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Research Article

HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHIC DETERMINATION OF OFLOXACIN IN PLASMA

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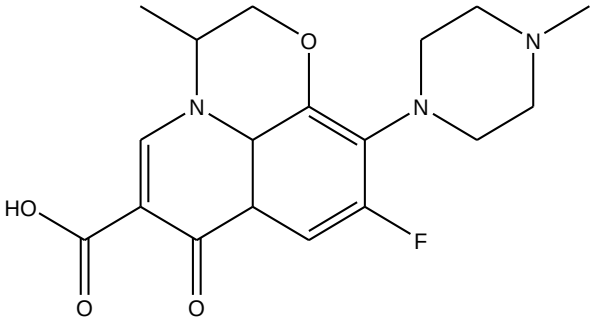
ABSTRACT

A new simple High Performance Thin Layer Chromatographic (HPTLC) method for determination of Ofloxacin in plasma has been developed and validated. A Simple precipitation method was carried out by using Trichloroacetic acid and a known amount of supernatant solution was spotted on precoated silica gel 60 F254 plates using a Camag Linomat IV autosampler.. Detection and quantitation were performed without using an internal standard. The mobile phase selected was n-butanol: Methanol: Ammonia (6:1:3 v/v/v) with UV detection at 295 nm. The calibration curves of Ofloxacin in methanol and in plasma were linear in range 100-600 ng. The limit of quantization for Ofloxacin in human plasma was 100 ng and no interference was found from endogenous compounds. The recovery of Ofloxacin from human plasma using the described precipitation procedure was about 88.1047%. The method provides a direct estimate of the amount of Ofloxacin present in human plasma.

Key words: Ofloxacin, High Performance Thin Layer chromatography, Trichloroacetic acid, Densitometry.

INTRODUCTION

The fluoroquinolones are a series of synthetic antibacterial agents which are used for the treatment of a variety of bacterial infections. They have demonstrated activity against a wide range of Gram-positive and Gram-negative organisms and have proved useful against micro-organisms that are resistant to other antibacterial agents [1, 2]. Ofloxacin a fluorinated carboxyquinolone, is the racemate, (±)-9-fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7oxo-7H-pyrido[1,2,3-de]-1,4-benzoxazine-6-carboxylic acid[3].



Ofloxacin exhibits a marked bactericidal effect by inhibiting DNA gyrase a type II topoisomerase, and topoisomerase IV,which is an enzyme necessary to separate replicated DNA, thereby inhibiting cell division. In vitro studies on bactericidal activity against Mycobacterium tuberculosis have suggested that ofloxacin is likely to be the most useful drug in the early stages of treatment and in preventing the emergence of resistance to other drugs [4]. Its favorable pharmacokinetic features include good oral absorption and lack of metabolism resulting in less drug interactions. There is negligible presystemic metabolism. Maximum serum concentration (Tmax) are usually achieved in under 2h (0.5-1.6h).The plasma elimination half-life is about 6-7h. Ofloxacin exhibits low plasma protein binding (20-25%). Very high concentrations of the drug are achieved in serum and most body fluids, tissues including saliva, nasal secretions, tears, blister fluid, bronchial secretions, and sputum[5].

Literature survey reveals A bio-analytical HPLC method for the simultaneous quantification of ofloxacin, ciprofloxacin and moxifloxacin in human plasma [6]. HPLC and fluorescence detection of ofloxacin in human plasma [7]. Reports are available for Simple and rapid high-performance liquid chromatography method for the determination of ofloxacin concentrations in plasma and urine [8], HPLC seperation & quantitation of ofloxacin enantiomers in rat microsomes [9] and Determination of the antibacterial ofloxacin in human urine and serum samples by solid-phase spectrofluorimetry [10], Simple Extraction and Determination of Ofloxacin in Human Plasma by High – Performance Liquid Chromatography with Fluorescence Detector [11] Stability indicating HPTLC method of analysis of ofloxacin [12],HPLC with UV detection of ofloxacin in plasma [13]. No work has been reported for the determination of the Ofloxacin in plasma by HPTLC method. The proposed method is optimized and validated.

EXPERIMENTAL

Reagents

A reference standard of Ofloxacin was obtained from CIPLA. (Nasik, Maharashtra). N-butanol, methanol, ammonia, (all AR grade) were used for for developing TLC plates. Trichloro acetic acid was used for precipitation.

Preparation of standard solutions

5 mg of Ofloxacin was diluted with methanol final volume of 10 ml in volumetric flask. Standard solutions were obtained by diluting the stock solutions to concentrations ranging from 2 to 120 ug/ml.

Preparation of plasma samples

To 0.5 ml of plasma, 0.5 ml of Ofloxacin working standard (Final concentrations: 100,200,300,400,500,600 ng), 0.5 ml methanol and 0.5 ml of trichoro acetic acid (10 % w/v) were added to a glass tubes. Each sample was vortex mixed for 3 min and centrifuged (2500 rpm for 15min). Unknown plasma samples were prepared in an identical manner except for the addition of Ofloxacin.

Instrumentation and chromatographic conditions

A remi cyclomixer was used for mixing and vortexing the samples. The 20-µl aliquots of the samples were spotted onto TLC plate in the form of bands of width of 6 mm with space between bands of 5 mm, with a 100 µL sample syringe (Hamilton, Bonaduz, Switzerland) on precoated silica gel aluminium plate 60 F254 (10 ×10) with 250 µm thickness (E. MERCK, Darmstadt, Germany) using a CAMAG Linomat 5 sample applicator (Switzerland). The slit dimensions 5 mm × 0.45 mm and scanning speed of 20 mm/sec was employed.

The linear ascending development was carried out in 10 cm × 10 cm twin trough glass chamber (CAMAG, Muttenz, Switzerland) using mobile phase n-butanol: Methanol: Ammonia (6:1:3 v/v/v). The optimized chamber saturation time for mobile phase was 10 min. The length of chromatogram run was 9 cm and development time was approximately 20 min. Tthe drug had an Rf value of 0.40 and

was separated from other components in plasma. TLC plates were dried in a current of air with the help of a hair drier. Densitometric scanning was performed on CAMAG thin layer chromatography scanner at 295nm for all developments operated by WINCATS software version 1.4.2. The source of radiation utilized was deuterium lamp emitting a continuous UV spectrum between 200 to 400 nm.

Quantitation

The quantization of the chromatograms was performed using the ratio of the peak area of the unknown to that of a standard. A representative standard curve of Ofloxacin was obtained by plotting the area under the peak of Ofloxacin against the concentration over the range 100-600ng. The minimum quantifiable concentration of Ofloxacin in human plasma samples was 100 ng/ spot.

Method validation

Linearity of HPTLC method was constructed by analysis of six solutions containing different concentration of Ofloxacin. The linearity of the detector response was tested by spotting standards in triplicate for each concentration ranging between 100 to 600 ng. The data were best fitted by a linear equation mx+ b= y The recovery of Ofloxacin from plasma was determined by comparing peak areas obtained from plasma spiked with Ofloxacin at concentrations of 200,300 and 400 ng/spot with the peak areas obtained from standards. The intra-day precision was completed by analyzing plasma samples in triplicate spiked with Ofloxacin at 200,300 and 400 ng/spot on the same day. The inter-day precision was determined by analyzing 200,300 and 400 ng/spot standards simultaneously with unknown plasma daily for 5 days and also by comparing with the calibration curve. Stability studies for ofloxacin were made on spiked plasma samples having concentrations of 100 and 400 ng. Freeze thaw stability of the spiked quality control samples was determined after three freeze thaw cycles stored at -28 °C ± 5 °C. Short term stability of the spiked quality control samples was determined for a period of 5 hours stored at room temperature.

RESULTS AND DISCUSSION

The peak area was observed to be dependent on the amount of the standard, ofloxacin and a linear relationship (r2=0.996) was found between the peak areas of ofloxacin at various concentrations over the range 100–600 ng (fig 1). The solvent system used for development of the plates produced no interference peaks in the area under the curve, and all other compounds were distinctly separated (fig.2). The RF value of ofloxacin under the conditions used was found to be 0.40±0.05 and spots were quantified at a wavelength of 295 nm. The accuracy, precision and reliability of the procedure were ascertained by adding known concentrations of drug to drug-free plasma and analyzing five samples of each concentration by the method described for precipitation (Table 1). The recovery of ofloxacin in the precipitation procedure from 0.5 ml of plasma was found to be 96.66 % (n=3) given in (Table 2). The intra-day and inter- day precisions are given in (Table 3, 4.) ofloxacin was shown to be stable through three freeze–thaw cycles, during storage at -28°C ± 5°C, and in the short term stability 5hr at room temperature and stock solution stability 5hr and 30 min., the results obtained were precise and accurate [5].

Table 1: Accuracy and precision of a HPTLC method for the determination of Ofloxacin in plasma.

Concentration Added [ng/spot]	Concentration detected (mean±SD, n=5) [ng/spot]	C.V. [A] (%)	Accuracy [B] (%)
200	189.036 ± 14.63	7.74	94.51
300	280.88 ± 11.99	4.27	93.62
400	378.77 ± 15.07	3.98	94.69

[A] **Coefficient of variation.**

[B] **After correction for recovery.**

Table 2: Recovery study data of a HPTLC assay for Ofloxacin in plasma.

Level	Concentration [ng spot^{-1}]	Mean amount [ng spot^{-1}]		Mean recovery [%]	RSD [%]
		Plasma (Mean ± S.D.)	Solution (Mean ± S.D.)		
1	200	181.41 ± 10.113	185.27 ± 11.920	97.91	5.57
2	300	288.68 ± 15.311	301.115 ± 14.798	95.87	5.30
3	400	386.61 ± 16.502	401.85 ± 9.192	96.21	4.27
Average mean recovery [%]			96.66		

Table 3: Intra-day of analysis of Ofloxacin.

Concentration Added (ng/spot)	Mean amounts of drug found (Mean ± S.D.) (ng/spot)	C.V. (%)
200	174.21 ± 7.863	4.51
300	275.51 ± 10.433	3.79
400	376.74 ± 13.625	3.62

Table 4: Inter-day of analysis of Ofloxacin

Concentration Added (ng/spot)	Mean amounts of drug found [ng/spot]			Mean from 3 days ± S.D.) [ng/spot]	RSD [%]
	Day1	Day2	Day3		
200	173.70	163.73	176.75	171.39 ± 6.811	3.97
300	278.27	263.72	266.68	269.55 ± 7.690	2.85
400	376.73	380.51	397.19	384.81 ± 10.886	2.83

Table 5: Stability study data of HPTLC assay for ofloxacin in plasma.

Procedure	Concentration of ofloxacin 100 [ng spot^{-1}]		400 [ng spot^{-1}]	
	Mean	R.S.D. %	Mean	R.S.D. %
Freeze and thaw(n=5)	91.84	6.49	93.58	4.47
Short term(n=3)	92.71	4.90	93.85	3.23
Stock solution(n=3)	94.74	2.65	91.54	2.82

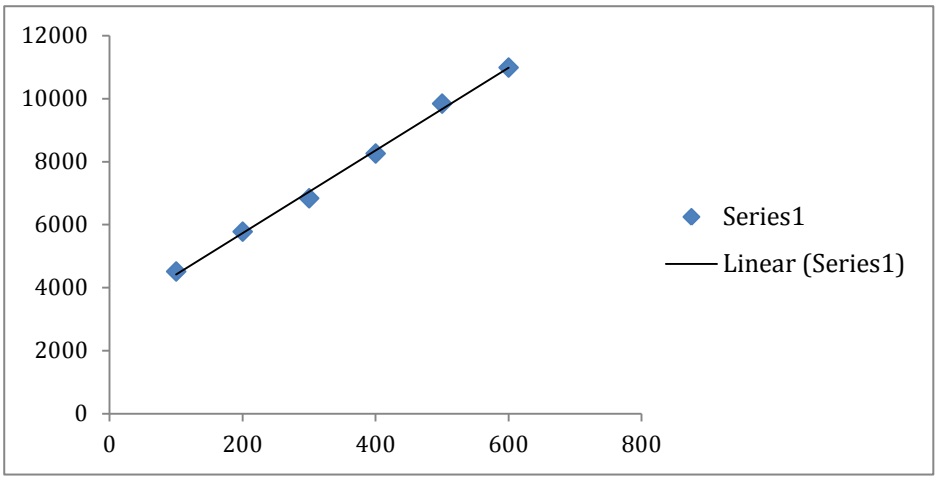


Fig. 1: Graph of linearity of ofloxacin in plasma.

a **b**

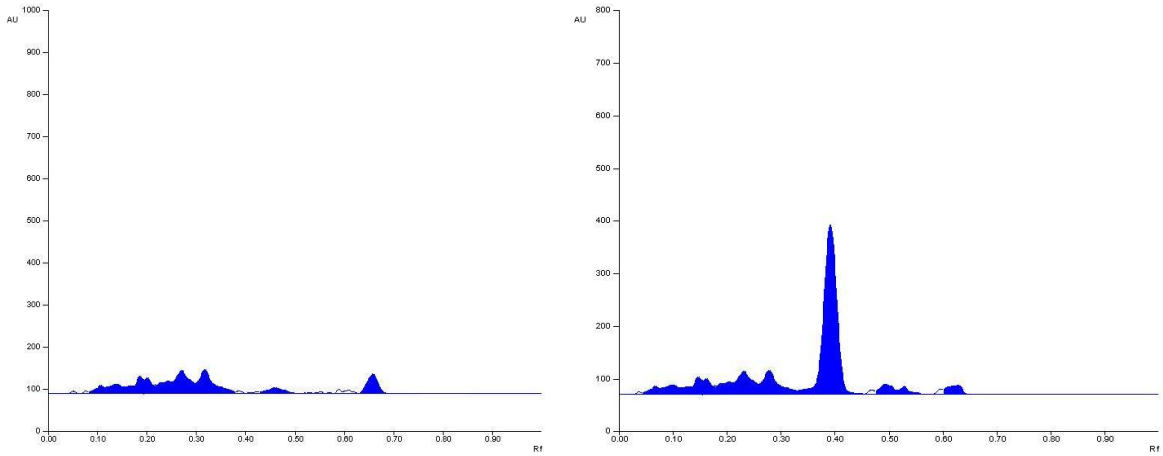


Fig. 2:- Typical Chromatogram of (a) Blank human plasma (b) Ofloxacin extracted from human plasma.

CONCLUSIONS

This HPTLC method for quantification of ofloxacin in human plasma is accurate, precise, rapid, and selective. It is a simple, practical, and economical alternative for studies of the bioavailability, bioequivalence, and pharmacokinetics of this drug in human plasma.The advantages of the method are that it uses a small amount of sample (20 µL), the volume of mobile phase used is approximately 10 mL per plate, detection of the plates takes approximately 7 min.

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