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Note

Determination of flumequine and its 7-hydroxy metabolite by reversed-phase high-performance liquid chromatography

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Flumequine (9-fluoro-6,7-dihydro-5-methyl-1-oxo-1H,5H-benzoquinolizine-2-carboxylic acid, I) (Fig. 1) is a quinolone derivative active against a broad spectrum of Gram-negative bacteria, some of which are associated with or responsible for urinary tract infections. Some chemical, microbiological and metabolic aspects of I have been published [1,2].

Recently, Harrison et al. [3] reported a high-performance liquid chromatographic (HPLC) method for the determination of I and a major metabolite, 7-hydroxyflumequine (II) (Fig. 1) in human plasma and urine. This method, based on anion exchange, uses a mobile phase adjusted to pH 9.0, which is very critical for a silica chemically bonded phase. However, this method needs a relatively large plasma sample size (1 ml). The method described here for the measurement of both I and II in human plasma and urine is sensitive and selective, and the plasma sample size necessary for a measurement is only 200 μl.

EXPERIMENTAL

Reagents and materials

All chemicals and solvents used were of analytical-reagent grade.

I and II were supplied by Riker/3M Labs. (Pithiviers, France). Quinaldic acid

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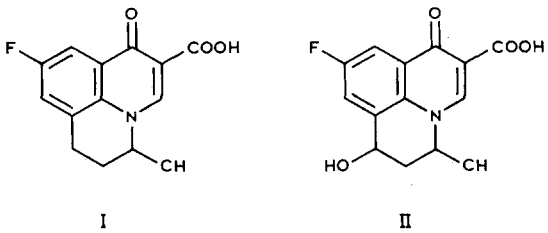

I II

Fig. 1. Structures of I and II.

(internal standard) was purchased from EGA-Chemie (Steinheim/Albuch, F.R.G.).

Drug-free human urine and plasma were obtained from healthy volunteers.

Chromatographic conditions

The HPLC system consisted of a Merck-Hitachi 655A-11 solvent delivery pump (Merck, Darmstadt, F.R.G.), an injection valve with a 20-μl sample loop (Model 7125, Rheodyne, Cotati, CA, U.S.A.) and a UV–visible detector operating at 254 nm (0.08 or 0.16 a.u.f.s.) (Merck, LMC System SM 25).

The mobile phase was 10^{-2} *M* KH_2PO_4–methanol (45:55, v/v) adjusted to pH 3 with H_3PO_4. It was filtered through an HVLP 0.6-μm microfilter (Millipore, Bedford, MA, U.S.A.) and pumped at 1.2 ml/min through a reversed-phase column (room temperature) pre-packed with LiChrosorb RP-18 (HIBAR RT 250-4, 5 μm; Merck).

The dead volume of the column was measured by injection of sodium nitrate solution.

Extraction procedures

The method of Harrison et al. [3] used for the extraction of I and II from plasma, was modified as follows: to 200 μl of plasma were added 2.4 μg of quinaldic acid (30 μl of a solution of 0.08 mg of quinaldic acid in 1 ml of methanol obtained by dilution of a stock solution of 0.8 mg of quinaldic acid in 1 ml of methanol), 0.5 ml of 0.1 *M* sodium hydroxide solution and 5 ml of chloroform. After mixing for 10 min on a mechanical shaker and centrifuging for 5 min at 1000 *g*, the organic phase was discarded. To the aqueous phase were added 0.5 ml of 0.6 *M* hydrochloric acid and 5 ml of chloroform. The tubes were shaken and centrifuged and the aqueous phase was discarded. The organic layer was evaporated at 30°C under a stream of nitrogen. The residue was then dissolved in 150 μl of mobile phase and 20 μl were injected.

The urine samples were extracted according to the method of Harrison et al. [3] (extraction with chloroform after addition of 1 ml of 0.5 *M* phosphate buffer, pH 6.0), except that the sample size was 250 μl. The evaporation temperature was maintained below 35°C. Moreover, all the glass tubes were systematically washed with sulphochromic acid before use.

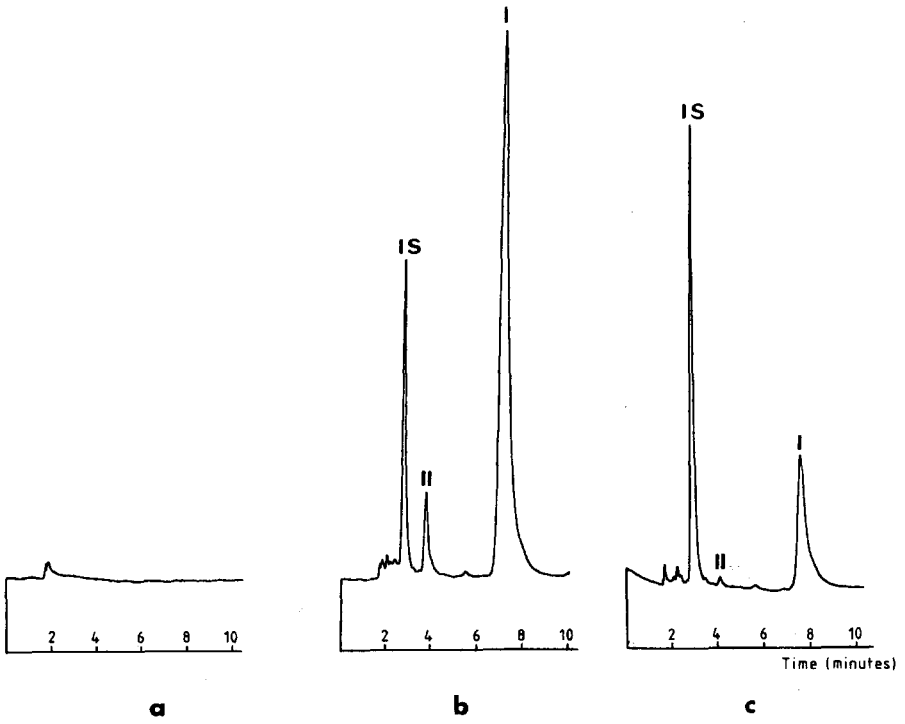

Fig. 2. Chromatographic profile obtained by analysis of (a) drug-free plasma, (b) plasma spiked with 20 μg/ml I and 2 μg/ml II and (c) plasma of a healthy volunteer who had received a single oral dose of I. IS = internal standard.

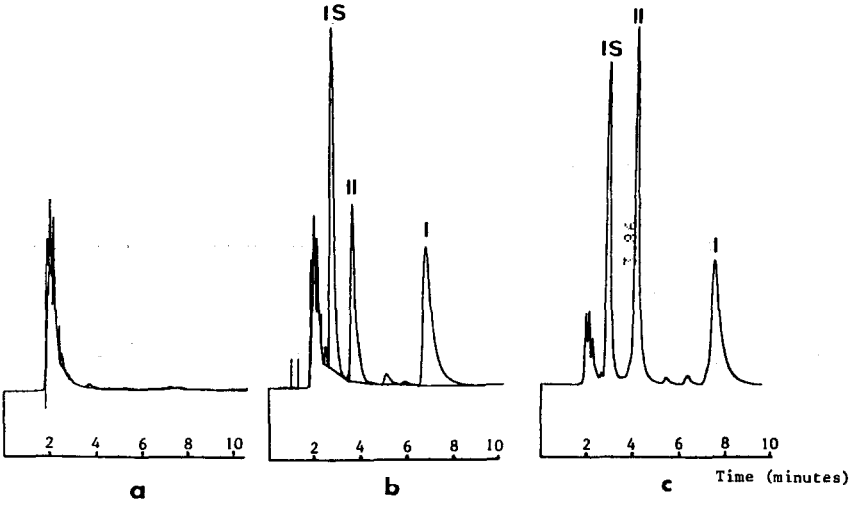

Fig. 3. Chromatographic profile obtained by analysis of (a) drug-free urine, (b) urine spiked with 20 μg/ml of both I and II and (c) urine of a healthy volunteer who had received a single oral dose of I. IS = internal standard.

TABLE I

RESULTS OF THE HPLC ASSAY

k' = capacity factor; α = selectivity factor; R_s = resolution; IS = internal standard.

Parameter	Plasma	Urine
k'_{I}	3.1	3.4
k'_{II}	1.87	1.86
k'_{IS}	1.4	1.39
$\alpha_{II, IS}$	1.33	1.34
$\alpha_{I, II}$	1.66	1.83
$R_{s\ II, IS}$	2.47	2.66
$R_{s\ I, II}$	4.1	5.3

RESULTS AND DISCUSSION

No endogenous sources of interference were observed. The chromatograms obtained on analysis of an extract of I, II and quinaldic acid-free human plasma and urine are illustrated in Figs. 2a and 3a, respectively, and those obtained on analysis of plasma and urine spiked with I, II and quinaldic acid are shown in Figs. 2b and 3b, respectively. Figs. 2c and 3c show the results for plasma and urine

TABLE II

INTRA- AND INTER-ASSAY COEFFICIENTS OF VARIATION (C.V.) FOR I AND II IN PLASMA AND URINE

	I				II			
	Plasma		Urine		Plasma		Urine	
	C (μg/ml)	C.V. (%)	C (μg/ml)	C.V. (%)	C (μg/ml)	C.V. (%)	C (μg/ml)	C.V. (%)
Intra-assay	10 (n=6)	4.3	20 (n=10)	3.7	2.5 (n=6)	4.9	20 (n=10)	3.7
	40 (n=8)	3.1	80 (n=10)	1.1	5 (n=8)	2.6	80 (n=10)	2.1
Inter-assay (n=5)	10	3.6	20	3.9	2.5	4.6	20	6.6
	40	1.4	80	1.1	5	4.1	80	1.0

TABLE III

ACCURACY AND PRECISION OF THE HPLC ASSAY FOR I AND II IN PLASMA AND URINE

Sample	Concentration of drug added (μg/ml)		Concentration found (mean ± S.D., n=3) (μg/ml)	
	I	II	I	II
Plasma	15	2	14.77 ± 0.58	2.11 ± 0.088
	45	7.5	45.02 ± 0.437	7.59 ± 0.357
Urine	16	16	15.82 ± 0.312	16.69 ± 0.79
	70	70	67.93 ± 2.9	69.23 ± 2.1

extracts from a healthy volunteer who had received a single 800-mg oral dose of flumequine (Apurone).

The HPLC results are given in Table I.

The intra- and inter-assay coefficients of variation (C.V.) for replicate analyses of I and II in plasma and urine are shown in Table II. The intra- and inter-assay coefficients of variation did not exceed 4.9% and 6.6%, respectively.

The accuracy and precision of the method were determined for two concentrations of I and II (Table III). Calibration graphs were constructed by plotting peak-area ratios (drug to internal standard) versus drug concentrations (μg/ml). The instrument responses and the concentrations were linearly related for both I and II over ranges 2.5–50 μg/ml for I in plasma ($y=0.18x+0.15$; $r=0.999$), 0.5–10 μg/ml for II in plasma ($y=0.12x+0.011$; $r=0.999$) and 10–100 μg/ml for both I and II in urine ($y=0.062x-0.011$; $r=0.999$ for I; $y=0.04x-0.054$; $r=0.998$ for II).

For I and II, the limits of detection were lower than 0.5 μg/ml (signal-to-noise ratio of 6) and 0.1 μg/ml (signal-to-noise ratio of 4), respectively (determined in plasma).

CONCLUSION

In comparison with the method of Harrison et al. [3], the main advantages of the method described here are the lack of damage to the HPLC column and the smaller sample size. The plasma and urine volumes required in our method are 200 and 250 μl, respectively, compared with 1 ml for the method proposed by Harrison et al. [3]. Considering the extraction procedure, which permits the extract to be dissolved in variable volumes, and the sensitivity of the method, these volumes could be reduced further.

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