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Eco-friendly based method development and validation for Estimation of Vibegron by using UV-Spectrophotometric Method

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ABSTRACT

On December 23, 2020, the US Food and Drug Administration (FDA) authorized Vibegron, also known as GEMTESA, to treat people with overactive bladder (OAB) who exhibit urge urine incontinence, urgency, and frequency of urination. The UV spectrophotometry method is used in this study to determine the drug's dose form in tablets. Additionally, the new method's greenness was assessed. Using methanol as a solvent, the UV technique was created and verified at 249 nm. The new UV spectrophotometric method's ecological impact was evaluated using the greenness assessment tools.For VBG, UV detection reveals linearity between 2.0 and 10.0 µg/mL. The percentage of recovery indicates that there is no interference from the excipients, and the value of % RSD within the limit showed that the procedure is accurate and robust. It was discovered that the analytical method validation parameters matched the acceptance criteria of the ICH Q2(R1) guideline. The method's environmental friendliness in terms of solvent usage, chemical compounds, energy consumption, and waste formation was validated by the results of the greenness evaluation. A trustworthy evaluation of the environmental and health risks connected to chemical use is necessary to determine how green an analytical procedure is.

Key words: Vibegron, UV Spectrophotometry method, Method validation, ICH Guideline, Greenness Assessment

I. INTRODUCTION

The FDA authorized the Vibegrone medication in December 2020 for the treatment of overactive bladder. In the absence of a confirmed infection or other discernible disease, overactive bladder (OAB) is a common urological illness characterised by a collection of urine symptoms, mainly urinary urgency, which is typically accompanied by increased frequency, nocturia, and occasionally urge urinary incontinence. **[Error! Reference source not found., 2, 3]**

Vibegron[(S)-N-(4-(((2S,5R)-5-((R)-hydroxy(phenyl)methyl)pyrrolidin-2-yl)methyl)phenyl)-4-oxo-4,6,7,8-tetrahydropyrrolo[1,2-a]pyrimidine-6-carboxamide] **(Figure 1)**specifically activating β3 receptors in the bladder detrusor muscle, Vibegrone, a novel β3 adrenergic receptor agonist, causes the muscles to relax and the bladder's capacity to increase. Vibegron is a good choice for long-term OAB therapy since it has better tolerability, fewer anticholinergic side effects, and a once-daily dosage schedule than earlier medications. **[4]**

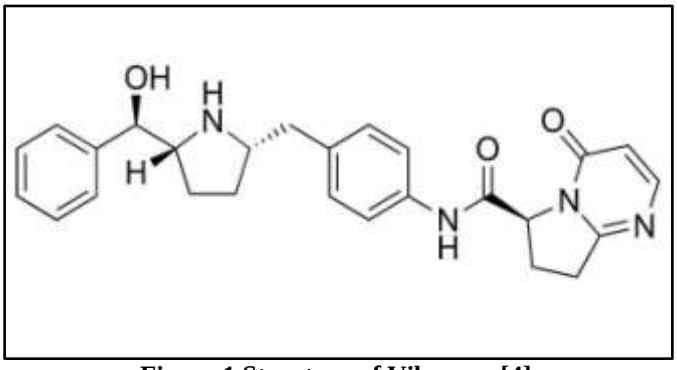

Figure 1 Structure of Vibegron [4]

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The growing acceptance and popularity of Green Analytical Chemistry (GAC) can be attributed to its origins. GAC is increasingly being used by researchers to improve analyst safety and reduce their environmental impact**[5, 6].**GAC's primary goal is to lessen or completely do away with the use of hazardous chemicals in analytical procedures while maintaining method efficacy in order to protect human health and the environment**[7, 8].** High performance liquid chromatography (HPLC) is the analytical technique most commonly used in pharmaceutical quality control (QC) to characterize active pharmaceutical ingredients (APIs) and their impurities in pharmaceutical formulations and biological fluid **[9].**UV spectroscopic methods are becoming more and more popular due to their benefits, which include speed, cost, convenience of use, precision, reproducibility, and the capacity to examine a wide range of components without requiring a lot of sample preparation. These methods enable the accurate identification of different constituents in pharmaceutical compositions, which makes them ideal for routine analysis.**[10, 11, 12]**

No UV-Spectrophotometric approach has been documented with its greenness assessment of VBG, according to a thorough literature review **[13–19].**

The objective of this study is to develop a reliable and accurate analytical technique for figuring out how much VBG is in a formulation. It offers an economical and effective method for simultaneous analysis, making a substantial contribution to pharmaceutical research, upholding quality control, and streamlining accurate dosage calculation.

II. EXPERIMENTAL MATERIALS

2.1 Reagents and chemicals

The source of the VBG reference standard was Ami Lifescience Pvt. Ltd. in Karkhadi, Vadodara, Gujarat. In this procedure, methanol (AR grade) was utilized as the solvent. Prior to use, every piece of glassware was calibrated.

2.2 Instrumentation

A UV-Visible double beam spectrophotometer (Make: SHIMADZU, Model: UV 1900I) with a pair of 1 cm matched quartz cells that is PC enabled (software: UV Probe).

2.3 Sample and standard preparation

To make 100.0 µg/mL of VBG, 10 mg of VBG was precisely weighed, transported to a 100 mL volumetric flask, dissolved, and the volume was adjusted with methanol. The standard solution was then diluted with methanol to provide various VBG concentrations ranging from 2.0 to 10.0 µg/mL.

III. METHOD VALIDATION

Accuracy, precision, assay, and detection and quantification limitations were all verified for the method. The International Conference Harmonization's (ICH) suggested guidelines were followed for validating the approach. **[20]**

3.1 Stability of analytical solution

Both solutions were examined over the course of 48 hours at room temperature to show the stability of the standard and sample solutions during analysis. The findings showed that neither solution significantly degraded throughout the specified time frame. Therefore, it was determined that both solutions were stable at room temperature for 48 hours.

3.2 Linearity

The suggested method's linearity was evaluated by scanning concentrations at five equidistance levels (n=6), ranging from 2.0 to 10.0 µg/mL. For the UV approach, a concentration vs. absorbance graph was created, and a regression equation was produced.

3.3 Precision

3.3.1 Repeatability

Six distinct solutions with the same concentration (6 µg/ml of vibegron) made from a single stock solution were analyzed to verify the method's repeatability.

3.3.2 Intraday and Interday precision

Three distinct levels of VBG—low (2.0 µg/ml), medium (6.0 µg/ml), and high (10 µg/ml)—were analyzed three times intraday on the same day and three times interday on different days. For precision, the percentage RSD value of the absorbance-related results was stated.

3.4 Accuracy (Recovery study)

It was ascertained by using the conventional addition approach to calculate the recovery of VBG. Accuracy was assessed by spiking the API to the placebo at 80%, 100%, and 120% recovery trials.

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3.5 Robustness

The method's robustness was assessed by exposing it to small changes in each of the procedure conditions separately, such as a change in wavelength (± 0.2 nm) between 248.80 and 249.20 nm.

3.6 Assay

To create a 1° stock solution with a strength of 750 µg/ml of medicine, tablet powder equal to 75 mg of drug was precisely weighed, dissolved with a few ml of methanol, filtered, and built up with methanol up to 100 ml. To create a 2° stock solution with a strength of 75 µg/ml of medication, the proper volume (1 ml) was precisely pipetted out and diluted with 10 ml of methanol. To create a test solution with a strength of 6 µg/ml of medication, 0.8 ml was carefully pipetted out of a stock solution that was at 2° and diluted with 10 ml of methanol. Absorbance was determined at the appropriate wavelength (249 nm) after test solutions were scanned. Additionally, it was discovered that concentration might be used to compute purity. The assay process was carried out three times.

3.7 LOD (Limit of Detection) and LOQ (Limit of Quantification)

Ten duplicates of standard solutions containing VBG were analyzed in order to determine the developed method's limit of detection and limit of quantification.

The LOD and LOQ calculated as

$$LOD=3.3\times\frac{SD}{Slope}$$

$$LOQ=10\times\frac{SD}{Slope}$$

Where, SD = ten replicates of absorbance
Slope = the mean slope of the 6 calibration curves

IV. RESULTS AND DISCUSSION

4.1 Selection of wavelength:

The standard solution of VBG was scanned between 200-400 nm and VBG showed absorbance maxima at 249.00 nm. **(Figure 2)**

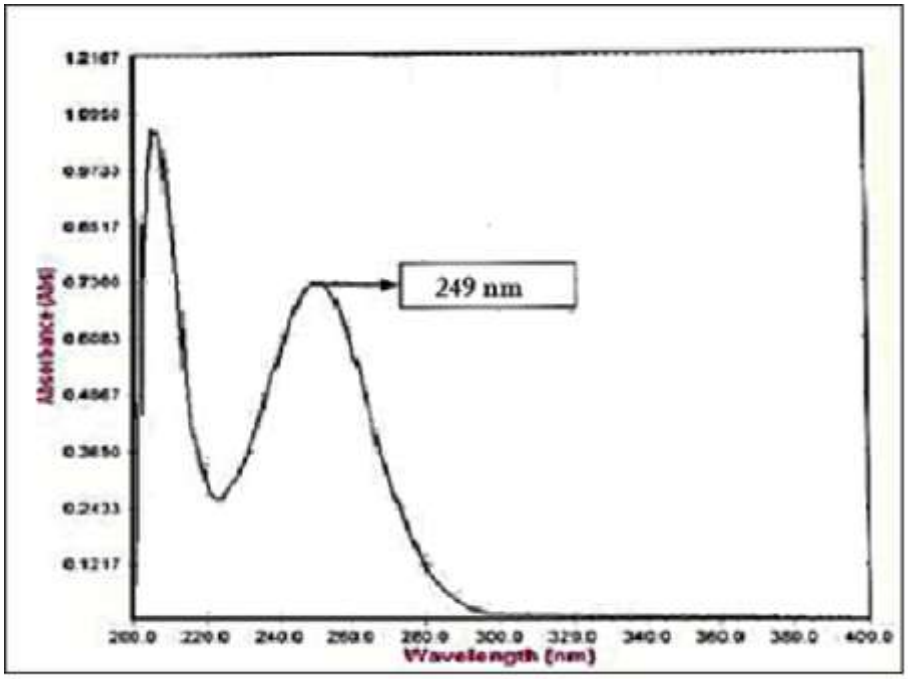

Figure 2: Wavelength detection of drug by UV method

4.2 Linearity

Figure 3 shows the linear UV overlain spectra over 2.0–10.0 µg/mL using the regression line equation y = 0.0717x + 0.0207 with r2 = 0.9986. (Fig. 4) Table 1 provided a summary of the linearity results.

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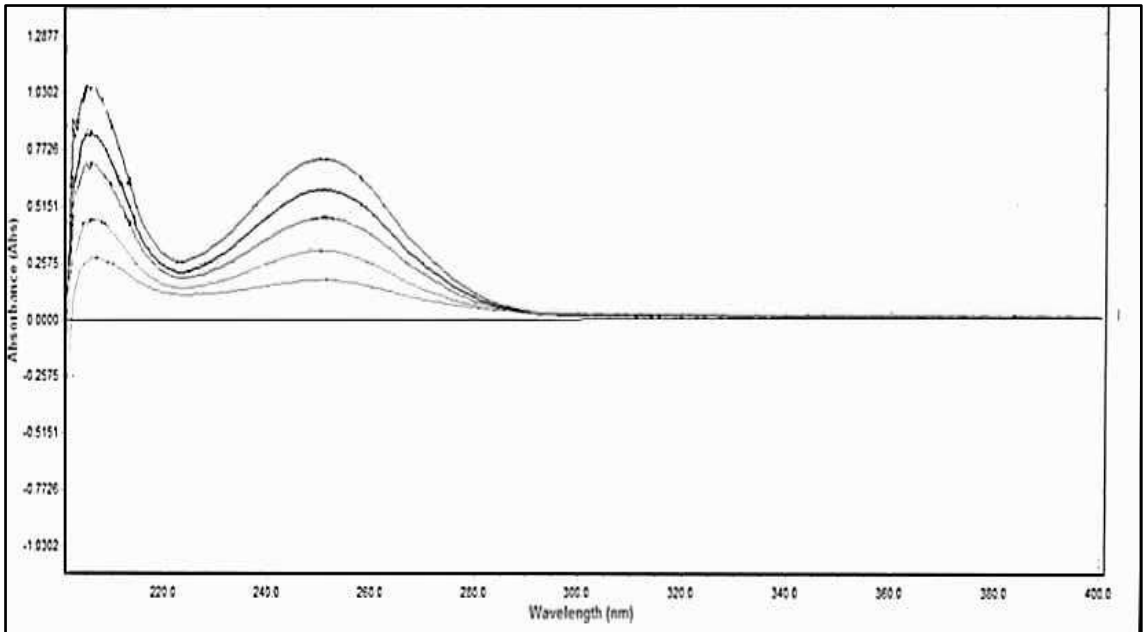

Figure 2 Overlain spectra of VBG (2.0 – 10.0 µg/mL)

Table 1 Linearity of VBG*(n=3)

Sr. no.	Conc. (µg/ml)	Mean of absorbance ± SD (n=3)	% RSD
1	2.0	0.155 ± 0.002	0.37
2	4.0	0.315 ± 0.005	0.18
3	6.0	0.462 ± 0.005	0.33
4	8.0	0.589 ± 0.004	0.35
5	10.0	0.734 ± 0.004	0.28

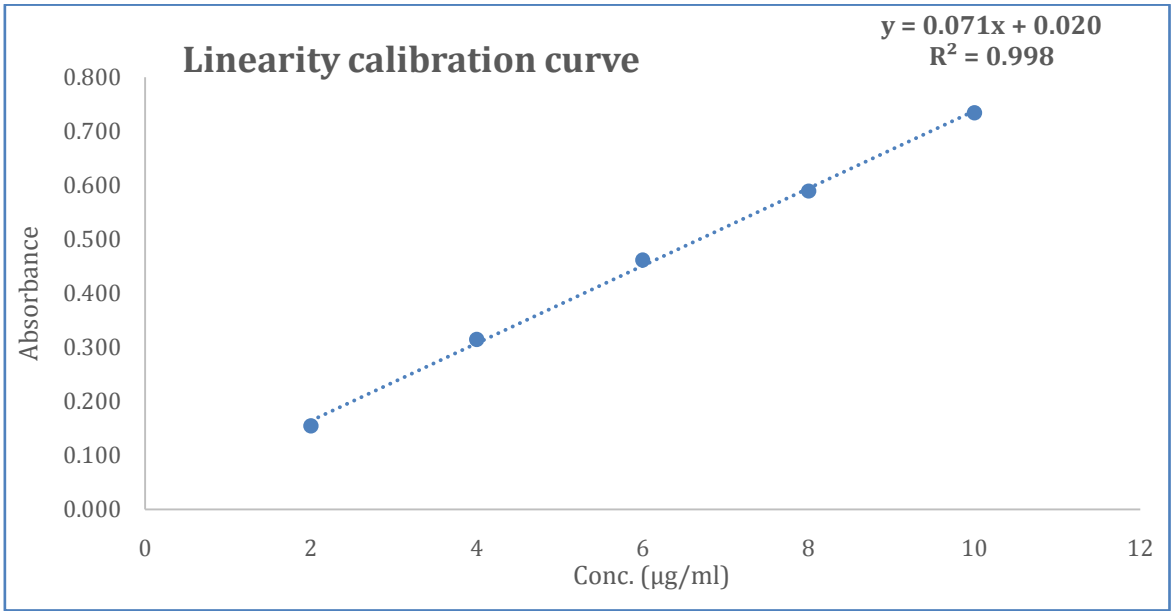

Figure 3Calibration curve of VBG

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4.3 Precision

4.3.1 Repeatability

The suggested approach was used to estimate six replicates of VBG (6 µg/ml). Repeatability values are tabulated in **Table 2.**

Table 2 Repeatability of VBG *(n=6)

Concentration(µg/ml)	Absorbance	Mean ± SD	% RSD
6.0	0.460	0.459 ± 0.005	1.17
	0.452		
	0.466		
	0.462		
	0.453		
	0.458		

Discussion: The percentage RSD was 1.17 percent, falling within the acceptable range. (% RSD < 2)

4.3.2 Intraday and Interday

The method's precision was demonstrated by the % RSD value, which was less than 2.0%. In Table 3, the VBG precision data is displayed.

Table 3 Intraday precision data for estimation of VBG*(n=3)

Conc. (µg/ml)	Mean Abs. ± SD	% RSD	Mean Abs. ± SD	% RSD
	Intraday		**Interday**	
2.0	0.152 ± 0.003	1.74	0.156 ± 0.002	1.28
6.0	0.463 ± 0.005	0.99	0.464 ± 0.004	0.87
10.0	0.735 ± 0.004	0.48	0.741 ± 0.003	0.46

Discussion: The precision of the devised approach was indicated by the precision study's % RSD value, which was less than 2.

4.4 Accuracy

According to the results described in Table 4, the recovery percentage, which was determined to be between 98% and 102%, validates the correctness of the devised UV approach.

Table 4 Recovery study of VBG*(n=3)

Level (%)	Std. conc. Spiked (µg/ml)	Total Conc. (µg/ml)	Absorbance	Total conc. Found (µg/ml)	Spiked conc. (µg/ml)	% Recovery	± SD	% RSD
80	3.2	7.2	0.541	7.26	3.26	101.77	± 1.29	1.28
100	4.0	8.0	0.592	7.97	3.97	99.20		
120	4.8	8.8	0.653	8.82	4.82	100.39		

Discussion: The percentage recovery indicates that the excipients are not interfering.

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4.5 Robustness
Table 5 provided a summary of the findings.

Table 5 Robustness (Change in wavelength)data of VBG *(n=3)

Drug	(λ Max 249 nm ± 0.2 nm)	Mean Abs.	% RSD
Vibegron (6 µg/ml)	λ Max 1 (248.80 nm)	0.454	0.16
	λ Max 2 (249.20 nm)	0.453	

Discussion: The robustness of the developed method was demonstrated by the absence of any notable alterations.

The devised UV technique was shown to be robust when the percentage RSD value for all robustness parameters was less than 2%.

4.6 Assay

The assay of VBG was analyzed by estimation from GEMTESA (VBG – 75mg) formulation was used. The % assay results were summarized in **Table 6** and were found between the acceptance criteria 98 % to 102 %.

Table 6 Analysis data of synthetic mixture*(n=3)

Drug	Labeled / Taken amount (mg)	Amount found (mg)	% Assay	% RSD
Tablet (GEMTESA)	75	74.55	99.40	0.71

Discussion: The % assay shows that there is no interference from excipients and the proposed method can successfully apply to analysis of commercial formulation containing VBG.

4.7 LOD (Limit of Detection) and LOQ (Limit of Quantitation)
The obtained LOD and LOQ results are presented in **Table 7.**

Table 7 LOD and LOQ

Parameters	VBG
LOD (µg/ml)	0.95
LOQ (µg/ml)	2.89

Discussion: The LOD and LOQ for VBG conformed to be 0.95µg/ml and 2.89µg/ml, respectively.

V. GREENNESS ASSESSMENT OF SUGGESTED METHOD

Use the Analytical Eco Scale (AES), National Environmental Method Index (NEMI), Green Analytical Procedure, Green Analytical Procedure Index (GAPI), and software-based analytical greenness index (AGREE) equipment to evaluate a product's environmental friendliness by utilizing the greenness profile aspects of any analytical investigation method.**[[21], [22]]**

NEMI

The NEMI evaluation tool offers a thorough conclusion regarding the environmental effects of the studied strategy that is easily comprehended by merely examining the pictogram due to its user-friendly interface and straightforward design. Persistent, bioaccumulative, toxic (PBT), hazardous, corrosive, and waste are the four main

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terms that characterize the profile criteria. Depending on how effectively the approach satisfies that condition, each quadrant is either green or blank. The approach satisfies the selection requirement for that quadrant when it is filled in with green. The two quadrants are thus green when the overall profile of the suggested UV spectrophotometric approach is examined. Given that methanol for UV spectroscopy is not a PBT list category chemical and that the waste produced is less than 50 g or ml per sample, the recommended method passes both of the greenness profile's quadrants.**Table 8**. **[[23], [24], [25], [26]]**

Eco Scale Assessment

A semi-quantitative tool for assessing the environmental friendliness of analytical techniques, the Eco-Scale was presented by the authors. Penalty points are assigned by this instrument based on factors such as energy consumption, waste generation, and the amount and toxicity of chemical compounds. The total number of penalty points is then subtracted from 100 to display the best green analysis. A score of 50 to 75 is considered ordinary, and a score of 75 or higher is deemed green. The investigation's criteria included waste volume, instruments, and solvents. The approach received a score of 82, as shown in Table 10. Based on these findings, the eco-scale tool indicates that the new process is more ecologically friendly.**[[27], [28], [29]]**

GAPI

GAPI is a well-known and extensively used greenness matrix for assessing the environmental impact of analytical procedures. It is a quick, dependable, and user-friendly tool that offers useful data on an approach's environmental friendliness. A great semi-quantitative tool is the GAPI. The GAPI diagram is used to assess and quantify the environmental impacts of each stage of an analytical process. Depending on the method used, the pictogram is colored red, yellow, or green to highlight the overall green nature of the analytical process from sample collection through result interpretation. Five pentagrams that each represent a different step in the analytical process make up the output of the GAPI diagram, which is shown in Table 8. Three red pentagrams indicate areas of concern, while the GAPI index of the UV spectrophotometry method produces four green and five yellow pentagrams. The recommended green approach is typically regarded as environmentally friendly based on the GAPI pentagrams. Even though it uses a caustic solvent that produces red pentagrams, it uses less energy, produces less waste, and takes fewer than three steps for sample preparation.**[[30]]**

AGREE

The environmental friendliness of the UV spectrophotometric approach was represented by the AGREE numerical value, as shown in Table 8. A number nearer one denoted a greener method, while a lower value showed regions where the environmental effect might be improved. Green analytical chemistry principles were used by the AGREE software to evaluate the greenness profile of the UV spectroscopic technique. When a technique or methodology receives a score of 0.7, it is considered environmentally benign and complies with green analytical chemistry principles. Reducing or eliminating hazardous materials, reducing waste, optimizing energy consumption, and enhancing health and safety are all included in a score **[[31], [32], [33][11]]**.When simply the assessment of greenness is considered, the findings generated using RGB 12 and AGREE, which correspond to the GAC concepts, respectively, should be seen as largely consistent. Algorithms determined that the approach was the most environmentally friendly.

Table 8 Assessment of greenness values of proposed analytical method by different tools.

Assessment tool	UV method
Eco Scale Assessment	Methanol – 4 Instrument - 1 Energy used 0 Waste 3 Occupational hazard 10 Total penalty points 18 Analytical eco-scale 82

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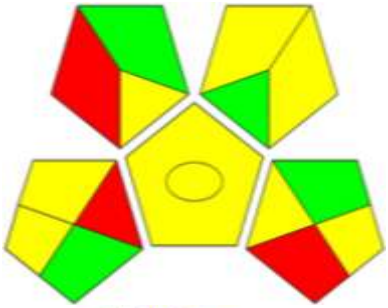
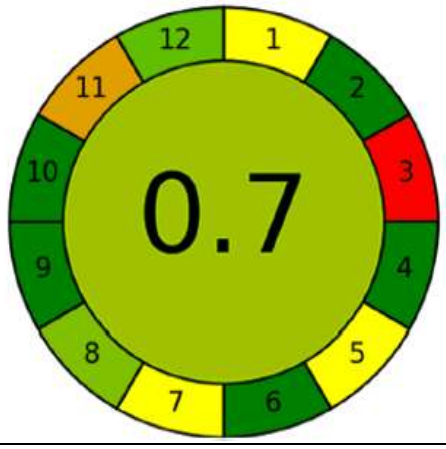
NEMI

GAPI



AGREE



VI. CONCLUSION

A validated UV technique has been developed to determine VBG in dosage form. For routine drug analysis utilizing prescription dose forms, it therefore performs admirably. Because of its simplicity, the procedure can be used in labs without sophisticated analytical equipment, which can be costly, time-consuming, and challenging. Given the possibility of VBG's global expansion, the proposed method might be advantageous for national quality control labs in poorer countries. Overall, the UV spectroscopic approach demonstrates how green analytical chemistry can lessen the analytical method's environmental impact without sacrificing analytical performance. Researchers may help create a more sustainable and environmentally conscious scientific community by using such eco-friendly techniques. The GAC ideas are enhanced and supplemented by the RGB 12 algorithm, a specific tool for method evaluation.

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