

<https://doi.org/10.48047/AFJBS.6.2.2024.2383-2394>



African Journal of Biological Sciences

Journal homepage: <http://www.afjbs.com>



Research Paper

Open Access

Validated spectrophotometric methods for the determination of fluoroquinolone antibiotic: Gemifloxacin mesylate in pharmaceutical formulations using ceric (IV) ammonium sulfate

Ragaa El Sheikh, Ayman A. Gouda*, Ahmed El Sayed, Moataz S. Mahmoud, Ahmed F. Abdel Allem, Nesma M. Jumaa, Noha E.M. Elsaify, Nehal S.A. Soliman, Asmaa El Sayed

Chemistry Department, Faculty of Science, Zagazig University, Zagazig, 44519, Egypt

Corresponding author: Ayman A. Gouda

Email: aymangouda77@gmail.com

Article History

Volume 6, Issue 2, Apr-Aug 2024

Received: 18 July 2024

Accepted: 5th August 2024

Published: 24 August 2024

doi: [10.48047/AFJBS.6.14.2024.2383-2394](https://doi.org/10.48047/AFJBS.6.14.2024.2383-2394)

Abstract: Background: A validated, sensitive, precise, and dependable spectrophotometric technique has been developed to accurately detect the concentration of gemifloxacin mesylate (GMF) in pure and dosage forms. The methods utilise ceric(IV) ammonium sulfate (Ce(IV)) as an oxidising agent and 3 specific dyes: amaranth (AM), methylene blue (MB), and methyle orange (MO). The ways depend on the oxidation process of GMF using a plentiful quantity of ceric(IV) ammonium sulfate in acidic circumstances. The residual ceric(IV) ammonium sulfate is measured by subjecting it to a chemical reaction with preset amounts of dyes, AM, MB, and MO. The absorbance is measured at λ_{max} of 520, 664 and 510 nm for AM, MB, and MO dye, 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 0.9990 to 0.9997, were observed under optimal conditions. These associations remained consistent throughout concentration ranges of 1.0-10, 1.0-12, and 1.0-14 $\mu\text{g/ml}$. The limits of detection (LOD) of 0.30, 0.29, and 0.28 $\mu\text{g/ml}$ for AM, MB, and MO methods, respectively. The calculated molar absorptivity values are 2.789×10^4 , 2.390×10^4 and $1.7436 \times 10^4 \text{ L.mol}^{-1}\text{.cm}^{-1}$ using AM, MB and MO methods, respectively. The accuracy and precision of the approaches' have been evaluated for measurements conducted within a single day as well as measurements conducted over multiple days. The methods were successfully applied to the assay of gemifloxacin mesylate in tablets and the results were statistically compared with those of the reference method by applying Student's *t*-test and *F*-test. No significant interference was observed with the usual pill excipients.

Keywords: Gemifloxacin mesylate; Ceric(IV) ammonium sulfate; Dyes; Spectrophotometry, Method validation, Dosage forms.

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. Gemifloxacin mesylate (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.

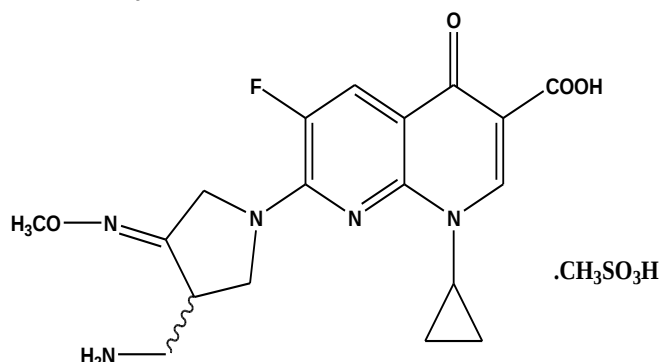


Figure 1. The chemical structure of gemifloxacin mesylate (GMF)

The literature review reveals that there are only a few published ways for determining GMF in dosage forms. These techniques include Densitometric thin-layer chromatography (TLC) [4], chromatography [5-7], electrochemistry [8-10], and spectrofluorimetry [11, 12, 35, 45]. 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 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.

Ceric(IV) ammonium sulfate has been widely used as an effective analytical reagent in spectrophotometric methods for the determination of many pharmaceutical compounds [37-41]. Ceric(IV) ammonium sulfate is a strong oxidant, and it has not been applied for the assay of GMF in pure form and tablets.

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 ceric(IV) ammonium sulfate as an environmentally friendly agent, in conjunction with AM, MB, and MO 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 [42].

EXERIMENTAL

Equipment

A Shimadzu UV-1601 UV/Vis. spectrophotometer (Sweden) equipped with a 10 mm quartz cell was utilised 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.

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.

Pure GMF and dosage forms

The pure GMF was provided by Al-Obour Pharmaceutical & Chemical Industries Company in Egypt. Its potency was measured to be $99.60 \pm 0.74\%$. 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 0.1 M HCl (0.2 mL). 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 1 week at 4°C.

A stock solution of 5.0×10^{-3} mol/L ceric(IV) ammonium sulfate (E-Merk, Darmstadt, Germany) was freshly prepared by dissolving 316.2 mg of cerium(IV) sulphate in the least amount of H_2SO_4 (2.0 mol/L) then completed to the mark in a 100 mL calibrated flask with the same acid and kept in a dark bottle and a refrigerator when not in use. A stock solution of 2.0 mol/L H_2SO_4 was prepared by adding 10.8 mL of concentrated acid (Merck, Darmstadt, Germany, 98%, Sp. Gr. 1.84) to bidistilled water, cooled to room temperature, transfer to 100 mL with measuring flask, diluted to the mark and standardized as recorded [43]. All standard solutions were stored in a refrigerator when it was not in use.

A stock solution of AM, MB, or MO (Sigma-aldrish, 90% dye concentration) 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.

Recommended procedures

Different aliquots (0.1-1.0 mL), (0.1-1.2 mL) and (0.1-1.4 mL) of a standard 100 µg mL⁻¹ GMF solution using AM, MB and MO methods, respectively, were transferred into a series of 10 mL calibrated flasks followed by adding 1.5 mL of 2.0 mol L⁻¹ H_2SO_4 and 1.0 mL of (5.0×10^{-3} mol L⁻¹) Ce(IV) solution. The flasks were stoppered and the contents were mixed well and the flasks were kept aside for 5.0 min with occasional shaking. Finally, 1.0 mL of (200 µg mL⁻¹) AM, MB or MO dye solution was added to each flask and mixed well, and then the volume was diluted to the mark with bidistilled water. The color intensity of dyes were measured after 5.0 min against reagent blank solution treated similarly omitting the drug, at their corresponding λ_{max} 520, 664 or 510 nm, respectively. The concentration of unknown was determined in each case from calibration graph which obtained by plotting the concentration of GMF against absorbance or computed from the regression equation derived using Beer's law data.

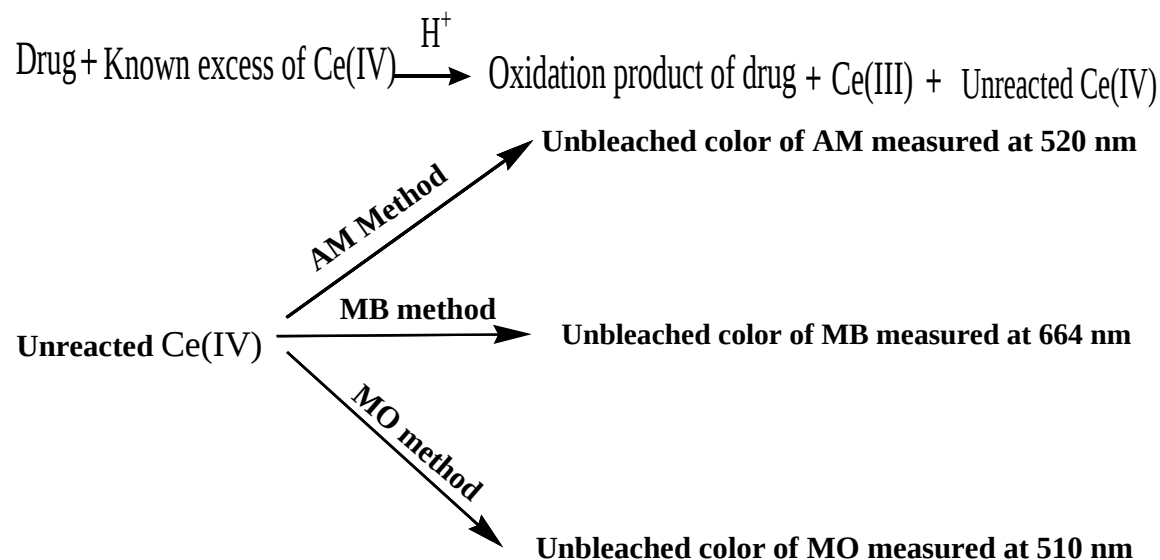
Procedure for dosage forms

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 minutes 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

In an acidic atmosphere, oxidizing agents cause a permanent conversion of many colors into compounds that lack color [44]. The proposed spectrophotometric method for the determination of GMF is indirect and involves two steps. The 1st stage involved the GMF oxidation utilising an excessive amount of Ce(IV) in acidic solution. In the 2nd stage, the surplus remaining Ce(IV) was quantified by reacting it with a specified amount of AM, MB, and MO dyes, and subsequently absorbance measuring at their respective $\lambda_{\max}$. 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 bleaching properties of Ce(IV) oxidant on dyes, leading to the fading of color caused by the oxidative breakdown of the pigment.



Scheme 1. The recommended chemical route for the proposed spectrophotometric approaches involves the utilization of Ce(IV) and dyes.

Analytical parameters optimization

The optimum conditions for the assay procedures and color development for each method have been established by varying the parameters one at a time, keeping the others fixed and observing the effect produced on the absorbance of the colored species.

Effect of acid type and concentration

The GMF and ceric(IV) ammonium sulfate conducted a reaction in several acidic solutions, such as HCl, H₂SO₄, H₃PO₄, HNO₃ and CH₃COOH. Exceptional results were obtained in a sulphuric acid (H₂SO₄) medium. An investigation was conducted to examine the influence of H₂SO₄ concentration. The H₂SO₄ concentration was modified between 0.25–3.0 ml of H₂SO₄ (2.0 mol/l) with keeping the ceric (IV) ammonium sulfate and GMF concentrations are constant. The results indicated that when utilizing a volume of 0.5–1.5 ml of H₂SO₄ with a concentration of 2.0 mol/l, the absorbance values produced in the presence of GMF were almost indistinguishable. When the volume of acid was less than 0.5 ml, the reaction proceeded at a slower rate and did not reach completion. Therefore, a fixed amount of 1.5 ml of H₂SO₄ (2.0 mol/l) was used as shown in Figure 3.

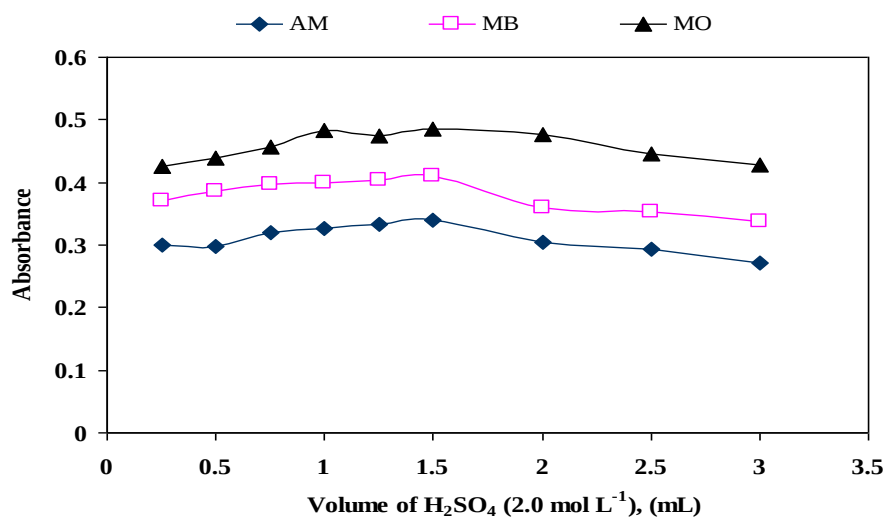


Figure 3. Effect of volume of H_2SO_4 (2.0 mol L^{-1}) on the absorbance of 10 $\mu\text{g/ml}$ GMF with Ce(IV) (5.0×10^{-3} mol L^{-1}) and (200 $\mu\text{g/ml}$) AM, MB and MO dyes.

Effect of ceric (IV) ammonium sulfate

To ascertain the optimal concentration of ceric (IV) ammonium sulfate, several volumes of ceric (IV) ammonium sulfate (5.0×10^{-3} mol/L) in the range of 0.25-3.0 ml were treated with a consistent quantity of dye in H_2SO_4 solution. The investigation found that the greatest absorbance value was achieved by utilizing 1.0 ml of ceric (IV) ammonium sulfate (5.0×10^{-3} mol/L) (Figure 4).

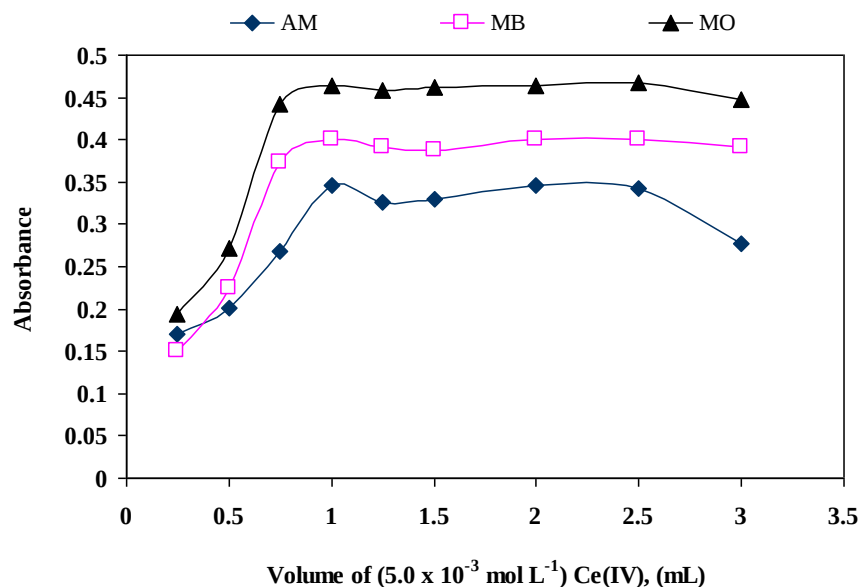


Figure 4. Effect of volume of Ce(IV) (5.0×10^{-3} mol L^{-1}) on the reaction product of GMF (10 $\mu\text{g/ml}$) with Ce(IV) and dyes in H_2SO_4 medium.

Effect of dye

A study was carried out to ascertain the ideal volume of AM, MB, and MO dyes at a concentration of 200 $\mu\text{g/ml}$ 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 1.0 ml of dye solution (Figure 5).

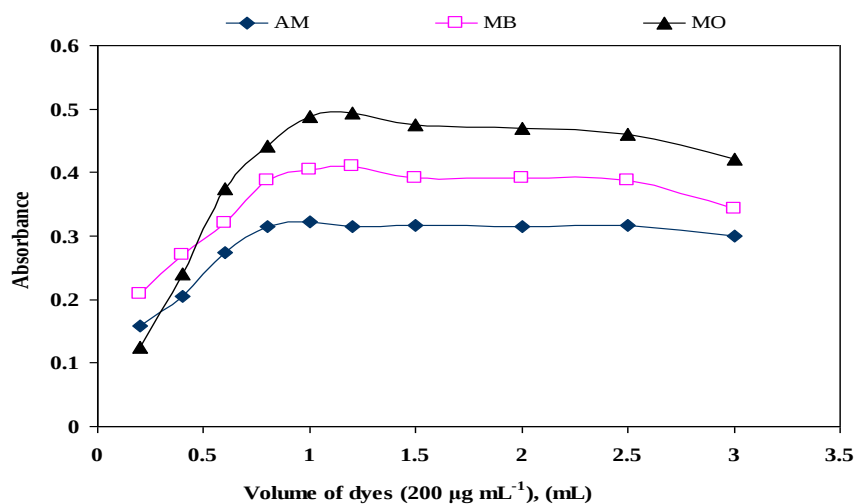


Figure 5. Effect of volume of dyes (200 µg/ml) on the reaction product of GMF (10 µg/ml) with Ce(IV) ($5.0 \times 10^{-3} \text{ mol L}^{-1}$) in H_2SO_4 medium.

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 20-60 °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 5.0 minutes is required in order to completely remove the dye pigment using the remaining Ce(IV). The dye absorbance that did not undergo a reaction remained consistent for a minimum of 8.0 hours following this time period.

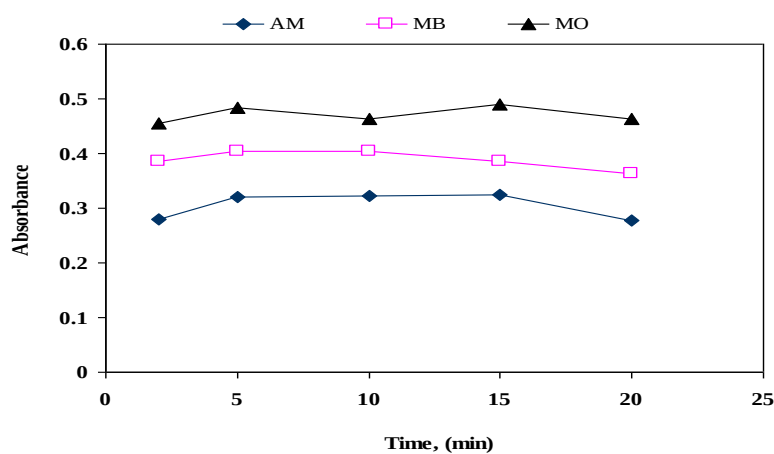


Figure 6. Effect of time on the reaction product of GMF (10 µg/ml) with Ce(IV) ($5.0 \times 10^{-3} \text{ mol L}^{-1}$) and dyes (200 µg/ml) in H_2SO_4 (2.0 mol L^{-1}) medium.

Impact of the sequence of addition

The optimal sequence of addition was as follows: GMF, H₂SO₄-Ce(IV) 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 1.0-10, 1.0-12 and 1.0-14 µg/ml using AM, MB, and MO methods, respectively. To get accurate outcomes, the Ringbom concentration range [45] was established. The detection (LOD = 3s / k) and (LOQ=10 s / k) quantitation limits for the suggested procedures were determined. The variable "s" represents the standard deviation of 10 repeated measurements of the reagent blank, whereas "k" represents the sensitivity, which is defined as the slope of the calibration graph. The resulting LOD values of 0.30, 0.29, and 0.28 µg/ml and LOQ values was determined to be 1.0, 0.97, and 0.93 µg/ml for the AM, MB, and MO 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	MO
Beer's law limits, µg/ml	1.0-10	1.0-12	1.0-14
Ringboom limits, µg/ml	3.0-8.0	3.0-10	3.0-12
Molar absorptivity, x 10 ⁴ L/mol.cm	2.789	2.390	1.7436
Sandell sensitivity, ng/cm ²	17.41	20.31	27.84
Regression equation ^a			
Intercept (a)	0.0041	-0.0342	0.00005
Standard deviation of intercept (S _a)	0.012	0.021	0.008
Slope (b)	0.0577	0.062	0.0369
Standard deviation of slope (S _b)	0.009	0.015	0.01
Correlation coefficient, (r)	0.9990	0.9997	0.9995
Mean ± SD	100.05±1.0	99.27±0.91	99.30±1.04
RSD%	1.0	0.91	1.04
RE%	1.05	0.96	1.10
Limit of detection, µg/ml	0.3	0.29	0.28
Limit of quantification, µg/ml	1.0	0.97	0.93
Calculated <i>t</i> -value ^b	0.81	0.63	0.53
Calculated <i>F</i> -value ^b	1.83	1.51	1.98

^a $A = a + bC$, where C is the concentration in µg mL⁻¹, 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

In 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. In order to evaluate the exactness and correctness of the proposed methods, it is possible to examine smaller values of the relative standard deviation (RSD%) and percentage

relative error (RE%). 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	3.0	99.20	0.70	-0.80	2.976±0.021
	6.0	99.70	0.94	-0.30	5.982±0.056
	9.0	100.60	1.23	0.60	9.054±0.111
MB	2.0	99.30	0.57	-0.70	1.986± 0.012
	4.0	99.90	0.80	-0.10	3.996 ± 0.034
	8.0	99.20	1.10	-0.80	7.936 ± 0.092
MO	2.0	99.00	0.67	-1.00	1.980 ± 0.014
	6.0	100.20	0.92	0.20	6.012± 0.058
	10	100.50	1.25	0.50	10.05 ± 0.132
Inter-day					
AM	3.0	99.50	0.47	-0.50	99.50
	6.0	100.20	0.66	0.20	100.20
	9.0	100.70	1.10	0.70	100.70
MB	2.0	99.80	0.63	-0.20	1.996 ± 0.013
	4.0	99.00	0.96	-0.10	3.960 ± 0.04
	8.0	100.60	1.30	0.60	8.048 ± 0.11
MO	2.0	99.60	0.64	-0.40	1.992 ± 0.013
	6.0	99.10	0.76	-0.80	5.946 ± 0.047
	10	99.60	1.40	-0.40	9.960 ± 0.146

^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 strength of the method, the acid 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 0.55-2.20%. 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 0.72% to 2.30%, whereas the inter-instruments RSD% varied from 0.45% to 2.40%, indicating that the suggested methodologies were reliable and consistent. The information is presented in Table 3.

Table 3. Robustness and ruggedness results of the developed methods. (n=3).

Methods	Nominal amount concentration (µg/ml)	RSD%			
		Robustness		Ruggedness	
		Variable alerted ^a			
		Acid volume	Reaction time	Different analysts	Different instruments
AM	3.0	1.04	0.58	0.72	0.60
	6.0	1.52	1.35	1.45	1.70
	9.0	1.95	2.10	2.15	2.40
MB	2.0	1.0	0.65	1.10	1.20
	6.0	1.75	1.40	1.40	1.60
	8.0	2.10	2.20	2.30	2.20
IC	2.0	0.80	0.55	1.20	0.45
	6.0	1.750	1.10	1.60	1.20
	10	2.0	1.80	2.10	2.0

^a Volume of (2.0 mol L⁻¹) H₂SO₄ is (±0.2 mL) and reaction time is (±2.0 min) (after adding Ce(IV)) were used.

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 utilising the following equation:

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

The variable C_F represents the total GMF concentration that was discovered. C_T 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 4, indicate that the accuracy of the suggested techniques remained not influenced by the different substances added to the formulations. Table 4 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 4. Application of the developed methods for the determining GMF in dosage forms.

Sample	Taken ($\mu\text{g/ml}$)	Added ($\mu\text{g/ml}$)	Recovery ^a (%)			Reported method [26]
			AM	MB	IC	
Flobiotic tablets	4.0	-	99.20	99.10	99.50	
		2.0	99.50	99.40	100.20	
		4.0	100.80	98.80	100.70	
		6.0	99.0	100.60	99.20	
Mean $\pm$ SD ^a			99.63 $\pm$ 0.81	99.48 $\pm$ 0.79	99.90 $\pm$ 0.68	99.40 $\pm$ 0.71
RSD% ^a			0.81	0.79	0.68	0.71
Variance			0.66	0.62	0.46	0.50
t-value ^b			0.48	0.17	1.14	
F-value ^b			1.32	1.24	1.09	
GemiQue tablets	4.0	-	99.90	99.40	98.70	
		2.0	99.00	100.50	99.30	
		4.0	98.80	99.00	100.20	
		6.0	100.60	100.60	100.0	
Mean $\pm$ SD ^a			99.45 $\pm$ 0.83	99.95 $\pm$ 0.80	99.65 $\pm$ 0.69	99.84 $\pm$ 0.52
RSD% ^a			0.83	0.80	0.69	0.52
Variance			0.70	0.64	0.47	0.27
t-value ^b			0.89	0.26	0.55	
F-value ^b			2.59	2.37	1.74	

^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).

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 ceric(IV) ammonium sulfate as an environmentally friendly brominating agent and have been thoroughly validated in compliance with the International Council for Harmonisation (ICH) criteria.

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