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COMPARATIVE ANALYSIS OF METHODS FOR THE DETECTION AND QUANTITATIVE DETERMINATION OF ETHYL ALCOHOL IN THE BODY

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The amount of ethanol in the blood of individuals was studied during the stages of resorption and elimination. Determination of alcohol was carried out by theoretical calculation using the Widmark's formula and by gas chromatographic method. As a result of comparing the obtained data, deviations caused by various factors were recorded. The combined use of these two methods will allow competent specialists to predetermine and more comprehensively assess the degree of alcohol intoxication.

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***Keywords*:** alcoholism, biological environment research, gas chromatography method, application of Widmark's formula, fight against alcoholism.

Introduction. Alcoholism is not only a social, but also an anti-state issue that requires timely intervention, extensive resources and strategy, necessitating contemporary approaches and means for systematic intervention and measures, including modern toxicochemical investigation and interpretation toolset, for the purpose of detection and determination of alcohol in the biospecimen with gas chromatographic instrumental method.

Using Widmark's formula, which is used to determine the amount of ethyl alcohol in the body during toxicochemical analysis, it is possible to calculate the concentration of alcohol in the blood *in vivo* at any moment of time, as well as the amount of alcohol taken, necessary to reach the given concentration, which, due to certain factors, may differ from the results of an *in vitro* study [1].

The Widmark modeling approach is a best practices method for super-imposing multiple alcohol doses ingested at various times with alcohol elimination rate adjustments based on individual body factors.

However, during the examination of problematic circumstances and the case, due to clarifications of certain prerequisites and professional guidance and observations, that calculation can realistically provide considerable support.

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Ethyl alcohol has a special role among alcohols due to its toxicological significance. Ethanol is not considered an absolute poison, but the abuse of beverages containing ethyl alcohol can lead to chronic alcohol dependence, alcoholism, in which the consequences of intoxication (poisoning), morally and materially, are so heavy for society that alcoholism has become not only a social, but also an anti-state evil, the solution of which requires long time, considerable resources and funds.

As a result of numerous studies, it has been found that addiction to psychoactive substances is considered a polygenic disease, when, against the background of a genetic predisposition, the passive activity of the function of the "brain reward system" is born, which is responsible for the limbic system, with various structures under the cerebral cortex, and a lack of dopamine in this system, one of the neurotransmitters involved, can promote depression and hence alcoholism. It is a chronic disease in its own way, which is caused by the abuse of systematic use of alcoholic beverages, it is manifested by physical and mental dependence, mental and social degradation, pathology of internal organs, metabolism, central and peripheral nervous system. A person's behavior changes under the influence of alcohol, antisocial behavior is inevitably manifested, road accidents and violators of traffic rules, the majority of disorderly persons showing aggression and intolerance are under the influence of ethyl alcohol [2]. Alcohol is a depressant that can impair a person's ability to operate a motor vehicle; determining blood alcohol concentration (BAC) is therefore one of the most prevalent forensic chemical analyses performed for criminal and medical purposes [3]. For example, a recent review article evaluating 69 epidemiological studies found that 52% of driving-related fatalities and 35% of driving-related injuries were associated with positive alcohol tests [4]. They are characterized by immoral behavior, including the destruction of the family, seizing alcoholic and material values from the family, a propensity to commit crimes, acquire severe or incurable diseases, disorders of the digestive and cardiovascular system, cirrhosis of the liver, leave a generation with mental and physical disabilities. As a result of long-term use of alcohol (chronic intoxication), a toxic lesion of the brain occurs: severe functional disorders of the nervous system (alcoholic psychoses, hallucinations – "white fever", which causes a clouding of consciousness, visual and auditory hallucinations of a threatening nature, agitation, somatic and nervous disorders.

Ethyl alcohol has a numbing toxicodynamic effect on the central nervous system and weakens the process of agitation. When taking ethyl alcohol, first there is a pleasant irritation, and then depression of the central nervous system. The anesthetic effect of ethanol depends on the rate of absorption, distribution, elimination and excretion phases, blood concentration, and tolerance. Alcohol enters the body through the gastrointestinal tract and as a result of inhalation of its vapors. The resorption phase lasts on average 1–3 *h*. Resorption begins already in the mouth and esophagus, but a significant part of alcohol is absorbed in the stomach and intestines. Ethyl alcohol is quickly absorbed in the gastrointestinal tract, about 20% in the stomach, and 80% in the small intestine. Absorption is affected by the nature of the food: food rich in protein, fat and starch slows down the process, the rate of which depends on the amount and concentration of alcohol ingested, as well as the contents of the stomach. Consumption of alcohol on an empty stomach leads to its

rapid absorption into the blood and after 40–90 *min* (about 1 *h* on average) the maximum concentration is found in the blood, and in the case of a full stomach – after 1.5–2.5 *h*. Alcohol is a small polar molecule that accumulates in water-rich areas of the body, and does not significantly diffuse into fatty tissues. Following ingestion, alcohol is absorbed slowly in the stomach and rapidly in the small intestines. The rate of alcohol absorption is affected by the rate of gastric emptying, which in turn is influenced by various factors such as food ingestion [5]. It should be noted that the ratio of ethanol concentrations in urine and blood in the resorption phase never exceeds one, and in the elimination phase is always greater than one.

Each of the express methods for the determination of alcohol can have a permissible error limit. Research to determine the exact value of ethyl alcohol in the biological environment, blood and urine is crucial not only from a toxicological and forensic point of view, but also in the implementation of the functions of the law enforcement system and especially investigative bodies, in the course of ensuring a comprehensive investigation of alleged crimes, of the circumstances to be examined within the framework of the initiated criminal proceedings, or for the assessment of a person's mental and physical condition during illegal activities, which is directly proportional to the amount of ethyl alcohol in the blood [6].

During the examination of alcohol in a person's exhaled air, depending on the examination method, there may be circumstances that lead to possible errors and affect the results of the study. The first incorrect result can be obtained from fixed alcohol, the amount of alcohol that can be adsorbed on the esophageal mucosa as a result of taking a drug containing alcohol beforehand. The second error may be caused by the presence of some substances in the surrounding environment. The presence of significant concentrations of acetone, gasoline and other volatile substances in the air leads to the distortion of the determination of alcohol in the exhaled air of the person being examined.

On the other hand, the low amount of ethyl alcohol found in a blood sample taken after the incident under investigation cannot be evaluated and attributed to the sober state of the person at the time of the incident or the amount of endogenous ethyl alcohol in the body (the concentration of which is below 1 *mg*/*L* and is in equilibrium with its metabolites). Therefore, for a full examination of problematic circumstances, it is very necessary to find out the amount of ethyl alcohol in blood and urine samples taken from the person's body at the same time. Through comparative analysis of these values, the possible presence of alcohol intoxication at the time of sampling and at the time of the incident can be determined. Therefore, for this complex analysis, it is extremely important and necessary to have reliable and sensitive research methods in accordance with modern international standards for the determination of alcohol in blood and urine, in order to provide an exhaustive assessment of the question posed.

Widmark calculations are the most commonly used alcohol calculations to estimate [7]:

a) the amount of alcohol consumed, based on blood alcohol concentration (BAC);

b) BAC at a set time after consumption of a known amount of alcohol. These calculations are vital in forensic casework [8].

The Widmark model is the most used in forensic medicine and by traffic officers, due to its simplicity and the fact that it adjusts quite well the alcohol elimination phase. However, in some situations the interest is not in estimating the level of alcohol at the present time, but in past temporal points (e.g. forensic science trying to estimate the level of alcohol of the driver at the exact time of a car accident that happened some time ago: minutes, hours, ...). In this scenario, it would not be reasonable to estimate the level of alcohol in the past by the decreasing linear trend. The linear regression model works well in a local environment of the lab test, but it does not when going backwards [5].

It should be noted that to determine theoretically the degree of alcohol intoxication, Widmark's formula is used, which makes possible to estimate the amount of ethyl alcohol in the body after taking an alcoholic drink, specifying in advance the type of alcohol taken, the amount, the duration of the period from the moment of alcohol intake to sampling, as well as taking into account the fact of the elimination of ethyl alcohol from the blood, which according to the average statistical data from literature, ranges from 0.1–0.15 *g/L*.

However, it should be stated that it may slightly differ from the results of an *in vitro* research, because the amount of alcohol in the body, particularly in the blood sample, depends on a number of factors: weight, tolerance, gender, age, health and physical condition of the organism, characteristics of the organism as well as the number of drinks taken, the nature of the contents of the stomach, the amount of fluids taken during that period, etc.

Measuring a person's BAC, therefore furnishes important evidence in forensic and legal medicine when alcohol-related crimes are investigated.

The use of efficiency, specificity and sensitivity standards of the methods used for the detection and determination of ethyl alcohol in a biological environment, including proper sampling and storage under appropriate conditions, can minimize and even exclude false positive or false negative research results that are certain to be accepted in toxicological chemistry. It should be noted that in classical gas chromatographic research, obtaining alkyl nitrites, which are more volatile than ethyl alcohol contained in biological objects, and injecting them into the gas chromatograph may be accompanied by possible risks of negative effects caused by the human factor, leading to a false negative result (or absence of alcohol intoxication, sober state). And the Mokhovo–Shinkarenko method of using indicator tubes to determine alcohol intoxication is in some sense inapplicable for obtaining an exhaustive assessment using toxicochemical studies, unless other confirmatory methods are used.

In order to avoid the above-mentioned risks, in addition to the screening methods, a modern gas chromatography method combined with an automatic sample injection and evaporation system was used during this research.

Materials and Methods. Within the framework of this research, a remarkable pilot study was conducted with the participation of a group of ten volunteers. The "object" of the research was 10 young people aged 20–45 from the Yerevan City, two persons (No. 2 and No. 3) were female, the rest were male. They took enough energy-rich food (BBQ and other snacks) with 52% ethyl alcohol distilled

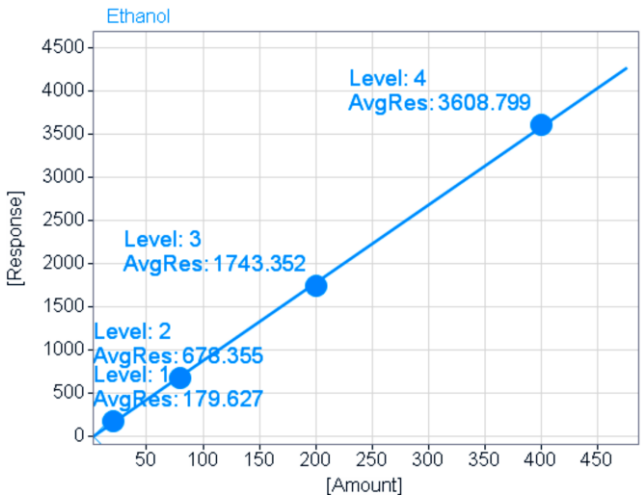
vodka made at home for about an hour, 2 people drank wine, and about an hour after taking the last glass of vodka, venous blood sampling was performed. In order to eliminate ethyl alcohol in the blood and to assess alcohol intoxication, the 2nd sampling was performed 2 *h* after the first. Vacutainers with a green head (sterile vacuum test tube) filled with lithium-heparin, with a capacity of 2 *mL*, size 13×75 *mm* were used for sampling.

Investigations were carried out for the detection and determination of ethanol in blood samples by two different methods: with the Mokhovo–Shinkarenko alcohol-indicator tube, using the oxidation-reduction reaction and instrumental gas chromatographic methods.

As mentioned above, this method is considered quantitatively sensitive in the case of a negative result, it is reliable and a confirmatory method may not be necessary, but the disadvantage of this method is its lack of specificity.

In all 20 samples examined by us, the alcohol-indicator test tubes recorded a positive result – the presence of alcohol.

As a second and confirmatory method, the determination of ethyl alcohol in a blood sample was performed by a gas-chromatographic method with an autosampler combined with an evaporation system (Agilent GC 7820A, FID, 7697A Head Space) under the following conditions: column – DB-ALC2; length – 30 *m*; diameter – 0.32 *mm*; phase thickness – 1.20 *μm*; N_2 was used as a mobile phase, nitrogen flow – 25 *mL*/*min*; hydrogen flow – 30 *mL*/*min*; air flow – 400 *mL*/*min*; chamber and detector temperature – 40°C and 250°C, respectively; evaporator temperature – 110°C; heating temperature in the evaporation system – 70°C; loop temperature – 80°C [9]. For quantification, the ethanol calibration solution concentration range was chosen to be the range of ethanol solutions with concentrations from 0.2 *g*/*L* to 4.0 *g*/*L*, and certified standard solutions of 20 *mg*/*dL*, 80 *mg*/*dL*, 200 *mg*/*dL*, and 400 *mg*/*dL* were used. Retention time RT was 1.72, calibration curve correlation coefficient was 0.999808 (see Figure).



Calibration curve of ethanol analysed by gas-chromatography method.

Results and Discussion. The results of the analysis of the blood samples by the gas chromatography method are presented in Table, where in the 5th column the concentrations of a blood sample in the body of individuals were theoretically calculated using the Widmark formula. During the calculation, the following assumptions were theoretically accepted: one-time introduction of ethanol into the body, the distribution volume of ethanol was 0.66 *L*/*kg* (it varied from 0.6 to 0.7 *L*/*kg*), the release of ethyl alcohol per hour was assumed to be 0.15‰ (in females: 0.1‰), and a certain amount of ethyl alcohol combined with food in the stomach should be ignored.

The amount of ethyl alcohol in the blood by gas chromatographic method

Member's number	Member's height, *cm*	Member's weight, *kg*	Amount of alcohol taken, *g*	The amount of ethyl alcohol in the blood 1 *h* later theoretically **, ‰	The amount of ethyl alcohol in the blood usage of the last cup of alcohol, ‰	
					after 1 *h*	after 3 *h*
1	170	59	39.45	0.86	0.348	0.15
2*	161	61	11.36	0.18	0.1499	0.04
3*	165	61	23.67	0,48	0.171	0.024
4	172	72	39.45	0.68	0.312	0.049
5	173	72	47.34	0.84	0.282	0.045
6	170	74	23.67	0.33	0.0399	0.02
7	193	82	47.34	0.72	0.342	0.174
8	182	85	47.34	0.69	0.353	0.154
9	173	100	47.34	0.56	0.336	0.036
10	185	106	47.34	0.52	0.339	0.105

Note: * – female representatives; ** – in the theoretical calculation, it was assumed that the stomach was empty and ethyl alcohol was introduced into the body at once.

According to the study, a significant deviation of the expected amount of ethanol was found in the blood samples of persons with number 2 and 6, which could be in one case due to the low concentration of alcohol taken (12% wine), and in another case because of the metabolic activity of the other person's physically prepared organism, respectively, due to the rapid progress of elimination and excretion. According to the Table, the amount of ethyl alcohol in the blood samples of people when theoretically calculated using Widmark's formula differs significantly from experimentally obtained data, the reason of which can also be the deficit of alcohol in the stomach, i.e. the neglect of the alcohol fixed in the food and the deviation in the calculation of the time of alcohol intake into the body and the time before sampling, because the full volume of alcohol intake was not carried out at once, but over a certain period of time, finally, the average statistical features of the organism were accepted in the calculation and individual physiological and health reactions were ignored.

Conclusion. Thus, our study showed deviations from the Widmark formula caused by various factors. Consequently, the effectiveness of applying this formula will be expressed especially in the clarification of such circumstances, when the results of the examination and the circumstances of the problematic case presented by a person in a drunken state are contradictory.

As mentioned above, alcoholism is an evil that is not limited to the problems created around a person or group of persons, who have become addicted to having positive emotions as a result of alcohol use or to avoid the sad reality, it includes the social and public environment that surrounds them and in a certain way expresses the state of the country culture. The problems created by alcoholism are universal, and the complex struggle of social and health policies directed against it should include the reduction of the presence of alcohol, in particular, the price and taxes of alcoholic beverages, restrictions on marketing and sales, measures against driving under the influence of alcohol, the review of human value systems and a set of cumulative means of strengthening and therapeutic interventions, in which the effectiveness and reliability of toxicochemical studies play an important role.

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ՕՐԳԱՆԻԶՄՈՒՄ ԷԹԻԼ ՍՊԻՐՏԻ ՀԱՅՏՆԱԲԵՐՄԱՆ ԵՎ ՔԱՆԱԿԱԿԱՆ ՈՐՈՇՄԱՆ ՄԵԹՈԴՆԵՐԻ ՀԱՄԵՄԱՏԱԿԱՆ ՎԵՐԼՈՒԾՈՒԹՅՈՒՆ

Ուսումնասիրվել է հետազոտվող անձանց արյան մեջ էթանոլի քանակությունը ռեզորբցիայի և էլիմինացիայի փուլերում: Ալկոհոլի որոշումն իրականացվել է Վիդմարկի բանաձևով տեսական հաշվարկի և գազքրոմատոգրաֆիական անալիզի մեթոդով: Ստացված տվյալների համեմատության արդյունքում արձանագրվել են շեղումներ, որոնք պայմանավորված են տարբեր գործոններով: Այս երկու մեթոդների համատեղ կիրառությունը իրավասու մասնագետներին թույլ կտա կանխորոշել և առավել բազմակողմ գնահատել ալկոհոլային հարբածության աստիճանը:

Н. А. КИРАКОСЯН, С. Г. ПЕТРОСЯН, Л. Ю. СААКЯН

СРАВНИТЕЛЬНЫЙ АНАЛИЗ МЕТОДОВ ОБНАРУЖЕНИЯ И КОЛИЧЕСТВЕННОГО ОПРЕДЕЛЕНИЯ ЭТИЛОВОГО СПИРТА В ОРГАНИЗМЕ

Количество этанола в крови испытуемых изучали на стадиях резорбции и элиминации. Определение спирта проводили по формуле Видмарка, теоретическому расчету и методу газохроматографического анализа. В результате сравнения полученных данных были зафиксированы отклонения, вызванные различными факторами. Совместное использование этих двух методов позволит компетентным специалистам заранее определять и более комплексно оценивать степень алкогольного опьянения.