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Short communication

Simultaneous determination of theophylline, enoxacin and ciprofloxacin in human plasma and saliva by high-performance liquid chromatography

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Abstract

A simple reversed-phase high-performance liquid chromatographic method has been developed for the simultaneous determination of theophylline, ciprofloxacin and enoxacin in plasma and saliva. The biological fluid samples were extracted with methylene chloride-isopropyl alcohol prior to isocratic chromatography on a Waters C_{18} μBondapak column. Ultraviolet detection was carried out at 268 nm. The assay is linear for ciprofloxacin and enoxacin (0.05–10 μg/ml), and theophylline (0.1–20 μg/ml). The assay can be used to investigate the interaction of these two fluoroquinolones with theophylline.

1. Introduction

Theophylline (1,3-dimethylxanthine) is a bronchodilator that frequently is prescribed for the management of asthma and chronic obstructive pulmonary disease. Although it is used widely, theophylline has a narrow margin of safety between therapeutic and toxic concentrations. Enoxacin and ciprofloxacin are second-generation fluoroquinolone antibiotics with a broad spectrum of antibacterial activity and low toxicity. However, they have potent competitive inhibitory effects on theophylline metabolism, causing elevated plasma theophylline concentrations and potential toxicity [1]. Although many methods have been described for the determination of the quinolones [2–6], and for theophylline [7–8], no HPLC assay has been reported to measure simultaneously enoxacin, ciprofloxacin, and theophylline in plasma or saliva samples.

This paper describes an isocratic reversed-phase HPLC method to determine simultaneously these three drugs in the same human plasma or saliva sample. This assay can be used to

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investigate drug interactions involving theophylline and these two fluoroquinolones.

2. Experimental

2.1 Reagents

Ciprofloxacin was donated by Miles (Pharmaceutical Division, West Haven, CT, USA). Difloxacin was kindly supplied by Abbott Laboratories (North Chicago, IL, USA). Enoxacin was a gift from Rhône–Poulenc Rorer Pharmaceutical (Collegeville, PA, USA). Theophylline, 1-methylxanthine, 3-methylxanthine, 7-methylxanthine, 1-methyluric acid, 1,7-dimethyl xanthine, 1,3-dimethyluric acid, hypoxanthine, theobromine, caffeine, potassium phosphate, sodium phosphate, and tetrabutylammonium hydroxide were purchased from Sigma (St. Louis, MO, USA). Methanol, methylene chloride, and isopropyl alcohol were obtained from Baxter Health Care Corporation (Muskegon, MI, USA). All solvents were HPLC grade.

2.2. Apparatus

The apparatus used for the HPLC analysis was a Waters Associates 600 E multisolvent delivery system (Milford, MA, USA), equipped with a Waters 715 Ultra WISP autosampler, and a Waters 484 tunable absorbance detector. Peak ratios were recorded with a 3390A HP Integrator (Hewlett-Packard Company, Avondale, PA, USA). Separation was carried out at room temperature on a reversed-phase Waters Associates C_{18} μBondapak column (300 × 3.9 mm, 10 μm particle size). A guard column (Guard-Pak Holder and μBondapak C_{18} insert from Waters, Milford, MA, USA) was used to reduce contamination of the analytical column.

2.3. Sample preparation

In a 15-ml glass centrifuge tube, 0.5 ml plasma or saliva and 50 μl of internal standard (difloxacin, 0.05 μg/ml) were added and vortexed briefly. A 0.5 ml volume of 0.1 *M* phosphate buffer (pH 7.4), 4 ml of methylene chloride, and 1 ml isopropyl alcohol were added to the sample and vortex-mixed for 30 s. The tubes were gently shaken for 30 min in an electric shaker and then centrifuged for 20 min at 1500 *g*. The upper aqueous layer was removed by aspiration with transfer pipettes and the lower organic layer was transferred to a fresh tube and evaporated under N_2 at 45°C. A 500-μl aliquot of mobile phase was added to the tubes and 50 to 200 μl of the sample was injected into the column.

2.4. Chromatography

The mobile phase was a methanol–salt solution. Potassium dihydrogenphosphate 5.44 g (0.08 *M*) was dissolved in distilled water, and 4 ml tetrabutylammonium hydroxide (6 m*M*) was added. The mixture was diluted with distilled water to yield 1 l of solution. The pH was adjusted to 2.5 with 85% (w/v) phosphoric acid, and 350 ml methanol was added. This mixture was filtered prior to use and purged with helium throughout the chromatography run. A flow-rate of 2 ml/min was employed with ultraviolet detection at 268 nm, which provided optimal absorbance for all three analytes.

2.5. Assay interference

To investigate the possibility of interference by other methylxanthines, known amounts of 1-methylxanthine, 3-methylxanthine, 7-methylxanthine, 1-methyluric acid, 1,7-dimethylxanthine, 1,3-dimethyluric acid, hypoxanthine, theobromine, and caffeine were added to blank plasma samples. These samples were treated according to the described sample preparation. In addition, since metabolites of the quinolones were not available, enoxacin (400 mg) or ciprofloxacin (750 mg) were given orally to a healthy male volunteer on separate study days to determine whether metabolites were detectable in blood and saliva samples, which were collected at timed intervals and stored at −20°C until analysis.

2.6. Calibration curves

Known amounts of theophylline, enoxacin, and ciprofloxacin in the range 0.05–20 μg/ml were added to blank plasma and saliva samples. These samples were treated according to the described sample preparation. The ratios of the peak-heights for each drug standard to that of the internal standard were plotted against the concentrations of each drug to construct calibration curves, which were analyzed by unweighted least-squares linear regression.

2.7. Drug disposition procedure

Theophylline (300 mg), enoxacin (400 mg) and ciprofloxacin (500 mg) were given orally to a healthy male volunteer. Blood was withdrawn after 0, 1/2, 1, 2, 3, 4, 6, 8, 10, 12, 23, 24 h. Paraffin was used to stimulate saliva secretion, and saliva samples were collected at the same time as each blood sample. The plasma and saliva were stored at −20°C until analysis.

3. Results and discussion

Fig. 1 shows a typical chromatogram of plasma and saliva obtained with this procedure. The retention times for theophylline, enoxacin, ciprofloxacin, and difloxacin were approximately 4.3, 5.2, 7.1, 8.8 min, respectively.

The extraction procedure has good efficiency for plasma and saliva, with theophylline extracting better than ciprofloxacin and enoxacin (Table 1). Difloxacin extraction recovery was 94.7 ± 0.2% for plasma, and 95.6 ± 0.3% for saliva. The within-day and day-to-day coefficients of variation were good for all three drugs (Table 2). Calibration curves were linear over the range 0.05–10 μg/ml for enoxacin and ciprofloxacin, and over the range 0.1–20 μg/ml for theophylline. The detection limit was 0.05 μg/ml for enoxacin and ciprofloxacin, and 0.1 μg/ml for theophylline. The following equations were derived from the calibration curves: $y = 0.613x - 0.079$ ($r = 0.9997$), and $y = 0.638x - 0.059$ ($r = 0.9998$) for theophylline in plasma and saliva, respectively; $y = 0.882x - 0.180$ ($r = 0.9997$), and $y = 0.868x - 0.166$ ($r = 0.9997$) for enoxacin in plasma and saliva, respectively; $y = 0.907x - 0.126$ ($r = 0.9996$), and $y = 0.887x - 0.09$ ($r = 0.9994$) for ciprofloxacin in plasma and saliva, respectively. The assay was used to demonstrate the disposition of theophylline, enoxacin, and ciprofloxacin in a healthy volunteer (Fig. 2).

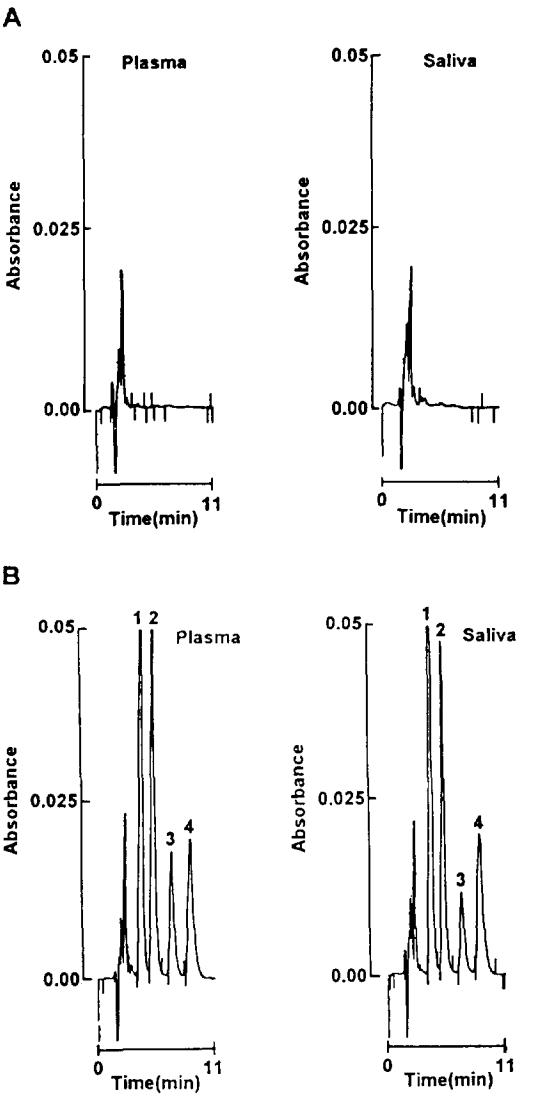

Fig. 1. Chromatogram of blank plasma and saliva samples (A), and plasma and saliva samples from a drug-dosed volunteer (B). Peaks: 1 = theophylline; 2 = enoxacin; 3 = ciprofloxacin; 4 = difloxacin. UV detection at 268 nm, 50 μl sample injected.

Table 1
Extraction recovery for theophylline, enoxacin and ciprofloxacin in human plasma and saliva

Concentration (μg/ml)	Recovery (mean ± S.D., $n = 8$) (%)		
	Theophylline	Enoxacin	Ciprofloxacin
Plasma			
0.75	85.7 ± 1.1		
10.0	85.5 ± 0.5		
20.0	84.1 ± 2.0		
0.4		73.2 ± 2.2	73.1 ± 2.4
2.0		75.9 ± 1.4	70.4 ± 2.4
10.0		79.9 ± 1.6	76.7 ± 1.4
Saliva			
0.75	82.8 ± 1.8		
10.0	88.9 ± 0.3		
20.0	90.7 ± 1.6		
0.4		57.5 ± 3.6	69.1 ± 2.7
2.0		67.7 ± 1.5	67.8 ± 1.2
10.0		79.5 ± 1.1	78.1 ± 1.4

Table 2
Accuracy and precision of the assay for theophylline, enoxacin and ciprofloxacin in human plasma and saliva

Drug	Spiked concentration (μg/ml)	Within-day ($n = 8$)		Between-day ($n = 6$)	
		Mean (μg/ml)	C.V. (%)	Mean (μg/ml)	C.V. (%)
Plasma					
Theophyllinee	0.75	0.71	5.0	0.83	10.1
	10.0	11.02	1.0	9.91	2.9
	20.0	19.08	1.7	21.19	3.0
Enoxacin	0.4	0.32	3.5	0.34	6.9
	2.0	1.89	2.3	1.80	7.1
	10.0	10.22	2.1	9.46	4.0
Ciprofloxacin	0.4	0.34	5.0	0.38	8.1
	2.0	1.94	3.3	1.83	4.6
	10.0	10.64	1.9	9.93	3.3
Saliva					
Theophylline	0.75	0.68	3.7	0.66	7.5
	10.0	9.62	7.1	9.90	6.9
	20.0	17.40	3.0	21.40	4.8
Enoxacin	0.4	0.43	3.2	0.34	9.5
	2.0	1.70	6.3	1.91	8.8
	10.0	8.50	3.0	10.05	4.3
Ciprofloxacin	0.4	0.44	5.1	0.33	9.4
	2.0	1.92	8.1	1.86	6.5
	10.0	9.00	3.1	10.60	3.9

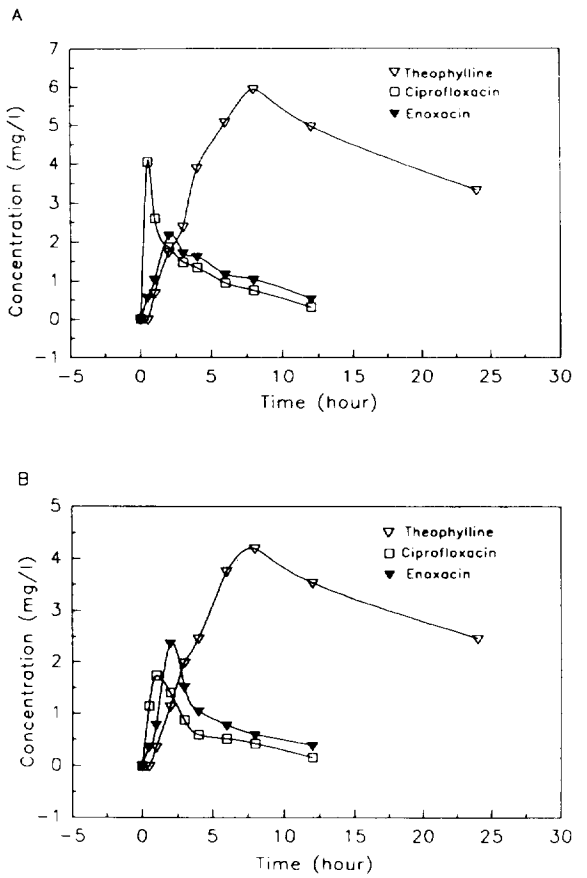

Fig. 2. Concentration–time curves of theophylline, enoxacin, and ciprofloxacin in plasma (A), and in saliva (B) from a volunteer following simultaneous oral administration of 300 mg theophylline, 400 mg enoxacin, and 500 mg ciprofloxacin.

To exclude potential interference from xanthine-related substances, we demonstrated that with the exception of caffeine and 1,7-dimethylxanthine all of the xanthine-related substances investigated do not interfere with this assay. Caffeine may interfere with the measurement of enoxacin, and 1,7-dimethylxanthine may interfere with the determination of theophylline. The plasma samples obtained following single dose administration of enoxacin or ciprofloxacin do not appear to contain detectable quantities of quinolone metabolites.

So far, there is only one report of an HPLC method to determine simultaneously a fluoroquinolone and theophylline [9]. Compared with that assay, the assay that we have developed has several advantages. Theophylline and both ciprofloxacin and enoxacin can be determined simultaneously with the same extraction procedure. This assay has less variability and the limit of detection is lower, which makes the assay more sensitive than the previously reported assay. In addition, levels in saliva show a good correlation with plasma.

In summary, this new assay procedure provides an economical and accurate method for investigating the interaction between theophylline and these two fluoroquinolone antibiotics. Although we have not done so, it could be adapted for studies with other fluoroquinolones including difloxacin, which is used as the internal standard in this assay.

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