Journal of Chromatography, 416 (1987) 207–211
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 3548

Note

Assay of ciprofloxacin and norfloxacin in serum and urine by high-performance liquid chromatography

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(First received October 17th, 1986; revised manuscript received November 28th, 1986)

The quinolones are a new class of antibiotics with a broad antibacterial spectrum. Norfloxacin shows great promise in the treatment of, for instance, complicated urinary tract infections and enteric infections. Ciprofloxacin will be an alternative for treatment of problematic infections, especially where *Pseudomonas aeruginosa* is involved, its great advantage over other antipseudomonal agents being that oral treatment is available.

Assays of these two compounds by high performance liquid chromatography (HPLC) have been reported earlier. However, the assays described for norfloxacin [1–6] involve rather elaborate sample preparation. The methods for ciprofloxacin [5–11] are sometimes simpler and easier to perform but often use laborious extraction procedures. We have developed an assay system based on direct injection of serum and urine samples and have found it suitable for assay of both norfloxacin and ciprofloxacin in serum and urine.

EXPERIMENTAL

Standard preparations

Norfloxacin (kindly given by Astra Läkemedel, Sweden) and ciprofloxacin (kind gift of Bayer Sverige, Sweden) were dissolved in 0.01 *M* sodium hydroxide and in distilled water, respectively, to make stock solutions of 1 mg/ml.

Chemicals

Freshly distilled, deionized water was used throughout the procedure. Acetonitrile (E. Merck, Darmstadt, F.R.G.), orthophosphoric acid (E. Merck) and

0378-4347/87/$03.50

tetrabutylammonium hydroxide, 40% (Sigma, St. Louis, MO, U.S.A.) were analytical grade.

Chromatographic conditions

A Waters ALC/GPC 204 liquid chromatograph with a U6K manual injector and a Varian Fluorichrome detector was used. Excitation was at 278 nm, emission at 445 nm. The stationary phase was 5-μm Nucleosil C_{18} (Macherey-Nagel, Düren, F.R.G.), slurry-packed in a stainless-steel column (20 cm×4 mm I.D.). The mobile phase was acetonitrile–0.025 *M* orthophosphoric acid adjusted to pH 3.0 with tetrabutylammonium hydroxide (11:89, v/v). Separations were performed at ambient temperature and the flow-rate was 1.5 ml/min. Emitted fluorescence was recorded with a Scintag recorder (Waters Assoc., Gothenburg, Sweden).

Sample preparations

Separate stock standard solutions of norfloxacin and ciprofloxacin were diluted with distilled water or pooled human serum to known concentrations. Serum assays were run against standards of 0.1 and 1.0 μg/ml and urine assays against standards of 10.0 and 100.0 μg/ml. Samples were also obtained from patients receiving treatment with norfloxacin or ciprofloxacin.

Serum samples were injected without any pretreatment except addition of an equal volume of distilled water which facilitated the passage through 0.6-μm Millipore filters. The volume injected onto the column was 10 or 20 μl (for samples with concentrations below 0.05 μg/ml). Urine samples were diluted with distilled water, the degree of dilution depending on the expected or known concentration

TABLE I

RECOVERY OF CIPROFLOXACIN AND NORFLOXACIN FROM SERUM AND URINE IN THE ASSAY PROCEDURE

Compound	Sample	Known concentration (mg/l)	Found concentration (mg/l)	Recovery (%)
Ciprofloxacin	Serum	1.00	0.97	96.9
		0.50	0.48	96.6
		0.10	0.09	90.0
Ciprofloxacin	Urine	100.0	102.6	102.6
		50.0	49.8	99.6
		10.0	10.4	104.1
Norfloxacin	Serum	1.00	0.98	97.8
		0.50	0.52	104.0
		0.10	0.10	98.5
Norfloxacin	Urine	100.0	97.6	97.6
		50.0	48.5	97.0
		10.0	9.6	96.2

in the sample under assay, filtered through 0.6-μm filters and 10 μl were injected.

RESULTS

In the chromatographic system described above, norfloxacin and ciprofloxacin had retention times of 2.8 and 3.1 min, respectively. The peaks were well separated from visibly fluorescent endogenous material in serum and urine, and the recovery, as judged by comparison with water solutions of standards, was quantitative (Table I). Figs. 1 and 2 show chromatograms obtained from analysis of samples from patients on treatment with either of the two quinolones, taken before administration and at various time-points after.

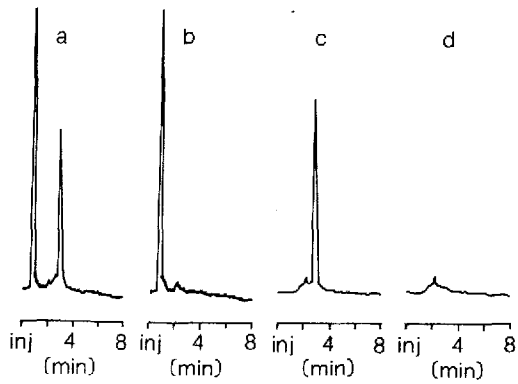

Fig. 1. Chromatograms of samples obtained from a patient receiving 400 mg of norfloxaclin orally. (a) Serum sample (2.58 μg/ml) 4 h after administration; (b) serum blank; (c) urine sample (48.4 μg/ml) 5 h after; (d) urine blank. Recorder was set at 1 mV attenuation for serum samples and 10 mV for urine samples. Fluorimeter expansion varied between $\times 10$ and $\times 100$.

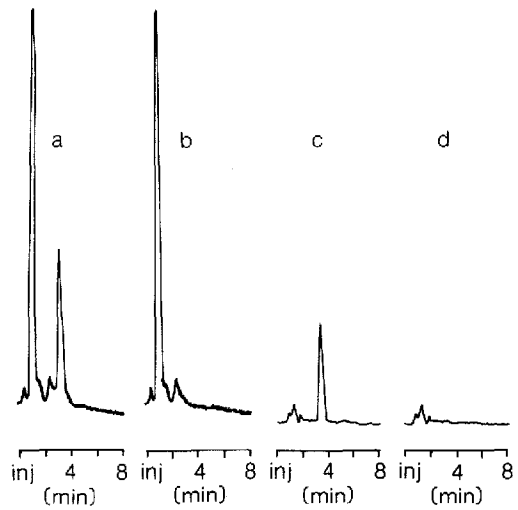

Fig. 2. Chromatograms of samples obtained from a patient treated with 500 mg of ciprofloxacin orally. (a) Serum sample (0.30 μg/ml) 7 h after intake; (b) serum blank; (c) urine sample (9.1 μg/ml) 12 h after; (d) urine blank. Recorder and fluorimeter settings as in Fig. 1.

TABLE II

INTRA- AND INTER-ASSAY IMPRECISION OF THE CHROMATOGRAPHIC ASSAY FOR CIPROFLOXACIN AND NORFLOXACIN IN SERUM AND URINE

Sample	Concentration (μg/ml)	Intra-assay C.V. (%)		Inter-assay C.V. (%)	
		Ciprofloxacin	Norfloxacin	Ciprofloxacin	Norfloxacin
Serum	0.10	3.9	3.5	4.6	4.8
	1.00	2.7	2.4	3.4	3.3
Urine	10.0	3.1	2.7	3.6	3.9
	100.0	1.9	1.5	2.4	2.0

TABLE III

SPECIFICITY OF PROCEDURE FOR CIPROFLOXACIN AND NORFLOXACIN IN SERUM AND URINE

Drugs found not to interfere with assay.

Antibiotics	Other drugs
Trimethoprimsulphamethoxazole	Salicylic acid
Netilmicin	Digoxin
Metronidazole	Furosemide
Benzyl penicillin	Paracetamol
Cloxacillin	Prednisolone
Doxycycline	Warfarin
Cefuroxime	Potassium chloride
Erythromycin	Dextropropoxyphene
	Multivitamins (A, B_1, B_2, C, D_3)

The imprecision of the assay was checked by running eight separate analyses of the same sample on the same day and on separate days. As shown in Table II, the serum assays had coefficients of variation (C.V.) below 5% at the concentrations tested and the assays in urine had coefficients of variation below 4%.

The sensitivity of the assay allows determination of concentrations as low as 0.01 μg/ml for both compounds, which is adequate for clinical and pharmacokinetic purposes.

The specificity of the procedure was checked by testing samples from ten patients receiving treatment with other drugs and antibiotics (Table III) in the assay system. No interference was found from any of these compounds.

DISCUSSION

The procedure described uses a chromatographic system that is similar to other assays [5–7,9,10] for determination of ciprofloxacin, and we have found it very suitable also for norfloxacin. Compared with most chromatographic methods previously reported for norfloxacin and ciprofloxacin determinations, which involve extractions and, in some cases, back-extractions, this assay procedure is much

easier to perform since it entails virtually no sample pretreatment. An internal standard is therefore not needed, as shown also by the excellent precision of the assay. Naturally, it is also practical to have the identical methodology for drugs of the same chemical class, and possibly other quinolone antibiotics can also be assayed in this system, an assumption that is supported by the recent assay by Groeneveld and Brouwers [5], where also ofloxacin and perfloxacin were chromatographed. It can be argued that injection of untreated serum onto a chromatographic column is not ideal since this will mean a shorter column life, and this is certainly a valid point. However, this disadvantage must be weighed against the simplicity of the assay. In our hands, columns have lasted for an average of 250 injections of serum samples (much longer for urine samples) and since we pack our own columns at a rather low cost this is considered reasonable. Column life can be substantially prolonged by use of a guard column, and this is recommended if the more expensive ready-packed commercial types of column are used.

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