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Research Article

DEVELOPMENT AND VALIDATION OF RP-UPLC METHOD FOR ESTIMATION OF OFLOXAXCIN IN THEIR DOSAGE FORM

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ABSTRACT

This research manuscript describes simple, sensitive, accurate, precise and repeatable RP-UPLC method for the determination of Ofloxacin (OFL) in Tablet dosage form. The sample was analyzed by reverse phase C18 column (Purospher Star 100×2.1 mm,Merck Specialities) as stationary phase and Phosphate Buffer: Acetonitrile (85:15, v/v) as a mobile phase and P^H of 3.0 adjusted by Triethylamine and orthophosperic acid at a flow rate of 0.4 ml/min. Quantification was achieved with PDA detector at 294 nm. The retention time for Ofloxacin was found to be 1.644 minute. The linearity for the drug was obtain in the concentration range 2.5-17.5 µg/ml with mean accuracies and 99.13 ± 0.41 for Ofloxacin. The method was successfully applied to pharmaceutical formulation because no chromatographic interferences from Tablet excipients were found. The method retained its accuracy and precision when the standard addition technique was applied.

Keywords: Tablet dosage forms, Ofloxacin, Method validation, RP-UPLC

INTRODUCTION

Ofloxacin (OFL), an antimicrobial drug chemically is (RS)-9-fluoro-3-methyl-10-(4-methylpiperazin-1-yl)-7-oxo-2, 3-dihydro-7H-pyrido [1, 2, 3,-de]-1, 4 benzoazeine-6-carboxylic acid (fig.1), drug are official in (Indian pharmacopeia, British Pharmacopeia and United States Pharmacopeia.) The OFL is widely used in treatment of microbial infections. Literature search reveals that various analytical methods like UV-visible spectrophotometry (Jadhav G.P.,et al.,1998; Maliwal D., et al.,2008; Mashru R.C.,et al., 1998;) conductometry(Tuncel M.,et al.,1992;) HPLC(Kasabe A. J.,et al.,2009; Krishnan.,et al.,2002; Argekar A. P.,et al.,1996; Zhong L,et al.,2007; Du Y. X.,et al.,1994; Xu J.,et al 1993; Ohkubo T.,et al.,1992;), and LC-MS (Tuerk J.,et al.,2006;)have been reported for OFL in their individual and combined dosage forms with other drugs. There is no reported method for estimation of OFL in their tablet dosage form by RP-UPLC. This prompted the present work. The aim of the present work is to develop a simple, rapid, accurate and precise RP-UPLC method for estimation of OFL in their marketed formulation which is more rapid, sensitive, accurate and precise method than the RP-HPLC method.

MATERIALS & METHODS

Apparatus

The chromatography was performed on a Water (Acquity) RP-UPLC instrument equipped with PDA detector and Em-power 2 software, Purospher Star C18 column (100mm × 2.1 mm id, 2µm particle size, Marck, Germany) was used as stationary phase. Mettler Toledo analytical balance (Germany), an ultrasonic cleaner (Frontline FS 4, Mumbai, India) were used in the study.

Reagents and materials

Ofloxacin bulk powder was obtained from Nirlife, Healthcare division of Nirma. Ahmedabad, India. The commercial fixed dose combination product was procured from the Nirlife. Acetonitrile, Methanol, KH_2PO_4 (Finar Reagent, Ahemedabad, India) used were of HPLC grade. Whatman filter paper no. 41. (Whatman International Ltd., England) were used in the study.

Chromatographic Condition

Estimation was achieved by using Purospher Star C18 column (100mm × 2.1 mm id, 2µm particle size, Marck, Germany) as stationary phase with Phosphate Buffer: Acetonitrile (85:15, v/v) as a mobile phase and P^H of 3.0 adjusted by Triethylamine and orthophosperic acid at a flow rate of 0.4 ml/min and detection wavelength was 294nm in PDA detector

Preparation of mobile phase

0.68 gm Dihydrogen orthophosphate was weighed accurately in 1000mL volumetric flask. To it add about 70mL of Water and sonicate further make up the volume up to mark with water, further this buffer solution is mixed with 150 ml of actonitrile in 1000 ml volumetric flask to make a mobile phase ratio buffer: acetonirile (85:15%v/v) and adjust p^H 3 by using ortho phosphoric acid and triethylamine this mobile phase used as a diluents also was used throughout study

Preparation of standard stock solution (100µg/mL)

An accurately weighed Ofloxacin (5 mg) were transferred into two different 100 mL volumetric flask , dissolved in 50 mL methanol and sonicate after this diluted up to mark with methanol to get concentration of Ofloxacin(50µg/mL)

Preparation of standard working solution (10µg/mL)

Accurately weighed Ofloxacin (5 mg) were transferred to 100 mL volumetric flask, dissolved in 50 mL methanol and diluted up to mark with methanol to get concentration of Ofloxacin (50µg/mL)

Preparation of calibration curve

Aliquots (0.5, 1, 1.5, 2, 2.5, 3, and 3.5 ml) of standard working solutions 2.5, 5, 7.5, 10, 12.5, 15, and 17.5 µg/ml Ofloxacin, each were transferred in a series of 10 ml volumetric flasks, and the volume was made up to the mark with mobile phase. Each solution was injected under the operating chromatographic condition as described above and responses were recorded. Calibration curves were constructed by plotting the peak areas versus the concentration (fig.3), and the regression equations were calculated. Each response was average of three determinations.

Preparation of Marketed sample solutionfor Assay

For determination of the content of Ofloxacin in marketed Tablet Take 360 mg powder from Tablet and transferred to 100 mL volumetric flask, dissolved in mobile phase and sonicated for 30 min. The solution was filtered through Whatmann filter paper No. 41 and residue was washed with mobile phase. The solution was diluted up to the mark with mobile phase. Accurately measured 2.0 mL of solution was transferred to 10 mL volumetric flask, diluted up to the mark with mobile phase to get final working concentration of (10 µg/mL). A sample solution was injected under the operating chromatographic condition as described above and responses were recorded (fig. 2). The analysis procedure was repeated three times with Tablet formulation.

Method Validation

The method was validated in compliance with ICH guidelines. (Q 2 B)

Accuracy (recovery study)

The accuracy of the method was determined by calculating the recoveries of Ofloxacin by the standard addition method. Known amounts of standard solutions of Ofloxacin were at added at 80, 100 and 120 % level to prequantified sample solutions of Ofloxacin (5 µg/ml). The amounts of Ofloxacin were estimated by applying obtained values to the respective regression line equations.

Method precision (repeatability)

The precision of the instrument was checked by repeatedly injecting (n=6) solutions of Ofloxacin (10 µg/ml drug) without changing the parameters.

Intermediate precision (reproducibility)

The intraday and interday precisions of the proposed method was determined by estimating the corresponding responses 3 times on the same day and on 3 different days over a period of one week for 3 different concentrations of standard solutions of Ofloxacin (5, 7.5 and 10 µg/ml). The results were reported in terms of relative standard deviation (% RSD).

Limit of detection and Limit of quantification

The limit of detection (LOD) and limit of quantitation (LOQ) of the method were determined by standard deviation of response and slope method.

RESULTS AND DISCUSSION

To optimize the RP-UPLC parameters, several mobile phase compositions were tried. A satisfactory Estimation and good peak symmetry Ofloxacin were obtained with a mobile phase comprising of Phosphate Buffer: Acetonitrile (85:15, v/v) and P^H of 3.0 adjusted by Triethylamine and orthophosperic acid at a flow rate of 0.4 ml/min to get better reproducibility and repeatability. Quantification was achieved with PDA detection at 294 nm based on peak area. The retention time for Ofloxacin were found to be 1.644 min, (Fig.2). Linear correlation was obtained between peak area versus concentrations of Ofloxacin(Fig.3) in the concentration ranges of concentration range of 2.5-17.5 µg/ml with mean accuracies 99.13 ± 0.41 for Ofloxacin The mean recoveries obtained were 99.13 ± 0.41 % for Ofloxacin, Table 2 which indicates accuracy of the proposed method. The % RSD values for Ofloxacin were found to be <2 %, which indicates that the proposed method is repeatable. The low % RSD values of interday (0.148-0.244%) and intraday (0.078-0.529%) variations for Ofloxacin reveal that the proposed method is precise. LOD values for Ofloxacin were found to be 0.0255µg/ml and LOQ values for Ofloxacin were found to be 0.077µg/ml (Table 1). These data show that the proposed method is sensitive for the determination of Ofloxacin. The results of system suitability testing are given in (Table 3). The amount of Ofloxacin present in the marketed sample were determined by fitting the responses into the regression equations of the calibration curve for Ofloxacin, and the results obtained were comparable with the corresponding labeled claim (Table 4).

Table 1: Regression analysis data and summary of validation parameter for the Proposed RP-UPLC method

Parameters	RP-UPLC method
	Ofloxacin
Concentration range (µg/ml)	2.5-17.5
Slope	23429
Intercept	-3149
Correlation coefficient	0.998
LOD[a] (µg/ml)	0.025
LOQ[b] (µg/ml)	0.077
Accuracy	99.13 ± 0.41
Repeatability (% RSD[c], n = 6)	0.414
Precision (%RSD)	
Intraday (*n* = 3)	0.078-0.529%
Interday (*n* = 3)	0.148-0.244%

a=Limit of Detection, b=Limit of Quantitation, c=relative standard deviate

Table 2: Recovery data for the proposed method

Drug	Level	Amount of sample taken (µg/ml)	Amount of standard spiked (%)	Mean % Recovery ± S.D[d]. (n=6)
Ofloxacin	I	5	80 %	99.58±0.14
	II	5	100 %	99.86±0.30
	III	5	120 %	99.5±1.0

s.d=Standard deviation

Table 3: System suitability test parameters for Ofloxacin for the proposed RP-UPLC method

Parameters	Ofloxacin ± RSD (n = 6)
Retention time (min)	1.65 ± 0.161
Tailing factor	1.26 ± 0.225
Theoretical plates	6857 ± 0.937

Table 4: Analysis of marketed formulation of Ofloxacin by proposed RP-UPLC method (n = 3)

Tablet	Label claim	Amount found	% Label claim ± S. D.(n=3)
	Ofloxacin	Ofloxacin	Ofloxacin
I	50.0	99.87	99.87±1.03

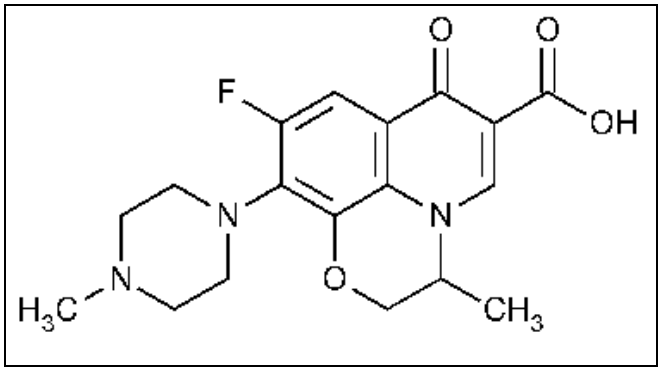

Fig. 1: Structure of Ofloxacin

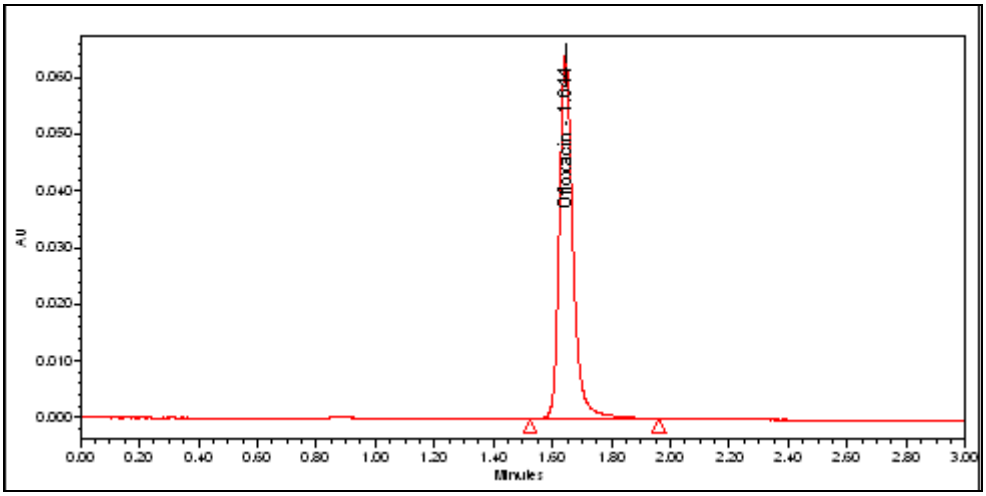

Fig. 2: Chromatogram of standard solution (10.0µg/mL) of Ofloxacin (RT 1.644 min) in methanol by RP-UPLC method

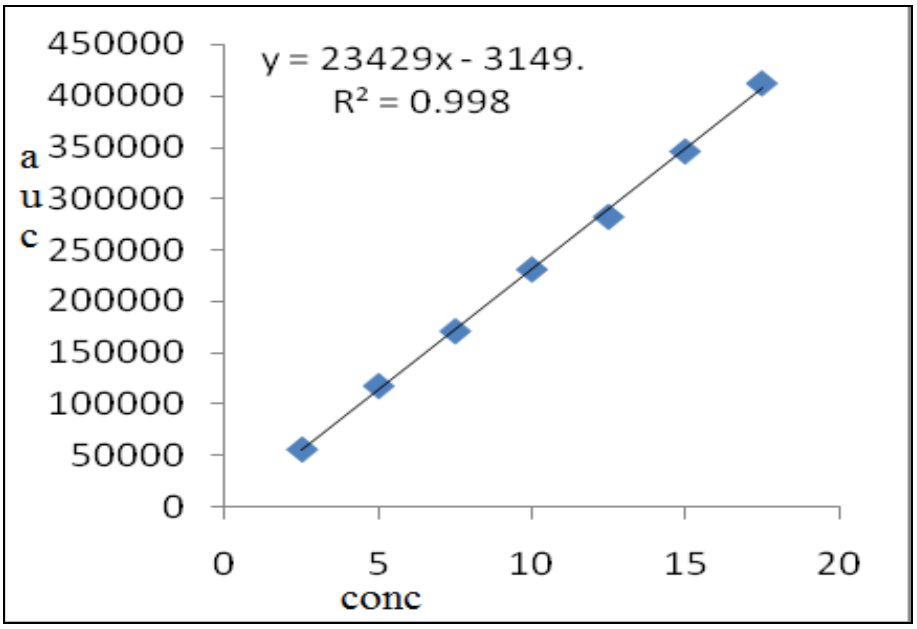

Fig. 3: Linearity of Ofloxacin

CONCLUSION

In this proposed method the linearity is observed in the concentration range of 2.5-17.5 µg/ml with co-efficient of correlation, and (r^2) = 0.998 for Ofloxacin, at 294 nm. The proposed method is simple, accurate, precise, specific, and sensitive and has ability to estimate drug in Tablet. The method is suitable for routine analysis of Ofloxacin in tablet. The simplicity of the method allows for application in laboratories that lack sophisticated analytical instruments such as LC–MS. The prime importance was given to

develop less time consuming and simple RP-UPLC-PDA method. The RP-UPLC method developed meets the system suitability criteria, peak integrity and resolution for the parent drug. Detection and quantification limits achieved, describe the method is very sensitive. High recoveries and acceptable % CV values confirm established RP-UPLC method is accurate and precise. The analytical results demonstrate the ability of the developed method to assay Ofloxacin. Assay results found from the study show that the method is successfully applied for the analysis of Ofloxacin in Tablet. Hence, the method is recommended for routine quality control analysis of Ofloxacin.

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