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Simultaneous determination of moxifloxacin and ofloxacin in physiological fluids using high performance liquid chromatography with ultraviolet detection

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ABSTRACT

A novel, sensitive and validated RP-HPLC–UV method was developed for simultaneous determination of moxifloxacin and ofloxacin using timolol maleate as internal standard in physiological fluids. Different experimental parameters were optimized and validated according to international guidelines. Complete separation of the analytes was achieved with Kromasil 100-5C18 analytical column (250 mm × 4.6 mm × 5 μm), methanol and 0.05% trifloroacetic acid (TFA) (38:62 v/v) were used as mobile phase, pumped at flow rate of 1.1 ml/min in isocratic phase, column oven temperature maintained at 45 °C and detection wavelength of 290 nm. Protein precipitation method was applied to extract the drugs from human plasma and bovine aqueous humor samples using methanol as precipitating solvent. This method is linear in concentration range of 0.018–100 μg/ml for moxifloxacin and 0.014–20 μg/ml for ofloxacin. The recoveries of the method were 97.52 and 97.39% in human plasma for MX and OFN respectively, while in aqueous humor 94.48% for MX. The LOD values in plasma were found to be 10.0 and 8.00 ng/ml for MX and OFN respectively, while their respective LOQ values were 18.0 and 14 ng/ml. In aqueous humor the LOD and LOQ for MX were 16.0 and 24 ng/ml respectively. In future, this method will be used to study the pharmacokinetic profile of moxifloxacin and ofloxacin in biological fluids and pharmaceutical products.

1. Introduction

Moxifloxacin (MX), (Fig. 1), is fourth generation floroquinolone. Chemically, it is 1-cyclopropyl-6-fluoro-8-methoxy-7-[(4aS,7aS)-octahydro-6H-pyrrolo [3,4-b]pyridin-6-yl]-4-oxo-1,4-dihydro-3-quinolinecarboxylic acid [1]. MX is active against a wide range of microbes and has efficacy against both streptococci and staphylococci. It also has a reasonable activity against gram negative ocular pathogens [2]. MX has much better penetration into the ocular tissues than any other fluoroquinolones, the mean aqueous humor concentration of moxifloxacin (0.67 ± 0.50 μg/ml) is higher than besifloxacin (0.13 μg/ml ± 0.58) and gatifloxacin (0.13 ± 0.08 μg/ml) [3,4]. MX is therefore frequently used to treat and prevent various bacterial keratitis, conjuctivitis and endophthalmitis [5].

Ofloxacin (OFN), (Fig. 1), is a broad spectrum second generation floroquinolone, prescribed to treat a number of bacterial infections. Chemically, OFN is (±)-9-fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7H-pyrido[1,2,3-de]-1,4-benzoxazine-6-carboxylic acid [6]. Like other floroquinolones, ofloxacin is also efficacious against both Gram positive and Gram negative bacteria, and so approved for the treatment of gastrointestinal, respiratory and urinary tract infections [7]. OFN is also used to treat different ocular infections [8].

Fluoroquinolones attach to protein/DNA complex involving DNA gyrase and topoisomerase IV, thus interferes with the replication of DNA into daughter cells [9]. Various spectrophotometric [10,11] and HPLC techniques [1,12–17] have been reported for the analysis of moxifloxacin alone and/or in combination with other drugs in various pharmaceutical products, human plasma and aqueous humor. Similarly various analytical methods are also available for the determination and estimation of ofloxacin in plasma, blood, pharmaceutical products and aqueous humor using spectrophotometric [18] and HPLC techniques [8,19–25].

Simultaneous determination of MX and OFX in physiological fluids has been reported using HPLC coupled with fluorescent detector [26,27] and UPLC–MS/MS detection [28,29]. These equipments are generally expensive and also the complex techniques

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involved would add up to cost of the analysis. Using these expensive techniques for routine clinical/pharmaceutical analysis of these fluoroquinolones would be not cost-effective. In most of the developing countries, research laboratories, pharmaceutical industries and clinical settings generally use HPLC coupled with UV detector. It is thus required to develop a validated HPLC–UV method for the simultaneous determination of both these drugs in human plasma and aqueous humor samples. To our knowledge so far, no simultaneous method is reported for MX and OFX using HPLC–UV detection.

The objective of this proposed method is to analyze the aforementioned drugs simultaneously using timolol maleate as internal standard (IS). This HPLC–UV method is simple, fast, reliable, sensitive and cost-effective for the analysis of moxifloxacin and ofloxacin in physiological fluids and pharmaceutical products. This method is validated and optimized for various experimental conditions such as mobile phase composition, flow rate, internal standard, column oven temperature, sample size and detector wavelength [30]. This method will be used to study the pharmacokinetic profile of MX and OFN and for the analysis of both these drugs in pharmaceutical dosage forms. Along with this, we can also quantify MX in aqueous humor using this method.

2. Materials and methods

2.1. Chemicals and reagents

Moxifloxacin (MX) (purity 99.9%) (Dr. Raza Pharma, Pvt., Ltd., Peshawar) and Ofloxacin (OFX) (purity 99.9%) (Saydon Pharmaceutical Industries Pvt., Ltd., Peshawar) and internal standerd Timolol maleate (purity 97.5%) (Schazo Pharma. Pvt., Ltd., Lahore), were kindly supplied by these local pharmaceuticals. HPLC grade methanol, acetonitrile, dichloromethane, ethanol and triethylamine were acquired from Sigma–Aldrich (Oslo, Norway) while trifloroacetic acid (TFA) and diethyl ether were purchased from Scharlau chemie (Spain). Water purification was carried out with Millipore ultra-pure water system (Milford, USA).

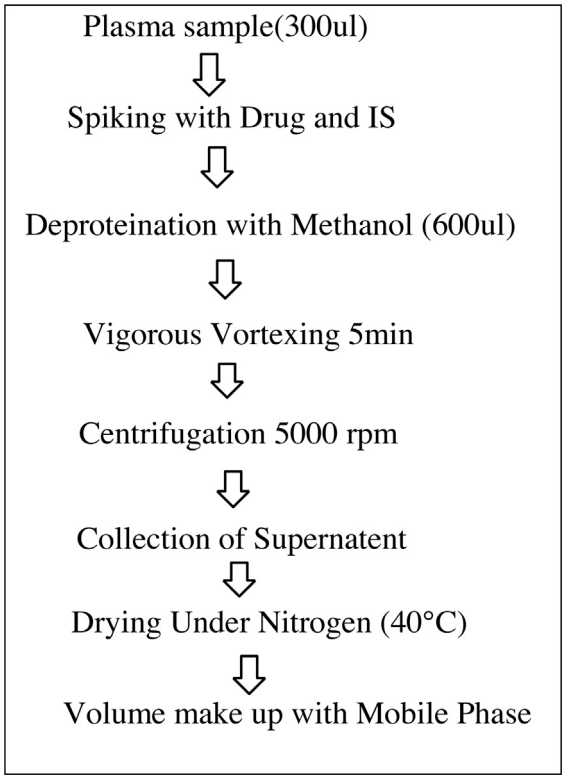
2.2. Apparatus and chromatographic conditions

The chromatographic apparatus consisted of Perkin-Elmer HPLC system (series 200; Norwalk, USA), equipped with a pump, online vacuum degasser, manual injector (Rheodyne 7725i), column oven, and UV–vis detector. The acquired data was evaluated using Perkin-Elmer Totalchrom chromatography work-station (version 6.3.1), coupled with HPLC system through network chromatography interface (NCI) 900. The analysis was performed using Kromasil 100-5C18 analytical column (250 mm × 4.6 mm × 5 μm), which was protected by Perkin Elmer pre-column guard cartridge C18 (30 mm × 4.6 mm, 10 μm; Norwalk, USA). Methanol and 0.05% trifloroacetic acid (TFA), (38:62 v/v) were used as mobile phase, which was pumped at flow rate of 1.1 ml/min in isocratic phase, column oven temperature maintained at 45 °C and detection was performed at 290 nm. Sample volume of 50 μl was injected to the HPLC system manually.

2.3. Preparation of standard solutions

Stock solutions of the working standards (moxifloxacin, ofloxacin), each of 100 μg/ml were prepared in methanol and then stored in amber colored containers at −20 °C. These stock solutions were then further diluted with the mobile phase (methanol and 0.05% TFA) to obtain various dilutions in concentration range of 0.010–100 μg/ml for moxifloxacin and 0.010–20 μg/ml for ofloxacin while the IS concentration was kept constant at 1 μg/ml in each dilution.

Table 1
Scheme for sample preparation.



2.4. Preparation of samples

2.4.1. Spiked plasma

Blood samples were collected in EDTA tubes from healthy human volunteers at Department of Pharmacy, University of Peshawar, after obtaining informed consent. Blood samples after collection were centrifuged at 5000 rpm for 5 min at 4 °C. The plasma was separated and stored at −20 °C. At the time of sample preparation, the plasma was thawed at room temperature and spiked with moxifloxacin and ofloxacin standard solutions to get various dilutions in range of 0.018–100 μg/ml for moxifloxacin and 0.014–20 μg/ml for ofloxacin. Precipitation method was applied for the sample preparation and methanol was used as protein precipitant. Samples were prepared in accordance with scheme set in Table 1, and 50 μl reconstituted sample was then injected to the HPLC system.

2.4.2. Aqueous humor

The aqueous humor was collected from bovine eyes, stored in tightly closed containers at −20 °C till the time of analysis. Samples for injection were prepared according to scheme set in Table 1.

2.4.3. Extraction procedure

Protein precipitation method was applied to extract moxifloxacin and ofloxacin from plasma and aqueous humor samples. Moxifloxacin and ofloxacin in their respective concentrations and IS solution (1 μg/ml) were added to 300 μl of plasma/aqueous humor samples. This mixture was then vortexed for 2 min. Then 600 μl of methanol was added to both the plasma and aqueous humor samples and vortexed vigorously for 5 min to precipitate proteins. All the samples were then centrifuged at 8000 rpm for 5 min at 0 °C. The supernatant obtained was then transferred to another eppendorf tube and dried under nitrogen at 40 °C. After drying the residue was