

DEVELOPMENT AND VALIDATION OF A ROBUST UV SPECTROPHOTOMETRIC METHOD FOR THE QUANTITATIVE ANALYSIS OF ANASTROZOLE IN BULK AND COMMERCIAL FORMULATIONS

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ABSTRACT

This study reports the development and validation of a novel, cost-effective UV-visible spectrophotometric method for the quantitative estimation of anastrozole in both bulk and tablet dosage forms. The method utilizes a double-beam UV-visible spectrophotometer, with maximum absorbance observed at 210 nm using a solvent mixture of phosphate buffer saline (pH 7.4) and methanol in a 9:1 ratio. Validation was performed in accordance with ICH Q2(R1) guidelines, assessing parameters such as linearity, precision, accuracy, limit of detection (LOD), and limit of quantification (LOQ). The method demonstrated excellent linearity in the concentration range of 4–20 µg/mL with a correlation coefficient (R^2) of 0.9996. Accuracy results ranged from 98.04% to 100.43%, and precision studies showed low relative standard deviation (RSD), indicating high reproducibility. Sensitivity analysis yielded LOD and LOQ values of 0.5723 µg/mL and 1.7344 µg/mL, respectively. Application of the method to Femistra tablets confirmed the anastrozole content to be consistent with the labeled claims. The results establish this method as a simple, sensitive, precise, and reliable analytical approach for routine quality control of anastrozole in pharmaceutical preparations.

Keywords: Anastrozole, UV-visible spectrophotometer, Correlation coefficient, λ_{max} , Assay, ICH[Q2(R1)]

INTRODUCTION

Anastrozole (ANS) is a nonsteroidal compound that functions as an inhibitor of estrogen production. Structurally similar to paclitaxel, it is classified as a fourth-generation aromatase inhibitor and acts by selectively and reversibly inhibiting aromatase, an enzyme complex belonging to the cytochrome P450 family, present in several tissues such as the breast, liver, and premenopausal ovaries (1). Aromatase is responsible for catalyzing the final steps in estrogen biosynthesis, specifically the conversion of androstenedione and testosterone into the estrogens estrone and estradiol (2). In estrogen-dependent breast cancers, ANS exhibits the potential to impede tumor growth. Due to its importance in cancer treatment, it is crucial to develop a reliable and accurate method for the analysis of ANS (3). Accurate and consistent analytical methods are imperative for confirming the quality, safety, and efficacy of ANS-based pharmaceutical formulations. UV spectrophotometry is particularly suitable for compounds with chromophores that absorb in the UV range, making it an ideal choice for ANS, which exhibits absorption in the ultraviolet region (4). This method's non-destructive nature and simplicity make it an attractive option for routine pharmaceutical analysis. This essay aims to discuss the development and validation of a UV spectrophotometric method for the analysis of ANS as per the ICH guidelines. The successful implementation of a validated

UV spectrophotometric method for ANS not only facilitates quality control during pharmaceutical manufacturing but also supports research and development efforts. The ability to accurately quantify ANS concentrations (conc.) in pharmaceutical formulations contributes to the overall understanding of drug behavior and aids in the optimization of dosage forms

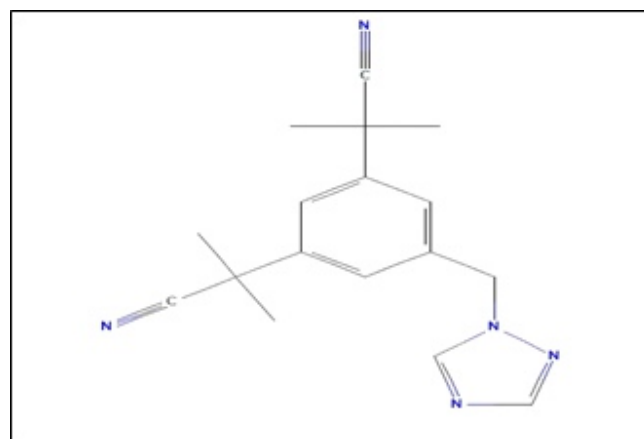


Fig. 1 Chemical structure of ANS.

ANS gift sample was procured from Natco Pharma Limited, Chemical Division, Telangana, India. A Shimadzu model UV-1900 i double-beam UV-visible spectrophotometer equipped with two identical quartz cells featuring a 1 cm light path and integrated with Shimadzu LabSolutions UV-Vis software was employed for spectrum recording and absorbance (Abs) measurements. Additionally, analytical precision was ensured through the use of a Mettler Toledo

electronic weighing balance with a sensitivity of 0.1mg, a DELUX model 101 digital pH meter, and a Sonica model 2200 MH sonicator. These state-of-the-art instruments were carefully selected to facilitate accurate and reliable data acquisition in the experimental procedures.

METHOD

Stock solution

A precisely measured quantity of 10 mg of ANS was wisely placed into a 10 mL volumetric flask. The flask was then filled to its mark with a blend of pH 7.4 PBS and methanol in a 9:1 ratio. To achieve a clear and homogeneous solution, the mixture underwent sonication using a bath sonicator. This process ensured thorough dissolution and uniform dispersion of ANS in the solvent mixture, setting the stage for subsequent analyses or applications (5).

Scanning and determination of maximum wavelength (λ_{max})

To determine the λ_{max} for the drug ANS, a solution containing the drug at a conc. of 100 $\mu\text{g/mL}$; in a mixture of pH 7.4 PBS and methanol in a 9:1 ratio was subjected to scanning using a spectrophotometer across the wavelength range of 200-400 nm, with a blend of pH 7.4 PBS and methanol in a 9:1 ratio serving as the blank (6).

Generating a calibration curve

A precise calibration curve for ANS was methodically developed by assessing the Abs of ANS solutions spanning conc. from 4 to 20 $\mu\text{g/mL}$. These solutions were created from carefully prepared stock solutions in a mixture of PBS (pH 7.4) and methanol. Triplicate measurements at 210nm were conducted to establish the calibration curve, effectively depicting the correlation between Abs (y-axis) and ANS conc. (x-axis).

Validation of Methods

The ICH Q2(R1) guideline provides a comprehensive framework for validating analytical procedures, ensuring that the method is appropriate and reliable for its intended application. Validation ensures the reliability and integrity of a measurement process, ensuring that it generates accurate and valid results (7). Method validation, as a systematic procedure, verifies the suitability of an analytical method for a particular test, and the insights gained from this validation process play a crucial role in defining the overall quality and dependability of analytical outcomes. Consequently, method validation stands

as an indispensable component of sound analytical practices. The method validation process involved an assessment of the following parameters: (8)

Linearity: Ensuring linearity in the method validation process is essential, as it confirms that the analytical method yields results balanced to the conc. of the analyte across a specified range. In validating our proposed method, we diligently prepared standard solutions with conc. of 4, 8, 12, 16, and 20 $\mu\text{g/mL}$ of ANS from a stock solution. Each conc. was meticulously analyzed in triplicate, ensuring the accuracy and reliability of the measurements (9).

Precision: Precision studies were carried out to evaluate the dependability of the suggested analytical method. **Repeatability** was judged by analyzing six reproduces of a 12 $\mu\text{g/mL}$ conc. Intraday precision was established by measuring the Abs of the 12 $\mu\text{g/mL}$ drug solution at three different times within a single day. Likewise, Interday precision was evaluated by following the same procedure on three different days. The outcomes were expressed as the p %RSD (10).

Accuracy: Accuracy refers to how closely the measured results align with the true values of the analyte. To evaluate this, a recovery study was performed by carefully preparing solutions at three concentration levels 80%, 100%, and 120% relative to the test solution.. Subsequently, the Abs of each of these solutions was measured in triplicate to ensure robust and reliable results (11, 12).

Repeatability: Repeatability was assessed through the analysis of six samples with identical drug conc. (12 $\mu\text{g/mL}$). The resulting Abs values were utilized to calculate both the standard deviation (SD) and RSD, providing insights into the consistency and precision of the analytical method (13).

Robustness and ruggedness: Robustness and ruggedness of the analytical method were assessed by varying parameters such as wavelength range. The robustness was scrutinized through systematic changes, while ruggedness was evaluated by multiple analysts operating under consistent operational and environmental conditions. The results were quantified in terms of the percentage (% RSD) (14).

LOD and LOQ: The LOD and LOQ followed the principles outlined in the ICH guidelines. The LOD, representing the minimum detectable conc. of an analyte in a sample, was computed using the formula:

$$\text{LOD} = 3.3 * \sigma / s$$

Similarly, the LOQ, signifying the lowest analyte conc. that can be quantitatively determined with acceptable levels of repeatability precision and trueness, was calculated using

$$LOQ = 10 * \sigma / s$$

In these formulas, σ denotes the SD (n=3) of the reagent blank determination, and s represents the slope of the calibration curve. This approach ensures a robust and standardized methodology for establishing the detection and quantification limits in analytical procedures (15, 16).

Assay of ANS

To prepare the sample stock solution, the contents of twenty Femistra tablets, each containing 1 mg of ANS, were weighed to obtain a quantity equivalent to 10 mg of anastrozole. This powdered sample was transferred into individual 10 mL volumetric flasks, to which approximately 1 mL of methanol was added. The volume was then brought up to the mark with phosphate buffer saline (PBS, pH 7.4), resulting in a stock solution of anastrozole with a concentration of 1000 μ g/mL. For the preparation of the working solution, 0.10 mL of this stock solution was accurately measured and diluted to 10 mL in a volumetric flask, producing a final concentration of 100 μ g/mL of ANS. This solution was utilized for absorbance measurements, and the recorded results were used for further analysis. ANS content analysis was conducted on sample solutions from marketed formulation, each with a conc. of 8 μ g/mL. These solutions were assayed against a reference standard (8 μ g/mL) to determine the ANS content in the respective marketed brands.

$$\text{Content of ANS (\% per tablet)} = \frac{A_s}{A_{st}} \times \frac{W_{st}}{w_s} \times \frac{w}{p} \times 100$$

Here, " A_s " represents the absorbance of the marketed sample solution, while " A_{st} " signifies the absorbance of the ANS reference standard solution. The variables " W_s " and " W_{st} " stand for the weights of the generic sample powder and ANS reference standard powder in milligrams (mg), respectively. The parameter " W " denotes the mean weight of the tablet in milligrams (mg).

RESULT AND DISCUSSION

Determination of λ_{max}

The ANS stock solution, with a conc. 1000 μ g/mL, underwent additional dilution to achieve a conc. of 100 μ g/mL by a blend of PBS (pH 7.4) and methanol (9:1 ratio). Subsequently, the resulting solution's Abs was scanned within the 200-400 nm range using a UV spectrophotometer. The observed λ_{max} was identified at 210 nm, as depicted in Figure 2. The robust linearity of the calibration curve of the drug.

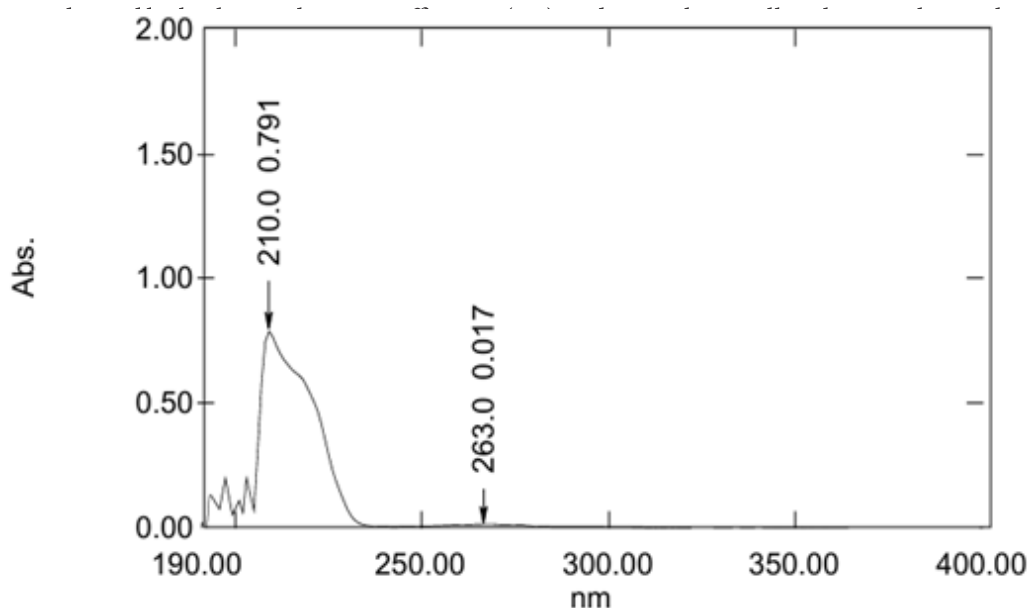


Fig. 2 Absorption spectrum of ANS showing maximum Abs at 210 nm.

Conc. (µg/mL)	Abs	$y = 0.0401x - 0.0016$ $R^2 = 0.9996$
0	0	
4	0.165	
8	0.314	
12	0.471	
16	0.637	
20	0.807	

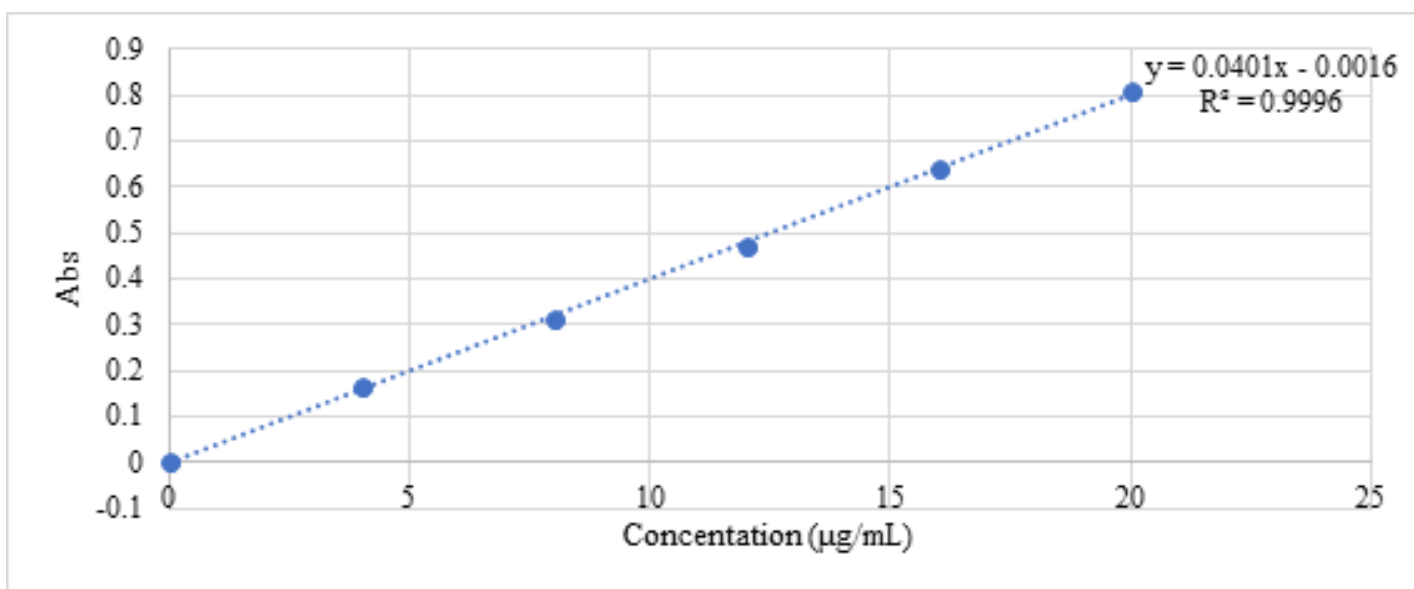


Fig. 3 Calibration curve for ANS within a blend of PBS (pH 7.4) and methanol 9:1 ratio, with measurements conducted at 210nm

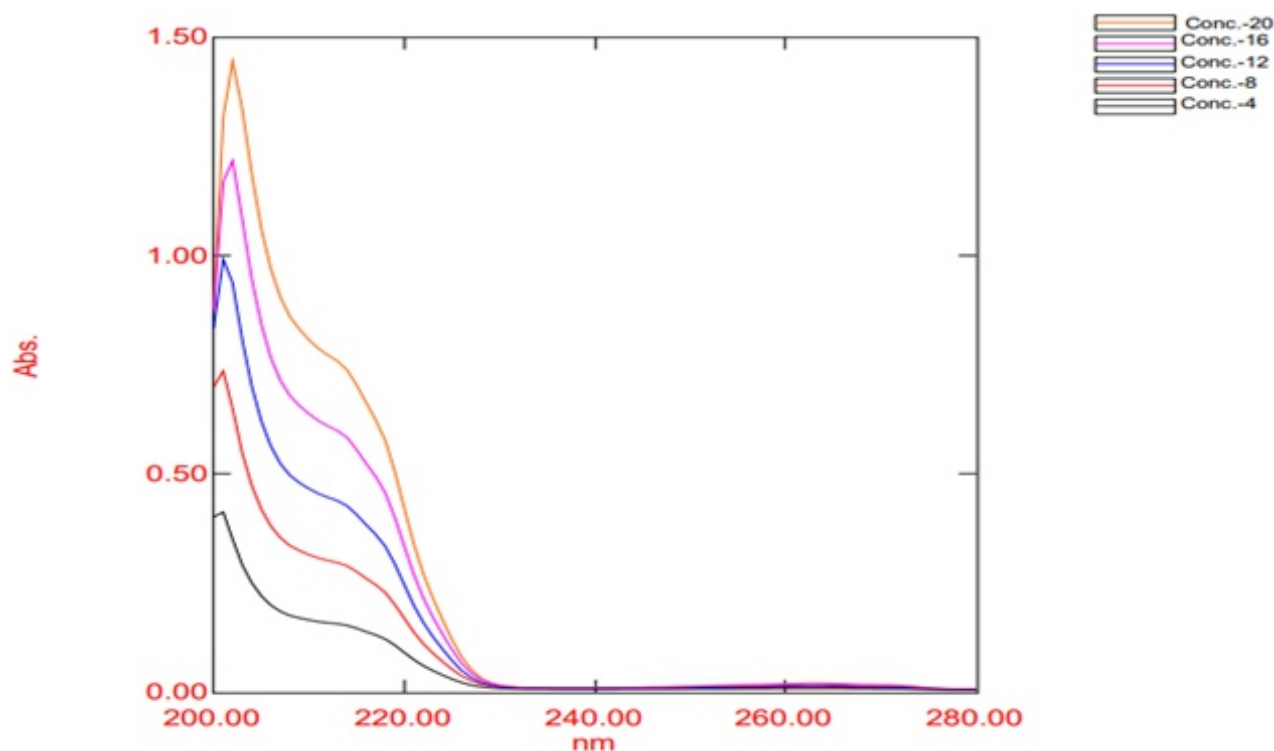


Fig. 4 Superimpose the spectra of ANS in a PBS with a pH of 7.4 and methanol using a 9:1 ratio.

Precision (Intraday and Interday Study)

Intraday precision

Intraday precision was evaluated by conducting three analyses of the drug at a defined Conc. on the same day, with breaks of 3 hours, specifically at 10:00 am, 1:00 pm, and 4:00 pm. The %RSD values were calculated and found to be within the range of 0.652%, confirming the reproducibility of the method.

Interday precision

Interday precision was assessed by analyzing samples on three successive days. The %RSD values, calculated for the experiment, consistently fell within the range of 0.857%, affirming the reproducibility of the method. The precision results underscore the reliability and consistency of the current methodology, indicating its robustness. Detailed precision outcomes are provided in Tables 2 and 3. Furthermore, the proportion of relative variance was computed to provide additional insight into the method's precision

Table 2 Intraday Precision

Conc. (µg/mL)	Abs (10:00 am)	Abs (1:00 pm)	Abs (4:00 pm)	Statistical Analysis
12	0.476	0.483	0.469	Mean = 0.475 SD = 0.003103 % RSD = 0.652
12	0.467	0.477	0.473	
12	0.474	0.479	0.475	
12	0.472	0.482	0.476	
12	0.469	0.480	0.478	
12	0.474	0.484	0.477	
Mean	0.472	0.480	0.474	
SD	0.003405	0.002639	0.003266	
%RSD	0.721	0.548	0.688	

Table 3 Interday Precision.

Conc. (µg/mL)	Abs (Day 1)	Abs (Day 2)	Abs (Day 3)	Statistical Analysis
12	0.476	0.470	0.464	Mean = 0.466 SD = 0.004002 % RSD = 0.857
12	0.467	0.463	0.458	
12	0.474	0.471	0.468	
12	0.472	0.462	0.455	
12	0.469	0.460	0.463	
12	0.474	0.472	0.460	
Mean	0.472	0.467	0.461	
SD	0.003405	0.004472	0.004131	
%RSD	0.721	0.957	0.894	

Accuracy

Accuracy was assessed through recovery studies using the standard addition method at three concentration levels, 80%, 100%, and 120% of the drug concentration. Known amounts of the standard were added to previously analyzed samples, and the percentage recovery was subsequently calculated to determine the method's reliability. The findings from the recovery experiment confirm the lack of interference from common pharmaceutical additives and excipients. The mean recovery values,

spanning from 98.04% to 100.43%, underscore a strong performance well within the defined limits. A comprehensive presentation of the recovery study results is available in Table 4, aligning consistently with the expected criteria.

Table 4: Evaluation data of the accuracy study

%Recovery Level	Average %Recovery	%RSD
80%	98.61	0.567
100%	98.04	0.318
120%	100.43	0.247897735

Repeatability

The instrument's repeatability was confirmed by measuring the Abs of six samples at a consistent Conc. of 16µg/mL but in varying working solvents. The SD and %RSD were found to be within acceptable limits, as detailed in Table 5. Validating the repeatability of the methodology is pivotal for the routine analysis of drug content in both bulk and formulated samples. The results obtained indicate that there were no significant variations in outcomes upon repeating the methodology.

Table 5 Results of repeatability studies

Conc. (µg/mL)	Abs	Statistical Analysis
16	0.637	Mean = 0.642 SD = 0.004844 %RSD = 0.753
16	0.649	
16	0.648	
16	0.642	
16	0.639	
16	0.641	

Robustness

Robustness studies were carried out with the presumption that slight variations in any of the variables would not significantly influence the outcomes. The study confirmed the dependability of the proposed method during routine analyses and indicated that modifications in instruments did not produce any noteworthy impact on the obtained results. The observed RSD was determined to be consistently below 2%, well within the specified limit, as depicted in Table 6.

Table 6 Findings from robustness investigations and subsequent statistical analyses

Conc. (µg/mL)	Abs	Statistical Analysis
Wavelength: 210 nm		Mean = 0.472 SD = 0.003405 %RSD = 0.721
12	0.476	
12	0.467	
12	0.474	
12	0.472	
12	0.469	
12	0.474	
Wavelength: 211 nm		Mean = 0.459 SD = 0.003162 %RSD = 0.688
12	0.454	
12	0.457	
12	0.461	
12	0.463	
12	0.459	
12	0.46	

Ruggedness

Ruggedness assessment was conducted by analyzing samples at a Conc. of 12 µg/mL with different analysts. The resulting Abs readings were recorded, and the outcomes were expressed as the %RSD. Ideally, a %RSD less than 2 is desirable, as depicted in Table 7. The ruggedness analysis demonstrated that there were no significant changes in the reliability and repeatability of the methodology, even when different analysts were involved.

Table 7 Findings from ruggedness investigations and subsequent statistical analyses.

Conc. (µg/mL)	Abs	Statistical Analysis
Analyst 1		Mean = 0.461 SD = 0.003386 %RSD = 0.734
12	0.465	
12	0.459	
12	0.469	
12	0.456	
12	0.461	
12	0.474	
Wavelength: 211 nm		Mean = 0.466 SD = 0.003559 %RSD = 0.762
12	0.47	
12	0.464	
12	0.471	
12	0.468	
12	0.462	
12	0.465	

LOQ and LOD:

The determined values for the LOQ and LOD were 1.7344 µg/mL and 0.5723 µg/mL, respectively.

Table 8 Assay of laboratory mixture by UV-Visible Spectrophotometer.

Drug	Label claim (mg)	Quantity found (mean ± SD, mg)	%Estimation (mg/tab)	%RSD
ANS	1	0.992±0.008	99.2	0.80

Table 9 Validation parameters.

Validation parameter	Result
Absorption maxima (λ max)	210nm
Linearity Range	4-20 µg/mL
Regression Equation	y = 0.0401x - 0.0016
Intercept	0.0016
Slope (m)	0.0401
Correlation Co-efficient (R ²)	0.9996
%RSD for Intraday Precision	0.652
%RSD for Interday Precision	0.857
Repeatability (%RSD)	0.753
LOD	0.5723
LOQ	1.7344

CONCLUSION

In accordance with ICH Q2(R1) guidelines, the analytical method was successfully validated, fulfilling all predefined acceptance criteria. The thorough evaluation confirms that the method exhibits essential parameters such as specificity, precision, linearity, accuracy, robustness, and the ability to indicate stability. Compared to HPLC, the UV-visible spectrophotometer is not only simpler but also more cost-effective. Its ease of use and user-friendly design make it a superior choice compared to previously documented methods. As a result, it can be confidently asserted that this analytical method is well-suited for its intended purpose.

DECLARATIONS

Funding

None

CONFLICTS OF INTEREST

The authors declare that they have no conflict of interest.

ETHICS APPROVAL

Not applicable

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