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Rapid liquid chromatographic assay of ciprofloxacin in human aqueous humor

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Abstract

A simple, selective and sensitive method has been developed to determine ciprofloxacin in human aqueous humor. Separation of ciprofloxacin was carried out with pipemidic acid as internal standard using a Novapak C_{18} reversed-phase cartridge column (100 × 8 mm i.d., particle size 4 μm) and a mobile phase consisting of methanol–acetonitrile–citric acid (0.4 M) (3:1:10, v/v/v) at a flow rate of 1 ml min^{-1}. The column effluent was monitored with fluorescence detection at 278 nm (excitation) and 450 nm (emission) after direct injection. The retention times were 4.88 min for pipemidic acid and 7.52 min for ciprofloxacin. The within-day and day-to-day reproducibilities were less than 7% for ciprofloxacin at 0.1 and 1 μg ml^{-1} ($n = 6$). The mean recovery from aqueous humor was found to be 101.37 ± 6.7% for ciprofloxacin at 0.1 μg ml^{-1} ($n = 6$) and the detection limit corresponding to a signal-to-noise ratio of 2.5:1 was 250 pg ml^{-1}. The method was shown to be suitable for determining ciprofloxacin levels in human aqueous humor samples.

Keywords: Ciprofloxacin; Aqueous humor; High performance liquid chromatography

1. Introduction

Ciprofloxacin (Fig. 1) is a fluoroquinolone antibiotic with a broad spectrum of activity used in the prophylaxis and treatment of a wide range of bacterial infections [1]. Recently, there has been interest in a single drug substituting combinations for treatment of ocular infections. For example, in a prospective clinical study of bacterial keratitis, a single-drug topical treatment with 0.3% ciprofloxacin yielded a 91.9% clinical success rate [2]. Although these data are extremely encouraging, such a treatment regimen has several

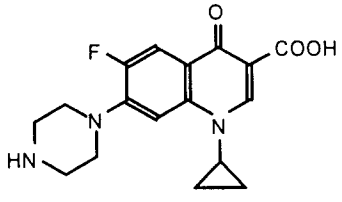

Fig. 1. Structure of ciprofloxacin.

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features that make it sub-optimal. Thus, to correlate ciprofloxacin levels related to dosing regimen in the human aqueous humor after topical administration, it is necessary to assess the efficacy of single-drug treatment.

Ciprofloxacin in body fluids, such as urine, plasma, sputum and saliva, has been determined by HPLC with ultraviolet or fluorescence detection [3–10] or by microbiological methods [11,12]. Highly specific and sensitive HPLC methods are preferable to more time-consuming and less specific microbiological methods. In the latter, active metabolites or co-administered antibiotics can interfere to give higher values for the drug. Microbiological methods also suffer from poor reproducibility and accuracy [11,12]. Although, most of the published LC methods for human specimens were carried out using a reversed-phase column with UV detection, a high level of background UV absorption and interfering peaks usually occurred.

To the authors' knowledge, no method has so far been reported for the determination of ciprofloxacin in human aqueous humor. This paper describes a HPLC method for the determination of ciprofloxacin in human aqueous humor after direct injection. HPLC with fluorescence detection confers high sensitivity, specificity and rapidity on the method.

2. Materials and methods

2.1. Chemicals and reagents

HPLC-grade methanol and acetonitrile were purchased from Baker (Phillipsburg, NJ, USA) and analytical-grade citric acid from Sigma (St. Louis, MO, USA). Pipemidic acid was purchased from Sigma and ciprofloxacin was kindly donated by Bayer (Istanbul, Turkey). Stock solutions of ciprofloxacin (1 mg ml^{-1}) and pipemidic acid (1 mg ml^{-1}) were prepared in HCl (0.01 N) and in NaOH (0.2 M), respectively. Standard solutions of ciprofloxacin containing pipemidic acid (1 $\mu g\ ml^{-1}$) as internal standard were prepared by diluting the stock solutions to final ciprofloxacin concentrations of 0.05, 0.1, 0.2, 0.5, 1, 2, 5 and 10 $\mu g\ ml^{-1}$ with water daily. Aqueous humor standard containing 0.1 $\mu g\ ml^{-1}$ ciprofloxacin and 1 $\mu g\ ml^{-1}$ pipemidic acid was prepared in the same way as the samples. Stock solutions and samples were stored at −25°C until analysis and all solutions were protected from light because of the light sensitivity of pipemidic acid.

To determine the concentration of ciprofloxacin in samples, a calibration curve of the peak-area ratio of ciprofloxacin to pipemidic acid versus the concentration of ciprofloxacin in distilled water was plotted. A calibration curve prepared in drug-free aqueous humor was not possible because of the lack of adequate blank aqueous humor.

2.2. Chromatography

The LC system consisted of a Model PU-980 HPLC pump (Jasco, Tokyo, Japan), a Model 7125 injector (Rheodyne, Cotati, CA, USA), a Model 486 UV/Vis detector, a Model 470 fluorescence detector and a Model 746 data module (Waters, Milford, MA, USA). The separation was performed on a Novapak C_{18} cartridge (100 × 8 mm i.d., particle size 4 μm) (Waters) compressed in a Radial-Pak cartridge holder (RCM 8 × 10, Waters) in conjunction with a pre-column module (Guard-Pak, Waters) containing a Novapak C_{18} insert. The mobile phase consisted of methanol–acetonitrile–citric acid (0.4 M) (3:1:10, v/v/v) at a flow rate of 1 ml min^{-1} at ambient temperature. The UV detector was set to 275 nm, and the fluorescence excitation and emission wavelengths were 278 and 450 nm, respectively.

2.3. Sample preparation

To a glass screw-capped tube, 50 μl of aqueous humor sample, 350 μl of distilled water and pipemidic acid (1 $\mu g\ ml^{-1}$) as an internal standard were added. The tube was vortex mixed for 30 s and 20 μl of solution were injected on to the column. All analyses were performed in duplicate.

2.4. Recovery

The recovery of ciprofloxacin was determined by comparison of an aqueous humor standard

with a standard solution containing the same concentration in distilled water. The recovery was expressed as the mean of six replicates (mean ± SD).

2.5. Collection of aqueous humor samples from humans

Patients with an age range from 45 to 80 years were administered topical ciprofloxacin drops (0.3%) (Ciloxan; Alcon Laboratories, Ft. Worth, TX, USA) before their scheduled lens extraction surgery. None of the patients had any ocular pathology other than cataracts. Informed consent was given by all patients.

Two drug instillation schedules were used so that ciprofloxacin was instilled at one drop every 15 min for five doses followed by one drop every 30 min for three doses in the first group ($n = 6$) and one drop every 15 min for five doses followed by one drop every 30 min for nine doses in the second group ($n = 6$). Instilling was done by a nurse to ensure compliance. Aqueous humor samples were drawn by paracentesis 30 min after the last dose.

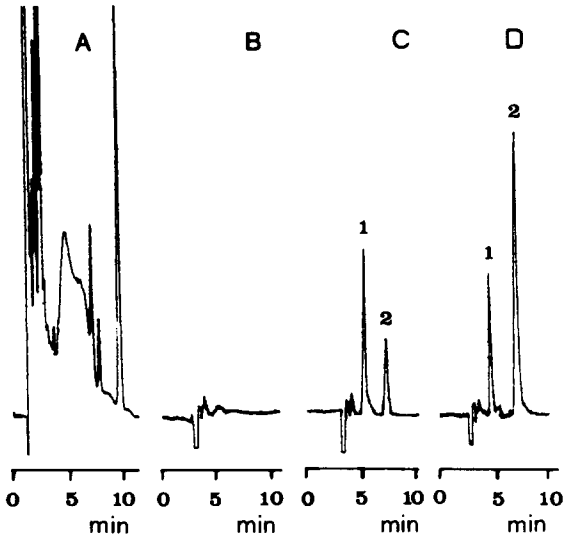

Fig. 2. Representative chromatograms of (A, B) blank aqueous humor, (C) spiked aqueous humor containing 0.1 $\mu g\,ml^{-1}$ ciprofloxacin and (D) a patient's aqueous humor containing 1.54 $\mu g\,ml^{-1}$ of ciprofloxacin. Chromatograms were monitored with (A) UV detection at 275 nm, AUFS = 0.02, and (B–D) fluorescence detection at 278 nm (excitation), 450 nm (emission). The signal gain of the fluorescence detector was constant at 10. Sample preparation and chromatographic conditions were as described in the text. Peaks: 1, pipemidic acid (internal standard); 2, ciprofloxacin.

3. Results and discussion

The selection of a suitable detection procedure is important for optimizing the chromatographic conditions. For ciprofloxacin, essentially two types of detection can be used: UV spectrophotometry and fluorescence. Figs. 2A and B show the simultaneously recorded UV and fluorescence chromatograms obtained after direct injection of a drug-free aqueous humor sample. A high level of background UV absorption and interfering peaks occurred when a UV detector was used. However, the interfering peaks disappeared with fluorescence detection. Because of this observation, fluorescence detection was used in the study. Pipemidic acid and ciprofloxacin appeared as well resolved peaks with retention times of 4.88 and 7.52 min, respectively (Figs. 2C and D).

The mean recovery from aqueous humor was found to be 101.37 ± 6.7% for ciprofloxacin at 0.1 $\mu g\,ml^{-1}$ ($n = 6$). The calibration curve for ciprofloxacin was linear over the concentration range 0.05–10 $\mu g\,ml^{-1}$ ($r = 0.9998$). In order to assess the accuracy of the method, the reproducibility for both day-to-day and within-day variations was determined by analysis of two replicate samples containing 0.1 and 1 $\mu g\,ml^{-1}$ of ciprofloxacin. The relative standard deviations ($n = 6$) were less than 7% in each case. The detection limit corresponding to a signal-to-noise ratio of 2.5:1 was 250 $pg\,ml^{-1}$ for ciprofloxacin.

Ciprofloxacin levels in aqueous humor after repetitive topical administration of 0.3% ciprofloxacin are given in Table 1. The concentrations were between 0.49 and 4.94 $\mu g\,ml^{-1}$, indicating almost a 10-fold difference between the highest and the lowest concentrations. The mean aqueous humor ciprofloxacin level of the first group in which ciprofloxacin was instilled for a period of

Table 1
Aqueous humor level of ciprofloxacin for 12 subjects following repetitive instillation of 0.3% ciprofloxacin eyedrops

Subject No.	Instillation period (h) [a]		Concentration of ciprofloxacin (μg/ml)
1	3		0.49
2	3		0.52
3	3		1.61
4	3		0.49
5	3		1.29
6	3		0.35
		Mean	0.79
		SD	0.48
7	6		1.54
8	6		4.94
9	6		2.04
10	6		4.43
11	6		1.83
12	6		1.26
		Mean	2.67
		SD	1.45

[a] For subjects 1–6, ciprofloxacin was instilled one drop every 15 min for five doses followed by one drop every 30 min for three doses; for subjects 7–12, ciprofloxacin was instilled one drop every 15 min for five doses followed by one drop every 30 min for nine doses.

3 h was found to be $0.73 \pm 0.54\ \mu g\ ml^{-1}$. When instillation period was extended to 6 h (the second group), the level increased to $2.67 \pm 1.45\ \mu g\ ml^{-1}$. These concentrations are greater than the MICs against most aerobic bacteria [13]. The results demonstrate the usefulness of the present method for the determination of ciprofloxacin in aqueous humor samples.

In conclusion, a fast, sensitive and reliable method was developed for the determination of ciprofloxacin in human aqueous humor samples to permit correlation studies between dosage regimen and drug levels in human aqueous humor as an indicator of the clinical efficacy of ciprofloxacin.

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