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Note

Simple method for the determination of L-5-hydroxytryptophan in plasma by high-performance liquid chromatography

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Tissue levels of serotonin can be elevated by administration of its immediate precursor, L-5-hydroxytryptophan (5-HTP) [1], which, in contrast to the amine, is able to cross the blood–brain barrier This technique has been used in therapy and 5-HTP has been administered for therapeutic purposes in epilepsy [2], schizophrenia [3], depression [4,5] and Lesch–Nyhan syndrome [6].

Thus the estimation of 5-HTP levels in plasma of humans and animals is of interest 5-HTP was determined by many fluorimetric methods [7–11] and by high-performance liquid chromatography (HPLC) with fluorimetric [12–17] and electrochemical [18–20] detection However, only a few studies reported plasma levels in humans [21–23] and in animals [24]

This paper describes a simple HPLC method for the determination of 5-HTP in plasma and some results after oral administration to rats

EXPERIMENTAL

Reagents and materials

The solvents used were all of HPLC grade (LiChrosolv, Merck, Darmstadt, F R.G or Carlo Erba, Milan, Italy) Water was twice distilled using a glass distillation apparatus and filtered through on a 0 45-μm membrane (Type HAWP, Millipore) The 5-HTP standard was prepared in our laboratories, the other reagents were all of analytical grade

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High-performance liquid chromatography

The apparatus used was a Varian Model 5000, equipped with a UV-100 detector, Vista Model 401 printer–plotter computer integrator and Valco automatic injection valve. The column (250 mm × 4 mm I.D.) was packed with LiChrosorb RP-NH_2, 10 μm. The temperature of the column was 18°C. The mobile phase was 0.01 *M* monobasic potassium phosphate–acetonitrile (21:79, v/v). The flow-rate was 1.0 ml/min, the volume injected was 10 μl and the detection wavelength was 230 nm.

Standard solution

A standard solution of 5-HTP was prepared at a concentration of 500 μg/ml in water and stored at 4°C. The solution was diluted in the mobile phase to a final concentration of 1–100 μg/ml and a 10-μl aliquot was injected into the chromatograph. The calibration graph was obtained by adding known amounts of 5-HTP to rat plasma. These standards were treated as described below.

Assay procedure

To 1 ml of plasma was added 1 ml of acetonitrile. The mixture was stirred on a vortex mixer for 1 min and centrifuged at 3000 *g* for 10 min. A 10-μl aliquot of the supernatant was injected into the chromatograph.

Quantitative analysis

The 5-HTP content in plasma was determined by external calibration. No internal standard was used owing to the very simple procedure.

Animal study

Male Sprague–Dawley rats (Charles River), weighing 200 g and fasted overnight, were used. The animals were treated orally with 200 mg/kg 5-HTP dissolved in 0.167 *M* hydrochloric acid (5 ml/kg). Blood samples were withdrawn before and 0.5, 1, 2, 3, 4 and 6 h after the administration.

RESULTS AND DISCUSSION

Fig. 1 shows typical chromatograms of 5-HTP in plasma obtained under our experimental conditions. The 5-HTP peak is not fully resolved from an endogenous component in the plasma. However, peak areas can be correctly integrated with a computer integrator. In our hands, the retention time of 5-HTP was found to be 6.70 ± 0.19 min and that of the endogenous component was 7.20 ± 0.22 min (mean ± S.D.). Some precision problems can arise if this method is used without a computer integrator. Under the same conditions the retention times of serotonin and tryptophan were 4.53 and 5.68 min, respectively.

In order to check the validity of the method proposed a known amount of 5-HTP was added to rat plasma, and its recovery was determined by HPLC

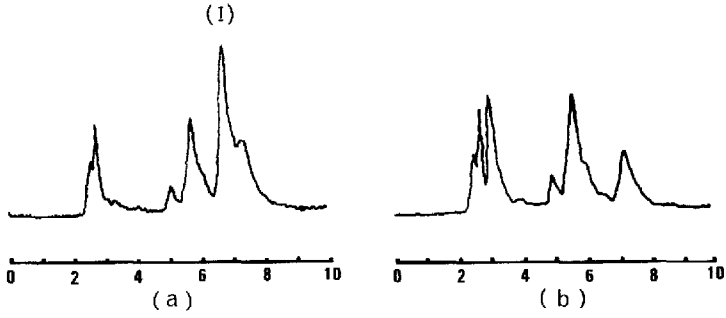

Fig 1 High-performance liquid chromatograms obtained from (a) plasma spiked with 5-hydroxytryptophan (I) (40 μg/ml) and (b) drug-free plasma Chromatographic conditions are as described in Experimental

TABLE I

RECOVERY OF 5-HYDROXYTRYPTOPHAN ADDED TO RAT PLASMA

Concentration (μg/ml)		Recovery (mean ± S D , $n=5$) (%)
Added	Found	
2 00	1 92	96 00 ± 4 90
10 00	9 80	98 00 ± 4 58
25 00	25 12	100 48 ± 3 45
75 00	75 76	101 01 ± 3 12

As reported in Table I, the recovery of 5-HTP from plasma was 96 00–101 01%. The accuracy was within 4 0% (Table I) The calibration graph was linear over the range 1–100 μg/ml, the relationship between 5-HTP plasma concentrations in this range and the peak areas is expressed as $y=2616x-774$, where x is the concentration of 5-HTP injected expressed as μg/ml and y is the peak area The correlation coefficient was $r=0\ 99947$ The detection limit of 5-HTP was estimated to be 1 0 μg/ml, at a signal-to-noise ratio of ca 6 1. The day-to-day reproducibility obtained from five determinations showed values between 3 35 and 5 56%, the within-day reproducibility, also obtained from five determinations, showed values between 3 09 and 5.10% The day-to-day reproducibility was similar throughout the time the column was used

The results of bioavailability studies in animals are reported in Table II The mean pharmacokinetic parameters were obtained with a structural model of two exponentials after extravascular bolus without lag time model using peeling algorithm (Siphar program). The mean values are: C_{max} (maximum concentration) = 36 37 ± 6.22 μg/ml; T_{max} (time to reach maximum concentration) = 1 0 h; $t_{\frac{1}{2}elim}$ (elimination half-life) = 1 59 h; $t_{\frac{1}{2}abs}$ (absorption half-life) = 0 36 h; $AUC_{0-\infty}$ (area under the curve) = 138 06 mg h l^{-1}, total clearance/F = 0 29 l h^{-1}, distribution volume/F = 0 66 l

The method described is very useful to study the pharmacokinetics of

TABLE II

PLASMA LEVELS OF 5-HYDROXYTRYPTOPHAN IN RATS AFTER ORAL ADMINISTRATION (200 mg/kg)

Time after administration (h)	Plasma level (mean ± S D , $n=4$) (μg/ml)
0 5	32 81 ± 5 41
1	36 37 ± 12 45
2	33 30 ± 8 60
3	27 23 ± 3 72
4	13 19 ± 9 29
6	4 55 ± 3 27

5-HTP in animals and humans This method can be greatly improved both in specificity and sensitivity by using a fluorescence or electrochemical detector instead of a UV spectrophotometer, in agreement with the results of other methods [15–20], if the 5-HTP has to be measured in very low amounts and in different biological tissues

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