



Eco-friendly approach for determination of moxifloxacin in pharmaceutical preparations and biological fluids through fluorescence quenching of eosin Y

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Abstract

An easy, verified spectrofluorimetric approach was established for the investigation of moxifloxacin in pure forms, pharmaceutical preparations, and biological fluids. The approach involves forming a binary complex of moxifloxacin and eosin Y in an acetate buffer with a pH of 3.6. The highest quenching of eosin Y with moxifloxacin occurs at 545 nm. Several factors, such as pH, buffer type and concentration, and eosin Y concentration, were carefully studied. The calibration graph showed a linear relationship between fluorescence intensity and moxifloxacin concentrations between 0.2 and 10 $\mu\text{g mL}^{-1}$ with a correlation coefficient of 0.998. It was determined that the detection and quantification limits were 0.0322 $\mu\text{g mL}^{-1}$ and 0.0976 $\mu\text{g mL}^{-1}$, respectively. The impact of common excipients was investigated, but no interferences were discovered. Standard forms of moxifloxacin, pharmaceuticals, and biological samples have all been studied using the established methodology. The method, which successfully complied with ICH requirements, was used for the analysis of moxifloxacin in its pure form, pharmaceutical dosage forms, and biological samples. The percentage recoveries obtained were ranged from 99.50 to 102.50% for pharmaceutical preparations and from 100.50 to 102.50% for human blood plasma and urine.

Keywords Moxifloxacin · Eosin Y · Spectrofluorimetry · Pharmaceutical products · Biological fluids

Introduction

Fluoroquinolones are among the most effective antibacterial drugs available to humans. They have antibacterial efficacy against Gram-positive and Gram-negative bacteria via inhibiting DNA gyrase, as well as mycobacteria, mycoplasmas, and rickettsias [1].

Moxifloxacin is a fourth-generation 8-methoxy fluoroquinolone that was created to treat community-acquired pneumonia and infections of the upper respiratory tract. Gram-negative pathogens, Gram-positive cocci, aerobic

intracellular bacteria, atypical species, and anaerobic bacteria are all targets [2, 3].

Several methods for determining moxifloxacin in pharmaceutical formulations and biological fluids have been documented. These methods include HPLC [4–9], differential spectrophotometry [10], UV–Vis spectrophotometry [11, 12], capillary electrophoresis [13], differential pulse polarography [14], differential pulse voltammetry [15], and spectrofluorimetric methods [16, 17].

The published methods for determining moxifloxacin are costly, require expensive reagents, and have a limited range of application to biological materials, low sensitivity, and interferences. Therefore, it is crucial to create a straightforward and affordable method for finding moxifloxacin in pharmaceutical formulations and biological fluids.

In comparison, only a few spectrofluorimetric methods for determining moxifloxacin in pharmaceutical formulations and biological materials have been documented. For the determination of moxifloxacin using eosin Y, no spectrofluorimetric approach has been published. The goal of this study is to develop a spectrofluorimetric method for quantifying moxifloxacin in pharmaceutical formulations and

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biological fluids that is simple, sensitive, and cost-effective. The proposed method relies on the formation of a binary complex between moxifloxacin and eosin Y in acidic conditions, and the intensity of their fluorescence is evaluated at 545 nm following excitation at 301 nm.

The developed spectrofluorimetric method is superior to the earlier reported methods in its better sensitivity, shorter measurement time and little cost. In addition, the proposed procedure is very simple, as the sample is dissolved in water and the formed ion pair was directly measured in the aqueous solution without the need for extraction with organic solvents. As a result, no volatile solvents but only water is used throughout the work which make the method meet the requirements of green chemistry.

Experimental

Instrument

The Fluorescence Spectrometer (Perkin Elmer LS 45) is outfitted with an emission grating monochromator, an excitation, a 150 Watt Xenon discharge lamp, and a 1 cm quartz cell. The slit widths for excitation and emission were both adjusted to 10 nm. Anthracene/Naphthalene, p-terphenyl, Ovalene, E11, Tetraphenyl butadiene, and Rhodamine were among the Certified Reference materials used to calibrate the instrument. The instruments utilized in this study included a JENWAY 3505 pH metre, an OHUAS Corporation USA digital analytical balance, a SONICOR SC-101TH sonicator, and a microcentrifuge.

Materials and reagents

In this study, analytical-grade reagents and compounds were used. A pharmaceutical company provided the standard moxifloxacin medication, and marketable descriptions were obtained on the local market. Dissolving 0.0138 g of Eosin Y disodium salt (Merck, Darmstadt, Germany) in 100 mL of distilled water yielded 4.0×10^{-4} mol L⁻¹ of eosin Y disodium salt solution. A known volume of 0.1 mol L⁻¹ sodium acetate and 0.1 mol L⁻¹ acetic acid were combined to create an acetate buffer (0.1 mol L⁻¹), and the pH was then adjusted using a pH meter.

Pharmaceutical formulations

Marketable products containing 400 mg per tablet of moxifloxacin (Fotiflox, manufactured by Helix Pharma (Pvt) Ltd., Karachi, Pakistan; Xefecta, manufactured by Hilton Pharma (Pvt) Ltd., Korangi Industrial Era, Karachi, Pakistan; Moxifo, Moxifo made by Tabros Pharma (Pvt) Ltd., L-20/B, Sector 22, F.B. Industrial Area, Karachi, Pakistan, and Oxaquin made by

Highnoon Laboratories Ltd., Multan Road, Lahore-Pakistan) were purchased from the local pharmaceutical shop.

Standard drug solutions

The standard solution for moxifloxacin ($100 \mu\text{g mL}^{-1}$) was prepared by dissolving 0.005 g of moxifloxacin in 50 mL of distilled water. By diluting the necessary volume of the standard solution with distilled water, different working solutions of the required concentrations were created. When refrigerated, the typical stock solution remained stable for one week.

Procedure in general

The necessary volume of $100 \mu\text{g mL}^{-1}$ standard moxifloxacin solution was added to a series of volumetric flasks along with 1.0 mL of acetate buffer solution (pH 3.6) and 1.0 mL of eosin Y (4.0×10^{-4} mol/L). Using distilled water, the volume of the resultant solution was increased to 10 mL. The relative fluorescence intensity was assessed at 549 nm following excitation at 300 nm in comparison to a reagent blank.

Procedure for tablets

A carefully weighed amount of powdered pharmaceutical tablets, equivalent to $100 \mu\text{g mL}^{-1}$ moxifloxacin, was weighed, dissolved in distilled water, and the volume was completed to 50 mL with distilled water. Different volumes of sample solution equivalent to 0.2, 0.4, and $0.6 \mu\text{g mL}^{-1}$ were obtained and evaluated in 10 mL volumetric flasks using the same approach as indicated above under “[Procedure in General.](#)”

Applications to biological samples

Separately, 1.0 mL of blood plasma and 1.0 mL of urine were combined with 2.5 mL of the $100 \mu\text{g mL}^{-1}$ standard moxifloxacin solution. The plasma and urine samples were then deproteinized using 6.0 mL of acetonitrile. The mixed solution was centrifuged for 10 min at 3000 rpm. The clear liquid was transferred to a 50 mL volumetric flask, and the required volume of distilled water was added afterward. The same process was used to assess the volume of this solution that would produce 0.2, 0.4, and $0.6 \mu\text{g mL}^{-1}$ of moxifloxacin.

Results and discussion

Eosin Y is a green-fluorescent acidic dye with a yellowish-red color. In acidic environments, the fluorescence of Eosin Y is quenched by the formation of a stable combination with

cationic medicines [18]. Eosin Y has been widely employed in the development of precise and accurate analytical procedures for determining a wide range of pharmaceutical substances using spectrofluorimetric and/or spectrophotometric measurements [19–23].

At 545 nm emission and 300 nm excitation wavelengths, the eosin Y solution's fluorescence peak was seen. Eosin Y's fluorescence was significantly reduced by the addition of moxifloxacin (Fig. 1). Collisional or dynamic quenching and static quenching are the two key types of quenching process. Static quenching occurs when a fluorophore and quencher form a non-fluorescent complex before excitation of fluorophore [24]. The proposed reaction occurs on the mechanism of static quenching as moxifloxacin form an ion-pair complex with eosin Y before excitation of eosin Y (Fluorophore). The fluorescence of Eosin Y was found to be quenched through the formation of a stable non-fluorescence ion-pair complex with moxifloxacin.

Optimization of the reaction conditions

Several characteristics were thoroughly examined to determine the best circumstances for the complex development. The pH of the solution, the concentration of eosin Y, the solvents employed for dilution, the reaction stability, and the effect of temperature were all examined.

The influence of pH and buffer concentration

The impact of pH was observed in the pH range of 2.8 to 4.0. At pH 3.6, the greatest ΔF was measured (Fig. 2). Because a pH greater or lower than 3.6 can cause the quenching effect to gradually diminish, pH 3.6 was chosen. The effects of other buffers, including McIlvaine, acetate, citrate, and phosphate, were also examined. The acetate buffer yielded the highest ΔF value. The effect of different acetate buffer concentrations was also investigated, and it was discovered

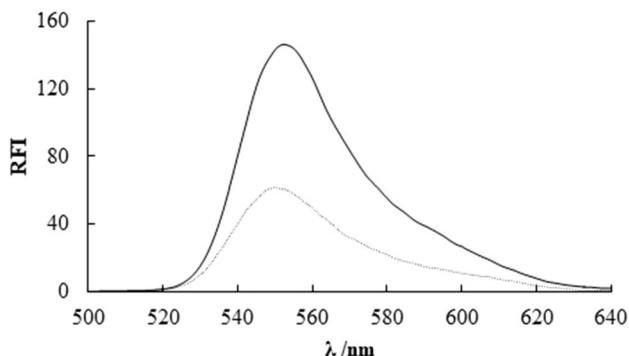


Fig. 1 Emission spectra of eosin Y (4.0×10^{-4} mol L $^{-1}$) at 545 nm and (.....) its reaction product with moxifloxacin ($10 \mu\text{g mL}^{-1}$) after excitation at 301 nm

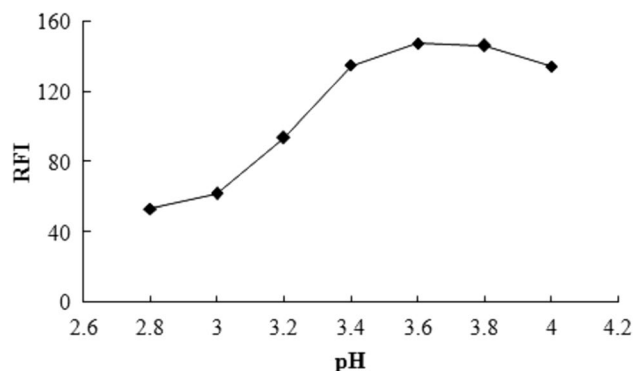


Fig. 2 Effect of pH on the reaction of moxifloxacin with eosin Y. Conditions; $10 \mu\text{g mL}^{-1}$ moxifloxacin; 1.0 mL of pH (2.8–4.0) acetate buffer; 1.0 mL of eosin Y (4.0×10^{-4} mol/L); diluted to 10 mL

that a 0.1 mol L^{-1} acetate buffer solution with a pH of 3.6 generated the most quenching. Investigations on the impact of acetate buffer volume (pH 3.6) produced the best results when using 1.0 mL of 0.1 mol L^{-1} acetate buffer.

The effect of eosin Y concentration

Eosin Y concentrations ranging from 1.0×10^{-4} to 6.0×10^{-4} mol L $^{-1}$ were examined for their effects. Eosin Y concentrations of 4.0×10^{-4} mol L $^{-1}$ show the highest fluorescence intensity (ΔF) (Fig. 3). The influence of eosin Y volume was also tested, and the maximum ΔF value was found with 1.0 mL of 4.0×10^{-4} mol L $^{-1}$ eosin Y solution.

Diluting solvent's effect

The ion-pair complex of MOX and eosin Y was diluted in a variety of solvents including acetone, chloroform, dichloromethane, dioxane, ethanol, methanol, and distilled water (Fig. 4). With distilled water, the highest ΔF values were

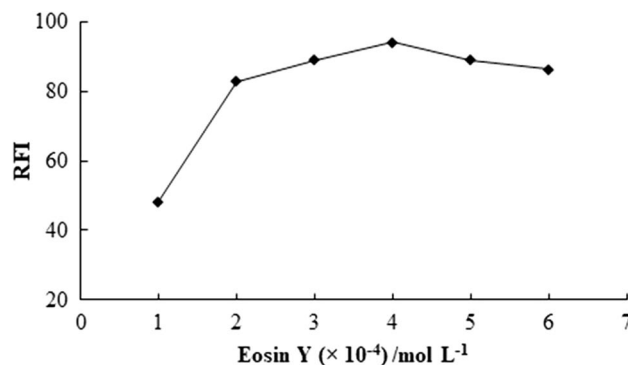


Fig. 3 Effect of eosin Y concentration on fluorescence intensity. Conditions; $10 \mu\text{g mL}^{-1}$ moxifloxacin; 1.0 mL of pH 3.6 acetate buffer; 1.0 mL of eosin Y (1.0×10^{-4} to 6.0×10^{-4} mol L $^{-1}$); diluted to 10 mL

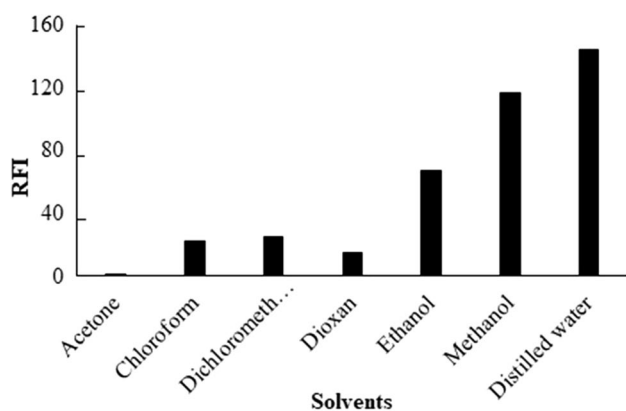


Fig. 4 Effect of diluting solvent on the reaction of moxifloxacin with eosin Y

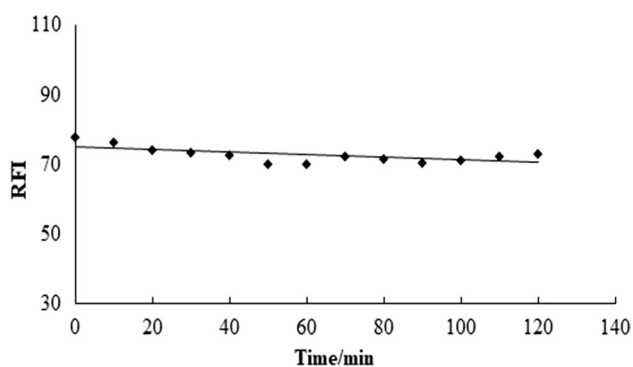


Fig. 5 Effect of time on the fluorescence intensity. Conditions; $10 \mu\text{g mL}^{-1}$ moxifloxacin; 1.0 mL of pH 3.6 acetate buffer; 1.0 mL of eosin Y 4.0×10^{-4} mol/L; diluted to 10 mL

attained. As a result, throughout the analysis, distilled water is used as a solvent.

The product's stability

The influence of time on the stability of the ion-pair complex generated between MOX and eosin Y was investigated. The product's fluorescence intensity was monitored at 10-min intervals for up to 2 h. According to the findings (Fig. 5), the substance remains stable for up to two hours after dilution.

Eosin Y and moxifloxacin reaction stoichiometry

The Job's method of continuous variations [25] was used to calculate the molar ratio between the moxifloxacin and Eosin Y using 4.0×10^{-4} mol L^{-1} master equimolar solutions. The ratio of Eosin to moxifloxacin was found to be 1:1 (Fig. 6). The results of Job's approach studies conformed to the postulated reaction mechanism [26, 27]. The complex was produced by electrostatic interaction between Eosin

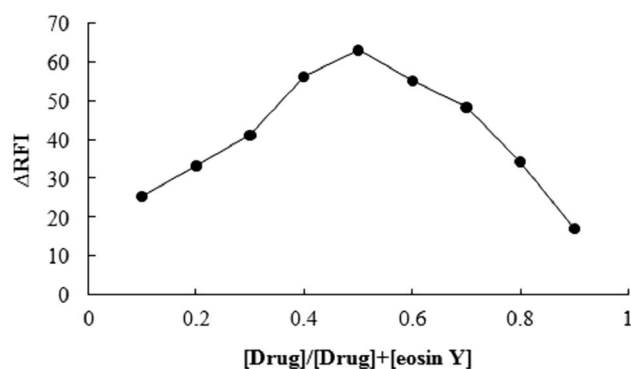


Fig. 6 Job's method plot for determination of the molar ratio of moxifloxacin and Eosin Y using 4.0×10^{-4} mol L^{-1} equimolar solutions

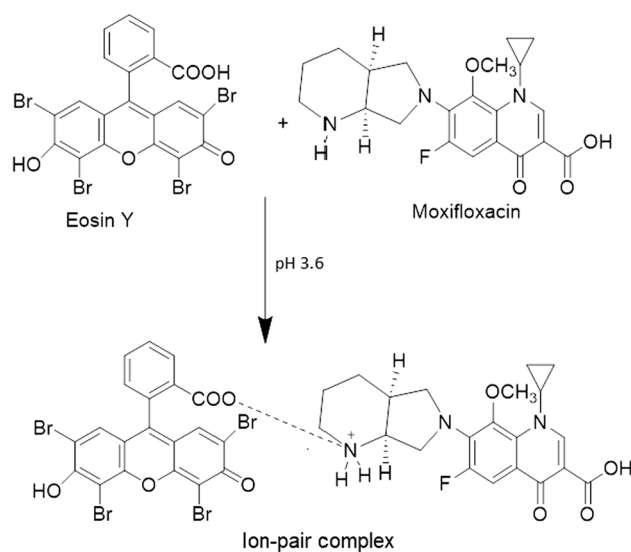


Fig. 7 Proposed mechanisms for the reaction between moxifloxacin and eosin Y

Y's acidic carboxylate group and the moxifloxacin tertiary amine. Figure 7 depicts the proposed reaction process.

Temperature influences

The combination was heated for 15 min in an electrical thermostatic water bath at 50, 60, 70, 80, 90, and 100 °C to determine the impact of temperature. The mixture was then cooled and diluted to 10 mL with distilled water. The quenching effect is reduced as the temperature rises. As a result, more testing was done at the ambient temperature.

Analytical figures of excellence

The dye's fluorescence quenching rises in a linear relationship with moxifloxacin concentration. Under optimal

experimental conditions, the calibration graph of fluorescence intensity versus moxifloxacin concentration was linear in the range of 0.2–10 $\mu\text{g mL}^{-1}$, with an exceptional correlation coefficient of 0.992 (Fig. 8). The limit of detection (LOD) was estimated using the formula $\text{LOD} = 3.3 \sigma/S$, where S is the slope of the calibration curve and σ is the standard deviation of the intercept. The LOD was found to be 0.0322 $\mu\text{g mL}^{-1}$. The limit of quantification (LOQ) was calculated using the formula $\text{LOQ} = 10/S$ and determined to be 0.0976 $\mu\text{g mL}^{-1}$. The results of calculating a number of analytical parameters using the linear regression equation are displayed in Table 1. In Table 2, the proposed method's sensitivity was compared to that of other approaches in the literature. The results reveal that the proposed method has a higher sensitivity than the previously described methods.

The method's reliability

The method's precision was verified by measuring moxifloxacin in standard and commercial formulations in triplicate at three distinct concentrations (0.2, 0.4, and 0.6 $\mu\text{g mL}^{-1}$). Table 3 shows the results for the standard form, while Table 4 shows the results for pharmaceutical formulations. The percent recovery obtained ranged from 101.29% to 102.71% for the standard and from 98.83% to 102.50% for pharmaceutical formulations with smaller relative standard deviations, demonstrating the reproducibility of the suggested procedure.

The developed method's repeatability and reproducibility were assessed by looking at intra-day and inter-day precision. To evaluate intra-day precision, three separate standard moxifloxacin concentrations were examined in triplicate during the course of the same day at three different times. The identical solutions were examined on three successive days for inter-day precision. Table 5 summarizes the findings.

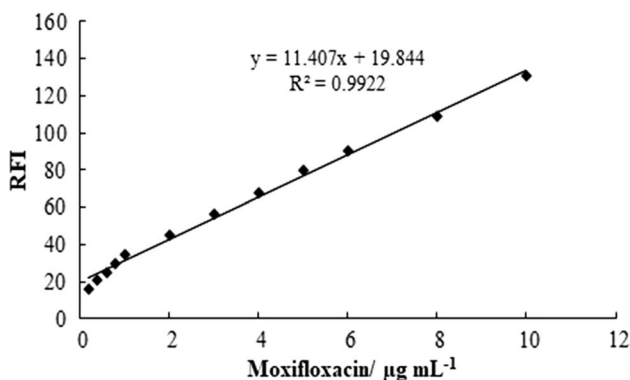


Fig. 8 Linear range of moxifloxacin. Conditions; 0.2–10 $\mu\text{g mL}^{-1}$ moxifloxacin; 1.0 mL of pH 3.6 acetate buffer; 1.0 mL of eosin Y 4.0×10^{-4} mol/L; diluted to 10 mL

Table 1 Analytical parameters for the moxifloxacin assessment using spectrofluorimetry

Characteristics	Values
wavelength of excitation (nm)	301
emission spectrum (nm)	545
Range of linearity, ($\mu\text{g mL}^{-1}$)	0.2–10
LOD ($\mu\text{g mL}^{-1}$)	0.0322
LOQ ($\mu\text{g mL}^{-1}$)	0.0976
Standard deviation (SD) ($\mu\text{g mL}^{-1}$)	6.95×10^{-3}
Regression formula	$Y = 11.40x + 19.84$
Slope, (b)	11.40
Intercept, (a)	19.84
coefficient of correlation (r^2)	0.992
RSD (%)	3.42

The lower RSD values imply that the established method is repeatable and reproducible.

The proposed method's accuracy was tested using a standard addition method with four distinct brands of pills containing 40 mg of moxifloxacin: Oxaquin, Fotiflox, Moxifo, and Xefecta. The known quantity of tablet solutions was mixed with three different concentrations of standard moxifloxacin solutions and analysed using the procedure specified. The percentage recoveries ranged from 98.50 to 101.16 percent and were calculated by comparing the findings before and after the addition of standard moxifloxacin solution (Table 6).

Selectivity

Interferents may be present in a real sample, which might reduce or amplify the fluorescence signal of the analyte under investigation. The effects of glucose, sucrose, lactose, ascorbic acid, Mg-stearate, starch, and fructose, all of which are commonly found in pharmaceutical products, were investigated. The given process was used to generate and analyse a sample containing a set dose of moxifloxacin ($0.2 \mu\text{g mL}^{-1}$) and varied concentration ratios of excipients of 1:1, 1:2, 1:4, 1:6, 1:8, 1:10, 1:20, 1:40, and 1:100. The criterion for error was set at 5%. There has been no evidence that any of these excipients create interference (Fig. 9).

Application of the developed method

The proposed method was used to determine the active components of moxifloxacin in four distinct pharmaceutical formulations with great effectiveness. The results were comparable to the quantities on the labels (Table 7). The new approach was also successfully used to determine moxifloxacin in spiked human plasma and urine due to the absence of excipient interference. The percent recoveries for plasma

Table 2 Comparison of the proposed method and other reported methods for the determination of moxifloxacin levels

Methods	Linear range	Limit of detection (LOD)	References
Spectrofluorimetry	0.2–5 $\mu\text{g mL}^{-1}$	0.016 $\mu\text{g mL}^{-1}$	[17]
Spectrophotometry	0.803–12.846 $\mu\text{g mL}^{-1}$	9.3 ng mL^{-1}	[28]
Liquid chromatography	0.2–2.0 $\mu\text{g mL}^{-1}$	0.05 $\mu\text{g mL}^{-1}$	[29]
Spectrofluorimetry	0.2–10 $\mu\text{g mL}^{-1}$	0.0322 $\mu\text{g mL}^{-1}$	Present method

Table 3 The current method's accuracy and precision utilises pure moxifloxacin

Amount taken ($\mu\text{g mL}^{-1}$)	Amount found ($\mu\text{g mL}^{-1}$)	Recovery (%) \pm RSD
0.20	0.205	102.71 \pm 1.95
0.40	0.405	101.29 \pm 2.16
0.60	0.610	101.68 \pm 1.17
	Mean =	101.89
	SD =	0.006644
	RSD =	1.7628

The averages of three distinct analyses were used to arrive at the final results. RSD is the relative standard deviation

Table 4 The proposed method's accuracy and precision for detecting moxifloxacin in pharmaceutical preparations

Pharmaceutical formulations (400 mg/tablet)	Amount taken ($\mu\text{g mL}^{-1}$)	Amount found ($\mu\text{g mL}^{-1}$)	%Recovery \pm RSD
Oxiquin	0.2	0.199	99.50 \pm 1.58
	0.4	0.410	102.50 \pm 2.84
	0.6	0.601	100.16 \pm 2.21
Fotiflox	0.2	0.205	102.50 \pm 4.84
	0.4	0.410	102.50 \pm 2.39
	0.6	0.601	100.16 \pm 2.34
Moxifo	0.2	0.201	100.50 \pm 1.98
	0.4	0.404	101.00 \pm 4.59
	0.6	0.596	99.33 \pm 4.03
Xeficta	0.2	0.200	100.00 \pm 2.45
	0.4	0.405	101.25 \pm 3.15
	0.6	0.593	98.83 \pm 3.05

The averages from three different analyses make up the results. Relative standard deviation is referred to as RSD

Table 5 Interday and Intraday precision of the proposed Eosin spectrofluorimetric method

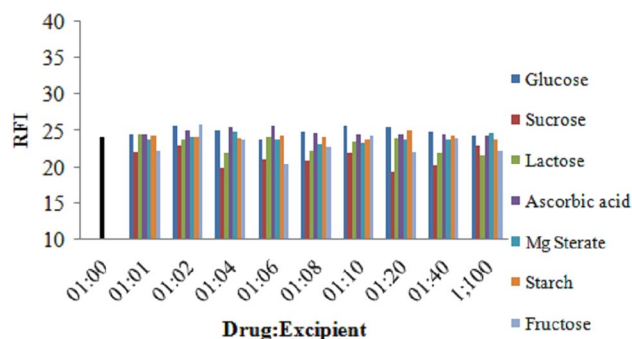
(Moxifloxacin)	Conc. level ($\mu\text{g mL}^{-1}$)	Intraday precision % Recovery* \pm RSD	Interday precision % Recovery* \pm RSD
1	0.2	99.67 \pm 2.10	103.41 \pm 1.18
2	0.4	100.94 \pm 2.43	100.59 \pm 1.48
3	0.6	98.17 \pm 3.50	100.35 \pm 2.29

*Average of three determinations

Table 6 % of moxifloxacin recovered in commercial formulations using the standard addition technique

Pharmaceutical preparations (400 mg/tablet)	Amount added ($\mu\text{g mL}^{-1}$)	Amount found ($\mu\text{g mL}^{-1}$)	%Recovery \pm RSD
Oxiquine	0.2	0.202	101.00 \pm 2.55
	0.4	0.403	100.75 \pm 1.74
	0.6	0.605	100.83 \pm 1.77
Fotiflox	0.2	0.197	98.50 \pm 3.18
	0.4	0.401	100.25 \pm 1.74
	0.6	0.592	98.67 \pm 1.48
Moxifo	0.2	0.201	100.50 \pm 2.12
	0.4	0.398	99.50 \pm 1.90
	0.6	0.607	101.16 \pm 1.80
Xeficta	0.2	0.199	99.50 \pm 3.21
	0.4	0.397	99.25 \pm 1.90
	0.6	0.594	99.00 \pm 1.33

The averages of three different analyses make up the results

**Fig. 9** Effect of excipients on fluorescence intensity. Conditions; 0.2 $\mu\text{g mL}^{-1}$ moxifloxacin; excipients (1:1 to 1:100), 1.0 mL of pH 3.6 acetate buffer; 1.0 mL of eosin Y 4.0×10^{-4} mol/L; diluted to 10 mL

samples varied from 100.50% to 101.50%, while those for urine samples ranged from 101.25% to 102.50% (Table 8).

Conclusions

A spectrofluorimetric approach that is relatively simple, selective, and economical was devised for the analysis of moxifloxacin in pure form, pharmaceutical formulations,

Table 7 Analyzing the moxifloxacin active ingredients used in pharmaceutical formulations

Brand names	Active ingredients (mg/tab)		<i>t</i> -test value (4.303)
	Label value	Obtained value	
Oxiquine	400	403.33 ± 2.05	2.53
Fotiflox	400	405.32 ± 1.89	1.63
Moxifo	400	400.43 ± 2.52	2.28
Xeficta	400	399.35 ± 3.14	1.83

The outcomes are the medians of three different analyses;

Table 8 Moxifloxacin determination in spiked human plasma and urine

Sample	Taken ($\mu\text{g mL}^{-1}$)	Found ($\mu\text{g mL}^{-1}$)	% Recovery ± RSD
Human plasma	0.2	0.201	100.50 ± 2.73
	0.4	0.406	101.50 ± 2.16
	0.6	0.605	100.83 ± 2.91
Urine	0.2	0.205	102.50 ± 2.27
	0.4	0.405	101.25 ± 1.27
	0.6	0.610	101.67 ± 1.86

Each result is the average of three independent triplicate analyses

and biological fluids. In comparison to previously published methods, it was discovered that the novel methodology had a wider linear range and lower detection and quantification limits. These characteristics made the devised approach more useful than previously published methods for quantifying moxifloxacin active components in pharmaceutical formulations, human blood plasma, and urine samples. Finally, the study was unaffected by excipients commonly seen in medicinal preparations.

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Declarations

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

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