

SEROTONIN (5-HYDROXYTRIPTAMINE) IN URINE BY FLUORIMETRY – FAST - CODE Z15610

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).

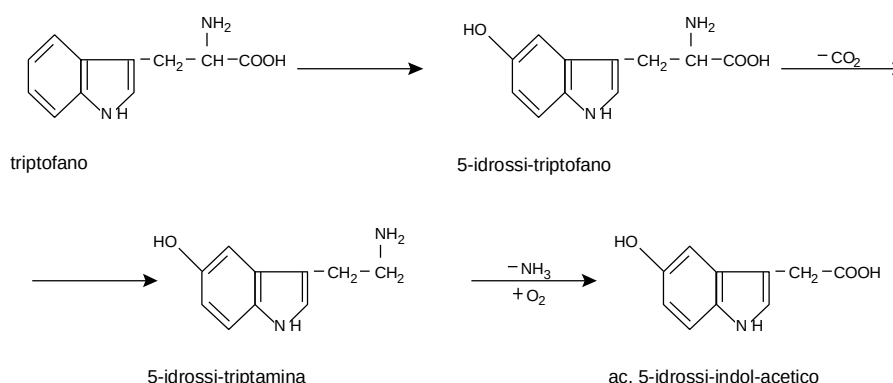


Figura 1: Metabolismo del triptofano.

PATHOLOGY

Carcinoid tumors excrete increased quantities of serotonin causing carcinoid syndrome. The major features of this syndrome are diarrhoea, 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.



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CE

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 urine by Fluorimetry - FAST	10/2024
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TECHNICAL FEATURES

Principle of the Method :

Serotonin or 5-hydroxytryptamine or 5-HT is analysed after purification with Clean-up Columns and suitable reagents. 50 µl of the eluate is directly injected into HPLC.

Recovery : >98 %

Sensitivity (LLOD) : 2 µg/l

Minimum concentration analyzable (LLOQ): 5 µg/l

Linearity: 5 – 7500 µg/l

Accuracy intra serie (relative error %) :

Ci	Cs
25 µg/l	125 µg/l
12,0%	2,7%

Accuracy inter serie (relative error %) :

Ci	Cs
25 µg/l	125 µg/l
13,0%	3,7%

Reproducibility intra serie (coefficient of variation %):

CI	Cm	Cs
5 µg/l	75 µg/l	250 µg/l
5,4%	2,0%	1,5%

Reproducibility inter serie (coefficient of variation %):

CI	Cm	Cs
5 µg/l	75 µg/l	250 µg/l
8,9%	4,4%	2,4%

Coefficient of correlation R² + Dev Std

0,9917 ± 0,0049

Normal Values in 24 h Urine:

26,6 – 182 µg/24h

Components of the kit :

Reagent A – Conditioning Solution N° 1, **1 x 120 ml**

Reagent B – Conditioning Solution N° 2, **1 x 110 ml**

Reagent C – Diluting Solution, **1 x 75 ml**

Reagent D – Wash Solution N° 1, **1 x 120 ml**

Reagent E – Wash Solution N° 2, **1 x 110 ml**

Reagent F – Eluting Solution, **1 x 120 ml**

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

Reagent H – Internal Standard Solution, **1 x 10 ml**

Reagent I – Stabilizing Solution, **1 x 40 ml**

Urine Calibrator lyophil., 1 x 5 ml

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

Clean-up Columns, 100 pcs

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.

Store at 2-8 °C

Code Z15516 (packed separately – see data sheets)

Minimum Instrumental equipment required :

Isocratic HPLC System with loop of 5 µl

24 h Urine Collection Procedure and storage:

24-hour urine must be collected into a container with 5 ml (Child) or 10 ml (Adult) of HCl 5 M for each urine litre. After collection, 10 ml of urine should be delivered to the lab with the indication of the total diuresis. **Laboratory should verify that the delivered urine has a pH between 2.5 and 3.5. If the pH is > 7 the sample may not be suitable for testing.** Delayed analyses require sample freezing at -20°C or less. Stable over 2 months.

Pre-analytic step :

Defrost and analyse the sample rapidly.

PREANALYTICAL PROCEDURE

Solution Test at 220 µg/l Preparation

Pipette in a tube:

- 750 µl of H₂O HPLC grade
- 200 µl of **Reagent G – Test Solution**
- 50 µl of **Reagent H – Internal Standard Solution**

Vortex for 10 seconds

INJECTION :

Inject 5 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 : Clean-up Columns Conditioning

Pipette in Clean-up Columns:

- 1,0 ml of **Reagent A – Conditioning Sol. N° 1** and discharge the percolated liquid
Don't let the column be dry!
- 1,0 ml of **Reagent B – Conditioning Sol. N° 2** and discharge the percolated liquid
Don't let the column be dry!

STEP 2 : Calibrator, Samples and Controls Preparation

Pipette in a tube:

	Calibrator	Samples	Controls
Reagent C – Diluting Sol.	750 µl	750 µl	750 µl
Calibrator	200 µl		
Samples		200 µl	
Controls			200 µl
Reagent H – Internal Standard Sol.	50 µl	50 µl	50 µl

Vortex for 10 seconds

STEP 3 : Samples Loading

Pipette in Clean-up Columns the samples prepared in **STEP 2:**

- 1,0 ml of **Calibrator**,
- 1,0 ml of **Sample**,
- 1,0 ml of **Control**

STEP 4: Clean-up Columns Washing

Pipette in Clean-up columns:

- 1,0 ml of **Reagent D – Wash Sol. N° 1** **Discharge the percolated liquid.**
- 1,0 ml of **Reagent E – Wash Sol. N° 2** **Discharge the percolated liquid.**

STEP 5 : Eluting

Pipette in Clean-up Columns:

- 1,0 ml of **Reagent F – Eluting Solution** **Collect the percolated liquid.**

Vortex for 10 seconds

STEP 6 : Take 200 µl of eluate and add 400 µl of **Reagent I – Stabilizing Solution**

Vortex for 10 seconds

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

INJECTION:

- Inject 5 µl of this solution in the chromatographic system.

SEROTONIN in URINE - Warnings

REAGENT G : TEST SOLUTION

SEROTONIN	About 880 µg/l
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SPECTROFLUORIMETRIC DETECTOR PARAMETERS

λ OF EXCITATION	285 nm
λ OF EMISSION	344 nm
GAIN	2 - Medium

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 new analytical C₁₈ 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 30 ml of a solution of **H₂O : Methanol (80 : 20 v / v)** at flow of 1,0 ml/minute. **Don't recycle the washing solutions.** Condition the column with the mobile phase at a flow of 1,0 ml/minute 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	5 µl
FLOW	1,0 ml/min.
PRESSURE	About 80 bar

ACCESSORIES AND CONSUMABLES

CODE	DESCRIPTION	PACKAGING
Z15516	Urine Calibrator lyophil. for Serotonin	4 x 5 ml
Z15519	Urine Control lyophil. for Serotonin, Levels 1 and 2	2 x 5 x 5 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
S29057U	Standard glass vials of 2 ml with screw cap	1 x 100 Pcs

SEROTONIN IN URINE

(Reference Chromatograms)

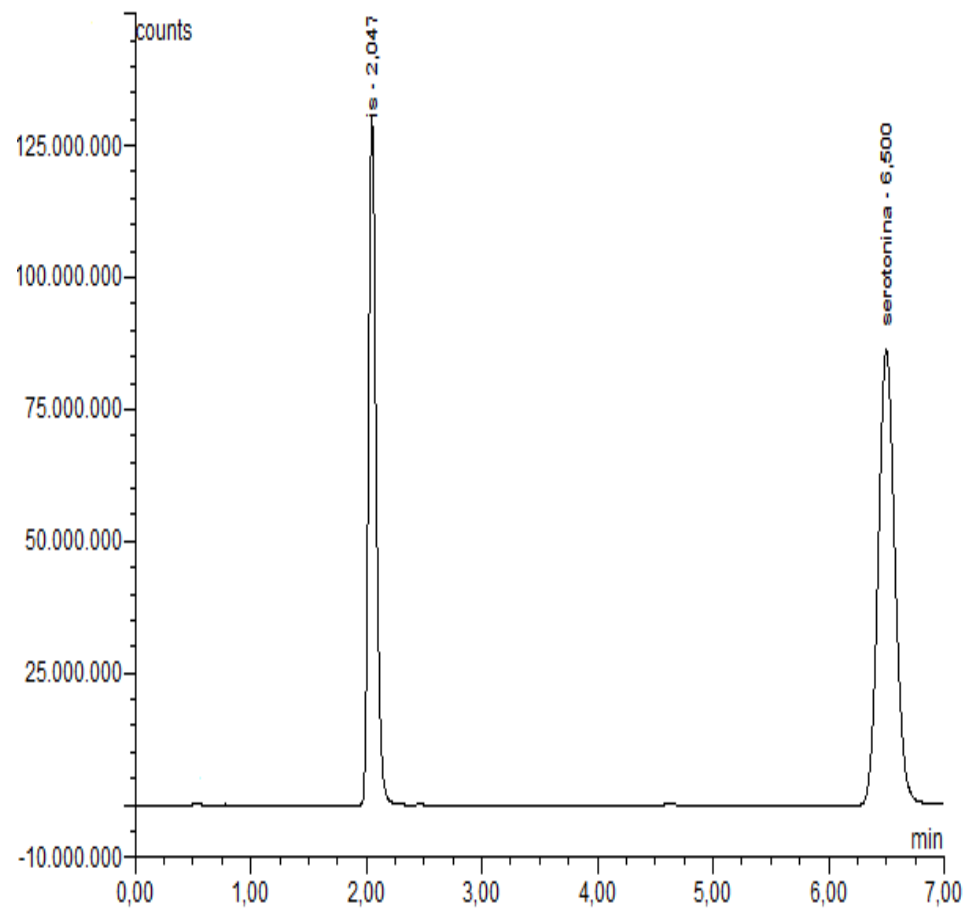


Fig. 2 :	Test Solution
	R.T. 2.0 Internal Standard
	R.T. 6.5 Serotonin

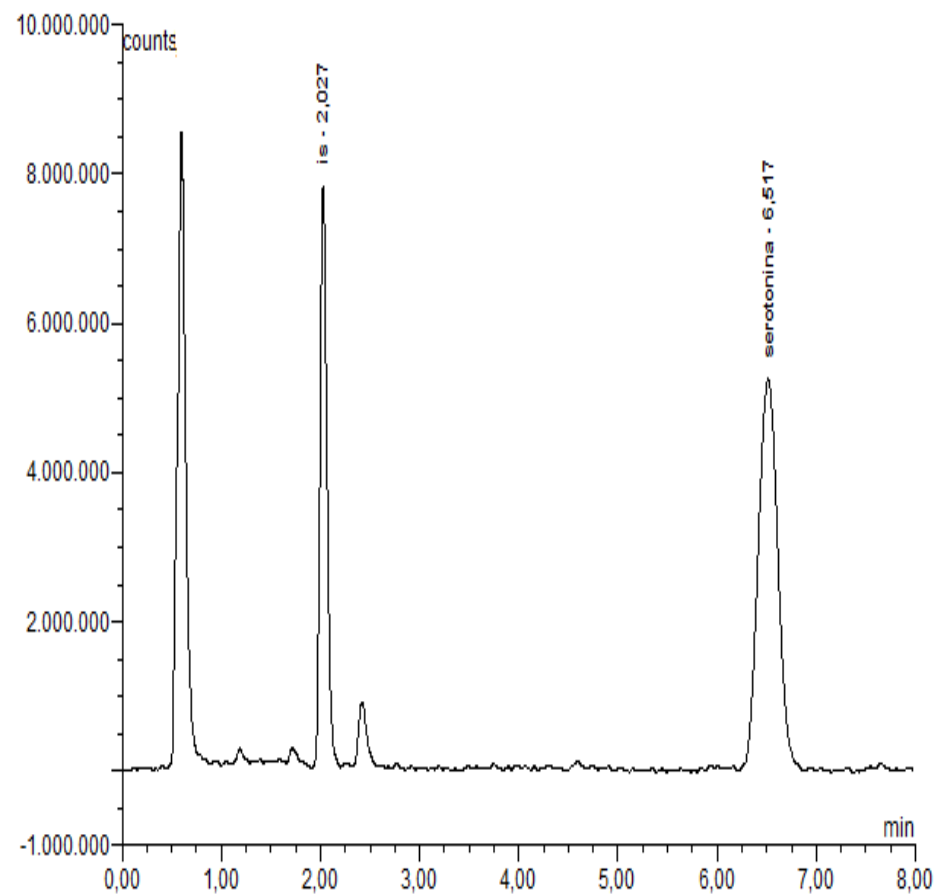


Fig. 3 :	Urine Calibrator lyophil.
	R.T. 2.0 Internal Standard
	R.T. 6.5 Serotonin 285,9 µg/l

SEROTONIN IN URINE

(Reference Chromatograms)

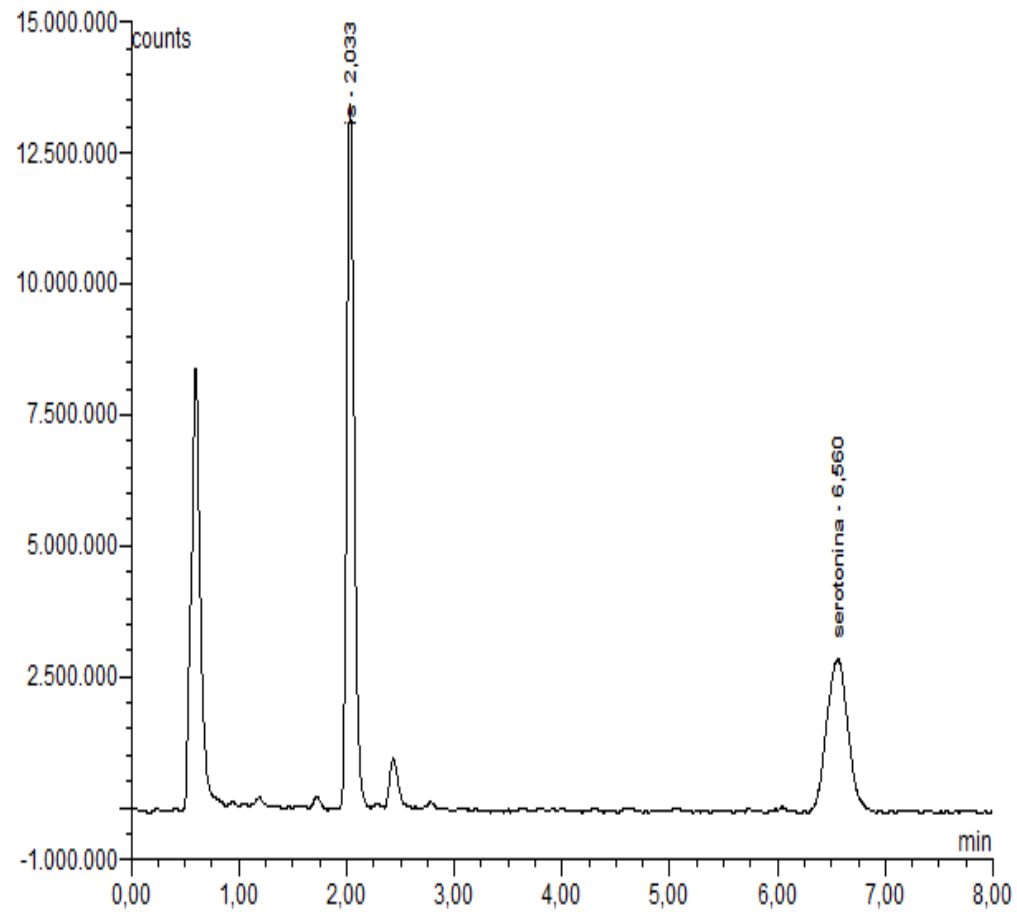


Fig. 4 :	Urine Control lyophil. Level 1
	R.T. 2.0 Internal Standard
	R.T. 6.5 Serotonin 149,0 µg/l

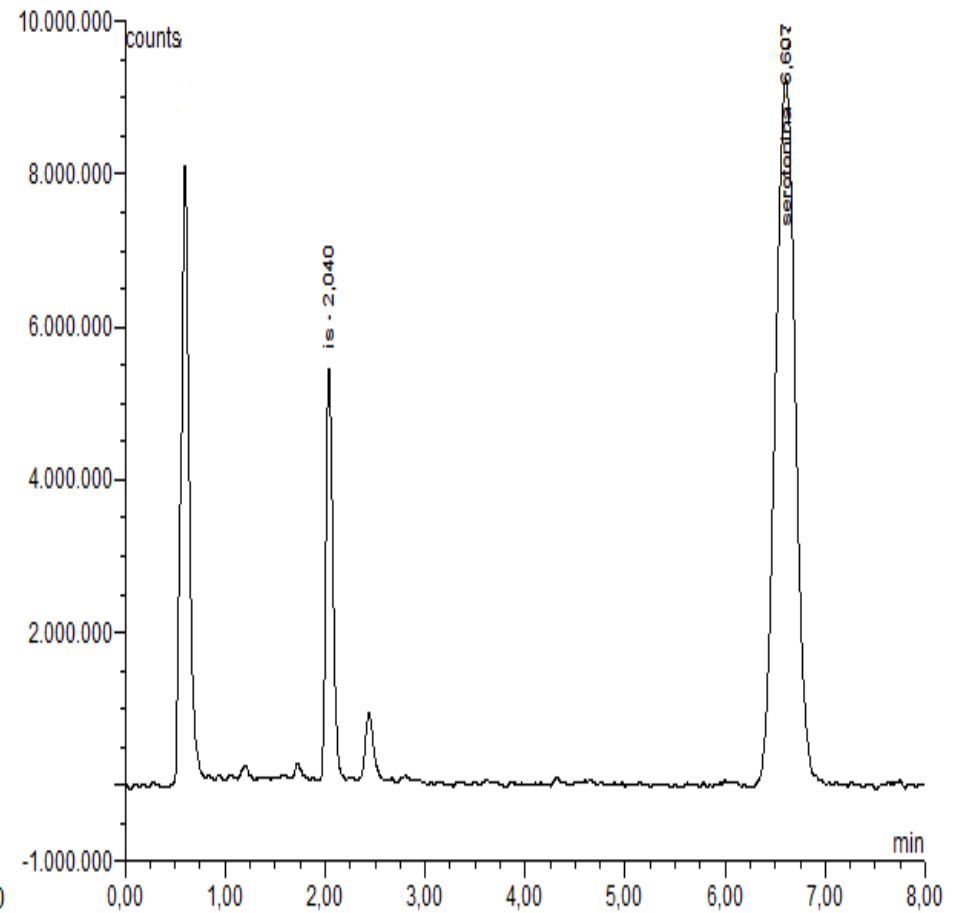


Fig. 5 :	Urine Control lyophil. Level 2
	R.T. 2.0 Internal Standard
	R.T. 6.6 Serotonin 463,9 µg/l