

SEROTONIN (5-HYDROXYTRIPTAMINE) IN SERUM BY FLUORIMETRY - FAST CODE Z15110

BIOCHEMISTRY (synthesis and metabolism)

Serotonin (M.W.=176,22) is a biological compound that presents an indolic group as structural domain. It derives from the bio-transformation (hydroxylation and decarboxylation) of tryptophan. The steps in serotonin production are: formation of 5-hydroxytryptophan by tryptophan hydroxylase and production of 5-hydroxytryptamine (or serotonin) by aromatic L-aminoacid decarboxylase. Serotonin catabolism is caused by monoamine oxidase, forming 5-hydroxyindolacetic acid that is excreted by urine as free molecules or linked with glucuronic acid. (fig.1).

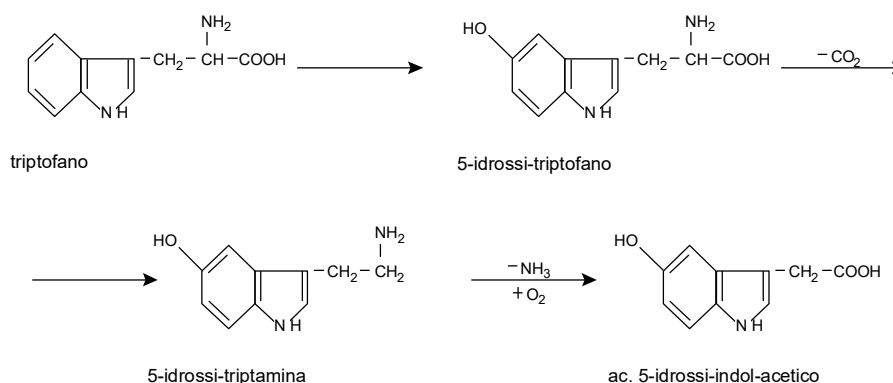


Figura 1: Metabolismo del triptofano.

PATHOLOGY

Carcinoid tumors excrete increased quantities of serotonin causing the carcinoid syndrome. The major features of this syndrome are diarrhea, paroxysms of flushing, bronchospasm and cardiac disease. Diagnosis can be established by measuring the urinary excretion of 5-hydroxyindolacetic acid and/or by the assay of plasma serotonin.

 EUREKA S.R.L. LAB DIVISION
Via M. D'Antona 28
60033 Chiaravalle (AN) - Italy
Tel. +39 071 7450790
eureka-support@sentinel.it
www.eurekakit.com



This product fulfills all the requirements of Directive 98/79/EC on in vitro diagnostic medical devices (IVD). The declaration of conformity is available upon request.

N° 003	Serotonin in serum by Fluorimetry - FAST	04/2025
--------	--	---------

TECHNICAL FEATURES

Principle of the Method :

Serotonin or 5 hydroxytryptamine or 5 HT is separated treating the serum/plasma with a suitable precipitant. After addition of a stabilization solution, 50 µl of the eluate is directly injected into HPLC.

Recovery : >98 %

Sensitivity (LLOD) : 3 µg/l

Minimum Concentration Analyzable (LLOQ): 10 µg/l

Linearity : 2,0 – 5.000 µg/l

Normal Values: 80 – 160 µg/l

Accuracy intra serie (relative error %) :

Ci	Cs
50 µg/l	150 µg/l
7,2%	3,5%

Accuracy inter serie (relative error %) :

Ci	Cs
50 µg/l	150 µg/l
10,0%	3,8%

Reproducibility intra serie (coefficient of variation %):

CI	Cm	Cs
40 µg/l	100 µg/l	200 µg/l
8,2%	5,5%	2,7%

Reproducibility inter serie (coefficient of variation %):

CI	Cm	Cs
40 µg/l	100 µg/ml	200 µg/ml
10,8%	6,7%	3,1%

Coefficient of correlation R² + Dev Std 0,9911 ± 0,0021

Components of the kit :

All the components are ready-to-use. Stability: 3 years at room temperature (≤ 20° C).
The Calibrator is stable 36 months at 2–8 °C.

Reagent A – Deproteinization Solution with Internal Standard, **1 x 40 ml**

Reagent B – Stabilization Solution, **1 x 20 ml**

Reagent C – Test Solution, **1 x 5 ml**

Store at 2-8 °C

Plasma Calibrator Iyophil., **1 x 2 ml**

Code Z15016 (packed separately – see data sheets)

Reagent M – Mobile Phase, **3 x 500 ml**

Minimum Instrumental equipment required:

Isocratic HPLC System with loop of 10 μ l
Fluorimetric Detector λ_{EX} =285 nm λ_{EM} =344 nm

Plasma Collection Procedure and serum storage:

Tubes suitable for sampling are those that promote the coagulation process, such as those containing separator gel, micronized silica, kaolin, etc. The use of tubes with anticoagulants (e.g., citrate, heparin, EDTA) must be avoided. After venous collection, the tube must be gently inverted 5 to 6 times to activate the coagulation process. Centrifugation must be performed no earlier than 1 hour and no later than 2 hours after collection, following the manufacturer's instructions for the tube to ensure complete separation of the cellular component from the serum. The obtained serum must be transferred to appropriate storage tubes (PET, PE, etc.) and stored at -20 °C until analysis. Under these conditions, the sample remains stable for at least 2 months from collection.

NOTE: If a blood sample collected with an anticoagulant arrives at the laboratory, it can still be analyzed by centrifuging it at less than 150 rpm or allowing it to settle spontaneously. This is because 5-HT (Serotonin) is primarily contained in platelets, which remain suspended in plasma only if centrifuged at a speed lower than 150 rpm.

Pre-analytic step:

Defrost and analyse the sample rapidly.

WARNING:

Increased or decreased number of blood platelets can cause false pathological values of serotonin. The ratio between plasma serotonin/platelets count can avoid these misinterpretations of the results.

PREANALYTICAL PROCEDURE

Test Solution Preparation.

Pipette in a tube :

- 900 µl of H₂O HPLC grade
- 100 µl of **Reagent C – Test Solution**

Vortex for 10 seconds

INJECTION :

- Inject 10 µl of this solution in the chromatographic system.

Verify that the Test Solution has retention time similar to fig. 2. If the Test is all right you can start with the analytical procedure; if not, check the functionality of the analytical system.

Important : Don't use this solution to calibrate!

ANALYTICAL PROCEDURE

STEP 1 : Calibrator, Samples and Controls Preparation

Dispense in a suitable tube :

	Blank	Calibrator	Sample
Water HPLC grade	200 µl		
Calibrator		200 µl	
Sample			200 µl
Reagent A – Deproteinization Sol. + internal standard	400 µl	400 µl	400 µl

Warning: deproteinize directly on Vortex for at least 10 seconds

STEP 2 : Centrifuge at 14.000 rpm for 10 min.

STEP 3: Pipette in a tube 200 µl of clear supernatant and add 200 µl of **Reagent B – Stabilization Solution.**

Vortex for 10 seconds

N.B.: at this step, the sample is stable 3 days at 2-8 °C

INJECTION :

- Inject 10 µl of this solution in the chromatographic system.

SEROTONIN in SERUM - Warnings

REAGENT C : TEST SOLUTION

SEROTONIN	About 3.000 µg/l
------------------	------------------

FLUORIMETRIC DETECTOR PARAMETERS

λ	285 nm
λ	344 nm
GAIN	1 x 100

HPLC COLUMN PROTECTION

To save the analytical column Reverse Phase POROSHELL 120 EC C-18 4,6 x 50 mm, 2,7 µm, the use of Javelin Colfilters (1 x 10 pcs.) cod. S90199511 is obligatory.
--

HPLC COLUMN CONDITIONING

Install a <u>new</u> analytical C ₁₈ Reversed-Phase column POROSHELL 120 EC-C18 (50 x 4,6 mm, 2,7 µm). Disconnect the detector and filter 30 ml of H₂O : Methanol (20 : 80 v / v) set flow of 1,0 ml/minute. Don't recycle the washing solutions. Flux a solution of H₂O : Methanol (80 : 20 v / v) at flow of 1,0 ml/minute for 1 hour. Don't recycle the washing solutions. Condition the column with the mobile phase at a flow of 1,0 ml/minute for 30 minutes and discharge the first 20 ml. Finally inject the Chemical Standard and verify the quality of the HPLC run. It is NOT possible to make analysis at recycling phase. If room temperature is > 20 °C store the Mobile Phase at 2-8 °C between an analytical session and another.
--

COLUMN CLEANING

Disconnect the detector. Flux 15 ml of H₂O : Acetonitrile or Methanol (80 : 20 v / v) set flow of 1,0 ml/minute and discharge. Flux a solution of H₂O : Acetonitrile or Methanol (30 : 70 v / v) for 30 minutes discharge it. It is recommended to perform the washing of Analytical Column at the end of each analytical run. Store the column in a solution of H₂O : Acetonitrile or Methanol (30 : 70 v / v).

INJECTION NEEDLE WASHING

Wash with a solution of Water / Ethanol or Methanol or Acetonitrile (80 : 20 v/v).
--

HPLC PARAMETERS

LOOP	10 µl
FLOW	1,0 ml/min.
PRESSURE	About 130 bar

ACCESSORIES AND CONSUMABLES

CODE	DESCRIPTION	PACKAGING
Z15016	Calibrator in serum for Serotonin	4 x 2 ml
Z15019	Control in serum for Serotonin, Levels 1 and 2	2 x 5 x 2 ml
Z699975902	Poroshell 120-EC-C 18 (50 x 4,6mm –2,7 µm) Analytical Column	1 Pc
S90199511	Javelin Colfilters	1 x 10 Pcs
S51843550	Clear glass vials with reduced volume from 1,5 ml to 15 µl	1 x 100 Pcs
S51820717	Caps for Clear glass vials with reduced volume from 1,5 ml to 15 µl	1 x 100 Pcs

SEROTONIN in SERUM (Reference Chromatograms)

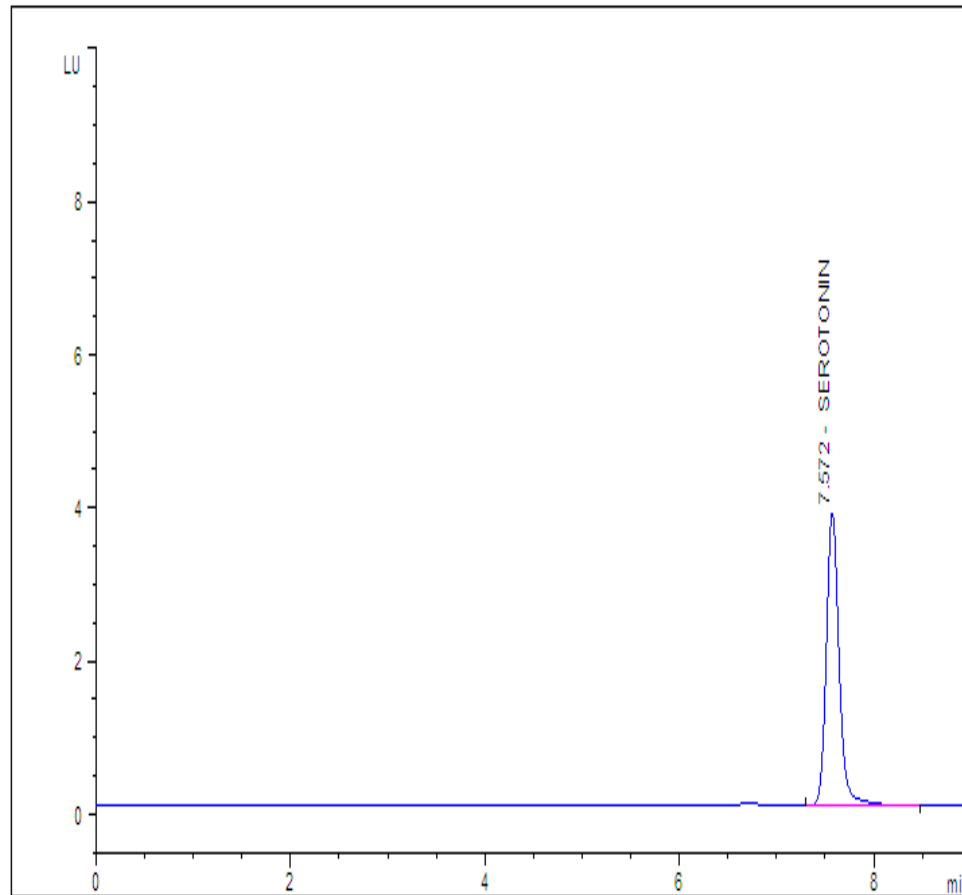


Fig. 2	Test Solution
	R.T. 7.57 Serotonin

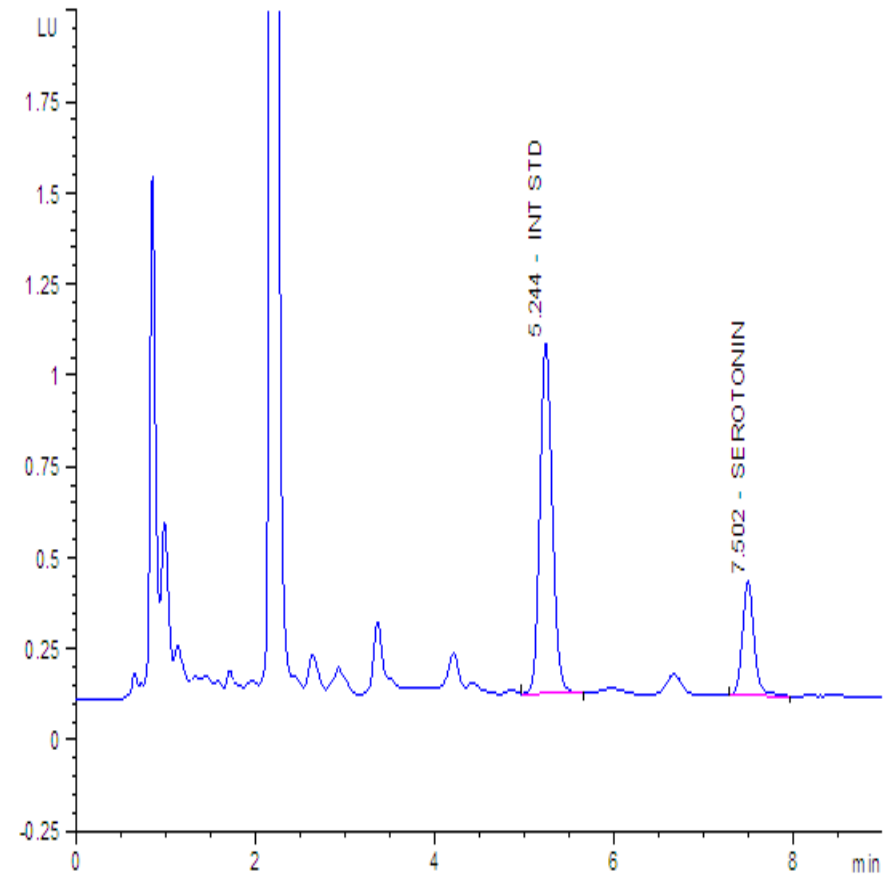


Fig. 3	Calibrator in serum
	R.T. 5.24 Internal Standard
	R.T. 7.50 Serotonin 174,5 µg/l

SEROTONIN IN SERUM

(Reference Chromatograms)

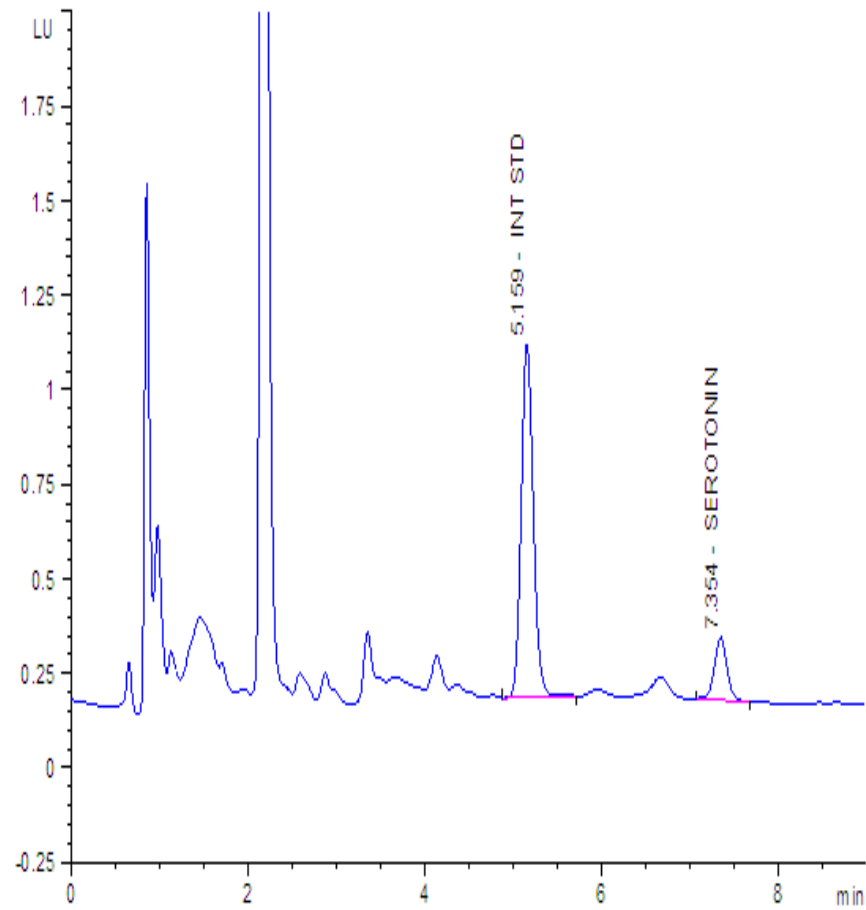


Fig. 4 *Control in serum Level 1*

R.T.	5.15	Internal Standard	
R.T.	7.35	Serotonin	101,7 µg/l

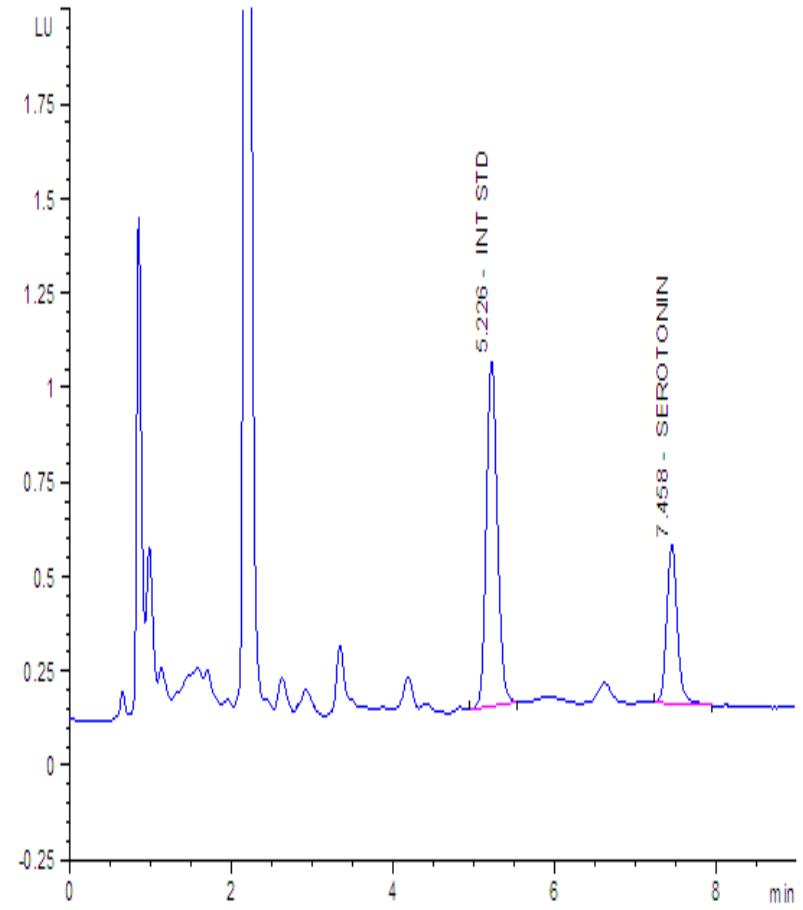


Fig. 5 *Control in serum Level 2*

R.T.	5.22	Internal Standard	
R.T.	7.45	Serotonin	253,9 µg/l