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Note

Method for the determination of tryptophan in serum and cerebrospinal fluid

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Recently, high-performance liquid chromatography (HPLC), coupled with fluorimetric detection, has been shown to be useful for the analysis of indoles in physiological samples [1]. In this paper, a simple and rapid method is described based on the HPLC—fluorimetric system for the determination of tryptophan (Trp) in serum and cerebrospinal fluid (CSF).

EXPERIMENTAL

Equipment

The system consisted of a Spectra Physics 740B high-pressure pump, a Valco 7000 p.s.i. injection valve, a 100 mm × 4.6 mm I.D. column for the serum samples and a 150 mm × 4.6 mm I.D. column for the CSF samples, both packed with Nucleosil C_{18} (5 μm particle size), and a Schoeffel FS 970 fluorescence detector. The excitation wavelength was 282 nm; a 370-nm cut-off filter was used on the emission side. The mobile phase was a 10 mmol/l acetate solution (pH 4.0) containing 14% (v/v) methanol.

An Amicon Model 12 ultrafiltration cell and Diaflo XM50 filters were used for the ultrafiltration of serum.

Chemicals

Trp and α-methyltryptophan (αMTrp) were purchased from Sigma (St. Louis, Mo., U.S.A.). Standard solutions of Trp and αMTrp were prepared in water and stored at +4°. All other chemicals used were of an analytical grade.

Procedure

Human serum was obtained from healthy volunteers; human lumbar CSF was obtained from subjects under various neurological investigations.

To 1.2 ml of serum were added 45.1 nmol αMTrp and 0.1 ml of a 1.0%

sodium dodecylsulfate solution. The mixture was shaken and allowed to stand for 10 min. Thereafter, 1.2 ml of a 20% trichloroacetic acid solution was added. The mixture was shaken and centrifuged at 2000 *g* for 5 min. The supernatant was transferred to a clean tube and 10 μl were injected into the chromatographic system.

For the analysis of free Trp the albumin-bound fraction was separated by ultrafiltration. Serum (3.0 ml) was pipetted into the ultrafiltration cell. The system was rinsed with 5% carbon dioxide in nitrogen and a pressure of 75 p.s.i. was applied. When the first drop had passed, 50 μl of the filtrate were collected. A 10-μl aliquot was injected into the chromatographic system.

The CSF samples were shaken and 60 μl injected into the chromatographic system.

Quantitation was achieved by preparing standard samples in water. The standard samples were analysed as described above for CSF and serum.

RESULTS AND DISCUSSION

The chromatograms (Fig. 1) from the analysis of Trp (free and total) in human serum and CSF showed that no interfering peaks are present. The determination of total serum Trp involved precipitation of serum proteins. To correct for losses, αMTrp was used as an internal standard. For the analysis of the free Trp portion in serum, the ultrafiltrate was injected into the system without further treatment. Only a small volume (<5%) was allowed to pass the filter in order not to affect the equilibrium of free and bound Trp. The CSF required no treatment prior to injection.

The data from the determination of the reproducibility of individual analyses (Table I) showed that the total serum Trp and CSF Trp could be determined with experimental errors of less than 4%. The determination of the free Trp portion in serum showed a greater variability (11%), induced by the ultra-

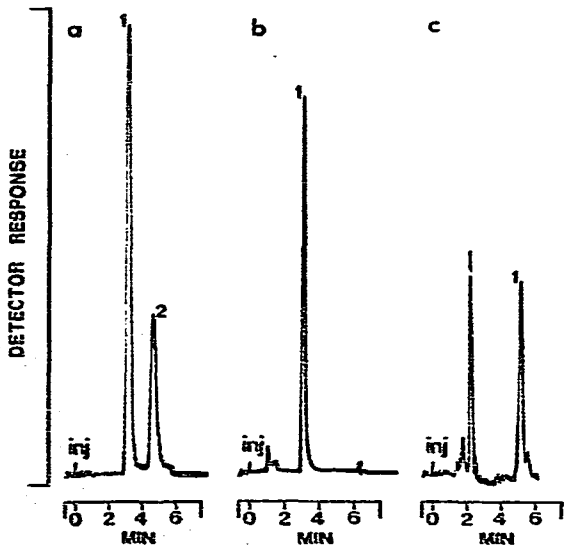

Fig. 1. Chromatograms from the analysis of (a) total serum Trp, (b) free serum Trp, and (c) CSF. Conditions were as given in the Experimental section. 1 = Trp; 2 = αMTrp.

TABLE I

REPRODUCIBILITY OF INDIVIDUAL ANALYSES

	Amount (nmol/ml ± S.D.)	%	*n*
CSF	2.04 ± 0.6	3.0	10
Serum (free)	17.9 ± 2.0	11	8
Serum (total)	68.3 ± 2.2	3.3	10

filtration. The levels reported here are in agreement with those in earlier publications [2].

In conclusion, the sensitivity and specificity of the HPLC—fluorimetric system allowed a simple and rapid method to be developed for the determination of Trp in serum and CSF.

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