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Note

Determination of sodium flavodate in body fluids by high-performance liquid chromatography

Application to clinical pharmacokinetic studies

L. ZECCA, L. GUADAGNI and S.R. BAREGGI*

Istituto di Farmacologia, IV Cattedra, Via Vanvitelli 32, 20129 Milan (Italy)

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Sodium flavodate (disodium-5-phenyl-5,7-dioxyacetate-benzo-γ-pyrone, Pericel®, LIRCA (Farmaceutici, Limito, Milan, Italy) is a synthetic hydrosoluble derivative of natural flavonoids (Fig. 1) [1]. Sodium flavodate (SF) has been shown to reduce capillary fragility and permeability [1—3].

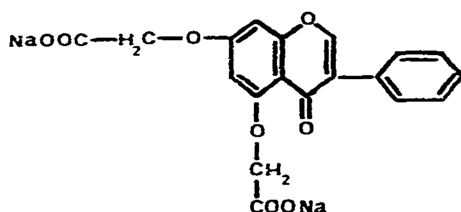

Fig. 1. Chemical structure of sodium flavodate.

Whereas gas and liquid chromatographic methods for the quantitation of flavonoids in natural products, such as quercitine in tea leaves and soy beans, have been described, no methods for the determination of these compounds in plasma and/or urine can be found in the literature [4—9].

In the present work we describe a high-performance liquid chromatographic (HPLC) method for the determination of SF in plasma and urine and its application to the study of SF pharmacokinetics in man.

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EXPERIMENTAL

Reagents and standard solutions

Sodium flavodate was supplied by LIRCA, and 1-naphthylacetic acid (NAA, internal standard) was obtained from Fluka (Buchs, Switzerland). Stock solutions at a concentration of 1 mg/ml were prepared in distilled water containing 1 mg/ml $NaHCO_3$. Working standard solutions, at a concentration of 100 μg/ml, were prepared by dilution in water.

Methanol, phosphoric acid, hydrochloric acid and diethyl ether were analytical grade reagents (Merck, Darmstadt, G.F.R.).

Apparatus and chromatographic conditions

A Perkin-Elmer (Norwalk, CT, U.S.A.) series 2/2 high-pressure liquid chromatograph equipped with a Perkin-Elmer LC-75 variable-wavelength UV detector and Autocontrol system was used.

The column was a LiChrosorb RP-18 (10 μm particle size, 25 cm × 4.0 mm I.D.) from Merck. The mobile phase was prepared by mixing 60 volumes of methanol with 40 volumes of 0.1% phosphoric acid for plasma and 57:43 for urines. A flow-rate of 1.3 ml/min and a wavelength of 268 nm were used. This wavelength was chosen as this gives the best signal-to-noise ratio on the UV spectrum recorded by the Autocontrol system.

Samples were introduced via a syringe into a Rheodyne 7105 (Berkeley, CA, U.S.A.) injection valve.

Procedure

Into a 12-ml tube were placed 1 ml of plasma or urine, 300 μl of hydrochloric acid (18.5%), 100 μl of the internal standard solution and 7 ml of diethyl ether, and the tubes were shaken on a reciprocal shaker for 10 min. After centrifugation at 2000 rpm for 15 min, 6 ml of the organic phase were transferred to other 12-ml tubes and evaporated to dryness under a gentle flow of nitrogen at room temperature. The residues were reconstituted with 100 μl of the mobile phase and 10—20 μl were injected into the chromatograph.

In addition to the unknown samples, plasma and urine calibration standards containing 0.1, 0.5, 1, 3, 5, and 10 μg of SF and 10 μg of NAA were prepared and processed as above. A calibration curve was constructed by plotting the SF concentrations versus the ratio of SF to the NAA peak heights for both plasma and urine.

Typical chromatograms of plasma and urine extracts are shown in Figs. 2 and 3.

Recovery

Percentage recovery was calculated by comparing the peak heights of SF standards prepared in mobile phase solutions with those obtained by injecting plasma and urine calibration standards at the same concentrations.

Subjects and pharmacokinetic measurements

Six healthy male volunteers (23—33 years, 59—90 kg) took two tablets containing 500 mg of sodium flavodate with 100 ml of water at 8.00 a.m. after 12

TABLE I

PLASMA CONCENTRATIONS (μg/ml) AFTER ORAL ADMINISTRATION OF 1 g OF SODIUM FLAVODATE

Subjects	Weight (kg)	Hours after administration							AUC 0—24 h ($\mu g\ h\ ml^{-1}$)
		0.5	1	2	4	8	10	24	
RP	62	2.20	1.01	0.70	0.51	0.04	0.06	<0.05	6.01
MS	68	1.70	0.51	0.52	0.45	0.30	0.35	0.25	8.56
PV	90	0.45	0.50	0.55	0.55	0.20	0.20	<0.05	4.52
SQ	72	0.85	0.73	0.55	0.48	0.25	0.12	<0.05	4.60
LG	59	3.00	3.41	2.00	1.50	0.72	0.45	0.18	17.52
LZ	77	1.39	0.77	0.56	0.38	0.30	0.23	0.16	6.96
Mean ± S.E.	71.3 ± 4.5	1.60 ±0.40	1.15 ±0.46	0.81 ±0.24	0.64 ±0.17	0.36 ±0.08	0.23 ±0.06	0.12 ±0.03	8.04 ±1.994

h of fasting. Blood samples were drawn 0.5, 1, 2, 4, 6, 10 and 24 h later and 0—12 h and 12—24 h urines were collected. Blood samples were immediately centrifuged and the plasma transferred and stored at —30°C until analysis.

The following pharmacokinetic parameters were calculated from results in Table I. Elimination constant rate (K_e) and half-life were determined by linear regression analysis of the concentrations between 6 and 24 h. The experimental area under the curve ($AUC^{0\to n}$) was measured by the trapezoidal rule and $AUC^{0\to\infty}$ by adding the extrapolated term $C(n)/K_e$ to $AUC^{0\to n}$, where $C(n)$ is the last determined plasma concentration.

RESULTS

Chromatograms of blank plasma and urine extracts do not show interfering peaks and both SF and NAA peaks were well separated. Retention times of SF and NAA were 6.0 and 7.5 min, respectively, for plasma, and 7.5 and 9.5 for urine analysis (Figs. 2 and 3). With this method, SF could be assayed quantitatively over a wide range of concentrations with a linear relationship between 0.1 and 20 μg/ml, and a recovery from either plasma or urine of 95 ± 7%. The limit of sensitivity of the method (50 ng/ml) was sufficient for pharmacokinetic studies of SF.

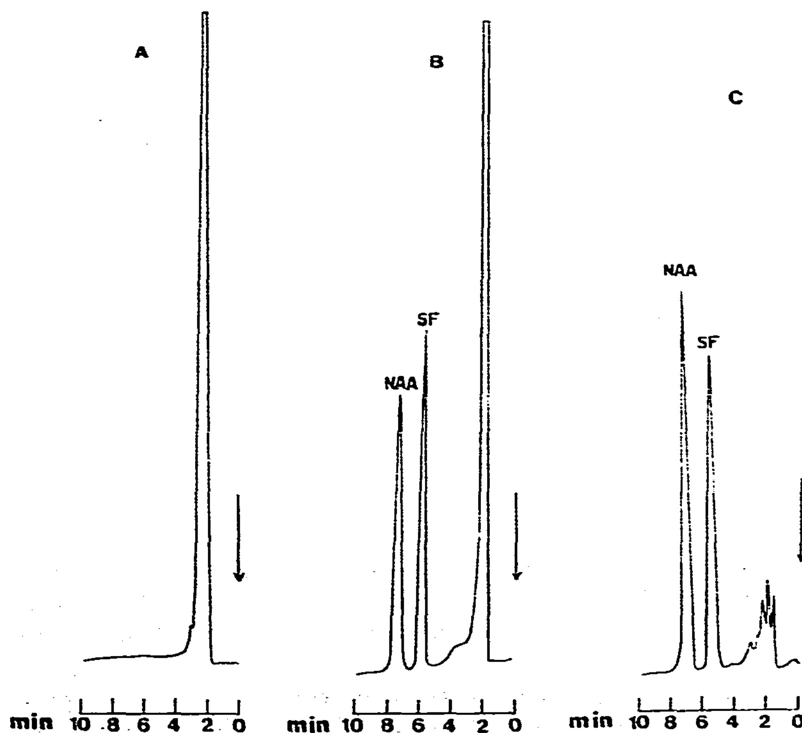

Fig. 2. Chromatograms of HPLC analysis of plasma extracts: (A) human control plasma; (B) human control plasma containing 5 μg/ml SF and 10 μg/ml NAA (internal standard); (C) plasma of a subject receiving 1 g of SF and containing 10 μg/ml NAA.

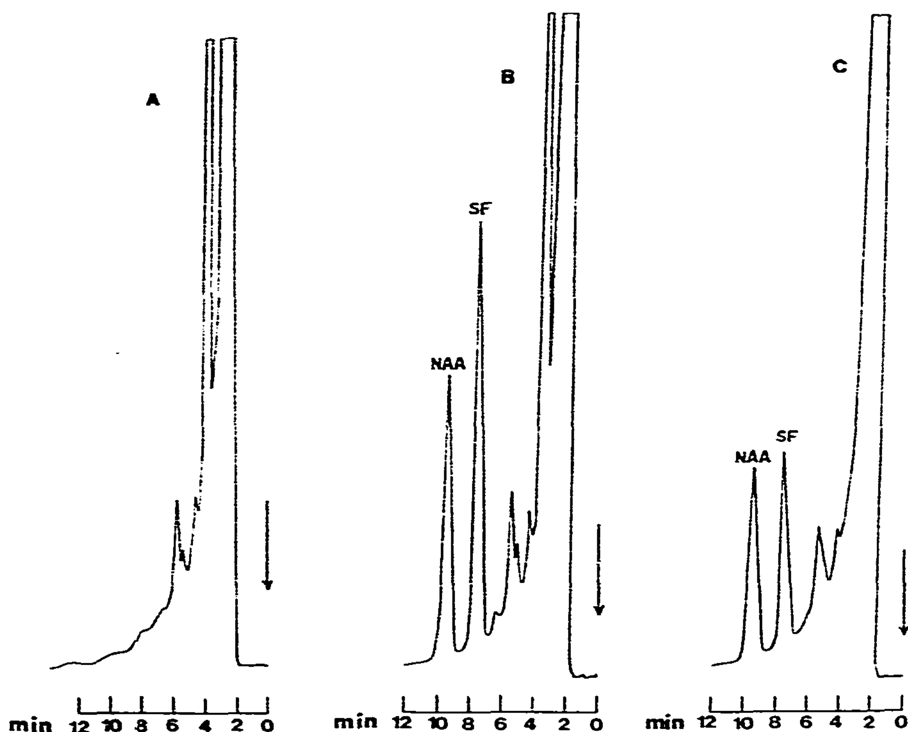

Fig. 3. Chromatograms of HPLC analysis of urine extracts: (A) human control urine; (B) human control urine containing 5 μg/ml SF and 10 μg/ml NAA (internal standard); (C) urine of a subject receiving 1 g of SF and containing 10 μg/ml NAA.

Peak concentrations (1.6 ± 0.4 μg/ml, $\bar{X}$ ± S.E.) were found 30 min after oral administration of SF. Thereafter, levels decayed biphasically with a mean elimination rate (K_e) of 0.057 and a half-life of 12.1 h (Fig. 1). The mean $AUC^{0\to\infty}$ was 10.2 ± 2.0 μg h ml^{-1}. A linear relationship was found between body weight and both peak plasma concentration and AUC ($r = 0.69$ and 0.64, $p < 0.05$).

The amount of unconjugated SF in urine was 4.6 ± 0.6 mg in the first 12 h and 0.9 ± 0.2 mg in the following 12 h. Thus, cumulative excretion of SF in the 24 h after dosing represents less than 1% of the administered dose.

DISCUSSION

A simple and sensitive HPLC method for the determination of sodium flavodate in plasma and urine is described. The method can be used for quantitative determination of SF and has been shown to be suitable for pharmacokinetic studies.

The HPLC method presents several advantages over the gas—liquid chromatographic (GLC) methods described for some natural flavones (e.g. quercitine) [8, 9]. The HPLC procedure is more sensitive and simpler than the GLC one, avoiding the necessity of derivatizing the hydroxyl and carboxyl groups. Moreover, for SF we found that the trimethylsilyl derivative used for the GLC

analysis of quercitine was unstable and therefore unsuitable for the GLC analysis of SF [8].

The determination of plasma concentrations of SF showed rapid absorption of the compound after oral ingestion, the peak concentration being observed at 0.5 h. Thereafter, the concentration decayed biphasically with a mean elimination half-life of 12 h. The peak concentration and the AUC for plasma values were quite low in view of the large dose administered. Since there are no data on the bioavailability of the drug after oral administration, the low levels might be interpreted to indicate poor absorption or a large volume of distribution.

In favor of this latter interpretation there are the results obtained by Gazave [2], who showed that SF is firmly bound to the lecithin of vascular membranes. If this were so, a large amount of the drug would be extracted from the circulation with the consequence of increasing the apparent volume of distribution of the drug.

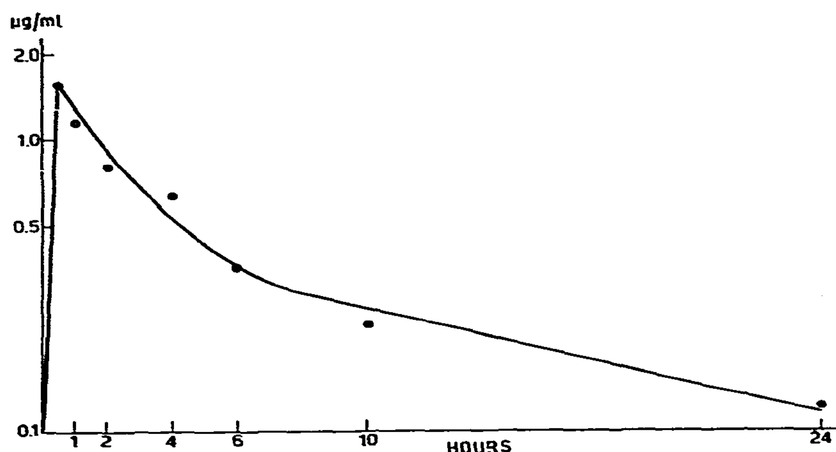

Fig. 4. Time—course curve of mean plasma levels of six subjects who took 1 g of SF orally.

The amount of unconjugated SF excreted in the urine in the first 24 h was less than 1% of the administered dose. This can be interpreted in the same way as the plasma levels, but, alternatively, one could suppose that SF was metabolized extensively and excreted as one or more metabolites. We have no data about how SF is metabolized and no metabolites have been identified so far in urine or other biological fluids, including bile. On the other hand, Gazave [2] suggested that SF could be rapidly transformed to active metabolites such as flavanol, but evidence supporting this is still incomplete. In our experiment, the chromatograms of urine extracts did not show at any time peaks that could be attributed to SF metabolites. Of course, this is not sufficient to disprove metabolism, since the procedure was not set up to determine these compounds.

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