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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF OFLOXACIN EYE DROP BY HPLC

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ABSTRACT

A simple, rapid, sensitive, specific, accurate, HPLC method was developed and validated as per ICH guidelines for the determination of ofloxacin in eye drop. Thermoseparation products C8 (250 cm × 4.6 mm i.d., 5 µm) column with a mobile phase consisting of Acetonitrile: Buffer in the ratio 35:65 v/v with a flow rate of 1.5 mL/min was used. Detection was carried out at 315 nm using UV detector. Validation parameters were performed to demonstrate system suitability, specificity, precision, linearity and range, accuracy, ruggedness and robustness. The method was linear over the concentration range of 50 – 300 µg/mL. The method showed good recoveries (99.8 – 103.73%) and the relative standard deviations of intra and inter-day assay were 0.554 and 0.677% respectively. The proposed method was found to be precise, accurate, selective and rapid for the determination of ofloxacin in quality control and in assay.

Key words: Ofloxacin, Formulation, Estimation.

INTRODUCTION

Ofloxacin (OFLX) [9-fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7H-pyrido [1,2,3-de]-1,4-benzoxacine-6-carboxylic acid] (Fig. 1) is a fluoroquinolone antibiotic considered to be a second-generation fluoroquinolone. The fluoroquinolone (quinolone) class of chemotherapeutic agents is considered to be a drug of last resort to treat life threatening bacterial infections[1,2]. It is a broad-spectrum antibiotic that is active against both Gram-positive and Gram-negative bacteria. It functions by inhibiting DNA gyrase, a type II topoisomerase, and topoisomerase IV[3-5] which is an enzyme necessary to separate replicated DNA, thereby inhibiting cell division.The fluoroquinolones interfere with DNA replication by inhibiting an enzyme complex called DNA gyrase. This can also affect mammalian cell replication. In particular, some congeners of this drug family display high activity not only against bacterial topoisomerases, but also against eukaryotic topoisomerases and are toxic to cultured mammalian cells and *in vivo* tumor models. Although the quinolone is highly toxic to mammalian cells in culture, its mechanism of cytotoxic action is not known. Quinolone induced DNA damage was first reported in 1986[6].

Recent studies have demonstrated a correlation between mammalian cell cytotoxicity of the quinolones and the induction of micronuclei[7-9]. As such some fluoroquinolones may cause injury to the

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chromosome of eukaryotic cells[10-15]. There continues to be considerable debate as to whether or not this DNA damage is to be considered one of the mechanisms of action concerning the severe and non abating adverse reactions experienced by some patients following fluoroquinolone therapy[16-17]. Different techniques have been proposed for the determination of the drug. OFLX has been determined by potenciometry, conductometry, differential-pulse voltammetry, adsorptive stripping voltammetry and high-performance liquid chromatography (HPLC)[18-26].

EXPERIMENTAL

Apparatus

The analysis was performed by using thermoseparation products, pump with spectra series R 100, detection with spectra series UV 100, with the loop volume of 10 µL. Column used in HPLC is of hyper clone, 250 cm × 4.6 mm 5 µ.

Reagents and solutions

Pure sample of ofloxacin and other ingredients were kindly supplied by, Radico Pharmaceuticals Company, Jaunpur, Uttar Pradesh. Acetonitrile and water used were of HPLC grade was supplied by Merck Pharmaceuticals. All other chemicals like mobile phase consists of Buffer : ACN (35 : 65), triethonalamine used were of AR grade.

Standard Preparation

150 mg of ofloxacin was weighed accurately and transferred into a 100 mL volumetric flask; 25 mL mobile phase was added and sonicated for 5 minutes and the volume was made up to the mark with the mobile phase. 1 mL of the resultant the solution was transferred into a 50 mL standard flask and volume was made with mobile phase and mixed well.

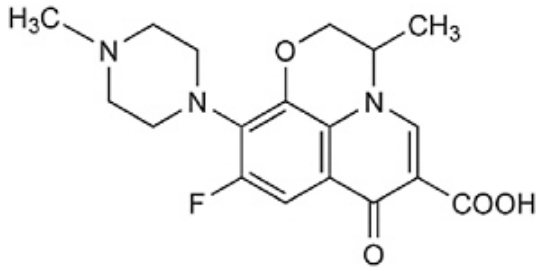


Fig. 1: Structure of ofloxacin

Sample preparation

1 mL of the ophthalmic solution was taken (each mL contains 3 mg of ofloxacin) in 50 mL volumetric flask and volume was made with mobile phase.

Linearity

Several aliquots of standard solutions of ofloxacin taken in different 10 mL volumetric flasks and diluted up to the mark with mobile phase such that the final concentration of OFLX was 60 – 300 µg/mL. Linearity was measured at 315 nm; peak area was recorded for all the peaks. The slope and intercept value for calibration curve was y = 8.6204 x + 23.506 coefficient correlation was found to be 0.999 for OFLX. The result shows that an excellent correlation exists between peak area and concentration of drugs within the concentration range indicated above. The calibration graph was presented in Fig. 2.

Regression analysis of the calibration curve for ofloxacin showed a linear relationship between the concentration and peak area with correlation coefficients higher than 0.9888 in all the curves assayed.

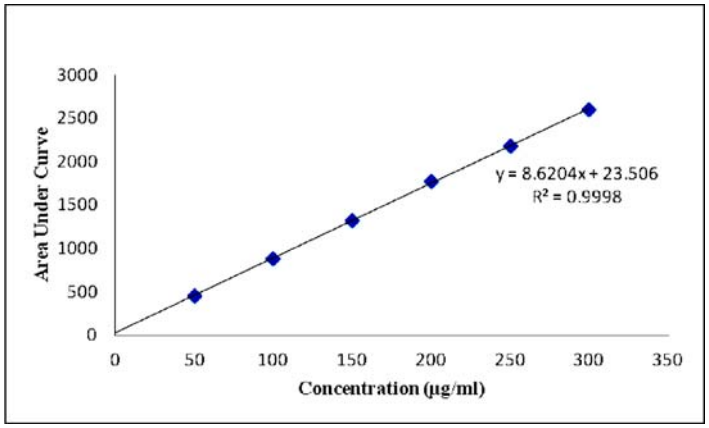


Fig. 2: Linearity profile of ofloxacin

Assay

20 µL of standard and sample solutions were injected into the column, from the peak area of OFLX chromatograph amount of drug present in samples were computed.

Method validation

Limit of Detection and Limit of Quantification

The Limit of Detection (LOD) and limit of Quantification (LOQ) of the developed method were determined by injecting progressively low concentrations of the standard solutions using the developed HPLC method. The LOD is the smallest concentration of the analyte that gives a measurable response (signal to noise ratio of 3). The LOD for ofloxacin was 7.327 µg/mL. The LOQ is the smallest concentration of the analyte, which gives response that can be accurately quantified (signal to noise ratio of 10). The LOQ was found to be 22.20 µg/mL.

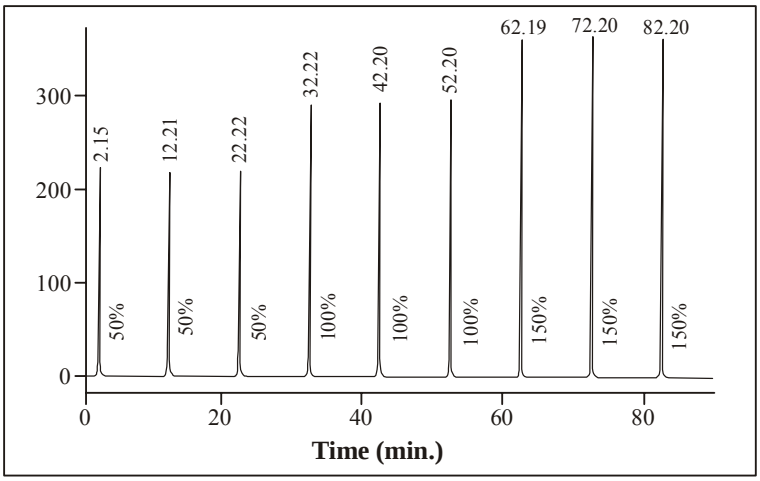
Recovery Studies

To study the accuracy and reproducibility of the proposed method recovery experiments were carried out. A fixed amount of pre-analyzed sample was taken and standard drug was added at 50%, 100%, and 150% levels. Each level was repeated three times. The contents of ofloxacin found by proposed method are shown in Table 1.

Table 1: Validation Parameters

Parameters	Ofloxacin
Linearity range	50-300 µg/Ml
Correlation coefficient	0.991-0.996
Slope	8.7916
Y Intercept	155.81
Precision (%)	101.5
Recovery (n = 3) (%)	99.8
Intra-day (n = 6) (% RSD)	0.554
Inter-day (n = 6) (% RSD)	0.677

The lower values of RSD of assay indicate the method is accurate. The mean recoveries of OFLX are in range of 99.8-100.78% that shows there is no interference from excipients. Analysis of formulation and recovery studies with chromatogram has been presented in Fig. 3.



Result Table – calculation Method Uncal

Peak No.	Reten. time	Area (mV. S.)	Height (mV)	WO5 (min.)	Area (%)	Height (%)
1	2.150	1521.0319	221.9586	0.1000	8.3261	8.4536
2	12.210	1553.7455	219.1185	0.1000	8.5051	8.3455
3	22.220	1541.2288	219.2393	0.1000	8.4366	8.3501
4	32.220	2038.8641	289.3146	0.1000	11.1607	11.0190
5	42.200	2027.7572	293.6410	0.1000	11.0999	11.1838
6	52.200	2034.2555	294.3012	0.1000	11.1354	11.2089
7	62.190	2513.8978	361.6489	0.1100	13.7610	13.7740
8	72.200	2522.6254	363.8473	0.1000	13.8087	13.8577
9	82.200	2514.9162	362.5265	0.1000	13.7665	13.8074
-	Total	18268.3223	2625.5958			

Column Performance Test Report

Peak No.	Reten. time	WO 5 (min)	Asymmetry (-)	Capacity (-)	Efficiency (th. P1.)	Eff./1 (t. p. /m)	Resolution (-)	Ret. Index (-)
1	2.150	0.100	2.500	1.15	2563	10253		0
2	12.210	0.100	2.667	11.21	82667	330669	59.223	0
3	22.220	0.100	2.667	21.22	273772	1095090	58.928	0
4	32.220	0.100	2.667	31.22	575642	2302569	58.870	0
5	42.200	0.100	2.667	41.20	987476	3949903	58.725	0

Fig. 3: Analysis of formulation and recovery studies with chromatogram

Table 2: System suitability parameters

Parameter	Ofloxacin
Calibration range (μg/mL)	50-300
Theoretical plates	2580
Resolution	2.25
Tailing factor	0.8
LOD (μg/mL)	7.32
LOQ (μg/mL)	22.20

Robustness

The robustness study was carried out at the wavelength of 315 ± 2 nm, at a flow rate of 1.5 ± 0.2 mL/ min and column temperature at 25 ± 0.2ºC.

RESULTS AND DISCUSSION

The UV-spectrum of the drug shows absorption band at 315 nm. Under the experimental conditions, the chromatogram showed a single peak of the drug around 10 min. Satisfactory resolution was obtained using the mobile phase system of Buffer: CAN (35 : 65) at a flow rate of 1.5 mL min^{-1}. The calibration curve was prepared by plotting the peak area of ofloxacin against drug concentration and was linear in the range of 60-300 μg/mL. Peak area and concentration were subjected to least square linear regression analysis to calculate the calibration equation and correlation coefficients. The regression equation was found as Y = 8.6204 x + 23.506 (r = 1, n = 7); (Y = ax + b), where Y is the peak area of ROP, a is the slope, b is the intercept and x is the concentration of the measured solution in μg mL^{-1}). Limit of detection (LOD) value was found as 7.327 μg mL^{-1} which is the concentration that yields a signal-to-noise ratio of 3 : 1. Limit of quantization (LOQ) value under the described condition was 22.20 μg mL^{-1}, which is the signal-to-noise ratio of 10:1. The repeatability of sample application and measurement of peak area were expressed in terms of % RSD which revealed intra-day (n = 6) and inter-day (n = 6, at 5 different day). The RSD values were found to be 0.554-0.677%, for intra- and inter-day variation, respectively, indicating good precision. To examine the accuracy of the method, recovery studies were carried out by standard addition method. The average percent recoveries obtained as 99.8-103.73% indicate good accuracy of the method. The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small variations in method conditions.

CONCLUSION

The HPLC assay developed for the qualification of ofloxacin was performed in mobile phase. The method was evaluated in a mass of facets, such as best condition, linear relation including coefficient of correlation, robustness, accuracy, reproducibility and precision. The result of specificity studies indicated no interference in the peak of ofloxacin which indicates no impurities. The method was found to be rapid. Hence it can be inferred that the developed method will be useful in routine laboratory analysis with a high degree of accuracy and precision.

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