

3-METHYL INDOLE (Skatole) IN URINE BY FLUORIMETRY – FAST –
Code Z60010

BIOCHEMISTRY

3-methyl indole or skatole (P.M. 131.172) is an organic compound whose chemical structure contains the indolic group. It is present in faeces and is derived from biotransformation (decarboxylation) of the amino acid **Tryptophan** is found in the digestive tract of mammals.

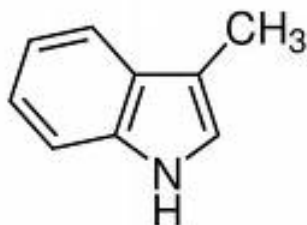


Fig. 1: 3-methylindole or Skatole

PATHOLOGIES

In the colon, there is a large bacterial flora that may be subject to changes that make it harmful to health.

When this flora is balanced and useful to the body, a balance that is called "eubiosis" occurs. When, however, unwanted bacteria appear, they alter the balance and create the "intestinal disbiosis", unfortunately a very common condition.

If the intestinal flora is not balanced, the amino acids derived from undigested proteins undergo a decarboxylation process that produces toxic amines, such as 3-methyl indole, resulting from tryptophan.

PROCESSING OF PROTEINS

Protein	Amine produced
arginine	agmatina
cystine and cysteine	mercaptan
histidine	histamine
lysine	cadaverine
ornithine	putrescine
tyrosine	tyramine
tryptophan	indole and skatole

Many of these amines are powerful vasoconstrictive poisons. It should also be noted that 3-methyl indole is responsible for the particularly odor of faeces.

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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.

N° 012	3-Methylindole in urine by Fluorimetry - FAST	09/2025
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TECHNICAL FEATURES

Principle of the Method:

The 3-Methylindole is analyzed by treating the urine with a suitable diluent and the resulting solution is directly injected into HPLC.

Recovery of the Method : > 95 %

Sensitivity of the Method : < 0.1 µg/l

Dynamic Range of the Method : 0.1 – 100 µg/l

CV% : 5.8 %

Reference Values:

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

Components of the kit :

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

Reagent A – Buffer Solution, 1 x 100 ml

Reagent B – Test Solution, 1 x 2 ml

See Warnings

Reagent C – Internal Standard Solution, 1 x 400 µl

Supplied separately as Box 3/3, to be stored at – 25 °C / –15 °C

Urine Calibrator Liophyl., 5 x 1 ml

Packed separately as Box 2/3 – see technical data sheet.

Reagent M – Mobile Phase, 4 x 500 ml

Minimum Instrumental equipment required:

Isocratic HPLC System with loop of 20µl
Fluorimetric Detector $\lambda_{EX}=280\text{ nm}$ $\lambda_{EM}=360\text{ nm}$
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 at **40 µg/l**

Dispense in a tube :

- 900 µl of H₂O HPLC grade
- 100 µl of **Reagent B - Test Solution**
- 10 µl of **Reagent C - Internal Standard Solution**

Vortex for 10 sec.

INJECTION :

- Inject 20 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

Dilute the **Reagent C – Internal Standard Solution** 1:10 with Water HPLC grade, preparing the volume sufficient to cover the run.

STEP 1 : Dispense in a tube

	Calibrator	Sample
Reagent A – Buffer Solution	950 µl	950 µl
Sample		50 µl
Calibrator	50 µl	
Reagent C - Internal Standard Solution (diluted 1:10 in Water HPLC grade)	20 µl	20 µl

Vortex for 10 sec.

N.B.: at this step, the sample is stable 2 days at 2-8 °C and 3 months at -20 °C

INJECTION :

- Inject 20 µl of the solution into the chromatographic system.

3-METHYLINDOLE IN URINE - Warnings

REAGENT B : TEST SOLUTION

3-METHYLINDOLE	400 µg/l
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FLUORIMETRIC DETECTOR PARAMETERS

λ_{EX}	280 nm
λ_{EM}	360 nm
GAIN	1 x 100
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 µm, the use of Javelin Colfilters (1 x 10 pcs.) cod. S90199511 is obligatory.

HPLC COLUMN CONDITIONING

Install a new analytical C₁₈Reversed-Phase column POROSHELL 120 EC-C18 (50 x 4,6 mm, 2,7 µ).
Disconnect the detector and filter 30 ml of **H₂O : Methanol or Acetonitrile (20 : 80 v / v)** set flow of 1,0 ml/minute. **Don't recycle the washing solutions.** Flux 15 ml of a solution of **H₂O : Acetonitrile or Methanol (20 : 80 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 1,5 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 possible to make analysis at recycling phase, as long as you have the foresight to filter the same mobile phase with 0.22 µ filter before each analytical run. 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 **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 (20 : 80 v / v)** for 30 minutes discharge it. When you re-use the column, flux 15 ml di **H₂O : Acetonitrile or Methanol (20 : 80 v / v)** at flow of 1,0 ml/minute before a new conditioning with the Mobile Phase. **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 (20 : 80 v / v)**.

INJECTION NEEDLE WASHING

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

PARAMETRI HPLC

LOOP	20 µl
FLOW	1 ml/min
PRESSURE	About 110 bar

ACCESSORIES AND CONSUMABLES

CODE	DESCRIPTION	PACKAGING
Z60016	Calibrator for 3-Methylindole in urine	4 x 1 ml
Z60019	Control for 3-Methylindole 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 Pc
S90199511	Javelin Colfilters	1 x 10 Pcs
S29057U	Standard glass vials of 2 ml with screw cap	1 x 100 Pcs

3-METHYLINDOLE IN URINE (Reference Chromatograms)

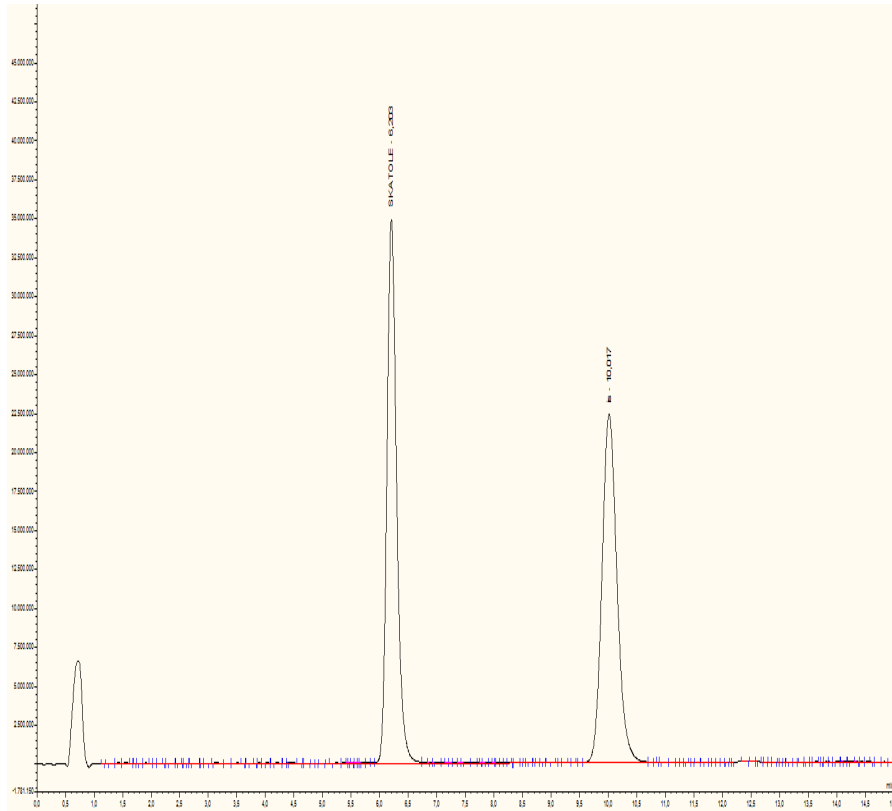


Fig. 2 :	Test Solution
	R.T. 6.20 3-Methylindole
	R.T. 10.02 IS

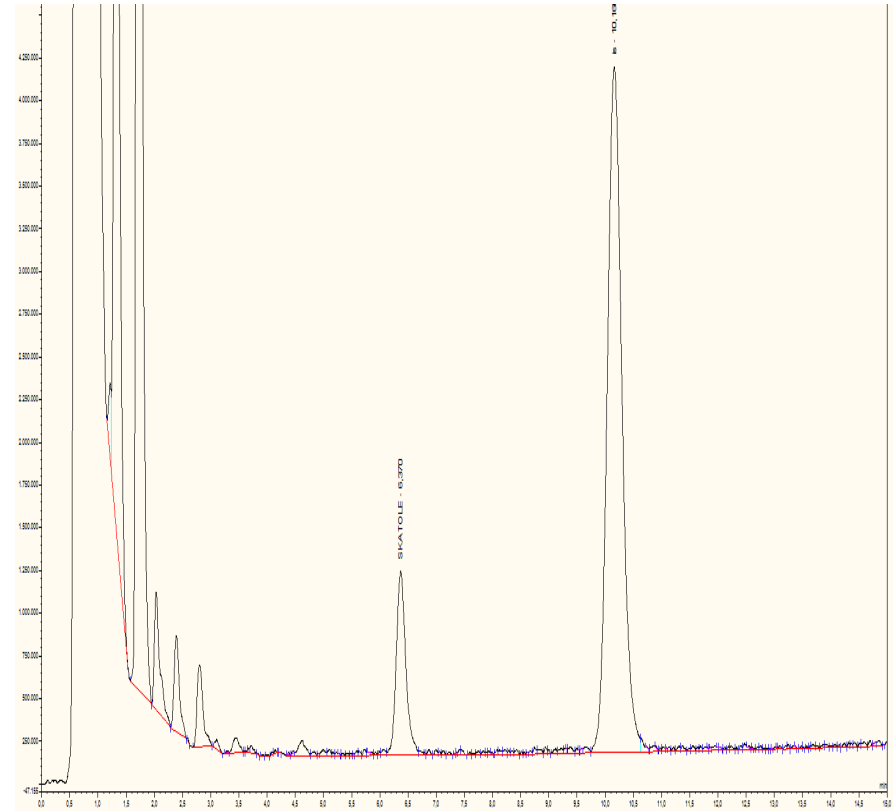


Fig. 3 :	Calibrator in urine
	R.T. 6.37 3-Methylindole 30.7 µg/l
	R.T. 10.16 IS

3-METHYLINDOLE IN URINE (Reference Chromatograms)

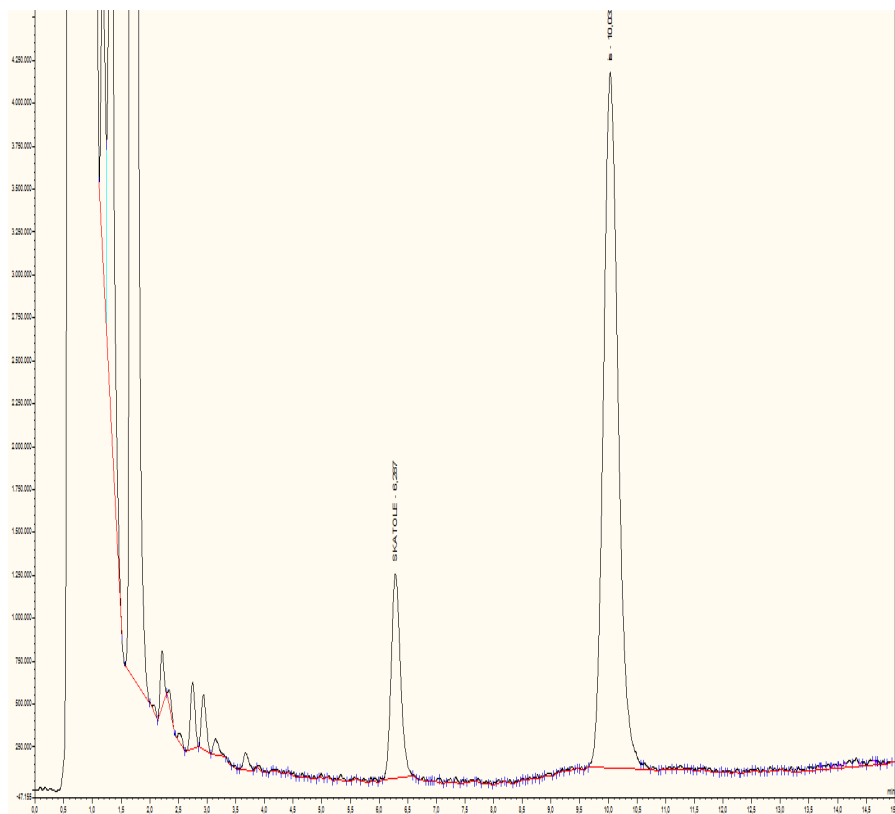


Fig. 4 :	Low Control
	R.T. 6.29 3-Methylindole 32.5 µg/l
	R.T. 10.03 IS

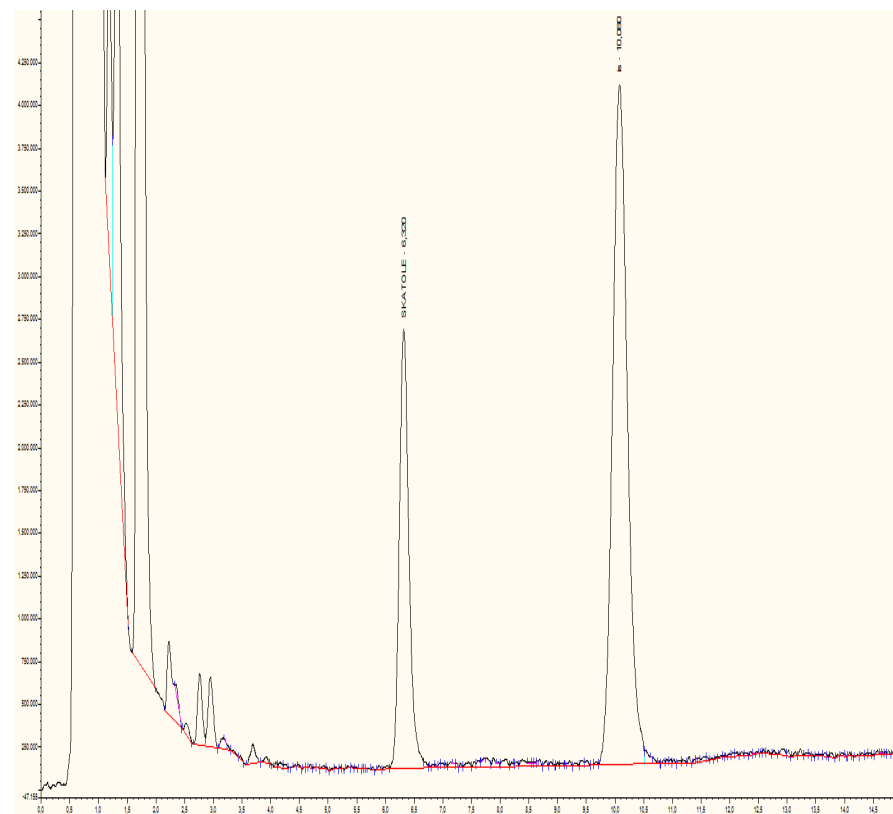


Fig. 5 :	High Control
	R.T. 6.32 3-Methylindole 73.5 µg/l
	R.T. 10.08 IS