

A RAPID HPLC ASSAY FOR THE DETERMINATION OF PLASMA SEROTONIN. USE IN DIAGNOSIS OF CARCINOID TUMOR.

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INTRODUCTION

The development of a reversed-phase HPLC assay, requiring 400 μ l of plasma, utilizing a single-step extraction and a chromatographic separation of 8.0 min, for the determination of plasma serotonin (5-HT) is described.

5-HT is one of the vasoactive substances secreted by carcinoid tumor and its measure is an important tool to increase the sensitivity of the diagnosis for 5-HT overproduction.

We developed a specific and accurate liquid chromatographic method with amperometric detection after a simple preparation step in plasma.

METHODS

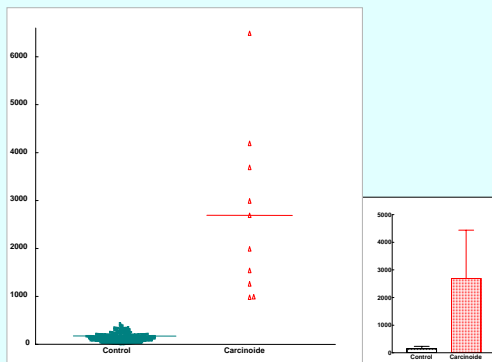
An isocratic system consisted of a Model P200 pump (Spectra Series) and a Model LC 4B amperometric detector with a glassy carbon electrode at a potential of + 700 mv (Bioanalytical System) was used.

The mobile phase consisted of phosphate / citric acid buffer (0.15 M pH 4.5) containing acetonitrile 8 % v/v that was pumped at 0.8 ml/min through a 250 X 4.6 mm C 18 analytical column with 5 μ m beads. (Waters Associates).

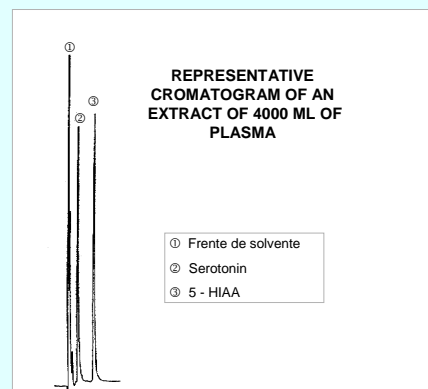
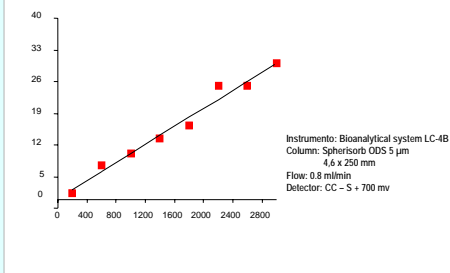
PROCEDURE

Blood was collected by venipuncture into plastic tubes containing EDTA K3 as anticoagulant, samples were kept at room temperature and centrifuged to obtain plasma.

10 μ l of 12 M perchloric acid were added to 400 μ l of plasma to deproteinize the sample and after vortex-mixing, the tubes were centrifuged at 3000 RPM and 4° C for 10 minutes; 20 μ l were injected into the chromatographic system.



PEAK HEIGHTS FOR SEROTONIN IN A PLASMA SAMPLE TO WHICH INCREASING AMOUNTS OF SMT HAD BEEN ADDED
EACH POINT IS THE AVERAGE OF TWO DETERMINATIONS



SPIKING RECOVERY

Two spiking solutions (A&B) were made to represent 4.0 and 20 μ g/ml respectively. A 50 μ l aliquot of each of these solutions were spiked into 1950 μ l aliquots of plasma. Then, the samples were desproteinized with HClO_4 without modifying the serum matrix.

Sample ng/ml	Spiking	Observed	Expected	% Recovery
120		120		
	A (4ng/ml)	210	220	95
	B (20ng/ml)	590	620	95

Within day precision (n=10) CV= 5 %
Day to day precision (n=10) CV= 12%

CONCLUSION

This HPLC method, with amperometric detection is simple, rapid, reliable and allows study serotonin metabolism in plasma and laboratory evaluation of patients with suspected carcinoid tumor