bulk and Formulation with Greenness Assessment

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ABSTRACT: The present study aimed to develop and validate a simple, environmentally sustainable, stability-indicating UV spectrophotometric method for simultaneous estimation of lenvatinib (LEN) and palbociclib (PAL) in bulk drugs and liposomal formulations. An Analytical Quality by Design approach using Central Composite Design was employed to optimize the analytical conditions. A methanol mixture (50:50, v/v) was selected as the optimum solvent. LEN and PAL showed maximum absorbance at 243 and 220 nm, respectively, with a common isosbestic point at 256 nm. The method was validated according to ICH Q2(R2) guidelines. Both drugs exhibited linearity over 2–10 µg/mL, with correlation coefficients above 0.99. Recovery values were close to 100%, while precision, ruggedness, robustness and repeatability studies showed %RSD values below 2%. The method demonstrated adequate sensitivity, with LOD/LOQ values of 0.48/1.46 µg/mL for LEN and 0.22/0.683 µg/mL for PAL. Forced degradation studies confirmed its stability-indicating capability under acidic, alkaline, oxidative, thermal and photolytic conditions. Greenness assessment using AGREE, AGREEprep, ComplexGAPI and BAGI indicated favorable environmental performance. The developed method is simple, economical, reliable and suitable for routine quality control and stability assessment of LEN and PAL formulations.

1. INTRODUCTION

Cancer remains one of the foremost public health challenges worldwide and continues to impose a substantial clinical and socioeconomic burden. According to recent global cancer statistics, nearly 20 million new cases and approximately 9.7 million cancer-related deaths were reported in 2022, underscoring the urgent need for more effective therapeutic and diagnostic strategies. Among all primary liver malignancies, hepatocellular carcinoma (HCC) accounts for the majority of diagnosed cases and is recognized for its aggressive biological behavior, high recurrence rate, and poor prognosis. Despite considerable advances in cancer diagnosis and treatment, the incidence and mortality associated with HCC remain alarmingly high, emphasizing the importance of developing improved therapeutic approaches and reliable analytical methods to support pharmaceutical research and quality assurance.[1]

Lenvatinib (LEN) is an orally administered multikinase inhibitor (Figure 1a) that selectively targets several receptor tyrosine kinases involved in tumour growth and angiogenesis, including vascular endothelial growth factor receptors (VEGFR-1, VEGFR-2 and VEGFR-3), fibroblast growth factor receptors (FGFR-1 to FGFR-4), platelet-derived growth factor receptor-α

(PDGFR-α), RET and KIT.[2] Through simultaneous inhibition of these signalling pathways, lenvatinib suppresses tumour vascularization and cellular proliferation, thereby improving therapeutic outcomes in several malignancies, particularly hepatocellular carcinoma, differentiated thyroid carcinoma and renal cell carcinoma.[3,4] Nevertheless, factors such as variable oral bioavailability, interpatient pharmacokinetic differences and treatment-related adverse effects may compromise its overall therapeutic efficacy. Consequently, there is increasing interest in developing advanced drug delivery systems together with robust analytical techniques capable of ensuring accurate drug quantification during formulation development and quality evaluation.[5,6]

Palbociclib (PAL) is a selective cyclin-dependent kinase 4 and 6 (CDK4/6) inhibitor (Figure 1b) that interrupts cell-cycle progression by preventing phosphorylation of the retinoblastoma (Rb) protein, ultimately arresting cells in the G1 phase of the cell cycle.[7,8] Clinically, PAL is extensively used in combination with endocrine therapy for patients with hormone receptor-positive and HER2-negative advanced or metastatic breast cancer, where it has demonstrated significant improvements in progression-free survival.[9] Although its therapeutic benefits are well established, its pharmaceutical development remains challenging because of poor aqueous solubility, limited physicochemical stability and formulation-related constraints that may adversely influence drug delivery and therapeutic performance.[10,11]

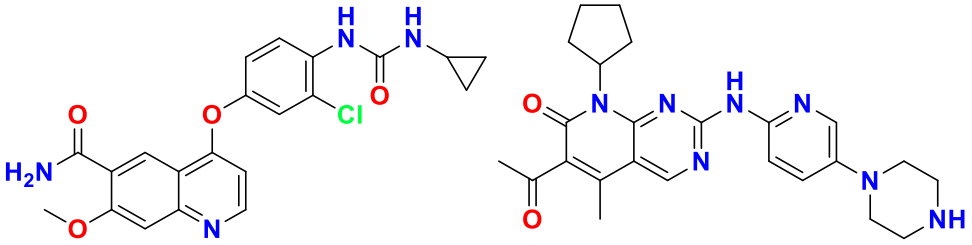


FIGURE 1: Chemical structures of (a) Lenvatinib and (b) Palbociclib

The simultaneous determination of multiple anticancer agents has become increasingly important in pharmaceutical research, particularly for the development and quality assessment of combination drug products. Reliable analytical methods are essential to accurately quantify each active pharmaceutical ingredient, ensure batch-to-batch consistency, evaluate formulation stability and support regulatory compliance.[12,13] However, the presence of chemically diverse compounds within a single dosage form often introduces analytical challenges because of differences in their physicochemical properties and spectral characteristics. Consequently, analytical procedures intended for combination formulations must be carefully optimized to achieve adequate selectivity, sensitivity and reproducibility while maintaining analytical efficiency.[14-16]

Several instrumental techniques, including reversed-phase high-performance liquid chromatography (RP-HPLC), ultra-performance liquid chromatography (UPLC) and liquid chromatography coupled with tandem mass spectrometry (LC–MS/MS), have been reported for the individual determination of LEN or PAL. Although these techniques provide excellent analytical performance, their routine application is frequently limited by expensive instrumentation, extensive sample preparation, higher solvent consumption and increased operational costs.[17] In addition, most published methods have focused on the analysis of the individual drugs rather than their simultaneous estimation in a combined pharmaceutical system. A comprehensive review of the available literature further revealed the absence of a validated UV spectrophotometric method for the simultaneous determination of LEN and PAL. This lack of a simple and economical analytical procedure highlights the need for an alternative method that can be readily implemented in routine pharmaceutical quality control laboratories.

In recent years, the principles of Green Analytical Chemistry (GAC) have gained considerable attention owing to the growing emphasis on minimizing the environmental impact of analytical practices. Modern analytical method development is no longer confined to achieving high analytical performance but also aims to reduce hazardous solvent consumption, energy requirements and waste generation without compromising data quality. Consequently, the environmental sustainability of analytical procedures has become an important criterion during method selection and regulatory evaluation. To quantitatively assess the greenness of analytical methods, several established assessment tools, including AGREE, AGREEprep, ComplexGAPI and BAGI, have been introduced. These complementary metrics provide a systematic evaluation of environmental performance throughout different stages of the analytical workflow and facilitate the development of more sustainable analytical strategies.[18,19]

Among the available analytical techniques, UV-visible spectrophotometry continues to be one of the most practical and widely adopted approaches for routine pharmaceutical analysis because of its operational simplicity, rapid analysis, affordability and broad accessibility across quality control laboratories. When appropriately optimized and validated, UV spectrophotometric methods are capable of providing reliable quantitative results for drug assay, formulation evaluation and stability studies while requiring minimal sample preparation and instrumentation.[20,21] Despite these advantages, no published report describing the simultaneous UV spectrophotometric estimation of LEN and PAL in a combined formulation could be identified from the available literature.

Therefore, the present study aims to design and validate a simple, accurate, precise UV spectrophotometric method for the simultaneous estimation of Lenvatinib and Palbociclib in accordance with ICH guidelines[22,23]. The developed method is intended to support formulation development, routine quality control analysis and stability evaluation of combined anticancer drug products.

MATERIALS AND METHODS

2.1. Materials:

Lenvatinib (purity ≥98.0%) was procured from Yarrow Chem Pvt Ltd, Mumbai-421201 and Palbociclib (Purity ≥98.0%) was a gift sample from CDYMAX pharma Pvt. Ltd. Methanol (99%) was procured from Merck Life Science Pvt. Ltd. (Bengaluru, Kranataka). Millipore water was obtained from the Milli-Q apparatus, Model: VOE-WPS-ECO. All reagents were of analytical grade.

2.2. Instrumentation

Shimadzu UV-Visible spectrophotometer Model:UV-1900 with quartz cuvette (1cm).UV Probe, version: 2.4.3 software was used. Analytical balance, ultrasonic bath and standard volumetric glassware were employed throughout the study.

2.3. Selection of solvents

Several analytical solvents, including methanol, distilled water, ethanol and different methanol–water mixtures, were initially evaluated during preliminary trial-and-error experiments to identify a suitable solvent system for UV spectrophotometric analysis. The selection was based on the solubility of both LEN and PAL, spectral characteristics and the absence of interfering absorbance. Among the solvents investigated, a methanol:water mixture (50:50, v/v) provided complete drug solubility, well-defined absorption spectra and consistent analytical performance. Therefore, this solvent system was selected for subsequent method development and validation.

2.4. Green evaluation software

To examine the environmental profile of the developed UV spectrophotometric method, four greenness assessment tools were employed: Complex Green Analytical Procedure Index (ComplexGAPI, version 0.2 beta), Analytical GREEnness Calculator (AGREE, version 0.5, 2020), AGREEprep, and Blue Applicability Grade Index (BAGI).[24-26]

2.5. Preparation of standard and working solution of Lenvatinib and Palbociclib

Individual primary stock solutions of LEN and PAL were prepared by accurately weighing 10 mg of each drug and dissolving it separately in a methanol-water mixture (50:50, v/v) in 10 mL volumetric flasks to obtain a concentration of 1000 µg/mL. From each primary stock solution, 1 mL was withdrawn and diluted to 10 mL with the same solvent system to produce secondary stock solutions containing 100 µg/mL of each drug. Subsequently, 1 mL of the respective secondary stock solution was further diluted to 10 mL using methanol-water (50:50, v/v), yielding working standard solutions with a final concentration of 10 µg/mL. These freshly prepared working solutions were used throughout the method development and validation studies.

2.6. Selection of Wavelength

The UV absorption spectra of LEN and PAL were recorded over the wavelength range of 200–400 nm. LEN exhibited a λmax at 243 nm, whereas PAL showed maximum absorbance at 220 nm.

2.7. Analytical Method Development and Optimization

2.7.1. Optimization of the Method using DoE Software

DoE approach was employed to systematically optimize the UV spectrophotometric method by evaluating the influence of selected analytical parameters on the measured responses. A Central Composite Design (CCD) comprising two independent factors and three levels was implemented using Design-Expert® software (Version 13.0, Stat-Ease Inc., USA), generating nine experimental runs to establish the optimum analytical conditions and ensure method robustness.[27,28]

2.8. Method Validation

The developed method was subjected to validation in accordance with the ICH Q2(R2) guideline,with assessment of critical analytical performance characteristics, including linearity, specificity, selectivity, accuracy, precision, robustness, ruggedness, repeatability and the LOD and LOQ.

2.8.1. Linearity

The linear response of the proposed UV spectrophotometric method was evaluated by measuring the absorbance of LEN and PAL at different concentration levels. Standard solutions were prepared over the concentration range of 2–10 μg/mL for both analytes and their absorbance values were recorded under the optimized analytical conditions. Calibration plots were constructed by plotting absorbance against concentration and linear regression analysis was performed to obtain the regression equations and correlation coefficients for assessing method linearity.[29]

2.8.2. Specificity and Selectivity

Specificity and selectivity are essential validation parameters for ensuring the accurate estimation of LEN and PAL in pharmaceutical analysis. These parameters were evaluated to confirm the absence of interference from the solvent or other extraneous components. A blank solution containing the optimized methanol:water (50:50 v/v) solvent system was scanned over the wavelength range of 200–400 nm and no interfering absorbance was observed at the selected analytical wavelengths.

2.8.3. Sensitivity

Method sensitivity was evaluated by determining the detection and quantification limits for both analytes. The limit of detection (LOD) was defined as the lowest concentration capable of producing a detectable analytical response, whereas the limit of quantification (LOQ) represented the lowest concentration that could be quantified with acceptable accuracy and precision. These values were derived from the calibration plot using the standard deviation of the response (σ) together with the slope of the regression equation (S), as expressed below:

$$LOD = 3.3 \times \sigma/S$$

$$LOQ = 10 \times \sigma/S$$

where σ corresponds to the standard deviation of the response and S is the slope of the regression line.

2.8.4. Precision

The reproducibility of the proposed UV spectrophotometric method was established through intraday and interday precision studies. Three quality-control concentrations (2, 6 and 10 μg/mL) of both LEN and PAL were selected for evaluation. For intraday precision, each concentration was analyzed at different time points on the same day, whereas interday precision was assessed by repeating the analysis on two consecutive days. The variability among replicate measurements was calculated as %RSD.

2.8.5. Accuracy

Method accuracy was established through recovery studies employing the standard addition technique at 80%, 100% and 120% of the target concentration. Predetermined amounts of LEN and PAL standards were spiked into the sample matrix and the percentage recovery was calculated at each level. The mean recovery values were used to confirm the accuracy and reliability of the developed UV spectrophotometric method.

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2.8.6. Ruggedness

Ruggedness of the developed method was evaluated by performing the analysis under normal operating conditions using the same instrument by two independent analysts. The consistency and reproducibility of the method were assessed by calculating the %RSD of the obtained absorbance values.

2.8.7. Robustness

Robustness of the developed method was evaluated by deliberate variations in the analytical wavelength (±2 nm) while keeping all other experimental conditions constant. The effect of these minor changes on the analytical performance was assessed by calculating the %RSD, thereby confirming the reliability and robustness of the proposed method.

2.8.8. Repeatability

Repeatability was evaluated by analyzing a standard solution containing LEN (6 µg/mL) and PAL (6 µg/mL) six consecutive times under identical experimental conditions. The repeatability of the developed method was assessed by calculating the %RSD of the recorded absorbance values.

2.9. Forced Degradation Study

To confirm the stability-indicating performance of the developed analytical method, LEN and PAL were subjected to a series of forced degradation studies following ICH Q1A(R2) guidance. Individual standard solutions were challenged under acidic (0.1 N HCl), basic (0.1 N NaOH), oxidative (30% H_2O_2), thermal and photolytic conditions. Acidic, alkaline and oxidative degradation experiments involved treatment with the respective reagents at 80°C for 2 h. Thermal stress was applied by heating the solutions in a water bath maintained at 80°C, while photolytic stress was induced by UV irradiation for 2 h. After completion of the stress treatments, UV spectra were recorded between 200 - 400 nm and degradation of the analytes was assessed by comparing the spectral responses obtained under each condition.[30]

2.10. Preparation of Liposomes

Dual drug-loaded liposomes containing LEN and PAL were fabricated using the conventional thin-film hydration method. Soy lecithin and cholesterol were dissolved in a chloroform:methanol mixture (2:1, v/v) within a clean, dry round bottom flask. LEN and PAL (0.1% w/v each) were incorporated into the organic phase, and the mixture was gently stirred until a clear solution was obtained. Organic solvents were removed under reduced pressure using a rotary evaporator maintained at 45 °C, producing a uniform lipid film on the inner surface of the flask. To eliminate traces of residual solvent, the film was further vacuum-dried for 1 h. Hydration was then carried out with 30 mL of phosphate-buffered saline (PBS, pH 7.4) at 40–60 °C while rotating at 60 rpm for 30 min to generate multilamellar vesicles. The dispersion was subsequently probe-sonicated for 10 min to reduce vesicle size and obtain uniformly dispersed unilamellar liposomes. The prepared formulations were characterized for particle size, polydispersity index (PDI) and zeta potential, and stored at 2–8 °C until further analysis.

2.11. Application of the Method to Nanoformulation

The developed UV spectrophotometric method was applied for the simultaneous estimation of . LEN and PAL in the prepared liposomal formulation. An accurately measured quantity of the liposomal dispersion equivalent to the required drug content was transferred to a volumetric flask, diluted with the optimized solvent system and sonicated to ensure complete disruption of the liposomes and drug extraction. The resulting solution was suitably diluted to the desired working concentration and the drug content was determined using the UV spectrophotometric method.

III. RESULTS

The simultaneous estimation of LEN and PAL was successfully accomplished using the developed UV spectrophotometric method, which was systematically optimized through the QbD approach and validation in accordance with ICH guidelines.

3.1. Selection of wavelength

A UV Spectrophotometer was used to analyze and scan . LEN and PAL (10**µ**g/ml) within the range of 400 to 200 nm to determine the Lambda maxima. LEN exhibited highest absorbance at 243 nm and. PAL exhibited highest absorbance at 220 nm. (as shown in figure 2) The particular wavelengths coincided with previously documented findings.

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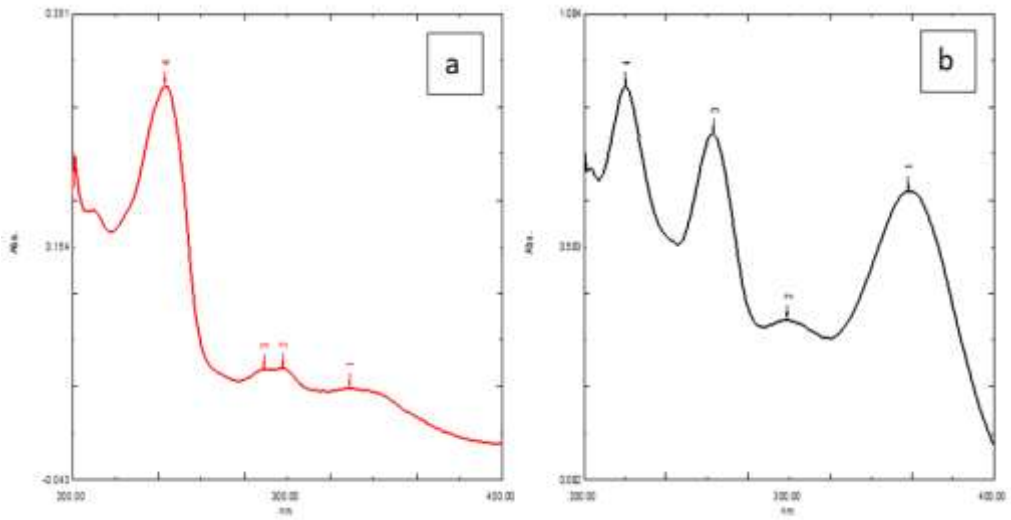


Figure 2: λmax of (a) Lenvatinib observed at 243 nm with 0.283 abs and (b) Palbociclib at 220 nm with 0.928 abs

3.2. Isosbestic Point Method

The isosbestic point method was employed for the simultaneous estimation of LEN and PAL using UV–Visible spectrophotometry. Standard stock solutions of LEN and PAL were prepared separately in the selected solvent and scanned in the wavelength range of 200–400 nm. The individual absorption spectra showed that LEN exhibited a maximum absorbance at 243 nm, while PAL showed a maximum absorbance at 220 nm. The overlay spectra of both drugs revealed a common wavelength at 256 nm, which was identified as the isosbestic point, indicating equal absorptivity of both drugs at this wavelength.(as shown in figure 3)

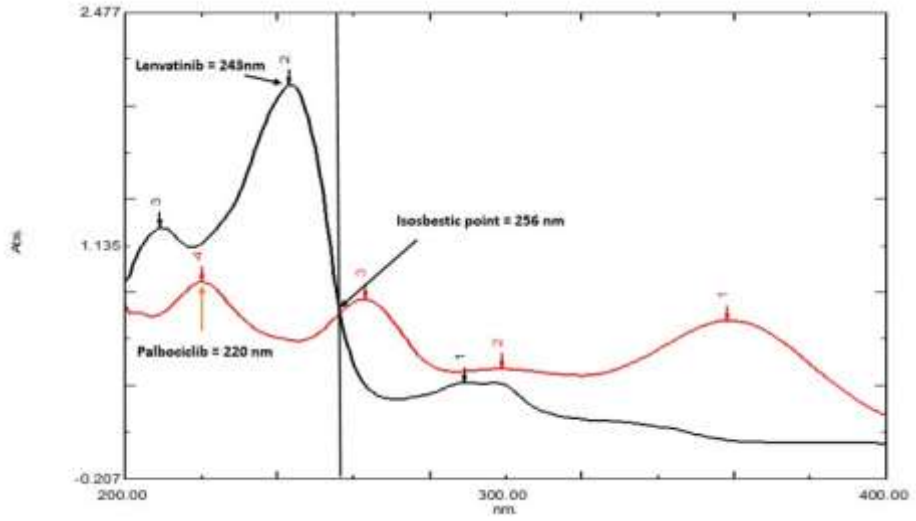


Figure 3: Isosbestic point of Lenvatinib and Palbociclib at 256 nm

3.3. Optimization of Analytical Parameters Using Central Composite Design (CCD)

The analytical method was optimized using a CCD under the Design of Experiments (DoE) approach with an alpha value of 1. The objective was to determine the optimum levels of solvent ratio (A) and scanning speed (B) to enhance method performance and robustness. The selected independent variables and their corresponding levels employed in the CCD are presented in Table 1.

TABLE 1. UV-spectrophotometric factors and levels for Central Composite Design

Independent Variable	Levels		
	(Low)	(Medium)	(High)
Solvent Ratio (A)	40%	50%	60%
Scanning Speed (B)	-1	0	+1

A total of nine experimental runs, including a center point, were generated by the CCD to evaluate the effect of the selected factors on the analytical responses (Table 2).

TABLE 2 : Projected combination of factors based on the Central Composite experimental design (CCD)

Run	Factor 1 A:Solvent ratio %	Factor 2 B:Scanning speed	Response 1 R1:Absorbance (243nm)	Response 2 R2:Absorbance (220nm)
1	60	0	0.198	0.759
2	40	0	0.214	0.823
3	60	1	0.178	0.786
4	60	-1	0.213	0.711
5	50	0	0.275	0.911
6	40	-1	0.216	0.944
7	40	1	0.215	0.856
8	50	1	0.281	0.931
9	50	-1	0.278	0.905

Following completion of the experimental trials, the obtained data were subjected to statistical analysis using Design-Expert® software. Analysis of variance (ANOVA) was performed for both LEN and PAL responses. The obtained statistical parameters, including p-values, adjusted R^2 values, and adequate precision values, were found to be within acceptable limits, confirming the suitability and significance of the developed models ($p < 0.05$), as summarized in Supplementary Table 1A and 1B.

The model F-values of 42.07 for LEN and 9.59 for PAL indicated a strong correlation between the experimental and predicted data with a low likelihood of noise influencing the results. Model adequacy and reliability were further verified through diagnostic plots, including predicted versus actual plots, Box–Cox plots, and Cook's distance plots (Figure 4), all of which demonstrated satisfactory model performance. Furthermore, two-dimensional contour plots (Figure 5a and 5d), three-dimensional response surface plots (Figure 5b and 5e) and overlay plots (Figure 5c and 5f) were constructed to elucidate the effects and interactions of the investigated variables on the measured responses.

Graphical optimization revealed that the response surface plots (Figures 5b and 5e) exhibited an increase in absorbance with increasing solvent ratio and scanning speed within the studied design space. The optimized operating conditions were identified

as a methanol-water ratio of 50:50 (% v/v) coupled with a moderate scanning speed, which provided the desired analytical performance for the simultaneous estimation of both LEN and PAL.

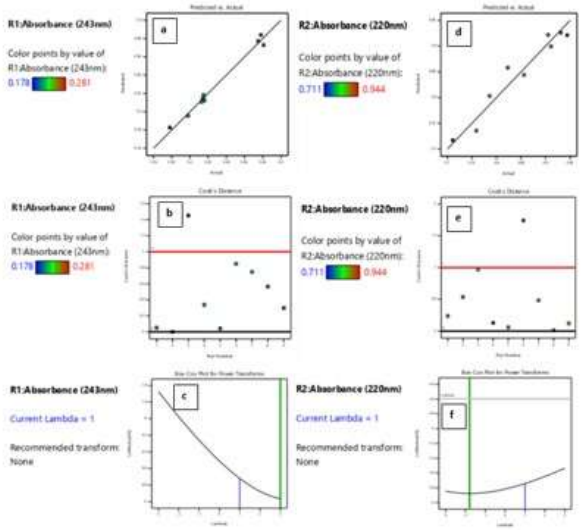


FIGURE 4: (a) Predicted versus Actual,(b) Cook's distance plot, (c) Box-Cox plots of Lenvatinib and (d) Predicted versus Actual,(e) Cook's distance plot, (f) Box-Cox plots of Palbociclcib

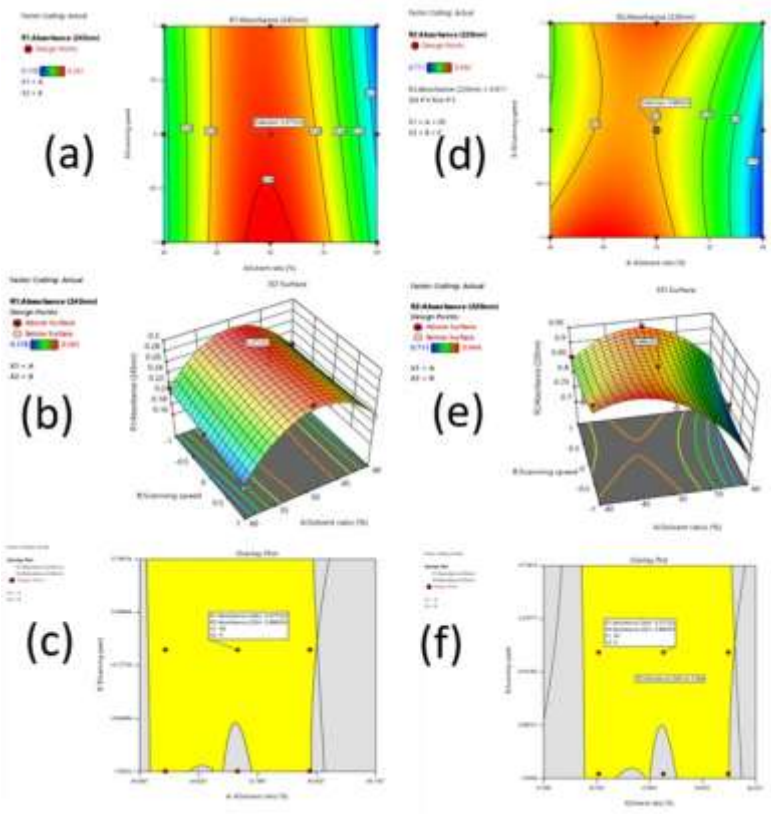


FIGURE 5 : (a,d) 2D contour plots of LEN and PAL, (b,e) 3-D contour plots of LEN and PAL and (c,f)Overlay plot of LEN and PAL depicting the design space generated from the central Composite Design (CCD) illustrating the optimal operating region

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iv. Method Validation

In compliance with ICH Q2(R2) guidelines, validation parameters including selectivity,specificity, linearity, precision, accuracy, LOD, LOQ, robustness, ruggedness and repeatability were thoroughly evaluated.

4.1.Linearity

Linearity of the proposed method was evaluated by assessing the correlation between analyte concentration and absorbance. A set of five solutions of LEN and PAL individually was prepared from a secondary stock solution. The resulting absorbance values were employed to generate a calibration curve from which the regression equation and correlation coefficient were calculated.(shown in figure 6 and Supplementary figure S1)

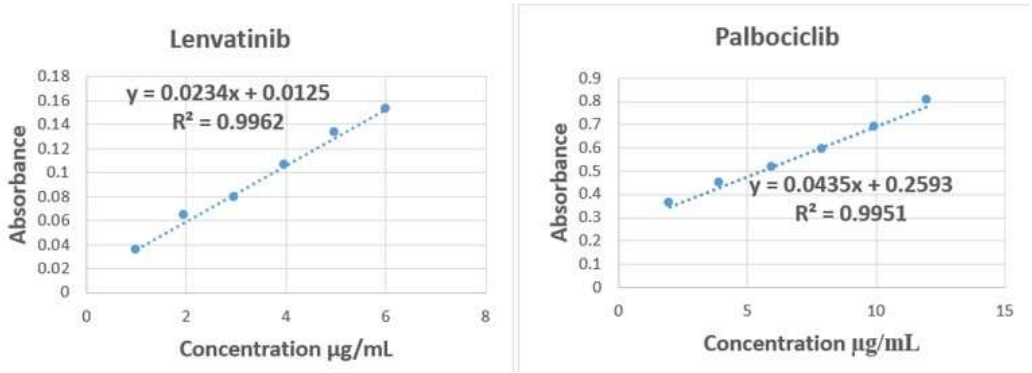


FIGURE 6: Linearity of Lenvatinib and Palbociclib having regression coefficient 0.996 and 0.9951 respectively

4.2. Specificity and Selectivity

The selectivity of the developed UV spectrophotometric method was demonstrated by its ability to accurately quantify LEN and PAL in the absence of interference from the solvent system. LEN and PAL exhibited distinct absorption maxima at 243 nm and 220 nm, respectively, while a well-defined isosbestic point was observed at 256 nm, confirming the suitability of the selected wavelengths for simultaneous estimation. Furthermore, the optimized solvent system showed no measurable absorbance at the selected analytical wavelengths, thereby confirming the specificity and selectivity of the proposed method.

4.3.Sensitivity

The developed UV-spectrophotometric method exhibited excellent sensitivity, as indicated by the low LOD and LOQ. The LOD values LEN and PAL were found to be 0.48 µg/mL and 0.22 µg/mL, respectively, whereas the corresponding LOQ values were 1.46 µg/mL and 0.683 µg/mL. These findings indicate that the proposed method is sufficiently sensitive for the reliable detection and accurate quantification of the two analytes, confirming its suitability for routine pharmaceutical quality control.

4.4.Precision

The precision of the developed UV spectrophotometric method was evaluated through intraday and interday studies to assess its reproducibility under normal analytical conditions. The %RSD values for both LEN and PAL were found to be below 2%, demonstrating excellent repeatability and intermediate precision. These results confirm the reliability and precision of the proposed method for regular analysis. (as shown in table 3 A and 3B)

TABLE 3 A: Intraday precision data of Lenvatinib and Palbociclib by UV method

Precision	Conc. (µg/ml)	LEN			PAL		
		Mean Absorbance	Mean SD	%RSD	Mean Absorbance	Mean SD	%RSD
Morning	2	0.193	0.0005	0.298	0.146	0.002	1.578
	6	0.479	0.001	0.208	0.343	0.001	0.336
	10	0.884	0.0005	0.065	0.544	0.0005	0.106

Afternoon	2	0.195	0.0005	0.295	0.147	0.0005	0.390
	6	0.480	0.001	0.208	0.350	0.001	0.329
	10	0.886	0.0005	0.065	0.546	0.0005	0.105
Evening	2	0.195	0.00057	0.295	0.148	0.0005	0.389
	6	0.470	0.002	0.491	0.338	0.001	0.340
	10	0.881	0.00057	0.065	0.543	0.0005	0.106

TABLE 3 B: Interday precision data of Lenvatinib and Palbociclib by UV method

Precision	Concentration (µg/ml)	LEN			PAL		
		Mean Absorbance	Mean Standard Deviation	%RSD	Mean Absorbance	Mean Standard Deviation	%RSD
Interday-1	2	0.192	0.001	0.520	0.144	0.001	1.202
	6	0.475	0.002	0.437	0.339	0.002	0.612
	10	0.888	0.0005	0.112	0.545	0.002	0.381
Interday-2	2	0.201	0.0005	0.286	0.160	0.001	1.082
	6	0.491	0.0005	0.117	0.368	0.0005	0.156
	10	10	0.881	0.0005	0.065	0.544	0.0005

4.5. Accuracy

Accuracy was performed for range 80%, 100% and 120%. The data is reported in table 4. All the %RSD for 80%, 100% and 120% are within the specification of less than 2%. The developed method demonstrated acceptable accuracy for both analytes. Accuracy of the analytical method was evaluated using the standard addition approach. The obtained percentage recovery ranged from 99.91–100.24% for LEN and 99.95–100.32% for PAL, while the corresponding percentage relative standard deviation (%RSD) values were between 0.56–0.63% and 0.19–0.29%, respectively. These results complied with the acceptance limits specified by ICH guidelines, confirming the reliability of the method.

TABLE 4 : Accuracy data for Lenvatinib and Palbociclib

Excess dug Level % (LEN)	Amount of analyte taken (µg/ml)	Amount of Standard analyte added (µg/ml)	Total amount (µg/ml)	Mean Standard Deviation	% RSD	% Recovery
80%	2	1.5	3.5	0.0005	0.156	98.91
100%	2	2	4	0.001	0.274	100.55
120%	2	2.5	4.5	0.001	0.215	99.56
Excess dug Level % (PAL)	Amount of analyte taken (µg/ml)	Amount of Standard analyte added (µg/ml)	Total amount (µg/ml)	Mean Standard Deviation	% RSD	% Recovery
80%	2	1.5	3.5	0.0005	0.228	100.03
100%	2	2	4	0.001	0.410	99.87
120%	2	2.5	4.5	0.001	0.189	101.13

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4.6.Ruggedness

Ruggedness was evaluated to determine the influence of analyst-to-analyst variation on the performance of the developed method. The %RSD values for both LEN and PAL were below 2%, indicating excellent reproducibility and consistency under normal operating conditions. These findings confirm that the proposed method is sufficiently rugged for routine pharmaceutical analysis.

4.7. Robustness

Robustness was assessed by introducing deliberate minor variations in the analytical wavelength to evaluate the reliability of the developed UV spectrophotometric method under slightly altered experimental conditions. The %RSD values remained below 2%, indicating that these small changes had no significant effect on the analytical performance. These findings confirm the robustness and suitability of the proposed method for routine pharmaceutical quality control.

4.8. Repeatability

The repeatability study demonstrated excellent precision, with %RSD values of 0.22% for LEN and 0.05% for PAL, both of which were below the acceptable limit of 2%. These findings confirm the repeatability and reliability of the developed UV spectrophotometric method for routine pharmaceutical analysis.

4.10. Forced Degradation Study

Forced degradation studies were carried out to understand how LEN and PAL behave under different stress conditions and to confirm that the developed UV method can reliably detect the drugs without interference. The samples were exposed to acidic, basic, oxidative, thermal and photolytic conditions. Noticeable changes in the spectra were observed under oxidative and Photolytic environments. Oxidative conditions caused more significant degradation, reflected by marked changes in absorbance. In comparison, acid, base and thermal exposure showed relatively moderate effects. (as shown in figure 7 and Supplementary table 2)

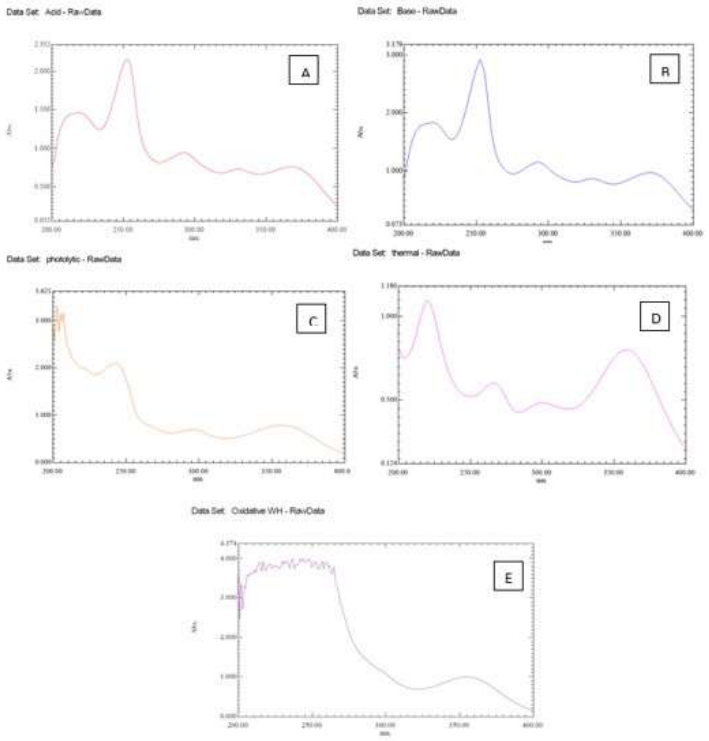


FIGURE 7: Forced degradation graphs of (a) Acid , (b) Base, (c) Photolytic, (d) Thermal and (e) Oxidative for the drug.

4.11. Liposome Preparation

LEN and PAL loaded liposomes were prepared using the thin-film hydration method. The optimized formulation exhibited favourable physicochemical characteristics, demonstrating the suitability of the formulation approach for liposomal drug delivery. Dynamic light scattering analysis revealed a nanosized vesicular system with an appropriate particle size (160nm), indicating its potential for enhanced cellular uptake and passive tumour targeting. The optimized formulation also exhibited a low polydispersity index (PDI) (0.428), suggesting a narrow particle size distribution, while the zeta potential value (-36 mV) showed excellent colloidal stability due to sufficient electrostatic repulsion between vesicles.(as shown in supplementary figure S2 and figure S3.)[31,32]

4.6. Assessment Methods for green analytical chemistry

The greenness and sustainability of the developed UV spectrophotometric method were comprehensively evaluated using AGREE, AGREEprep, NEMI, GAPI, Complex GAPI, White Analytical Chemistry (WAC), and BAGI tools (Figure 8). The AGREE assessment yielded a score of 0.72, indicating good compliance with the twelve principles of GAC and confirming the environmentally favorable nature of the developed method. Likewise, the AGREEprep evaluation produced a score of 0.71, demonstrating that the sample preparation procedure is simple, efficient, and associated with minimal environmental impact.

The NEMI pictogram showed compliance in three of the four quadrants, indicating the absence of persistent, bioaccumulative and toxic (PBT) chemicals, low waste generation, and non-corrosive analytical conditions. The only non-compliant quadrant corresponded to the use of a hazardous solvent, reflecting the presence of methanol in the analytical procedure. Nevertheless, the overall NEMI profile supports the environmental acceptability of the method.

The GAPI and Complex GAPI assessments exhibited predominantly green regions with only a few yellow and red segments, signifying minimal sample preparation requirements, low reagent consumption, reduced waste generation, and acceptable analytical complexity. These results further confirm the environmentally benign characteristics of the proposed method.

The BAGI assessment generated an overall score of 77.5, indicating satisfactory environmental performance while maintaining analytical efficiency and practical applicability for routine pharmaceutical analysis.

Furthermore, the White Analytical Chemistry (WAC) evaluation demonstrated excellent balance among analytical performance, environmental sustainability and practical efficiency. The method achieved scores of 94.0 for analytical performance (red component), 84.75 for environmental impact (green component) and 89.0 for practical and economic efficiency (blue component), resulting in an overall whiteness score of 89.25. These findings indicate that the method not only provides reliable analytical results but also maintains a high degree of sustainability and operational feasibility.

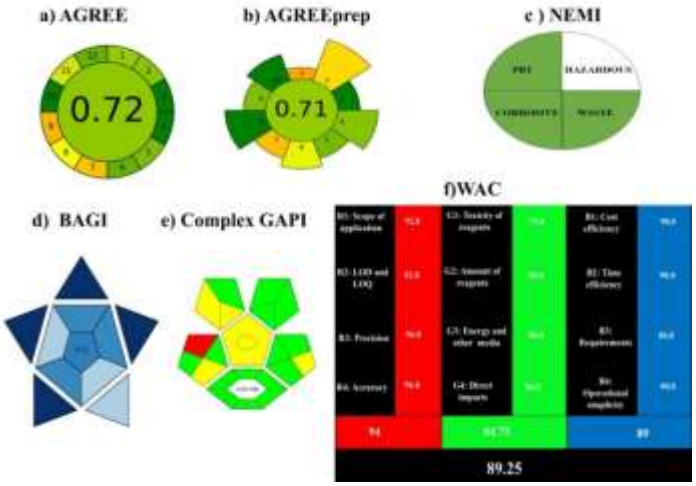


FIGURE 8: Greenness assessment of the UV spectrophotometric developed method using multiple Green Analytical Chemistry tools a) AGREE b) AGREEprep, c) NEMI, d) BAGI, e) Complex GAPI and f) WAC

Overall, the combined outcomes of AGREE, AGREEprep, NEMI, GAPI, Complex GAPI, BAGI, and WAC assessments confirm that the developed UV spectrophotometric method is environmentally friendly, economical, simple and suitable for routine quality control analysis while adhering to the principles of Green Analytical Chemistry and White Analytical Chemistry.

V. CONCLUSION

The development of the present UV spectrophotometric method using a CCD) based Design of Experiments (DoE) approach represents a significant advancement over conventional trial-and-error method development strategies. Systematic optimization of critical analytical parameters, including solvent ratio and scanning speed, enabled the establishment of a robust analytical design space and ensured reliable method performance. The CCD model demonstrated satisfactory statistical significance with acceptable ANOVA parameters, model F-values and adequate precision, confirming the suitability of the optimized conditions for simultaneous estimation of LEN and PAL.

The method was successfully developed based on the identification of a well-defined isosbestic point at 256 nm. LEN and PAL exhibited distinct absorption maxima at 243 nm and 220 nm, respectively, while the common isosbestic wavelength confirmed equal absorptivity and absence of spectral interference between the two drugs. This observation validates the applicability of the proposed method for simultaneous quantification of both analytes in a single analytical run.

The developed method exhibited excellent analytical performance, as evidenced by the high correlation coefficients ($r^2 = 0.9960$ for Lenvatinib and 0.9951 for Palbociclib) within the concentration ranges of 2-10 µg/mL and 2-10 µg/mL, respectively. The accuracy studies demonstrated recoveries close to 100%, while precision studies showed %RSD values below 2%, confirming the reproducibility and reliability of the method. Furthermore, the low LOD and LOQ values (0.48 and 1.46 µg/mL for Lenvatinib; 0.22 and 0.683 µg/mL for Palbociclib) indicate good sensitivity for routine pharmaceutical applications.

A notable strength of the present work is the comprehensive evaluation of stability indicating capability through forced degradation studies under acidic, alkaline, oxidative, thermal and photolytic stress conditions. Observable spectral changes under degradation conditions demonstrated the ability of the method to detect degradation-related alterations without compromising analyte estimation. Oxidative stress produced the most pronounced degradation effects, whereas thermal and photolytic conditions resulted in comparatively moderate changes. These findings confirm the suitability of the method for stability assessment and quality monitoring of pharmaceutical formulations containing Lenvatinib and Palbociclib.

Compared with previously reported chromatographic methods such as RP-HPLC, UPLC and LC-MS/MS, the proposed UV spectrophotometric method offers several practical advantages. It requires simpler instrumentation, shorter analysis time, lower operational cost and significantly reduced solvent consumption while maintaining acceptable accuracy, precision and sensitivity. These characteristics make the method particularly attractive for routine quality control laboratories and academic research settings where access to sophisticated analytical equipment may be limited.

An additional distinguishing feature of the present study is the comprehensive greenness assessment performed using multiple modern Green Analytical Chemistry metrics, including AGREE, AGREEprep, NEMI, ComplexGAPI, BAGI and White Analytical Chemistry (WAC). The AGREE score of 0.72, AGREEprep score of 0.71, BAGI score of 77.5 and WAC score of 89.25 collectively demonstrate the excellent environmental sustainability of the method. The use of a simple methanol-water solvent system, minimal reagent consumption, low energy requirements and reduced waste generation contributed substantially to the favorable green profile.

Overall, the developed CCD-optimized UV spectrophotometric method provides a reliable, economical, stability-indicating and environmentally sustainable analytical platform for the simultaneous estimation of Lenvatinib and Palbociclib. The method aligns with current regulatory expectations and emerging Green Analytical Chemistry principles, making it highly suitable for routine pharmaceutical analysis, formulation development and stability studies.

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VII. AUTHOR CONSENT

All authors confirm that they have read and approved the submitted version of the manuscript and consent to its submission to the journal.

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No external funding was received.

IX. DECLARATION OF COMPETING INTERESTS

The authors declare no known conflict of interest.

X. AUTHOR CONTRIBUTIONS

Shourya Jadhav: Conceptualisation, methodology development, experimental execution, data analysis, and manuscript drafting. **Bhaskar Kurangi**: Supervision, guidance throughout the project, critical review, and final manuscript approval. **Madhuri Sankpal**: Assistance in experimental work, data collection **Anjali Bawadekar:** technical support during the study, methodology development. **Priya Patil:**Assistance in Software, Manuscript review and editing

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