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Note

High-performance liquid chromatographic determination of 2-mercapto-propionylglycine (thiopronine) in urine

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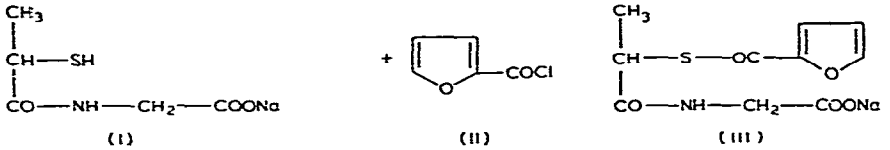
Thiopronine (2-mercapto-propionylglycine or 2-thiol-propionamidoacetic acid) is utilized as a hepatotropic [1—3], radioprotective [4] and mucolytic [5—7] drug. The substance has been detected in biological fluids using the amino group—free thiol method [8] or the radioactive ^{35}S-labelled compound [9].

This work describes a more specific and sensitive method suitable for bioavailability studies. Thiopronine (I) sodium salt reacts quantitatively in a pH 7.0 buffer with 2-furoyl chloride (II) to obtain 2-furoyl-thiopronine (III) [10]. Compound III is quantitatively determined by high-performance liquid



chromatography (HPLC). This determination is carried out on urine samples after administration of thiopronine. In this case the interfering substances present in urine are partially eliminated through percolation on a glass column.

EXPERIMENTAL

Materials

The solvents used are all of HPLC grade (LiChrosolv, from Merck, Darmstadt, G.F.R., or Carlo Erba, Milan, Italy). The water was previously bidistilled

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through a glass distiller and filtered on a 0.45-μm membrane (type FH, Millipore). The other reagents are all of analytical grade.

Chromatographic conditions

The high-performance liquid chromatograph is a Varian Model 5000, equipped with a Varian UV 50 detector, a Varian CDS 111L integrator, a Varian 9176 recorder, and a Rheodyne Model 7125 injector. The column used is packed with LiChrosorb RP-18 10 μm supplied by Brownlee Labs. (Santa Clara, CA, U.S.A.); the mobile phase is McIlvaine buffer pH 3.0—acetonitrile (80:20, v/v). The buffer at pH 3.0 is prepared by mixing 15.89 ml of 0.1 *M* citric acid with 4.1 ml of 0.2 *M* disodium phosphate. The flow-rate is 0.8 ml/min, pressure 37 bars, wavelength 290 nm, and the quantity injected is 20 μl.

Standards

Prepare a standard solution of thiopronine in distilled water at a concentration of 1000 μg/ml. From this solution prepare standard solutions in water or urine diluting 1 ml to 10 ml with water or urine. The standard solutions are stable for at least one week if kept at +4°C. To determine the calibration curve and method sensitivity prepare dilutions of thiopronine from 50 to 1 μg/ml in urine.

Procedures

Prepare a chromatographic glass column having an internal diameter of 10 mm, equipped with porous septum, packed with 2 g of LiChrosorb RP-18 with an average diameter of 40—60 μm (Merck). The column so prepared is washed with 5 ml of acetonitrile (LiChrosorb, Merck) and successively with 5 ml of distilled water; the washings are discarded. Apply to the column 1 ml of a standard solution of thiopronine in water or 1 ml of a standard solution in urine, and elute with 5 ml of distilled water. Add to the eluate (collected in a 15-ml capped test tube) 1 ml of buffer pH 7.0 (prepare a 0.5 *M* Na_2HPO_4 solution and adjust the pH to 7.0 with 85% H_3PO_4), stir on a Vortex for 1 min, and then add, under a hood, 1 drop of 2-furoyl chloride (Fluka, Buchs, Switzerland); stir again for 1 min. Add distilled water up to 10 ml, stir and filter on a 0.5-μm Millipore membrane. The solution is ready to be injected.

Quantitative evaluation

In the analysis of thiopronine levels in the urine, peak areas were compared with those of an aqueous solution of thiopronine standard. No internal standard was used owing to the very simple procedure.

Animal study

Male rats (Wistar albino, Morini), weighing 200 g and fasted overnight were used. The animals were treated intravenously or orally with a 100 mg/kg dose of thiopronine dissolved in water. Urine samples were collected 24 h after administration, stored at 4°C and analysed within a week.

RESULTS AND DISCUSSION

Fig. 1 shows a typical high-performance liquid chromatogram of compound III obtained from the reaction of sodium thiopronine and 2-furoyl chloride. Fig. 2 shows a typical chromatogram of thiopronine extracted from urine of treated rats. Fig. 3 shows the profile of urine of rats not treated and not passed through the glass column; and Fig. 4 shows the profile of urine of rats not treated but passed through the glass column. The importance of this passage is obvious to reduce interference between the peaks of substances present in the urine and the peak of thiopronine. The urinary recovery for thiopronine using the described procedure is 95.0 ± 4.3%. The mean inter-assay and intra-assay variability for the compound was 5.38 and 4.76, respectively

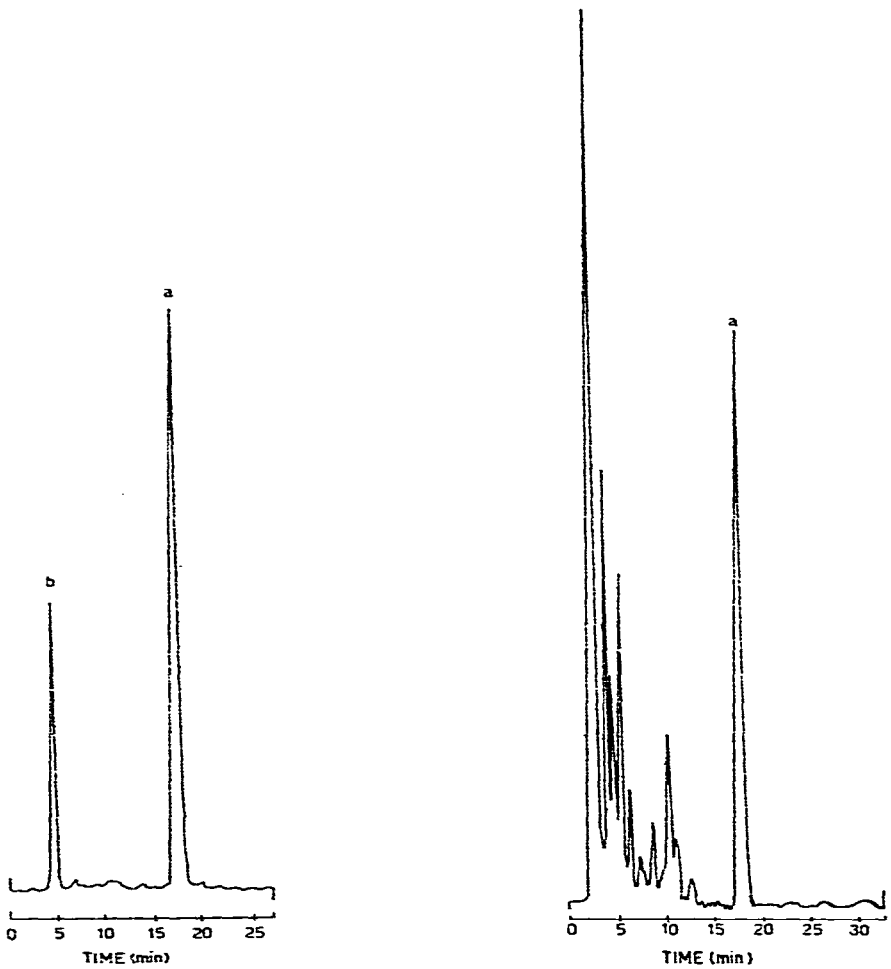

Fig. 1. Chromatogram of compound III prepared from water. a = 2-Furoyl-thiopronine (III), b = 2-furoyl chloride (II) in excess.

Fig. 2. Chromatogram of compound III obtained from urine of treated rat. a = 2-Furoyl-thiopronine (III).

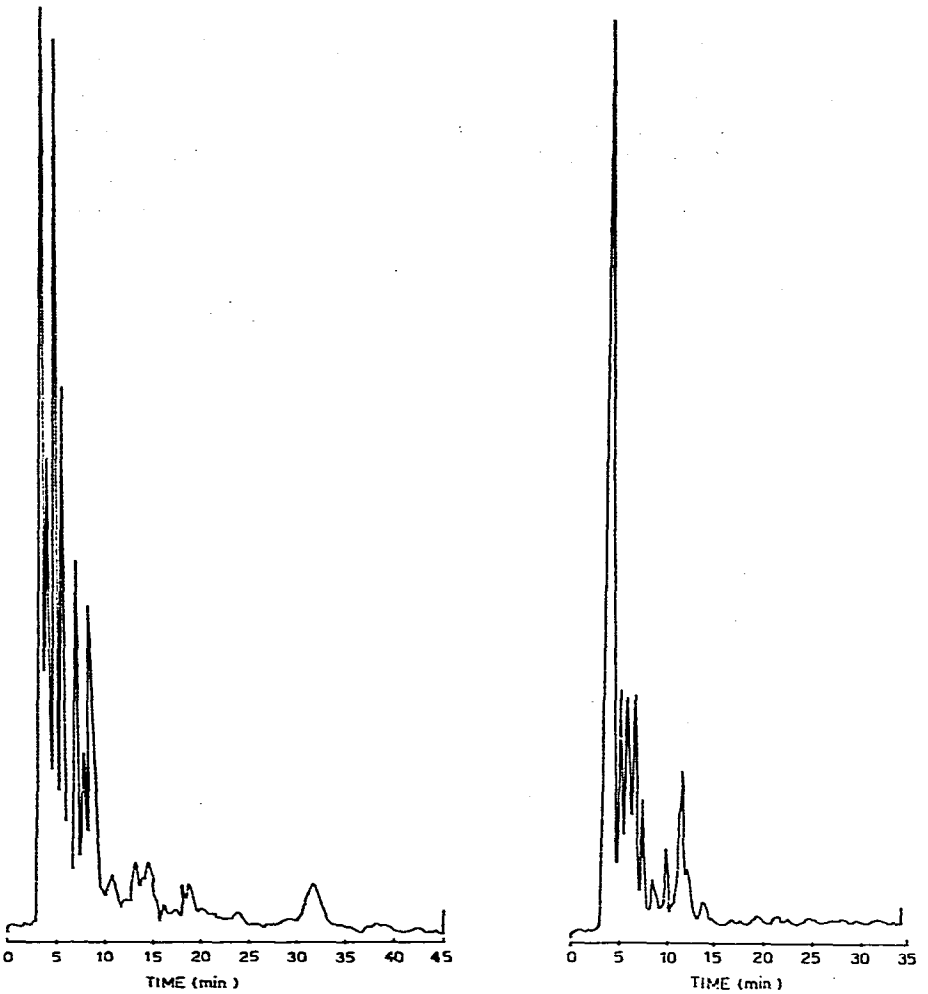

Fig. 3. Chromatogram of blank urines not passed through the glass column packed with 2 g of LiChrosorb 40—60 μm (Merck).

Fig. 4. Chromatogram of blank urines passed through the glass column packed with 2 g of LiChrosorb 40—60 μm (Merck).

(Table I). The minimum detectability of the drug with the described procedure is 1 μg/ml of urine.

The calibration curve for thiopronine shows a good linearity over the concentration range 1—50 μg/ml both in water and in urine. The relationship between thiopronine urine concentration in the range 1—50 μg/ml and the peak area is: peak area (instrument) = 3.0528 × concentration (μg/ml) + 0.03348. The correlation coefficient is 0.9999.

The first results of bioavailability studies on animals are reported in Table II. More extensive results on animals and on humans will be reported soon. In conclusion, the proposed method is suitable for a sensitive and reproducible quantitative evaluation of thiopronine in urine.

TABLE I

RECOVERY AND INTRA- AND INTER-ASSAY VARIABILITY DATA

Aliquots (1 ml) of control urine were spiked with 100 μl of standard solutions and treated as described in the Experimental section. Recoveries were determined from HPLC peak areas from one set of spiked samples. Intra-assay variability was determined from three sets of spiked samples that were extracted and analyzed in one day. Inter-assay variability was determined from three sets of spiked samples that were extracted and analyzed on three different days.

Amount added to 1 ml of urine (μg)	Recovery (%)	Inter-assay variability			Intra-assay variability		
		Amount found (μg/ml)		Coefficient of variation (%)	Amount found (μg/ml)		Coefficient of variation (%)
		Mean	± S.D.		Mean	± S.D.	
1	89	0.90	0.06	6.67	0.93	0.05	5.38
2	93	1.88	0.11	5.85	1.96	0.09	4.59
5	99	5.16	0.27	5.23	5.08	0.24	4.72
10	101	10.19	0.61	5.99	10.09	0.55	5.45
20	97	19.56	0.98	5.01	19.92	1.00	5.02
35	91	34.82	1.42	4.08	35.02	1.32	3.77
50	95	51.07	2.48	4.86	50.85	2.22	4.37
Mean ± S.D.	95.0±4.3			Mean: 5.38			Mean: 4.76

TABLE II

URINARY EXCRETION (0—24 h) OF THIOPRONINE AFTER INTRAVENOUS OR ORAL TREATMENT WITH 100 mg/kg IN THE RAT

Treatment	Rat No.	Weight (g)	Urine volume (ml)	Urinary excretion of thiopronine (0—24 h)		
				Concentration (μg/ml)	Total excretion (mg)	Excretion/administration (%)
Intravenous	1	300	14.5	415.42	6.024	20.08
	2	295	13.5	552.83	7.463	25.30
	3	300	13.8	480.45	6.630	22.10
Oral	4	255	14.0	4.59	0.064	0.25
	5	250	12.5	4.38	0.055	0.22
	6	285	13.5	1.61	0.022	0.08

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