

TRICHLOROETHANE IN URINE BY GC/MS Headspace
Code GC16010

BIOCHEMISTRY

Trichloroethane is an alkyl halide at room temperature, which is presented as a colorless, sweet smelling liquid. It's a very efficient solvent of organic compounds as its low polarity can dissolve many compounds without mixing with non polar solvents, and thus allows it to operate as a selective solvent and extractor. Trichloroethane is used in the metalworking industry as a cleaner and degreaser and in the removal of PVC waste from metals such as silver and copper. In the electronics industry it is used as a solvent of photoresist and as a co-solvent in the production of paints, inks, glues and adhesives.

Trichloroethane is absorbed by inhalation and only 4% is metabolized via CYP450 to Trichloroethanol and subsequently to trichloroacetic acid. 90% is exhaled.

Trichloroethane affects the human central nervous system presenting the classic symptoms of intoxication such as vomiting, confusion, unconsciousness and in severe cases death. The proposed method determines trichloroethane concentration by injecting urine sample previously treated in an appropriate solvent into GC-MS/ headspace. The levels of trichloroethane in a urine sample are determined at the beginning and end of the cycle.



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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° 004	Trichloroethane in urine by GC/MS- headspace	11/2024
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TECHNICAL FEATURES

Principle of the Method :

This method determines the trichloroethane urinary GC / MS headspace. The trichloroethane is first diluted and then is directly injected into GC.

Recovery : 100%

Sensitivity : 78 µg/l by MS

Dynamic Range of the Method: 80 – 25.000 µg/l by MS

Normal Value: < 595 µg/l exposed

CV%: 4,6% intra serie
5,1% inter serie

Components of the kit :

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

Reagent A – Diluting Solution, 1 x 180 ml

Reagent B – Test Solution / Chemical Standard, 2 x 2 ml

For the sampling of Reagent C, the cap sealing requires the use of 50 µl Hamilton syringe Cod. ZRE24533
Store at 2-8 °C

Minimum Instrumental equipment required:

GC/MS
Operational Computer

Optional Equipment:

Autosampler

Urine Collection Procedure:

At the end of the first half shifts, after emptying of the bladder at the beginning of the work shift collect 10 ml of urine in a glass vial sealed (with ferrules aluminum caps and Teflon coated). The transfer of urine in the container must be carried out in the shortest possible time.
Stable 7 days at 4 °C.
Stable 4 weeks at –20 °C.

PREANALYTICAL PROCEDURE

Preparation of Test Solution

Dispense in a vial for headspace of 10ml:

- 1980 µl of **Reagent A – Diluting Solution**
- 20 µl of **Reagent B – Test Solution (using the Hamilton syringe of 50 µl code ZRE24533)**

Inject 1000 µl of solution into gas chromatograph GC. 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 Calibration Standard Solution at 625 µg/l

Dispense in a tube of 10 ml with screw cap:

- 150 µl of **Methanol**
- 50 µl of **Reagent B – Chemical Standard (using the Hamilton syringe of 50 µl code ZRE24533)**

Close the cap ermetically

Vortex for 30 sec.

STEP 2: Preparation of Standard in urine

Dispense in a tube of 10 ml with screw cap:

- 950 µl of **Urine without 1,1,1-Trichloroethane**
- 50 µl of **Chemical Standard prepared in STEP 1 (using the Hamilton syringe of 50 µl code ZRE24533)**

Close the cap ermetically

Vortex for 30 sec.

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

To verify that the urine is effectively free from 1,1,1-Trichloroethane, to prepare a Blank.

Dispense in a tube of 10 ml with screw cap:

- 1600 µl of **Reagent A – Diluting Solution**
- 400 µl of **Urine without 1,1,1-Trichloroethane**

If the Blank urine presented the peak of 1,1,1-Trichloroethane, proceed to identify the quantitative difference between the areas / heights of the peaks of 1,1,1-Trichloroethane in urine enriched and blank.

N.B.: after the preparation of Standards immediately close the vial of solution Standard Chemicals with parafilm and immediately stored at 2-8 ° C.

FASE 3: Samples Preparation

Dispense in a tube of 10 ml for headspace:

	Calibration Standard of 625 µg/l	Unknown Samples	Controls
Calibration Standard prepared in STEP 1	400 µl		
Samples		400 µl	
Controls			400 µl
Reagent A – Diluting Solution	1600 µl	1600 µl	1600 µl

Close the cap quickly

Vortex for 30 sec.

- Inject 1 ml headspace Calibrator, any Control Levels and the unknown samples prepared and start the series according to the specifications of the system

N.B.: it is recommended to use a syringe for headspace autosampler **ONLY** for the injections of 1,1,1-Trichloroethane to prevent any carryover effects and / or interfering due to other types of methods.

TRICHLOROETHANE - Warnings

REAGENT B : TEST SOLUTION / CHEMICAL STANDARD

TRICHLOROETHANE	50.000 µg/l
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SET OF GAS-CROMATOGRAPH:

- Restek Rxi-5SilMS Column 30 m x 0,25 mm, 0,25 µm (conditioned)
- Injector's Temperature 200 °C
- Temperature 50 °C x 4,75 minutes + 40 °C/min up to 180 °C x 0 min (run 8 min)
- Helium Gas 1 ml/min
- Split Report Start: Initial ON Split 20

SET OF AUTOSAMPLER FOR HEADSPACE:

- Syringe Temperature: 90°C
- Mixer Temperature: 90°C (incubate x 10 min at 750 rpm)
- Mixer ON: 5 sec
- Mixer OFF: 2 sec
- Plunger Fill Speed: 500 ul/sec
- Plunger Injection Speed: 1000 ul/sec
- Fill Strokes: 0
- Viscosity Delay: 1sec
- Pre-Injection Delay: 0.1sec
- Post-Injection Delay: 0.1sec
- Syringe Flush Time: 180 sec
- GC Cycle Time (for prep ahead): 4 min

SET OF MASSA DETECTOR (4000):

- Range of Massa 50 – 130
- Operative Method in Electronic Impact (EI)
- Temperature of Massa: 190 °C
- Temperature of Manifold: 80 °C
- Temperature of Transfer Line: 270 °C
- Filament on: 1 minute

CONDITIONING OF RESTEK RXI-5SILMS 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)

ACCESSORIES AND CONSUMABLES

CODE	DESCRIPTION	PACKAGING
GC16020	Chemical Standard for Tetrachloroethane	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

(Reference Chromatograms/Spectrums GC-MS / Headspace)

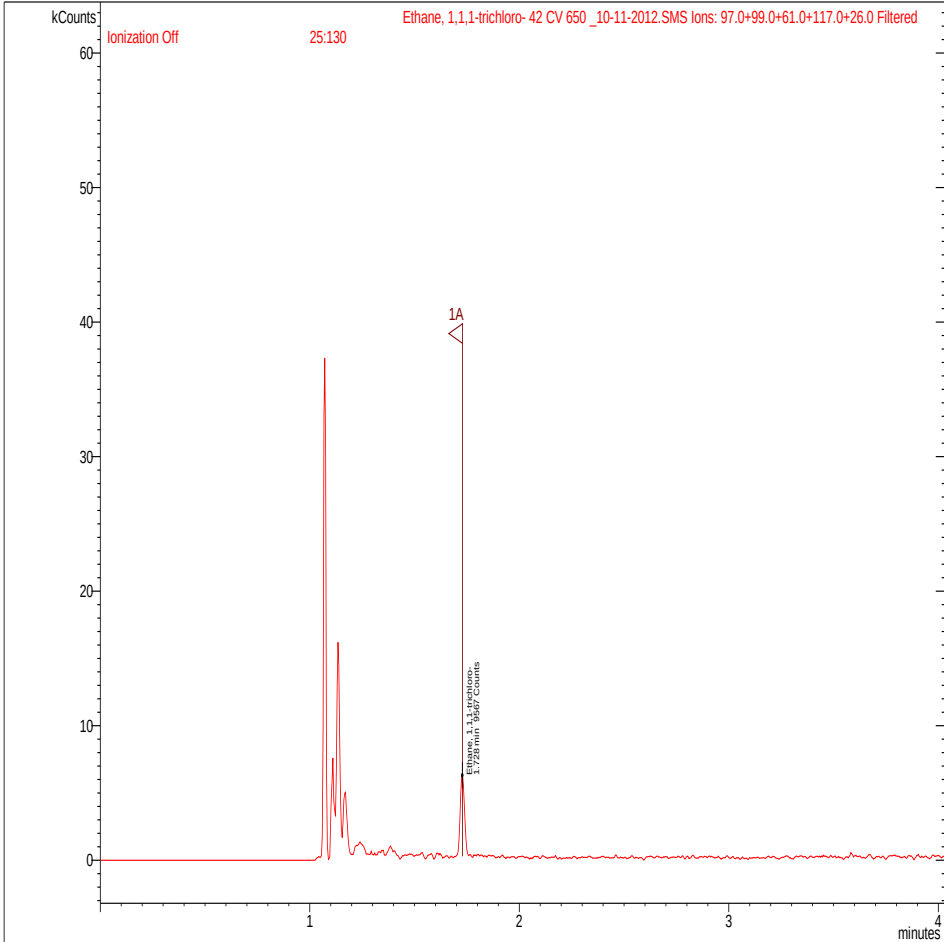


Fig. 1 :	Test Solution
	R.T. 1.7 TRICHLOROETHANE

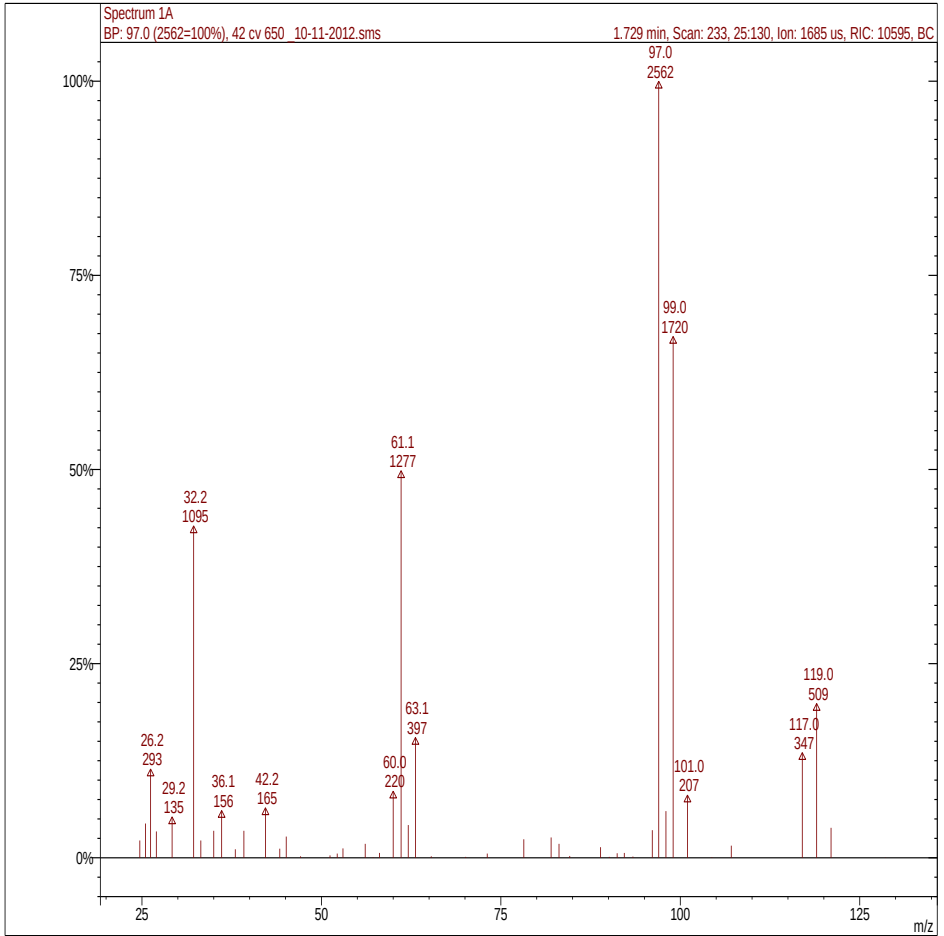


Fig. 2 :	Massa Spectrum of Trichloroethane
	MOLECULAR IONS : 97-99-61-117