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Note

Quantitative determination of flufenamic acid and its major metabolites in plasma by high-performance liquid chromatography

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The metabolic disposition of flufenamic acid, which is used therapeutically as an anti-inflammatory agent, has been studied in some detail by the use of the [^{14}C]-carboxyl-labelled drug[1]. 4′-Hydroxy- and 5-hydroxyflufenamic acids were found to be major metabolites after oral administration in man[1].

Unchanged flufenamic acid in plasma has been determined by fluorimetric assay[2–5], but no determinations of its metabolites have been successful except by the radio-labelling technique.

We have developed a method for the simultaneous quantitative analysis of flufenamic acid and its major metabolites in plasma using high-performance liquid chromatography.

EXPERIMENTAL

Materials

Flufenamic acid was obtained from a commercial source. 4′-Hydroxyflufenamic acid and 5-hydroxyflufenamic acid were synthesized in this laboratory. All other chemicals were of the highest grade available.

High-performance liquid chromatography

The liquid chromatograph was a Waters Assoc. (Milford, Mass., U.S.A.) Model ALC/GPC-204 instrument, equipped with a Model 6000A pumping system, a U6K loop injector and an ultraviolet (UV) detector (280 nm).

A stainless-steel column (30 cm × 4.0 mm I.D.) was pre-packed with microporous silica gel bound chemically with octadecyl chains (μBondapak C_{18}, Waters Assoc.).

The reversed-phase ion-pair chromatographic technique was used for the separation. The mobile phase consisted of water–ethanol (52:48) containing disodium hydrogen orthophosphate (0.1%, w/v) and tetrabutylammonium bromide (0.5%, w/v), adjusted to pH 7.8.

Extraction procedure and sample preparation

A 1-ml volume of plasma and 0.5 ml of 0.5 *N* hydrochloric acid saturated with ethyl acetate were placed in a test-tube and the solution was extracted with 6 ml of ethyl acetate for 10 min. After centrifugation at 2000 *g* for 10 min, 5 ml of the organic layer was transferred into another tube. The solvent was evaporated to dryness under reduce pressure, the residue was dissolved in 0.5 ml of ethanol and 10 μl of this solution were passed through 0.45-μm membrane filter was injected into the liquid chromatograph.

RESULTS AND DISCUSSION

The chromatogram of a sample extracted from rabbit plasma, which was prepared by adding an equal amount of flufenamic acid and its metabolites to produce a concentration of 4 μg/ml, is shown in Fig. 1.

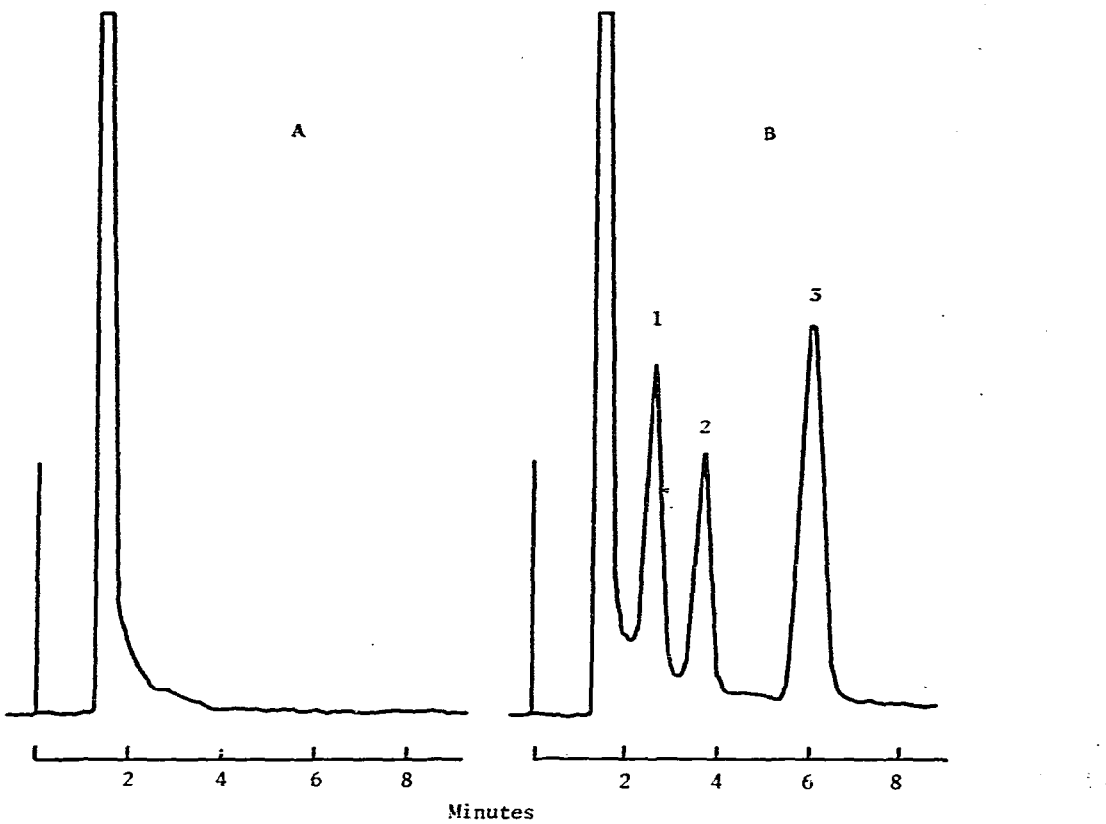

Fig. 1. (A) Chromatogram of a blank plasma extract. (B) Chromatogram of a plasma extract containing added flufenamic acid and its metabolites at concentrations of 4 μg/ml. Peaks: 1 = 4′-hydroxyflufenamic acid; 2 = 5-hydroxyflufenamic acid; 3 = flufenamic acid.

The values of log k' were −0.12 for 4′-hydroxyflufenamic acid, 0.19 for 5-hydroxyflufenamic acid and 0.51 for flufenamic acid. The technique separated the target compounds from the physiological compounds in rabbit plasma.

Calibration graphs for flufenamic acid, 4'-hydroxyflufenamic acid, and 5-hydroxyflufenamic acid in plasma were constructed over the range 1.0–10.0 μg/ml (Fig. 2). Three solutions of unchanged drug and its metabolites prepared in the same manner as the calibration standard solutions were analysed. The mean recoveries for the three compounds were 98.8 ± 2.0%, 97.0 ± 2.1% and 98.0 ± 0.8%, respectively.

Fig. 3 illustrates a typical chromatogram from plasma after oral administration of 200 mg of flufenamic acid to a rabbit, indicating that two metabolites appeared in the plasma. Table I gives the concentration of flufenamic acid, 4'-hydroxyflufenamic

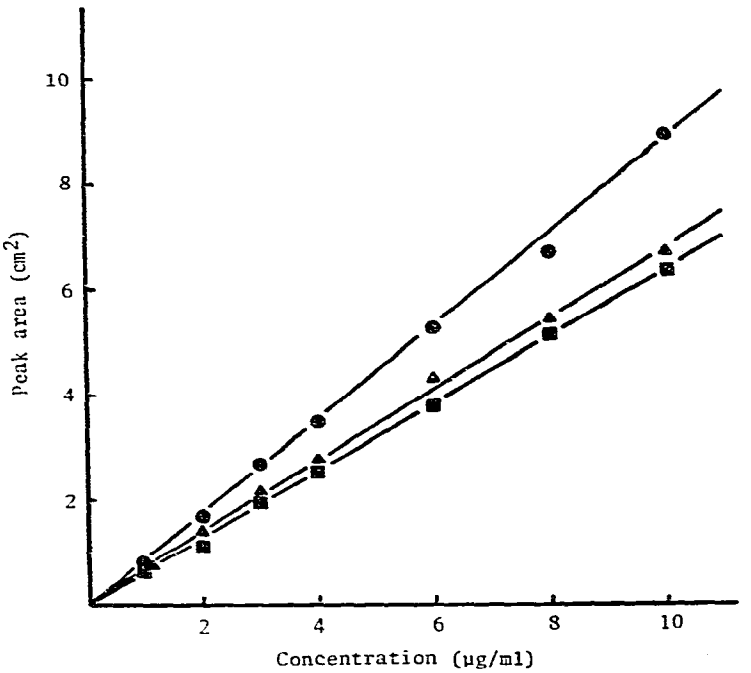

Fig. 2. Calibration graphs for the determination of flufenamic acid and its metabolites in rabbit plasma. ●, Flufenamic acid; ▲, 4'-hydroxyflufenamic acid; ■, 5-hydroxyflufenamic acid.

TABLE I

PLASMA LEVELS (μg/ml) OF FLUFENAMIC ACID AND ITS METABOLITES AFTER ORAL ADMINISTRATION OF 200 mg OF FLUFENAMIC ACID TO RABBITS

I = Flufenamic acid; II = 4'-hydroxyflufenamic acid; III = 5-hydroxyflufenamic acid. ND = Not detected.

Period after administration (h)	*Rabbit A*			*Rabbit B*		
	I	*II*	*III*	*I*	*II*	*III*
1	1.37	ND	1.94	0.39	ND	0.67
2	1.53	0.05	2.14	4.68	0.08	3.97
4	2.34	0.23	3.65	2.14	ND	2.97
6	1.35	0.31	1.59	5.96	0.20	5.98
12	1.55	0.14	1.54	3.87	0.43	5.42
24	1.96	0.55	3.51	2.20	0.56	4.15
32	0.89	0.29	1.94	1.53	0.53	3.01

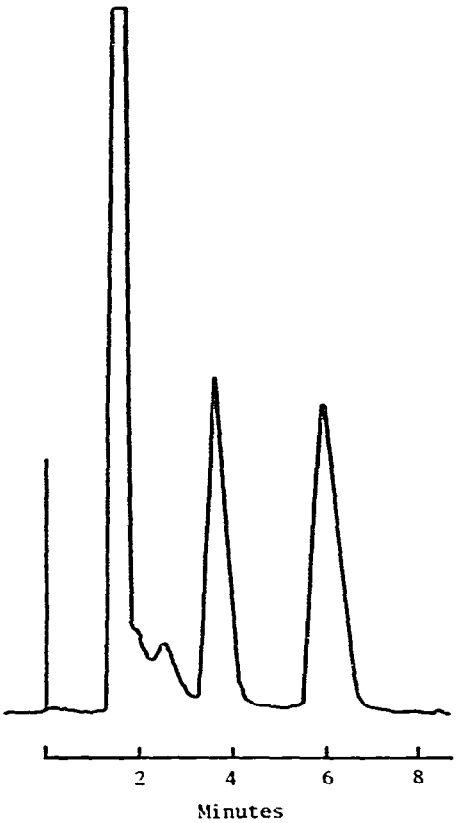

Fig. 3. Typical chromatogram of a plasma extract after oral administration of 200 mg of flufenamic acid to a rabbit (6 h after administration to rabbit B).

acid and 5-hydroxyflufenamic acid as a function of time found in plasma from two rabbits.

Glazko[1] reported 5,4′-dihydroxyflufenamic acid as another metabolite in human urine, but we could not detect it in rabbit plasma. Studies on the metabolic disposition of flufenamic acid in other species of animals are in progress.

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REFERENCES

1 A. J. Glazko, *Ann. Phys. Med.*, 9 (1967) 23.
2 H.-D. Dell and R. Kanp, *Arch. Pharm. Ber. Dtsch. Pharm. Ges.*, 303 (1970) 785.
3 W. Schmollack and U. Wenzel, *Pharmazie*, 29 (1974) 583.
4 Y. Hattori, T. Arai and E. Fujihira, *Chem. Pharm. Bull.*, 18 (1970) 1063.
5 H.-D. Dell, J. Fiedler and B. Washe, *Arzneim.-Forsch.*, 27 (1977) 1312.