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A Validated Spectrofluorimetric Method for the Determination of Moxifloxacin in Its Pure Form, Pharmaceutical Preparations, and Biological Samples

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This research work presents a simple, sensitive, selective, economic, and widely applicable and interferences-free spectrofluorimetric method estimating moxifloxacin in the pure form, commercial formulations and biological samples. The method is based on the reaction of moxifloxacin with Ce(IV) in an acidic medium to generate fluorescent active species Ce(III). The excitation and emission of the fluorescent species are 250 and 362 nm, respectively. Different variables that might influence the oxidation of moxifloxacin, including the Ce(IV) concentration and volume, the effect of temperature and the heating time, the type of acids and its concentration were analyzed and boosted. The linearity was observed in the concentration range of 0.2 – 5.0 μg mL^{-1} with a correlation coefficient of 0.9991. The limit of detection and the limit of quantification were calculated and observed to be 0.016 and 0.056 μg mL^{-1} respectively. The effects of the common excipients and some co-administrated drugs usually used in the determination of moxifloxacin were investigated, and no interferences were noted. The planned method has been successfully practical for the analysis of moxifloxacin in its pure form, in pharmaceutical products and in biological samples. The obtained percent recoveries ranged from 95.50 to 101.37% for pharmaceutical products and from 95.15 to 103.18% for human blood plasma and urine.

Keywords Moxifloxacin, spectrofluorimetry, cerium(IV), urine, blood plasma

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Introduction

Antibiotics are extensively applied for the cure or monitoring of severe microbial infectious diseases by preventing their manner of action, whether bacteriostatically or bacteriocidically, from stopping millions of lives.

Recently, the utilization of various antibiotics has been reduced, as a result of the development of bacterial resistance mechanisms, which was the most serious challenging problem for researchers in the improvement of novel generations of synthetic types of antibiotics and overcome in the mid of 1980s by preparing Quinolones. They were active mainly against various aerobic types of Gram-positive and Gram-negative microbes, but experienced restricted use due to evolving resistance.[1]

It has been altered and restructured, leding to the formation of fluoroquinolones, a fluorinated quinolones having fluorine atom at the C-6 position and persistently progressing in expanding their spectrum of activity.[2] They are the most significant newer generation of antimicrobial agents, and are in advance phases of development. They are most significantly used in human medicines and broadly against Gram-positive and Gram-negative microbes, like mycobacteria, mycoplasmas, rickettsias, and also used in metal complexes responding as antioxidant and anticancer compounds.[3,4]

Moxifloxacin, amongst the advance synthetic fourth generation broad spectrum fluoroquinolones, mainly clinically used, that is getting greater attention as resistance develops as compared to the other antibiotics, and is presented as the monohydrochloride salt of 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. The mechanism of action involves the inhibition of enzyme topoisomerase and gyrase, which is essential in plasmid DNA replication, transcription and recombination.[5]

Moxifloxacin differs from other fluoroquinolones in a sense that it has a methoxy group at the C-8 position and a diazobicyclononyl moiety at C-7, which increase activity against Gram-positive species of bacteria, potency, spectrum of activity, half-life and decrease phototoxicity and resistant mutant respectively. The drug is rapidly and essentially emerged, with peak plasma concentrations reached within 1 – 4 h and a long half-life (11.4 – 15.6 h), administering it suitable for a once daily dose.[6-8] Shortly, it is broadly used to treat against aerobic, anaerobic Gram-positive and Gram-negative bacteria, and also used for the treatment of respiratory-tract infections and community acquired pneumonia.[9]

A short, but updated and relative, literature survey has been reported for the quantification of moxifloxacin such as HPTLC,[10] HPLC with a fluorescence detector,[11,12] electroanalytical methods,[13,14] a differential pulse polarographic method,[15]

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LC-MS,[16] RP-HPLC,[17,18] capillary electrophoresis,[19] spectrophotometry,[20,21] and spectrofluorimetric methods.[22,23]

Recently, various earlier researchers have organized studies on the determination of moxifloxacin in commercial products and biological fluids under different developed methods, but most of these methods faces some problems like a lack of sensitivity, interferences, has a narrow linear range and limited applicability to biological samples.

The present work was an attempt to develop a simple, economic, precise and interference-free method for the determination of moxifloxacin in pharmaceutical preparations and biological samples, which is based on the reaction of moxifloxacin with Ce(IV) in an acidic medium to generate a fluorescent active species Ce(III). Furthermore an increase in the fluorescence intensity was proportional to the amount of moxifloxacin added. Under optimized conditions, the fluorescence intensity was proportional to the concentration of moxifloxacin in the range of 0.2 – 5.0 μg mL^{-1} with a limit of detection of 0.016 μg mL^{-1}.

Experimental

Instrument

A Perkin Elmer spectrofluorimeter (LS 45) was used to measure the fluorescence intensity, equipped with a 150-W xenon lamp, and a grating monochromator of a 1 × 1 cm matched quartz cell. The instrument was first calibrated against the set 6BF, the Certified Reference Materials, which contain anthracine/naphthalene, ovalene, *p*-terphenyl, E11, tetraphenylbutadiene and rhodamine. Digital Analytical Balance (OHAUS Corporation USA), Digital Water Bath, LAbacon Model LWB-104, Single Hexode and Micro Centrifuge, Mod (mini) Lab Tech were also involved in the current research work.

Chemical reagents

All of the chemical reagents were of analytical grade type and were used throughout the work without further purification. Cerium sulfate tetra hydrate ($Ce(SO_4)_2{\cdot}4H_2O$) (Riedel-deHaën), sulfuric acid (95 – 97%) Merck Darmstadt, Germany was employed. Pure (standard) moxifloxacin was gifted by Heal Pharmaceutical Industry Pvt. Limited, W.33 Industrial East Hayatabad Peshawar Pakistan.

Commercial products containing 400 mg/tablet of moxifloxacin hydrochloride, Xefecta, Hilton Pharma (Pvt) Ltd., Plot No. 13 – 14, sector 15, Korangi Industrial Area, Karachi, Pakistan, Mofilox, Macter International Limited F-216 S.I.T.E Karachi Pakistan, Moksi Abbott laboratories (Pakistan) Ltd Landhi, Karachi), Mofest, Sami Pharmaceutical Company (pvt) Ltd., F-95, S.I.T.E., Karachi, Fotiflox, Helix Pharma (Pvt) Ltd., A.56, S.I.T.E., Karachi, and Moxiget, Getz Pharma (Pvt.) Ltd. Karachi Pakistan were purchased from a local medicinal store.

Preparations of reagents solutions

A cerium(IV) (100 μg mL^{-1}) solution was made by dissolving 0.01 g of cerium(IV) sulfatedihydrate in 10 mL of 4 mol L^{-1} H_2SO_4 and transferred to 100 mL in a volumetric flask using distilled water.

Drug solution

A 100 μg mL^{-1} standard stock solution of drug was freshly prepared by dissolving 0.005 g of standard moxifloxacin in distilled water and completed the volume to 50 mL in a volumetric flask.

Sample preparations

Five tablets of each medication were weighed individually to obtain an average weight of a single tablet. The tablets were ground with the help of a pestle and mortar to obtain a fine powder. The powder equivalent to 100 μg mL^{-1} of moxifloxacin was prepared in distilled water, filtered and transferred to a 50-mL volumetric flask and completed the volume to the mark.

Preparation of biological sample

Urine and blood plasma were obtained from adult healthy volunteers.

General experimental procedure

The standard moxifloxacin solution in the concentration range of 0.2 – 5.0 μg mL^{-1} was mixed with 4.5 mL of a cerium(IV) solution (500 μg mL^{-1}) in test tubes. Then the mixture was heated at 60°C for 30 min on an electrical thermostatic water bath and diluted to 10 mL with distilled water in a volumetric flask. The mixture was then placed in a 1 × 1 cm quartz cell and the fluorescent intensity was carefully determined at an excitation wavelength of 250 nm and an emission wavelength of 362 nm against a blank solution. All measurements were made in triplicate.

Applications to pharmaceutical preparations

Proper volumes of the sample solutions were diluted with distilled water to give a moxifloxacin concentration within the working range and analyzed using the procedure described above and the actual moxifloxacin concentration in commercial formulations were calculated using the calibration equation.

Applications to spiked human plasma and urine samples

After 1.0 mL of plasma or urine and 2.5 mL of standard moxifloxacin solution (100 μg mL^{-1}) was mixed with 6.0 mL of acetonitrile for deproteinisation, the mixture was centrifuged by adjusting the centrifuge machine at 3000 rpm for a time period of 10 min. A clear liquid was poured into a 50-mL volume flask, and volume was completed to the mark with distilled water. Proper volumes (0.2, 0.4, 0.6 μg mL^{-1}) from this solution were investigated using the general procedure described above.

Results and Discussion

Optimization of reaction conditions

Various experimental parameters affecting the reaction, such as temperature, heating time, concentration and volume of Ce(IV) and type and concentration of acid, were carefully analyzed and optimized.

Effects of temperature and heating time

The reaction between moxifloxacin and Ce(IV) was slow at room temperature. Therefore, the effects of the temperature and heating time were investigated. The effect of the temperature was investigated in the range of 30 – 100°C and the heating time from 10 – 60 min. The highest fluorescence intensity was recorded by heating the reaction mixture at 60°C for 30 min. Upon heating above 60°C, the fluorescence intensity decreased due to the formation of a byproduct, which is why its intensity decreases (Fig. 1).

Effect of Ce(IV) concentration

The effect of the concentration of Ce(IV) was investigated in the range of 100 – 700 μg mL^{-1}. The maximum fluorescence intensity was obtained at 500 μg mL^{-1} Ce(IV), and above

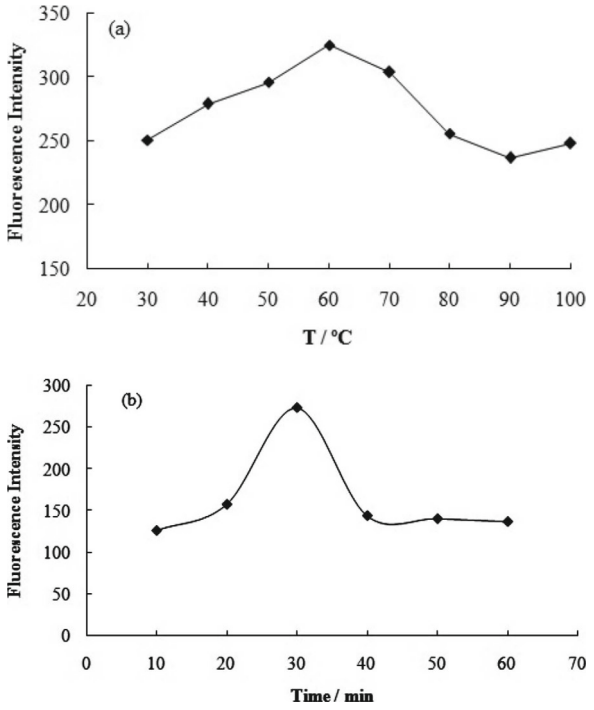

Fig. 1 Effect of the temperature (a) heating time (b) on the reaction of moxifloxacin with Ce(IV).

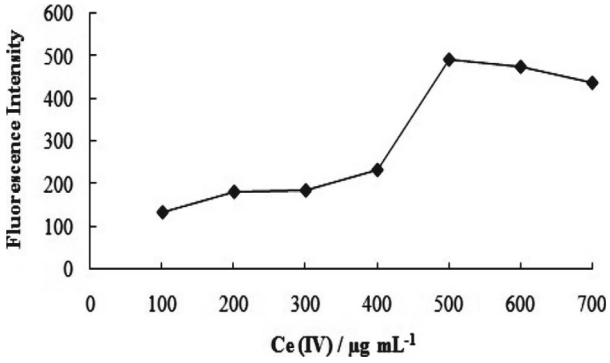

Fig. 2 Effect of the Ce(IV) concentration on the reaction of moxifloxacin.

500 µg mL^{-1} a small decrease was observed in the fluorescence intensity. This small decrease was due to an incomplete conversion of Ce(IV) to Ce(III) (Fig. 2). The influence of the volume of Ce(IV) was also analyzed in the range of 0.5 – 5.5 mL and 4.5 mL of 500 µg mL^{-1} a Ce(IV) solution gave the maximum fluorescence intensity.

Effect of acid type and concentration

The oxidation reaction of moxifloxacin with Ce(IV) was carried out in an acidic medium. For this purpose the Ce(IV) solution was prepared in various acids, such as HCl, HNO_3 and H_2SO_4' to find the most suitable acid. H_2SO_4 produced the maximum intensity. The concentration effect of H_2SO_4 was also examined in the range of 0.4 – 2.8 mol/L, 1.6 mol/L of H_2SO_4 recorded the highest fluorescence intensity (Fig. 3).

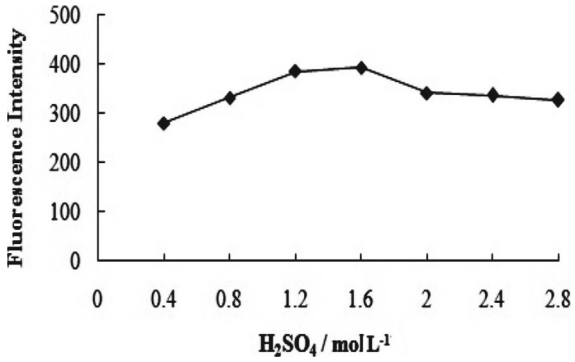

Fig. 3 Effect of the acid concentration on the reaction of moxifloxacin with Ce(IV).

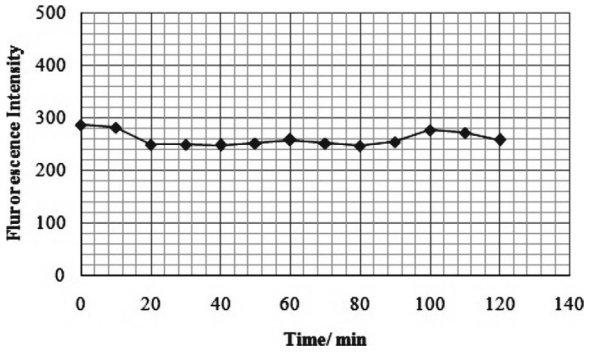

Fig. 4 Effect of the time on the stability of the reaction of moxifloxacin with Ce(IV).

Stability of fluorophore

The time period for the product stability was analyzed for up to 2 h after dilution. The result showed that no change in the fluorescence intensity was observed, and the product was stable for 2 h after dilution (Fig. 4).

Analytical parameters

The fluorescence intensity of moxifloxacin-Ce(IV) system increased linearly with the concentration of moxifloxacin. The calibration plot showed linearity in the concentration range of 0.2 – 5.0 µg mL^{-1} with an excellent correlation coefficient of 0.9991 (Fig. 5). The limit of detection and the limit of quantification were calculated and found to be 0.016 and 0.056 µg mL^{-1} respectively. The important analytical parameters, such as the excitation and emission wavelengths, linear regression equation, intercept, slope, correlation coefficient and relative standard deviation, are given in Table 1. The method sensitivity is compared with other reported methods in Table 2. The results show that the proposed method is more sensitive than already reported methods.

Effect of interferences

The selectivity of the recommended method was studied by inspecting the interferences effect of commonly used excipients like sucrose, fructose, glucose, starch, sorbitol, Mg-stearate, lactose and ascorbic acid that might be added during the synthesis of pharmaceutical tablets for different specific health purposes. To a constant amount of moxifloxacin, these excipients were added in different concentrations in ratios of

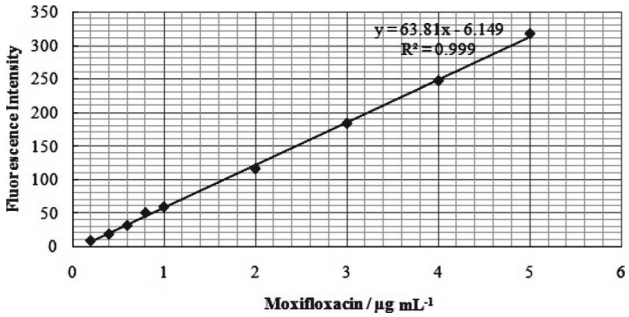

Fig. 5 Effect of the moxifloxacin concentration on fluorescence intensity. Conditions: 0.2 – 5.0 µg mL^{-1} moxifloxacin, 4.5 mL cerium(IV) (500 µg mL^{-1}), heated at 60°C for 30 min, diluted to 10 mL, λ_{emis} 362 nm.

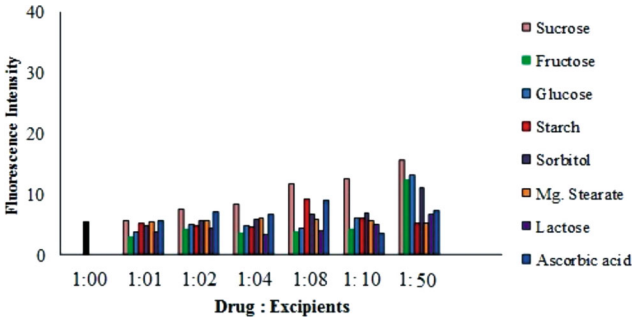

Fig. 6 Effect of common excipients on the determination of moxifloxacin.

Table 1 Related analytical characteristics for the proposed spectrofluorimetric method for the evaluation of moxifloxacin

Characteristics	Value
Excitation wavelength/nm	250
Emission wavelength/nm	362
Linearity range/µg mL^{-1}	0.2 – 5.0
LOD/µg mL^{-1}	0.016
LOQ/µg mL^{-1}	0.056
Standard deviation (SD)/µg mL^{-1}	0.005
Regression equation	$Y = 63.818X - 6.149$
Slope, *b*	63.818
Intercept, *a*	–6.149
Correlation coefficient, r^2	0.9991
RSD, %	2.74

Table 2 Comparison of the proposed method with other reported methods for the spectrofluorimetric determination of moxifloxacin

Method	Linear range	Limit of detection (LOD)	Reference
HPLC	0.2 – 2.0 µg mL^{-1}	0.05 µg mL^{-1}	24
Spectrophotometry	1 – 20 µg mL^{-1}	0.588 µg mL^{-1}	25
HPLC method	3 – 1300 µg L^{-1}	1.0 µg L^{-1}	26
Spectrophotometry	0.80 – 12.8 µg mL^{-1}	9.3 ng mL^{-1}	27
Spectrofluorimetry	0.2 – 5.0 µg mL^{-1}	0.016 µg mL^{-1}	Present method

1:1, 1:2, 1:4, 1:6, 1:8, 1:10 and 1:50 and analyzed by the proposed method. None of the excipient was found to produce interferences at lower concentrations. Only sucrose produced non-significant interferences at higher concentration (Fig. 6).

Similarly, some co-administrative drugs like metronidazole, mefenamic acid, famotidine, ibrufen, paracetamol, omeprazole, cetirizine and doxycycline were also checked in the analysis of moxifloxacin. Solutions containing 0.2 µg mL^{-1} of moxifloxacin and different concentrations of these drugs in the ratio of 1:1 to 1:10 were examined by the advanced method. The result shows that these co-administrative drugs do not produce any interference (Fig. 7).

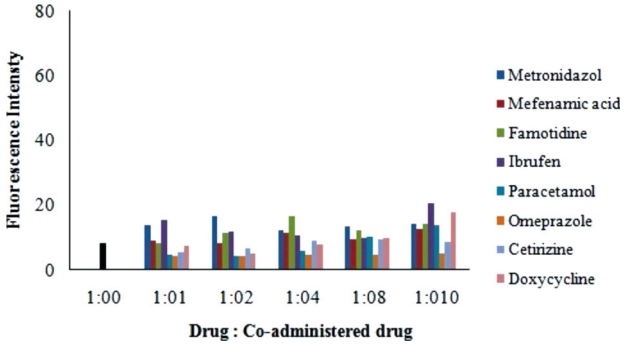

Fig. 7 Effect of co-administrated drugs on the determination of moxifloxacin.

Table 3 Precision and accuracy of reference moxifloxacin solution

Amount taken/ µg mL^{-1}	Amount found/ µg mL^{-1}	Recovery, % ± RSD
0.2	0.199	99.82 ± 3.46
0.4	0.392	98.18 ± 0.34
0.6	0.591	98.50 ± 1.46
	Mean	98.83
	SD	0.869
	RSD	0.879

Each result is the average of separate triplicate analysis.

Reliability of the method

The precision and accuracy of the proposed method were tested by ascertaining moxifloxacin in the standard form, and in commercial formulations for each concentration within the calibration limits in triplicate. The results are tabulated in Table 3 for the pure form and in Table 4 for pharmaceutical preparations. The obtained % recoveries ranged from 98.18 to 99.82% for the pure form and 95.50 to 101.37% for pharmaceutical preparations with a small value of the relative standard deviation, indicating the reproducibility of the method. The accuracy of the developed method was analyzed by applying the standard addition method, analyzing six different medicinal brands of drugs including Xefecta, Mofilox, Moksi, and Mofest, Fotiflox and Moxiget, each consisting 400 mg/tab of moxifloxacin. The percent recoveries obtained ranged from 98.47 to 103.25% (Table 5). This good percent recoveries

Table 4 Precision and accuracy of the proposed method for determination of moxifloxacin in commercial brands

Pharmaceutical formulations (400 mg/tablet)	Amount taken/ μg mL^{-1}	Amount found/ μg mL^{-1}	Recovery, % ± RSD
Xefecta	0.2	0.19	97.78 ± 3.60
	0.4	0.39	98.43 ± 0.12
	0.6	0.60	101.37 ± 1.56
Mofilox	0.2	0.19	99.86 ± 1.59
	0.4	0.39	97.98 ± 0.75
	0.6	0.59	98.88 ± 0.34
Moksi	0.2	0.19	99.50 ± 0.48
	0.4	0.39	98.82 ± 1.73
	0.6	0.57	96.35 ± 1.96
Mofest	0.2	0.19	95.50 ± 0.58
	0.4	0.39	97.79 ± 0.42
	0.6	0.59	98.80 ± 2.73
Fotiflox	0.2	0.19	98.03 ±1.49
	0.4	0.39	98.18 ± 0.38
	0.6	0.59	99.30 ± 0.47
Moxiget	0.2	0.19	98.63 ± 1.42
	0.4	0.39	98.34 ± 0.73
	0.6	0.58	97.99 ± 0.74

Each result is the average of separate triplicate analysis.

Table 5 Recovery of moxifloxacin from commercial brands by the developed method (standard addition method)

Commercial brands (400 mg/tablet)	Added amount/ μg mL^{-1}	Found amount/ μg mL^{-1}	Recovery, % ± RSD
Xefecta	0.2	0.20	101.74 ± 2.89
	0.4	0.40	102.33 ± 0.18
	0.6	0.60	101.89 ± 0.32
Mofilox	0.2	0.19	99.14 ± 2.11
	0.4	0.39	101.39 ± 1.95
	0.6	0.59	99.15 ± 1.97
Moksi	0.2	0.19	98.47 ± 1.21
	0.4	0.39	100.89 ± 1.61
	0.6	0.59	100.11 ± 0.17
Mofest	0.2	0.20	102.36 ± 1.34
	0.4	0.40	103.25 ± 0.44
	0.6	0.58	99.09 ± 1.11
Fotiflox	0.2	0.19	98.53 ± 1.61
	0.4	0.39	100.91 ± 0.85
	0.6	0.60	101.59 ± 0.35
Moxiget	0.2	0.19	99.62 ± 0.86
	0.4	0.39	101.02 ± 0.77
	0.6	0.59	100.78 ± 0.36

Each result is the average of separate triplicate analysis.

obtained shows the high accuracy of the method for the determination of moxifloxacin in pharmaceutical products.

Application of the developed method

The developed method was effectively used for evaluating the active components of moxifloxacin in six different prescriptions. The results achieved by the developed method were comparable with the label claims (Table 6). Due to the high sensitivity and absence of interference from commonly used excipients and co-administrative drugs, the proposed method was also be applied to determine the moxifloxacin in spiked human plasma and urine samples. The percent recovery for plasma is 97.18 – 103.18% and 95.17 – 102.65% for urine sample (Table 7).

Table 6 Evaluation of active ingredients of moxifloxacin in medicinal brands

Brand name	Active constituents (mg/tab) ± t-test value		
	Branded value	Obtained value	(4.303)
Xefecta	400	396.11 ± 4.12	1.63
Mofilox	400	394.78 ± 3.53	2.51
Moksi	400	392.78 ± 6.61	1.89
Mofest	400	389.22 ± 6.51	2.86
Fotiflox	400	394.89 ± 2.52	3.51
Moxiget	400	392.78 ± 1.35	2.26

Each result is the average of separate triplicate analysis.

Table 7 Examination of moxifloxacin in spiked human plasma and urine samples

Sample	Taken/μg mL^{-1}	Found/μg mL^{-1}	Recovery, % ± RSD
Human plasma	0.2	0.20	103.18 ± 4.22
	0.4	0.38	97.18 ± 2.56
	0.6	0.60	101.26 ± 3.67
Urine	0.2	0.20	102.65 ± 4.22
	0.4	0.40	101.59 ± 3.45
	0.6	0.57	95.17 ± 2.22

Each result is the average of separate triplicate analysis.

Conclusions

A simple, fast, selective and low-cost spectrofluorimetric method was developed for the determination of moxifloxacin in the pure form, pharmaceutical preparations and biological fluids (human blood plasma and urine). The developed method was found to be precise, accurate and to have a low limit of detection and quantification than reported methods. The instrument and reagents used in the developed method is inexpensive and easily available, and does not need any tedious extraction procedure. These advantages make the developed method a valuable alternative to already present methods for the determination of active ingredients of moxifloxacin in pharmaceutical preparations and human blood plasma and urine samples. There are no interferences from commonly used excipients, and the method can be used for the quantification of moxifloxacin in general research and quality control laboratories.

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