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CORELATIONS AND INTERFERENCES OF BLOOD ALCOHOL LEVELS MEASURED BY CORDEBARD AND GASCROMATOGRAPHY-HEADSPACE METHODS

Popa Marius Florentin[1], Neculai-Cândea Lavinia[1], Comănici Ștefan-Radu[2]

[1] University "Ovidius" of Constanta, Faculty of Medicine
[2] County Clinical Emergency Hospital of Constanta

Lavinia Neculai-Cândea

email: lavinia_candea@ymail.com
phone: +40 740359292

ABSTRACT

Ethanol (ethyl alcohol) is an organic compound belonging to the alcohol class, being the main alcohol found in alcoholic beverages. It is a volatile, flammable and colorless liquid, with a characteristic odor, being considered one of the oldest drugs consumed by humans. Taking into account the prevalence of alcohol intoxication among drivers, and also among criminal offenders, it was deemed necessary to accurately determine the blood alcohol level detected in the biological samples taken from the suspects, considering the justice system requirements regarding the application of retributive measures in direct proportion to the detected blood alcohol level. The method currently used in Romania is the modified Cordebard Nitrochromic method, which comes with certain limitations, such as the variable homogeneity of the samples that induces sampling errors, thus resulting in blood alcohol levels below the real values. For these reasons, it is deemed necessary to align the methods of ethyl alcohol dosing at a national level and to the analytical requirements of the European Union exigencies, imposing the need to adopt a new method that eliminates as many sources of error as possible, a viable candidate at the moment being the Gascromatographic-Headspace method.

Keywords: Alcohol, penal law, Cordebard, GC-HS, DUI, traffic accidents

Introduction

Ethanol, one of the oldest drug consumed, has been used constantly since the beginning of civilization as a beverage, medicine or for religious and social purposes. The first alcoholic beverages were those made by the process of natural fermentation, the most widely known ones being wine and beer. Distillation first appeared during the 8th - 9th centuries, but whiskey production became common in the late 1500s.

Chronic, voluntary intoxication through repeated and sometimes massive consumption of alcoholic beverages is a social scourge ("toxic pandemic"), ethanol being included in the category of "social drugs".

Advances in the study of alcohol toxicity do not seem to simplify things, but on the contrary, complicates them, new discoveries being made about the harmful effects it produces on the human body.

Ethanol (ethyl alcohol) is an organic compound belonging to the alcohol class, being the main alcohol found in alcoholic beverages. It is a volatile, flammable and colorless liquid with a characteristic odor. Ethanol chemical formula is C2H5OH, which can be written CH3 - CH2 - OH, being structurally composed of an ethyl radical, linked to a hydroxyl group, being sometimes

symbolized as EtOH. Ethanol is mostly obtained by fermentation of sugars by means of yeasts, or by petrochemical processes. It is an addictive psychotropic drug, causing a characteristic acute intoxication, known as drunkenness, when consumed in large quantities. (1)

Due to the contribution of alcohol consumption in antisocial acts, traffic accidents, etc., it is particularly useful to establish the blood alcohol level as soon as possible to the moment of committing the deed.

The demonstration of the state of alcoholic influence belongs to the health network, respectively forensic medicine entities. For the court to be able to properly resolve the cases criminal cases which involve alcohol consumption, a blood alcohol level test must be executed, which can be later corroborated with other evidence.

The toxic action of alcohol is manifested primarily on the Central Nervous System, initially producing a condition improperly called "arousal", followed by a phase of depression. The first phase, improperly called "excitation", which corresponds to an alcohol level between 0.50-1.50 g/dL, is the result of a depressive action on the upper nervous centers, leading to excessive and unrestrained activity of the lower nervous centers, thus giving free rein to instinctual, uncontrolled behavior. Euphoria, expansiveness, and the false impression of increased brain activity occur. In the second phase, called the "forensic phase" corresponding to an alcohol level between 1.00-2.50g/dL. Mentally, a state of disorientation and confusion sets in, in some case culminating with hallucinations and illusions. This is the phase in which aggressive acts, thefts, rapes, murders and accidents occur. The last phase, the phase of "deep intoxication", which corresponds to alcohol between 2.50-4.50 g/dL, is characterized by anesthesia, narcosis, coma and the abolition of reflexes, which can end in respiratory collapse and death. (2,3,4)

Probably the most fatal effect of alcohol consumption is indirectly represented by traffic accidents, caused by alcohol intoxicated drivers. After numerous studies, the conclusion is that alcohol consumption, even at low doses, significantly affected driving-related skills such as vision, braking behavior, and vigilance, with lower response to external stimuli. It has been shown by virtual reality simulations that alcohol consumption, due to the disinhibition caused at the central nervous level, induced a more adventurous driving style in most subjects (60%) compared to their style before alcohol consumption. (5) In Europe, drunk driving is thought to be responsible for 10 000 deaths each year. (6)

The methods of dosing blood alcohol are constantly being improved, especially over the last period of the last century and the begging of the current century. For almost half of the last century the Widmark method has been considered the best regarding blood alcohol dosing, a method that has been used with great success by the romanian forensic network. Technical advances permitted the adoption of the NICLOUX SULFOCROMIC method in later years, then in a more recent period the modified Cordebard method was used, which also comes with certain limitations, which require the adoption of a new, more accurate method, namely the GC-HS method. (4,7)

Considering the data presented above, the maximum accuracy determination of blood alcohol levels is very important, taking into account the different psychotropic effects that alcohol has, depending on the degree of blood alcohol, different punitive methods being applied for different blood alcohol concentrations.

Material and method

Material

Between February 2019 and February 2020, blood samples belonging to 80 drivers were analyzed for blood alcohol concentration at the Constanta County Forensic Medicine Service

The biological samples were analyzed by the Cordebard method, modified after Banciu and Droc., and by the Gascromathographic-Headspace method.

Method

Cordebard method

Method principle: Ethyl alcohol is cold oxidized with potassium dichromate in nitric acid medium to acetic acid. Excess potassium dichromate is titrated iodometrically.

Working technique: In a vial with a

ground-glass stopper, measure 5 ml of distillate, 3 ml of 0.13333 N potassium dichromate and 4 ml of nitric acid (d = 1.42). Allow to react for 15 minutes, then add 20 ml of 1% potassium iodide solution. Leave to darken for 5 minutes, then titrate the released iodine with sodium thiosulphate N/25 (in the presence of starch as an indicator).

At least two distillate samples and two distilled water control samples are processed in parallel.

GC-HS Method (gascromatographic-headspace)

Method principle: The ethyl alcohol separated from the biological sample in the head space device is introduced into the chromatographic column in which then migrates according to the affinity for the stationary phase of the column and is transported to the detector. The signal generated in the detector is visualized as a peak on the chromatogram, and depending on the calibration curve and the area of the peak obtained in the analysis of the sample, the concentration of ethyl alcohol in that sample is determined.

Working tehnique: Place the following in the 20 mL headspace vial: 250 µL sample, 1750 µL 0.4 g g isopropanol solution in 1M ammonium sulphate solution.

Specificity of the method

Under the said conditions the method is specific for ethanol, obtaining a well-defined retention time for ethanol and isopropanol.

Depending on the type of column used for both ethyl alcohol and isopropanol, specific retention times were obtained:

• ethyl alcohol - 1,361 min. (DB-ALC1 column), 1.311 min. (DB-ALC2 column); • isopropanol - 1,550 min. (DB-ALC1 column), 1.457 min. (column DB-ALC2). (Figure 1)

The DB-ALC1 column has a stationary phase thickness of 1.8 µm (compared to 1.2 µm for the DB-ALC2 column) and will increase the retention time of the analyte in the column.

The resolution obtained was always > 3.5 which indicates a very good separation of the peaks.

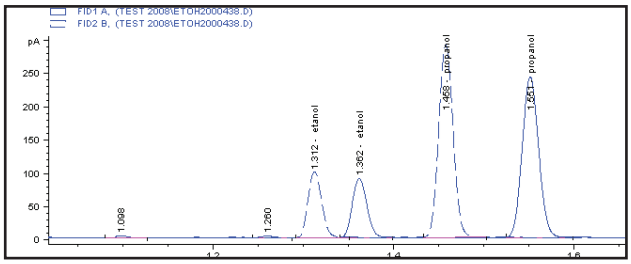

Figure 1. Chromatgram – analysis of a 1 g‰ ethanol solution)

Table 1. Calibration curve values

tR (min)	Signal	Ethanol solution concentration (g‰)	Peak area method
2 1.362	DB-ALC1 detector FID-1A Column	0.5	71.95158
		0.8	121.20863
		1.0	154.96611
		1.5	224.55360
		2.0	301.76907
		3.0	453.08987
		4.0	608.08527

Linearity

To evaluate the linearity, a calibration curve consisting of 7 points was established. For each point on the curve, three solutions were prepared, which were analyzed under the same conditions and the average of the determinations was calculated.

The characteristic parameters of the curves made when using the DB-ALC1 column and the FID-1A detector are presented in Table 1.

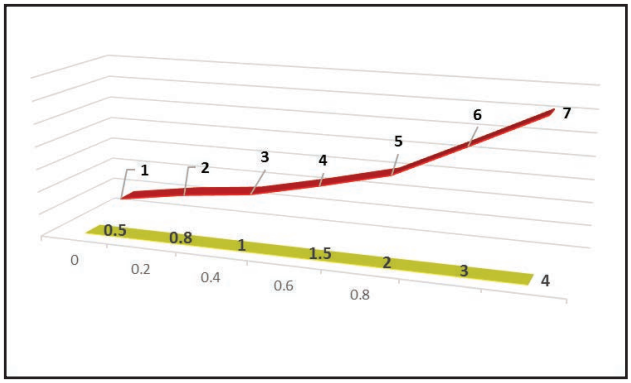

Figure 2. Calibration curve for DB-ALC1 column, FID-1A detector

The equations of the characteristic lines of the two calibration curves are:

- for the curve made on the DB-ALC1 column, FID-1A detector y = 152.09 · conc - 1.5847 R2 = 0.99958
- for the curve made on the DB-ALC2 column, detector FID-2B y = 158.84 - conc - 2.3269 R2 = 0.99953.

To determine the accuracy and precision of the method, the standards were run as samples, obtaining concentrations close to the theoretical ones, the percentage of retrieval (accuracy) ranging between 99.37% and 107.7%. These retrieval percentage values emphasize the accuracy and precision of the method developed. (Figure 2)

Table 2. Results obtained in the evaluation of the method accuracy and precision, using water diluted ethanol solution

tR (min)	Mean peak area	Theoretical Concentration (g%»)	Determined concentration (g%»)	Retrieval (%)
	76,73072	0,5	0,53854	107,70%
1,361	153,04187	1,0	1,02518	102,51%
1,361	227,03665	1,5	1,53140	102,09%
1,360	457,00775	3,0	2,96672	98,89%
1,361	618,54785	4,0	3,97495	99,37%

The obtained results show that on the studied concentration range (0.5-4.0 g ‰) the method is linear and exact. (Table 2)

Results

The results of the blood alcohol level on a batch of 80 samples taken during a 13 month period were evaluated.

An increased incidence of biological sampling in autumn and spring was observed, with a minimum incidence during the summer. (Figure 7)

Of the 80 determinations performed between 2019 and 2020, by the GC-HS method, 7.79% had a blood alcohol level below 0.8 g ‰ and 92.21% had a blood alcohol level above 0.8 g ‰, limit considered criminal in the case of drivers, according to the Romanian legislation. (Figure 6)

Comparative determinations were also performed on the same batch of samples, analyzed in duplicate, with Cordebard and GC-HS methods.

The average alcohol concentration observed were 1.59 g / dL, and 1.89 g / dL , the lower being the value obtained by the Cordebard method (0.60 St.dev), and the higher by GC-HS method (0.69 St.dev). (Figure 3)

The minimum alcohol concentration determined by both methods was 0.17 g / dL, while for the maximum concentration a large difference was observed, the Cordebard method returning a maximum blood alcohol level of 3.3 g / dL, while the GC-HS method returned a maximum value of 3.78 g / dL. (Figure 4, Figure 5)

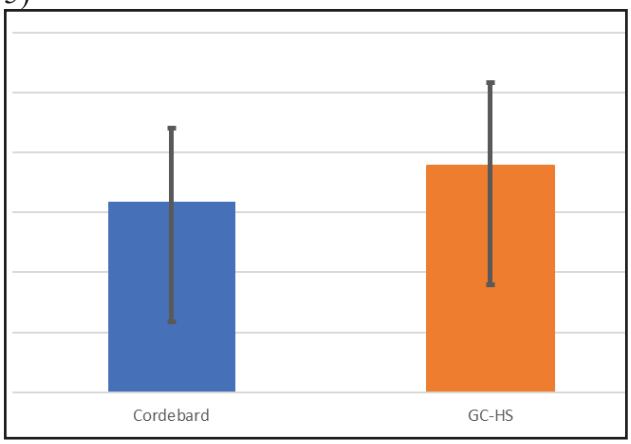

Figure 3. Mean blood alcohol level(standard deviation)

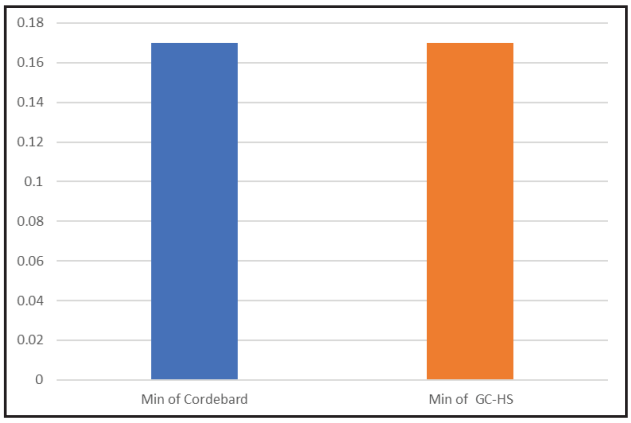

Figure 4. Minimum blood alcohol level value by each method (g/L)

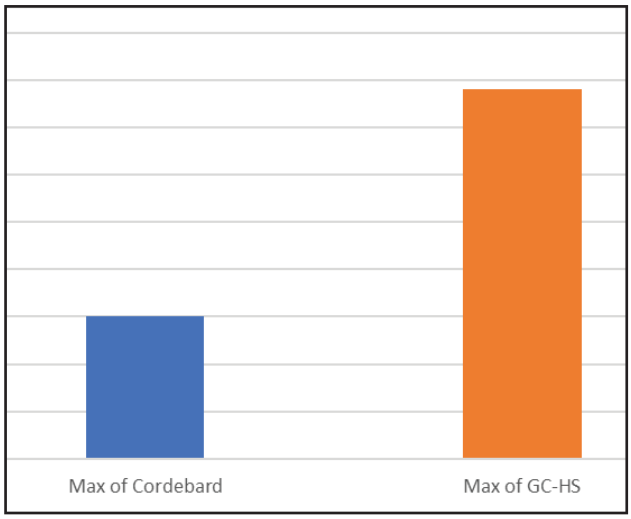

Figure 5. Maximum blood alcohol level by each method(g/L)

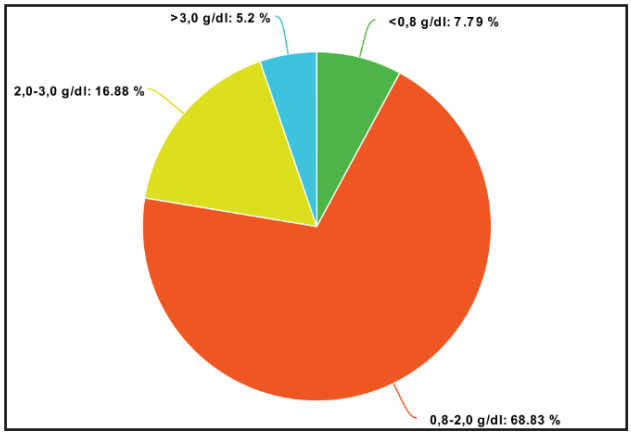

Figure 6. BAL percent distribution grouped by penal classification intervals

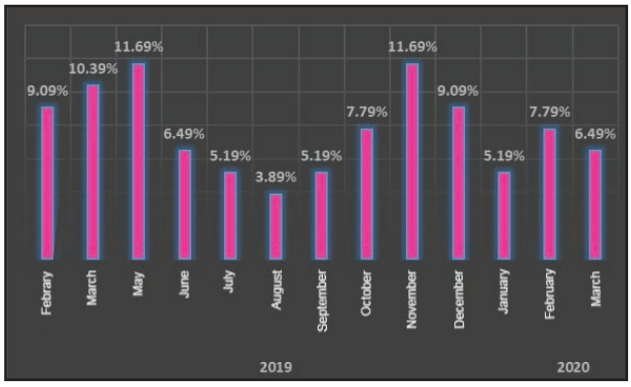

Figure 7. Testing incidence by month

Discussions

In this retrospective study, an increased rate of biological sampling was observed in the spring and autumn seasons, with a minimum of sampling in summer. One way to explain the increased incidence is the autumn harvest and subsequent period in which alcoholic beverages (wine, brandy) are produced, especially in the Constanta's neighboring localities belonging to the county.

A surprising result is the low rate of biological sampling during the summer, Constanta County, and especially the city of Constanta being the most popular tourist destination for this time of year. A possible explanation for this result may be the presence of traffic police crews in greater numbers than in the rest of the year, which discourages driving under the influence of alcohol, drivers fearing having more chances of being caught and suffering the legal consequences of drinking and driving.

We also cannot comment on the real incidence of alcohol consumption among drivers, because in most cases a value below 0.4 g/L alcohol in expired air, detected by the Draeger alcohol meter, does not impose the biological sampling obligation.

A visibly increased average blood alcohol concentration can be observed in the case of determinations by the GC-HS method compared to the Cordebard method, taking into account the standard deviation, with the calculation of the error interval.

It is observed from the result of the performed statistics that the minimum level of determined blood alcohol levels is the same by both methods, the discrepancies between the blood alcohol levels increasing directly proportional to the degree of alcoholic imbibition, thus observing a difference of 0.45 g/L of the maximum blood alcohol level in favor of GC-HS versus Cordebard method.

A maximum incidence of blood alcohol levels of 0.8-2.0 g/L is observed, this being explained by the police not taking biological samples at a result below 0.4 g/L of pure alcohol in the expired air recorded by the Draeger device. At values equal or greater than 0.4 g/L, when biological sampling is required, the result of determinations by the Coredebard or GC-HS method is approximately twice the value of the breath alcohol test.

Limitations

Our study was performed on a relatively small batch of cases, the explanation being the relatively new introduction of blood alcohol dosing by the GC-HS method. The option to provide a new study on a larger batch remains open for us, after allowing a period of time to pass, so that our institution will be provided with new samples to test.

Although we have presented a real picture of the proportions of alcohol in the biological samples taken from drivers under the influence of alcohol, the exposure of a real image, corresponding to the incidence of alcohol consumption while driving cannot be proven through this study, in the absence of sampling from drivers detected with less than 0.4 g/L blood alcohol level using the Draeger device.

Conclusions

Taking into account the obvious high variation rate of the blood alcohol levels between the two methods used on the same biological material, it is necessary to standardize the dosing methods at a national level. In the absence of this uniformity, serious judicial errors can occur, which can derail the normal life course of citizens detected driving under the influence of alcohol, with serious criminal consequences, including imprisonment. The criminal/misdemeanor consequences are mainly differentiated according to the degree of alcoholic imbibition of the detected person driving under the influence of alcohol, so the same offender can be included in the misdemeanor range by dosing the blood alcohol by the Cordebard method, while a determination done by the GC-HS method on the same blood sample, taken from the same person, can include the offender in the criminal punishment range.

The lack of an uniform method of blood alcohol measurement at a national level would clearly influence the punitive methods aplications corectness intended by the lawmaker, offenders which are tested by determination with GC-HS method being obviously disadvantaged compared to those who benefit from testing by the Cordebard method, and at the same time the possible victims of road accidents caused by drivers under the influence of alcohol would be disadvantaged by a determination of the culprit's blood alcohol by the Cordebard method, while the determination by the GC-HS method would benefit them.

Consequently, we believe that adoption of the GC-HS method at national level to be a priority, this method being grafted by fewer limits, with results closer to the real blood alcohol level of the tested subjects than the other methods available at the moment.

References

1. Dermengiu D. Patologie medico-legală. Bucuresti: Editura Viata Medicala Romaneasca; 2002.
2. Vladimir B. Tratat de medicina legala. Bucuresti: Editura Medicaid; 1995.
3. Iftenie V, Dermengiu D. Medicina legala. 3 ed: C.H. Beck; 2019.
4. Popa M, Neculai-Candea L. Compendiu de Medicina Legala. Constanta: Ovidius University Press; 2019.
5. Zhao X, Zhang X, Rong J. Study of the Effects of Alcohol on Drivers and Driving Performance on Straight Road. Mathematical Problems in Engineering. 2014 2014/02/23;2014:607652.
6. European Commission. Saving 20000 lives on our roads. Luxemburg: European Commission, 2003. Report No.: Contract No.: Tech. Rep. COM 2003/311, European Road Safety Action Programme.
7. Vasiliu T, Popovici N. Dozarea alcoolului dupa Widmark si explicarea factorilor introdusi de el. Revista de Medicina Legala. 1937;II(2).