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Quality by Design-Assisted Green UV-Spectrophotometric Method Development and Validation for the Quantitative Estimation of Letrozole

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Letrozole, a potent aromatase inhibitor, is commonly used in treating hormone-responsive breast cancer. With the rising incidence of breast cancer and increased demand for anticancer agents, this study aims to develop a green, cost-effective, and precise UV-spectrophotometric method for Letrozole estimation using Quality by Design (QbD) and Green Analytical Chemistry (GAC) approaches. A UV-spectrophotometric method was developed using an eco-friendly solvent system to improve the solubility and analytical efficiency of Letrozole. Method optimization was carried out using a QbD to ensure robustness and sustainability. The method was validated as per ICH Q2(R1) guidelines for linearity, accuracy, precision, specificity, and robustness, confirming its suitability for routine analysis. The developed method exhibited excellent linearity within the concentration range of 2–10 µg/mL, with a correlation coefficient (R^2) of 0.9994. The limit of detection (LOD) and limit of quantification (LOQ) were determined to be 0.870 µg/mL and 2.021 µg/mL, respectively. Precision, robustness, and ruggedness assessments demonstrated percent relative standard deviation values below 2%, indicating high method reproducibility and reliability. The mean recovery values ranged from 99.71% to 102.40%, confirming the method's accuracy. Furthermore, the assay of marketed formulations yielded consistent results, supporting the suitability of the method for routine quality control applications. A novel, simple, eco-friendly, and QbD guided UV-spectrophotometric method was successfully developed and validated for the estimation of Letrozole in bulk and pharmaceutical dosage forms. The method is suitable for routine analytical applications due to its precision, accuracy, and environmental sustainability.

Keywords: Quality by Design (QbD); Design of Experiment; UV-Spectrophotometry; Letrozole; Green Analytical Chemistry.

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Introduction

Cancer remains a leading global health challenge, causing significant mortality and societal impact, particularly among women. Utilizing 2022 GLOBOCAN data, this study examines global incidence, regional patterns, and future projections, focusing on the ten most common cancers and their implications for prevention and control (1). Building upon this, an analysis of GLOBOCAN 2020–2024 data highlights pronounced disparities in cancer outcomes across regions. Mortality-to-Incidence Ratios (MIRs) are notably higher in low-Human Development Index (HDI) areas, underscoring the significant influence of socioeconomic factors such as healthcare spending and education on cancer prognosis. These findings emphasize the urgent need for targeted interventions to address and reduce these disparities. (2).
Breast cancer—being the most prevalent cancer among women worldwide—manifests in three primary subtypes: hormone receptor-positive/HER2-negative, HER2-positive, and triple-negative. Each subtype exhibits distinct prognoses and treatment responses, with triple-negative breast cancer associated with a higher risk of recurrence and limited targeted therapies, highlighting the necessity for subtype-specific management strategies. (3)
Anticancer drugs are classified based on their mechanisms of action, including DNA-interactive agents, antimetabolites, antitubulin agents, molecular targeting agents, hormones, monoclonal antibodies, and other biological agents. Despite advancements in targeted therapies, classical cytotoxic agents remain integral to many treatment regimens. Consequently, there's a growing need for precise analytical methods to detect and quantify these drugs across various matrices, have become indispensable due to their high sensitivity and specificity, facilitating accurate monitoring of anticancer drug levels in pharmaceutical formulations, biological samples, and environmental contexts. (4)
Letrozole is a third-generation, non-steroidal aromatase inhibitor used to treat hormone receptor-positive breast cancer in postmenopausal women, Figure 1. It works by competitively binding to the aromatase enzyme, inhibiting the conversion of androgens to estrogens, thereby reducing estrogen levels that can promote the growth of certain breast cancers. (5)
A review of the literature indicates that, several UHPLC-MS/MS HPLC methods have been developed for the quantification of letrozole and biological samples, (6-11) while letrozole formulation also estimated by chromatographic method (12-16). There is a scarcity of studies focusing on UV spectrophotometric methods, particularly those employing Design of Experiments (DoE) for method optimization. Moreover, existing UV methods often lack comprehensive greenness

assessments, highlighting the need for environmentally friendly analytical techniques (17-20). Therefore, developing a robust, QbD-based UV spectrophotometric method, optimized through DoE and evaluated for its environmental impact, is essential to enhance analytical efficiency and sustainability in letrozole determination.

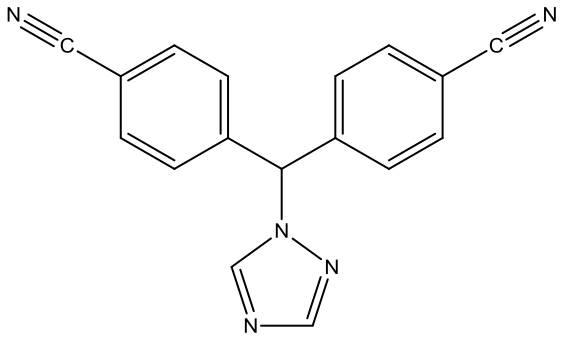

Figure 1. Chemical structure of Letrozole.

QbD is a systematic approach that integrates quality into analytical method development by emphasizing thorough process understanding and risk management. Utilizing Design of Experiments (DoE), QbD facilitates the identification and control of critical method parameters, leading to robust and reliable analytical procedures. This methodology enhances method performance, ensures consistency, and aligns with regulatory expectations for pharmaceutical analysis.

Design of Experiments (DoE) is a structured approach that systematically investigates the relationships between independent variables (e.g., solvent composition, pH, wavelength) and dependent variables (e.g., absorbance, linearity, sensitivity) in UV spectrophotometric method development. By varying these input factors methodically, DoE facilitates the identification of optimal conditions, enhances method robustness, and ensures reliable analytical performance. This approach is particularly valuable in developing precise and reproducible UV-based analytical methods (21).

Green Analytical Chemistry (GAC) aims to develop analytical methods that are environmentally friendly and safe for human health. This involves minimizing the use of hazardous reagents, reducing waste and energy consumption, and simplifying procedures. To assess the environmental impact of analytical methods, tools like the Analytical GREEnness (AGREE) calculator have been developed. AGREE evaluates methods based on the 12 principles of GAC, providing a comprehensive score and visual representation of a method's greenness. Such tools are instrumental in guiding the development of sustainable analytical procedures, including UV spectrophotometric methods (22).

Experimental section

Materials and Instrumentation

The absorbance measurements were conducted using a Shimadzu UV-1800 spectrophotometer. Prior to analysis, samples underwent sonication in an ultrasonic bath to ensure uniformity. Sample weights were precisely determined using a Sartorius BSA224S-CW electronic balance.

Chemicals and reagents

Analytical-grade methanol was used as the solvent, and the diluent was prepared using deionized water generated by a Milli-Q® Direct system (Millipore, USA) at KLE College of Pharmacy, Belagavi. Letrozole was kindly provided by Neon Laboratories Limited, Mumbai.

Method Development

Analytical method development is a systematic process aimed at creating reliable procedures for the identification, quantification, and analysis of chemical substances. In UV-Visible spectrophotometry, this involves selecting suitable solvents, determining optimal wavelengths, and establishing calibration curves to ensure accurate measurements.

Determination of Solvent and Wavelength

The UV-spectrophotometric method for Letrozole analysis was developed by selecting methanol as the primary solvent due to its high solubility for the drug, while a methanol: deionized water (20:80 v/v) mixture was used for further dilutions. This ratio was selected based on preliminary solubility and spectral clarity studies, which showed optimal drug solubility and stable baseline absorbance at this composition. To identify the optimal wavelength for analysis, a solution containing 10 µg/mL of the compound was scanned across the UV range of 200–800 nm. The resulting spectra indicated the maximum absorbance wavelength of 240 nm for Letrozole. The method's parameters, including solvent composition and scanning speed, were optimized using Design of Experiments (DoE) within a QbD framework to ensure robustness and reliability.

Preparation of Letrozole Standard Solution

A standard stock solution of Letrozole was prepared by accurately weighing 10 mg of the drug and dissolving it in 10 mL of methanol to achieve a concentration of 1000 µg/mL. For further dilution, the solution of methanol: deionized water (20:80 v/v) to obtain a final concentration of 100 µg/mL.

Preparation of Working Standard Solution

Appropriate aliquots of the 100 µg/mL stock solution were diluted with a methanol: deionized water (20:80 v/v) mixture to prepare concentrations of 2, 4, 6, 8, and 10 µg/mL. The absorbance of each solution was measured at 240 nm, the λmax for Letrozole.

Quantification of Letrozole in Tablet Formulation

To analyze Letrozole in tablet dosage form, twenty tablets were weighed and finely powdered. An amount equivalent to 10 mg of Letrozole was accurately weighed and transferred into a 10 mL volumetric flask, followed by the addition of methanol. The mixture underwent ultrasonic vibration for 25-30 minutes and was then filtered through Whatman filter paper. Appropriate dilutions were made to achieve a concentration of 2.5 µg/mL. The percent purity was calculated (19).

Optimization of Methodology Using QbD Principles

Analytical QbD is a structured approach to developing robust analytical methods. It begins with defining the Analytical Target Profile (ATP), which specifies the method's intended purpose and performance criteria. Subsequently, Critical Method Parameters (CMPs) are identified and assessed for their impact on Critical Method Attributes (CMAs) through risk analysis. The combination of CMPs that consistently yields desired CMAs defines the Design Space (DS), providing a multidimensional framework for method operation.

Define Analytical Target Profile (ATP)

The Analytical Target Profile (ATP) was established to guide the development of a robust, green UV spectrophotometric method for the quantitative estimation of Letrozole in pharmaceutical formulations. The method aims to achieve accurate and precise assay results, with performance criteria defined as follows: mean recovery within 98–102% for accuracy, relative standard deviation (%RSD) not exceeding 2% at the 100% assay concentration for precision, and a validated specification range spanning 80–120% of the test concentration, corresponding to 4.8–7.2 µg/mL.

Critical quality attributes (CQA)

In this study, absorbance at 240 nm was identified as the primary Critical Quality Attribute (CQA), as it directly reflects the method's ability to accurately quantify Letrozole. Linearity, precision, and accuracy were also considered supporting CQAs to ensure overall method reliability.

Risk assessment

Risk assessment was conducted to identify factors that could impact the Critical Quality Attributes (CQAs) of the developed UV-spectrophotometric method. An Ishikawa (Fishbone) diagram was used to categorize potential sources of variation, including solvent composition, scanning speed, instrument parameters, and analyst handling. Based on this assessment, solvent ratio and scanning speed were identified as high-risk factors and selected as Critical Method Parameters (CMPs) for further optimization using Design of Experiments (DoE).

Design space

The design space was established using Central Composite Design (CCD) and response surface methodology in Design-Expert® software. The Method Operable Design Region (MODR) was identified based on the combined effect of solvent composition and scanning speed on absorbance at 240 nm. The optimal region ensured consistent method performance and robustness, supporting reliable Letrozole quantification within the defined parameter range.

Control strategy

The control strategy was based on the MODR, focusing on maintaining solvent composition, scanning speed, and wavelength within optimized limits to ensure method robustness and alignment with the ATP (23).

Software- Assisted Optimization of Experimental Methods

Design of Experiments (DoE) is a structured approach used to plan, conduct, and analyze experiments efficiently by varying multiple factors simultaneously. It includes key stages such as screening, optimization, and robustness testing, aiming to identify critical variables, optimize outcomes, and ensure method reliability with minimal experiments and it includes following steps.

Determining Experimental Intentions

The objective of the study was to develop a robust UV-spectrophotometric method for the quantitative estimation of Letrozole using a QbD approach. Based on the chemical structure and UV absorbance properties of Letrozole, a detection wavelength of 240 nm and a methanol: deionized water (20:80 v/v) solvent system were selected for method development.

Determining Experimental Variables and Levels

In this study, solvent composition and scanning speed were selected as independent variables based on preliminary trials and literature. These controllable factors were evaluated at three levels using Design of Experiments (DoE) to assess their effect on the absorbance of Letrozole.

Determining Analytical Responses

Responses are dependent variables influenced by factor levels and selected based on study objectives. In this study, absorbance at 240 nm was selected as the primary analytical response, as it directly reflects the method's ability to quantify Letrozole accurately. This response was used to evaluate the effect of selected factors and optimize method performance using statistical tools. (24).

Determining the Experimental Design and Model

In UV spectrometry, Central Composite Design (CCD) was applied to optimize the UV-spectrophotometric method by evaluating the effect of solvent composition and scanning speed on Letrozole's absorbance. The design included center and axial points to explore potential interactions and quadratic effects, enabling efficient modeling of the response surface and identification of optimal conditions. (25).

Method Validation

The UV-Spectrophotometric method was validated according to the ICH Q2(R1) guidelines, using various validation parameters including specificity, selectivity, linearity, LOD, LOQ, precision, robustness, ruggedness, and accuracy, as detailed below.

Selectivity and Specificity

Selectivity is the ability of an analytical method to accurately measure an analyte in the presence of interferences. It is assessed by analyzing chromatographic blanks within the analyte's time window (26). To design an effective chromatographic system for analyzing a pharmaceutical active ingredient, it's crucial to

understand the drug's degradation potential, possible interference from degradants or precursors, and any interference from chemicals used in sample preparation or excipients. Degradation may occur due to factors like hydrolysis, oxidation, UV irradiation, heat, or light (27).

Linearity and Range

Linearity was assessed in the range of 2–10 µg/mL. The calibration curve showed a correlation coefficient (R^2) of 0.999. The intercept was statistically insignificant ($p > 0.05$), indicating minimal bias. A residual plot in Figure 4D. demonstrated homoscedasticity, confirming consistent variance across concentrations. Standard solutions were prepared in a methanol: deionized water (20:80 v/v) system, and their absorbance was measured at 240 nm.

Limit of Detection and Limit of Quantification

The Limit of Detection (LOD) and Limit of Quantification (LOQ) were determined based on the instrument's sensitivity using linearity experiments. The LOD is the lowest detectable concentration (signal-to-noise ratio 1:3), while the LOQ is the lowest reliably quantifiable concentration (signal-to-noise ratio 1:10). Values for Letrozole was calculated using calibration standards and the formulas LOD = 3 x σ/S and LOQ = 10 x σ/S, where σ is the standard deviation of the regression line's y-intercept and S is the slope of the calibration curve.

Precision

Precision reflects the consistency of test results when the method is repeatedly applied to multiple samples. It is measured by analyzing a series of standards or samples from a homogeneous lot, calculating the relative standard deviation (% RSD) using the standard deviation (SD) and mean values. The raw data is documented in the approved format, with acceptance criteria specified in the study plan. (26)

Robustness and Ruggedness

Robustness refers to an analytical method's ability to remain unaffected by small, intentional variations in parameters, indicating its reliability under normal conditions. Ruggedness assesses reproducibility across different conditions such as analysts, instruments, and laboratories. Key factors tested include flow rate, temperature, pH, and detection settings, depending on the analytical technique used.

Accuracy

Accuracy is determined by comparing the measured value to a known concentration. In this study, it was evaluated using recovery studies at 80%, 100%, and 120% levels by spiking samples and analyzing them with a UV spectrophotometer. Percentage recovery was calculated from six replicate trials (28).

Assay

The assay percentage was determined using a marketed formulation of Letrozole and the result remained within the acceptable limits (19).

Eco-Conscious Analytical Approach for Letrozole

The greenness of the developed UV-spectrophotometric method for Letrozole was evaluated using AGREE software, based on the 12 principles of Green Analytical Chemistry. The method achieved an overall AGREE score of 0.78, demonstrating good environmental sustainability. High scores were obtained for minimal sample and reagent use (0.98)**,** energy efficiency (1.0)**,** and complete avoidance of derivatization (1.0)**,** due to the method's simple design and low resource requirement. The solvent system used methanol: deionized water (20:80 v/v) supported moderate waste reduction (0.67) and ensured operator safety (0.8)**.** Additional strengths included reduced sample treatment (0.6)**,** preference for renewable reagents (1.0)**,** and multi-analyte compatibility (0.9)**,** making the method versatile and eco-friendly. While limitations were observed in in-situ analysis (0.33)**,** automation (0.5)**,** and use of non-toxic reagents (0.53)**,** the method overall aligns well with the principles of sustainable and green analytical development. (22).

Results and Discussion

Method Development

A methanol: deionized water (20:80 v/v) was used for solvent development, with Letrozole exhibiting maximum absorbance at 240 nm at medium scanning speed. Method optimization was carried out QbD principles, and the finalized parameters are listed in Table 1.

Table 1. UV analytical parameters and analytical target for the proposed method.

Sr No.	Parameters/ATP	Description
1	Analytical Technique	UV Spectroscopy
2	Instrument	UV-Spectrophotometer
3	Instrument Model	Shimadzu
4	Make	UV-1800
5	Software	UV-Probe
6	Target Compound	Letrozole
7	Solvent System	Methanol: deionized water
8	Wavelength	240nm
9	Sample Matrix	Letrozole marketed formulation
10	Performance Characteristics,	LOD, LOQ, precision, Accuracy, Assay

Method Optimization through QbD Framework

The method was systematically optimized using a QbD approach, as detailed in the methodology. Key steps included as below.

Analytical Target Profile (ATP)

The ATP defines the desired method performance based on scientific understanding. It was established through literature and drug profile review, targeting a fast, reliable, and economical UV spectrophotometric method for Letrozole. Table 1. outlines the ATP.

Critical Quality Attributes (CQAs)

CQAs are key analyte characteristics that must stay

within limits to ensure method quality. For this UV method, drug absorbance was the main CQA.

Critical Method Parameters (CMPs)

CMPs including detection wavelength, solvent composition, and scan speed were selected based on their impact on absorbance response, sensitivity, and method robustness. These were identified through initial screening studies and FMEA risk ranking.

Risk Assessment Using Fishbone Diagram

Fishbone (Ishikawa) diagram was used to assess potential risks and root causes affecting method performance. Parameters such as solvent type, wavelength, scan speed, sampling interval, and slit width were identified as significant risks (Figure 2).

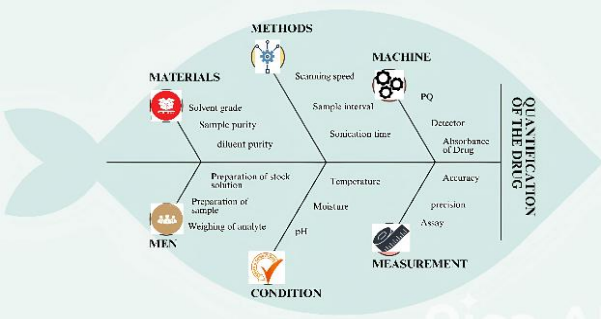

Figure 2. Ishikawa fishbone diagram.

To further prioritize risk factors, a Failure Mode and Effects Analysis (FMEA) was performed. Method parameters were scored based on severity, occurrence, and detectability. Wavelength, solvent composition, and scanning speed were identified as high-risk CMPs and selected for experimental evaluation using DoE. A heat map (Figure 3) was used to visualize risk prioritization.

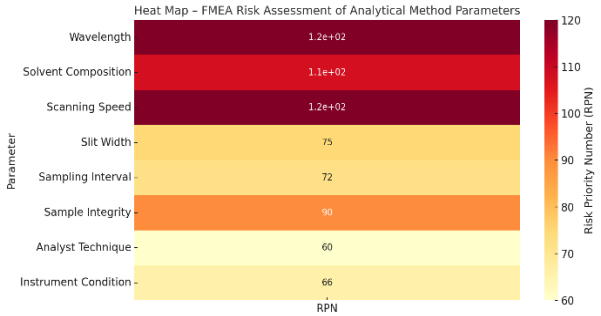

Figure 3. Heat map risk assessment of analytical method.

Optimization of the method through Design of Experiments software

The method was optimized using a Central Composite Design (CCD) in Design-Expert® Software v13 (Stat-Ease Inc., USA) in Table 2. Targeting scanning speed and solvent composition. A concentration of 2 µg/mL in methanol: deionized water (20:80 v/v), representing the lower end of the validated linearity range, was selected for DoE trials to ensure method robustness at low analyte levels. The CCD used an alpha (α) value of 1, indicating a face-centered design with axial points placed at the same level as the factorial points. Factor levels were set as: scanning speed – low (100 nm/min), medium (200 nm/min), high (300 nm/min); and solvent composition (methanol: water) – low (10:90 v/v), medium (20:80 v/v), high (30:70 v/v). Optimal conditions were selected based on maximum absorbance and desirability function. ANOVA confirmed the quadratic model's significance ($p < 0.05$, $F = 820.50$, adj. $R^2 = 0.9981$) These results support the use of a quadratic model to describe the relationship between scanning speed, solvent composition, and absorbance.

Table 2. CCD-Based experimental design matrix and optimization layout.

Run	Factor 1 A: Solvent ratio (%)	Factor 2 B: Scanning Speed	Response 1 R1: Absorbance (240)
1	30	0	0.285
2	20	0	0.285
3	20	1	0.284
4	10	-1	0.249
5	10	0	0.251
6	30	1	0.285
7	20	-1	0.283
8	30	-1	0.285
9	10	1	0.249

Figure 4 presents several graphical tools used to evaluate model adequacy and reliability. Figure 4a (Box-Cox plot) indicates an optimal lambda value of 1, confirming that the original, untransformed data is suitable for analysis and supports the assumptions of the quadratic model. Figure 4b (Cook's Distance plot) shows that all data points fall below the threshold, indicating the absence of influential outliers and confirming the reliability of the experimental runs. Figure 4c (Residuals vs. Predicted plot) displays a random scatter of residuals around zero, suggesting a good model fit with no systematic error or bias.

Figure 5a (2D Contour plot) illustrates that absorbance increases with higher methanol concentration, while scanning speed has a minimal effect within the studied range. The optimal region is located toward the higher methanol ratio zone. Figure 5b (3D Surface plot) further supports these findings, showing a curved response surface that confirms a quadratic relationship between the factors and the response.

Figure 6 (Overlay plot) identifies the method's design space, where the desired absorbance is achieved. The yellow-shaded area represents the acceptable region, and the marked point indicates the optimal combination of solvent ratio and scanning speed for robust method performance.

Analytical Method Validation

Validating the experimental design is essential to evaluate both risk and reliability in method development, especially under QbD framework. This process confirms the suitability of the mathematical model for correlating variables and achieving targeted results within the defined design space.

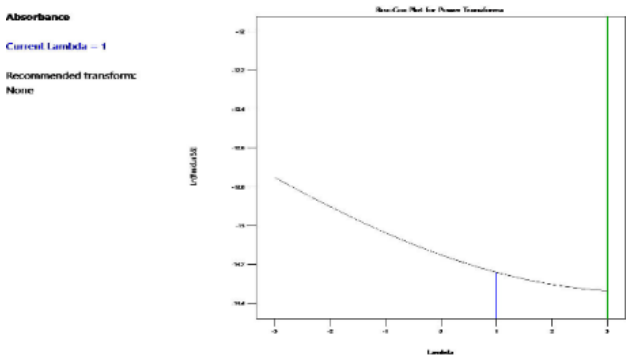

Figure 4(a) Box-Cox Plot.

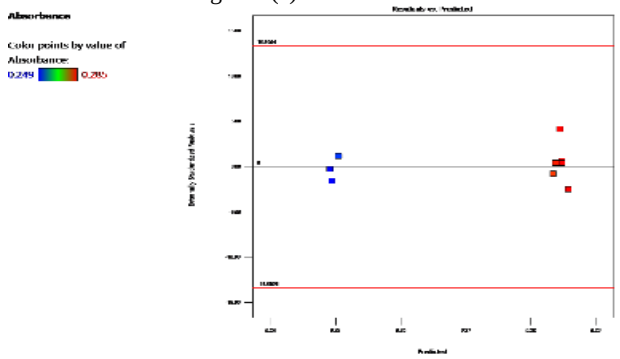

Figure 4(b) Cooks Distance plot Distance plot.

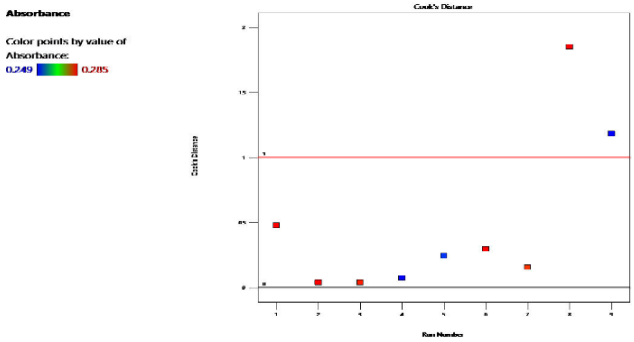

Figure 4(c) predicted vs. actual plot.

Figure 4. Graphics obtained from optimization model applied to the method development.

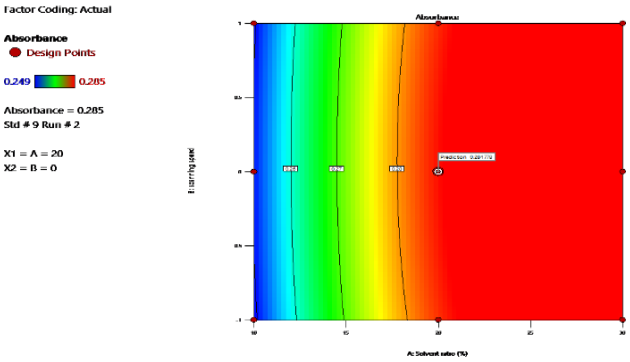

Figure 5(a) 2D contour plot

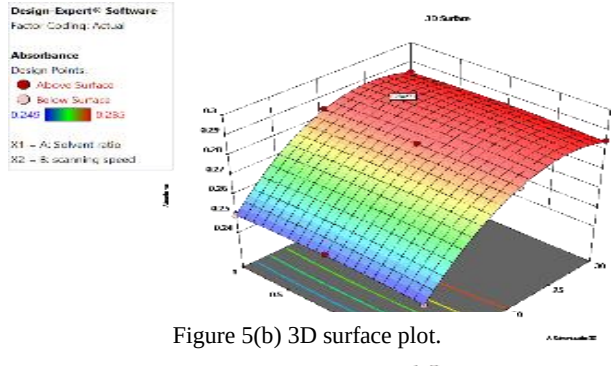

Figure 5(b) 3D surface plot.

Figure 5. Contour and surface plot obtained from method optimization model.

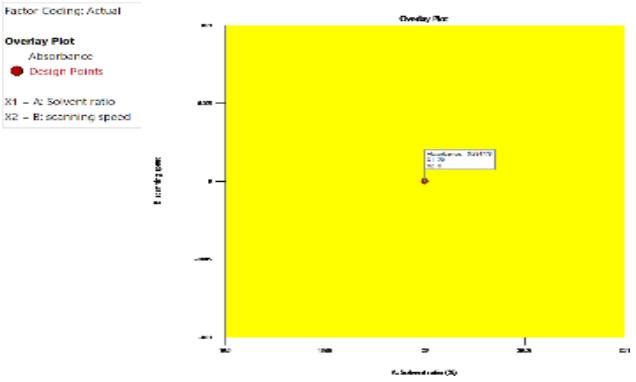

Figure 6. Overlay Plot obtained from method optimization model.

Specificity and Selectivity

The method showed no spectral interference at 240 nm, confirming its specificity for Letrozole, as supported by solvent and analyte spectra Figure 7 and 8.

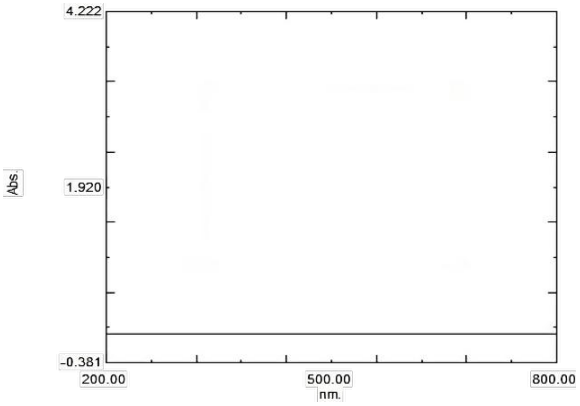

Figure 7. UV-Spectrum of Solvent used for method validation

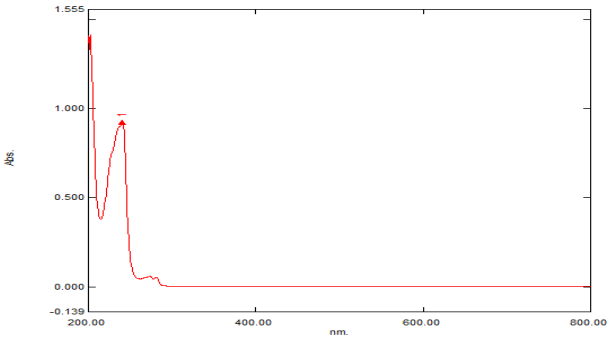

Figure 8. UV-Spectrum of Letrozole obtained during the method development.

Linearity and Range

Each line in the spectrum of Figure 9 represents Letrozole samples subjected to various concentration range dilution (Table 3). The observed differences in absorbance represent actual measured variations under these conditions. No vertical shift or graphical offset has been applied; the absorbance values are plotted as directly obtained from the spectrophotometric analysis.

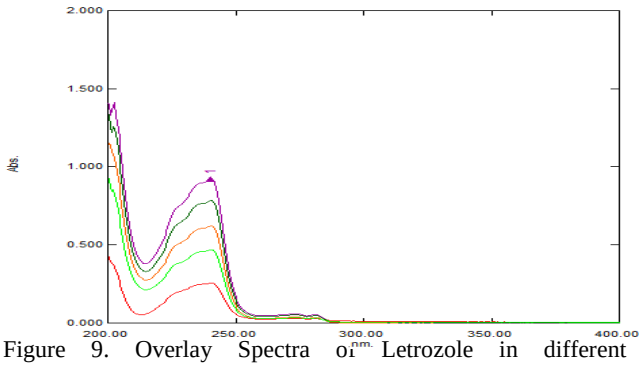

Figure 9. Overlay Spectra of Letrozole in different concentrations obtained during method development.

Figure 10 shows the calibration curve constructed for Letrozole over the concentration range of 2–10 µg/mL, with absorbance measured at its maximum wavelength. The linear regression equation ($y = 0.0968x + 0.0135$) and correlation coefficient ($R^2 = 0.9992$) indicate excellent linearity and suitability of the UV spectrophotometric method for quantitative analysis of Letrozole. This curve is essential for determining unknown concentrations in further analyses.

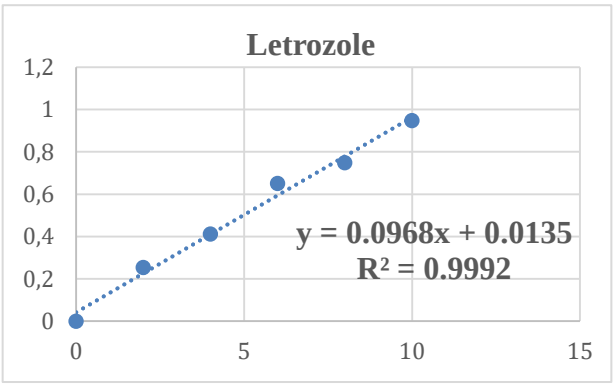

Figure 10. Calibration curve obtained for method linearity applied to Letrozole during validation of UV spectrophotometric method.

LOD and LOQ

The limit of detection (LOD) and limit of quantification (LOQ) for Letrozole were 0.870 µg/mL and 2.021 µg/mL, respectively.

Precision

Precision was confirmed with %RSD values under 2% across six replicates at 6 µg/mL, representing the assay-level concentration, demonstrating high reproducibility in Table 4.

Robustness and Ruggedness

The method remained unaffected by slight variations in wavelength and testing conditions, with %RSD within acceptable limits as in Tables 5. Confirming its robustness and ruggedness.

Accuracy

Recovery studies confirmed accuracy, with values falling within the accepted range at all levels Table 6.

Assay

The assay value for Letrozole was found to be 101.11%, indicating high method precision and reliability for quantifying Letrozole in marketed formulation in Table 6.

Table 3. Linearity data obtained for UV spectrophotometric method applied to Letrozole quantification

No.	Concentration (µg/ml)	Absorbance of Letrozole (240)
1	0	0
2	2	0.214
3	4	0.412
4	6	0.601
5	8	0.779
6	10	0.978

Table 4. Intraday and Interday precision data for UV spectrophotometric assay of Letrozole.

Concentration (µg/ml)	Intraday precision	%RSD	Intraday precision	%RSD
6	Morning	0.511	Interday-1	0.511
6	Afternoon	0.088	Interday-2	0.160
6	Evening	0.064	Interday-3	0.079

Table 5. Robustness and Ruggedness data obtained from validation of UV-spectrophotometric method applied to Letrozole.

Sr no.	Parameter	Method	%RSD
		238	0.381
I.	**Robustness**	240	0.301
1.	(λ max)	242	0.189
II.	**Ruggedness**	**(µg/ml)**	
1.	Change in Analyst	6	0.580
2.	Change in Instrument	6	0.119

Table 6. I. Accuracy and II. Assay data obtained from validation of UV spectrophotometric methpd applied to quantification of Letrozole.

Sr No.	Level (%)	Conc. (µg/ml)	Mean Recovery (%)	SD	%RSD
I.	80	4.8	99.71	0.008	0.160
	100	6.0	102.71	0.005	0.801
	120	7.2	99.80	0.001	0.198
II.	-	2.5	101.11	-	-

Greenness Evaluation of the Developed Method

Based on the greenness scoring scale (ranging from 0.00 to 1.00), values between 0.75 and 1.00 represent an excellent green method, scores from 0.50 to 0.74 are considered acceptable, while those below 0.50 indicate low environmental friendliness. As shown in the Figure 11. The developed analytical method achieved a greenness score of 0.78, classifying it as an excellent green approach.

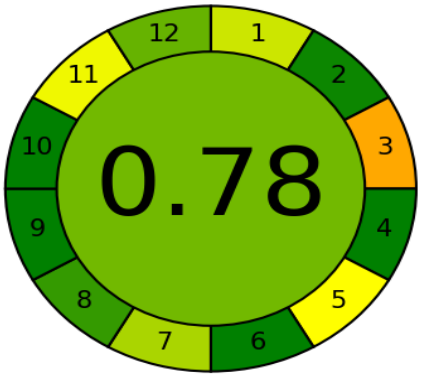

Figure 11. Greenness score for the UV spectrophotometric method applied to quantification of Letrozole.

Conclusions

The developed method is characterized by its simplicity, accuracy, precision, sensitivity, and cost-effectiveness, making it highly suitable for the quantification of Letrozole using a Quality by Design (QbD) framework. The systematic approach enhanced method robustness by identifying and minimizing risk factors to achieve the Critical Analytical Attributes (CAA). Key variables such as solvent ratio and scanning speed were optimized using experimental design and supported by statistical analysis, ensuring method reliability. The method exhibited excellent linearity across the range of 2–10 µg/mL for Letrozole, with percent recoveries between 99.71% to 102.40%, and %RSD below 2% for all validation parameters, confirming its precision and accuracy. The percent purity by assay was 101.11%. Additionally, the high greenness score (0.78) achieved through AGREE assessment indicates that the method aligns well with green analytical chemistry principles, offering a more environmentally sustainable alternative for routine analysis.

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Conflict of interest

The authors declare no conflicts of interest.

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