https://doi.org/10.48047/AFJBS.6.2.2024.2395-2408

African Journal of Biological Sciences

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

Research Paper Open Access

Evaluation of the Greenness Profiles of Spectrophotometric Methods for Determination of Moxifloxacin Hydrochloride in Pharmaceutical Formulations

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Article History

Volume 6, Issue 2, Apr-Aug 2024

Received: 16 July 2024

Accepted: 5th August 2024

Published: 24 August 2024

doi: 10.48047/AFJBS.6.2.2024.2395-2408

Abstract: Background: An accurate, precise, user-friendly, sensitive and validated spectrophotometric method has been developed for the estimation of moxifloxacin hydrochloride (MXF) in both pure form and dosage forms. In these methods N-bromosuccinimide (NBS) is used as an environmentally friendly oxidizing agent in conjunction with three selected dyes: amaranth (AM), methylene blue (MB) and indigocarmine (IC). The methods employ excess amounts of NBS under acidic conditions to oxidize moxifloxacin hydrochloride. Using predetermined quantities of dyes AM, MB and IC the unreacted NBS is determined by reacting it chemically. Absorption was measured at 520 nm, 664 nm, 610 nm for AM, MB and IC dyes 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 were valid within a concentration range of 1.0-18 µg/ml, 1.0-14 µg/ml, and 1.0-20 µg/ml respectively. AM method was found to have an LOD of 0.30 µg/ml while MB had an LOD of 0.29 µg/ml and IC had an LOD of 0.30 µg/ml. 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. No significant interference was observed with the usual pill excipients. Besides, the suggested processes' environmental impact was evaluated through three evaluation techniques specifically designed to measure environmental friendliness: the Analytical Greenness Metric (AGREE), the Green Analytical Procedure Index (GAPI) and Analytical Eco-Scale (AES). These assessments conclusively verified that the approaches have a diminished detrimental impact on the environment

Keywords: *Moxifloxacin hydrochloride; N-bromosuccinimide, Spectrophotometry, Method validation, Pharmaceutical formulations, Greenness assessment tools.*

Introduction

Moxifloxacin hydrochloride (MXF) belongs to the class of broad-spectrum antibacterial compound known as fluoroquinolones [1]. This therapeutic approach is mostly utilised for the management of acute bacterial sinusitis resulting from the presence of susceptible microorganisms, acute bacterial chronic bronchitis, mild to moderate community intravenous pneumonia, as well as skin and soft tissue infections, with minimal occurrence of sequelae. MXF, a novel iteration of antibacterial fluoroquinolone, exhibits robust antimicrobial efficacy, favourable clinical outcomes, and minimal toxicity. Within the field of ophthalmology, MXF is administered in the form of eye drops for the purpose of treating conjunctival infections caused by bacteria that are susceptible to its effects, as well as for preventing infection subsequent to ocular procedures [2]. MXF can be described as 1-cyclopropyl-7-[(S,S)-2,8-diazabicyclo[4.3.0]non-8-yl]-6-fluoro-8-methoxy-1,4-dihydro-4-oxo-3 quinoline carboxylic acid monohydrochloride [3].

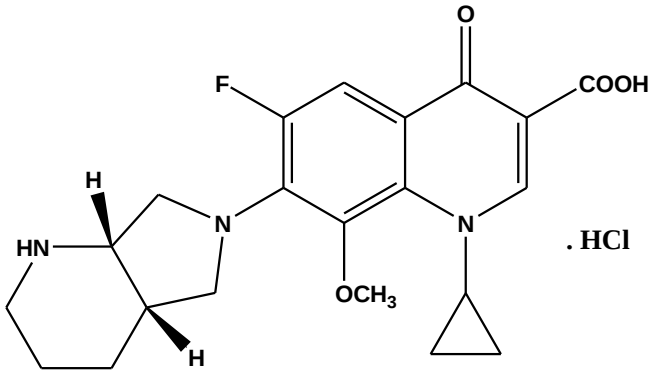


Figure 1. The chemical structure of moxifloxacin hydrochloride (MXF)

The literature review reveals that there are only a few published ways for determining MXF in dosage forms. These techniques include spectrophotometry [4-26], spectrofluorimetry [27-32] chromatography [33-38] and electrochemistry [39-43]. 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 MXF in its dosage forms.

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 [44-47].

The aim of this project is to develop innovative spectrophotometric methods that are simple, highly sensitive, accurate, and affordable for measuring MXF in pure state and dosage forms. The proposed approaches employ NBS as an environmentally friendly agent, in conjunction with AM, MB, and 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 MXF. 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 [48]. 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

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 MXF and dosage forms

The pure MXF was provided by Sabaa, Kahira Company, Egypt. Its potency was measured to be 99.70±0.79%. Avelox ® tablets containing 400 mg of MXF per tablet were purchased from Bayer, Germany, while Moxiflox tablets containing 400 mg of MXF per tablet were obtained from EVA Pharm. & Chem. Ind. Company, Egypt. These tablets were bought from local market.

Standard solutions preparation

To create a standard solution of MXF, 1.0 mg of pure MXF 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 weak at 4°C. A stock solution of NBS from Sigma-Aldrich with a concentration of 200 µg/ml was generated by dissolving around 0.002 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 [49]. Potassium bromide, *KBr (1.0% w/v)* solution was made by dissolving 1.0 g of KBr in 100 ml bidistilled water. HCl (Merck, Darmstadt, Germany, Sp. gr. 1.18, 37%) solution (5.0 mol/l) 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 [50] 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 (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

Varying volumes from 0.05-1.6 ml of MXF 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 water. Each flask was filled with 1.5 ml of HCl (5.0 mol/l), 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 milliliters 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 λmax 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 MXF were accurately measured and subsequently ground into a fine powder. The exact mass of the powdered tablets, equivalent to 10 mg MXF, 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 MXF (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 [51]. The presented methods depend on the reaction between MXF 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 genetically modified food (MXF) 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 MXF concentration and the rise in absorbance at the particular λ_{max}.

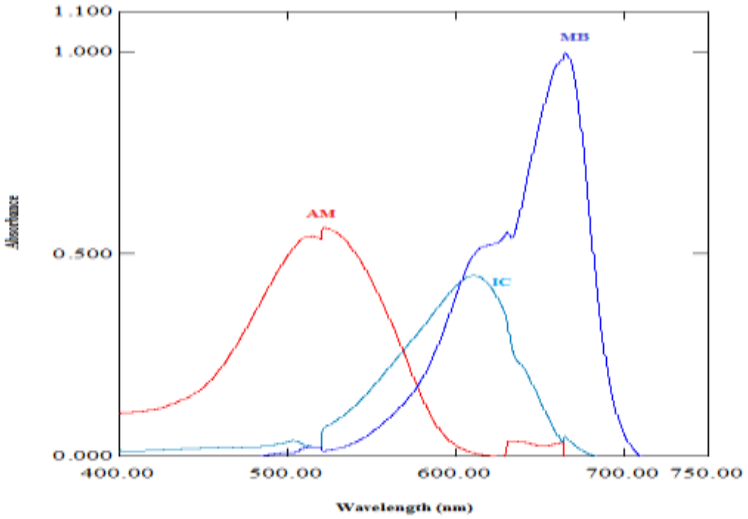
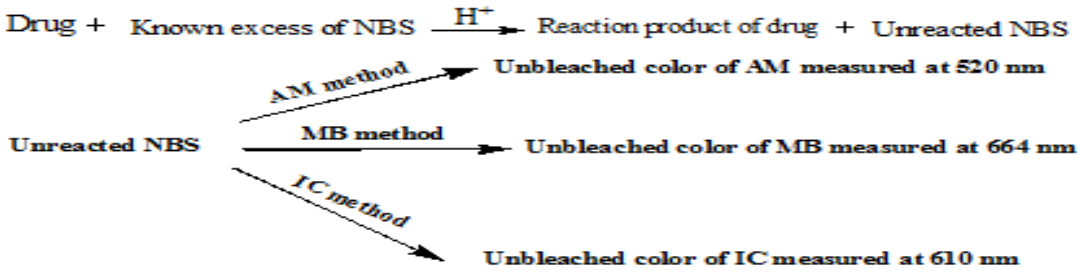


Figure 2. The absorption spectra for the unbleached AM, MB, and IC dyes.

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. Moreover, it is specifically used to introduce bromine atoms into alkenes at the allylic position [52]. The reaction included of 2 consecutive stages. The 1st stage involved the MXF bromination utilising an excessive amount of NBS in HCl solution. 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 MXF 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.



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

Analytical parameters optimization

Identification and quantification of acid type and concentration

The MXF 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 mol/l) with keeping the NBS and MXF 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 mol/l, the absorbance values produced in the presence of MXF were almost indistinguishable. When the volume of acid was less than 1.5 ml, the reaction proceeded at a slower rate and did not reach completion. Therefore, a fixed amount of 1.5 ml of HCl (5.0 mol/l) was used as shown in Figure 3.

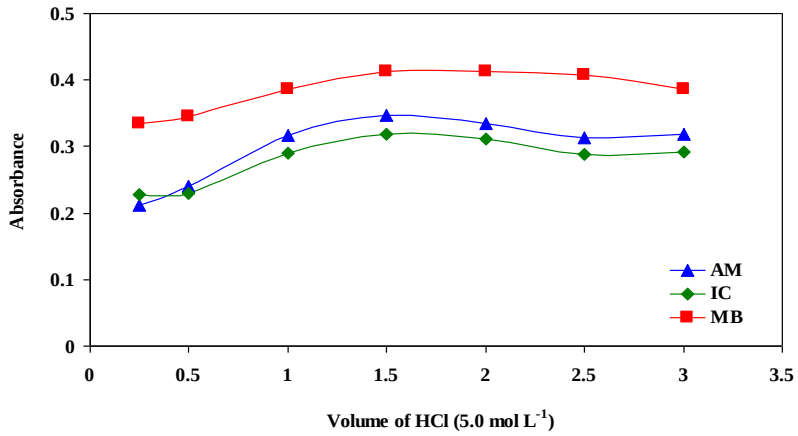


Figure 3. The impact of the HCl volume (5.0 mol/l) on the oxidation product of MXF with NBS and dyes.

Effect of NBS

To ascertain the optimal concentration 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 4).

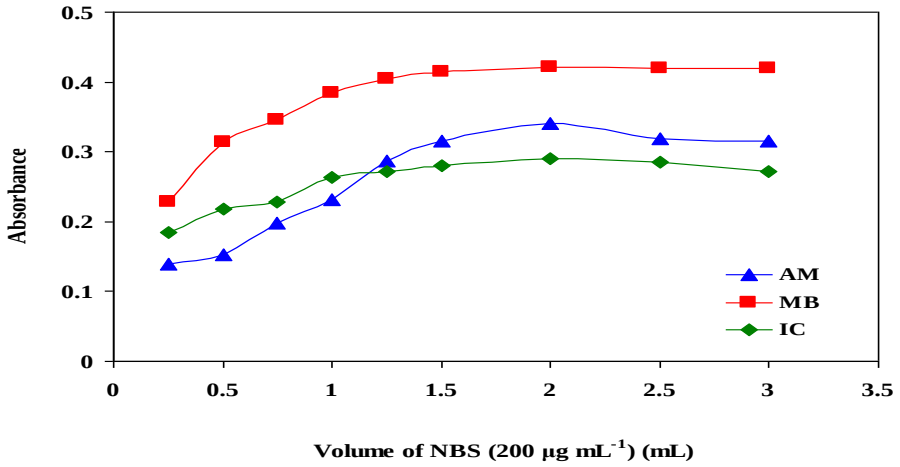


Figure 4. The impact of the NBS volume (200 µg/ml) on the oxidation product of MXF with NBS and dyes in HCl medium.

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.

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 5).

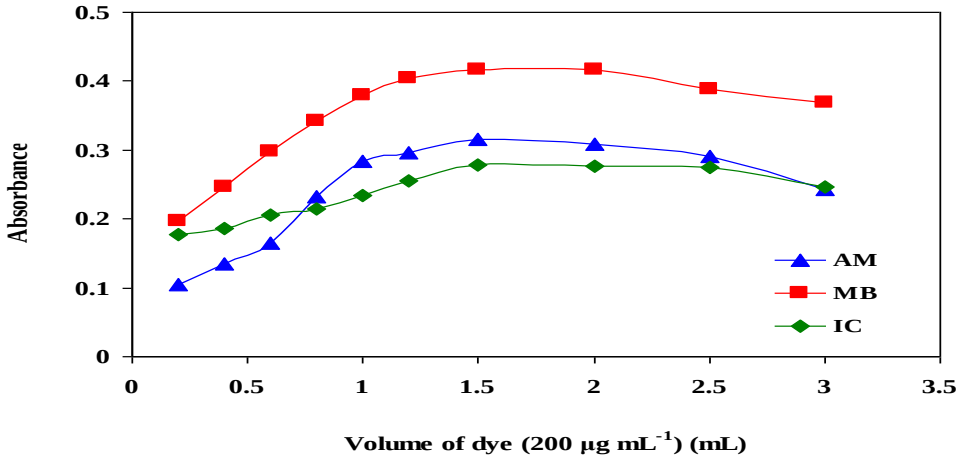


Figure 5. The impact of the dye volume (200 µg/ml) on the oxidation product of MXF with NBS and dyes in HCl medium.

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 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 minutes 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.

Impact of the sequence of addition

The optimal sequence of addition was as follows: MXF, 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 MXF concentration. This association was determined under ideal circumstances. The MXF concentration ranges were 0.5-12, 0.5-16 and 0.5-10 µg/ml using AM, MB, and IC methods, respectively. To get accurate outcomes, the Ringbom concentration range [53] 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 0.15, 0.15, and 0.14 µg/ml and LOQ values was determined to be 0.5, 0.5, and 0.47 µg/ml for the AM, MB, and IC techniques, respectively. The suggested approaches were assessed for validity by statistical analysis [54], comparing the results derived by these approaches with the reported method [15]. Based on the results of the Student's t-test and variance ratio F-test (Table 2), there is no statistically significant distinction between the suggested approach and the method provided in reference [15] in terms of accuracy and precision.

Table 2. Analytical and regression parameters of the developed approaches for determining MXF.

Parameters	**AM**	**MB**	**IC**
Beer's law limits, µg/ml	0.5-12	0.5-16	0.5-10
Ringboom limits, µg/ml	1.0-10	1.0-14	1.0-8.0
Molar absorptivity, x 10^4 L/mol.cm	1.3885	1.2949	2.229
Sandell sensitivity, ng/cm^2	31.54	33.82	19.65
Regression equation[a]			
Intercept (a)	0.013	0.017	0.0023
Standard deviation of intercept (S_a)	0.075	0.045	0.019
Slope (b)	0.0256	0.0229	0.0508
Standard deviation of slope (S_b)	0.034	0.025	0.031
Correlation coefficient, (r)	0.9998	0.9991	0.9995
Mean ± SD	99.30±1.04	99.90±1.20	99.40±1.08
RSD%	1.04	1.20	1.08
RE%	1.09	1.26	1.13
Limit of detection, µg/ml	0.15	0.15	0.14
Limit of quantification, µg/ml	0.50	0.50	0.47
Calculated *t*-value [b]	0.68	0.31	0.50
Calculated *F*-value [b]	1.73	2.31	1.87

[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 MXF concentrations. A study was performed on 6 similar samples. The results obtained from this investigation are concisely summarized in Table 3. 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 3. 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 3. 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	99.60	0.80	-0.40	2.988 ± 0.025
	6.0	100.50	1.10	0.50	6.03 ± 0.07
	9.0	100.30	1.80	0.30	9.027 ± 0.171
MB	5.0	99.40	0.63	-0.60	4.97 ± 0.033
	10	99.20	1.20	-0.80	9.92 ± 0.125
	15	99.60	1.53	-0.40	14.94 ± 0.24
IC	3.0	99.00	0.73	-1.0	2.970±0.023
	6.0	99.50	1.1	-0.50	5.970±0.057
	9.0	100.1	1.7	0.10	9.009±0.16
	Inter-day				
AM	3.0	99.50	0.90	-0.50	2.985 ± 0.028
	6.0	100.40	1.30	0.40	6.024 ± 0.082
	9.0	99.70	1.25	-0.30	8.973 ± 0.118
MB	5.0	99.30	1.04	-0.70	4.965 ± 0.054
	10	100.80	1.60	0.80	10.08± 0.169
	15	100.70	2.05	0.70	15.105 ± 0.325
IC	3.0	100.4	0.59	0.40	3.012 ± 0.019
	6.0	98.80	1.0	-1.2	5.928 ±0.062
	9.0	99.30	1.9	-0.70	8.937 ± 0.180

[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 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 MXF concentration. The techniques used were found to be unaltered, as indicated by the RSD% lying in the range of 0.48-2.30%. 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 0.54% to 2.30%, whereas the inter-instruments RSD% varied from 0.60% to 2.20%, indicating that the suggested methodologies were reliable and consistent. The information is presented in Table 4.

Table 4. Robustness and ruggedness results of the developed methods. (n=3).

Methods	**Nominal amount concentration (µg/ml)**	**RSD%**			
		Robustness		**Ruggedness**	
		Variable alerted [a]			
		NBS volume	Reaction time	Different analysts	Different instruments
AM	3.0	0.48	0.50	0.60	0.68
	6.0	1.90	1.10	1.42	1.55
	9.0	1.85	1.60	1.40	2.10
MB	5.0	1.05	0.78	1.20	0.60
	10	1.70	1.15	1.80	1.60
	15	2.30	1.70	2.30	1.90
IC	3.0	0.59	0.90	0.54	0.75
	6.0	1.90	1.70	1.10	1.20
	9.0	2.30	2.10	1.90	2.20

[a] Volume of (200 µg/ml) NBS is (2.0±0.2 mL) and reaction time is (5.0±2.0 min) 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 three separate MXF concentrations to a preset quantity of MXF in tablets (which had already been examined). The final concentration was subsequently established using the recommended protocols. The determination was replicated three times at each level, and the recovery was calculated utilising the following equation:

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

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 5, 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 [15] by calculating Student's t-test and F-value at a 95% confidence level for 5 degrees of freedom [54]. 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 5. Application of the developed methods for the determining MXF in dosage forms.

Sample	**Taken (µg/ml)**	**Added (µg/ml)**	**Recovery [a] (%)**			**Reported method [17]**
			AM	**MB**	**IC**	
Flobiotic tablets	4.0	-	100.10	100.20	99.10	
		2.0	99.70	99.0	100.10	
		4.0	99.10	99.20	99.70	
		6.0	99.0	100.30	100.30	
Mean ± SD[a]			99.48±0.52	99.68±0.67	99.80±0.53	99.50±0.94
RSD% [a]			0.52	0.67	0.53	0.94
Variance			0.27	0.45	0.28	0.88
t-value [b]			0.04	0.35	0.62	
F-value [b]			3.30	1.96	3.14	
GemiQue tablets	4.0	-	99.80	99.30	99.0	
		2.0	99.20	99.50	100.40	
		4.0	100.80	100.30	99.10	
		6.0	99.40	99.0	98.80	
Mean ± SD[a]			99.80±0.71	99.53±0.56	99.33±0.73	99.60±0.70
RSD% [a]			0.71	0.56	0.73	0.70
Variance			0.51	0.31	0.53	0.49
t-value [b]			0.45	0.44	0.60	
F-value [b]			1.04	1.58	1.08	

[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).

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 colour-coded on a scale that goes from red to yellow to green. This colour 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 [55, 56]. The proposed methods were subjected to this approach, and the resulting pictogram, as shown in Figure 6(a), received a favourable 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 [55]. This method utilises five pentagrams to illustrate the ecological consequences of the innovative methodology. The stages are categorised by colour, 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 coloured yellow, and just three were coloured red, as shown in Figure 6(b). The revolutionary methods clearly had a negligible impact on the environment.

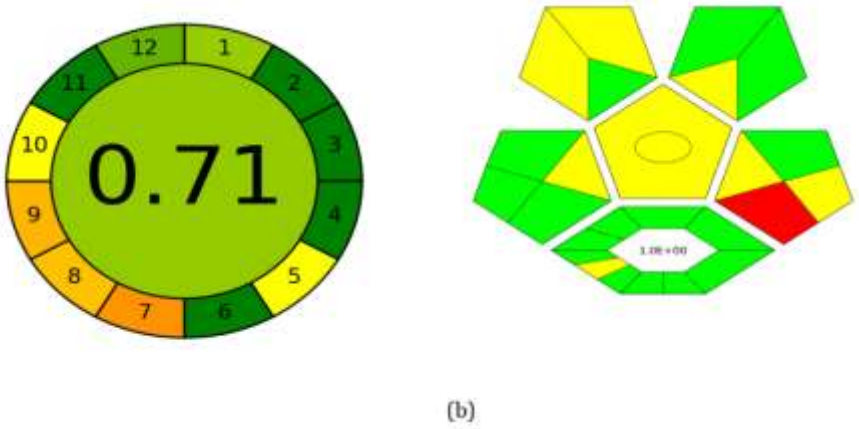


Figure 6. Greenness evaluation tools of the proposed approaches (a) AGREE and (b) GAPI.

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 recognised for its user-friendly interface, straightforwardness, and efficiency [55]. The most efficient green analysis, as per this approach, entails reducing the quantity of detrimental liquids, diminishing waste generation, and minimising energy usage. Thus, the quantities of reagents, waste production, energy consumption, and risks are evaluated to determine their respective penalty scores [56, 57]. 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 MXF 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 Harmonisation (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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