Plasma tryptophan and tyrosine concentrations: determination using high performance liquid chromatography and fluorometric detection

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Overview

Tryptophan is an essential amino acid and the precursor for the synthesis of the indoleamine, serotonin, or 5-hydroxy-tryptamine. Tyrosine, a nonessential amino acid, is synthesized from phenylalanine and the precursor for catecholamine synthesis (dopamine, norepinephrine, and epinephrine). This assay takes advantage of the natural fluorescence of tyrosine and tryptophan and uses HPLC with fluorescent detection to rapidly quantitate their concentrations in blood. An aliquot of plasma is deproteinized with perchloric acid, the supernatant automatically injected into a HPLC reversed phase column, and the fluorescent emission quantitated. Application of this technique may be extended to other body fluids.

Reagents

7.5 N Perchloric acid (Mallinckrodt, Paris, KY, USA). Bring 64.4 ml 70% perchloric acid to 100 ml final volume with distilled water.

Mobile phase: *0.01 M sodium acetate* (Fisher Scientific, Fair Lawn, NJ, USA) pH 4.0, and 6% methanol, HPLC Grade (Fisher Scientific, Fair Lawn, NJ, USA). Dissolve 1.64 g sodium acetate in 1500 ml distilled water. Adjust pH to 4.0 with glacial acetic acid (Fisher Scientific, Fair Lawn, NJ, USA). Add distilled water to a final volume of 1880 ml. Filter and degas by suction. Add 120 ml HPLC grade methanol.

Standard solutions

Tyrosine stock standard. 100 μg/ml L-tyrosine 0.1% ascorbic acid (Fisher Scientific, Fair Lawn, NJ, USA). Dissolve 12.0 mg L-tyrosine methyl ester hydrochloride (Sigma, St. Louis, MO, USA) and 100 mg ascorbic acid in 100 ml distilled water. This solution should be aliquoted and frozen.

Tryptophan stock standard. 100 μg/ml L-tryptophan (Sigma, St Louis, MO, USA) 0.1% ascorbic acid (Fisher Scientific, Fair Lawn, NJ,

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Modified from Anderson, G.M., Young, J.G., and Cohen, D.J. (1979). Rapid liquid chromatographic determination of tryptophan, tyrosine, 5-hydroxyindoleacetic acid and homovanillic acid in cerebrospinal fluid. *J. Chromatogr.* **164**, 501–505.

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USA). Dissolve 10 mg L-tryptophan and 100 mg ascorbic acid in 100 ml distilled water. This solution must be aliquoted and frozen.

Working standard. 10 μg/ml L-tyrosine, 10 μg/ml L-tryptophan, 0.285 N perchloric acid. Use 1 ml of 100 μg/ml L-tyrosine, 1 ml of 100 μg/ml L-tryptophan and 0.38 ml of 7.5 N perchloric acid. Bring to a final volume of 10 ml with distilled water. This must be made fresh daily.

Procedure

1. Blood is collected in heparinized tubes, centrifuged at 4°C, and plasma separated from the red cells.
2. 250 μl of plasma is mixed with 10 μl of 7.5 N perchloric acid and centrifuged at 8,800g for 5 min.
3. Inject 25 μl onto HPLC.
4. HPLC system:
   Column (Bio Rad, Richmond, CA, USA) Bio-Sil ODS-5S, 250 mm × 4 mm
   Flow rate 1.0 ml/min, pressure of 2,500-3,000 PSI
   Retention times of 3.9 min for tyrosine and 12.7 min for tryptophan.
5. Fluorescence detector:
   Hewlett Packard (Los Angeles, CA, USA) Programmable Fluorescence Detector HP1046
   Optimum wavelengths:

Tyrosine:	Excitation wavelength 225 nm
	Emission wavelength 302 nm
Tryptophan:	Excitation wavelength 225 nm
	Emission wavelength 350 nm

Discussion

The fluorometric method of Denckla and Dewey[1] or revised procedures[2-4] for the assay of tryptophan are most commonly used for the determination of tryptophan concentrations in biological materials. Tryptophan and tyrosine have also been quantitated using amino acid analyzers, ion exchange chromatography, or post-column derivatization on an HPLC.[5-7] All of these methods are sensitive, but they are time-consuming and require relatively large sample volumes.

The development of reversed phase HPLC has improved the analytical methods and shortened the time for quantitative determination of amino acids. Recently, methods using liquid chromatography with fluorometric detection have been reported for the separation of tryptophan and its metabolites in biological materials.[8-11] Fluorometric measurements offer advantages over other commonly used detection systems in terms of sensitivity and selectivity. Moreover, since few compounds possess natural fluorescence, interferences are not encountered as often as with the less selective detection systems. The method we describe here is rapid and simple, selective and sensitive, and uses only a small amount of sample.

The deproteinization process has been recognized as a prerequisite step for amino acid analysis of plasma samples. Different methods have been used to deproteinize biological fluids.[12] In the method we describe here, the choice has been perchloric acid. We have obtained similar results using 5-sulfosalicylic acid. Both tryptophan and tyrosine analysis have been consistent and reproducible. This assay is able to detect 0.5 nmol/ml of tyrosine and tryptophan (equivalent to 12.5 pmoles applied to the HPLC). Values obtained by this method in normal healthy adults on regular diets have been 40–65 nmol/ml and 30–55 nmol/ml for tyrosine and tryptophan, respectively. In parallel with the samples, a pooled plasma sample with a known amount of tyrosine and tryptophan is run to check the consistency and reproducibility of the procedure. Coefficient of variation is <2%.

The stock standard solution of tryptophan is unstable over time unless

stored at −80°C. At this temperature the standard can be stored indefinitely.

If unable to change wavelengths during a run, a good wavelength to use for emission wavelength for both tyrosine and tryptophan is 335 nm.

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