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Research Article

DEVELOPMENT AND VALIDATION OF SPECTROFLUORIMETRIC METHOD FOR THE ESTIMATION OF MOXIFLOXACIN IN PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

Objective: A simple, sensitive, rapid, precise, and accurate Spectrofluorimetric method has been developed for estimation of Moxifloxacin in its pure and pharmaceutical dosage forms.

Method: Moxifloxacin showed good fluorescence intensity in double distilled water and so Double distilled water was selected as a solvent for estimation of Moxifloxacin. The optimized excitation (λ_{ex}) and emission (λ_{em}) wavelength were 296 nm and 471 nm respectively with 2.5nm slit width for Moxifloxacin determination.

Result: The calibration curves were found to be linear between fluorescence intensity and drug concentration in the range of 20-60 ng/ml with coefficients of determination above 0.999 for all the analyte. The limits of detection (LOD) and limit of quantification (LOQ) were found in the range of 0.0023 and 0.0070 ng/ml respectively. The method recoveries were higher than 99%.The % RSD value of intra- and interday variation coefficients were observed less than 2%.The developed method was validated in terms of linearity, precision, accuracy, limit of detection and quantification, robustness as per International Conference on Harmonization Q2 (R1) guidelines.

Conclusion: This method is simple, accurate and rapid; those require no preliminary separation and therefore can be used for routine analysis of Moxifloxacin in quality control laboratories.

Keywords: Spectrofluorimetric, Fluorescence, Excitation and Emission.

INTRODUCTION

Moxifloxacin is oral 4th generation fluoroquinolone class antibiotic drug. Chemically, it is 1-cyclopropyl-7-(2,8-diazabicyclo [4,3,0] nonane)-6-fluoro-8-methoxy-1,4-dihydro-4-oxo-quinoline carboxylic acid (Fig.1)and that has potent and enhanced antimicrobial activity against Gram positive and maintained activity against Gram negative anaerobes. It is used in the treatment of lungs infections, urinarytract infections, respiratory tract infections, cutaneousallergy, pneumonia, abdominal and eye infections caused by bacteria[1-3].

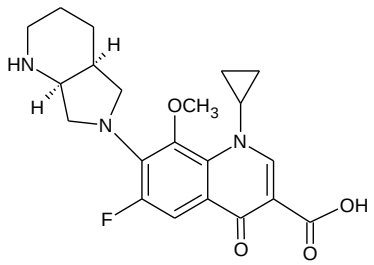

Fig. 1: Chemical structure of Moxifloxacin

The literature survey reveals that Moxifloxacin can be estimated by various methods like UV-spectrophotometric [4], by HPLC[5-9] and by HPTLC[10]individually or with other drugs in bulk drugs and in human plasma. However, there is no any Spectrofluorimetric method has been reported for the estimation of Moxifloxacin without derivatization [11]. The present work describes the fast, economic, specific and selective Spectrofluorimetric method for the estimation of Moxifloxacin in pharmaceutical dosage forms. There were numbers of trial performed in different solvent like 1N HCl, 1N NaOH, Methanol and distill water. Among the all double distill water was shown good intensity and no interference compared to other solvent so proposed method is direct and based on the measurement fluorescence intensity of Moxifloxacin in water at room temperature.

Pharmaceutical grade of Moxifloxacin was kindly gifted by Claris Lifescience Ltd (Ahmadabad, India). A tablet formulation was purchased from the local market (MAHAFLOX containing Moxifloxacin 400 mg). All the reagents used in this method were of analytical grade and water was double distilled water. Spectrofluorimetric analysis was performed on Perkin Elmer LS 55 Fluorescence Spectrofluorimeter with xenon discharge lamp (20KW), two automatic monochromators, Photomultiplier tube as detector; Software (FL Win LAB) and quartz cuvette was used. All weights were taken on shimadzu electronic balance AUX 220.

The standard solution of Moxifloxacin was prepared by weighing 10 mg of drug poured in 10 ml amber colour volumetric flask containing of double distill water (1000µg/ml) and further diluted 0.1 ml solution in amber colour volumetric flask containing 10ml of water to get a concentration of 10µg/ml, from the above solution take 1ml solution in 100 ml amber colour volumetric flask containing distilled water to get a final concentration of 100 ng/ml. The concentration of 100ng/ml was used for optimization of validation parameters.

From the above solution pipette out 2.0, 3.0, 4.0, 5.0 and 6.0 ml stock solution in 10 ml amber colour volumetric flask and make up the volume up to 10 ml with double distilled water. Then take the spectra of above all solutions and the r^2 value was found to be 0.999. (Fig.3)

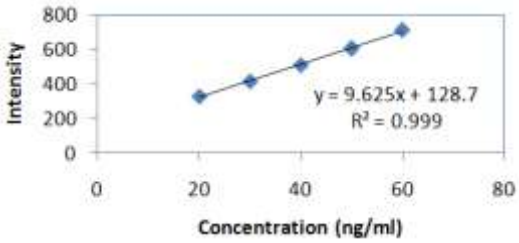

Fig. 3: Calibration Curve for Moxifloxacin

The first step involved in fluorimetric analysis was the selection of excitation and emission wavelength. The 50 ng/ml concentration solution was scanned in the region of 200 - 600 nm. Spectra of excitation and emission of Moxifloxacin was recorded. Keeping the emission wavelength constant, the excitation spectrum of

Moxifloxacin was measured in spectral measurement mode of the instrument. Similarly, the emission spectrum was again measured with the fixed excitation wavelength. The found constant excitation and emission wavelength for Moxifloxacin was 296 nm and 471 nm respectively [Fig.2 (a, b)].The method was validated as per ICH guideline.[12-13]

The linearity was calculated by linear regression analysis, using least square regression method. The calibration curve was plotted between the fluorescence intensities of the Moxifloxacin and concentrations of the calibration standards and the fluorescence intensity was measured at the fixed excitation and emission wavelength of 296 and 471 nm.

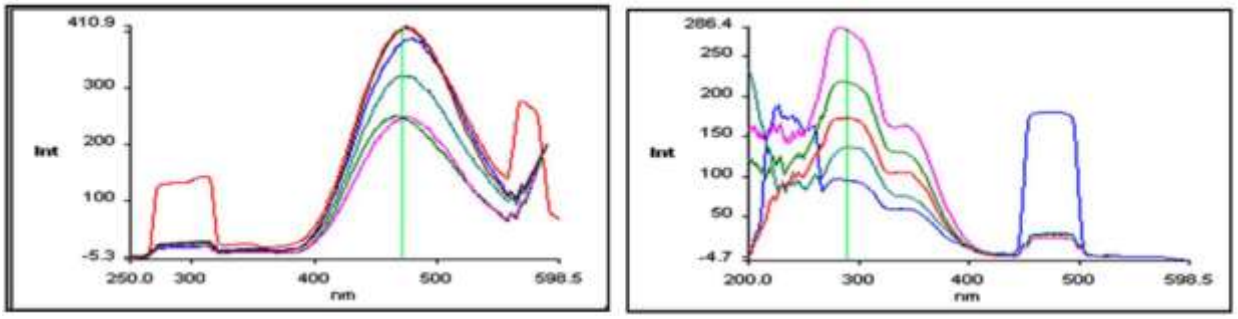

Fig. 2(a): Emission Spectra of Moxifloxacin (λ_{em} 471 nm) Fig. 2(b): Excitation Spectra of Moxifloxacin (λ_{ex} 296 nm)

The six replicate of prepared 50 ng/ml solution of Moxifloxacin taken from different stock solution and scanned at 200-800 nm for fluorescence intensity. The relative standard deviation (%RSD) was found to be less than 2 %, which indicates that the proposed method is repeatable.

Variation of results within the same day is called Intra-day precision. Intraday precision was determined by solution of Moxifloxacin three times in a day for three different concentrations (20, 30, 40 ng/ml). Mean intensity, S.D, and %R.S.D for fluorescence intensity were calculated for Moxifloxacin and summarized in Table 1.

Table 1: Summaries of Validation Parameters

Parameters	Result
Range	10 ng/ml – 70 ng/ml
Linearity	20 – 60 ng/ml
Regression equation	Y= 9.625x + 128.7
r^2 Value	0.999
Intraday precision (n=3)	0.691 – 1.227
Interday precision(n=3)	1.101 – 1.771
% Recovery	100.29 – 101.18
Assay (%)	99.916
LOD (ng/ml)	0.0023
LOQ (ng/ml)	0.0070
Robustness	Robust

Variation of results among different days is called Inter-day precision. Interday precision was determined by solution of Moxifloxacin for three days for three different concentrations (20, 30, 40 ng/ml). Mean % intensity, S.D, and %RSD for fluorescence intensity were calculated for Moxifloxacin and summarized in Table 1.

Ten tablets (MAHAFLOX 400 mg of Moxifloxacin per tablet) were accurately weighed and finely powdered. A quantity of the powder equivalent to 10 mg of Moxifloxacin was extracted by shaking with 20 ml of double distilled water, followed by another two extractions each with 10 ml of double distilled water. It was filtered on whatmann filter paper no. 42 to remove insoluble materials. The volume of filtrate was diluted to 100 ml with double distilled water (100µg/ml). It was further diluted according to the need and then analyzed following the proposed procedures. The nominal content of the tablets was calculated from the previously plotted calibration graph (Table 1).

To find the accuracy of the method, the recovery experiment was carried out using the standard addition method. Take the sample (20 ng/ml) and known amount of standard drug was added at 80%, 100% and 120 % level. The contents were reanalysed with the above described procedure (Table 1).

The sensitivity of the analytical method was evaluated by determining the Limits of Detection (LOD) and Limits of Quantitation (LOQ). The LOD and LOQ of the drugs were calculated using the following equation as per ICH guidelines.

LOD = 3.3 σ /S LOQ = 10 σ /S

Where, σ = Standard deviation of the response, S = Slope of the calibration curve.

The robustness of a method is its ability to remain unaffected by small changes in parameters like changes in emission wavelength. With this change in emission wavelength ± 2 nm. Take the spectra at 469 nm and 473 nm.

Linearity was assessed for Moxifloxacin by plotting calibration curves of the Intensity versus the concentration. The correlation coefficient (r^2) for Moxifloxacin was found to be 0.999. (Fig.3)The following equations for straight line were obtained for Moxifloxacin.

Linear equation for Moxifloxacin, Y= 9.625x + 128.7

The % recoveries were found to be in the range of 100.293-101.182 % for Moxifloxacin (Table 1). The precision of the method was determined by Intraday and inter-day precision and was expressed as the %RSD (Table 1), which indicate good method precision. The Limit of detection and Limit of quantification for Moxifloxacin was found to be 0.0023 ng/ml and 0.0070ng/ml respectively (Table 1).

The developed Spectrofluorimetric method for estimation of Moxifloxacin is simple, rapid, precise and accurate. The advantage of the present method is used as alternative to reference method (HPLC and other method) for determination of Moxifloxacin in pure and dosage forms in the industrial and research institutional laboratories.

REFERENCES

1. Drug bank: Moxifloxacin (DB00218), open drug data and drug target database, http://www.drugbank.ca/drugs/DB00218.
2. European Pharmacopoeia;7thedition, European Pharmacopoeia Commission, Volume 2, 2009: 1612.
3. British pharmacopoeia; 6th edition; British pharmacopoeia commission office, London, 2010, Vol I& II:1496
4. Motwani SK,ChopraS, Khar RK:Validated spectrophotometric methods for the estimation of Moxifloxacin in bulk and pharmaceutical Formulations.Journal of Pharmaceutical and Biomedical Analysis2007;68(2): 160-170.
5. DjurdjevicaP, CiricaA:Optimization of separation and determination of Moxifloxacin and its related substances by RP-HPLC. Journal of Pharmaceutical and Biomedical Analysis2009; 50: 117–126.

6. SmetJ, Boussery KA:Bio-analytical HPLC method for the simultaneous quantification of Ofloxacin, Ciprofloxacin and Moxifloxacin in human plasma. Journal of Chromatography2009;10:961–967.
7. VishwanathanK ,BartlettMG, Stewart JT: Determination of Moxifloxacin in human plasma by liquid chromatography electrospray ionization tandem mass spectrometry.Journal of Pharmaceutical and Biomedical Analysis 2002; 30: 961–968.
8. Al-Qaim FF, Pauziabdullah MD, Othman MR: Analysis of different therapeutic classes using Liquid chromatography – Mass Spectrometry in aquatic environment.International Journal of Pharmacy and Pharmaceutical Sciences 2012; 4(4):3-11.
9. Patel D, Patel M, Patel K:Simultaneous RP-HPLC Estimation of Moxifloxacin Hydrochloride and Ketorolac Tromethamine in Ophthalmic Dosage Forms. Asian Journal of Research in Chemistry2012; 5(5):697-700.
10. Khar RK, Ahmad FJ,Chopra S: Application of a validated stability-indicating densitometric thin-layer chromatographic method to stress degradation studies on Moxifloxacin.AnalChimActa2007;582: 75–82.
11. Juan A,Francisco JB:Spectrofluorimetric determination of Moxifloxacin in tablets, human urine and serum. Journal of Analytical and Biotechnical Science2011;5: 2322-25.
12. International Conference on Harmonization (ICH) of Technical Requirements for the Registration of Pharmaceuticals for Human Use, Validation of analytical procedures, ICH-Q2A, Geneva; 1995. 16.
13. Ravichandran V, Shalini S,Sundram KM, Harish R: Validation of analytical methods – strategies & importance.International Journal of Pharmacy and Pharmaceutical Sciences 2010; 2:18-22.