

REVERSE PHASE – HPLC METHOD FOR THE ANALYSIS OF OFLOXACIN IN PHARMACEUTICAL DOSAGE FORM.

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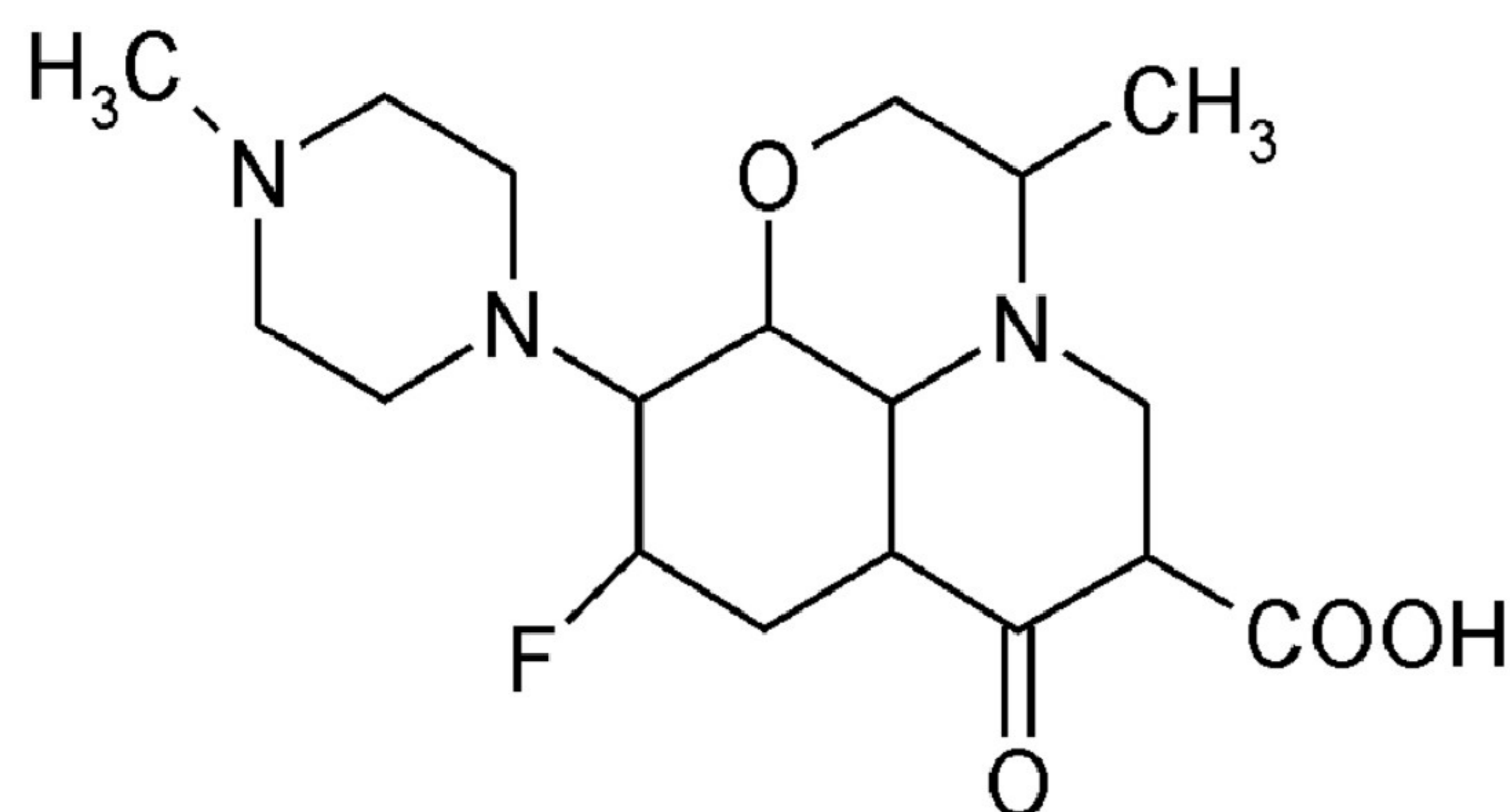
ABSTRACT

A simple rapid and reproducible high performance reverse phase liquid chromatographic method has been developed for the estimation of ofloxacin in bulk drug sample and pharmaceutical dosage form was developed using ss wako sil II. C₁₈, 250 x 4.6mm, 5µm column with mobile phase composition of acetonitrile and phosphate buffer 3:1 (PH 5), flow rate of 1.0 ml/min and UV detection at 295nm linearity was observed over concentration range of 10-50mcg/ml. The accuracy of the proposed method was determined by recovery studies and found to be 95-105%, the proposed method was validated and results conformed with ICH parameters.

KEY WORDS: Ofloxacin, RP - HPLC.

1. INTRODUCTION

Ofloxacin is chemically \pm 9-Fluoro -2,3 dihydro -3-methyl-10-(4-methyl-1-piperazinyl) -7-oxo-7H-pyridol (1,2,3 - di) -1,4 benzoxazine -6 - carboxylic acid.



Which is used for the treatment of urinary tract prostrate and skin or soft tissues infections caused by susceptible bacteria. A tablet formulation contain 200mg of ofloxacin is available (oflox, protec division of cipla Ltd)

A literature revealed that few methods are available like LC method for the estimation of ofloxacin in human plasma and its pharmacokinetic (Macek and Ptacek, 1989; Miyazawa, 1991; Krass and Hirrl, 1986) spectrophotometry (Patel, 1985; Bhatkar and Nagavankar, 1982). The aim of this study is to develop a simple, rapid, precise and accurate reverse phase HPLC method for the determination of ofloxacin in bulk drug samples or in pharmaceutical dosage form.

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2. EXPERIMENTAL

Instrumentation:

Quantitative HPLC was performed on a gradient high pressure liquid chromatograph (Shimadzu HPLC class-VP Series) with two LC-10AT VP Pumps, variable wavelength programmable UV/Vis detector SPD-10A VP system controller (Shimadzu), a disposable guard column LC-18 (Pelliguard)TM, LC-18, 2cm, SS wako sil II RPC-18 column (250x4.6mm, ID particle size 5µm) was used. The HPLC system was equipped with the software class-VP series version 6.01 (Shimadzu). The flow rate of mobile phase was maintained at 1ml/min and detection was carried at 295nm at room temperature.

Chemicals and Reagents:

Water of HPLC grade was collected from a milli-Q system potassium dihydrogen phosphate AR (Ranbaxy) and ortho phosphoric acid AR (Ranbaxy) mobile phase were purchased from the market.

Preparation of Mobile Phase:

A mixture of acetonitrile and 0.02 M potassium dihydrogen phosphate buffer (adjusted to pH 5.0 using orthophosphoric acid) in the ratio of 75 : 25 v/v was filtered through 0.45 µ membrane filter and then used as mobile phase and sonicated for 10 min.

Preparation of Standard Solution:

Standard stock solution of ofloxacin was prepared in mobile phase of concentration 500 µg/ml. The stock solutions were diluted to obtain working standard solution of concentration of 10µg/ml to 50 µg/ml. The resulting solutions were sonicated for 10 min was 100 µl was injected. The retention time for ofloxacin was found to be 2.32 min and for Tinidazole

3.04 min. The Linearity ranger for ofloxacin was found to be 10-50 µg/ml and for tinidazole 10-80 µg/ml.

Preparation of Sample Solution:

Oflox, tablets five in number were weighed. An amount equivalent of 5mg of ofloxacin was transferred into 10ml volumetric flask. The powder was first dissolved with a few drops of mobile phase and the volume then made upto 10 ml with mobile phase. The solution was filtered through membrane filter with pore size of 0.45 micron. The sample stock solution was adequately dilute to obtain ofloxacin concentration of 10 µg/ml. The resulting solution was sonicated for 10 min and 100 µl of the sample was injected. The peak area from the chromatogram was tabulated and the amount of ofloxacin present in the tablet formulation was determined from the linearity curve.

Table-1: Recovery Studies:

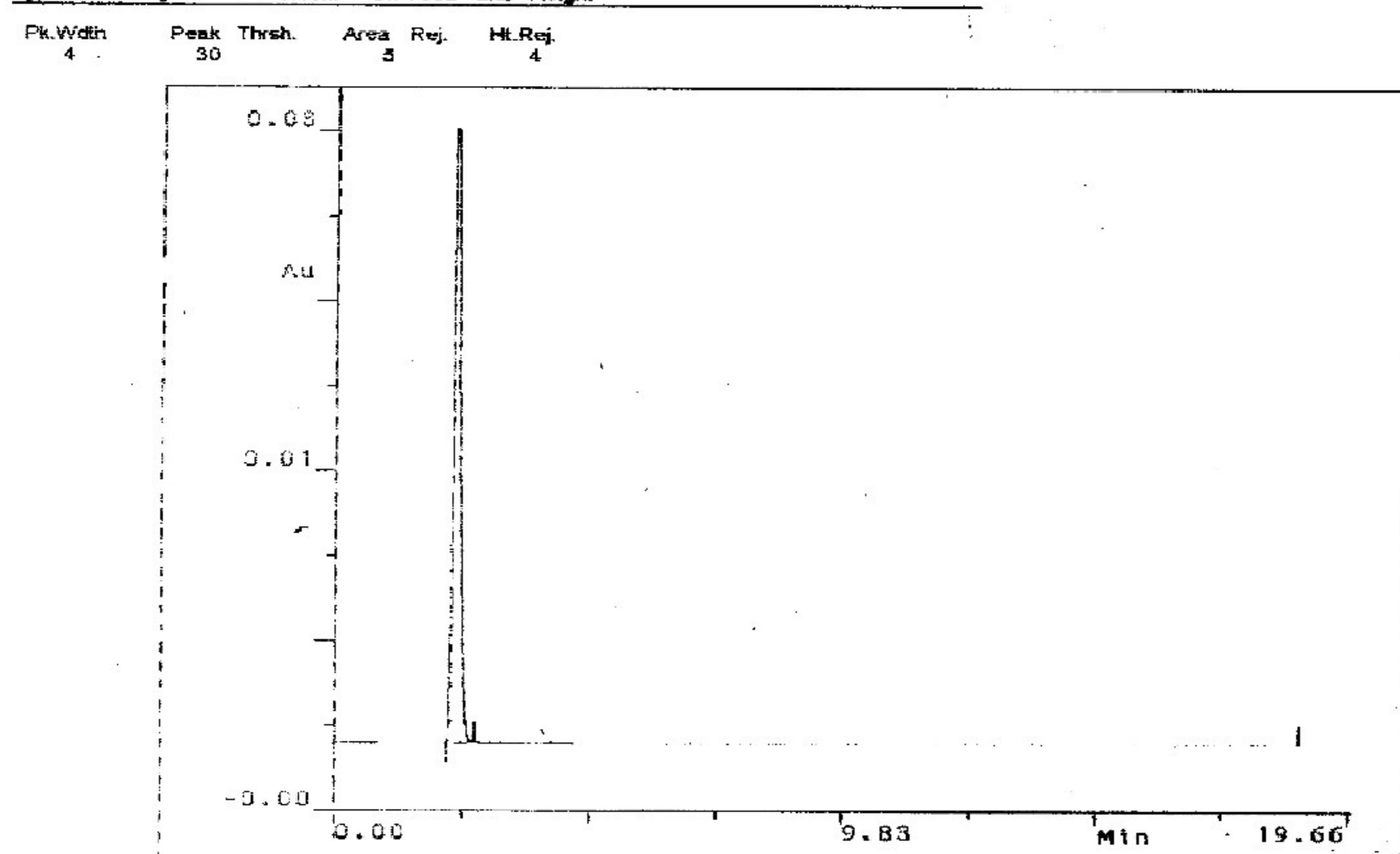
Drug	Amount Added	Amount Recovered	Average Recovery %
Ofloxacin	20	9.5	95
	30	15.9	103
	40	21.0	105

Table-2: System Suitability Parameter:

Parameter	Ofloxacin
Theoretical Plates	3925
Tailing Factor	0.3
Resolution	0.872
Calibration Range	10-50µg/mL

CHROMATOGRAM OF SAMPLE SOLUTION

Sample Name: pipeMet100 Date File: ...OMK02_13.DAT
 Method File: C:\WINDOWS\WINCHROM\FASHANEM\MET
 Detector: UV-VIS System: HPLC
 Date: 04 Jan 2005 Time: 22:06:16
 Run: ch1: 14
 Type of Analysis: Percent On Area and Height



NO.	Retn. Time	Height	Area	Height %	Area %	Peak Type
1	2.32	8543	1977552	100.0000	100.0000	BB
	9e+03		1977552			

3.RESULTS AND DISCUSSION

The proposed method was validated as per ICH parameters. Precision of the proposed HPLC method was carried out by injecting replicate of six of concentration 10 mg/ml and the precision of the proposed HPLC method was found to be 0.4% for ofloxacin. The low RSD values indicate that the proposed method had good precision. The precision of instrument was carried out by injecting replicate of six of concentration 10 mg/ml, which was found to be 0.12 for ofloxacin. Accuracy of the method was also determined. The average recovery of ofloxacin were 95-105%, respectively. The sample recovery in the formulation was in good agreement with the label claim. High percentage recovery showed that the method was free from interferences of the excipients used in the formulations. Ruggedness of the method was determined by carrying out the assay by different analysts on different days. The test results were found to be satisfactory with RSD for set of analysis on the same date being less than 0.8% and RSD between set of analysis on different days being less than 1.6% for ofloxacin. The percentage area on calculation was found to be 101-102% for ofloxacin. This shows that the result are reproducible. Robustness of the method was determined by carrying out the assay during which the mobile phase ratio and pH of mobile phase were altered slightly. The percentage recovery found to be 95-102% for ofloxacin. when mobile phase was alter slightly. System suitability parameters of ofloxacin are given in the Table 2. Assay of the ofloxacin in tablet dosage form was found to be 94.4% of ofloxacin.

4.CONCLUSION

The method was simple and had short runtime of 2.32 min, which makes the method rapid. The results of the study indicate that the proposed HPLC method was simple, precise, highly accurate, specific and less time consuming.

5.ACKNOWLEDGEMENTS

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