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Note

Quantitative determination of flufenamic acid in rat plasma and uterus by gas chromatography

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Flufenamic acid (FA) [N(α,α,α-trifluoro-*m*-tolyl)anthranilic acid] (Fig. 1) shows anti-inflammatory activity in the usual pharmacological tests at doses which have low toxicity in laboratory animals[1]. Its therapeutic properties, similar to those of aspirin and phenylbutazone, have led to its use in patients with rheumatoid arthritis[2].

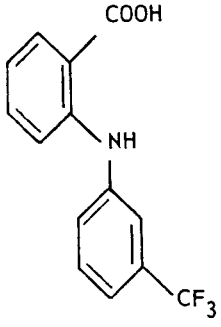

Fig. 1. Structural formula of flufenamic acid.

It is well known that FA and indomethacin inhibit biosynthesis of prostaglandins[3] and have a beneficial effect on primary dysmenorrhea in young women[4]. These reports induced us to determine FA in rat uterus and in plasma after intravenous and oral treatment. The metabolic disposition of FA has been studied in some detail using the [^{14}C]carboxyl-labelled drug[5]. We have developed a method for quantitative analysis of FA using gas–liquid chromatography (GLC).

Because of the strong polarity of FA it usually has to be derivatized to make it suitable for gas chromatographic analysis. In the gas chromatographic determination of acidic drugs in plasma it is often impossible to extract them from plasma in sufficient amounts without also extracting interfering compounds. Many methods for

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the derivatization of acidic drugs have been described[6,7] but the extraction and clean-up of the sample have attracted less attention[8,9].

EXPERIMENTAL

Chemicals and reagents

FA was obtained from Simes (Milan, Italy). The solvents used were of analytical-reagent grade (Carlo Erba, Milan, Italy). N-Nitroso-toluo-4-sulphomethylamide for diazomethane synthesis[10] was purchased from Fluka (Buchs, Switzerland). Diazomethane was prepared according to an earlier method[11].

Animals

Female Sprague Dawley rats (Charles River, Calco, Como, Italy) weighing 170–190 g were used in all experiments.

Drug administration

Intravenous treatment. FA was dissolved in diethylacetamide–Tween 80–water (0.1:1:3.9) and injected intravenously at a dose of 10 mg/kg. Control animals were given the same volume of the solvent mixture alone.

Oral treatment. Rats, fasted for 16 h before the experiment, were given FA, suspended in a constant volume of 0.5% carboxymethylcellulose (CMC) at a dose of 100 mg/kg. Control animals were given the same volume of vehicle.

At different intervals after drug administration, the animals were decapitated and blood was collected in heparinized test tubes. The uterus was removed with its tubes and stored in ice until analysis.

FA extraction procedure and derivatization

To 1 ml of plasma and 1 ml of 0.25 *M* acetate buffer (pH 4.35) 3 ml of benzene were added. Each sample was shaken mechanically for 10 min and centrifuged at 3500 *g* for 10 min. The extraction was repeated three times, and the combined benzene phases were evaporated to dryness under vacuum in a rotary evaporator. The residue was dissolved in 0.5 ml of methanol and methylated with 3 ml of an ether solution of diazomethane. The sample was dried in a rotary evaporator, and the residue dissolved in an acetone solution of the internal standard 3-chloro-6-aminobenzophenone (100 μg/ml).

For FA extraction from uterus, 0.25 *M* acetate buffer (pH 4.35) was added (1:8, w/v) to the tissue, which was homogenized in a glass–glass Potter homogenizer. 1 ml of the resulting suspension was extracted three times with 3 ml of benzene, and the procedure was continued as described for plasma.

Gas chromatography

Gas chromatographic analysis was carried out using a Fractovap 2300 gas chromatograph (Carlo Erba) equipped with a flame ionization detector (FID). The stationary phase was 3% OV-17 on Gas-Chrom Q (100–120 mesh) packed into a glass column (3 m × 2 mm I.D.). The column temperature was 230° and carrier gas (nitrogen) flow-rate was 40 ml/min. FID sensitivity was 1 μg/ml plasma and 5 μg/g uterus. For quantitative analysis the internal standard technique was used.

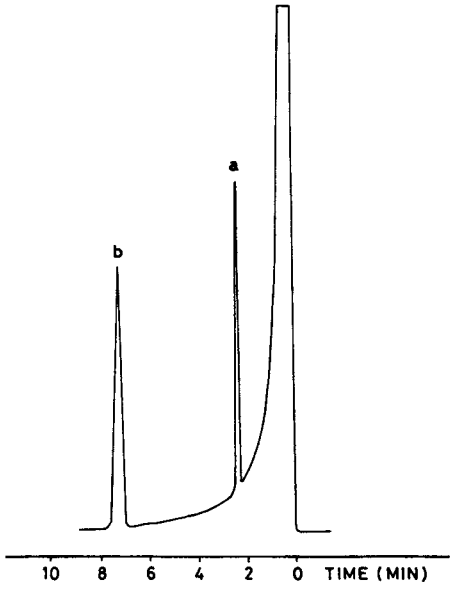

Fig. 2. Typical gas chromatogram of a plasma extract. (a) Flufenamic acid; (b) 3-chloro-6-amino-benzophenone.

RESULTS AND DISCUSSION

A typical gas chromatogram of plasma extract injection is given in Fig. 2. GLC coupled with mass spectrometry was employed to ascertain the identity of FA with the gas chromatographic peak. A mass spectrum of the characteristic gas chromatographic peak of FA is presented in Fig. 3.

The calibration graph for FA is shown in Fig. 4. The results show good linearity in the range examined. The recovery of FA from plasma and uterus is shown in Table I. Addition of FA to the plasma at concentrations ranging from

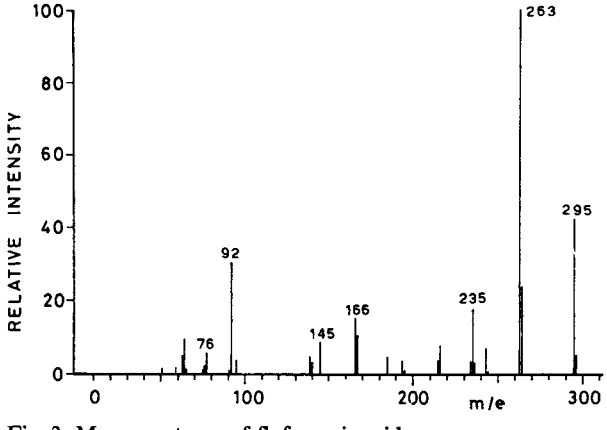

Fig. 3. Mass spectrum of flufenamic acid.

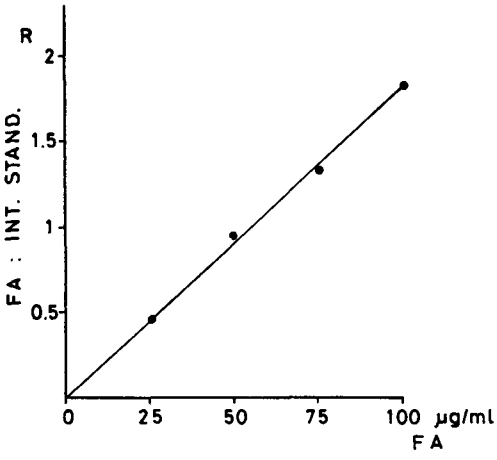

Fig. 4. Calibration curve of flufenamic acid. R = ratio of the peak areas of flufenamic acid and its internal standard.

10 to 100 µg/ml resulted in overall recovery of 98.2%. Addition of FA to the uterus (15 µg/ml) resulted in overall recovery of 90%.

FA levels in rat plasma and uterus after 10 mg/kg i.v. injection are given in Table II. The maximum concentration of FA in plasma and uterus was reached *ca.* 10 min after injection. Thereafter the concentrations progressively decreased to 6.7 µg/ml plasma measured 3 h after treatment.

TABLE I

RECOVERY OF FLUFENAMIC ACID STANDARD THROUGH A COMPLETE EXTRACTION PROCEDURE

Flufenamic acid	*Amount (µg)*			*Recovery (%)*
	Added	*Recovered**		
		Day 1	*Day 2*	
Plasma	10	9.90 ± 2.31	9.83 ± 2.31	98.6
	50	49.40 ± 0.87	49.70 ± 0.84	99.0
	100	97.27 ± 3.38	96.88 ± 3.81	97.0
Uterus	15	13.98 ± 1.07	13.05 ± 1.75	90.1

* Mean ±S.D. of four determinations.

TABLE II

PLASMA AND UTERUS LEVELS OF FA AFTER i.v. INJECTION OF 10 mg/kg TO RATS

Minutes after injection	*Plasma (µg/ml)*	*Uterus (µg/g)*
10	37.38 ± 2.172	14.6 ± 1.962
20	30.66 ± 1.662	10.49 ± 2.83
30	18.39 ± 1.68	6.30 ± 0.061
60	15.56 ± 1.067	5.25 ± 0.07
120	11.06 ± 0.7	<4
180	6.79 ± 0.555	<4

TABLE III

PLASMA AND UTERUS LEVELS OF FA AFTER ORAL ADMINISTRATION OF 100 mg/kg TO RATS

Hours after administration	*Plasma (μg/ml)*	*Uterus (μg/g)*
0.5	57.52 ± 3.28	20.18 ± 3.94
1	96.73 ± 28.69	28.18 ± 9.42
2	101.47 ± 10.79	21.79 ± 11
4	53.25 ± 19.96	15.90 ± 4.06
8	70.60 ± 11.51	17.13 ± 2.76
16	51.57 ± 10.7	12.66 ± 2.01
24	13.85 ± 8	<4

The disappearance curve of FA in uterus was similar to that in plasma. No drug was detectable in uterus 3 h after i.v. injection (the analytical limit of sensitivity is 5 μg/g).

Table III sets out the plasma and uterine levels after oral administration of FA. The curve of FA plasma disappearance after oral treatment is again similar to that found in the uterus, FA levels in the latter being lower than in plasma. These results show that FA reaches the uterus, where it may exert its inhibitory effect on prostaglandin synthesis[12]. FA determination in plasma and uterus using this procedure is faster than other methods, while the sensitivity is comparable with other GLC methods[6].

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