## BREATH ALCOHOL CONCENTRATION MEASUREMENTS IN ROMANIA

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**Rezumat.** Trasabilitatea concentrației de alcool din aerul expirat este un domeniu nou, de interes în România. Aproape 1700 de analizoare de alcool din aerul expirat au fost achizitionate de către Ministerul de Interne (Departamentul de Poliție) acum câțiva ani, ca urmare a unui Proiect European, pentru a echipa Departamentele Poliției din Europa de Est. De atunci, trasabilitatea măsurărilor efectuate cu ajutorul unor astfel de aparate a fost o prioritate în scopul asigurarii măsurărilor precise și, în mod special, acceptarea în instanțele de judecată. Măsurările efectuate în momente diferite și în locuri diferite sunt, astfel, direct legate la o referință comună. Aplicarea acestui concept de trasabilitate pentru măsurările de alcool din aerul expirat nu este ușoară, dar trasabilitatea trebuie să furnizeze rezultate calitative, prin tehnici analitice utilizate în laboratoarele de etalonare.

Cuvinte cheie: Trasabilitate, materiale de referință, concentrația de alcool din aerul expirat, metrologie.

Abstract. Traceability of breath alcohol concentration is a new field of interest in Romania. About 1700 breath alcohol analysers were purchased by the Ministry of the Interior (Police Department) a few years ago following a European project to equip East European Police Departments. Since then, the traceability of measurements performed by such instruments was a priority in order to ensure accurate measurements and especially acceptance in court. Measurements made at different times or in different places are thus directly related to a common reference. Applying the concept of traceability to breath alcohol measurements is not easy, but traceability has to provide qualitative results using analytical techniques used in calibration laboratories.

Key words: Traceability; reference materials; breath alcohol concentration; metrology.

### **1. INTRODUCTION**

Alcohol is a general term that designates a group of chemical organic substances with common properties. This family consist of ethanol, methanol, isopropanol and others. Due to its physicochemical and physiological properties ethanol is the most ingested substance from its alcohol group.

The word "alcohol" comes from an Arabian word that means "refined, subtle".

When is consumed, it passes rapidly to blood and through it, directly to whole body. Generally speaking, it is a stimulant; from medical point of view alcohol is a first-degree toxic substance for body cell and thus the human body reacts instantly to eliminate it.

Chemical formula of ethanol is  $C_2H_5OH$ , (C is carbon, the dash is a single bond, H is hydrogen, O

is oxygen). The chemistry of ethanol is largely that of its hydroxyl group. Ethanol for industrial use is most often made from petrochemicals, typically by the acid-catalyzed hydration of ethylene, represented by the chemical equation:

$$C_2H_4 + H_2O \rightarrow CH_3CH_2OH$$

In an older process, first practiced on the industrial scale in 1930 by Union Carbide, but now almost entirely obsolete, ethene was hydrated indirectly by reacting it with concentrated sulfuric acid to product ethyl sulfate, which was then hydrolyzed to yield ethanol and regenerate the sulfuric acid:

$$C_2H_4 + H_2SO_4 \rightarrow CH_3CH_2SO_4H$$
$$CH_3CH_2SO_4H + H_2O \rightarrow CH_3CH_2OH + H_2SO_4$$

### Alcohol chemistry



Ethanol for use in alcoholic beverages, and the vast majority of ethanol for use as fuel, is produced by fermentation: when certain species of yeast (most importantly, *Saccharomyces cerevisiae*) metabolize sugar in the absence of oxygen, they produce ethanol and carbon dioxide. The overall chemical reaction conducted by the yeast may be represented by the chemical equation:

$$C_6H_{12}O_6 \rightarrow 2 CH_3CH_2OH + 2 CO_2$$

### 2. ALCOHOL AND HUMAN BODY

Scientist from all over the world are studying new methods and technique to measure the alcohol concentration from human body, and international organisations issued standards which stands technological limits for newest devices.

Due to ethanol property of being volatile a certain quantity of alcohol, proportional with blood alcohol concentration, transfers from blood to lung alveoli, in the same way that  $CO_2$  goes from blood to the lung alveoli in order to be eliminated from human body.



Based on this phenomenon, it is possible to measure the alcohol concentration from deep breath sample with high accuracy.

An alcohol concentration of 0,3 % has a negative influence on reaction ability: week focus capacity; impaired cognition; predisposition for accidents increases; visual angle is narrowing – "tunnel effect vision", object seems to be farther then they are in reality.

Starting with 0,5 ‰ reflexes slow down; visual sensibility decrease and is difficult to appreciate the speed.

Coordination of movements is reduced; confusion increases and orientation is weaker; night vision is reduced very much. Increasing self-confident leads to excessive and dangerous ideas about the own capabilities, losing the control on his own personality.

The subject overreacts. Imminent dangers are recognized too late and the reactions are not fitting the situations

When somebody drinks alcoholic beverages, the alcohol passes into the stomach and small intestine.

The alcohol then diffuses through the stomach/ small intestine wall, passing via the capillaries into the bloodstream. Most of the alcohol is absorbed via the small intestine.

The alcohol continues to diffuse into the blood until a stable state is reached.



The alcohol is transported to the liver through the veins, where part of the alcohol that flows through the liver is continuously broken down, i.e. converted into  $CO_2$  and water and releasing energy.

The part of the alcohol, which is not broken down, passes via the bloodstream (arteries) to all parts of the body (including the brain) and is deposited in all "aqueous" tissues of the body. Part of the alcohol also flows back to the heart via the veins.

Alcohol in the bloodstream diffuses into the body's tissue in proportion to the latter's water content.

Because the water content varies from tissue to tissue, some tissues absorb more of the alcohol than others do; muscles consist of around 80 % water; fat contains approximate 20 % water.

This distribution process continues until an equilibrium is reached between the alcohol concentration in the blood and the alcohol concentration in the tissue.



In this stable state, no relative exchanges in alcohol concentration take place.

The alcohol concentration in the blood falls as alcohol in the liver is expelled from the body.

As the alcohol concentration in the bloodstream falls, alcohol diffuses back from the tissue into the blood.



This process continues until all the alcohol has been removed from the body.

The speed at which alcohol is broken down varies from one person to another and generally cannot be accelerated by drinking coffee, taking a shower etc. In alcoholics, the breakdown of alcohol may take place more quickly due to the fact that the enzymes in their liver are more active.

The concentration of alcohol in the blood is determined by the processes of absorption, distribution and breakdown. The increase in blood alcohol concentration over time, after consumption of alcohol, depends on various factors:

- duration and quantity of alcohol consumption;
- amount of food in the stomach;
- weight of the person;
- sex of the person.

Determination of alcohol concentration from end expiratory sample is based on the principle according to, at equilibrium, in a closed system, the concentration of a substance in gaseous phase is proportional with its concentration in liquid phase. This principle is known as **Henry's law**. When an *aqueous mixture (blood) of a volatile substance (alcohol)* reaches *equilibrium with air (in the lung)*, there will be a *fixed ratio* between the concentration of the substance in the air and its concentration in the solution.

This ratio (2100:1) remains constant for a *given temperature* (*body temperature is constant and* does not depend on the ambient pressure.

Currently, the breath alcohol value, 2100:1 (concentration of alcohol in the blood to the concentration of alcohol in the breath) is used by the producers of breath alcohol analysers and also by the legislative system as a fixed partition value.

### 3. METHODS FOR ANALYSIS OF ALCOHOL IN HUMAN BODY

Qualitative and quantitative methods for analysis of alcohol from biological samples are based on five analytical principles:

- chemical oxidation;
- enzymatic oxidation;

- gas chromatography;
- electrochemical oxidation;
- Infra Red spectrometry;
- magnetic resonance of 1H-MRS

### 3.1. Chemical oxidation method (chemical reactions method; wet chemistry method)

Erik M.P. Widmark developed the microdiffusion method for quantitative analysis of blood alcohol. The method consists of direct sampling of 100 mg of blood in S shape glass capillaries, from finger or from ear. The inner surface of capillaries is coated with a thick layer of potassium fluoride or oxalic potassium to prevent blood coagulation. Ethanol is oxidized to acetic acid with a mixture of dichromate and sulphuric acid in excess; the unoxidised quantity is determined by iodomentric titration with natrium tiosulfate.

The method was used in Scandinavia starting with 1930-1940 in forensic purposes and improved with different reagents.

# 3.2. Enzymatic oxidation method (alcohol dehydrogenation)

The enzymatic method used for alcohol detection from biological samples consist of direct analysis of the sample, diluted (100...1.000) times with a buffer solution (pH 9,6) before adding necessary quantities of enzyme and coenzyme (NAD<sup>+</sup> – nicotine amide ademyn dinucleotida). During reaction, NAD<sup>+</sup> reduces to NADH and the resulted quantity is proportional with the ethanol concentration. Complete oxidation of ethanol takes about 60 minutes at room temperature and the reduced coenzyme (NADH) is monitorised by a spectrophotometer at 340 nm wavelenght. The method improved during the time by automatisation.

### 3.3. Gas-chromatograph method

This method depends on physico-chemical properties of ethanol: chemical structure, volatility, boiling point and solubility in lipid / water. The method consist of partition of a volatile substance (ethanol) between an inert mobile phase (carrier gas  $-N_2$  or He) and a stationary liquid phase, which is deposit as a layer on an absorbent material used as support for liquid phase. The glass or metal column, 2 meters long and interior diameter of 3 milimeters is filled with a granular

material. The carrier gas  $(N_2)$  flows constantly through the column and at a certain pressure which allows volatile substance to interact or to establish equilibrium with stationary liquid phase, conducting thus to separation of molecules. After separation, the ethanol is measured quantitative and qualitative by a thermoconductometric TCD (Thermo Conductometric Detector) or by more performant detector FID (Flame Ionization Detector).

The Chromatographic HeadSpace consists of dilution of the sample (1:5 or 1:10) with an internal standard, in a vial maintained at 50 °C. After the equilibrium is reached vapours are automatically injected into the column by a syringe. For separation can be used either filled or capillary columns.

# 3.4. Electrochemical Oxidation Method (Electrochemical Sensors)

This new analytical principle was developed at early 1970's as result of cooperation between laboratories from Cardiff University and Medical Centre for Research – London.

The principle consists of electrochemical oxidation on a catalyst surface and is a detector (electrochemical fuel cell) for ethanol oxidation.

In order to perform measurement a sample containing 1.5 L of exhaled air is processed and displayed in approximate 15 seconds. A certain quantity of air intended to be measured, access the entrance of the sensor using a sampling system. The sensor consists of the following main parts (see the picture): electrolyte, working electrode (cathode) and a counting electrode (anode), as shown in figure 1.



Fig. 1. Electrochemical sensor.

The electrolyte and the material which electrodes are made from are selected in such a way as the alcohol intended to be measured is electrochemically converted at the cathode, generating thus a current that flows through the sensor:

$$C_2H_5OH + 3 H_2O \rightarrow 2 CO_2 + 12 H^+ + 12 e^-$$

Inside the sensor, at the anode, will take place a chemical reaction with the oxygen from the air:

$$3 \text{ O}_2 + 12 \text{ H}^+ + 12 \text{ e}^- \rightarrow 6 \text{ H}_2\text{C}$$

Electrochemical sensors have, besides very good selectivity, the following advantages:

 $\succ$  high sensitivity at very low concentration levels, on the 0,1 ppm ranges (parts per million);

➤ long lifetime (aproximative 5 years);

 $\triangleright$  high accuracy compared with simple and robust design;

 $\succ$  low energy consumption, integrating thus the sensors in portable devices.

Electrochemical sensors are not yet prepared to perform direct measurement, due to the fact that the current produced at its contacts is proportional with the total number of the molecules electrochemically converted into the sensor. In order to be able to determine the concentration from quantitative point of view is necessary to supply a definite gas quantity. The problem was solved using a sampling system.

# 3.5. Infra Red spectrometry method (infrared radiation absorption)

Infrared spectrometry is an non-destructive analytical method promoted at early 1960's.

The method is based on absorption of infrared radiations and on **Beer-Lambert** law for qualitative and quantitative analysis of alcohol samples taken from human body.

Beer-Lambert law states the relationship between concentration and infrared absorption and is expressed by the formula:

$$I = I_0 e^{-KC} \tag{1}$$

where: *I* is the intensity of electromagnetically radiation at detector;

- $I_0$  intensity of electromagnetically radiation generated by infrared source;
- K analyser's specific constant; depends on wavelength and measuring chamber length;
- C breath alcohol concentration.

An infrared detector, presented in figure 2, consists of:

l - infrared source;

2 – measuring chamber;

3 - lens;

4 - chopper with filters for narrow wavelength range;

5 – high sensivity photodetector;

6 – microprocessor.



Fig. 2. Infrared detector.

### 3.6. Magnetic resonance of 1H-MRS

The method studies physical interaction of alcohol with brain tunic. This contains proteic elements, called "receptors" which communicate neuronal activity, allowing us to move, to feel, to think and to remember. In order to function properly, the receptors must be positioned correctly in membrane. Alcohol adheres to membrane, braking off the normal position of receptors, which modify the normal function of the brain and lead to intoxication, tolerance, addiction, and alcohol problems. This adhesion phenomenon in known as "magnetisation transfer" effect. Alcohol exists in tissues in different molecular environments; is exist in intra- and extracellular fluids, case in which is named "free" alcohol and exists molecular state, interacting or adhering to the cell's membrane, case in which is named "bonded" alcohol. 1 H MRS technology (Proton's Magnetic Resonance) allows direct measurement only of "free" alcohol not of "bonded" alcohol. That means "bonded" alcohol is invisible for "1 H MRS" method. Although is not detectable by this method "bonded" alcohol is responsible for the effects on brain's functions.

Brain alcohol concentration is a sum of "free" and "bonded" alcohol. If the detected brain alcohol concentration is 90 % of the value measured from blood and breath means that 10 % of alcohol is "bonded". If the measured value is 50 % then 50% of alcohol is "bonded". This difference is very big, especially if is considered the fact that "bonded" alcohol is the most likely responsible of the effect of alcohol against brain's function; this is the reason of how important is to know exactly the properties of alcohol. Alcohol has different effects on different parts of the brain.

### 4. AIR: BLOOD PARTITION RATIO AND CONVERSION OF BLOOD ALCOHOL CONCENTRATIO – BAC IN BREATH ALCOHOL CONCENTRATION – BRAC

### 4.1. Air : blood partition ratio

It is known that partition ratio of ethanol between its concentration in blood and to the concentration in the exhale air is governed by **Henry's law**.

According to this law when a volatile chemical substance (ethanol) is dissolved in a liquid (blood), in a sealed recipient, in which is present air (alveolar air) equilibrium is established between the concentration of the volatile substance in air and its concentration in the aqueous mixture. This law is valid for well-defined values for pressure and temperature.

This law states that, at equilibrium, measuring ethanol concentration in gaseous phase is possible to determine its concentration in liquid phase. The following comparison can be used: recipient represents lungs, blood from lungs is like aqueous solution and breath is represented by gaseous phase above the liquid.

Unfortunately, Henry's law applies to the lung if the following three conditions are fulfilled:

 $\triangleright$  solution must be in a sealed recipient. Lungs are a dynamic system rather then a closed one;

 $\succ$  solution must be maintained at a known constant value. Lung's temperature is never accurately known and is changing constantly;

> pressure must be maintained at a constant value. The pressure inside the lungs is changing constantly, having lower values to allow inhaling and higher values to allow exhaling.

Recent research demonstrated that ethanol is not diffusing in alveolar space as it was believed.

In fact, diffusion occurs in aerial capillary of the lungs avoiding alveoli. Conclusion is that there is not a stable partition ratio for human body and cannot be predicted for sure any partition ratio for a certain person. In this case, 2100 :1 ratio will be incorrect in the most part of time.

Another problem is generated by the fact that during absorption (when ethanol is absorbed in blood stream of small intestine) the value of partition ratio drops to a smaller value when the abortion is completed.

Therefore, for each person, the partition ratio will change the value while the ethanol is absorbed and eliminated.

In addition, the hematocrit and water concentration from human body's blood is variable, changing partition ratio, leading to additional uncertainties.



**4.2. CONVERSION OF BLOOD ALCOHOL CONCENTRATION – BAC IN BREATH ALCOHOL CONCENTRATION – BRAC** 

Blood alcohol concentration of a subject, which drinks alcohol, can be calculated using the equation: Consumed alcohol quantity (g) BAC(mg/100 mL) =×100 Body weight (kg)× Widmark factor (2)

where: one millilitre of alcohol weights 0,79 grams; partition ratio between the concentration of the substance in the solution (blood) and its concentration in air (BAC:BrAC) is 2100:1.

Note that a traceability chain in this field is presented in figure 3.

### **5. CONCLUSIONS**

Breath alcohol analysers are widely accepted as legal measuring devices used for determination of mass concentration of alcohol from deep lung breath sample. Nowadays, Police Department from Ministry of Interior is using about 1700 electronic devices to measure breath alcohol concentrations.

Gas Concentration Department has started a project to prepare ethanol in air standard mixtures in order to assure the following control procedures:

- initial tests of new breath alcohol analysers;
- periodic verification;
- performance tests and calibrations.

During two years of sustained research activity there were prepared different concentrations of alcohol; their associated uncertainties have been evaluated according to the latest standards [1,2].

The results obtained show that the National Institute of Metrology's standards, prepared according to the European and International Standards and with the knowledge and equipment in existence in the INM laboratory, are of the required accuracy and can be used to transmit the measuring unit, mg/L, to breath alcohol analysers.

measurement results.

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