https://doi.org/10.48047/AFJBS.6.2.2024.2486-2498

African Journal of Biological Sciences

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

Research Paper Open Access

Utilization of Ceric Ammonium Sulfate and Dyes for Sensitive Spectrophotometric Determination of Moxifloxacin Hydrochloride in Pure Form and Pharmaceuticals Formulations

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

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: 10 August 2024

Accepted: 28 August 2024

Published: 28 August 2024

doi: 10.48047/AFJBS.6.2.2024.2486-2498

Abstract: A validated, sensitive, precise, and dependable spectrophotometric technique has been developed to accurately detect the concentration of moxifloxacin hydrochloride in pure and dosage forms. The methods utilise ceric(IV) ammonium sulfate (Ce(IV)) as an oxidising agent and 3 specific dyes: indigocarmine (IC), rhodamine B (RB) and orange G (OG). The ways depend on the oxidation process of moxifloxacin hydrochloride 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, IC, RB, and OG. The absorbance is measured at λmax of 610, 550 and 478 nm for IC, RB, and OG 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 1.0-8.0, 1.0-10, and 1.0-15 µg/ml. The limits of detection (LOD) of 0.29, 0.30, and 0.30 µg/ml for IC, RB, and OG methods, respectively. The calculated molar absorptivity values are 2.8262×10^4, 1.8855×10^4 and 1.4717×10^4 $L.mol^{-1}.cm^{-1}$ using IC, RB, and OG 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 moxifloxacin hydrochloride 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: *Moxifloxacin hydrochloride; Spectrophotometry; Method validation; Ceric(IV) ammonium sulfate; Dyes; Pharmaceuticals Formulations*

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].

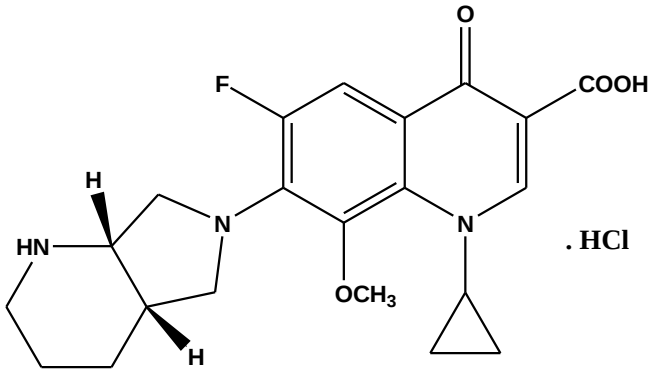


Figure 1. The chemical structure of moxifloxacin hydrochloride (MXF)

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.

Ceric(IV) ammonium sulfate has been widely used as an effective analytical reagent in spectrophotometric methods for the determination of many pharmaceutical compounds [44-48]. Ceric(IV) ammonium sulfate is a strong oxidant, and it has not been applied for the assay of MXF 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 MXF in pure state and dosage forms. The proposed approaches employ ceric(IV) ammonium sulfate as an environmentally friendly agent, in conjunction with IC, RB, and OG 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 [49].

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 5.0×10^{-3} mol/l ceric(IV) ammonium sulfate (E-Merk, Darmstadt, Germany) was freshly prepared by dissolving 316.2 mg of ceric(IV) ammonium 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 [50]. All standard solutions were stored in a refrigerator when it was not in use.

A stock solution of indigocarmine (IC), rhodamine B (RB) and orange G (OG) (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

A range of volumes, specifically 0.1-0.8 mL, 0.1-1.0 mL, and 0.1-1.5 mL, of a standard solution containing 100 µg/ml of MXF were transferred into a set of 10 mL calibrated flasks using a micro burette. The IC, RB and OG procedures were used for each corresponding volume range. Each flask was sequentially supplemented with 1.5 mL of 2.0 mol/l H_2SO_4, and 1.0 mL of (5.0×10^{-3} mol/l) Ce(IV) solution. The flasks were sealed with stoppers, their contents were combined, and then the flasks were set aside for a duration of 5.0 minutes while being shaken occasionally. Subsequently, a total of 1.0 mL of a solution containing 200 µg/ml of IC, RB or OG was added to each flask and thoroughly mixed. The volume was then adjusted to the desired level by diluting it with water. The absorbance of each solution was quantified at wavelengths of 610, 550, and 478 nm using the IC, RB and OG techniques, respectively, after a 5.0-min interval, relative to a reagent blank. A standard graph was created by graphing the absorbance against the drug concentration in all methods. The concentration of the unknown substance was determined either by reading it directly from the calibration graph or by calculating it using the regression equation generated from the data obtained through Beer's law.

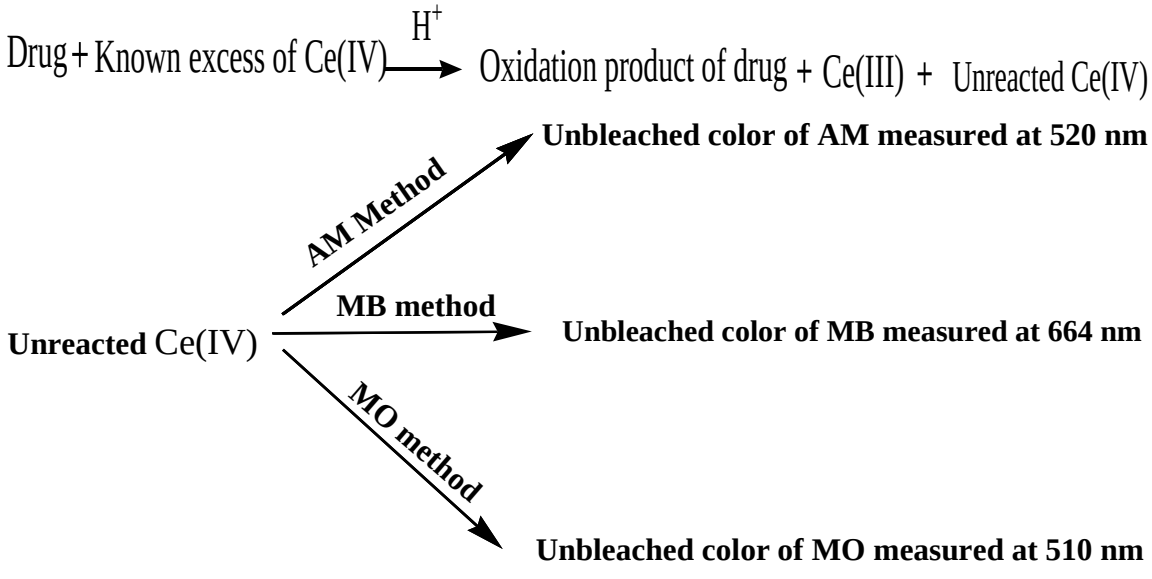
Procedure for dosage forms

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 proposed spectrophotometric method for the determination of MXF is indirect and involves two steps. The 1st stage involved the MXF 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 IC, RB and OG 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 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 MXF and ceric(IV) ammonium sulfate conducted a reaction in several acidic solutions, such as HCl, H_2SO_4, H_3PO_4, HNO_3 and CH_3COOH. Exceptional results were obtained in a sulphuric acid (H_2SO_4) medium. An investigation was conducted to examine the influence of H_2SO_4 concentration. The H_2SO_4concentration was modified between 0.25–3.0 ml of H_2SO_4 (2.0 mol/l) with keeping the ceric (IV) ammonium sulfate and MXF concentrations are constant. The results indicated that when utilizing a volume of 0.5-1.5 ml of H_2SO_4 with a concentration of 2.0 mol/l, the absorbance values produced in the presence of MXF 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 H_2SO_4 (2.0 mol/l) was used as shown in Figure 3.

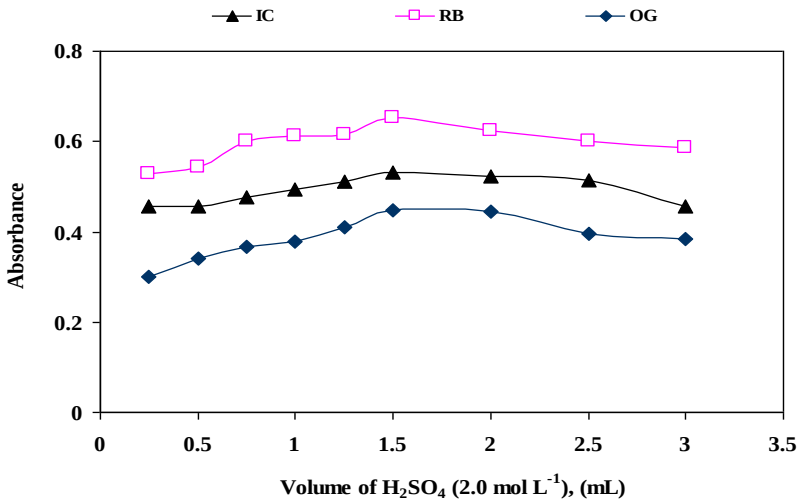


Figure 3. Effect of volume of H_2SO_4 (2.0 mol/L) on the absorbance of 10 µg/ml MXF with Ce(IV) (5.0 x 10^{-3} mol/L) and (200 µg/ml) IC, RB and OG dyes.

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).

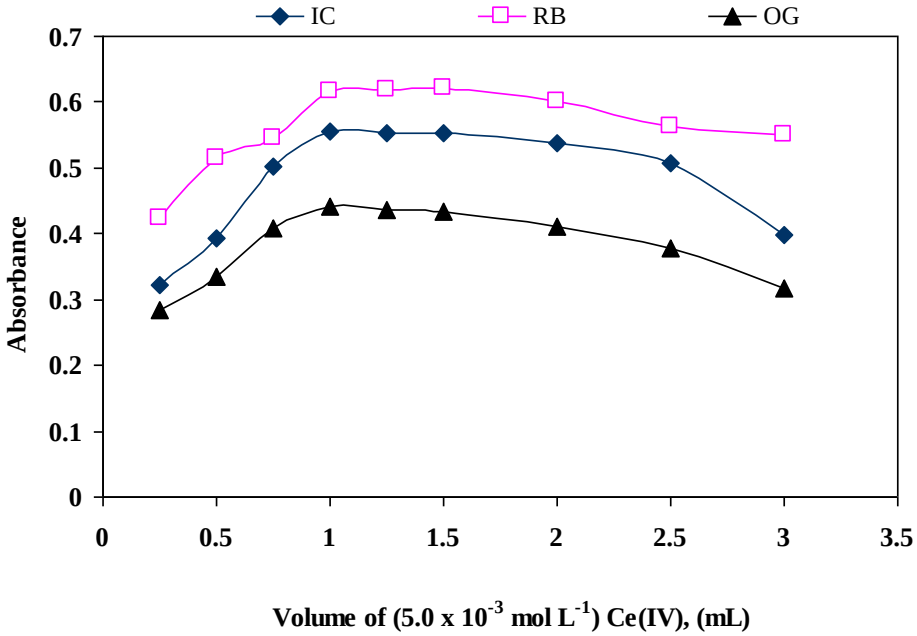


Figure 4. Effect of volume of Ce(IV) (5.0 x 10^{-3} mol/L) on the reaction product of MX (10 µg/ml) with Ce(IV) and dyes in H_2SO_4 medium.

Effect of dye

A study was carried out to ascertain the ideal volume of IC, RB, or OG 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 1.0 ml of dye solution (Figure 5).

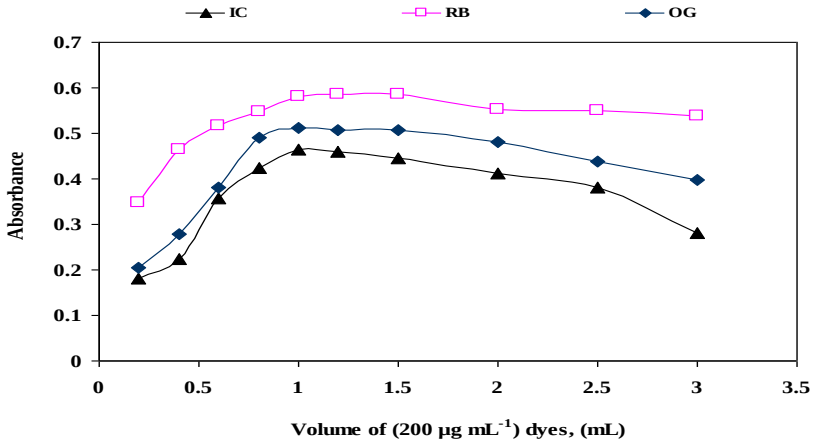


Figure 5. Effect of volume of dyes (200 µg/ml) on the reaction product of MXF (10 µg/ml) with Ce(IV) (5.0 x 10^{-3} mol/l) in H_2SO_4 medium.

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) (Figure 6). The dye absorbance that did not undergo a reaction remained consistent for a minimum of 8.0 hours following this time period.

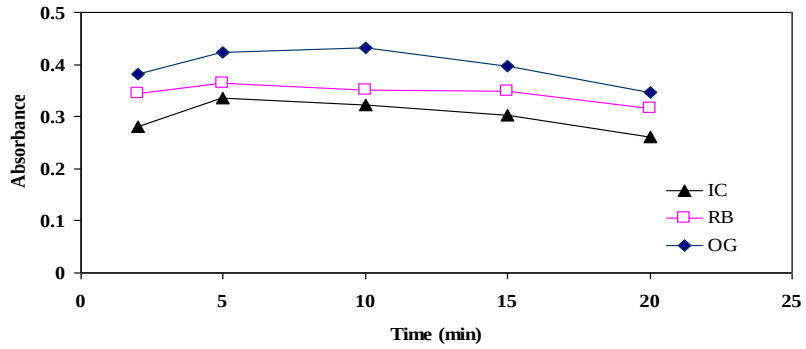


Figure 6. Effect of time on the reaction product of MXF (10 µg/ml) with Ce(IV) (5.0 x 10^{-3} mol/l) and dyes (200 µg/ml) in H_2SO_4 (2.0 mol/l) medium.

Impact of the sequence of addition

The optimal sequence of addition was as follows: MXF, H_2SO_4–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 MXF concentration. This association was determined under ideal circumstances. The MXF concentration ranges were 1.0-8.0, 1.0-10 and 1.0-15 µg/ml using IC, RB and OG methods, respectively. To get accurate outcomes, the Ringbom concentration range [52] 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 0.29, 0.30, and 0.30 µg/ml and LOQ values was determined to be 0.97, 1.0, and 1.0 µg/ml for the IC, RB and OG techniques, respectively. The suggested approaches were assessed for validity by statistical analysis [53], 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 1), there is no statistically significant distinction between the suggested approach and the method provided in reference [15] in terms of accuracy and precision.

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

Parameters	**IC**	**RB**	**OG**
Beer's law limits, µg/ml	1.0-8.0	1.0-10	1.0-15
Ringboom limits, µg/ml	3.0-6.0	3.0-8.0	3.0-12
Molar absorptivity, x 10^4 L/mol.cm	2.8262	1.8855	1.4717
Sandell sensitivity, ng/cm^2	15.49	23.22	29.75
Regression equation[a]			
Intercept (a)	-0.0432	-0.0073	-0.0103
Standard deviation of intercept (S_a)	0.013	0.017	0.015
Slope (b)	0.0818	0.0455	0.0369
Standard deviation of slope (S_b)	0.022	0.018	0.012
Correlation coefficient, (r)	0.9994	0.9992	0.9993
Mean ± SD	99.40±0.52	99.20±1.10	99.90±0.60
RSD%	0.52	1.10	0.60
RE%	0.55	1.16	0.63
Limit of detection, µg/ml	0.29	0.30	0.30
Limit of quantification, µg/ml	0.97	1.0	1.0
Calculated *t*-value [b]	0.71	0.83	0.45
Calculated *F*-value [b]	2.31	1.94	1.73

[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 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				
IC	2.0	99.10	0.60	-0.90	1.982±0.012
	4.0	99.00	0.50	-1.00	3.96±0.021
	6.0	100.20	0.80	0.20	6.012±0.051
RB	3.0	99.00	0.45	-1.0	2.97±0.016
	6.0	99.50	0.75	-0.50	5.97±0.051
	9.0	99.80	1.30	-0.20	8.982±0.12
OG	4.0	99.30	0.75	-0.70	3.972±0.025
	8.0	99.10	0.94	-0.90	7.928±0.075
	12	100.5	1.20	0.50	12.06±0.19
	Inter-day				
IC	2.0	99.40	0.38	-0.60	1.988±0.008
	4.0	99.20	0.48	-0.80	3.968±0.02
	6.0	100.30	1.15	0.30	6.018±0.073
RB	3.0	100.5	0.60	0.50	3.015±0.019
	6.0	99.20	0.96	-0.80	5.952±0.06
	9.0	99.70	1.2	-0.30	8.973±0.108
OG	4.0	99.00	0.38	-1.0	3.96±0.016
	8.0	99.70	0.75	-0.30	7.976±0.063
	12	98.80	1.1	-1.2	11.856±0.137

[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 MXF concentration. The techniques used were found to be unaltered, as indicated by the RSD% lying in the range of 0.50-2.50%. 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 0.80% to 2.30%, whereas the inter-instruments RSD% varied from 0.70% to 2.25%, 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
IC	2.0	1.25	0.90	1.20	0.80
	4.0	1.30	1.90	1.80	1.60
	6.0	2.0	2.10	2.30	1.90
RB	3.0	1.30	0.50	0.80	0.70
	6.0	1.80	1.10	1.20	1.20
	9.0	2.10	1.70	2.30	2.20
OG	4.0	1.0	0.60	0.80	0.90
	8.0	1.70	1.80	1.60	1.80
	12	2.30	2.50	1.80	2.25

[a] *Volume of (2.0 mol L^{-1}) H_2SO_4 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 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 3 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 MXF concentration that is founded in the tablet. C_P represents the pure MXF 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 MXF 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 [53]. 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 MXF in dosage forms.

Sample	**Taken (µg/ml)**	**Added (µg/ml)**	**Recovery [a] (%)**			**Reported method [15]**
			IC	**RB**	**OG**	
Avelox ® tablets	3.0	-	99.00	99.40	100.20	
		1.5	99.30	99.00	99.10	
		3.0	99.50	100.70	98.80	
		4.5	100.60	100.20	100.30	
Mean ± SD[a]			99.60±0.70	99.83±0.77	99.60±0.76	99.50±0.94
RSD% [a]			0.70	0.77	0.76	0.94
Variance			0.49	0.59	0.58	0.88
t-value [b]			0.19	0.62	0.18	
F-value [b]			1.80	1.49	1.53	
Moxiflox tablets	3.0	-	100.80	99.60	99.10	
		1.5	99.40	99.10	99.30	
		3.0	99.60	100.20	100.60	
		4.5	99.20	99.00	99.00	
Mean ± SD[a]			99.75±0.72	99.48±0.55	99.50±0.74	99.60±0.70
RSD% [a]			0.72	0.55	0.74	0.70
Variance			0.52	0.30	0.55	0.49
t-value [b]			0.33	0.30	0.22	
F-value [b]			1.06	1.62	1.12	

[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.

References

[1] Al Omari M.M.H., Jaafari D.S., Al-Souod K.A., Badwan A.A., Moxifloxacin hydrochloride, Profiles of Drug Substances, Excipients and Related Methodology, 39 (2014) 299-431.

[2] Vardanyan R., Hruby V., Antibacterial Drugs, Synthesis of Best-Seller Drugs, (2016) 645-667.

[3] United States Pharmacopeia, USP 46, National Formulary-41, USP Monographs, moxifloxacin hydrochloride, (2023), Rockville, DOI: https://doi.org/10.31003/USPNF_M54940_05_01

[4] Attimarad, M., Chohan, M.S., Balgoname, A.A., Simultaneous determination of moxifloxacin and flavoxate by RP-HPLC and ecofriendly derivative spectrophotometry methods in formulations, Int. J. Environ. Res. Public Health, 16(7) (2019) 1196.

[5] Ashour S., Spectrophotometric determination of moxifloxacin and methyldopa in pharmaceutical formulations using oxidative coupling reactions, 9th Edition of International Conference on Analytical Chemistry, March 26-28, (2018), Vienna, Austria Insights in Analytical Electrochemistry, Volume 4, DOI: 10.21767/2470-9867-C1-006

[6] Patel, P.B., Patel, S.A., Developmentand validation of spectrophotometric method for simultaneous estimation of moxifloxacin and prednisolone acetate in eye drop, Int. J. Pharm. Res., 9(1) (2017) 98–103.

[7] Kazan R.M.W., Seddik H., Aboudane M., A new spectrophotometric method for determination of moxifloxacin with 1-(2-hydroxyphenylazo)-2,7-dihydroxynaphthalene in bulk and pharmaceutical formulations, Int. J. Acad. Sci. Res., 5(2) (2017) 47- 56.

[8] Kazan R.M.W., Seddik H., Aboudane M., Development and validation of spectrophotometric method for determination of moxifloxacin HCl in bulk and pharmaceutical formulations, Int. J. Acad. Sci. Res., 5(2) (2017), 14- 24.

[9] Madhuri D., Chandrasekhar K.B., Devanna N., Somasekhar G., spectrophotometric estimation of moxifloxacin hydrochloride using osmium(OS-VIII) ions in micellar medium, Acta Ciencia Indica, XLII C (2) (2016) 73-80.

[10] Ashour, S., Bayram, R., Development and validation of sensitive kinetic spectrophotometric method for the determination of moxifloxacin antibiotic in pure and commercial tablets, Spectrochim. Acta A. Mol. Biomol. Spectrosc., 140 (2015) 216–222.

[11] Tirupathi, B., Venkateshwarlu, G., Spectrophotometric determination of drugs using chloramine-T and rhodamine-B dye, Int. J. Pharma Bio Sci., 6(4) (2015) 218–226.

[12] Zayed, M.A., Dakhly, H.A., Spectrophotometric determination of gemifloxacin mesylate, moxifloxacin. HCl and gatifloxacin sesquihydrate in pure and in pharmaceutical preparations, Egyp. J. Chem., 58(3) (2015) 349–364.

[13] Pradhan, N., Rajkhowa, H., Giri, H., Shrestha, B., Simultaneous spectrophotometric estimation of moxifloxacin hydrochloride and doxorubicin hydrochloride, Int. J. Pharmacy Pharm. Sci., 7(11) (2015) 21–26

[14] Abdel Wahed, M.G., El Sheikh, R., Gouda, A.A., Abou Taleb, S., Kinetic spectrophotometric determination of gemifloxacin mesylate and moxifloxacin hydrochloride in pharmaceutical preparations using 4-chloro-7-nitrobenzo-2-oxa-1,3-diazole, J. Spectrosc., 2014 (2014) 917234.

[15] Gouda, A.A., Amin, A.S., El-Sheikh, R., Yousef, A.G., Spectrophotometric determination of gemifloxacin mesylate, moxifloxacin hydrochloride, and enrofloxacin in pharmaceutical formulations using acid dyes, J. Anal. Methods Chem., 2014 (2014) 286379.

[16] Wahed, M.G.A., Sheikh, R.E., Gouda, A.A., Taleb, S.A., Kinetic spectrophotometric determination of some fluoroquinolone antibiotics in bulk and pharmaceutical preparations, Bull. Chem. Soc. Ethiop., 27(3) (2013) 329–346.

[17] Tirupathi, B., Venkateshwarlu, G., Spectrophotometric determination of drugs using N-bromosuccinimide and rhodamine-B dye, Int. J. ChemTech. Res., 5(5) (2013) 2243–2251.

[18] Venkatesan, S., Kannappan, N., Development and validation of a spectrophotometric method using vierordt's method for simultaneous estimation of moxifloxacin and cefixime in tablet formulation, Int. J. Pharmacy Pharm. Sci., 5(4),(2013) 768–772.

[19] Elbashir A.A., Ebraheem S.A.M., Elwagee A.H.E., Aboul-Enein H.Y., New spectrophotometric methods for the determination of moxifloxacin in pharmaceutical formulations, Acta. Chim. Slov., 60(1) (2013) 159-65.

[20] El-Hawary W.F., Al-Gethami F.Kh., Mutual spectrophotometric determination of moxifloxacin drug and iron(III) ions by formation of a complex compound, Eur. Chem. Bull., 2(1) (2013) 22-27.

[21] Abdellaziz, L.M., Hosny, M.M., Development and validation of spectrophotometric, atomic absorption and kinetic methods for determination of moxifloxacin hydrochloride, Anal. Chem. Insights, 6(1) (2011) 67–78

[22] Chaple, D.R., Bhusari, K.P., Spectrophotometric estimation of fluroquinolones as ion-pairs with bromocresol green in bulk and pharmaceutical dosage form, Asian J. Chem., 22(4) (2010) 2593–2598

[23] Sultan, M.A., New, simple and validated kinetics spectrophotometric method for determination of moxifloxacine in its pharmaceutical formulations, Arabian J. Chem., 2(2) (2009) 79–85.

[24] Al-Ghannam S.M., Atomic absorption spectroscopic, conductometric and colorimetric methods for determination of some fluoroquinolone antibacterials using ammonium reineckate, Spectrochim. Acta A. Mol. Biomol. Spectrosc., 69(4) (2008) 1188-94.

[25] Cirić, A., Jelić, R., Joksović, L., Jelikić- Stankov, M., Djurdjević, P., Determination of moxifloxacin in human plasma by derivative UV spectrophotometry in a micellar medium, Canadian J. Anal. Sci. Spectrosc., 52(6) (2007) 343–350.

[26] Motwani, S.K., Chopra, S., Ahmad, F.J., Khar, R.K., Validated spectrophotometric methods for the estimation of moxifloxacin in bulk and pharmaceutical formulations, Spectrochim. Acta A. Mol. Biomol. Spectrosc., 68(2) (2007) 250–256.

[27] Othman W.M., Al-Zoman N.Z., Darwish I.A., Almomen A., Farid N.F., Abdallah F.F., Saad S.S., Development and validation of synchronous spectrofluorimetric method for the simultaneous determination of duvelisib and moxifloxacin: greenness metric assessment and application to a pharmacokinetic study in rats, Methods Appl. Fluoresc. 12 (2024) 015010.

[28] Madbouly E.A., El-Shanawani A.A., El-adl S.M., Abdelkhalek A.S., Eco-friendly novel deconvoluted synchronous spectrofluorimetric approach for the determination of favipiravir, levodropropizine and moxifloxacin hydrochloride as an effective therapeutic combination for COVID-19; application in laboratory prepared mixtures and spiked human plasma, Spectrochim. Acta A. Mol. Biomol. Spectrosc., 309 (2024) 123823.

[29] Ibrahim N., Elzanfaly E.S., El Gendy A.E., Hassan S.A., Development, optimization, and validation of a green spectrofluorimetric method for the determination of moxifloxacin using an experimental design approach. Res. J. Pharmacy Technol., 14(4) (2021) 1880-1886.

[30] Khan M.N., Ali W., Shah Z., Idrees M., Gulab, Adnan, A validated spectrofluorimetric method for the determination of moxifloxacin in its pure form, Pharmaceutical Preparations, and Biological Samples, Anal. Sci., 36 (2020) 361-366.

[31] Khan M.N., Khan M., Jan M.N., Eco-friendly approach for the determination of moxifloxacin in pharmaceutical formulations and biological fluids based on fluorescence quenching of l-tryptophan, Luminescence, 39(2) (2024) e4664.

[32] Khan, M.N., Zaman, N., Mursaleen, M., Naz, F., Ullah, Z.,
Eco-friendly approach for determination of moxifloxacin in pharmaceutical preparations and biological fluids through fluorescence quenching of eosin Y, Anal. Sci., 38(12) (2022) 1541–1547.

[33] El-Yazbi A.F., Aboukhalil F.M., Khamis E.F., Elkhatib M.A.W., M.A. El Sayed, Youssef R.M., Simple simultaneous determination of moxifloxacin and metronidazole in complex biological matrices, RSC Adv., 12 (2022) 15694-15704.

[34] Vankalapati K.R. , Algete P. , Boodida S., A rapid RP-HPLC stability-indicating method development and validation of moxifloxacin hydrochloride-related substances in finished dosage forms, Biomed Chromatogr., 35(11) (2021) e5192.

[35] Ibrahim F.A., Elmansi H., Fathy M.E., Green RP-HPLC method for simultaneous determination of moxifloxacin combinations: Investigation of the greenness for the proposed method, Microchem. J., 148, (2019) 151-161

[36] Czyrski A., Sokół A., Szałek E., HPLC method for determination of moxifloxacin in plasma and its application in pharmacokinetic analysis, J. Liq. Chromatogr. Rel. Technol., 40(1) (2017) 8-12.

[37] Salkić, A., Otašević, B., Rašević, M., Zečević, M., Micellar liquid chromatographic method for determination of moxifloxacin and its impurities, Acta Chimica Slov., 70(3) (2023) 385–397

[38] Abdel Hameed, E.A., Abd El-Naby, Z.A., El Gindy, A., Roshdy S., Al Balawi A.N., Zaitone, S.A., Khairy, G.M., Two eco-friendly chromatographic methods evaluated by GAPI for simultaneous determination of the fluoroquinolones moxifloxacin, levofloxacin, and gemifloxacin in their pharmaceutical products, Separations, 9(11) (2022) 330.

[39] El-Yazbi, A.F., Aboukhalil, F.M., Khamis, E.F., ElKhatib M.A.W., El-Sayed, M.A., Youssef, R.M., Simple simultaneous determination of moxifloxacin and metronidazole in complex biological matrices, RSC Adv., 12(25) (2022) 15694–15704.

[40] Shehata M, Fekry AM, Walcarius A. Moxifloxacin hydrochloride electrochemical detection at gold nanoparticles modified screen-printed electrode, Sensors, 20(10) (2020)2797.

[41] Fekry A.M., Abdel-Gawad S.A., Azab S.M., Walcarius A., A sensitive electrochemical sensor for moxifloxacin hydrochloride based on Nafion/graphene oxide/Zeolite modified carbon paste electrode, Electroanal., 33(4) (2021) 964-974

[42] Homoda, A.M.A., Ionophore-based potentiometric PVC membrane sensors for the determination of moxifloxacin, Egy. J. Chem., 67(6) (2024) 387–394.

[43] Essa, W.A., Beltagi, A.M., Hathoot, A.A., Azzem, M.A.,
A sensitive voltammetric sensor for improved simultaneous determination of moxifloxacin hydrochloride and paracetamol, J. Electrochem. Soc., 167(16) (2020) 167509.

[44] El Sheikh R., Hassan W.S., Gouda A.A., Al Owairdhi A., Validated and sensitive spectrophotometric methods for estimation of rizatriptan benzoate in pharmaceutical formulations using cerium (IV) sulphate, Res. J. Pharm. Tech., 12, (2019), 2123-2130.

[45] El Shiekh R., Amin A.S., Hafez E.M., Gouda A.A., Spectrophotometric estimation of vardenafil HCl and tadalafil in pure forms and tablets using cerium(IV) ammonium sulphate. Der Pharm. Lett. 8, (2016), 153-165.

[46] El Sheikh R., Hassan W.S., Gouda A.A., El Gabry M.M., Utility of cerium (IV) ammonium sulphate as oxidizing agent for spectrophotometric assay of oxybutynin hydrochloride in pharmaceutical preparations, Int. J. Res. Pharmacy Pharm. Sci., 3(1), (2018), 09-16.

[47] El Sheikh R., Hassan W.S., Gouda A.A., Abdel Fattah G.M., Validated spectrophotometric methods for determination of donepezil hydrochloride in pharmaceutical formulations based on redox reaction with cerric(IV) ammonium sulphate, Int. J. Pharm. Sci. Rev. Res., 52, (2018), 66-74.

[48] El Sheikh R., Gouda A.A., Sadek L.A., New spectrophotometric method for determination of aripiprazole in pure form and pharmaceutical formulations using ceric (IV) ammonium sulphate, Int. J. Res. Pharmacy Pharm. Sci., 3(1), (2018), 259-267.

[49] International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use. ICH Harmonized Tripartite Guideline, Validation of Analytical Procedures: Text and Methodology Q2(R 1), Complementary Guideline on Methodology, London, November. (2005).

[50] Jeffery, G.H.; Bassett, J.; Mendham, J.; Denney, R.C.; Titrimetric analysis. In Vogel's textbook of quantitative inorganic analysis, 5th Ed., ELBS: London, (1989), p.286.

[51] Kolthoff, I.M.; Belcher, R.; Stenger, V.A.; Matsuyama, G.; Volumetric Analysis, III. Interscience Publishers, Inc., New York, USA, (1957), p. 504.

[52] Ringbom, A.; Accuracy of colorimetric determination I and II. Z. Anal. Chem. 115, (1993), 332-338.

[53] Miller, J.N.; Miller, J.C. Statistics and Chemometrics for Analytical Chemistry, 6th ed., Pearson Education Limited: Essex, England; (2010), p 202