Rom J Leg Med [18] 265 – 270 [2010]
DOI: 10.4323/rjlm.2010.265

Volatile congeners in alcoholic beverages: analysis and forensic significance

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Abstract: Beside ethyl alcohol, the major active component of alcoholic beverages, almost all drinks contain volatile and non-volatile substances called congeners. They have various pharmacological effects and are present in different concentrations depending on beverage type and manufacturing methods. We analyzed volatile congeners in five samples of Romanian home-made plum spirits collected from four Transylvanian counties were double distillation is used after fruits fermentation, by a GC method developed in the Institute of Legal Medicine from Freiburg. Volumetric concentration of ethanol ranged between 52 and 76%. Calibration with aqueous standards resulted in linearity with a correlation coefficient of over 0.995 for each congener. We found high amounts of isobutanol in our samples compared to beverages listed in the literature, influencing forensic opinion concerning the time of ingestion in correlation with concentration of 1.propanol. Another congener, usually absent in alcoholic beverages, 1-butanol, was constantly present in all our spirit samples, meaning a limitation of its use as a blood putrefaction marker.
Volatile congener composition of all our samples complied with EEC regulations with regard to fruit spirits.
Key words: volatile congeners, fruit spirits, forensic expertise

By definition, any drinkable liquid that contains from 0.5 percent to 95 percent ethyl alcohol is an alcoholic beverage. Although the major physiologically active component of most alcoholic beverages is ethyl alcohol, there is a remaining fraction of compounds called congeners. Even if quantitatively small, they play an important and often unnoticed role in the social use and of the alcohol abuse.

Congeners may be highly volatile compounds, like alcohols, acids, aldehydes, ketones and esters. Other components include carbohydrates, tannins, phenols, metals, coloring agents, minerals, histamine and other pharmacologically active substances. Congener content of commercial alcoholic beverages differs significantly for each type of beverage, wine and beer having appreciably higher amounts than distilled spirits [1].

While thousands of different volatile congeners may be found in various drinks at one time or another, several of them have been found to be constantly present: methyl alcohol, acetaldehyde, ethyl acetate, ethyl formate and the small aliphatic alcohols (n-propyl alcohol, isobutyl alcohol, n-butanol) make up the major volatile congener content of beers, wines and distilled spirits.

Material and methods

Aim of our study was to identify and quantitatively analyze alcohol volatile congeners in some Romanian home-made beverages (wine and spirits) of known source and composition, using a gas-chromatograph method with validation based on aqueous and serum standards. We analyzed 5 samples

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of home-made plum spirits originating from four different counties of Transylvania area: Bistrița (BN), Sălaj (SJ), Maramureş (MM) and Mureş (MS).

The characteristic process of obtaining the spirits in this area is double distillation after fermentation of fruits. We diluted the samples 1:100 in order to comply with the calibration standards. In some cases a second analysis with 1:1000 dilutions was necessary to fit the range of calibration. Measurements were performed in the Toxicological Laboratory of the Institute for Legal Medicine Freiburg, Germany using a Perkin – Elmer with head-space and flame ionization detector gas-chromatograph (HS-40). Carrier gas was Helium at a pressure of 120 kPa, oven temperature was progressively raised from 40ºC to 200ºC while injector temperature was 250ºC.

We worked with t-butanol as internal standard and we prepared controlled diluted samples with distilled water using automatic pipettes. Qualitative identification followed based on retention time in the column and appearance of the peak signal as compared to the standard solution with known substances. We calculated congeners concentration depending on signal's peak height through interpolation on the line resulted from five standard solutions (five-point line).

Therefore, proof of linearity was an essential part of the validation. Aqueous standards used for calibration was stored at a temperature range from +2 to +8 ºC and was within the expiration date on the vial label. We searched for the following volatile congeners of ethyl alcohol: methanol, acetone, n-buthyl alcohol, 2-buthyl alcohol, methyl-ethyl ketone, isobutyl alcohol, 2-methyl-1-butanol, isopentyl alcohol, n-propyl alcohol. Ethanol concentration was determined by gas-chromatograph-method in the toxicological laboratory of the Institute for Legal Medicine Tîrgu Mureş.

Results

Ethanol concentration in analyzed samples ranged between 52 and 76% v/v.

For all congeners we obtained linear correlation between known amounts of substance in aqueous solutions verified by accredited laboratories and peak height resulted during our analysis. We present below the linear regression equations and the corresponding correlation coeffiecient (r^2) for methanol, 1-propanol and isobutanol:

methanol: $y = 0.0427x + 0.0035$, $r^2 = 0.9967$
1-propanol: $y = 0.3055x + 0.0061$, $r^2 = 0.9982$
isobutanol: $y = 0.9169x + 0.0136$, $r^2 = 0.9971$

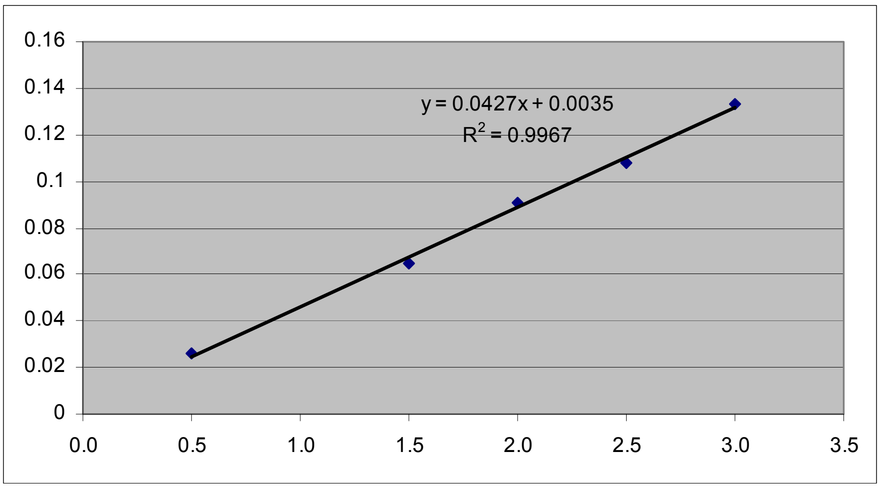

Figure 1 Calibration for methanol; linearity proved

The calibration curves for the above mentioned congeners are showed in figures 1 (methanol), 2 (1-.propanol) and 3 (isobutanol). Linearity, as an important part of the validation was proved by good correlation coefficients, $r^2 > 0.995$ in every case.

Before and after running the samples, we established accuracy of the method by analyzing control serums levels 1, 5 and 7. The results were within the confidence range specified by the manufacturer. Blank controls at the beginning and at the end of the analysis showed no peaks for congeners, demonstrating the absence of carry-over from previous and current assay.

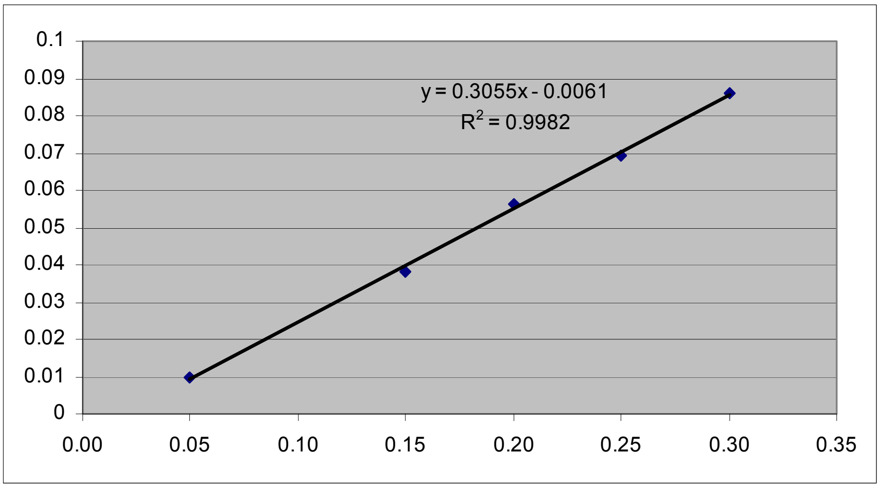

Figure 2 Calibration for 1-propanol; linearity proved

For the five samples we found methanol amounts between 554 – 4170 mg/l, 1-propanol between 76 – 1141 mg/l and isobutanol in the range of 147 – 1092 mg/l.

Different dilutions were needed to quantify all congeners. While 1-butanol was visible only at 1:100 dilution, for other compounds further dilution was necessary in order to fit the calibration range (chromatograms in figure 4 and figure 5).

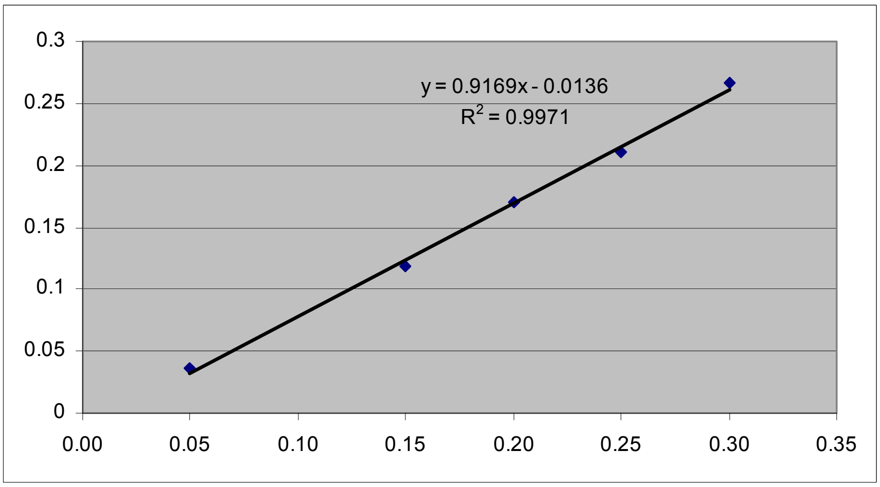

Figure 3 Calibration for isobutanol; linearity proved

The exact values per each sample are listed in table 1. In the table only constantly found congeners (in all 5 samples) are listed. Beside them, isopropanol was found in one sample, 2.butanol in three samples. For Methyl-ethyl-cetone (MEK) we found low peaks in all samples but the amounts calculated were below the limit of detection and quantification; for this reason we neglected these values.

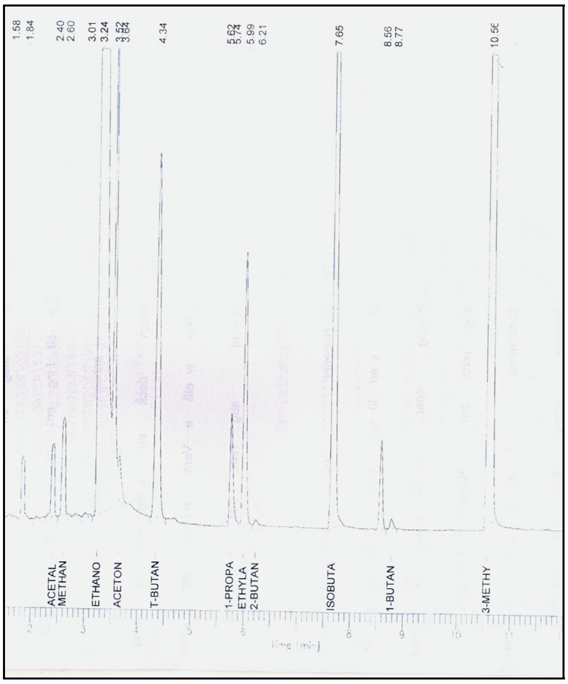

Figure 4 Sample 1 diluted 1:100

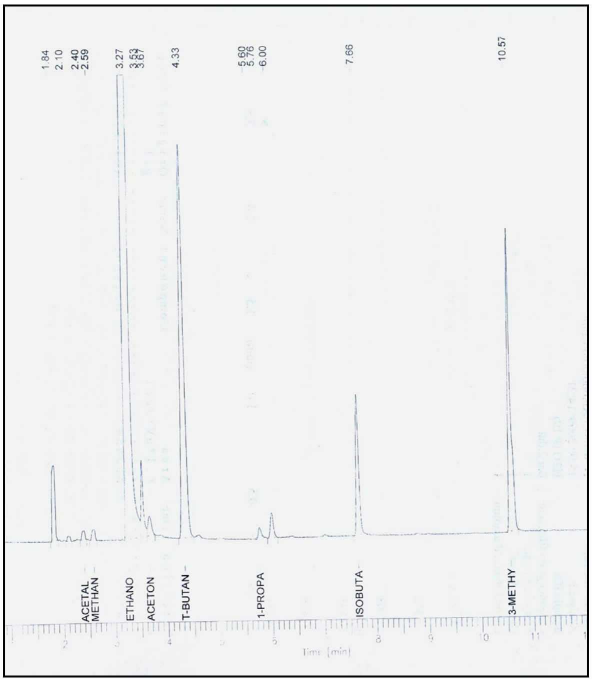

Figure 5 Sample 1 diluted 1:1000

	Ethanol (% v/v)	Methanol (mg/l)	1propanol (mg/l)	Isobutanol (mg/l)	1Butanol	3Methylbutanol (mg/l)	Acetone (mg/l)
Sample 1 (BN)	56	554	76	390	8	610	25
Sample 2 (SJ)	52	3710	700	1092	13	1235	40
Sample 3 (MM)	60	3558	480	390	74	767	37
Sample 4 (MS)	76	4170	1141	581	38	868	36
Sample 5 (MS)	53	4028	395	309	28	640	30

Table 1 Volatile alcohol congener content in samples of Romanian home-made plum spirits

Discussions

Complementary to ethyl alcohol more than forty different alcohols have been identified in alcoholic beverages [2, 3]. These alcohols and other volatile compounds are produced during fermentation or distillation, they contribute to the distinctive taste and bouquet of the beverage and it is thought they may contribute to the severity of hangovers and other toxic problems of drinking.

Vodka is considered the “cleanest” beverage, having less congeners and therefore causing less troubles than other drinks. When starch is the sugar source, a mixture of primary alcohols is produced, consisting mostly of isopentyl alcohol with smaller amounts of n-propyl alcohol, isobutyl alcohol and n-butyl alcohol [4]. The Council of European Communities (EEC) laid down since 1989 general rules on the definition, description and presentation of the spirit drinks.

Fruit spirits are produced exclusively by the alcoholic fermentation and distillation of fleshy fruit or must of such fruit, with or without stones, distilled at less than 86 % volumes so that the distillate has an aroma and taste derived from the fruits distilled, having a quantity of volatile substances equal to or exceeding 200 grams per hectolitre of 100 % volume alcohol, and having a maximum methyl alcohol content of 1000 grams per hectolitre of 100 % volume alcohol [5].

Concerns have been raised about the levels of higher alcohols in home-produced alcoholic beverages that might lead to an increased incidence of liver diseases in regions where there is a high consumption of such beverages. In a review study on the toxicity of higher alcohols and estimate tolerable concentrations in alcoholic beverages, a reasonable preliminary guideline level of maximum 1000 grams total volatile compounds per 100 liters of pure alcohol was proposed but uncertainties during the extrapolation of toxicological data between the different alcohols as well as unknown interactions between the different higher alcohols and ethanol represent limitation of a scientifically established recommendation [6]. In our samples of home-made plum spirits we found total volatile congeners within the range of 298 – 1318 g per hectoliter pure alcohol, only one drink exceeded the 1000 g/hl threshold.

Methyl alcohol (also known as: methanol, wood alcohol, wood spirit or colonial spirit) is the simplest, lowest molecular weight alcohol, yet it is the most toxic of all, due to it’s metabolic products – formaldehyde and formic acid [7]. There is a competition between ethyl alcohol and methyl alcohol for the metabolizing system and for this reason ethanol is being used as treatment of methanol poisoning, in combination with hemodialysis.

N-propyl alcohol (propanol) is colorless, toxic by inhalation, moderate skin irritant, a powerful narcotic and at very high dosages has produced cancer in rats (LD 50 was over 1800 mg/kg).

Isobutyl alcohol (isobutanol, 2-methyl-1-propanol) is a clear and sweet-smelling liquid which has narcotic properties at high concentrations; it causes central nervous system depression.

Acetaldehyde is colorless and has a fruity aroma; it is the most prevalent congener in rum and some bourbons; it has been demonstrated that it is psychoactive and has a depressing effect on aggressive behavior at doses that do not cause locomotor decrements [8]. We found in our samples of plum spirits concentrations between 250 – 400 mg/l, but results expressed according to European rules

(EEC Regulation 2870/2000 [9]) are within a range between 44.6-76.9 g/100 l pure ethanol from the analyzed beverages.

Characterization of alcoholic beverages based on chemical descriptors as an alternative to sensorial analysis is the aim of chemometrics. Concentration and ratio of some higher alcohols is different in principal types of whiskey: irish whiskey, scotch whiskey and bourbon (American whiskey). While the amount of 1-propanol is rather constant (170-270 mg/ml), the concentration of isobutanol differs in scotch whiskeys (around 200 mg/ml), irish whiskeys (90 mg/ml) and bourbons (400 – 600 mg/ml) [10, 11].

We obtained for Romanian home-made plum spirits concentrations of 1-propanol between 76-1141 mg/l. This wide range is partly explainable through ethanol content variation in home-made spirits compared to industrial produced beverages (whiskeys for instance constantly have 40% of pure ethanol). The content of 1-propanol at 100 l pure ethanol in the home-made spirits is of 13.6 – 150 g with an average of 90.6 g. For isobutanol we found concentrations between 58-210 g/100 l pure ethanol, with an average of 96 g/100 l. These amounts are higher than those found in plum brandies from Poland [12]. A study about the influence of yeast on fermentation process revealed that non-*Saccharomyces* yeasts were responsible for higher concentrations of esters and methanol, while *Saccharomyces cerevisiae* strains resulted in increased levels of higher alcohols. It was also found that isolated indigenous strains of *S. cerevisiae* synthesized relatively low amounts of higher alcohols compared to commercial cultures [13].

In the field of forensic medicine high alcohol congeners determination is relevant in cases where blood alcohol interpretation is necessary for drivers at the moment of accident/event – this blood alcohol content is useful for Justice. Usually there is a time interval between the moment of stop in traffic or accident and the moment of blood sample collection.

Declaration of the involved driver concerning alcohol consumption (including after-event drinking or short-time before event drinking) has to be examined by the forensic experts and compared to the toxicological results; in case on non-correlation, the declaration should be invalidated. Legislation in Romania stipulates that blood alcohol content recalculation and interpretation is performed by a commission consisting of a forensic medicine expert and a medico-legal toxicology expert. In Germany, the procedure is different: the initial interpretation is made in Court by a forensic medicine expert and if further toxicological analysis is required, like congeners determination, the Court asks for a toxicological expert's opinion as well. From alcohol congeners, 1-propanol and isobutanol are useful for confirmation or contradiction of the involved person's declaration about the type, amount and period of alcoholic beverage consumption.

Many experiments on animals or in vitro have been made and published concerning pharmacokinetics of fusel oils, establishing that the distribution volume for methanol, 1-propanol and 2-butanol is similar to the one for ethanol [14] while isobutanol distributes in a more larger one, including fatty tissue [15]. Elimination rates are different among congeners, for example half-time in blood is in average 2 ¼ hours for isobutanol and 3 ½ hours for 1-propanol [2], therefore low amounts of isobutanol may invalidate the assumption of recent alcohol ingestion.

The assertion is based also on the usually lower isobutanol concentration compared to 1-propanol in most alcoholic beverages [2, 16]. Our samples had relatively high isobutanol amounts, the isobutanol to 1-propanol ratio was over 1. This characteristic is important to be considered during blood alcohol interpretation expertise when local home-made "țuica" or "palinca" ingestion is declared.

Because blood samples have to be reanalyzed after a period of time (weeks or months), proper storage conditions must be ensured in order to avoid putrefaction. 1-butanol is considered a marker for putrefaction, since it is absent in most alcoholic beverages. However, in our samples of home-made Romanian plum spirits we found concentrations of 1-butanol between 8 – 74 mg/l, thus its presence signification in a blood sample must be interpreted carefully. Identification of methanol in the blood specimen is definitely meaning alcohol consumption before collecting and depositing the sample [14].

Conclusions

Samples of Romanian home-made plum spirits complied with European rules set by EEC with reference to ethanol concentration, maximum methanol content and minimum volatile compounds content.

Determination of alcohol congeners in alcoholic beverages and in the blood of the persons driving under influence of alcohol may be a useful tool in the practice of expertise for blood alcohol content interpretation.

Romanian home-made plum spirits in our study showed high amounts of isobutanol; this characteristic should be considered carefully when interpreting pharmacokinetics of ethanol and congeners in order to verify the defendant's declaration about period, amount and type of ingested alcoholic beverage. Because of the large variation in volatile congeners amounts, the ideal situation would be the defendant provides a sample of the home-made spirit claimed to be consumed, and this is analyzed together with the blood sample.

The presence of 1-butanol in all of our alcoholic beverage samples suggests a limitation of using this congener as putrefaction marker for the blood.

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