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Determination of Ofloxacin in Plasma by HPLC with UV Detection

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Abstract: A simple, sensitive isocratic method for detection and quantification of ofloxacin in plasma has been developed. The assay consisted of reversed HPLC with UV detection. Separation was achieved on a C18 column. The detection limit was 10 ng mL^{-1} in plasma. The validation data has been studied. This method provides sufficient sensitivity for pharmacokinetic studies and dose control in veterinary as well as in human medicine.

Key words: Determination, ofloxacin, plasma, UV-detection

INTRODUCTION

Ofloxacin (OFX) and some other fluroquinolones are gaining widespread acceptance in veterinary and human medicine, because of its broad spectrum activity against gram positive and negative, aerobes, mycoplasma, rickettsia and against multidrug-resistant microorganisms[1]. *In vitro* studies had shown that OFX has early bactericidal activity against *Mycobacterium tuberculosis*[2]. OFX is known to induce arthropathic lesions in young and growing animals, therefore, dosage regimens of fluroquinolones should not be extrapolated even from the adult of the same species[3]. In the other hand uncontrolled or boundless usage can lead to resistant microorganisms in human and veterinary infections. OFX has good antibacterial activity at considerably low plasma/tissue concentration. The Minimum Inhibitory Concentration (MIC_{90}) value of OFX against some of the common veterinary infections is set between 0.06 and 0.5 μg mL^{-1}[4]. Monitoring of OFX concentration in body fluids may be valuable to adjust the drug dosage to achieve MIC and protection from side effect such as arthropathic lesions. Several papers have described determination of OFX in biological fluid by HPLC with UV or fluorescence detection[5,6] or by microbiological methods[1]. Poor reproducibility and accuracy for the last method have been reported[7]. Currently, HPLC is the analytical method of choice for measuring fluroquinolones[8].

This study describes an isocratic HPLC method using UV detection, which provides adequate sensitivity for routine use and diminishing the time of sampling and chromatographic analysis.

MATERIALS AND METHODS

Standard and reagents: Ofloxacin and ciprofloxacin (Fig. 1) were kindly provided by Lorestan and Toliddaru Pharmaceutical Companies (Tehran, Iran), respectively. Methanol was HPLC grade and other chemical compounds were analytical grade, which purchased from Merck (Darmstadt, Germany). Double distilled water was used throughout the study.

Apparatus and chromatographic condition: The chromatographic apparatus were a waters HPLC system with a 510 pump, Rheodyne injector (7725i) and a waters 486 UV detector adjusted at 294 nm connected to a 746 data module integrator. Mobile phase consisted of KH_2PO_4 0.03 M solution and methanol (30:70) which was adjusted to pH=3.1 with formic acid. The stationary phase was a Novapak reversed phase column (C_{18}, 150×4 mm i.d., 4 μm). The mobile phase was prepared daily and delivered at a flow rate of 1 mL min^{-1}.

Solutions: A stock solution of OFX (50 μg mL^{-1}) and ciprofloxacin (IS) were prepared in methanol. Working standard solutions were prepared daily by diluting the stock solution with 1% HCl solution. All the stock solutions were stable at least four weeks when stored at -15°C.

Sample preparation: To 1 mL human plasma in a 10 mL glass screw-capped tube 100 μL of internal standard solution (10 μg mL^{-1}) and 100 μL of OFX working standard solution were added. The tubes were vortex-mixed for 30 sec and then 0.5 mL of zinc sulfate solution

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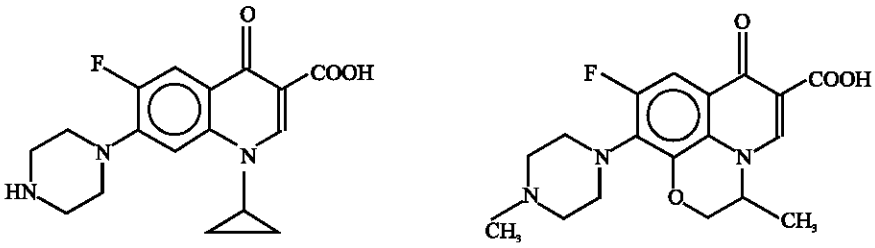

Fig. 1: Structure of ofloxacin and ciprofloxacin

(0.7 M) and 0.1 mL of 1 N sodium hydroxide solution were added. After mixing for 1 min and centrifuging at 2000 g for 10 min, supernatant was filtered by 0.45 μm sample filter (Millipore filter). A volume of 25 μL filtered supernatant was injected into HPLC column.

Calibration curve and recovery: Working standard solutions were added to thawed plasma each day to yield concentrations ranging from 0.025 to 2.5 μg mL^{-1}. Calibration curve for plasma were obtained by plotting peak area ratio of OFX and internal standard against OFX concentration. The recovery was calculated by comparing peak area ratio obtained from plasma samples (QC samples) with area ratio resulted after injecting standard solutions at the same theoretical concentration.

RESULTS

Under the chromatographic condition described OFX and IS peaks were well resolved with retention time of 5.17 and 6.67 min, respectively (Fig. 2). No interfering peak was observed at retention times of OFX and IS. The calibration curve for determination of ofloxacin in plasma was linear over range of 0.025-2.5 μg mL^{-1} and the corresponding regression equation was: Y = 0.00132(X)-0.0047, where, Y is the peak area ratio of OFX to internal standard and X is the ofloxacin concentration. The reproducibility of the method was determined by repeating three replicate plasma samples on each three-separate day. According to the intra-day and inter-day data good precision and accuracy were observed over the entire concentration range. The CV values for within run and between run variability were less than 10% for all seven selected concentrations (Table 1). The Limit of Quantification (LOQ) of the method, defined as the minimum concentration that could be measured with CV<10% was found to be 25 ng mL^{-1}. The Limit of Detection (LOD) with a S/N ratio of 3:1 was 10 ng mL^{-1} in

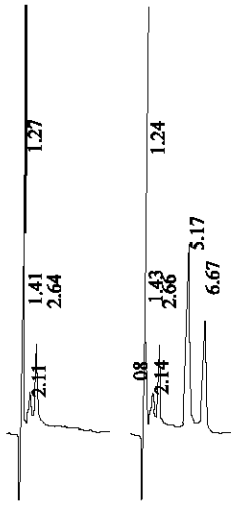

Fig. 2: Representative chromatograms of plasma blank (A) and plasma containing 1 μg L^{-1} ofloxacin (5.17) and 1 μg mL^{-1} internal standard (6.67) (B)

Table 1: Intra-day and inter-day data for spike ofloxacin in plasma

Added concentration (ng mL^{-1})	Determined concentrations (mean±SD, ng mL^{-1})	CV (%)
Intra-day (n=3)		
25	25.30±2.21	8.73
50	52.04±4.53	8.64
100	107.04±5.93	5.53
250	253.06±6.73	2.66
500	489.44±9.56	1.95
1000	978.25±16.43	1.68
2500	2533.65±25.89	1.02
Inter-day (n=3)		
25	24.60±2.45	9.95
50	49.08±4.69	9.55
100	103.06±8.12	7.88
250	254.18±19.55	7.69
500	505.44±30.79	6.09
1000	993.69±38.45	3.86
2500	2519.15±59.65	2.37

plasma. The recovery of drug determined by comparing the peak area for OFX from spiked plasma and a standard solution of OFX in 1% HCl solution with the same

Table 2: Recovery (%) of ofloxacin at three concentrations

Added concentration (ng mL^{-1})	Determined concentration (mean±SD, ng mL^{-1})	CV (%)	Recovery (%)
250	201.60±5.5	2.73	80.64
500	435.50±8.96	2.06	87.10
1000	895.22±18.33	2.05	89.52

concentration. Three-concentration range, i.e. 0.25, 0.5 and 1 μg mL^{-1} were investigated for recovery studies. The mean of recovery from the plasma for OFX was 85.75% (Table 2).

Stability of QC samples was evaluated at -15°C for 3 months, at room temperature for 24 h and after three freeze-thaw cycles. In all of the conditions, samples preserved their potency (>95%) during the mentioned period.

DISCUSSION

The present technique is able to measure concentration of OFX in order to monitoring of drug concentration in body fluid, determination of drug level in plasma for dose regulation and bioavailability studies. In previous published methods the assay was achieved by long liquid-liquid extraction procedures[8-11]. A survey of these papers revealed that some of them are quite complex and lengthy[10,11]. In the present method no extraction needed because involving simple step like deporteination /dilution with adding zinc sulfate. A clean chromatogram was resulted using zinc sulfate which is suitable deproteination agent for OFX and probably other fluroquinolones. Although short sampling procedure is reflected by Chandra and Hemanth method, which used perchloric acid as deporteination agent[5]. In the other hand, in a majority of published method such as Chandra, expensive florescent detector was used, a facility that is not commonly available in every laboratory. Because these methods are labor intensive, requiring extensive sample clean-up procedures, they cannot be used for routine screening of numerous samples. Present method is adequately sensitive for routine use with UV detection. If necessary, the sensitivity of the method can be increased by raising the injection volume. Short elution time and easy sampling method will able us to analyze the large amount samples in short period.

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