

FORMIC ACID IN URINE BY DETECTOR MS-HEAD SPACE - Code GC74010

BIOCHEMISTRY

Methanol is used in the production of cellulose, synthetic resins, formaldehyde, acetic acid and fuel. Methanol is distributed uniformly in tissues and blood, it is metabolized in the liver and excreted in urine as **Formaldehyde, Formic Acid and CO₂**.

The major toxicity of methanol is not due to the molecule but to metabolite, Formic Acid.

Alcohol dehydrogenase is the first metabolic pathway that oxidizes Methanol into Formaldehyde. The subsequent oxidation of Formaldehyde to Formic Acid takes place through aldehyde dehydrogenase, an extremely fast reaction. In some patients, Methanol poisoning can not be determined by Formaldehyde either in tissues or in fluids.

Even industrial exposure to dichloromethane (used as a solvent, degreasing agent and to remove the paint) can cause an increase in Formic Acid in urine because it metabolises two ways: in one the cytochrome P450 is involved leading to the formation of Carbon monoxide (CO), in the other you have the formation of **Formaldehyde → Formic Acid → CO₂**.

TOXICITY

After 6-18 hours from the time of exposure visual disturbances (pupillar oedema) and metabolic acidosis are observed: they are caused mainly by Formic Acid which is also the main cause of optic neuritis.

The dosage of methanol is only useful if it's done early, while the determination of formates is more useful and can be used as an index of the intoxication's severity.



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 and 332/00 on in vitro diagnostic medical devices (IVD). The declaration of conformity is available upon request.

N° 003	Formic Acid in urine by detector MS-head space	11/2025
--------	--	---------

TECHNICAL FEATURES

Principle of the Method :

This method enables you to determine, after derivatization, urinary formic acid in GC/MS head space.

Recovery : 100%

Sensitivity : 1,0 mg/l

Dynamic Range of the Method : 1,0 mg/l – 1.000 mg/l

Reference Values : not Exposed < 17 mg/l
Exposed < 75 mg/l

CV% 4,42% intra serie
2,73% inter serie

Components of the kit :

All the reagents are ready to use and stable 3 years at 2 – 8 °C

Reagent A – Diluting Solution, 1 x 100 ml

Reagent B – Derivatization Solution, 1 x 200 ml

Reagent C – Test Solution/Chemical Standard, 1 x 1 ml

For the sampling of Reagent C, the cap sealing requires the use of 100 ul Hamilton syringe Cod. ZRE24563
Store at 2-8 °C

Reagent D – Internal Standard Solution, 1 x 2,5 ml

Minimum Instrumental equipment required:

GC/ECD or GC/MS
Autosampler for headspace
Operational Computer

Urine Collection Procedure:

At the end of the work shift collect 10 ml of urine. The transfer of the sample in the container must be carried out as soon as possible. The sample is stable 7 days at 2-8° C if the analysis is delayed freeze sample at – 20° C where is stable for 1 month.

PREANALYTICAL PROCEDURE

Test Solution Preparation

Dispense in a vial of 10 ml for headspace:

- 950 µl of **Water HPLC grade**
- 50 µl of **Reagent D – Internal Standard Solution**
- 50 µl of **Reagent C – Test Solution/Chemical Standard**

Close vials hermetically and mix at Vortex for 5 sec.

- Add 1 ml of **Reagent A – Diluting Solution**
- Add 2 ml of **Reagent B – Derivatization Solution**

Close vials hermetically and mix at Vortex for 5 sec.

Wait 20 minutes at Room Temperature

IMPORTANT: This Calibration Standard Solution is prepared from time to time

Inject 200 µl of solution into gas chromatograph GC. Verify that the retention times of formic acid and internal standard are between 1,0 and 3,0 minutes.

Test is all right you can start with the analytical procedure; if not, check the functionality of the analytical system. N.B.: the test solution should not be used to calibrate the samples.

ANALYTICAL PROCEDURE

STEP 1 : Preparation of solution of Calibration Standard at **73,2 mg/l**

Dispense in a tube:

- 1980 µl of **H₂O HPLC grade**
- 20 µl of **Reagent C – Test Solution/Chemical Standard**

IMPORTANT: This Calibration Standard Solution is prepared from time to time

STEP 2 : Preparation of Calibrator and Samples

Dispense in vials of 10 ml for headspace:

	Calibration Standard	Control	Sample
Calibration Standard	1000 µl		
Control		1000 µl	
Sample			1000 µl
Reagent D – Internal Standard Sol.	20 µl	20 µl	20 µl
Reagent A – Diluting Sol.	1000 µl	1000 µl	1000 µl
Reagent B – Derivatization Sol.	2000 µl	2000 µl	2000 µl

Close vials hermetically and mix at Vortex for 5 sec.

Wait 20 minutes at Room Temperature

STEP 3 : Inject 200 µl in GC

Prepare work list and start the series according to the system's specifications.

FORMIC ACID IN URINE BY DETECTOR MS-HEAD SPACE - Warnings

REAGENT C : TEST SOLUTION/CHEMICAL STANDARD

Formic Acid	7.320 mg/l
-------------	------------

SET OF AUTOSAMPLER COMBIPAL:

- Temperature Syringe: 70°C
- Temperature Mixer: 60 °C (incubate x 1 minute at 750 rpm)
- Mixer ON: 5sec
- Mixer OFF: 2sec
- Plunger Fill Speed: 200 ul/sec
- Plunger Injection Speed: 500 ul/sec
- Fill Strokes: 0
- Viscosity Delay: 3sec
- Pre-Injection Delay: 0.1sec
- Post-Injection Delay: 0.1sec
- Syringe Flush Time: 60 sec

SET OF GAS-CROMATOGRAPH:

- VF-5ms 30m x 0,25mm, 0,25µm column (conditioned)
- Injector Temperature 200°C
- Splitting Ratio 1:20
- Gas Helium Flow 1,5 ml/min
- Isotherm Column Oven: 40°C x 2 minutes + 40 °C/min up till 220 °C x 2,5 minutes (run 9 minutes)

SET OF MASSA DETECTOR:

- Massa Range: 30-120
- Massa Temperature: 180°C
- Transfer Line Temperature: 270°C
- Manifold Temperature: 80°C
- Filament ON: 0,1 min

CONDITIONING OF VF-5 MS COLUMN

Follow the manufacturer prescription.
Do not condition column/s if connected to the Massa Detector.

CLEANING OF THE COLUMN

Disconnect the detector. Keep the column at the highest temperature for the recommended time.
(See the manufacturer 's instructions)

NB: It is recommended to use a syringe for autosampler for HeadSpace exclusively for formic acid injections to prevent drag effects and/or interference caused by other types of methods.

ACCESSORIES AND CONSUMABLES

CODE	DESCRIPTION	PACKAGING
GC74020	Chemical Standard for Formic Acid	1 x 2 ml
ZRE13623	Rxi-Sil 5MS (30 m x 0,25 mm) ID, DF=0,25 Capillary column	1 Pc
SCP8944	VF-5ms (30 m x 0,25 mm) ID DF=0,25 column	1 Pc
S51834475	Glass vial of 10 ml for headspace	1 x 100 Pcs
S80100165	Magnetic caps with teflon and sylicon septa for headspace vials	1 x 100 Pcs
S50404669	New Easy Grip Manual Crimper for 20 mm	1 x 100 Pcs



FORMIC ACID IN URINE

(Reference Chromatograms)

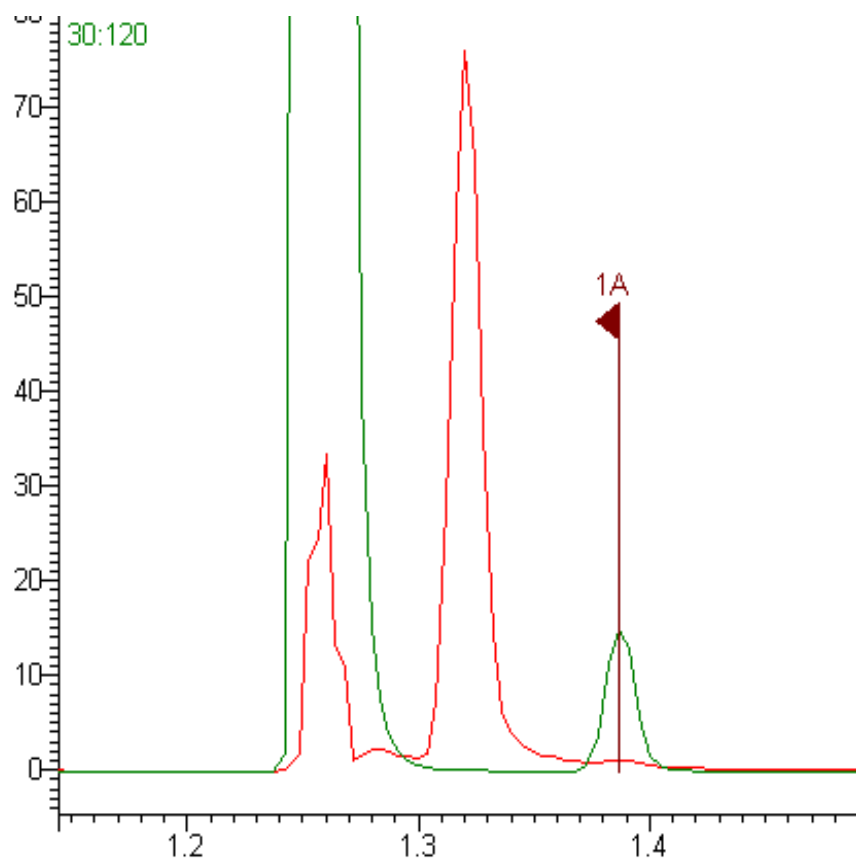


Fig. 1 :	Test Solution
	R.T. 1.32 Internal Standard
	R.T. 1.39 Formic Acid

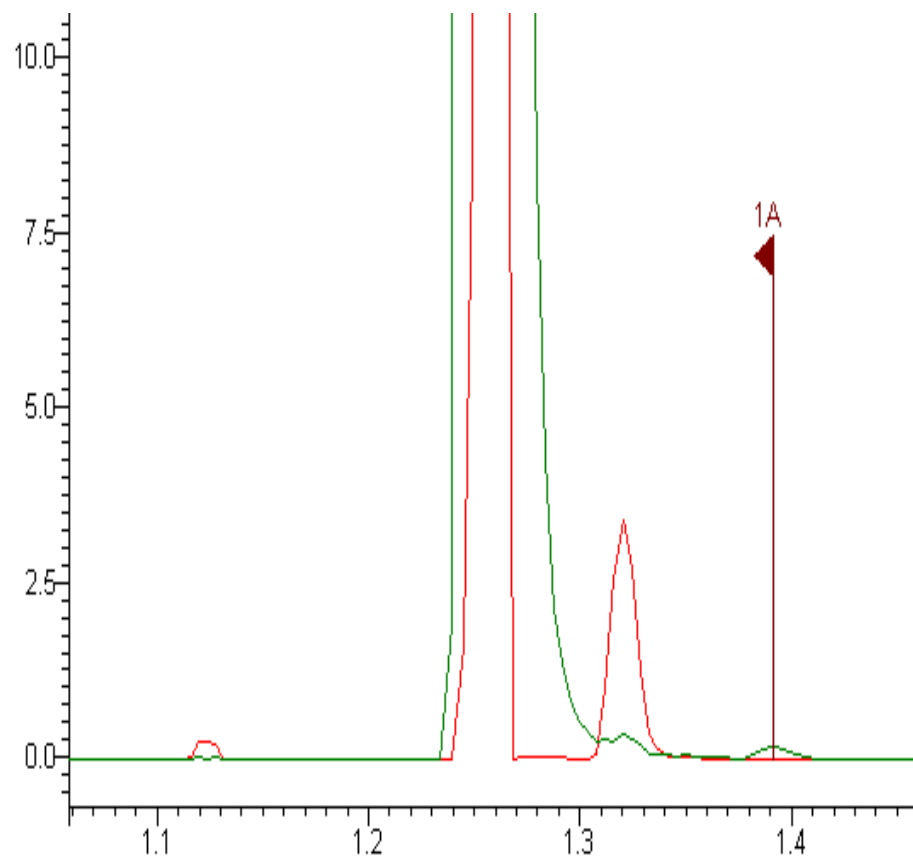


Fig. 2 :	Urine Sample
	R.T. 1.32 Internal Standard
	R.T. 1.39 Formic Acid

FORMIC ACID IN URINE (Reference Chromatograms/Spectra)

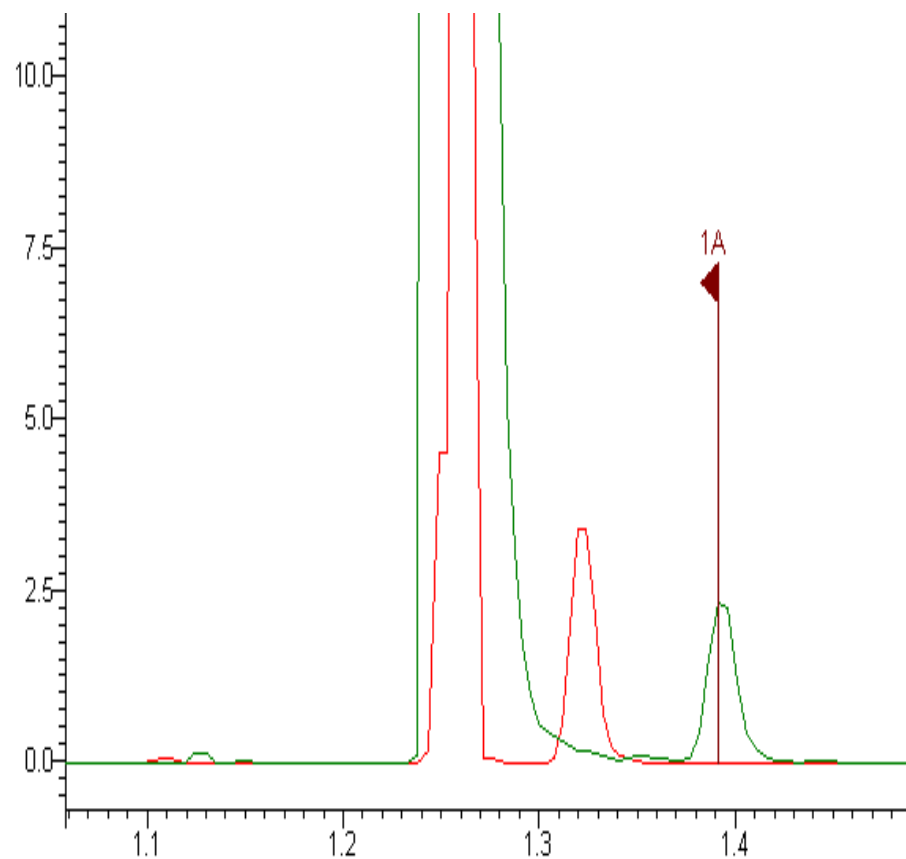


Fig. 3 :	Chemical Standard
	R.T. 1.32 Internal Standard
	R.T. 1.39 Formic Acid

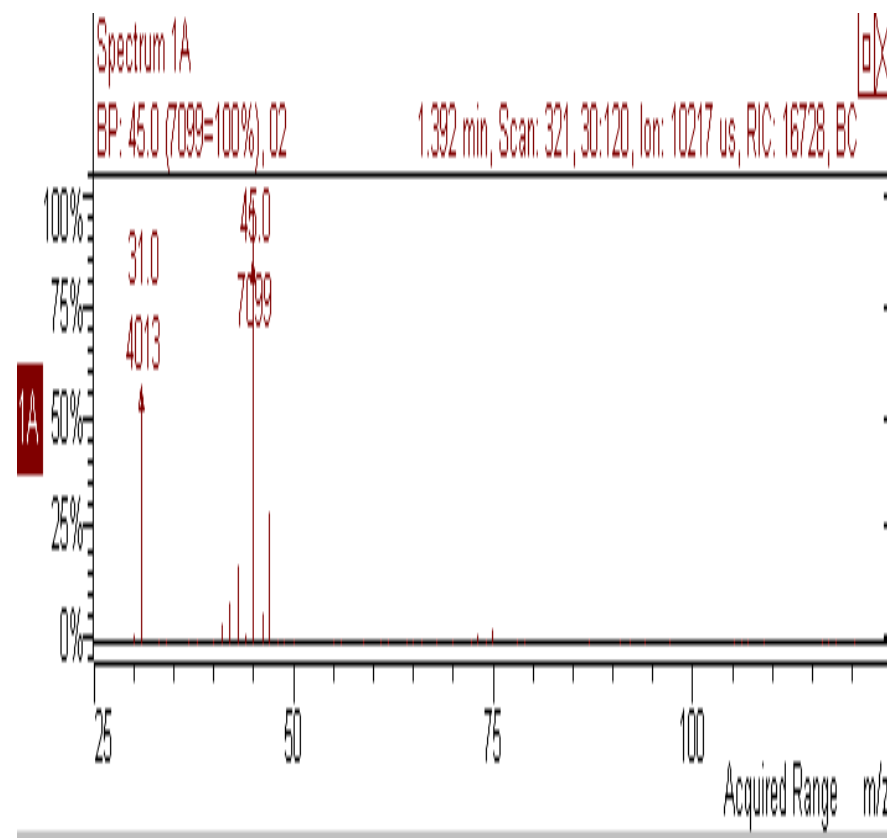


Fig. 4 :	Spectrum of Formic Acid
	MOLECULAR IONS: 74-75