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Short Communication

Simultaneous assay of fluoroquinolones and theophylline in plasma by high-performance liquid chromatography

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ABSTRACT

A sensitive and selective reversed-phase high-performance liquid chromatographic method for the determination of theophylline in plasma simultaneously with either ciprofloxacin, enoxacin or norfloxacin has been developed. It involves extraction of plasma with chloroform–isopropanol or dichloromethane–isopropanol prior to chromatography on a Spherisorb ODS2 column. The mobile phase is 15% acetonitrile in a phosphate buffer (pH 3.0) containing tetrabutylammonium hydrogen sulphate (4.4 m*M*) as an ion-pairing agent. Ultraviolet detection is carried out at 280 nm. Run time is less than 10 min for all three separations. The assays have been used to determine the effect of plasma concentrations of fluoroquinolone on theophylline clearance.

INTRODUCTION

The fluoroquinolones [*e.g.* enoxacin (Enox), ciprofloxacin (Cipro) and norfloxacin (Norflox)] are a group of antibiotics that have been shown to be effective against a wide range of infections. In particular they provide an effective oral treatment for gram-negative organisms such as *Pseudomonas aeruginosa*. However, a number of authors have shown that certain fluoroquinolones have a pharmacokinetic interaction with methylxanthines, due to metabolic inhibition [1,2]. This is particularly important with theophylline (Theo), which has a narrow margin of safety between therapeutic concentrations and toxic concentrations. A number of assays for fluoroquinolones have been reported [3–7], but none allow the measurement of both fluoroquinolone and Theo in the same plasma sample. The analytical procedures described here allow simultaneous determination of Theo and either Enox, Cipro or Norflox and hence can be used to establish plasma concentration–effect relationships for fluoroquinolone inhibition of Theo disposition.

EXPERIMENTAL

Instrumentation

Chromatography was performed using a Beckman (High Wycombe, Bucks., UK) 110B single headed solvent delivery module, Kratos (Ramsay, NJ, USA) Model 757 UV detector and a Hewlett-Packard (Avondale, PA, USA) Model 3393A integrator.

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Reagents

Ciprofloxacin hydrochloride monohydrate was a gift from Bayer UK (Newbury, Berks., UK) and enoxacin sesquihydrate was a gift from Rhône-Poulenc (Vitry sur Seine, France). Norfloxacin, theophylline, β-hydroxypropyl theophylline, tetrabutylammonium hydrogen sulphate and orthophosphoric acid (85%) were purchased from Sigma (Poole, Dorset, UK). Freshly distilled, deionised water was used throughout the procedure. Acetonitrile was HPLC grade, all other solvents were analytical grade.

Chromatography

The stationary phase was a Spherisorb S5 ODS2 column (Hicrom, 25 cm × 4.9 mm I.D.) fitted with a Spherisorb ODS 10 mm × 4.9 mm I.D. guard column (HPLC Technology, Macclesfield, Cheshire, UK). The mobile phase was acetonitrile–salt solution (15:85, v/v). The salt solution contained KH_2PO_4 (4.54 g/l), $Na_2HPO_4 \cdot 2H_2O$ (5.94 g/l) and $(n\text{-}C_4H_9)_4N^+HSO_4^-$ (1.49 g/l). The mobile phase was adjusted to pH 3.0 using 85% phosphoric acid immediately prior to use. Separation was achieved using a flow-rate of 1.3 ml/min at room temperature; the detector wavelength was 280 nm. Retention times were as follows: Theo 3.3 min, β-hydroxypropyl theophylline 4.2 min, Enox 4.5 min, Norflox 5.5 min, and Cipro 6.2 min.

Extraction

Ciprofloxacin and theophylline. To 100 μl of plasma was added 100 μl of phosphate buffer (pH 7.4) and 50 μl of the internal standard solution (20 μg/ml β-hydroxypropyl theophylline in phosphate buffer pH 7.4). This was extracted using 5 ml chloroform–propan-2-ol (95:5, v/v) by shaking on a rotary mixer for 15 min. The mixture was centrifuged at 800 *g* for 5 min to separate the layers; the aqueous layer was aspirated off and discarded. The organic layer was evaporated under nitrogen at 45°C and the residue reconstituted by sonication with 100 μl of mobile phase. A 25-μl aliquot was injected *via* a Rheodyne Model 7125 on to the HPLC column. Calibration lines were drawn from analyses performed with 100 μl of standard solutions, containing both Theo and Cipro in phosphate buffer (pH 7.4), added to 100 μl of blank plasma and 50 μl of internal standard. These mixtures were treated as above. The calibration lines were drawn as peak-height ratios of drug to internal standard *versus* concentration.

Enoxacin and theophylline. To 100 μl of plasma was added 100 μl of saturated sodium bicarbonate solution and 50 μl of internal standard (Cipro, 20 μg/ml in saturated sodium bicarbonate solution). This was extracted with 5 ml of dichloromethane–propan-2-ol (90:10, v/v) as described above. Calibration curves were drawn from analyses performed with 100 μl of standard solutions containing Enox and Theo in saturated sodium bicarbonate added to blank plasma and 50 μl of internal standard — this mixture was treated as above.

Norfloxacin and theophylline. To 100 μl of plasma was added 100 μl of phosphate buffer pH 7.4 and 50 μl internal standard (10 μg/ml β-hydroxypropyl theophylline in phosphate buffer pH 7.4). This was extracted with 5 ml dichloromethane–propan-2-ol (80:20, v/v) as described above. Calibration curves were drawn from analyses performed with 100 μl of standard solutions containing both Theo and Norflox in phosphate buffer (pH 7.4) added to 100 μl of blank plasma and 50 μl of internal standard, and treated as above.

Interaction experiments in rats

Male Sprague–Dawley rats were cannulated in the carotid artery and jugular vein. Serial blood samples (250 μl) were taken from the carotid artery following intravenous administration of 6 mg/kg Theo to animals that had constant plasma concentrations of fluoroquinolone, maintained by intravenous infusion. Plasma (100 μl) was separated and extracted as described above. Concentrations were estimated from calibration curves obtained from standard solutions extracted under the same conditions.

TABLE I

EXTRACTION EFFICIENCY FOR THEO WITH CIPRO, ENOX OR NORFLOX

Concentration (mg/l)	Recovery (mean ± S.D.) (%)			
	Theophylline	Fluoroquinolone		
		Ciprofloxacin[a]	Enoxacin[b]	Norfloxacin[c]
0.5	87.4 ± 3.5[a]	80.1 ± 12.4		
1.0	90.9 ± 8.1[a]	79.5 ± 6.9		
5.0	87.9 ± 8.1[a]	88.9 ± 9.4		
0.5	83.2 ± 6.7[b]		58.2 ± 2.7	
2.0	80.2 ± 6.5[b]		55.2 ± 1.6	
20.0	75.3 ± 7.0[b]		58.6 ± 2.2	
1.0	95.1 ± 9.1[b]			88.7 ± 7.5
2.0	87.3 ± 5.4[b]			93.9 ± 7.4
10.0	85.2 ± 6.1[b]			84.3 ± 5.0

[a] $n = 5$.
[b] $n = 6$.

TABLE II

WITHIN-DAY AND BETWEEN-DAY VARIABILITY (COEFFICIENT OF VARIATION %)

Concentration (mg/l)	Variability (%)							
	Theophylline		Fluoroquinolone					
	W	B	Ciprofloxacin		Enoxacin		Norfloxacin	
			W[a]	B[b]	W[c]	B[d]	W[c]	B[d]
1.0	7.9[a]	20.0[b]	13.2	9.1				
5.0	8.0[a]	9.0[b]	12.5	17.1				
10.0	4.2[a]	6.0[b]	11.2	13.7				
0.5	8.2[c]	17.9[d]			3.9	21.4		
2.0	6.3[c]	10.7[d]			2.0	16.5		
20.0	6.0[c]	14.0[d]			1.9	11.0		
1.0	13.7[c]	11.4[d]					14.6	20.4
2.0	8.4[c]	10.0[d]					4.3	12.6
10.0	7.6[c]	9.1[d]					8.2	18.5

W = within day. B = between day.
[a] $n = 5$.
[b] $n = 12$.
[c] $n = 6$.
[d] $n = 10$.

RESULTS

Ciprofloxacin with theophylline in plasma

The extraction procedure showed good efficiency, with both Theo and Cipro recovered at 80% or more (Table I). The within-day variability was good for both compounds; the between-day variability was greater at lower concentrations than at high (Table II). The assay was linear for both compounds at a detection wavelength of 280 nm over the range 0.2–20 μg/ml (the limit of detection was taken to be 0.5 μg/ml, as the variability was too great at lower concentrations). Fig. 1A shows a typical chromatogram of samples extracted as above.

Enoxacin with theophylline in plasma

The extraction procedure showed constant recovery over the concentration range studied, with Theo extracting better than Enox (Table I). The variability was good for both compounds; within-day replicates showed a coefficient of variation of less than 9%; the between-day variability was greater at lower concentrations than at high (Table II). The assay was linear at a detection wavelength of 280 nm for both compounds over the concentration range 0.5–20 μg/ml (the limit of detection was taken to be 0.5 μg/ml). Fig. 1B shows a typical chromatogram of samples extracted as above.

Norfloxacin with theophylline in plasma

The extraction procedure showed good efficiency for both Norflox and Theo — recovery was greater than 84% in all cases (Table I). The variability was good for both compounds; both within- and between-day variability was greater at lower concentrations than at high (Table II). The assay was linear at a detection wavelength of 280 nm for both compounds over the concentration range 1.0–20 μg/ml. The limit of detection was taken to be 1.0 μg/ml for Theo, as there was a small interfering peak just in front of Theo on the chromatogram. The limit of detection for Norflox was 0.5 mg/ml. Fig. 1C shows a typical chromatogram of samples extracted as above.

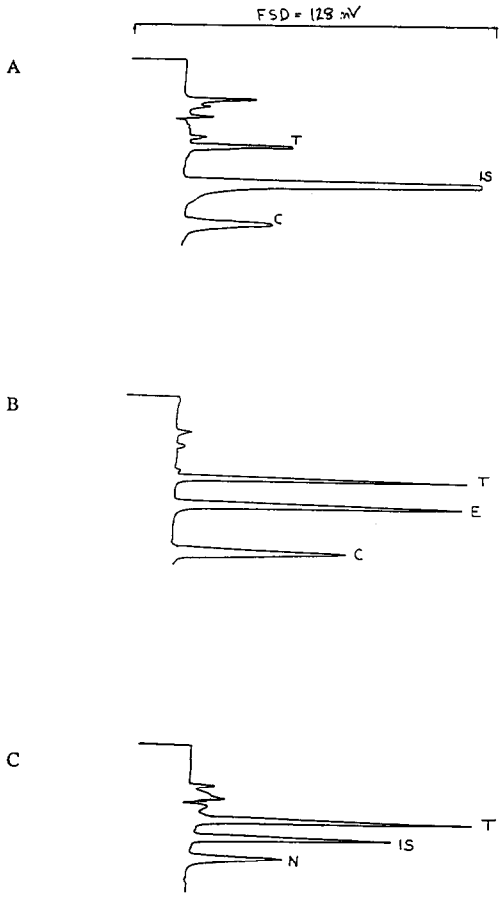

Fig. 1. Typical chromatograms obtained from the Cipro–Theo (A), Enox–Theo (B) and Norflox–Theo (C) assays. T: theophylline (2.1 mg/l, A; 1.1 mg/l, B; 4.8 mg/l, C); C: ciprofloxacin (3 mg/l); E: enoxacin (16 mg/l); N: norfloxacin (1.3 mg/l); I.S.: internal standard (β-hydroxypropyl theophylline). Chart speed 5 mm/min.

Application of assay methodology

The assays have been used to study the interaction of each of the three fluoroquinolones with Theo. Figs. 2A and B show the plasma concentration–time profile for Theo when administered alone or in the presence of Enox (0.5 or 15 mg/l). Similar studies have been carried out with Norflox and Cipro, both of which also decrease the clearance of Theo when maintained at steady-state concentrations.

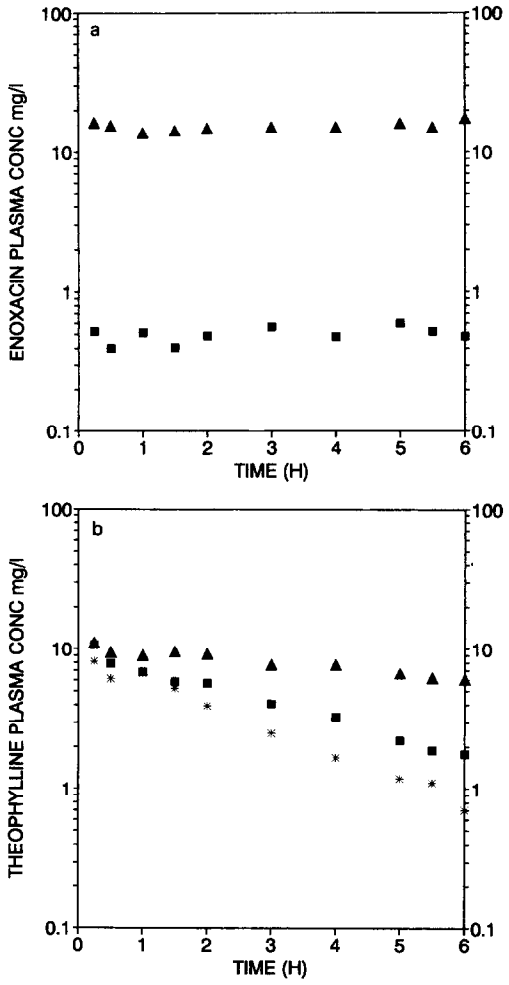

Fig. 2. (A) Steady state plasma concentrations of enoxacin, and (B) theophylline plasma concentration–time profiles in the presence of enoxacin. (*) Control, (■) 0.5 mg/l enoxacin, (▲) 15 mg/l enoxacin.

DISCUSSION

The procedures described use an UV–HPLC chromatographic system that is similar to other assays, but is capable of simultaneously measuring fluoroquinolone with theophylline. Assay sensitivity is 0.2–1 μg/ml, which is more than adequate to assess the relationship between plasma concentrations of fluoroquinolone and changes in Theo pharmacokinetics. The extraction efficiency for Theo is *ca.* 80–90%, while recovery of the fluoroquinolones varies between 55–85%, with each individual assay showing good reproducibility (C.V. < 10%) over the concentration ranges studied. None of the fluoroquinolones studied effected Theo extraction. Within- and between-day variability was good for all three assays. The assays are straightforward and are designed to analyse small plasma volumes (100 μl). Use of a solvent extraction method minimises any interfering peaks and prolongs the working life of the HPLC column. The assays procedures have been used to investigate the comparative inhibitory properties of three fluoroquinolones towards Theo disposition. By using fluoroquinolones in a steady-state infusion approach, plasma concentration–inhibition relationships have been established (Davis, *et al.* [8]) and Enox was found to be clearly more potent than either Norflox or Cipro, both of which had similar potencies.

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