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DETERMINATION OF CIPROFLOXACIN IN HUMAN GINGIVAL CREVICULAR FLUID BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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SUMMARY

A simple and selective high-performance liquid chromatographic (HPLC) method has been developed for determination of ciprofloxacin in human gingival crevicular fluid (GCF). GCF samples were collected with paper strips. Ciprofloxacin was extracted from the pooled strips with methanol–water, 50:50 (*v*/*v*), and separated on a cartridge column (Radial-pak C_{18}, 100 mm × 8 mm, 10 μm) with acetonitrile–sodium dihydrogen phosphate, 2:8 (*v*/*v*), as mobile phase at a flow rate of 2 mL min^{-1}. The effluent was monitored with a fluorescence detector at 280 nm (excitation) and 455 nm (emission). The retention times of ciprofloxacin and internal standard quinine sulphate were 4.55 and 13.25 min, respectively. The within-day and day-to-day precision were less than 9% for ciprofloxacin at 0.05, 0.1 and 0.4 μg mL^{-1} ($n = 6$), the within-day and day-to-day accuracy values were in the range 96.33–102.67% for ciprofloxacin at the concentrations given above and the detection limit corresponding to a signal-to-noise ratio of 3:1 was 3 ng mL^{-1}. This method was suitable and selective for determination of ciprofloxacin levels in human GCF.

INTRODUCTION

Gingival crevicular fluid (GCF) plays an important role in the oral defence mechanism and drugs excreted in GCF may be used advantageously for treatment of periodontal diseases [1]. Systemic antibiotics have been used in the therapy of the periodontitis for a long time [2]. Previous studies suggested that GCF levels of antibiotics can be used as an indicator of treatment response [3]. Ciprofloxacin is a widely prescribed fluoroqui-

nolone in infections. It has low MIC value for many oral periodontal pathogens, for example its MIC_{90} value is 0.01 μg mL^{-1} for *Actinobacilus actinomycetemcomitans* (A.a) [4]. Because of the efficient distribution of ciprofloxacin into the tissues after oral administration, it is expected that ciprofloxacin penetrates the GCF [5]. Knowing the level of ciprofloxacin in GCF may be useful in the management of periodontal infections. Ciprofloxacin levels in biological specimens such as plasma, urine, and aqueous humour have previously been determined by high-performance liquid chromatography (HPLC) with fluorescence detection [6–10]. In a recent study, gingival fluid ciprofloxacin levels were measured by HPLC [11], but detailed experimental methods for extraction of ciprofloxacin from GCF and for validation of ciprofloxacin determination in GCF have not been reported. In this study our objective was to establish an HPLC method for the determination of ciprofloxacin in human GCF.

EXPERIMENTAL

Chemicals

HPLC-grade acetonitrile was purchased from Baker (Phillipsburg, NJ, USA) and analytical-grade phosphoric acid from Merck (Darmstadt, Germany). Ciprofloxacin was obtained from Bayer (Istanbul, Turkey). Quinine sulphate and sodium dihydrogen phosphate were purchased from Sigma (St Louis, MO, USA).

Chromatography

Chromatography was performed with a Varian (Walnut Creek, CA, USA) model 9002 HPLC pump, a Varian model 9100 autosampler, a Waters (Milford, MA, USA) model 470 fluorescence detector and a Waters model 746 data module. Compounds were separated on a Waters Radialpak, C_{18} cartridge column (100 mm × 8 mm i.d., particle size 10 μm) and precolumn (C_{18}). The mobile phase was acetonitrile–0.1 M, pH 3.9, sodium dihydrogen phosphate buffer, 2:8 (*v*/*v*), at a flow-rate of 2 mL min^{-1} at ambient temperature. The excitation and emission wavelengths were set to 280 nm and 455 nm, respectively. All solutions were prepared in type-1 water (Simplicity 185 water system; Millipore, Bedford, MA, USA). GCF samples were collected by using paper strips (Periopapers; Ora Flow, Plainview, NY, USA) and the total volume of the strips was measured by use of a Ora Flow Periotron 8000 measuring apparatus.

Standard Solutions

Stock solutions of ciprofloxacin (1 mg mL^{-1}) and quinine sulphate (1 mg mL^{-1}) were prepared in HCl (0.01 M) and stored at −25°C. Standard solutions were prepared daily by diluting the stock solutions with mobile phase to concentrations of 0.01, 0.05, 0.1, 0.25, and 0.5 μg mL^{-1} ciprofloxacin containing 2 μg mL^{-1} quinine sulphate as internal standard.

Calibration Plot

Calibration plots were constructed for ciprofloxacin by calculating the peak area ratio of ciprofloxacin to quinine sulphate and plotting the ratio against the amount of standard. For assay of ciprofloxacin in GCF construction of a calibration plot for drug-free GCF was not possible, because of a lack of blank GCF. The calibration curve standards were therefore prepared in mobile phase.

Sample Collection

Five patients (two women and three men) participated in study. Subjects were administered a 500-mg single oral dose of ciprofloxacin. All volunteers were sufferers from peritonitis who had not received antibiotic and anti-inflammatory drugs nor received periodontal therapy in the previous three months. GCF samples were obtained 2, 4, and 7 h after drug administration. The procedure was approved by the ethical committee of Hacettepe University, Faculty of Medicine. Written informed consent was obtained from the patients.

GCF samples were collected with strips. Selected areas were isolated with cotton rolls and gently air dried. Standardized strips were used for GCF sampling, according to a method described previously [12]. To minimize the risk of evaporation, paper strips were damped with GCF residues and immediately transferred to the measurement apparatus, which has previously been calibrated with distilled water according to the manufacturer's instructions. GCF volume was converted to μL by means of a computer program. Paper strips were placed in sterile Eppendorf tubes and firmly sealed after GCF collection. All strips were frozen at −25°C until assay.

Sample Preparation in GCF

GCF samples from 30 paper strip samples per subject were pooled and the total volume of these strips was used as the sample volume. Total strip volumes were in the range 3.25–7.55 μL. Quinine sulphate (20 μL was

taken from 100 μg mL^{-1} quinine sulphate solution) as internal standard was added to the paper strips then methanol–water, 50:50 (*v*/*v*), was added until the final volume of the mixture for extraction was 1 mL. The mixture was shaken on a vortex mixer for 5 min. Liquid phase was transferred to another tube. After centrifugation at 10000*g* for 5 min, 100 μL was injected into the column and the concentration was calculated from the calibration plot. For determination of ciprofloxacin concentration in the GCF the estimated value was divided by the sample volume. All analyses were performed in duplicate.

Validation

The method was validated for recovery, accuracy, precision, limit of quantification (*LOQ*), limit of detection (*LOD*), linearity, and selectivity.

The extraction recovery in GCF sample was measured by comparing peak area ratio from an extracted spiked sample with the peak area from direct injection of an aqueous solution containing the same amount of drug ($n = 6$). The recovery was calculated as the mean ± *SD* of the six replicates.

The precision and accuracy were determined in mobile phase at three concentrations (0.05, 0.1, and 0.4 μg mL^{-1}). Precision reflected the variation in results from replicate analysis of the same sample. Intra-assay precision was determined on the same day with six replicates. Inter-assay precision was determined in six different days with six replicates. Precision was calculated as relative standard deviation (*RSD*), calculated by use of the equation:

$$RSD = 100 \times SD/\text{mean}.$$

The accuracy was the percentage difference (bias%) between measured mean concentrations and the corresponding nominal concentrations. The value of accuracy was calculated by use of the equation:

$$\text{Accuracy} = 100 - ((\text{nominal concentration} - \text{interpolated concentration}) \times 100/\text{nominal concentration})$$

The *LOD* was the lowest ciprofloxacin concentration that could be detected. *LOD* was expressed as a concentration for which the signal-to-noise ratio (*S*/*N*) was equal to 3. The *LOQ* was the lowest ciprofloxacin concentration that could be quantified in a sample within the range of precision. *LOQ* of the assay was evaluated as the concentration for which *S/N* was equal to 10.

The selectivity of the method was described as the resolution of the ciprofloxacin peak from other peaks.

RESULTS AND DISCUSSION

Analysis of Ciprofloxacin in Human GCF

Peaks of ciprofloxacin and the internal standard were well resolved in the aqueous and extracted samples and no interfering peak was observed in the samples. The retention times of ciprofloxacin and quinine sulphate were 4.55 and 13.25 min, respectively (Fig. 1). Figure 1A shows the peak-free chromatogram obtained after direct injection of a drug-free GCF sample; Figure 1B shows the chromatogram obtained from a patient GCF sample.

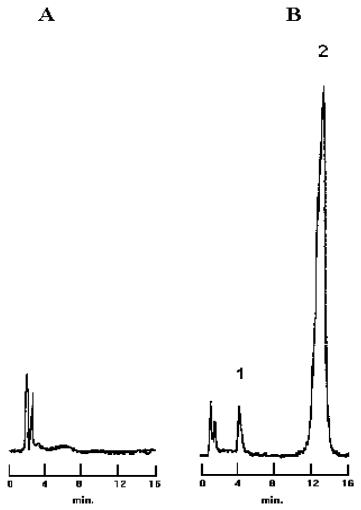

Fig. 1

Representative chromatograms of (A) drug-free GCF, (B) a patient's GCF sample containing 1.95 μg mL^{-1} ciprofloxacin. Peaks: 1, ciprofloxacin; 2, quinine sulphate (internal standard, 2 μg mL^{-1})

Assay Validation for Samples

The calibration curve for ciprofloxacin was linear over the concentration range 0.01–0.5 μg mL^{-1} ($y = -0.0055 + 2.29x$, $r = 0.9998$); the standard errors in the slope and intercept of the calibration curve were 0.0525 and 0.0346, respectively. The method was validated in this range. The lo-

wer *LOD* was 3 ng mL^{-1} ciprofloxacin (signal-to-noise ratio = 3) and the *LOQ* was 10 ng mL^{-1} (*RSD* = 9.87%, *n* = 6).

The mean recovery (±*SD*) from GCF was 101.57 ± 5.72% for ciprofloxacin at a concentration of 0.05 μg mL^{-1} (*n* = 6). Within-day and day-to-day precision and accuracy were calculated for samples containing 0.05, 0.1, and 0.4 μg mL^{-1} ciprofloxacin (*n* = 6) (Table I). Precision (*RSD*) was less than 9% at these concentrations. Experimental results are in the range of the acceptability for precision and accuracy [13].

Table I

Inter-assay and intra-assay precision and accuracy for ciprofloxacin in GCF (*n* = 6)

Added (μg mL^{-1})	Measured ± *SD* (μg mL^{-1})	*RSD* (%)	Accuracy (%)
Inter-assay (precision and accuracy)			
0.05	0.048 ± 0.002	4.24	96.33
0.1	0.110 ± 0.008	8.32	101.83
0.4	0.390 ± 0.008	4.23	98.75
Intra-assay (precision and accuracy)			
0.05	0.049 ± 0.028	4.07	97.00
0.1	0.103 ± 0.079	6.39	102.67
0.4	0.410 ± 0.011	5.34	102.50

Method Application

The method described was applied to the analysis of GCF samples collected from five volunteers. GCF drug levels after oral administration of 500 mg ciprofloxacin were higher than the MIC_{90} value for many periodontal pathogens. Levels of ciprofloxacin in GCF are given in Table II. After 2 h the concentration of ciprofloxacin in GCF is 338 times the MIC_{90} value for A.a. Results also show that the range of ciprofloxacin concentrations in GCF is 1.58–4.91 μg mL^{-1} at all time points.

Table 2

Ciprofloxacin levels (mean ± *SD*) in human GCF sample after oral administration of 500 mg ciprofloxacin (*n* = 5)

Time (h)	Ciprofloxacin levels in human GCF (μg mL^{-1})
2	3.38 ± 0.53
4	3.08 ± 0.57
7	2.14 ± 0.56

CONCLUSION

Analysis of ciprofloxacin in GCF has not previously been validated. This study describes a simple, sensitive, and reliable HPLC–fluorescence method enabling determination of ciprofloxacin in human GCF samples.

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