Bull. Chem. Soc. Ethiop. **2025**, 39(2), 227-242.
DOI: https://dx.doi.org/10.4314/bcse.v39i2.4

ISSN 1011-3924
Printed in Ethiopia
Online ISSN 1726-801X

EVALUATION OF THE GREENNESS PROFILES OF INDIRECT SPECTROPHOTOMETRIC METHODS FOR ESTIMATION OF GEMIFLOXACIN MESYLATE IN PURE AND DOSAGE FORMS UTILIZING N-BROMOSUCCINIMIDE AS A GREEN REAGENT

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(Received August 22, 2024; Revised October 19, 2024; Accepted October 24, 2024)

ABSTRACT. A validated, sensitive, user-friendly, precise, and dependable spectrophotometric technique has been developed to accurately detect the concentration of gemifloxacin mesylate in pure and dosage forms. The methods utilize N-bromosuccinimide as an eco-friendly oxidizing agent in acidic circumstances. The residual N-bromosuccinimide is measured by subjecting it to a chemical reaction with preset amounts of dyes, amaranth, methylene blue, and indigocarmine and the absorbance is measured at λ_{max} of 520, 664 and 610 nm, respectively. The analytical technique was implemented and validated by thoroughly examining and optimizing various factors that could potentially disrupt the reaction. Significant linear relationships, characterized by correlation coefficients ranging from 0.9993 to 0.9996, were observed under optimal conditions. These associations remained consistent throughout concentration ranges of 1.0-18, 1.0-14, and 1.0-20 µg/mL. The limits of detection (LOD) of 0.30, 0.29, and 0.30 µg/mL for amaranth, methylene blue, and indigocarmine methods, respectively. The accuracy and precision of the approaches' have been evaluated. No significant interference was observed with the usual pill excipients. In addition, the environmental impact of the suggested processes was assessed using three evaluation tools specifically designed to measure environmental friendliness: the Analytical Greenness Metric, the Green Analytical Procedure Index and Analytical Eco-Scale.

KEY WORDS: Gemifloxacin mesylate, N-bromosuccinimide, Spectrophotometry, Method validation, Dosage forms, Greenness assessment tools.

INTRODUCTION

Gemifloxacin mesylate (GMF) is a fourth-generation fluoroquinolone that has a broader range of effectiveness against bacteria, especially those that are resistant to prior fluoroquinolones, such as staphylococcus and streptococcus infections. GMF is a fluoroquinolone antibacterial drug that has the ability to effectively combat both gram-positive and gram-negative bacteria [1-3]. GMF is named 7-[(4Z)-3-(aminomethyl)-4-(methoxyimino)pyrrolidin-1-yl]-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxylic acid; mesylate.

The literature review reveals that there are only a few published ways for determining GMF in dosage forms. These techniques include chromatography [4-7], electrochemistry [8-10], and spectrofluorimetry [11, 12, 28, 36]. Based on our current knowledge, various techniques have been recorded for quantifying the quantity of GMF in commercial pharmaceutical products using a spectrophotometric method [13-36]. However, the previously documented techniques were either not sensitive enough or required a lot of effort and relied on expensive instrument that is

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not typically available in many laboratories. Hence, it was beneficial to develop innovative, straightforward, and cost-effective spectrophotometric methods for quantifying the concentration of GMF in its dosage forms.

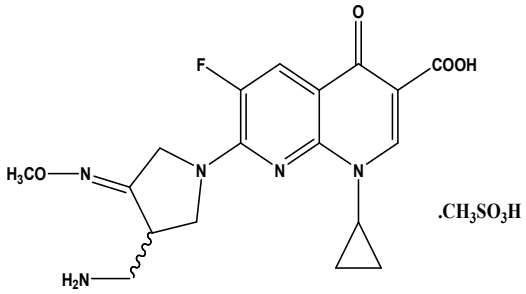


Figure 1. The chemical structure of gemifloxacin mesylate (GMF).

N-Bromosuccinimide (NBS) is a reagent that is environmentally benign and is utilized as a highly effective agent for oxidation and bromination. The spectrophotometric determinations using NBS were conducted by directly measuring the chromogenic derivative of the medication or indirectly by measuring the leftover NBS using color-producing reagents [37-40].

The aim of this project is to develop innovative spectrophotometric methods that are simple, highly sensitive, accurate, and cost-effective for measuring GMF in pure state and dosage forms. The proposed approaches employ NBS as an environmentally friendly agent, in conjunction with amaranth (AM), methylene blue (MB), and indigocarmine (IC) dyes. The existence of frequently employed additives at typical values frequently observed in pharmaceutical formulations did not have an impact on the assay of GMF. The suggested methods have been statistically verified for their accuracy, precision, sensitivity, selectivity, robustness, and ruggedness in accordance with the guidelines provided by the ICH [41]. The environmental impact of the suggested processes was assessed using three evaluation tools specifically designed to measure environmental friendliness: the Analytical Greenness Metric (AGREE), the Green Analytical Procedure Index (GAPI) and Analytical Eco-Scale (AES).

EXERIMENTAL

Chemicals and reagents

All chemicals, reagents and solvents used in this investigation were of exceptional purity analytical reagent. Furthermore, each solution was consistently generated at regular intervals. The experiment utilized double-distilled pure water. Hydrochloric acid (HCl) (Sp. gr. 1.18, 37%) was purchased from (Merck, Darmstadt, Germany. NBS and KBr were purchased from (Sigma-Aldrich, St. Louis, MO, USA). AM, MB, or IC (90% dye concentration) were obtained from (Sigma-Aldrich, St. Louis, MO, USA).

Pure GMF and dosage forms

The pure GMF potency was measured to be 99.60 ± 0.74%. It was provided by Al-Obour Pharmaceutical & Chemical Industries Company in Egypt. Flobiotic tablets containing 320 mg of GMF per tablet were purchased from Hikma Pharm. & Chem. Ind. Company in Egypt, while GemiQue tablets containing 320 mg of GMF per tablet were obtained from Obour Pharm. & Chem. Ind. Company, Egypt. These tablets were bought from local market.

Standard solutions preparation

To create a standard solution of GMF, 1.0 mg of pure GMF was dissolved in 0.2 mL HCl (0.1 M). The solution was then diluted to a final volume of 10 mL using bidistilled water in a 10 mL volumetric flask. The standard solutions remained stable for at least one weak at 4 °C.

A stock solution of NBS with a concentration of 200 µg/mL was generated by dissolving around 0.02 g of NBS in smallest possible quantity of bidistilled water possible in a 100 mL measuring flask. Afterwards, the solution was diluted with distilled water to the specified level and calibrated [42].

KBr (1.0%, w/v) solution was made by dissolving 1.0 g of KBr in 100 mL bidistilled water. HCl solution (5.0 M) was made by diluting 43 mL of concentrated HCl with bidistilled water to a final volume of 100 mL. The solution was then standardized according to the suggested procedure [43] before being used. All standard solutions were stored in a refrigerator when it was not in use.

A stock solution of AM, MB, or IC was prepared by dissolving exactly 112 mg of dye in bidistilled water to obtain a concentration of 1000 µg/mL. The solutions were subsequently diluted to the desired volume using a 100 mL calibrated flask. The solution was subsequently diluted to 200 µg/mL of dye.

Instruments and apparatus

A Shimadzu UV-1601 UV/Vis spectrophotometer (Sweden) equipped with a 10 mm glass cell was utilized for measuring absorbance. It exhibits exceptional precision in wavelength measurement, boasting an ±0.2 nm accuracy. The device scanning speed is 200 nm/min., and 2.0 nm bandwidth. It can cover wavelengths ranging from 200 to 900 nm.

Recommended procedures

Varying volumes from 0.1-2.0 mL of GMF solution (100 µg/mL) were put into a series of 10 mL measured flasks. The volumes were adjusted to a total of 5.0 mL by adding an appropriate amount of bidistilled water. Each flask was filled with 1.0 mL of HCl (5.0 M), 2.0 mL of NBS solution (200 µg/mL), and 1.0 mL of KBr (1.0%, w/v). The flasks were sealed with stoppers, the contents of the flasks were combined and shaken occasionally, and set aside for 5.0 min. Ultimately, 1.5 mL of (200 µg/mL) AM, MB, and IC solution were added and mixed thoroughly. Subsequently, the volume was modified to the intended level by diluting it with bidistilled water. The quantification of absorbance for each solution was performed at λ_{max} 520, 664, and 610 nm using the AM, MB, and IC procedures, respectively. The duration of the measurement was 3.0 minutes, and it was compared to a reagent blank. A conventional graph was generated by plotting the absorbance against the drug concentration in all methodologies.

Procedure for dosage forms

A total of 20 tablets of GMF were accurately measured and subsequently ground into a fine powder. The exact mass of the powdered tablets, equivalent to 10 mg GMF, was dissolved in 10 mL of 0.1 M HCl in a 100 mL volumetric flask. The solution was agitated for a duration of 5.0 min and subsequently separated by passing it through a Whatman No. 42 filter paper. The filtrate was mixed with distilled water in a 100 mL measuring flask till it attained the desired volume, which produced a stock solution of GMF (100 µg/mL). The spectrophotometric techniques were employed to analyze this solution. Subsequently, an appropriate segment was examined utilizing the specified methodologies previously described. Determine the precise quantity of dosage forms by applying the appropriate regression equation.

RESULTS AND DISCUSSION

Absorption spectra

The presented methods depend on the reaction between GMF with an abundant quantity of NBS, then the quantification of NBS through its interaction with a predetermined amount of AM, MB, and IC dye. The measurement of absorbance at wavelengths of 520, 664, and 610 nm is thereafter conducted (Figure 2). These methods employ the bleaching property of NBS on the dyes, causing the dyes to lose color as a result of their oxidative destruction. As the amount of GMF increases, the concentration of NBS decreases due to depletion, resulting in a parallel decline in its concentration. When a fixed quantity of dye is added to solutions with decreasing levels of NBS, there is a proportional rise in the dye level. Consequently, there is a clear relationship between the GMF concentration and the rise in absorbance at the particular λ_{max}.

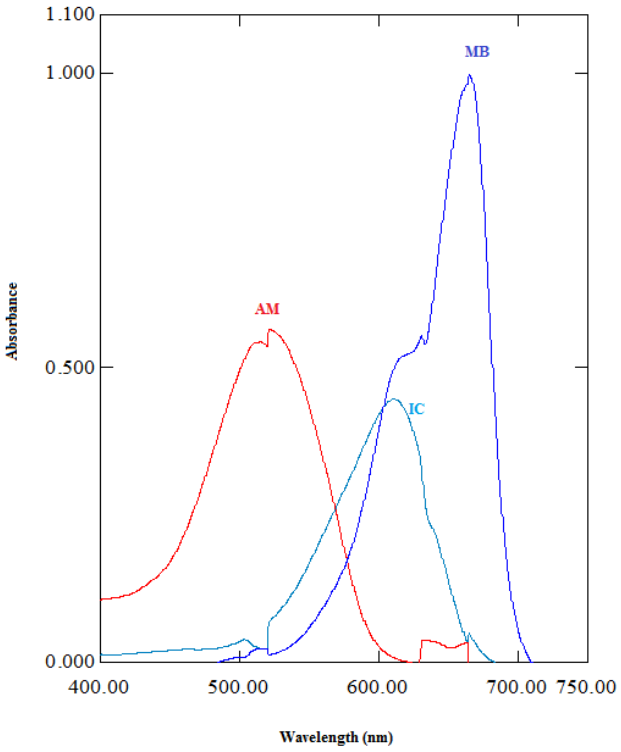
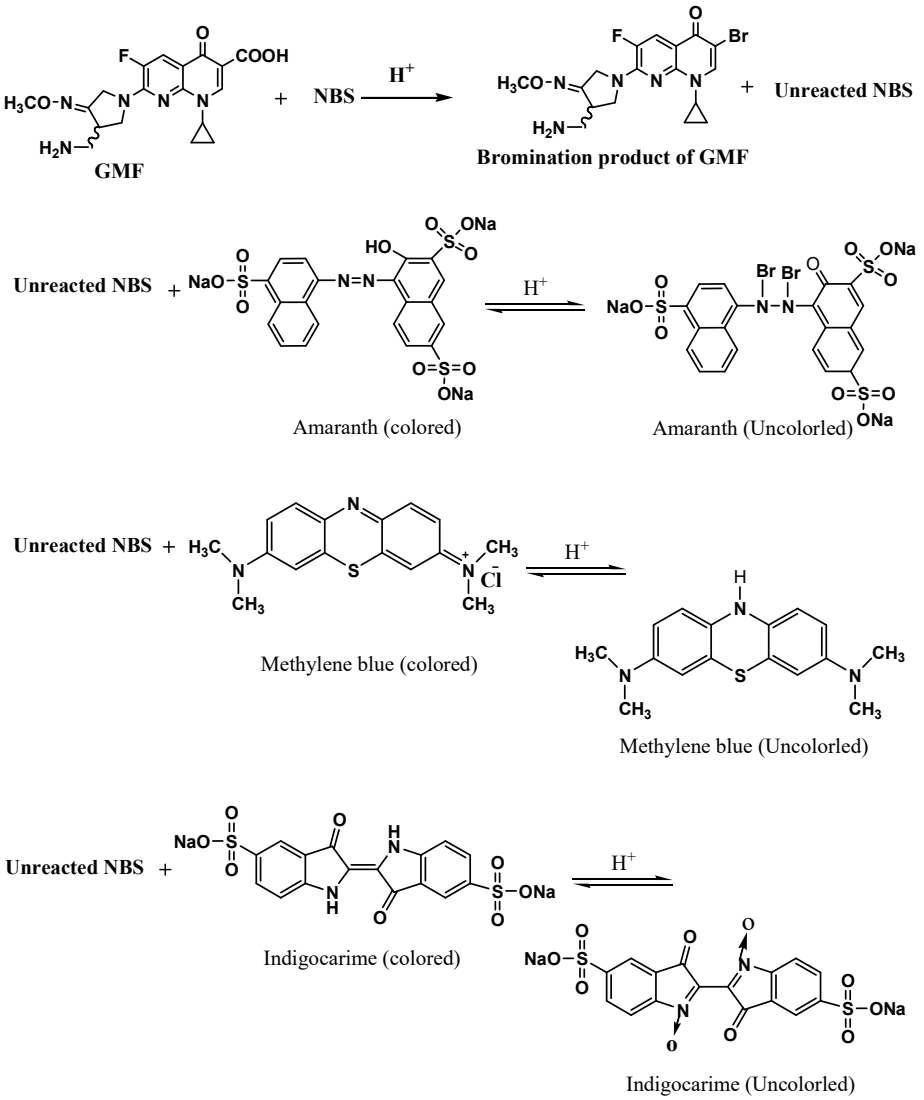


Figure 2. The absorption spectra for the unbleached AM, MB, and IC dyes.



Scheme 1. The recommended chemical route for the proposed spectrophotometric approaches A, B and C involves the utilization of NBS and dyes.

Chemistry of the reaction

NBS is a highly effective material that can oxidize or brominate other chemicals. It is widely used in the examination of various pharmaceutical drugs and is regarded the main organic compound containing bromine for this purpose [37-40]. Moreover, it is specifically used to introduce bromine atoms into alkenes at the allylic position [44]. The reaction included of 2 consecutive stages. The 1st stage involved the GMF bromination utilizing an excessive amount of NBS in HCl solution. The bromination of GMF will take place in position α to the carbonyl group [38]. In the

2nd stage, the surplus remaining NBS was quantified by reacting it with a specified amount of AM, MB, and IC dyes, and subsequently absorbance measuring at their respective λ_{max}. The spectrophotometric approaches' proposed reaction was illustrated in Scheme 1. The absorbance demonstrated a direct correlation with the GMF concentration in all operations. The aforementioned methods employ the whitening properties of NBS on dyes, leading to the fading of color caused by the oxidative breakdown of the pigment.

Analytical parameters optimization

Identification and quantification of acid type and concentration

The GMF and NBS conducted a reaction in several acidic solutions, such as HCl, H_2SO_4, HNO_3, and CH_3-COOH. Exceptional results were obtained in a HCl medium. An investigation was conducted to examine the influence of HCl concentration. The HCl concentration was modified between 0.25–3.0 mL of HCl (5.0 M) with keeping the NBS and GMF concentrations are constant. The results indicated that when utilizing a volume of 1.0-3.0 mL of hydrochloric acid (HCl) with a concentration of 5.0 M, the absorbance values produced in the presence of GMF were almost indistinguishable. When the volume of acid was less than 1.0 mL, the reaction proceeded at a slower rate and did not reach completion. Therefore, a fixed amount of 1.0 ml of HCl (5.0 M) was used.

Effect of NBS

To ascertain the optimal volume of NBS, several volumes of NBS (200 µg/mL) in the range of 0.25-3.0 mL were treated with a consistent quantity of dye in HCl solution. The investigation found that the greatest absorbance value was achieved by utilizing 2.0 mL of NBS (200 µg/mL) (Figure 3(a)).

Effect of dye

A study was carried out to ascertain the ideal volume of AM, MB, and IC dyes at a concentration of 200 µg/mL that would yield the greatest color intensity. A study was carried out to investigate the impact of dye volume, ranging from 0.25 to 3.0 mL, for each dye. According to the investigation, the oxidation products achieved the greatest level of color intensity when employing 1.5 mL of dye solution (Figure 3(b)).

Effect of KBr

The impact of KBr volume was investigated within the range of 0.5–3.0 mL. A 1.0 mL of KBr (1.0%, w/v) solution was selected as the optimal amount to accelerate the oxidation process.

Effects of temperature and duration of mixing

A study was carried out to analyze the influence of temperature on a series of sample and blank solutions. The solutions underwent temperature variations, ranging from 25-50 °C, through immersion in a water bath. The most elevated degree of color intensity was attained at 25 ± 2 °C. It has been found that raising the temperature does not produce consistent results. Consequently, the influence of time on the completion of oxidation process at time ranging from 2.0-20 min was investigated. The experiment demonstrated that maintaining contact for a duration of 5.0 min at a temperature of 25 ± 2 °C consistently produced robust and reproducible absorbance measurements. It was determined that a standing period of 3.0 min is required in order to

completely remove the dye pigment using the remaining NBS. The dye absorbance that did not undergo a reaction remained consistent for a minimum of 10 hours following this time period.

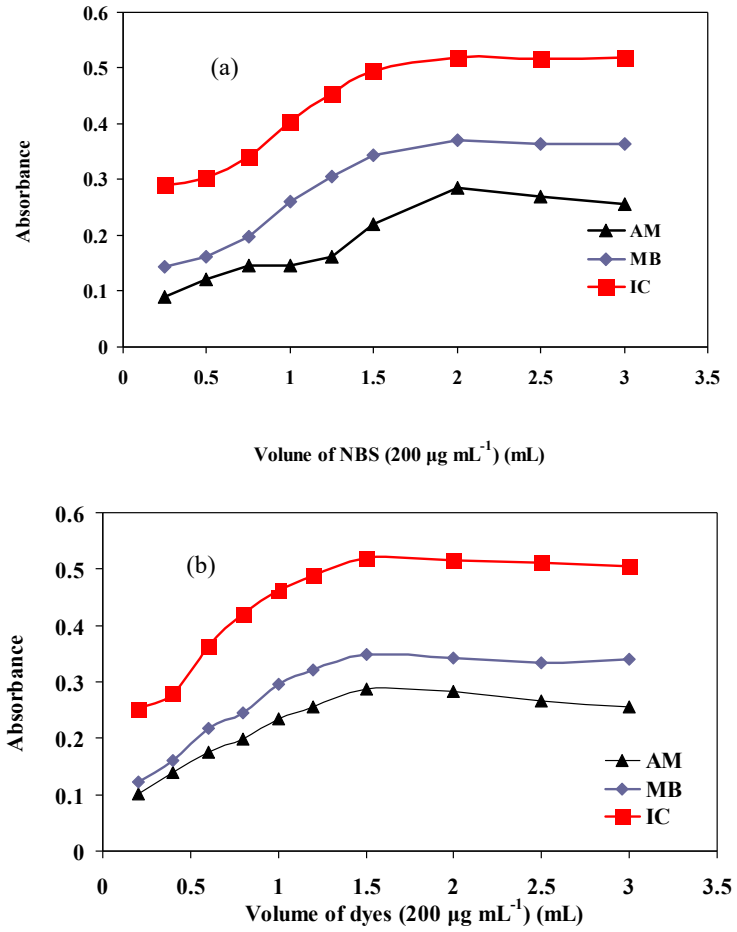


Figure 3. The impact of the (a) NBS volume (200 µg/mL) and (b) dye volume (200 µg/mL) on the oxidation product.

Impact of the sequence of addition

The optimal sequence of addition was as follows: GMF, HCl, NBS, KBr, and lastly, the dye. When the experimental conditions were the same, the alternate sequences showed lower absorbance values.

Method validation

Linearity and sensitivity

An evident correlation was noted between the absorbance at the maximum wavelength and the GMF concentration. This association was determined under ideal circumstances. The GMF

concentration ranges were 1.0-18, 1.0-14, and 1.0-20 µg/mL using AM, MB, and IC methods, respectively. To get accurate outcomes, the Ringbom concentration range [45] was established. The detection (LOD = 3s/b) and (LOQ = 10s/b) quantitation limits for the suggested procedures were determined. The variable "s" represents the standard deviation of 10 repeated measurements of the reagent blank, whereas "b" represents the sensitivity, which is defined as the slope of the calibration graph. The resulting LOD values of 0.30, 0.29, and 0.30 µg/mL and LOQ values was determined to be 1.0, 0.97, and 1.0 µg/mL for the AM, MB, and IC techniques, respectively. The suggested approaches were assessed for validity by statistical analysis [46], comparing the results derived by these approaches with the reported method [19]. Based on the results of the student's t-test and variance ratio F-test (Table 1), there is no statistically significant distinction between the suggested approach and the method provided in reference [19] in terms of accuracy and precision.

Table 1.Analytical and regression parameters of the developed approaches for determining GMF.

Parameters	AM	MB	IC
Beer's law limits, µg/mL	1.0-18	1.0-14	1.0-20
Ringboom limits, µg/mL	3.0-16	3.0-12	3.0-18
Molar absorptivity, x 10^4 L/mol.cm	1.0392	1.281	1.2387
Sandell sensitivity, ng/cm^2	46.72	37.90	39.19
Regression equation[a]			
Intercept (a)	0.0149	0.0153	0.0079
Standard deviation of intercept (S_a)	0.025	0.042	0.033
Slope (b)	0.0172	0.0199	0.0233
Standard deviation of slope (S_b)	0.032	0.025	0.021
Correlation coefficient, (r)	0.9996	0.9993	0.9995
Mean ± SD	100.10 ± 0.80	99.30 ± 0.50	99.90 ± 0.72
RSD%	0.80	0.50	0.72
RE%	0.84	0.53	0.78
Limit of detection, µg/mL	0.30	0.29	0.30
Limit of quantification, µg/mL	1.0	0.97	1.0
Calculated *t*-value[b]	1.03	0.75	0.65
Calculated *F*-value[b]	1.17	2.19	1.06

[a]$A = a + bC$, where C is the concentration in µg/mL, A is the absorbance units, a is the intercept, b is the slope. [b]The theoretical values of t and F are 2.571 and 5.05, respectively at confidence limit at 95% confidence level and five degrees of freedom ($p = 0.05$).

Accuracy and precision

To evaluate the accuracy and precision of the proposed methodologies, we generated and analyzed solutions that had 3 different GMF concentrations. A study was performed on 6 similar samples. The results obtained from this investigation are concisely summarized in Table 2. To assess the accuracy and precision of the suggested approaches, one can analyze smaller values of the percentage relative error (RE%) and relative standard deviation (RSD%), respectively. The results obtained from this investigation are concisely summarized in Table 2. The processes inter-day and intra-day accuracy and precision were evaluated and the results indicate that the suggested methodologies have exceptional levels of accuracy and precision, suggesting great repeatability and reproducibility.

Table 2. Intra-day and inter-day accuracy and precision of the developed approaches.

Method	Taken (µg/mL)	Recovery %	Precision RSD % [a]	Accuracy RE %	Confidence limit [b]
	Intra-day				
AM	5.0	99.20	0.54	-0.80	4.96 ± 0.028
	10	100.60	0.78	0.60	10.06 ± 0.082
	15	99.50	1.10	-0.50	14.925 ± 0.172
MB	4.0	99.70	0.61	-0.30	3.988 ± 0.026
	8.0	99.40	0.98	-0.60	7.952 ±0.082
	12	99.30	1.25	-0.70	11.916 ± 0.156
IC	6.0	98.90	1.40	-1.10	5.934 ± 0.087
	12	99.40	0.90	-0.60	11.928 ± 0.113
	18	99.10	1.30	-0.90	17.84 ± 0.243
	Inter-day				
AM	5.0	99.30	0.65	-0.70	4.965 ± 0.034
	10	99.10	1.05	-0.90	9.91 ± 0.11
	15	100.50	1.25	0.50	15.075 ± 0.198
MB	4.0	99.10	0.45	-0.90	3.964 ± 0.019
	8.0	99.60	0.86	-0.40	7.968 ± 0.072
	12	100.50	1.35	0.50	12.06 ± 0.171
IC	6.0	99.50	0.80	-0.50	5.97±0.051
	12	100.30	0.64	0.30	12.036 ± 0.081
	16	99.30	1.40	-0.70	15.888 ± 0.234

[a]RSD%, percentage relative standard deviation; RE%, percentage relative error. [b]Mean ± standard error, confidence limit at 95% and five degrees of freedom (t = 2.571).

Robustness and ruggedness

To evaluate the robustness of the method, the NBS volume was deliberately altered by a slight value (2.0±0.2 mL) and the time was intentionally varied within a range of 5.0±2.0 min. The analysis was performed using altered parameters, employing three separate levels of GMF concentration. The techniques used were found to be unaltered, as indicated by the RSD% lying in the range of 0.45-2.30%. The robustness of the approaches was measured by computing the RSD% of the procedure conducted by 3 distinct analyzers and utilizing 3 various instruments. The intra-analyst RSD% varied from 0.65% to 2.10%, whereas the inter-instruments RSD% varied from 0.58% to 2.25%, indicating that the suggested methodologies were reliable and consistent.

Recovery studies and application

To assess the accuracy, reliability, and validity of the suggested methodologies, a recovery experiment was conducted utilizing the standard addition methodology. The experiment entailed introducing 3 separate GMF concentrations to a preset quantity of GMF in tablets (which had already been examined). The final concentration was subsequently established using the recommended protocols. The determination was replicated 3 times at each level, and the recovery was calculated utilizing the following equation:

$$\textbf{\% Recovery} = \frac{[C_T - C_F]}{C_p} \times 100$$

The variable C_T represents the total GMF concentration that was discovered. C_F represents the GMF concentration that is founded in the tablet. C_P represents the pure GMF concentration that was spiked to the tablet. The findings of this investigation, as shown in Table 3, indicate that the

accuracy of the suggested techniques remained not influenced by the different substances added to the formulations. Table 5 displays a statistical variance of the results gathered by analyzing GMF utilizing the proposed methods and the documented one [19] by calculating Student's t-test and F-value at a 95% confidence level for 5 degrees of freedom [46]. The results are in agreement with the stated label claim. Hence, there is no substantial disparity between the suggested approaches and the documented ones.

Table 3. Application of the developed methods for the determining GMF in dosage forms.

Sample	Taken (µg/mL)	Added (µg/mL)	Recovery [a] (%)			Reported method [26]
			AM	MB	IC	
Flobiotic tablets	4.0	-	99.10	100.20	99.30	
		2.0	99.40	99.50	101.20	
		4.0	100.90	98.90	99.20	
		6.0	99.0	100.50	100.40	
Mean ± SD[a]			99.60 ± 0.88	99.78 ± 0.72	100.03 ± 0.95	99.40 ± 0.71
RSD% [a]			0.88	0.72	0.95	0.71
Variance			0.77	0.52	0.90	0.50
t-value [b]			0.40	0.84	1.19	
F-value [b]			1.08	1.04	1.80	
GemiQue tablets	4.0	-	99.20	100.40	99.70	
		2.0	99.60	99.20	100.30	
		4.0	100.80	99.10	99.20	
		6.0	98.80	100.30	99.0	
Mean ± SD[a]			99.60 ± 0.86	99.75 ± 0.70	99.55 ± 0.58	99.84 ± 0.52
RSD% [a]			0.86	0.70	0.58	0.52
Variance			0.75	0.49	0.34	0.27
t-value [b]			0.53	0.23	0.83	
F-value [b]			2.78	1.81	1.26	

[a]Average of six determinations; SD: standard deviation; RSD%: percentage relative standard deviation. [b]Theoretical values of t and F are 2.571 and 5.05, respectively at confidence limit at 95% confidence level and five degrees of freedom (p = 0.05).

Comparison of the developed methods with other reported spectrophotometric methods.

The proposed methods were found to be superior compared to the published spectrophotometric methods [13-36] in terms of simplicity, sensitivity, cost-effectiveness and good estimation ranges. They do not require expensive reagents, and apparatus. No heating and extraction procedure is required. Comparison of the performance characteristics of the proposed methods and the reported methods is shown in Table 4.

Table 4. An analysis comparing different spectrophotometric methods used to determine GMF.

Reagent	λ_{max} nm	Concentration range (µg/mL)	LOD (µg/mL)	Molar absorptivity L/mol.cm	Ref.
UV- Spectrophotometry	250	3-15	0.69	NA	[13]
UV- Spectrophotometry	270	0.5-5	0.197	NA	[14]
UV- Spectrophotometry	267	10-70	NA	NA	[15]
UV- Spectrophotometry	268.5-258.5	2-12	NA	NA	[16]
Safranin O	525	3.0-15	NA	2.81×10^4	[17]
Methylene blue	650	4.0-20	NA	2.20×10^4	
Naphthol blue 12BR	620	2.0-10	NA	4.02×10^4	

Reagent	λ_{max} nm	Concentration range (µg/mL)	LOD (µg/mL)	Molar absorptivity L/mol.cm	Ref.
Azocaramine G	540	2.0-10	NA	4.15×10^{4}	
Fast Green dye (FGFCF)	625	30-100	NA	2.37×10^{3}	[18]
Brucine	520	40-80	NA	0.746×10^{3}	
Vanillin	500	10-40	NA	1.38×10^{3}	
BCG	420	1-16	0.23	2.1787×10^{4}	[19]
BCP	408	1-12	0.26	3.9244×10^{4}	
BTB	415	2-16	0.52	1.8904×10^{4}	
BPB	416	1-16	0.28	2.4457×10^{4}	
MO	422	3-30	0.87	0.9386×10^{4}	
Rose Bengal	575	9.71-53.40	1.90	1.861×10^{4}	[20]
Methyl Orange	427	10-80	0.2563	NA	[21]
Iodine	290	6.0-30	NA	NA	[22]
2,3-dichloro-5,6-dicyano-p-benzoquinone (DDQ)	470	2.0-10	NA	NA	
7,7,8,8-Tetracyanoquinodimethane (TCNQ)	840	2.5-12.5	NA	NA	
Tetracyanoethylene (TCNE)	420	1.0-5.0	NA	NA	
$KMnO_4$/NaOH	610	4.0-36	0.0778	1.21×10^{4}	[23]
Phosphomolybdic acid	794	5-27	4.49	1.8×10^{4}	[24]
Chloramine-T/ Rhodamine-B	557	NA	NA	NA	[25]
NBD-Cl/borate buffer	466	0.5-8.0	0.12	4.0892×10^{4}	[26]
$KMnO_4$/NaOH	610	2-20	0.42	1.067×10^{4}	[27]
Eosin	543	1-10	0.157	NA	[28]
Cerium (IV) sulfate/methyl orange	507	2–9	0.27	2.117×10^{4}	[29]
1,2-Naphthoquinone-4-sulphonate (NQS) in alkaline medium (pH 11)	411	5–30	1.04	7.523×10^{3}	
Fe(III) +2,2'-bipyridyl	520	6.4-32	NA	2.18×10^{3}	[30]
Ferric alum	460	5.1-25.6	NA	0.550×10^{3}	
Palladium/zero order	430	2.0 - 14	NA	1.365×10^{4}	[31]
Palladium/Ist derivative	480	1.0 - 10	NA	9.37×10^{4}	
Palladium/ 2nd derivative	500	1.0- 15	NA	1.59×10^{4}	
Chromium/zero order	545	2.0 - 20	NA	1.07×10^{3}	[32]
Chromium/Ist derivative	620	1.0 - 15	NA	7.01×10^{4}	
Chromium/2nd derivative	660	1.0- 25	NA	1.04×10^{4}	
Ninhydrine (DMF)	590	4.0-32	NA	9.68×10^{3}	[33]
Ascorbic acid (DMF)	530	8.0-40	NA	5.58×10^{3}	
p-Benzoquinone (PBQ)	400	9.0–72	NA	4.98×10^{3}	
Folin-Ciocalteu / NaOH	685	10-50	NA	NA	[34]
3-Methyl-2-benzothiazolinone hydrazone/ $FeCl_3$	617	10-100	NA	NA	
$FeCl_3$/1,10-phenanthorline	466	40-200	NA	NA	

Reagent	λ_{max} nm	Concentration range (µg/mL)	LOD (µg/mL)	Molar absorptivity L/mol.cm	Ref.
Ferric nitrate/HCl	471	NA	NA	NA	[35]
Palladium(II), eosin /methyl cellulose	530	2-22	NA	NA	[36]
NBS/HCl/AM	520	1.0-18	0.30	1.0392×10^4	The propose d work
NBS/HCl/MB	664	1.0-14	0.29	1.281×10^4	
NBS/HCl/IC	610	1.0-20	0.30	1.2387×10^4	

NA: not available.

Greenness evaluation of the proposed approaches

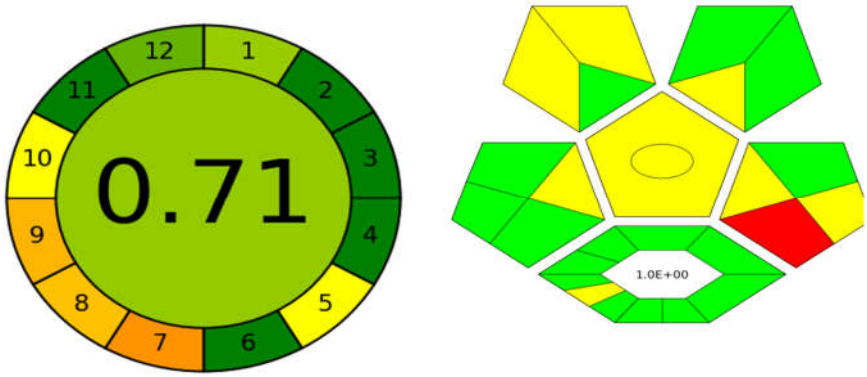
Not all analytical techniques possess equivalent levels of environmental sustainability; thus, it is necessary to evaluate the environmental impact of analytical approaches. Three distinct methodologies for assessing greenness were employed to measure the environmental friendliness of the created techniques.

AGREE

AGREE is assessed as a software that can be designed to be environmentally friendly, based on the concepts of Green Analytical Chemistry (GAC). These principles include various aspects, including sample preparation processes and operator safety. The 12 principles are arranged in a right-handed way, with each sector being color-coded on a scale that goes from red to yellow to green. This color scheme indicates the level of environmental friendliness that a method has. The score runs from 0 to 1, representing the spectrum from the lowest to the highest level of environmental friendliness. A number close to 1 indicates optimal performance, hence demonstrating the environmental sustainability of our processes [47, 48]. The proposed methods were subjected to this approach, and the resulting pictogram, as shown in Figure 4(a), received a favorable score of 0.71, suggesting that the developed method is ecologically friendly. One notable benefit of AGREE is its ability to clearly identify the robust and feeble aspects within the concepts of GAC. Furthermore, the overall score of the GAC bases is both dependable and instructive. AGREE is highly recommended due to its simplicity and flexibility in controlling the width of each component based on their respective relevance. Additionally, this tool is automated, allowing users to easily receive conclusions and information regarding the greenness profile of the approach with less effort.

GAPI

This method is regarded as a dependable instrument that offers a thorough ecological evaluation of the complete analytical process, beginning with collecting the sample and continuing through sample safeguarding, transportation, and preparation, till the end result is determined [47]. This method utilizes five pentagrams to illustrate the ecological consequences of the innovative methodology. The stages are categorized by color, ranging from green to yellow and finally to red, to indicate low, moderate, and high environmental effect, respectively. Applying this methodology to the developed methods showed that the developed methods are ecologically friendly. This is evident from the fact that six portions were coloured green, six were colored yellow, and just three were colored red, as shown in Figure 4(b). The revolutionary methods clearly had a negligible impact on the environment.



(a) (b)

Figure 4. Greenness evaluation tools of the proposed approaches (a) AGREE and (b) GAPI.

AES

The AES is a semi-quantitative way used to assess the environmental friendliness of analytical methods. It assigns penalty points to certain aspects of the techniques being evaluated. Compared to other techniques, it is recognized for its user-friendly interface, straightforwardness, and efficiency [47]. The most efficient green analysis, as per this approach, entails reducing the quantity of detrimental liquids, diminishing waste generation, and minimizing energy usage. Thus, the quantities of reagents, waste production, energy consumption, and risks are evaluated to determine their respective penalty scores [48, 49]. The penalty points associated with all the elements of the complete procedure were added together and then removed from a base value of 100. According to the report, a score > 75 indicated good ecofriendly analysis, a score < 50 suggested inadequate ecofriendly analysis, and a score ranged from 50 to 75 indicated acceptable ecofriendly analysis. The estimated Eco-Scale scores for the AM, IC, and MB procedures were 77, 77, and 75, respectively. These scores indicate the high level of environmental friendliness and least negative effects on human health.

CONCLUSION

Spectrophotometry can be beneficial in quality control laboratories for drug analysis and can offer maximum sensitivity without requiring costly equipment. The proposed spectrophotometric methods are deemed to possess high sensitivity, selectivity, cost-effectiveness, accuracy, and precision. They also eliminate the need for essential experimental components, arduous extraction procedures, and the use of toxic solvents, hence saving time. These methods effectively quantified the amount of GMF in its pure and dosage forms. The procedures employed utilize NBS as an environmentally friendly brominating agent and have been thoroughly validated in compliance with the International Council for Harmonization (ICH) criteria. The evaluation of the greenness profile was conducted using three distinct green metrics that were specifically developed to assess environmental friendliness: AGREEprep, GAPI, and AES. Therefore, it is clear that these methods have the potential to be considered as environmentally friendly options for analyzing genetically modified food in quality control laboratories.

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