ACADEMIA ROMÂNĂ
Revue Roumaine de Chimie
http://web.icf.ro/rrch/

Rev. Roum. Chim.,
2021, *66*(8-9), 741–746
DOI: 10.33224/rrch.2021.66.8-9.07

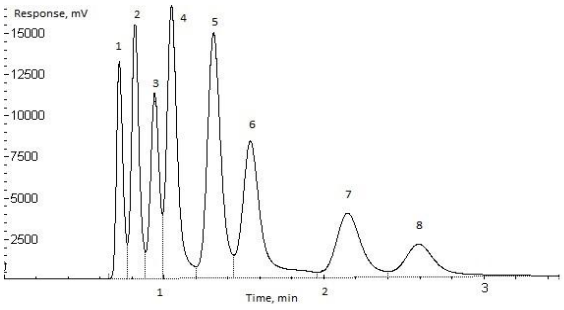
PRESUMPTIVE SCREENING OF MIXED ORGANIC SOLVENTS-DERIVED ALCOHOLS IN HUMAN BLOOD DURING THE FORENSIC GAS CHROMATOGRAPHIC DETERMINATION OF ALCOHOLS

Igor WINKLER,[a,*] Abdelmohsen M. SOLIMAN[b] and Iryna LEBEDINTSEVA[c]

[a] Bucovina State Medical University, 2 Teatralna Sq., Chernivtsi, 58000, Ukraine
[b] National Research Center of Egypt, El Buhouth St., Dokki, Cairo, 12622, Egypt
[c] Chernivtsi Regional Bureau of Forensic Medicine, 2 Chisinau St., Chernivtsi, 58000, Ukraine

Received February 28, 2021

A method of preliminary qualitative determination of some mixed organic solvents in the human blood during the routine forensic gas-chromatographic blood alcohol content analysis was tested.
It has been found that the alcohols present in the '646' and '647' thinners form volatile esters during the pre-treatment of blood samples according to the alcohol content determination protocol, and the head-space over the sample accumulates an ample amount of the alcohol-derived nitrite esters to form clear and distinct gas-chromatography responses of the thinners' components. Each thinner forms a distinctive peaks pattern, which makes it possible to assume preliminary the type of the thinner present in the blood sample. Since the methanolic peak of '647' thinner is much higher than that of '646' thinner, it can be used as the key distinguishing feature for the identification of the thinner type.



INTRODUCTION

The determination of alcohol content in the ante-mortem, post-mortem blood or other bio-samples is a routine part of forensic investigations and a regular traffic police practice worldwide. According to the legislation of Ukraine, gas chromatography (GC) is recognized in the juridical practice as a method which must be employed in forensic investigations.[1] The same or slightly modified approaches are used in many other countries where direct GC or derivative methods based on GC (*e.g.* chromato-mass spectrometry) are used regularly for this purpose. While other methods may be employed by the traffic police (including breath tests or a general examination performed by an authorized medical doctor), in the case of forensic investigations, GC remains the only method available for identification of alcohols in post-mortem samples.

According to the current legislation of Ukraine, GC determination is based on the analysis of the composition of esters formed as a result of interaction between an alcohol and nitrous acid (see reactions (1)-(2) below).[2-4]

It is important to emphasize that many advanced methods[5-7] can also be used in scientific research. However, the above-mentioned ester analysis is a legally recognized method that can be presented to state authorities or courts in Ukraine. This is a sensitive and selective method that is capable of identifying and quantifying C_1-C_5 alcohols with an accuracy of up to a tenth of a per mille.[2]

* Corresponding author: i.winkler@chnu.edu.ua

According to the procedure[2], a sample of blood, urine, or a tissue extract that may consist of some alcohols traces should be treated with a mixture of trichloroacetic acid and potassium nitrite. As a result, nitrous acid is formed (1), which then interacts with the alcohols in situ, transforming them into corresponding nitrite esters (2) (see the example below representing the chemical reactions in the case of ethanol).

$$CCl_3COOH + KNO_2 \rightarrow KCCl_3COO + HNO_2 \quad (1)$$

$$HNO_2 + C_2H_5OH \rightarrow C_2H_5ONO + H_2O \quad (2)$$

All these reactions should be performed in a tightly capped vessel to retain the just-formed alkyl nitrites inside. These esters are highly volatile, they evaporate actively, and a headspace probe taken from the vessel will contain a mixture of esters formed by the alcohols present in the sample and representing their real qualitative and quantitative composition. More details related to practical operations that should be done in the course of this analysis are provided in the next section.

In the context of wide utilization of the above-mentioned method in everyday forensic practice, it is interesting to investigate how other volatile organic compounds that could be present in the samples would manifest themselves in the results of GC investigation.

There are numerous multicomponent organic thinners available on the market. For example, the '646' and '647' mixtures are the most common in Ukraine. Both compositions are mixed distillates obtained from oil products. They consist of toluene (40-50%), butyl, propyl, ethyl and some other alcohols (10-20%), acetate esters, acetone (below 5%), and other minor volatile admixtures.[8,9] These mixtures are used widely in construction and repair practices for cleaning and degreasing surfaces before painting and/or thinning paints. Insufficient ventilation of the working area or failure to keep the necessary pause between the painting works and the beginning of regular usage of the just-painted items or rooms may cause inhalation of the solvent components followed by more or less severe intoxication. Such accidents are reported regularly in many countries.[10-12]

Each of the solvents' components can potentially be identified by direct GC or a derivative method such as GC-MS, but the corresponding experimental procedures[13,14] differ from those used for the forensic identification of alcohols. They are more complex and demand different lab equipment as well as extensive sample pretreatment, which require more time and resources.

Therefore, it should be clarified whether these solvents can manifest themselves, at least qualitatively, during the non-specific GC alcohols identification, and whether some of their components provide some GC responses, and to which of them they are related. Some well-detectable GC responses were recorded previously[15] but extra experiments were needed to obtain more exact details related to this issue. If the result is positive, it can be used as preliminary evidence of traces of the '646' or '647' thinners being present in the sample, which can further be identified and determined quantitatively by the corresponding specific methods. Since GC alcohols analysis is one of the most common procedures performed often and on a wide array of samples, interpretation of unexpected GC responses may be important in the context of possible MOS presence in the samples. This way, experts may assume possible intoxication with some MOS and employ other specific and quantitative methods to check this assumption.

EXPERIMENTAL

All chemical reagents used in this investigation were of the purity grades 65 (Pure Pharma Grade) or 35 (for Gas Chromatography) and were obtained from a local representative of "PanReac AppliChem." The '646' and '647' MOS were obtained from a local dealer of the company "Khimrezerv" supplying various stores with household and construction chemicals.

A control mixture of alcohols should consist of seven or eight C_1-C_5 alcohols (methanol, ethanol, *n*-propyl and isopropyl alcohols, *n*-butyl and isobutyl alcohols, isoamyl, and sometimes *n*-amyl alcohols) with the concentration of each component equal to 2 ‰. This mixture is intended for testing the GC, and it was prepared according to the methods provided in[2] before the experiments. The mixture was treated with trichloroacetic acid and potassium nitrite according to the officially approved procedure[2], and a 1 mL probe was taken manually from the headspace of the reaction tube and injected manually into the GC sampler. The standard conditions of the GC investigation of the alkyl nitrites mixture are given in Table 1.

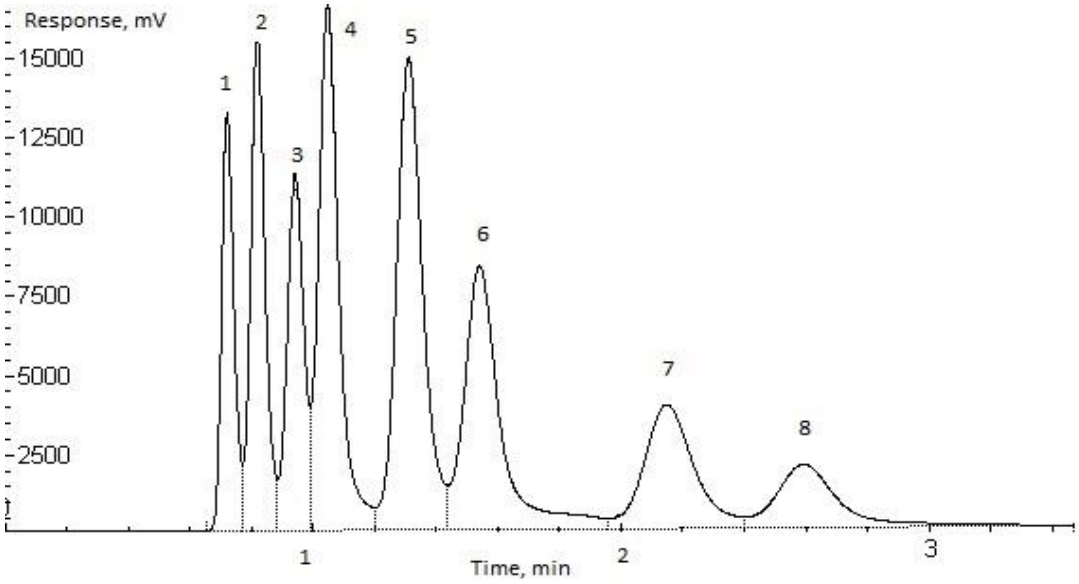


Fig. 1 – Typical GC image of eight homological alcohols manifesting themselves through the corresponding nitrite esters: methanol (1), ethanol (2); isopropyl alcohol (3); propanol (4); isobutyl alcohol (5); butanol (6); isoamyl alcohol (7), and amyl alcohol (8).

Table 1

Standard conditions for the GC investigation of the alkyl nitrites mixture (*)

Parameter	Value
Sampling method	Manual, by a micro-syringe
Column temperature, °C	75
Thermal regime	Isothermal
Injector temperature, °C	110
Detector temperature, °C	150
Detector type	Flame-ionization detector (FID)
Carrier gas (N_2) flux, mL/min	20
FID hydrogen flux, mL/min	60
FID air flux, mL/min	400

(*) - it should be emphasized that precisely these conditions must be used for this analysis as this is a legally approved method in Ukraine used in all official forensic investigations

A computer-controlled "Chromatec Crystal-2000M" device was used in all experimental investigations to separate and analyze the gas samples while the output data were analyzed, visualized, and recorded by standard software provided by the chromatograph manufacturer. The device was equipped with the packed 2.5 m long column filled with Chromaton T-AW with dimethylsilicone elastomer SE-30 (5 %) by Sigma Aldrich as the stationary phase.

A typical gas chromatogram of the control mixture is represented in Fig. 1. It can be seen that each of the alcohols present in the control mixture can be distinctively determined by a corresponding GC peak formed by its nitrous ester.

It should be understood that the chromatogram shown in Fig. 1 represents a qualitative pattern and it cannot be used for any quantification of the concentration of ethanol or other alcohols.

In order to do that, the internal standard must be chosen and all the peak areas must be normalized by its area. According to the approved GC method, an area of the propanol peak is used as a reference. This choice of the

internal standard is based on the fact that propanol is one of the closest homologs of ethanol and that normally it is not expected to be present in the human blood. On the other hand, it must be noted that propanol is formed during natural post-mortem decay of biological tissues and, therefore, it can be found in post-mortem blood along with other decay products of an alcoholic nature.[16,17] However, the concentration of propanol formed by the decay processes is much lesser than that of ethanol making it possible to use this compound as the internal standard with some precautions even in the case of analysis of post-mortem blood or bioliquids.[2,17]

Quantitative analysis of the alcohols content in a sample is performed according to the following procedure. 2 mL of the blood sample is mixed with 2 mL of the 4 ‰ aqueous solution of propanol to ensure the internal standard concentration 2 ‰. Then 1 mL of this mixture should be taken and put into a glass ampoule. 0.5 mL of the 30 % trichloroacetic acid should be added to the ampoule, then it must be capped and, finally, 0.3 mL of the 30 % aqueous solution of sodium nitrite should be injected inside. The alkyl esters are formed instantly according to the processes (1)-(2) (see above), they evaporate into the gas section of the ampoule, and so a headspace probe will consist of a mixture of esters representing the alcohols content in the initial blood sample.

Preliminary chromatograph calibration is performed in the same way as described above but with a series of preset ethanol (or other alcohol) solutions with known concentrations used instead of blood samples. A ratio between the alcohol peak area and the internal standard (propanol) peak is used to calibrate the device.

RESULTS AND DISCUSSION

The investigation of possible GC responses of the thinners' components within the regular blood ethanol content determination were carried out with the human blood samples containing a 0.2 ‰ admixture of MOS. This concentration corresponds to the initial state of alcohol intoxication, and it is more likely to be reached for a pathological state caused by inhalation of contaminated air.

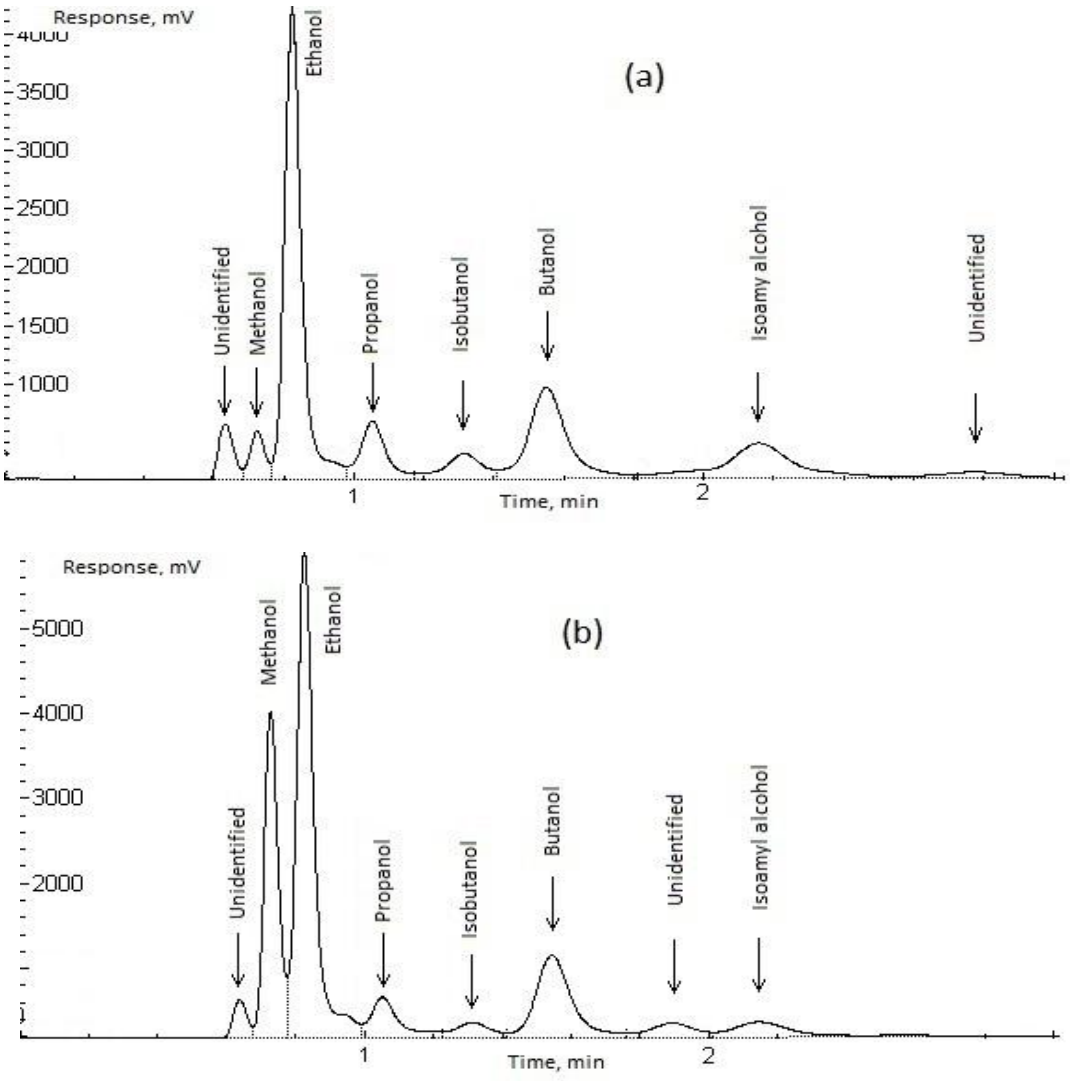


Fig. 2 – Chromatograms of the blood samples with a 0.2 ‰ admixture of '646' (a) and '647' (b) MOS.

An alcohol-free human venous blood sample (55 ml) has been taken from a male adult volunteer after receiving his verbal consent to participate in the present investigation. Based on the role, involvement and character of the human volunteer participation in this investigation, the Ethics Panel of the Bucovinian State Medical University, where the investigation project was performed, has approved this activity. The volunteer claimed that he had not consumed alcohol 3 days before the experiment. Then the blood was divided into 11 similar samples of 5 ml. One of them was used as a reference while the others were contaminated with '646' or '647' MOS (5 samples for each) in such a way to create the concentration of a thinner 0.2 ‰. All the samples were treated according to the above mentioned experimental procedure for the alcohol content determination in the human blood. The results for each MOS replicates were statistically processed and averaged.

The control chromatogram of the sample containing no MOS admixture was flat, without any peaks, and no chemicals were identified by the chromatogram analysis software, while some GC responses were found for the contaminated samples.

A typical chromatogram of the blood sample after adding an admixture of '646' or '647' MOS is shown in Fig. 2. It can be seen that numerous alcohols form the GC responses in both cases.

The methanol peak seems to be the most evident distinguishing feature between these solvents since it is rather weak for the '646' thinner, while in the case of the '647' mixture, its height is comparable with that of the highest peak of ethanol. Therefore, this distinction between the '646' and '647' methanol peaks can potentially be used to differentiate and identify these MOS in the GC alcohols content analysis.

However, a more exact approach requires quantitative determination of the components' peak areas normalized by the propanol peak area. An area of the propanol peak was taken as a reference and the areas of all other peaks where divided by the propanol one to get the normalized values. This quantification is presented in Table 2. It should be mentioned that the quantifications given below cannot be used for exact determination of the concentration of the MOS components in the blood because an accurate GC calibration using the combinations propanol/MOS component must have been performed for every compound to get their exact contents. However, our approach still can provide some approximate results showing relative concentrations of various MOS components.

That is why the quantitative data shown in Table 2 are indicative and can only be used to compare relative GC responses of different MOS components.

As seen from Table 2, six components have formed the well-detectable peaks when added to the pure blood samples. The ethanolic peaks were the largest while the peaks of butanol (in both cases), methanol ('647') and isoamyl alcohol ('646') also were larger than the reference propanolic peak. Therefore, the unexpected detection of GC responses of the above components in the human blood sample analyzed under the regular ethanol content determination protocol can be interpreted as a preliminary indication of a possible intoxication with '646' or '647' thinner.

Table 2

The averaged experimental and normalized peak areas registered in the chromatogram of the blood samples with an admixture of '646' (numerator) and '647' (denominator) MOS. The relative error for all peak areas was equal or below 15 %

Compound	Experimental peak area, mm^2	Peak area normalized by propanol
Methanol	1215.2/10974	**0.471/3.901**
Ethanol	14195/20658	**5.497/7.344**
Propanol	2582.1/2812.8	1/1
Isobutanol	1646.1/1282.9	0.637/0.456
Butanol	6129.6/7049.9	2.374/2.506
Isoamyl alcohol	4387.4/2113.5	1.699/0.751

It is our understanding that the methanol peak area can be useful for a presumptive distinguishing of a type of the thinner present in the blood. The higher the GC response of methanol, the more likely the presence of the '647' solvent, provided that other MOS components have also formed their responses.

However, different bioavailability, diffusion and metabolism rates of different alcohols may cause different GC profiles of contaminated blood. That is why the claim that a higher methanol response may be sufficient evidence of possible intoxication with the '647' solvent should be verified in further experiments involving blood samples taken from the patients suffering from real solvents intoxication.

CONCLUSION

Traces of the '646' and '647' MOS can be presumably identified in human blood samples taken for non-specific GC determination for a possible presence of alcohols. Methanol and isoamyl alcohol peaks act as evidence of a possible presence of these MOS, and the greater the normalized area of the former, the more probable the presence of the '647' MOS. A 0.2 ‰ admixture of any MOS in the human blood caused the well-detectable GC responses that were reliably recognized by the alcohol peak analysis software under the standard isothermal conditions of chromatography. It means that the presence of MOS in human blood in concentrations of the order of 0.2 ‰ can be preliminarily detected even without the application of a specialized chromatography of mass-spectra analysis method – just by analyzing the pattern and the areas of extra peaks appearing in the chromatograms. Then, more specific methods such as non-isothermic chromatography or GC-MS should be applied to improve preliminary non-specific detection of MOS and to quantify the exact contents of individual components.

ABBREVIATIONS

MOS – mixed organic solvent
GC – Gas chromatography
GC-MS – Gas chromatography – mass-spectrometry

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