RESEARCH PAPER

"Development and Validation of a UV Spectrophotometric Method for the Estimation of Ritonavir and Its Application in Solid supersaturated Self-Emulsifying Drug Delivery Systems (SEDDS) for Enhanced Oral Bioavailability"

Pallavi Pradeep Potadar[1], Dr. Bhuvaneshwari R. S.[1*]

[1]Department of Pharmaceutical Quality Assurance, KLE College of Pharmacy, Basavan Kudachi, Belagavi-591124, KLE Academy of Higher Education and Research, Belagavi, Karnataka, India

Corresponding author: Prof (Dr.) Bhuvaneshwari R. S. | Associate Professor, Department of Pharmaceutical Quality Assurance, KLE College of Pharmacy Belagavi, Basavan Kudachi, Belagavi- 591124, KLE Academy of Higher Education and Research (KAHER), Belagavi, Karnataka, India | Email: brsharannavar@klepharm.edu

ORCID ID: Prof (Dr.) Bhuvaneshwari R. S. - 0000-0001-9296-6782

Author Emails: Pallavi Pradeep Potadar - pallavipotadar830@gmail.com | Dr. Bhuvaneshwari R. S. - brsharannavar@klepharm.edu

ABSTRACT

The present study focuses on the development and validation of a UV spectrophotometric method for the quantitative estimation of a selected drug in bulk and/or formulation. The method was optimized by selecting an appropriate solvent system and determining the maximum absorbance wavelength (λmax) to ensure sensitivity and specificity. The developed method was validated in accordance with standard analytical parameters including linearity, accuracy, precision, limit of detection (LOD), and limit of quantification (LOQ). The calibration curve showed good linearity over the selected concentration range with a high correlation coefficient, indicating reliability of the method. The accuracy and precision studies confirmed the reproducibility of the method with minimal %RSD values. The sensitivity of the method was adequate as evidenced by low LOD and LOQ values. The proposed UV method is simple, rapid, cost-effective, and does not require complex sample preparation. Therefore, it can be effectively applied for routine quality control analysis of the drug in pharmaceutical formulations.

Keywords: Ritonavir, UV spectrophotometry, ICH Q2 (R2), Solid supersaturated SEDDS.

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1. INTRODUCTION

Human Immunodeficiency Virus the causative agent of acquired immunodeficiency syndrome, remains a major global health concern despite advances in antiretroviral therapy. Protease inhibitors play an important role in HIV treatment by inhibiting viral maturation. Ritonavir is a potent protease inhibitor widely used in combination therapy and also acts as a pharmacokinetic enhancer by inhibiting CYP3A4 and P-glycoprotein, thereby improving the bioavailability of co-administered drugs. Therefore, accurate quantification of Ritonavir in bulk and pharmaceutical formulations is essential for ensuring drug quality, efficacy, and safety [1].

Ritonavir is a potent antiretroviral drug belonging to the class of protease inhibitors used in the treatment of HIV infection. It inhibits the HIV protease enzyme, thereby preventing viral replication and maturation of infectious particles. Ritonavir is also widely used as a pharmacokinetic enhancer due to its ability to inhibit CYP3A4 enzymes, which improves the bioavailability of co-administered drugs. Because of its significant therapeutic role, accurate analysis of Ritonavir in pharmaceutical formulations is essential for ensuring quality, safety, and efficacy [2].

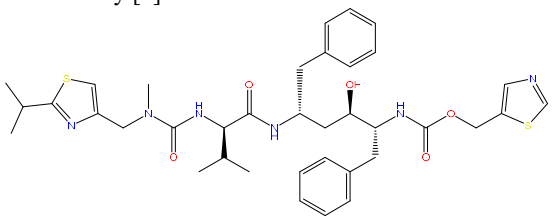


Figure 1: Chemical Structure of Ritonavir

Various analytical techniques such as UV-visible spectrophotometry, HPLC/UPLC, and LC–MS/MS have been reported for the estimation of Ritonavir. Among these methods, UV spectrophotometry is widely preferred because of its simplicity, rapid analysis, and cost-effectiveness. However, limitations such as spectral interference and inability

to distinguish degradation products restrict its application in stability and regulatory studies. To overcome these challenges, the Analytical Quality by Design (AQbD) approach offers a systematic and science-based strategy involving risk assessment, identification of critical quality attributes (CQAs), and application of Design of Experiments (DoE) to develop robust and reliable analytical methods [3].

Despite advancements in analytical method development, no QbD-based stability-indicating UV spectrophotometric method has been reported for the estimation of Ritonavir in Solid Supersaturated Self-Emulsifying Drug Delivery Systems (SS-SEDDS). Therefore, the present study aimed to develop and validate a simple, economical, robust, and stability-indicating UV spectrophotometric method based on AQbD principles for the estimation of Ritonavir in SS-SEDDS formulations [4].

2. METHODOLOGY

2.1 Materials:

Ritonavir was procured from Satyadeeptha Pharmaceuticals Ltd. Industrial Area, Humnabad, Bidar, Karnataka. India. All other chemicals and reagents used were of analytical grade and procured from KLE College of Pharmacy, Belagavi, India.

2.2 Instrumentation:

A Shimadzu UV-1900i spectrophotometer equipped with Lab Solutions software was used for all spectrophotometric measurements. Method optimization was performed using Design-Expert software (version 13.0, Stat-Ease Inc., Minneapolis, MN, USA). All measurements were performed at room temperature using double-distilled water as the solvent blank.

2.3 Selection of Solvent:

Various analytical solvents such as water, ethanol, methanol, and dimethyl sulfoxide (DMSO) were screened during the preliminary studies. Among these, methanol was selected as the suitable solvent for method development due to its superior solubility and better spectral clarity of Ritonavir.

2.4 Preparation of Stock Solution:

Accurately weighed Ritonavir (10 mg) was dissolved in methanol to prepare the primary stock solution. From this solution, 1 mL was further diluted to 10 mL with methanol to obtain the secondary stock solution. Working standard solutions in the concentration range of 4–20 µg/mL were prepared by appropriate dilution with water and sonicated for 5 minutes before analysis.

2.5 Selection of Wavelength:

Ritonavir was scanned in the wavelength range of 200–400 nm using a UV spectrophotometer to determine its maximum absorbance wavelength (λmax). Ritonavir showed maximum absorbance at 240 nm.

2.6 Method Validation

The developed method was validated according to ICH Q2 (R1) guidelines, assessing specificity, linearity, precision and accuracy, limit of detection (LOD), limit of quantification (LOQ), robustness, ruggedness, and repeatability [5,6].

2.6.1 Specificity and Selectivity

Specificity was assessed by possible interference from formulation excipients. No significant absorbance was observed at 239 nm or 231 nm in blank samples, confirming reliable selectivity for drug.

2.6.2 Linearity

Linearity of the developed method was evaluated over the concentration range of 4–20 µg/mL by constructing calibration curves of absorbance versus concentration. The regression equation and correlation coefficient (R^2) were determined to establish the linear relationship between concentration and absorbance.

2.6.3 Precision

Precision of the method was evaluated in terms of repeatability, intraday precision, and interday precision. Three different concentrations of Ritonavir were analyzed in triplicate on the same day for intraday precision and on three consecutive days for interday precision. The percentage relative standard deviation (%RSD) was calculated to assess the reproducibility and precision of the developed method.

2.6.4 Accuracy

Accuracy of the developed method was determined by recovery studies at three concentration levels, namely 80%, 100%, and 120% of the target concentration. The percentage recovery was calculated to evaluate the closeness of the measured values to the actual amount of Ritonavir present in the sample.

2.6.5 Sensitivity (LOD and LOQ)

Sensitivity of the developed method was evaluated by determining the limit of detection (LOD) and limit of quantification (LOQ). These parameters were calculated based on signal-to-noise ratios of approximately 3:1 for LOD and 10:1 for LOQ.

2.6.6 Ruggedness

Ruggedness of the developed method was evaluated by conducting the assay using different analysts and instruments under varied laboratory conditions. The percentage relative standard deviation (%RSD) was calculated to confirm the reproducibility and reliability of the method.

2.6.7 Robustness

Robustness of the developed method was evaluated by introducing deliberate minor changes in analytical parameters, including variation of ±2 nm from the λmax. The percentage relative standard deviation (%RSD) was calculated to assess the stability and reliability of the method under small procedural variations.

2.6.8 Repeatability

Repeatability was determined by measuring the absorbance of Ritonavir six times at 6 µg/mL. The %RSD was calculated to confirm consistent performance under the same operating conditions.

2.7 Forced Degradation Study

Forced degradation studies were performed to evaluate the stability-indicating capability of the developed method. Ritonavir was subjected to various stress conditions, including acidic hydrolysis (0.1 N HCl, 80°C, 2 h), alkaline hydrolysis (0.1 M NaOH, 80°C, 2 h), oxidative degradation (30% H_2O_2, 80°C, 2 h), and thermal degradation (40°C, 4 h). After treatment, the samples were diluted to 10 µg/mL with water and scanned in the wavelength range of 200–400 nm. Significant degradation was observed under the applied stress conditions, confirming the ability of the method to distinguish between degraded and non-degraded drug [7].

2.8 Preparation of Solid Supersaturated SEDDS of Ritonavir

Solid Supersaturated Self-Emulsifying Drug Delivery System (SS-SEDDS) of Ritonavir was prepared by dissolving the drug in a mixture of Clove oil, Tween 80 and PEG 400 under continuous stirring to obtain a clear and homogeneous liquid SEDDS. A suitable polymer (HPMC and Ethanol) was incorporated to maintain the supersaturated state and prevent drug precipitation. The prepared liquid formulation was then adsorbed onto a solid carrier (Neusilin) with continuous mixing to obtain a free-flowing powder. The resulting SS-SEDDS was dried, passed through a sieve, and stored in an airtight container for further evaluation and characterization [8,9].

2.9 Application of the Method to Solid Supersaturated SEDDS

The validated UV spectrophotometric method was successfully applied for the estimation of Ritonavir in the prepared Solid Supersaturated SEDDS formulation. An accurately weighed quantity of the formulation was dissolved in methanol, sonicated to ensure complete extraction of the drug, and suitably diluted for analysis. The absorbance was then measured at the selected λmax of Ritonavir using a UV spectrophotometer [10].

2.10 Greenness Assessment of the Developed Method

The sustainability of the developed UV spectrophotometric method for Ritonavir was evaluated using Complex GAPI, AGREE, and BAGI assessment tools. The overall evaluation confirmed that the method possesses minimal environmental impact along with good analytical efficiency and practical applicability [11].

Complex GAPI analysis was performed to assess the environmental impact of different stages of the analytical procedure, including sample preparation, solvent usage, instrumentation, energy consumption, and waste generation. The predominance of green and yellow zones in the pictogram indicated low solvent toxicity, minimal sample handling, and reduced energy requirements, confirming the eco-friendly nature of the method [12].

The AGREE metric was used to evaluate compliance with the twelve principles of Green Analytical Chemistry. The obtained AGREE score demonstrated that the developed UV spectrophotometric method is environmentally sustainable due to its low solvent consumption, simple instrumentation, and minimal energy demand [13].

Furthermore, the Blue Applicability Grade Index (BAGI) was applied to assess the overall sustainability of the method by integrating environmental, analytical, and practical aspects. BAGI considers factors such as solvent safety, waste generation, analyst safety, operational simplicity, and analytical performance. The high BAGI score obtained for the developed method indicated an excellent balance between analytical reliability and environmental sustainability. Overall, the greenness assessment confirmed that the proposed UV spectrophotometric method is simple, eco-friendly, robust, and suitable for routine estimation of Ritonavir in Solid Supersaturated SEDDS formulations [14].

3. RESULT AND DISCUSSION

3.1 Selection of Solvent and Wavelength

Methanol was selected as the suitable solvent after screening different solvents based on drug solubility and spectral clarity. Water was used as the blank throughout the experimental study. Ritonavir solution (10 µg/mL) was scanned in the wavelength range of 200–400 nm using a UV spectrophotometer, and the drug exhibited maximum absorbance (λmax) at 240 nm.

3.2 Method Validation.

Validation of analytical methods is essential to ensure regulatory compliance, scientific reliability, and adherence to quality control standards. The developed and optimized method was validated in accordance with ICH Q2 (R1) guidelines.

3.2.1 Specificity and Selectivity

Selectivity of an analytical method refers to its ability to accurately measure the analyte in the presence of potential interferences. The specificity of the developed UV spectrophotometric method was confirmed by the distinct absorbance of Ritonavir at 240 nm. No significant absorbance was observed at this wavelength in the solvent spectrum, indicating the selectivity of the method.

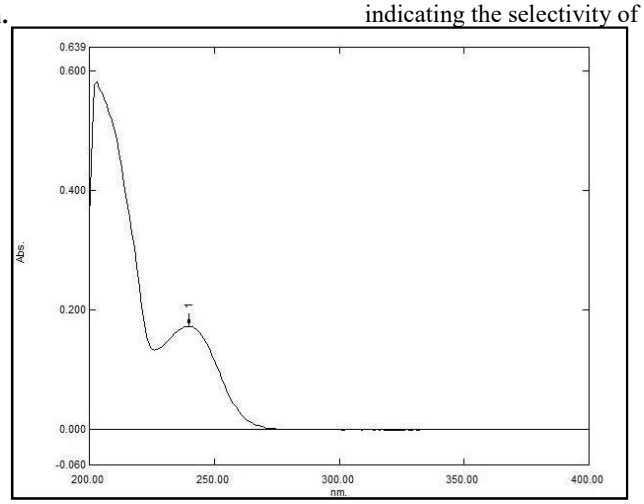


Figure.2. UV spectrum of Ritonavir

The UV spectrum shown in Figure 2 demonstrated that Ritonavir exhibited maximum absorbance (λmax) at 240 nm. The observed λmax value was found to be consistent with previously reported literature data.

3.2.2 Linearity

Method linearity indicates the ability of an analytical method to generate results that are directly proportional to the concentration of the analyte within a specified range. The developed UV spectrophotometric method for Ritonavir showed excellent linearity over the concentration range of 4–20 µg/mL with a high regression coefficient (R^2). Figure 3 presents the calibration curve of Ritonavir at 240 nm, demonstrating a direct proportional relationship between concentration and absorbance within the selected range.

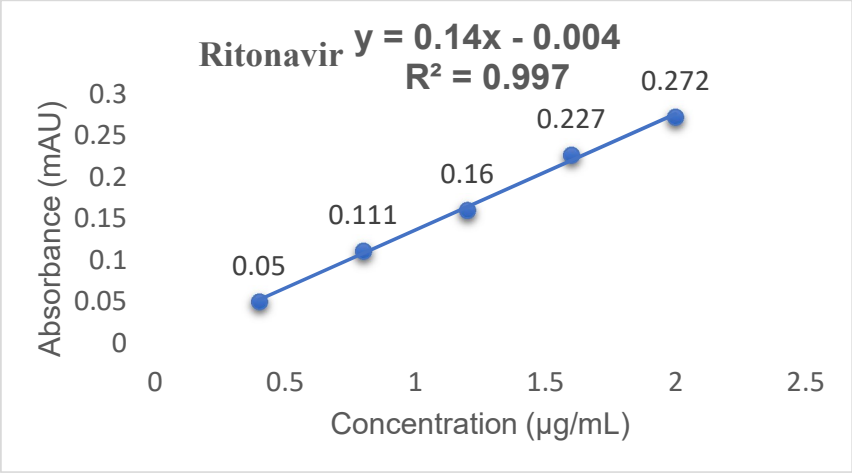


Figure.3. Standard Calibration Curve of Ritonavir

Table 1: Linearity and range data of Ritonavir

Study of Ritonavir (n = 3)				
Concentration (µg/mL)	**%RSD**	**Day 1**	**Day 2**	**Day 3**
4	0.92	0.188	0.186	0.184
8	0.57	0.372	0.369	0.367
12	0.41	0.556	0.553	0.551

3.2.3 System Precision

The precision of the developed UV spectrophotometric method was evaluated by intraday and interday precision studies. Intraday precision was determined by analyzing the absorbance at three different time intervals, namely morning, afternoon, and evening, within the same day, while interday precision was assessed over three consecutive days. The method exhibited good
precision and reproducibility, with all percentage relative standard deviation (%RSD) values found to be below 2%, indicating the reliability of the analytical method. The detailed results are summarized in Tables 2 and 3.

Table 2. Intraday Precision

Concentration ($\mu g/ml$)	**Absorbance**
4	0.195
8	0.344
12	0.513
16	0.728
20	0.860
R^2	0.997
LOD	0.094µg/mL
LOQ	0.286µg/mL

Table 3. Interday Precision

Study of Ritonavir (n = 3)				
Concentration (µg/mL)	**%RSD**	**Morning**	**Afternoon**	**Evening**
1	0.96	0.182	0.180	0.178
2	0.54	0.356	0.353	0.351
3	0.39	0.528	0.525	0.523

3.2.4 Accuracy

Accuracy of the analytical method represents the closeness between the measured value and the true value of the analyte. The percentage recovery of Ritonavir was found to be within the acceptable range of 98–102%, indicating satisfactory accuracy of the developed UV spectrophotometric method. Consistent recovery values at different concentration levels confirmed the reliability and suitability of the method for quantitative analysis. The detailed recovery results are presented in Table 4**.**

Table 4. Accuracy / Percentage Recovery Study

Level	Concentration (µg/mL)	Absorbance	% Recovery
80%	4	0.181	101.6%
		0.183	100.1%
		0.182	101.8%
100%	8	0.355	99.5%
		0.357	100.0%
		0.354	99.3%
120%	12	0.529	98.9%
		0.531	99.4%
		0.528	98.7%

3.2.5 Sensitivity

The sensitivity of the developed UV spectrophotometric method was evaluated by determining the limit of detection (LOD) and limit of quantification (LOQ). The LOD was calculated as 3.3 times the ratio of the standard deviation of the intercept to the slope of the calibration curve, while the LOQ was calculated as 10 times the same ratio. The obtained LOD and LOQ values were 0.094 µg/mL and 0.286µg/mL, respectively, indicating good sensitivity and suitability of the developed method for quantitative estimation of Ritonavir.

3.2.6 Robustness and Ruggedness

Robustness of the developed UV spectrophotometric method was evaluated by introducing deliberate minor variations in the analytical wavelength (240 ± 2 nm) to assess the stability and reliability of the method under small procedural changes. Ruggedness was determined by analyzing the same sample under varied laboratory conditions using two different analysts. Absorbance values for concentrations of 4, 8, and 12 µg/mL were recorded under each condition. The obtained percentage relative standard deviation (%RSD) values were below 2%, confirming the repeatability, reproducibility, ruggedness, and robustness of the developed analytical method. The detailed results are summarized in Tables 5 and 6.

Table.5. Ruggedness Study with Change in Analyst

Concentration (µg/mL)	Analyst 1 (%RSD)	Analyst 2 (%RSD)
4	0.48	1.12
8	0.42	0.68
12	0.21	0.46

Table.6. Robustness Study with Change in Wavelength

Concentration (µg/mL)	238 nm (%RSD)	242 nm (%RSD)
4	0.91	0.96
8	0.64	0.31
12	0.28	0.33

3.2.7 Repeatability

Intra-assay precision, also known as repeatability, reflects the ability of the developed analytical method to produce consistent results within a short period under identical experimental conditions. The obtained percentage relative standard deviation (%RSD) values were below 2%, indicating excellent repeatability and reliability of the developed UV spectrophotometric method. The detailed results are presented in Table 7.

Table 7. Repeatability Study of the Developed Method (n = 6)

Sr. no	Concentration (µg/mL)	Absorbance	%RSD
1	4	0.356	1.08
2	4	0.353	
3	4	0.355	
4	4	0.358	
5	4	0.354	
6	4	0.352	

3.2.8 Forced Degradation Study

Forced degradation studies demonstrated that Ritonavir was susceptible to acidic, alkaline, oxidative, and thermal stress conditions, with maximum degradation observed under alkaline and oxidative conditions. The results confirmed the stability-indicating nature of the developed UV spectrophotometric method by effectively distinguishing the degraded drug from the intact drug. The detailed degradation results are presented in Table 8.

Table 8. Results of Forced Degradation Study

Forced Degradation Condition	Degradation (%)
Acidic	14.26%
Alkaline	28.48%
Oxidative	98.72%
Photolytic (Sunlight)	23.15%

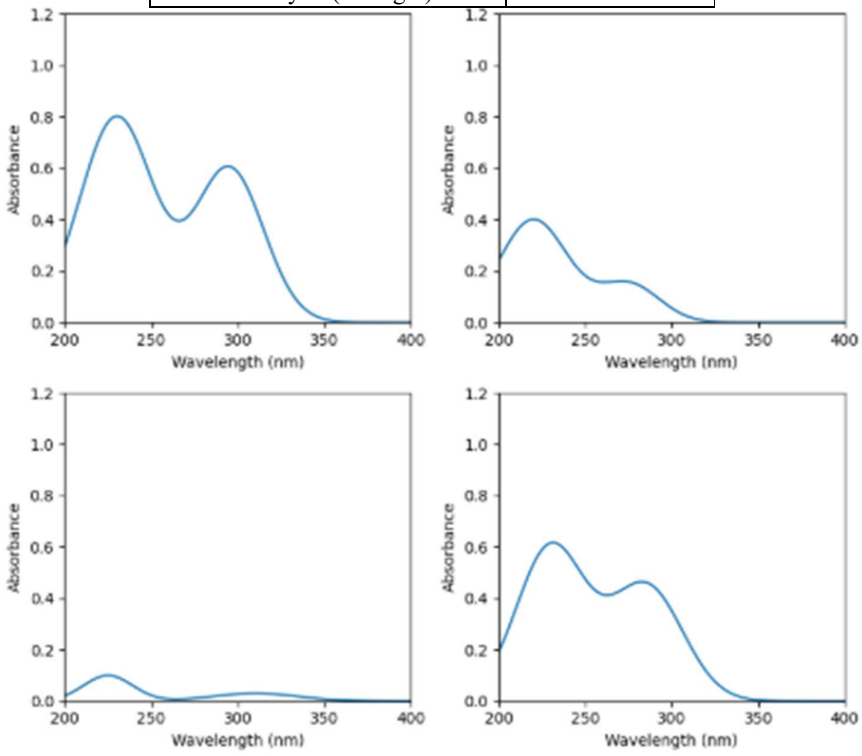


Figure. 4. Acidic, Basic, oxidative and photolytic, Forced Degradation assay of Ritonavir

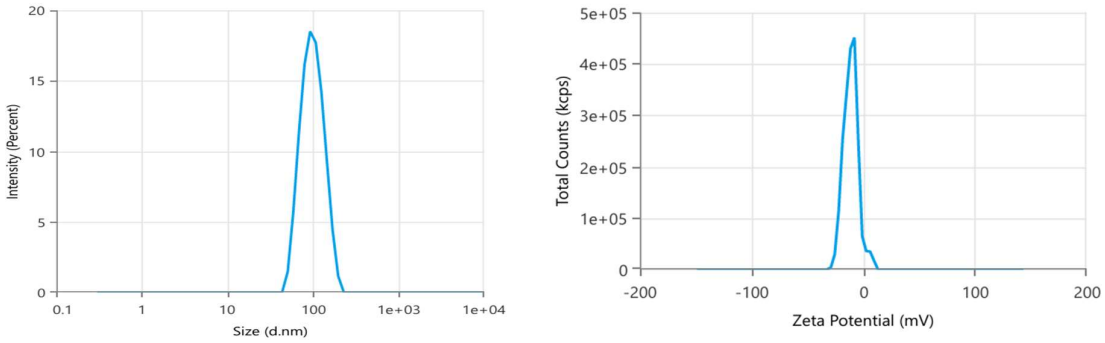
3.2.9 Estimation of Ritonavir loaded Solid supersaturated SEDDS

The developed and validated UV spectrophotometric method was successfully applied for the estimation of Ritonavir in the prepared Solid Supersaturated SEDDS formulation. An accurately weighed quantity of the formulation was dissolved in methanol, sonicated to ensure complete drug extraction, and suitably diluted for analysis. The absorbance was measured at 240 nm using a UV spectrophotometer. The assay results indicated satisfactory drug content, confirming the applicability and reliability of the developed method for routine estimation of Ritonavir in SS-SEDDS formulations (Table 9)

Table 9. Estimation and Characterization of Solid Supersaturated SEDDS

Formulation	Particle Size(nm)	PDI	Zeta Potential(mV)
Ritonavir in Solid supersaturated SEDDS	250	0.2184	-14.32

Figure. 5. Particle size & Zeta Potential of the Optimized formulation



3.3 Assessment of Method Greenness and Whiteness Attributes

The environmental sustainability of the developed UV spectrophotometric method for Ritonavir was evaluated using the Complementary Green Analytical Procedure Index (ComplexGAPI). The obtained pictogram demonstrated a predominance of green and yellow zones, indicating the eco-friendly nature of the analytical procedure. The green zones reflected the use of less hazardous solvents, low solvent consumption, and minimal waste generation, while the yellow zones were mainly associated with sample preparation and instrumental energy consumption. The low E-factor further confirmed reduced waste production and compliance with the principles of green analytical chemistry [15].

The environmental compatibility of the developed method was further assessed using the Analytical GREEnness (AGREE) metric, which evaluates adherence to the twelve principles of green analytical chemistry. The method showed a high AGREE score with a predominantly green pictogram, confirming its environmentally sustainable nature, low ecological impact, and suitability as a green analytical procedure [16].

Method whiteness was also evaluated using the Blue Applicability Grade Index (BAGI), which integrates analytical performance, environmental sustainability, and practical applicability into a single assessment tool. The obtained BAGI score indicated that the developed method possesses good robustness, operational simplicity, environmental safety, and analytical reliability, confirming its suitability for routine quantitative analysis [17].

Overall, the greenness and whiteness assessments confirmed that the developed UV spectrophotometric method effectively combines analytical efficiency with environmental sustainability and practical applicability. The method demonstrated low solvent consumption, minimal waste generation, good robustness, and ease of operation, making it suitable for routine pharmaceutical quality control and analysis of Ritonavir formulations [18]

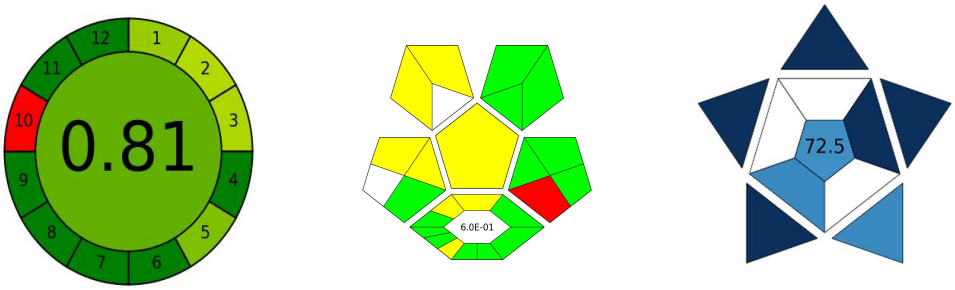


Figure.6. a) GAPI pictogram representing the greenness profile of the developed UV spectrophotometric method, b) AGREE pictogram representing the green analytical profile of the developed UV spectrophotometric method, c) BAGI pictogram representing the greenness profile of the developed UV spectrophotometric method.

Conclusions

A simple, accurate, precise, and stability-indicating UV spectrophotometric method was successfully developed and validated for the estimation of Ritonavir in Solid Supersaturated SEDDS formulations. The developed method exhibited excellent linearity, specificity, precision, accuracy, robustness, ruggedness, and sensitivity in accordance with ICH Q2 (R1) guidelines. Forced degradation studies confirmed the stability-indicating nature of the method by effectively distinguishing the intact drug from its degraded products under various stress conditions.

The validated method was successfully applied for the quantitative estimation of Ritonavir in Solid Supersaturated SEDDS formulations, confirming its suitability for routine pharmaceutical analysis and quality control applications. Furthermore, greenness and whiteness assessments using ComplexGAPI, AGREE, and BAGI tools demonstrated the environmentally sustainable and operationally efficient nature of the method. Overall, the developed analytical method provides a reliable, eco-friendly, and cost-effective approach for routine estimation of Ritonavir in Solid Supersaturated SEDDS formulations**.**

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Author Contributions

Author 1: Conceptualization, methodology, investigation, software, data acquisition, and preparation of the original draft manuscript.

Author 2: Project administration, supervision, review, and editing of the manuscript.

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The authors declare that they have no financial relationships or conflicts of interest related to the subject matter or materials presented in this manuscript.

Disclosure of conflicts

The authors state that there are no conflicts of interest or financial associations that may have affected the results or conclusions of this study.

Use of artificial intelligence (AI)-assisted technology

The authors confirm that no artificial intelligence (AI) tools were used in the writing or editing of the manuscript, and that no images were created or modified using AI.

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