

METHANOL IN URINE BY UV – FAST - Code Z45610

METABOLISM

Methanol is an aliphatic alcohol with PM=32,0.

Methanol, also known as methyl alcohol, carbinol, wood alcohol, wood naphtha or wood spirits, is a chemical compound with chemical formula CH_3OH (often abbreviated MeOH). It is the simplest alcohol, and is a light, volatile, colourless, flammable, poisonous liquid with a distinctive odour that is somewhat milder and sweeter than ethanol (ethyl alcohol). At room temperature it is a polar liquid and is used as an antifreeze, solvent, fuel, and as a denaturant for ethyl alcohol. It is also used for producing biodiesel via transesterification reaction.

The largest use of methanol by far, is in making other chemicals. About 40% of methanol is converted to formaldehyde, and from there into products as diverse as plastics, plywood, paints, explosives and permanent press textiles.

Methanol is toxic by two mechanisms. Firstly, methanol (whether it enters the body by ingestion, inhalation, or absorption through the skin) can be fatal due to its CNS depressant properties in the same manner as ethanol poisoning. Secondly, it is toxic by its breakdown (toxication) by the enzyme alcohol dehydrogenase in the liver by forming formic acid and formaldehyde which cause permanent blindness by destruction of the optic nerve. Fetal tissue will not tolerate methanol. Dangerous doses will build up if a person is regularly exposed to vapours or handles liquid without skin protection. If methanol has been ingested, a doctor should be contacted immediately. The usual fatal dose is 100-125 mL. Toxic effects take hours to start, and effective antidotes can often prevent permanent damage.

The initial symptoms of methanol intoxication are those of central nervous system depression: Headache, dizziness, nausea, lack of coordination, confusion, drowsiness and, with sufficiently large doses, unconsciousness and death. The initial symptoms of methanol exposure are usually less severe than the symptoms resulting from the ingestion of a similar quantity of ethyl alcohol.

Once the initial symptoms have passed, a second set of symptoms arises 10-30 hours after the initial exposure to methanol: blurring or complete loss of vision, together with acidosis. These symptoms result from the accumulation of toxic levels of formate in the bloodstream, and many progress to death by respiratory failure. The ester derivatives of methanol do not share this toxicity.

CHEMICAL ANALYSIS BY HPLC

The present method determinates the urine concentration of **Methanol** with derivatization and with HPLC scanning at $\lambda=385 \text{ nm}$.

It's important to note the small amounts of **Methanol**, due to bacterial fermentation, are always present in the urine of not exposed subjects: values of 0,5-1,0 mg/l do not represent a **Methanol** exposure index.

Urine samples must to be frozen quickly at -20 °C.



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° 004	Methanol in urine by UV - FAST	11/2025
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TECHNICAL FEATURES

Principle of the Method:

The Methanol is incubated at 40 °C for 30 minutes. After cooling, the derivatization solution is added and the solution is incubated at 70 °C for 30 minutes. After cooling the solution is diluted and directly injected into HPLC system.

Recovery : 100%

Sensitivity (LLOD) : 0,16 mg/l

Linearity: 0,5 – 120 mg/l

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

Accuracy intra serie (relative error %):

Ci	Cs
5 mg/l	30 mg/l
9,28%	3,52%

Accuracy inter serie (relative error %):

Ci	Cs
5 mg/l	30 mg/l
8,36%	4,07%

Reproducibility intra serie (coefficient of variation %):

C LLOQ	Cm	CA
0,5 mg/l	15 mg/l	60 mg/l
11,64%	4,12%	3,24%

Reproducibility inter serie (coefficient of variation %):

C LLOQ	Cm	CA
0,5 mg/l	15 mg/l	60 mg/l
9,98%	7,77%	4,09%

Coefficient of correlation R2 + Dev. std: 0,9960 ± 0,0010

Biological Index of Exposure (ACGIH/BEI) : 15 mg/l

Biological index in not exposed people : < 1 mg/l

Ref.: G Ital Med Lav Erg 2002;24:3 Suppl

<u>Components of the kit :</u>	All the components are ready-to-use. Stable 3 years at 2 – 8 °C.
Reagent A – Buffer Solution N° 1, 1 x 5 ml	
Reagent B – Reagent B, 1 x 3 ml	
Reagent C – Buffer Solution N° 2, 1 x 10 ml	
Reagent D – Derivatization Solution, 1 x 10 ml	
Reagent E – Stabilizing Solution, 1 x 10 ml	
Reagent F – Test Solution/Chemical Standard, 1 x 5 ml	
Reagent G – Diluting Solution, 1 x 40 ml	
Reagent I – Internal Standard Solution, 1 x 10 ml	
Reagent M – Mobile Phase, 4 x 500 ml	

Minimum Instrumental equipment required:

Isocratic HPLC System with loop of 10 µl.
Spectrophotometric Detector UV/VIS $\lambda=385$ nm
Chromatograms Recorder

Optional Equipment:

Autosampler
Operational Computer

Urine Collection Procedure:

After work shift, collect 10 ml of urine in a suitable test tube. Store urine samples at **-20°C**. Stable for 4 months.

PREANALYTICAL PROCEDURE

Preparation of Test Solution at 40 mg/l

Pipette in a glass tube with teflon cap:

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

This is a test solution at 40 mg/l.

Pipette in a glass tube with teflon cap:

- 50 µl of **Reagent A – Buffer Solution N° 1**
- 30 µl of **Reagent B**
- 100 µl of **Test Sol. at 40 mg/l**
- 100 µl of **Reagent I – Internal Std Solution**

Vortex for 10 sec.

- Incubate at 40 °C for 30 minutes

Cooling

Add:

- 100 µl of **Reagent C – Buffer Solution N° 2**
- 100 µl of **Reagent E – Stabilizing Solution**
- 100 µl of **Reagent D – Derivatization Solution**

Vortex for 10 sec.

- Incubate at 70 °C for 15 minutes

Cooling and vortex for 10 sec.

Pipette in a glass tube with teflon cap:

- 400 µl of **Reagent G – Diluting Solution**
- 100 µl of sample precedently derivatized.

INJECTION :

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

Verify that the Test Solution has retention time similar to fig. 1. If the Test is all right you can start with the analytical procedure; if not, check the functionality of the analytical system.

ANALYTICAL PROCEDURE

STEP 1 : Preparation of Chemical Standard at 40 mg/l.

Pipette in a glass tube with teflon cap:

- 900 µl of H₂O HPLC grade
- 100 µl of **Reagent F - Chemical Standard**

This is a Chemical Standard at 40 mg/l.

IMPORTANT : It is important to use standard solution just prepared.

STEP 2 : Preparation of Samples, Calibrator and Controls

Verify and bring the urine sample pH between 7,0 and 7,5.

Pipette in a glass tube with teflon cap:

	Blank	Standard	Sample
Water HPLC grade	100 µl		
Chemical Standard		100 µl	
Sample			100 µl
Reagent I – Internal Std	100 µl	100 µl	100 µl
Reagent A – Buffer Sol. N° 1	50 µl	50 µl	50 µl
Reagent B	30 µl	30 µl	30 µl

Vortex for 10 sec.

STEP 3 : Incubate at 40 °C for 30 minutes

Cooling at Room Temperature and **Vortex for 10 sec.**

STEP 4 : Derivatization Solution

Add:

- 100 µl of **Reagent C – Buffer Solution N° 2**
- 100 µl of **Reagent E – Stabilizing Sol.**
- 100 µl of **Reagent D – Derivatization Sol.**

Vortex for 10 sec.

STEP 5 : Incubate at 70 °C for 15 minutes

Cooling at Room Temperature and **Vortex for 10 sec.**

STEP 6 : Dilution:

Pipette in a glass tube with teflon cap:

- 400 µl of **Reagent G – Diluting Solution**
- 100 µl of sample precedently derivatized.

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.

N.B.: If the Blank presented the peak of Methanol, proceed to identify the quantitative difference between the areas / heights of the peaks of Methanol in Calibrators/Samples/Controls and blank.

METHANOL IN URINE BY UV - FAST - Warnings

REAGENT F : CHEMICAL STANDARD / TEST SOLUTION

Methanol	400 mg/l
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SPECTROFOTOMETRIC DETECTOR PARAMETERS

λ	385 nm
GAIN	0,001 AUFS
INTEGRATION TIME	10 sec.

HPLC COLUMN PROTECTION

To save the analytical column Reverse Phase POROSHELL 120 EC-C18 (50 x 4,6 mm, 2,7 μ M), 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 μ).
Disconnect the detector and filter 30 ml of H₂O: Acetonitrile (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 : Acetonitrile (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 ml/minute and discharge the first 20 ml. 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 (20 : 80 v / v) set flow of 1,0 ml/minute and discharge. Flux a solution of H₂O : Acetonitrile (70 : 30 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 (70 : 30 v / v).

INJECTION NEEDLE WASHING

H₂O / Ethanol or Acetonitrile (80 : 20 v / v).

HPLC PARAMETERS

LOOP	10 μ l
FLOW	0,6-1,5 ml/min.
PRESSURE	About 80-170 bar

FLOW SET

Time (min)	Flow (ml/min)
0	0.6
2	0.6
2.1	1.5
9	1.5
9.1	0.6

ACCESSORIES AND CONSUMABLES

CODE	DESCRIPTION	PACKAGING
Z05620	Chemical Standard for Methanol in urine	2 x 5 ml
Z699975902	Poroshell 120 EC C18 (50 x 4,6mm –2,7 u) 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



METHANOL IN URINE BY UV

(Reference Chromatograms)

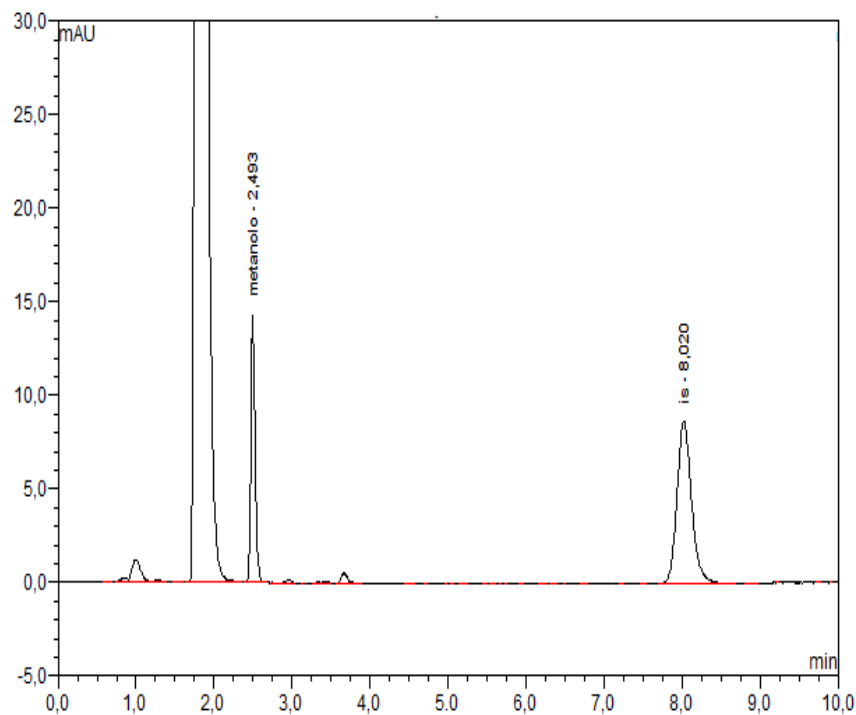


Fig. 1 : Test Solution

R.T. 2.49	Methanol	40 mg/l
R.T. 8.02	Internal Standard	

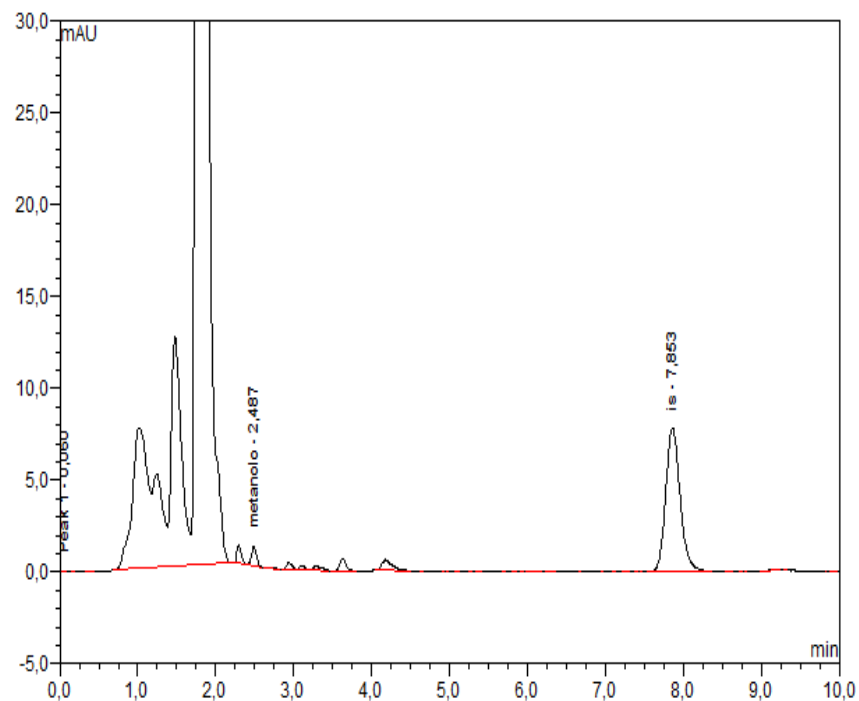


Fig. 2 : Urine Sample of not exposed subject

R.T. 2.48	Methanol	4,10 mg/l
R.T. 7.80	Internal Standard	

METHANOL IN URINE BY UV

(Reference Chromatograms)

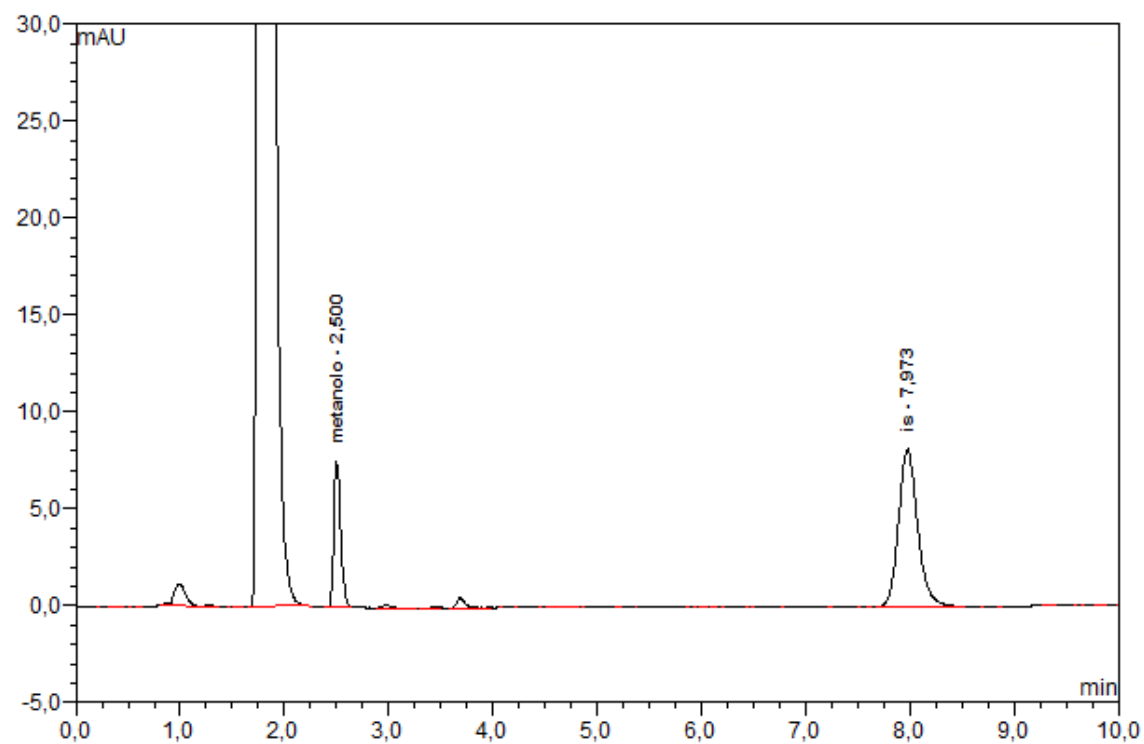


Fig. 3 :	Enriched Urine Sample
	R.T. 2.50 Methanol 20 mg/l
	R.T. 7.90 Internal Standard