

## INDICAN IN URINE BY FLUORIMETRY – FAST - Code Z60110

### **BIOCHEMISTRY**

Indican, or 3-indoxyl sulfate potassium salt, is an aromatic molecule derived from tryptophan metabolism. The latter is normally taken with the diet and is converted into indole by bacteria in the small intestine, so that it can be absorbed into the bloodstream. Subsequently, hepatic enzymes catalyze its hydroxylation, in order to allow the conjugation with the sulfate ion and finally the excretion in the urine as Indican.

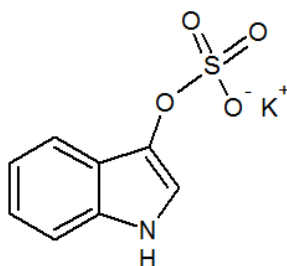


Fig.1 Indican

Since the small intestine is sparsely populated by those microorganisms that make up the human microbiota, the levels of urinary Indican are usually low in clinically healthy individuals, that is, in those who are in a condition of equilibrium called eubiosis. If there is, instead, an imbalance between micro-organisms, usually due to the proliferation of certain bacterial strains at the expense of others, a condition called dysbiosis is created. When this condition affects the small intestine, it is called fermentative dysbiosis, while if it is in the colon, it is called putrefactive dysbiosis, characterized by an increase of urinary skatole, another marker. In general, the symptoms of dysbiosis can be multiple: poor digestion, swelling, halitosis, increased susceptibility to infections, but also nervousness, anxiety, sleep disorders and fatigue.

The values of Indican present in the urine can undergo variations even in some conditions different from fermentative dysbiosis. In particular, they increase in case of steatorrhea due to pancreatic insufficiency treated with pancreatic enzyme extract, altered postprandial protein digestion and Hartnup disease, a rare genetic disease characterized by intestinal and renal malabsorption of some amino acids. Furthermore, high levels of this analyte are correlated with a rapid progression of chronic kidney disease in non-dialysate uremic patients. Oral antibiotics, on the other hand, can decrease the urinary Indican.

If the test is positive, the results should always be followed by a careful analysis of patient's clinical history and additional tests for the assessment of symptoms aetiology. It is recommended to repeat the test after 2/3 months, after following a treatment based on supplements and a correct diet.

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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.

Release N° 001	Indican in urine by Fluorimetry - FAST	March 2019
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## TECHNICAL FEATURES

### Principle of the Method:

The Indican is analyzed by treating the urine with a suitable diluent and the resulting solution is directly injected into HPLC.

**Recovery :** n.a.

**Sensitivity (LLOD) :** 0,17 mg/l

**Minimum Concentration analyzable (LLOQ):** 0,5 mg/l

**Linearity :** 0,5 – 400 mg/l

### **Accuracy intra serie (relative error %) :**

<b>Ci</b>	<b>Cs</b>
10 mg/l	60 mg/l
3,12%	2,33%

### **Accuracy inter serie (relative error %) :**

<b>Ci</b>	<b>Cs</b>
10 mg/l	60 mg/l
6,05%	1,98%

### **Reproducibility intra serie (coefficient of variation %):**

<b>C LLOQ</b>	<b>Cm</b>	<b>Cs</b>
0,5 mg/l	30 mg/l	100 mg/l
2,31%	2,85%	0,85%

### **Reproducibility inter serie (coefficient of variation %):**

<b>C LLOQ</b>	<b>Cm</b>	<b>Cs</b>
0,5 mg/l	30 mg/l	100 mg/l
4,77%	4,98%	2,99%

**Coefficient of correlation + Dev Std :** 0,9981 ± 0,0003

### **Reference Values:**

10 – 20 mg/l      low disbiosis  
20 – 40 mg/l      medium disbiosis  
> 40 mg/l          high disbiosis

*Note: each laboratory refers to its own ranges of normality*

### **Components of the kit :**

All the components are ready-to-use and stable 3 years at 2-8 °C.

**Reagent A** – Diluting Solution + Internal Std, 1 x 100 ml

**Reagent B** – Test Solution, 1 x 2 ml

**See Warnings**

**Urine Calibrator Liophyl.**, 5 x 1 ml

**Code Z60116 (packed separately – see data sheets)**

**Reagent M** – Mobile Phase, 2 x 500 ml

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**Minimum Instrumental equipment required:**

Isocratic HPLC System with loop of 10  $\mu$ l  
Fluorimetric Detector  $\lambda_{EX}=280$  nm  $\lambda_{EM}=394$  nm  
Chromatograms Recorder

**Optional Equipment:**

Autosampler  
Operational Computer

**Urine Collection Procedure:**

Collect 10 ml of urine in a suitable test tube.

## **PREANALYTICAL PROCEDURE**

Preparation of Test Solution

Dispense in a vial :

- 1000 µl of **Reagent A - Diluting Solution + Internal Standard**
- 20 µl of **Reagent B - Test Solution**

**Vortex for 5 sec.**

### **INJECTION :**

- Inject 5 µl of the solution into 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 :** Dispense in a vial

	<b>Calibrator</b>	<b>Sample</b>	<b>Controls</b>
<b>Reagent A – Diluting Solution + IS</b>	1000 µl	1000 µl	1000 µl
<b>Calibrator</b>	20 µl		
<b>Sample</b>		20 µl	
<b>Controls</b>			20 µl

**Vortex for 5 sec.**

**N.B.: at this step, the sample is stable 1 day at 2-8 °C**

### **INJECTION :**

- Inject 5 µl of the solution into the chromatographic system.

## INDICAN IN URINE - Warnings

### REAGENT B : TEST SOLUTION

INDICAN	100 mg/l
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### FLUORIMETRIC DETECTOR PARAMETERS

$\lambda_{EX}$	280 nm
$\lambda_{EM}$	394 nm
GAIN	1 x 10
INTEGRATION TIME	5 sec

### HPLC COLUMN PROTECTION

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

### HPLC COLUMN CONDITIONING

Install a new analytical C<sub>18</sub> Reversed-Phase column POROSHELL 120 EC-C18 (50 x 4,6 mm, 2,7  $\mu$ m).  
Disconnect the detector and filter 30 ml of H<sub>2</sub>O : Acetonitrile (20 : 80 v / v) at pH<3 and then 30 ml of a solution of H<sub>2</sub>O : Acetonitrile (80 : 20 v / v) at flow of 1,5 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 15 ml. Condition further on the column for 15 minutes also at recycling phase. Finally inject the Chemical Standard and verify the quality of the HPLC run.  
**And it is NOT possible to make analysis at recycling phase.** The signal that the mobile phase must be changed is given by the significant increase of the background noise of the detector Fluorimetric. 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 30 ml of H<sub>2</sub>O : Acetonitrile (80 : 20 v / v) and discharge. Flux a solution of H<sub>2</sub>O : Acetonitrile (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 H<sub>2</sub>O : Acetonitrile (30 : 70 v / v).

### INJECTION NEEDLE WASHING

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

### PARAMETRI HPLC

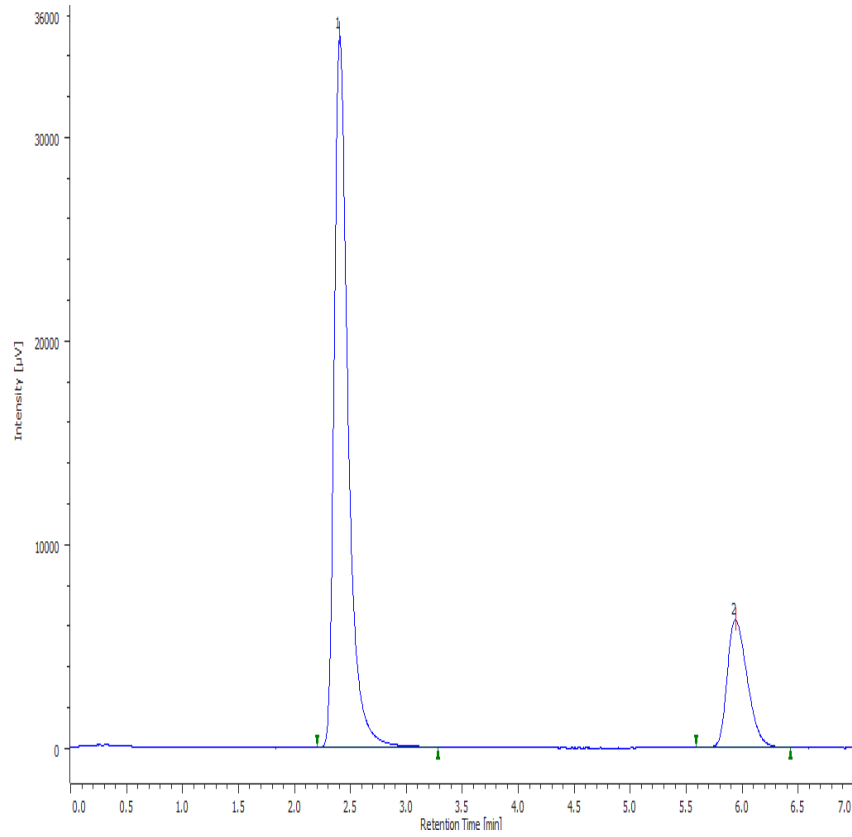
LOOP	10 $\mu$ l
FLOW	1 ml/min
PRESSURE	About 100 bar

### ACCESSORIES AND CONSUMABLES

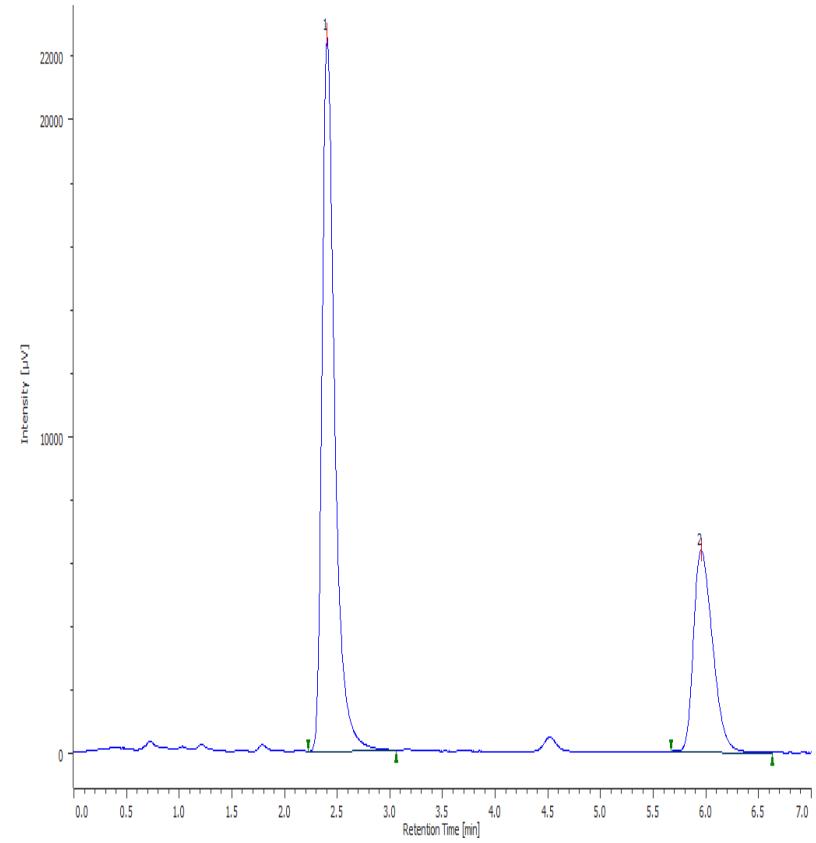
CODE	DESCRIPTION	PACKAGING
Z60116	Calibrator for Indican in urine	4 x 1 ml
Z60117	Control for Indican in urine – Level 1	5 x 1 ml
Z60118	Control for Indican in urine – Level 2	5 x 1 ml
Z60119	Control for Indican in urine – Levels 1 and 2	2 x 5 x 1 ml
Z699975902	Poroshell 120 EC C18 (50 x 4,6mm –2,7 u) Analytical Column	1 x 3 Pcs
S90199511	Javelin Colfilters	1 x 10 Pcs
S29057U	Standard glass vials of 2 ml with screw cap	1 x 100 Pcs



INDICAN IN URINE  
( Reference Chromatograms )

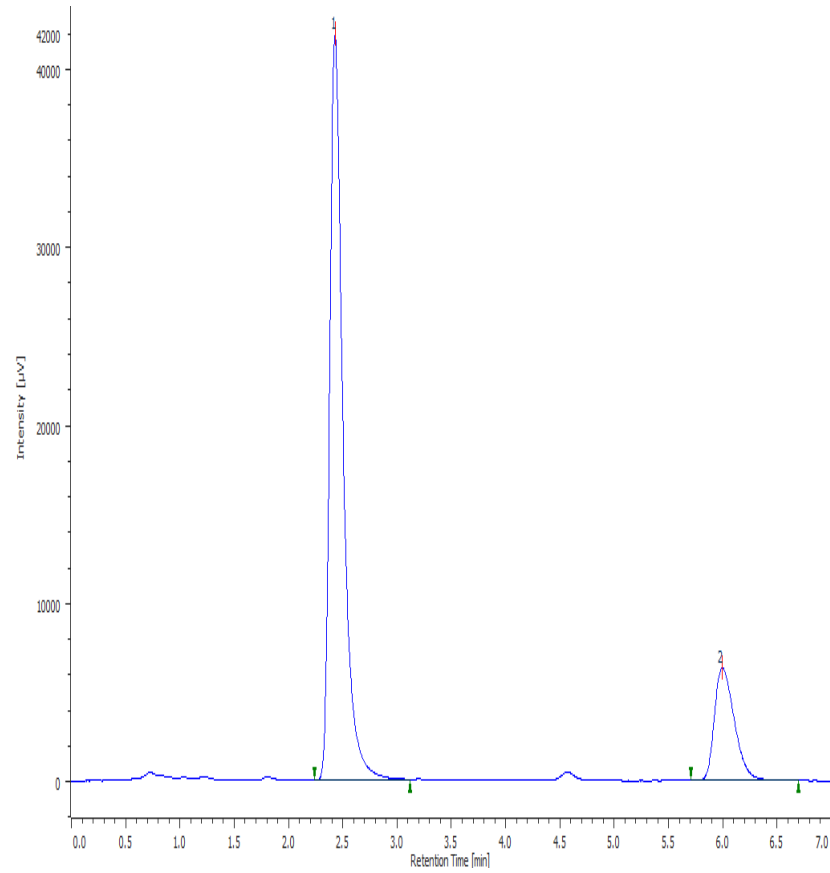


<b>Fig. 2 :</b>	<b>Test Solution</b>
	R.T. 2.40 Indican
	R.T. 5.93 IS

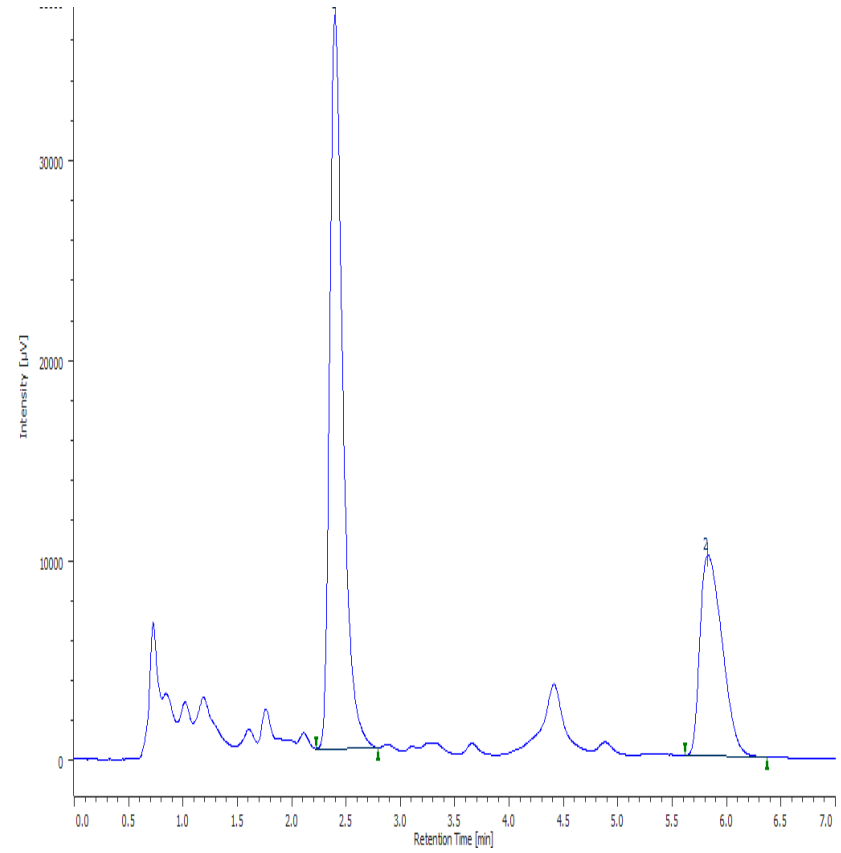


<b>Fig. 3 :</b>	<b>Urine Calibrator</b>
	R.T. 2.40 Indican 60 mg/l
	R.T. 5.95 IS

INDICAN IN URINE  
( Reference Chromatograms )



<b>Fig. 4 :</b>	<b>High Control</b>
	R.T. 2.42 Indican 110 mg/l
	R.T. 5.99 IS



<b>Fig. 5 :</b>	<b>Pathological Sample</b>
	R.T. 2.39 Indican 93 mg/l
	R.T. 5.82 IS