

ETHANOL (ETHYL ALCOHOL) in whole blood by GC/MS-headspace – CODE GC73010

BIOCHEMISTRY AND METABOLISM

Ethyl alcohol is an aliphatic alcohol PM=46,07.

Usually it is consumed orally and quickly absorbed through the stomach, small intestine and colon. The time necessary to complete the process of absorption is from 2 to 6 hours and varies as a result of different factors such as the presence of food and other liquids, the time employed for the ingestion of the drink and biological variability among individuals.

Because of its solubility in water and the low molecular weight, as soon as the alcohol is absorbed, it is quickly distributed in tissues and fluids of the organism, also passing through the encephalic and placental barriers. The proportion of alcohol concentration in some biological liquids has been determined experimentally by several authors (Fig. 1). It has been demonstrated that the maximum concentration is achieved approximately 20 minutes from consumption in blood saliva and expired air, while in urine the maximum concentration is achieved after approximately two hours.

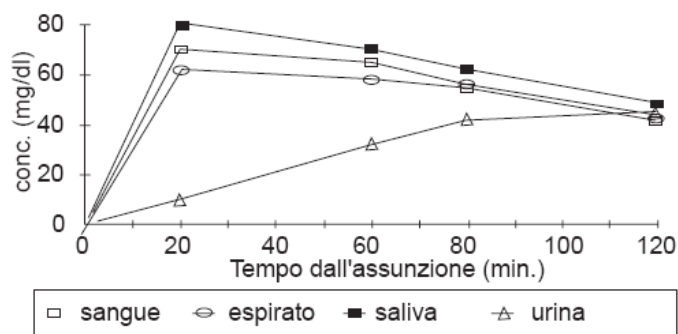


Fig. 1. Ethyl alcohol: concentration in the biological liquids

After absorption, Ethanol is mostly metabolized (90- 98%) by oxidation, at a speed that is directly proportional to the corporeal weight and constant in the time. The first reaction of oxidation occurs in the liver, where ethanol is broken down by an enzyme called alcohol dehydrogenase (ADH), which transforms ethanol into acetaldehyde; this is subsequently oxidized to acetic acid by the enzyme Aldehyde Dehydrogenase. The process of elimination does not have an exponential course, like most exogenous substances, but is pseudolinear. Following the completion of the absorption and distribution phase, when the concentration value is maximum C_0 , the relation between whole blood concentration C_t and time t is shown by the equation: $C_t = C_0 - kt$, with k between 10 and 25 mg/dL/h (Fig.2).

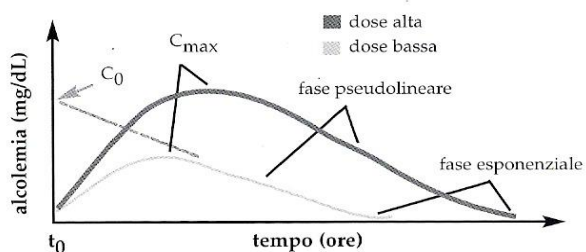


Fig.2. Urines and expired air represent also the main ways of elimination of the alcohol and its products of oxidation. The kinetic of elimination, altogether regulated from the equation of Michaelis Menten, assumes however a pseudolinear course after the completion of the phase of absorption and distribution.

About 2% of the consumed alcohol is excreted through the kidneys and lungs, and small amounts are also found in saliva and other organic liquids; it can go up as high as 10% in cases of massive ingestion. Urine concentration is a little bit higher than hematic concentration and alveolar concentration is approximately 0.05%. The concentration in plasma is approximately 10% higher than the whole blood.

ACUTE POISONING

The excessive consumption of alcohol provokes, in the short term, clinical situations of varied gravity that could require medical intervention, both for the direct effects of drunkenness and for indirect ones (e.g. car accidents).

The doctor could apply to the Laboratory for different reasons such as :

- to assess a possible cause of coma, in patient without cranial trauma;
- to assess the cause of coma in patient with trauma cranial (e.g. as a result of a car accident);
- to diagnose drunkenness in patient with doubtful symptomatology.

It has been shown there is a relationship between Blood Alcohol Concentration (BAC) and drunkenness. A blood level of 0.5% or more is commonly fatal. Levels of even less than 0.1% can cause intoxication, with unconsciousness often occurring at 0.3–0.4%. The amount of ethanol in the body is typically quantified by blood alcohol content (BAC), the milligrams of ethanol per 100 milliliters of blood. The table on the right summarizes the symptoms of ethanol consumption. Small doses of ethanol generally produce euphoria and relaxation; people experiencing these symptoms tend to become talkative and less inhibited, and may exhibit poor judgment. At higher dosages (BAC > 100 mg/dl), ethanol acts as a central nervous system depressant, producing at progressively higher dosages, impaired sensory and motor function, slowed cognition, stupefaction, unconsciousness, and possible death.

In America, about half of the deaths in car accidents occur in alcohol-related crashes. There is no completely safe level of alcohol for driving; the risk of a fatal car accident rises with the level of alcohol in the driver's blood. However, most drunk driving laws governing the acceptable levels in the blood while driving or operating heavy machinery set typical upper limits of blood alcohol content (BAC) between 0.05% to 0.08%. Italian Legislation does not consider alcohol consumption a crime except when the drinker has to drive (August 2003 Law 1, n. 214 - Art. 186). The suspicion of such crime authorizes the agents of highway patrol to carry out assessment of "psycho-physical alteration" due to the alcohol. Assessment that must be carried out using instruments and procedures determined by regulations (DPR 16 Decembers to you 1992, n. 495 - Art. 379). The procedures for the examination for the presence of alcohol in the blood are dictated by Article n°186 of the Rules of the Road.

In Italy the level of whole blood concentration for the state of drunkenness has been fixed to 0,5 g/l.

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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° 010	Ethanol in whole blood by GC/FID or GC/MS - headspace	07/2024
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TECHNICAL FEATURES

Principle of the Method:

The sample is transferred into vials for headspace, diluted and added of internal standard. Is then analyzed by GC / FID or GC / MS - headspace.

Recovery :

100%

Dynamic Range of the Method :

0,01 - 10 g/l

LOD:

0,003 g/l

LOQ:

0,01 g/l

Accuracy intraday (relative error %):

C LLOQ	Cm	Cs
0,01 g/l	0,5 g/l	2 g/l
9,2%	7,1%	5,9%

Accuracy interday (relative error %):

C LLOQ	Cm	Cs
0,01 g/l	0,5 g/l	2 g/l
10,5%	8,1%	6,1%

Precision intraday (coefficient of variation %):

C LLOQ	Cm	Cs
0,01 g/l	0,5 g/l	2 g/l
7,9%	6,8%	5,2%

Precision interday (coefficient of variation %):

C LLOQ	Cm	Cs
0,01 g/l	0,5 g/l	2 g/l
8,9%	7,6%	5,6%

Coefficient of Correlation + DS :

0,9981 $\pm$ 0,0025

Reference Values:

*Ref. Art. n° 186 of the Rule of the Road:
Guide in state of drunkenness*

0,5 g/l < Ethanol < 0,8 g/l:

pecuniary penalty from 500,00 to 2.000,00 euro and arrest till 1 month – additional administrative pecuniary penalty with driving licence suspension from 3 to 6 months;

0,8 g/l < Ethanol < 1,5 g/l:

pecuniary penalty from 800,00 to 3.200,00 euro and arrest till 3 months - additional administrative pecuniary penalty with driving licence suspension from 6 months to 1 year;

Ethanol > 1,5 g/l:

pecuniary penalty from 1.500,00 to 6.000,00 euro and arrest till 6 months - additional administrative pecuniary penalty with driving licence suspension from 1 to 2 years.

<u>Components of the kit :</u>	All the reagents are ready-to-use and stable 3 years at 2 – 8 °C.
Reagent A – Diluting Solution+ Internal Standard, 2 x 5 ml	<u>See Warnings</u> For the sampling the cap sealing requires the use of 100 ul Hamilton syringe Cod. ZRE24563
Reagent B – Test Solution, 1 x 3 ml	<u>See Warnings</u> For the sampling the cap sealing requires the use of 50 ul Hamilton syringe Cod. ZRE24533
Reagent C – Lyophilized whole blood, 3 bottles	<u>See Warnings</u>
Reagent D1 – Ethanol 0,5 g/l, 1 x 2 ml	<u>See Warnings</u> For the sampling the cap sealing requires the use of 50 ul Hamilton syringe Cod. ZRE24533
Reagent D2 – Ethanol 1 g/l, 1 x 2 ml	<u>See Warnings</u> For the sampling the cap sealing requires the use of 50 ul Hamilton syringe Cod. ZRE24533
Reagent D3 – Ethanol 2 g/l, 1 x 2 ml	<u>See Warnings</u> For the sampling the cap sealing requires the use of 50 ul Hamilton syringe Cod. ZRE24533
Reagent D4 – Ethanol 3 g/l, 1 x 2 ml	<u>See Warnings</u> For the sampling the cap sealing requires the use of 50 ul Hamilton syringe Cod. ZRE24533
Reagent D5 – Ethanol 4 g/l, 1 x 2 ml	<u>See Warnings</u> For the sampling the cap sealing requires the use of 50 ul Hamilton syringe Cod. ZRE24533
5 caps	<u>See Warnings</u> For the sampling the cap sealing requires the use of 50 ul Hamilton syringe Cod. ZRE24533 The caps of reagents D1-D2-D3-D4-D5 are to be replaced after 20 injections

Minimum Instrumental equipment required:

GC/MS or GC/FID Instrument
Operational Computer

Optional Equipment:

Autosampler for headspace

Whole Blood Collection Procedure:

Blood sampling for the determination of alcohol levels

Blood sampling for the determination of blood alcohol must necessarily take into account the following critical points:

- potential contamination due to the use of skin disinfectants containing ethanol;
- possible degradation phenomena that may encourage the formation of ethanol;
- sample manipulations (e.g. sierazione, centrifugation) these by altering its original characteristics;
- possible evaporation of alcohol from the sample after collection.

For these reasons the blood sampling for the determination of alcohol requires obligatorily the following:

- perform a blood test to determine blood alcohol content;
- disinfect the pickup area with disinfectant alcohol not;
- add sodium fluoride, or similar protection, the blood sample (100 mg of NaF₂ to 10 ml blood);
- avoid sierazione or centrifugation sample. It is to be noted, in this regard, that the determination of ethyl alcohol on blood derivatives (plasma, serum) can produce an overestimate relative to whole blood determination;
- use as much as possible useful volume of sample container (if necessary, change the container in relation to the volume of blood taken) in order to avoid evaporation phenomena;
- proceed immediately to the conservation of the sample and the counter sampling at temperature of -18/-22° C less than sample analyses performed within 4 h of collection, in which case it is sufficient, as soon as possible, at +4/+6° C.

If the analysis is not carried out within the 24-hour samples are frozen. Stable 4 weeks. The tube must be kept well capped.

(*) Ref: GUIDELINES FOR LABORATORIES FOR THE ANALYSIS OF SUBSTANCES OF ABUSE FOR TOXICOLOGICAL-FORENSIC AND MEDICO-LEGAL PURPOSES

Revision no. 3 of 1 March 2010 by the Quality Commission1 of the Italian Forensic Toxicologists Group (GTFI)

PREANALYTICAL PROCEDURE

Dilution of Test Solution 5 g/l

Pipette in a 10 ml vial for headspace:

- 75 µl di H₂O HPLC grade

Close hermetically the vials

- 25 µl di **Reagent B – Test Solution** with syringe Hamilton of 50 µl

Vortex for 10 sec.

Insert the vials in the headspace autosampler.

INJECTION :

- Inject 200 µl of this solution in GC/MS – headspace (diameter injector liner: 2 mm).

ANALYTICAL PROCEDURE

STEP 1 : Preparation of 5 Calibration Standards:

N.B.: THIS CALIBRATION STANDARD SOLUTION IS PREPARED FROM TIME TO TIME

The calibration curve should be carried out on 5 points + the blank so:

- Reconstitute the lyophilised whole blood (**Reagent C**) with 1 ml of Water HPLC grade (stir gently until all material is dissolved) – see Warnings

Seal the 10 ml vial and insert in the headspace autosampler:

- VIAL 1: 50 µl of Reconstitute whole blood + 50 µl of Water HPLC grade (**Calibrator at concentration of 0 g/l**)
- VIAL 2: 50 µl of Reconstitute whole blood + 50 µl of **Reagent D1 – Ethanol 0.5 g/l** (**Calibrator at concentration of 0.25 g/l**)
- VIAL 3: 50 µl of Reconstitute whole blood + 50 µl of **Reagent D2 – Ethanol 1 g/l** (**Calibrator at concentration of 0.5 g/l**)
- VIAL 4: 50 µl of Reconstitute whole blood + 50 µl of **Reagent D3 – Ethanol 2 g/l** (**Calibrator at concentration of 1.0 g/l**)
- VIAL 5: 50 µl of Reconstitute whole blood + 50 µl of **Reagent D4 – Ethanol 3 g/l** (**Calibrator at concentration of 1.5 g/l**)
- VIAL 6: 50 µl of Reconstitute whole blood + 50 µl of **Reagent D5 – Ethanol 4 g/l** (**Calibrator at concentration of 2.0 g/l**)
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N.B: apply 50 µl of lyophilised blood (and 50 µl of H₂O) with the pipette, seal the vials with caps for HeadSpace and add the reagent D1-D2-D3-D4-D5 using Hamilton syringe of 50 µl and from piercing the septum of the vials.

STEP 2 : Samples Preparation:

N.B: **Reagent A** is added with the Hamilton syringe of 100 µl by piercing the septum of the vials.

	VIAL 1 (prepared at STEP 1)	VIAL 2 (prepared at STEP 1)	VIAL 3 (prepared at STEP 1)	VIAL 4 (prepared at STEP 1)	VIAL 5 (prepared at STEP 1)	VIAL 6 (prepared at STEP 1)	Sample	Controls
VIAL 1	100 µl							
VIAL 2		100 µl						
VIAL 3			100 µl					
VIAL 4				100 µl				
VIAL 5					100 µl			
VIAL 6						100 µl		
Sample							100 µl	
Controls								100 µl
Reagent A – Diluting Sol. + Internal Std	100 µl	100 µl	100 µl	100 µl	100 µl	100 µl	100 µl	100 µl

Vortex for 10 sec.

Seal the vial and insert in the headspace autosampler.

INJECTION :

- Inject 200 µl of this solution in GC/MS – headspace (diameter injector liner: 2 mm).

ETHANOL IN WHOLE BLOOD - Warnings

REAGENT B : TEST SOLUTION

ETHANOL	20.0 g/l
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REAGENT C : LYOPHILIZED WHOLE BLOOD

RECONSTITUTION OF LYOPHILIZED WHOLE BLOOD: Remove metal seal and rubber stopper from the vial. Add exactly 1 ml of HPLC grade water to the vial. Replace the rubber stopper, allow to stand for 15 minutes. Shake before use by inverting the vial to dissolve the material to obtain a homogenous solution.

STORAGE AND STABILITY: The whole blood is stable 36 months from the date of preparation if stored at 2-8 ° C. After reconstitution are stable 30 days at 2-8 ° C and 6 months at -20 ° C. Do not use them after the expiration date.

PRECAUTIONS: This control human matrix must be treated with care and treated as potentially infectious.

REAGENT D1 : ETHANOL 0,5 g/L

ETHANOL	0.5 g/l
Aqueous solutions of ethyl alcohol are stable 12 months from the date of preparation if stored at 2-8 ° C. Do not use them after the expiration date.	

REAGENT D2 : ETHANOL 1,0 g/L

ETHANOL	1.0 g/l
Aqueous solutions of ethyl alcohol are stable 12 months from the date of preparation if stored at 2-8 ° C. Do not use them after the expiration date.	

REAGENT D3 : ETHANOL 2,0 g/L

ETHANOL	2.0 g/l
Aqueous solutions of ethyl alcohol are stable 12 months from the date of preparation if stored at 2-8 ° C. Do not use them after the expiration date.	

REAGENT D4 : ETHANOL 3,0 g/L

ETHANOL	3.0 g/l
Aqueous solutions of ethyl alcohol are stable 12 months from the date of preparation if stored at 2-8 ° C. Do not use them after the expiration date.	

REAGENT D5 : ETHANOL 4,0 g/L

ETHANOL	4.0 g/l
Aqueous solutions of ethyl alcohol are stable 12 months from the date of preparation if stored at 2-8 ° C. Do not use them after the expiration date.	

SET OF GAS-CROMATOGRAPH:

- Syringe Temperature: 60 °C
- Mixer Temperature: 50 °C (incubate for 20 minutes at 500 rpm)
- Mixer ON: 30 sec
- Mixer OFF: 30 sec
- Plunger Fill Speed: 200 µl/sec
- Plunger Injection Speed: 500 µl/sec
- Fill Strokes: 0
- Viscosity Delay: 3 sec
- Pre-Injection Delay: 0.1 sec
- Post-Injection Delay: 0.1 sec
- Syringe Flush Time: 120 sec

SET OF GAS-CROMATOGRAPH:

- VF-5ms Column 30 m x 0,25 mm, 0,25 µm (conditioned)
- Injector's Temperature 250 °C
- Split Ratio: 1:20
- Helium Gas 1,5 ml/min
- Column Oven Temperature: 40 °C x 6 min

SET OF FID DETECTOR:

- Temperature: 250 °C
- Range: 12
- Time constant: Fast

SET OF MASSA DETECTOR:

- Range of Massa 15 – 60
- Operative Method in Electronic Impact (EI)
- Temperature of Massa: 180 °C
- Temperature of Manifold: 80 °C
- Temperature of Transfer Line: 270 °C
- Filament on: 0.5 minutes

CONDITIONING OF VF-5 MS COLUMN

Follow the manufacturer prescription.
Do not condition column/s if connected to the Massa Detector.
At the beginning of each run to calibrate the masses and RF.

CLEANING OF THE COLUMN

Disconnect the detector. Keep the column at the highest temperature for the recommended time (see the manufacturer's instructions) with a speed of 30 °C/min.

N.B.: We recommend using a syringe for autosampler headspace ONLY for Ethanol injections to avoid any carry-over effects and / or interference caused by other types of methods.

ACCESSORIES AND CONSUMABLES

CODE	DESCRIPTION	PACKAGING
GC73016	Calibrator in whole blood for Ethyl Alcohol	6 x 1 ml/1 x 5 x 2 ml
GC73019	Control in whole blood for Ethyl Alcohol – Levels 1 and 2	4 x 1 ml/ 1 x 2 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

ETHANOL IN WHOLE BLOOD BY GC/FID or GC/MS – headspace

(Reference Chromatograms)

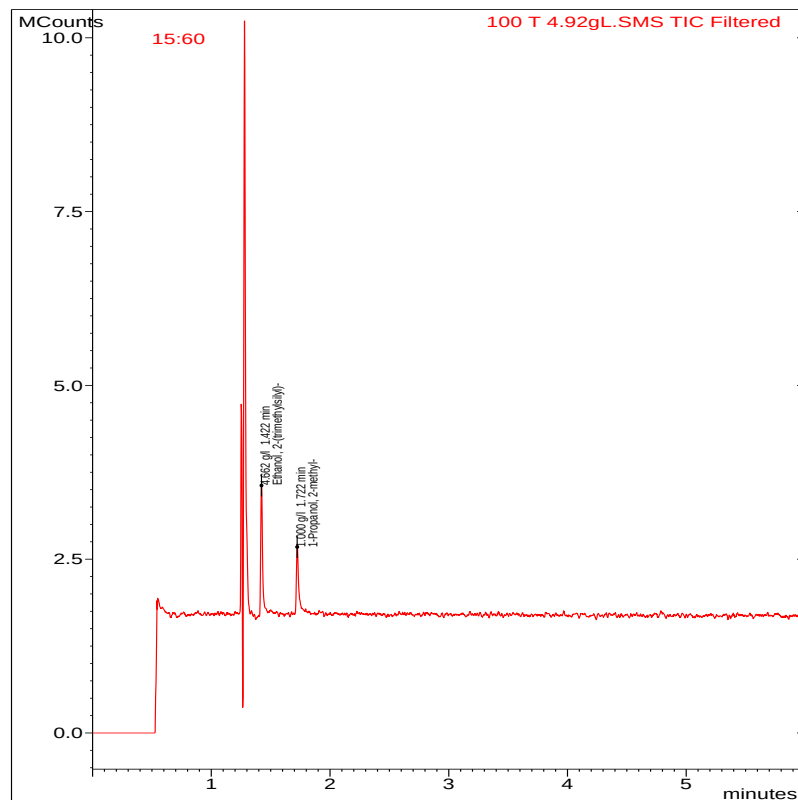


Fig. 3 :	Test Solution
	R.T. 1.42 Ethanol
	R.T. 1.72 Internal Standard

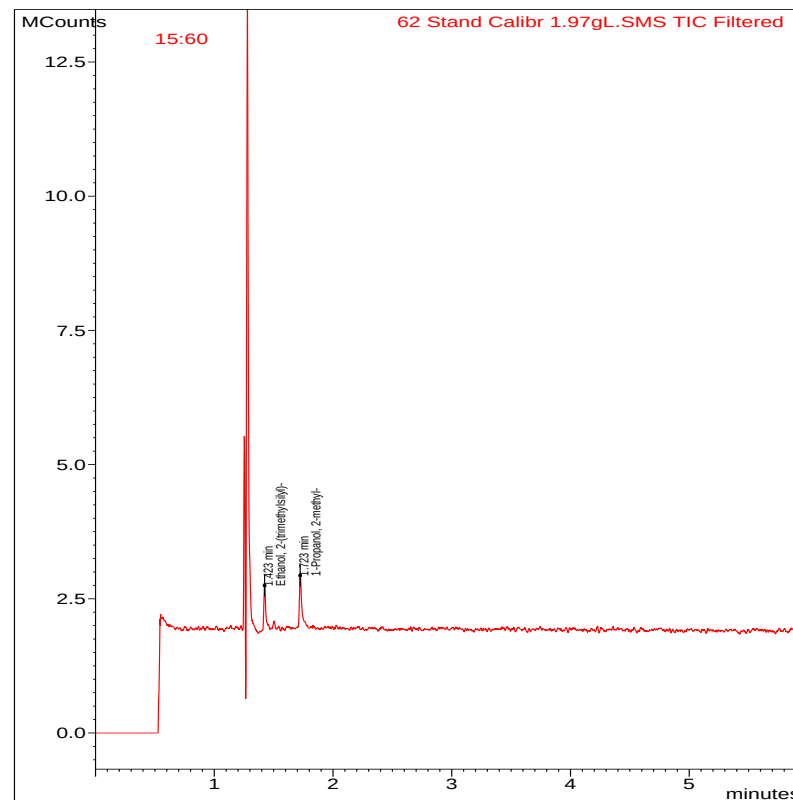


Fig. 4 :	Calibration Standard
	R.T. 1.42 Ethanol 1,97 g/l
	R.T. 1.72 Internal Standard

ETHANOL IN WHOLE BLOOD BY GC/FID or GC/MS – headspace

(Reference Chromatograms)

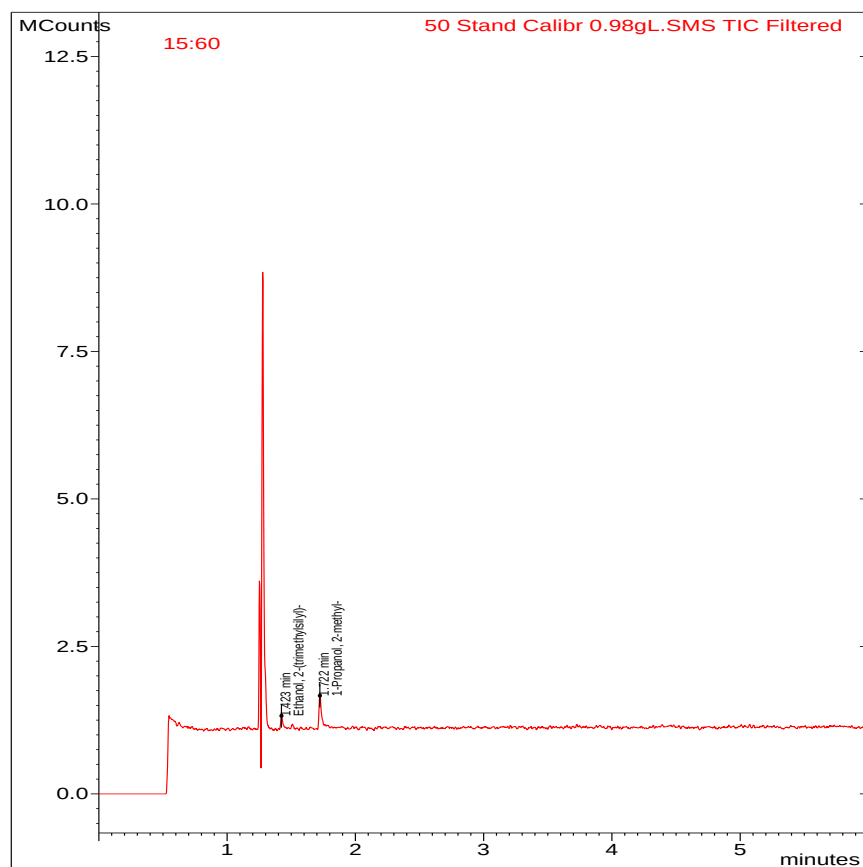


Fig. 5 :	Calibration Standard
	R.T. 1.42 Ethanol 0,98 g/l
	R.T. 1.72 Internal Standard

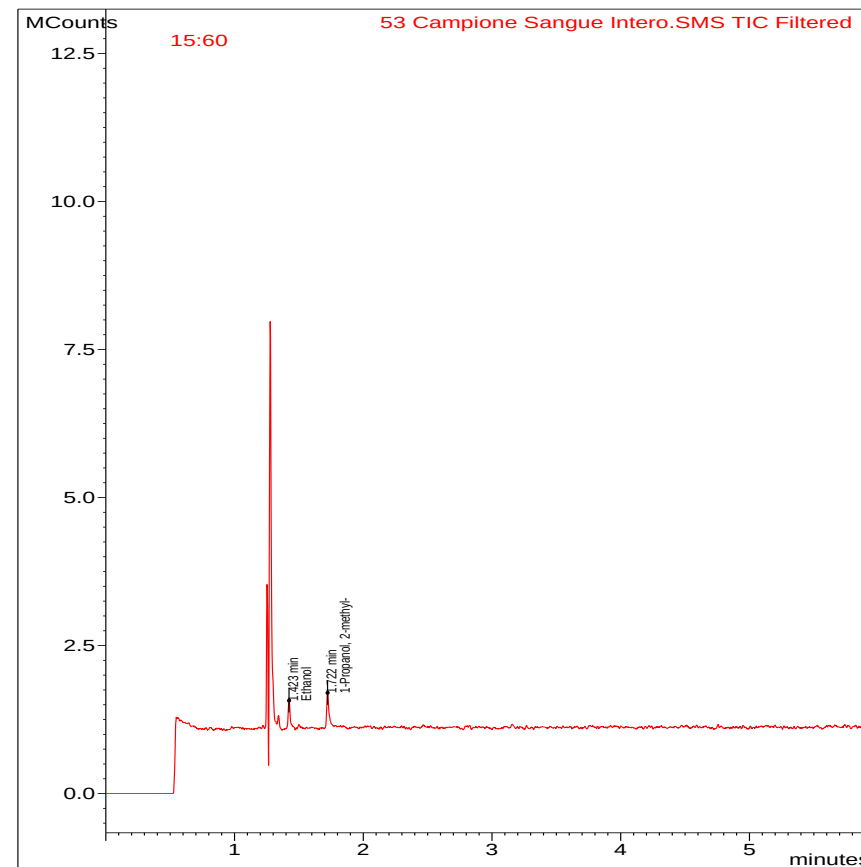


Fig. 6 :	Sample on whole blood
	R.T. 1.42 Ethanol 1,6 g/l
	R.T. 1.72 Internal Standard

ETHANOL IN WHOLE BLOOD BY GC/FID or GC/MS – headspace

(Reference Spectrums)

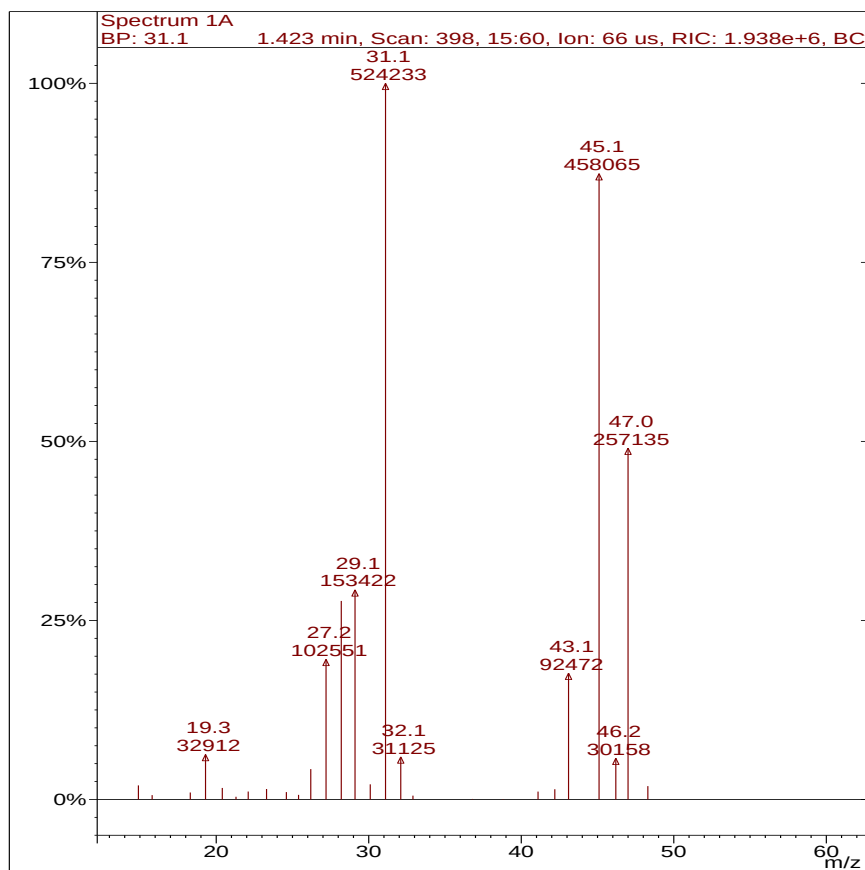


Fig. 7 :	Massa Spectrum of Ethanol
	MOLECULAR IONS: 31-29-45

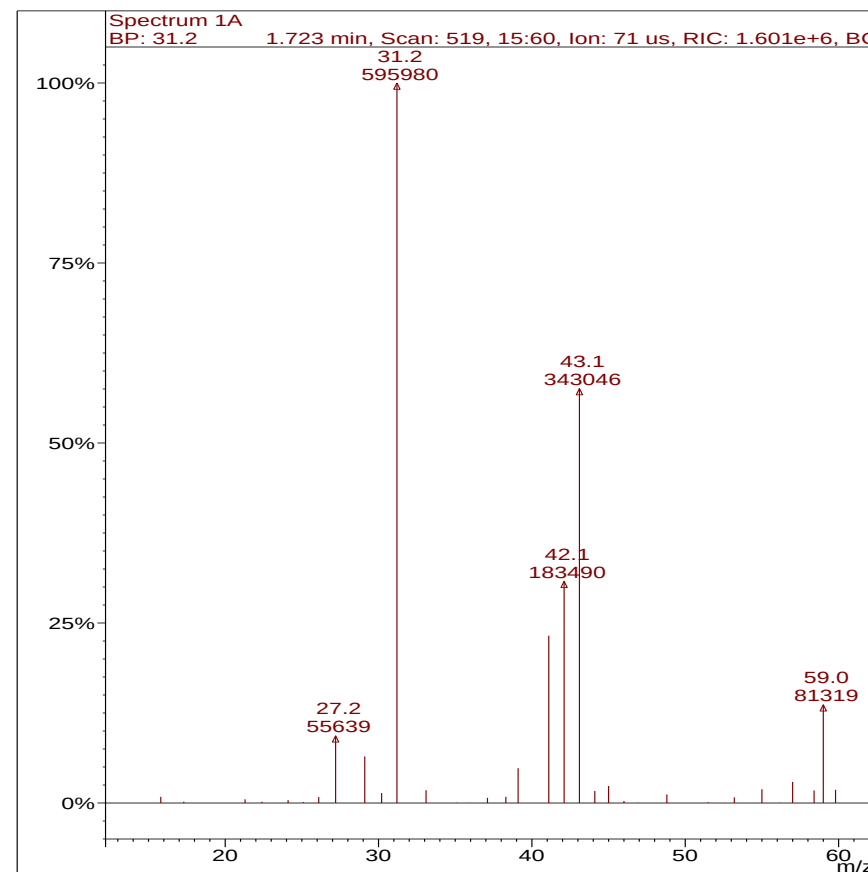


Fig. 8 :	Massa Spectrum of Internal Standard
	MOLECULAR IONS: 43-42-31