Destanoğlu O, Ateş İ. JOTCSA. 2019;6(1):21-28.

RESEARCH ARTICLE



Determination and Evaluation of Methanol, Ethanol and Higher Alcohols in Legally and Illegally Produced Alcoholic Beverages

Orhan DESTANOĞLU* 🖂 跑, İsmail ATEŞ 🖂 📵

Council of Forensic Medicine (ATK), Department of Chemistry, 34196 Bahçelievler, İstanbul, TURKEY

Abstract: In this paper, we demonstrated the results of ethanol, methanol and higher alcoholic contents of the legally and illegally produced alcoholic samples. For investigation, the samples, which were collected as evidence by officers from the crime scenes or illicit production sites, were sent to our laboratories by prosecutions. 96 Turkish Rakı samples, 8 beer samples, 1 wine sample, 101 other strong drink samples, thus totaling 206 samples, and 2 industrial ethanol samples were examined between years 2015-2017. Fast and reliable analysis of the alcoholic beverages, especially in terms of methanol concentration, has vital importance primarily due to the cases of death arose from metabolic acidosis after consumption of illicit alcoholic beverages with high concentration of methanol produced in clandestine laboratories. In the autumn of 2015, an outbreak of mass methanol poisonings took place and so many people died in Istanbul. An HS-GC-MS system was utilized for qualitative analysis of the higher alcohols and for scanning any volatile compound, whereas the determination of ethanol and methanol concentrations an HS-GC-FID system was performed. So, after all results were investigated in detail, mentioning the key points for evaluations, it was clearly described whether the drinks comply with criteria set by Turkish Food Codex Communiqué on Distilled Alcoholic Beverages. 89 of 96 Rakı samples and 90 of 101 strong alcoholic beverages were not definitely compliant with the Communiqué since they contained either tert-butanol, which is a denaturant, or high levels of methanol.

Keywords: Analysis, gas chromatography, methanol poisoning, alcoholic beverages, forensic chemistry.

Submitted: November 11, 2018. Accepted: January 13, 2019.

Cite this: Destanoğlu O, Ateş İ. Determination and Evaluation of Methanol, Ethanol and Higher Alcohols in Legally and Illegally Produced Alcoholic Beverages. Journal of the Turkish Chemical Society, Section A: Chemistry. 2019;6(1):21–32.

DOI: <u>http://dx.doi.org/10.18596/jotcsa.481384</u>.

*Corresponding author. destanoglu@itu.edu.tr

INTRODUCTION

Fermentation-based alcoholic beverage production has been carried out from ancient times throughout the world. However, as in Turkey, other countries have the issue of illegal alcoholic drink production, which is very serious. Informally produced alcohol consumption is very high in Eastern Europe, followed by South America and Africa (1).

According to the 2010 report of World Health Organization Global Information System on Alcohol and Health (WHO-GISAH), 30% of Turkish alcohol consumption is unrecorded. In 2010, consumption percentages to the type of alcoholic drink are 63% beer, 9% wine and 28% spirits, whereas in the year 2016, beer was consumed 57.6%, wine's consumption was 8.6% and of the spirits were 33.8%. 73% of total population is composed of adults (older than 15 years old) in Turkey and according to the 2016 statistics of WHO-GISAH, 11% of adult males consumed alcohol while this was 3% for females. In addition, 2010 and 2016 statistics of WHO-GISAH tell that males (15+) consumed 4.1 L and 3.7 L pure alcohol per year, respectively. For females, both years recorded the same value, 0.4 L. On the other hand, according to year 2016 data, only alcohol-consuming males had 29 L pure alcohol per year and females had 10.4 L pure alcohol. The data for the same year showed that daily consumed alcohol was 71.9 g for the males and 25.8 g for females. In the year 2016 in Turkey, it was reported that the ratio of alcohol-related deaths to the total was 2.1% for males and 0.5% for females (2,3).

For alcohol addicts, cheap alcoholic drinks are considered more attractive than the others. However, non-institutional markets sell alcoholic beverages at prices which are very much cheaper than the market value might be demanded by insensible customers. Increasing unemployment and economic burdens make alcohol addicts to be increasingly forced to find any cheaper drinks. However, there is a problem; ethyl alcohol which is not agricultural origin can be added into the cheap drinks produced by illicit means in clandestine laboratories, and some unscrupulous people add methanol into these drinks to increase the amount of alcohol and decrease the cost. The fact that methanol is cheaper than ethanol further decrease the cost of these counterfeit The beverages containing drinks. hiah concentration levels of methanol gives rise to serious health problems or deaths when consumed.

In addition, the number of cases of death attributed to consumption of traditionally fermented beverages, which are contaminated with methanol, increase in many countries including Nigeria, India, and Indonesia. The presence of methanol in traditionally fermented drinks is claimed to occur via microbial routes. Pectin methyl esterase-producing microbes are able to produce methanol from pectinecontaining fruits. In fact, during classical fermentation process, different types of microbes might produce a mixture of alcohols (4).

Methanol is metabolized to formaldehyde with alcohol dehydrogenase (ADH), followed by formic acid by aldehyde dehydrogenase quickly (5,6). These metabolites are very toxic. Methanol causes depression in the central nervous system and leads to toxicity, whereas formic acid is suppressively toxic. Therefore, these two must be considered together in toxicity cases. Formic acid is a strong cytotoxic molecule, inhibiting mitochondrial cytochrome c oxidase activation. Formic acid accumulation results in metabolic acidosis, greatly damaging the retinal surface and optical nerves (7,8). Excessive metabolic acidosis results in death. It is reported that toxic level of methanol changes between persons, but it is generally accepted that 10 - 20 mL methanol intake causes ocular

disruption or loss, while 30 – 100 mL of methanol leads to death. In addition, literature points out that methanol-intoxicated and survived patients had blood methanol concentration greater than 10 mg/dL (9). In addition, it was reported that the lethal methanolic dose is 20 – 60 g or 25 – 75 mL for a 60-kg adult, whereas ethanol dose is 300 g or 384 mL (10).

According to the UN and Turkish Food Codex Communiqué on Distilled Alcoholic Beverages, ethanol to be used in spirits must be of agricultural origin, at least 96% by volume, and methanol content must not be greater than 30 g $(0.038\%, d_{MeOH} = 0.792 \text{ g/mL})$ per hectoliter of 100% alcohol (11,12).

The Republic of Turkey, Tobacco and Alcohol Market Regulatory Authority released a definition named Regulation of the Production of Ethyl Alcohol and Methyl Alcohol and Procedures and about Domestic Principles and Foreian Commerce, and all types of non-alcoholic beverages and the beverages having equal to or greater than 60% ethyl alcohol are termed as "alcoholic mixtures" (13). If one or more of the following chemicals are added into ethyl alcohol or methanol to create color, smell, and flavor, the action is coined as "denaturation" and the chemicals added are termed as "denaturant": Thiophene, denatonium benzoate, tert-butanol, isopropanol, Color Index (CI) reactive red 24, methyl ethyl ketone, and diethylphthalate are most commonly used denaturants. In this "food alcohol" Regulation, term means agricultural ethyl alcohol (12,13). In addition, industrial ethyl alcohol is denaturated and released as such. Paragraph 20 of this Regulation tells that ethyl alcohol must be released to the market in six different ways: I) Domestic ethyl alcohol (food alcohol), II) Generic use ethyl alcohol (cleaning material, domestic fuel, etc, they are fully denatured ethyl alcohol for general uses), III) Medical ethyl alcohol (used in pharmacy stores and hospitals; they are not denatured), IV) Analytically pure ethyl alcohol for analysis purposes (as laboratory chemical), V) Ethyl alcohol with industrial input (for food and drug uses, packaged or bulk ethyl alcohol, and for cosmetics, chemical and other production industry uses, they are denatured, in packaged or bulk form), VI) Fuel bioethanol (for mixing with gasoline types, in denatured form to mix with unleaded gasoline) (13).

The denaturation process of ethyl alcohol is performed by adding the following chemicals into 100 liters of ethyl alcohol as calculated for absolute alcohol by considering the uses:

a) To release generic use ethyl alcohols, for full denaturation, 125 g of thiophene, 0.8 g of denatonium benzoate, 3 g of CI Reactive Red 24 (25% aqueous solution by weight), and 2 L of methyl ethyl ketone are added into 100 liters of

ethyl alcohol (calculated for at least 90% ethanol) calculated as absolute alcohol.

b) Including eau de cologne, cosmeticaimed agricultural ethyl alcohol samples add one of the following denaturant group. One denaturant type includes 0.8 g of denatonium benzoate and 78.0 g of tert-butanol, whereas the other type uses 0.5 kg of diethylphthalate and 78.0 g of tert-butanol.

c) 0.8 g of denatonium benzoate + 78.0 g of tert-butanol and 0.5 kg of diethylphthalate + 78.0 g of tert-butanol denaturant mixtures also are used for ethyl alcohol for industrial purposes.

d) Bioethanol as an additive to gasoline types are denatured by adding at least 1% gasoline by volume (13).

In this study, we investigated, and reported in detail, alcoholic drinks prepared by illegally or legally, their analysis methods, analysis results, and the suitability of an alcoholic beverage to the UN and Turkish Food Codex.

Headspace-Gas Chromatography-Mass А Spectrometry (HS-GC-MS) system was utilized for qualitative analysis of higher alcohols and volatile compound, whereas the any methanol determination of ethanol and concentrations were measured with а Headspace-Gas Chromatography-Flame Ionization Detection (HS-GC-FID) system. 96 rakı samples, 8 beer samples, 1 wine sample, 101 other strong drink samples, totaling to 206 samples, and 2 non-agricultural ethanol samples (used in manufacture of illegal alcoholic beverages) were thoroughly investigated.

MATERIALS AND METHODS

Instruments

The qualitative analyses of the alcoholic beverages were performed with a Perkin Elmer Clarus 680 gas chromatograph equipped with a Clarus SQ 8 T Mass Spectrometer and HS40 headspace (HS) autosampler. Separations were achieved using a Perkin Elmer Elite-FFAP capillary GC column (Crossbond Carbowax-PEG). The column was 30 m long, its i.d. was 0.25 mm, and df was 0.5 μ m. Helium was used as the carrier gas at 1 mL/min constant flow rate with an HS pressure of 35 psi. The HS oven set at 90 °C for 15 min. The temperature of HS needle was 100 °C.

Injections were made by adjusting needle time of the loopless HS. After the incubation time finished, pressure was applied to the vial with the needle for 1.0 min and then the gas was taken from vapor phase of the vial for 0.12 min. The temperature of transfer line was set at 110 °C. The GC oven temperature program was as follows: I) initially 40 °C for 8 min, II) elevated from 40 °C to 140 °C at rate of 10 °C min⁻¹ and held at 140 °C for 3 min, III) elevated from 140 °C to 240 °C at rate of 30 °C min⁻¹ and held at 240 °C for 3 min. Equilibration time of GC oven was 0.5 min. GC injector temperature was 150 °C during total analysis time was 21 min. Mass detection was performed at 200 °C and electron energy was 70 eV of EI+ source with both full scan between 10 – 300 amu for identification for 27 min.

Ethyl acetate, methanol, tert-butanol (2-methyl-2-propanol), ethanol, n-propanol, n-butanol, isobutanol (2-methyl-1-propanol), isoamyl alcohol (3-methyl-1-butanol) and anethole were analyzed both in the total ion chromatogram (TIC) and mass chromatograms for sensitive detection. Besides, standard solution analyses were performed to determine the retention times of the peaks (t_R). The t_R values and parent ions (m/z) of the analytes are given in Table 1. In addition to the targeted compounds, some other compounds were scanned if they were detected.

A TurboMass version 6.1.0.1963 software was utilized for data acquisition and instrumental control for GC and MS while headspace autosampler was controlled by computer using PerkinElmer HS Driver v2.5.0.0125 software.

For the quantitative determination of ethanol and methanol concentrations in alcoholic beverages, a Perkin Elmer Clarus 580 HS-GC-FID system was performed in the accredited alcoholmetry laboratory. In the HS part, the oven temperature was set to 70 °C for 15 minutes, needle and transfer line temperatures were set to 75 °C and 110 °C, respectively. Injection pressure was 30 psi. Carrier gas was He, and it was applied at 30 psi pressure.

Samples

Permission and sample collection

This study was conducted by permission of the Medicine Council of Forensic (ATK) Chairmanship, Education and Scientific Research Commission (decision number: 2018/737; date: September 18, 2018). After the samples of evidence were collected from the illicit laboratories or crime scenes by officers, the prosecutions sent the samples to our laboratories for examination.

Sample preparation

For qualitative scans, 0.1 mL of alcoholic beverage was taken and transferred into a vial of 22 mL.capacity The vial was tightly sealed with gas-tight polytetrafluoroethylene (PTFE)lined rubber septum cap. This sample was loaded into HS-GC-MS for analysis. For quantitative analysis, 1 mL of n-propanol (64 mg/dL) was transferred into a 22 mL-HS vial. Then, 0.2 mL of the alcoholic beverage was added. The vial was immediately capped tightly. Since ethanol concentration in the alcoholic beverages is very high, ethanol determination in the calibration range was conducted after appropriate dilution with deionized water. After applying to the HS-GC-FID system, the analysis was performed under aforementioned chromatographic conditions.

Table 1. The t_R and main ion (m/z) values of the analytes when HS-GC-MS system was employed.

				tert-butanol					
Ethyl acetate		methanol		(2-methyl-2-propanol)		ethanol		n-propanol	
t _R						t _R		t _R	
(min)	m/z	t _R (min)	m/z	t _R (min)	m/z	(min)	m/z	(min)	m/z
3.04	43	3.22	31	3.14	59	3.82	31	6.70	31
		isobutanol							
		(2-methyl-1-		isoamyl alcohol (3-methyl-1-					
n-butanol		propanol)		butanol)		anethole			
t _R						t _R			
(min)	m/z	t _R (min)	m/z	t _R (min)	m/z	(min)	m/z		
8.11	56	9.14	43	12.39	55	22.54	148		

RESULTS AND DISCUSSION

Depending on Communiqués and Regulations (vice versa), ethyl alcohols for medical, pharmaceutic, domestic, and food uses must not be denaturized. Tert-butanol is a denaturant that can be detected during alcoholic analysis with GC. Therefore, when tert-butanol is detected during alcoholic beverage analyses, the presence of denaturant must be noted. In addition, tert-butanol is an organic substance with anthropogenic source (14) and there is no tert-butanol's information about natural occurrence. Consequently, tert-butanol is not present in food alcohols.

In food alcohols, the major constituent is ethanol per 100% total food alcohol and when calculated with Communiqué criteria, methanol is present at 0.004% and higher alcohols are present at 6.5 x 10^{-5} %. Namely, methanol and higher alcohols are minor ingredients. When methanol or inappropriate ethanol is added to an alcoholic beverage, trace higher alcohols will be further diluted. In this study, methanol concentration was expressed as methanol to total value of methanol plus methanol concentration ratio. According to this approach, the formula below was derived. In this formula, m_{MetOH} and m_{EtOH} value is directly written from HS-GC-FID result (mg/dL).

$$Methanol in total alcohol (g/hL) = \frac{m_{MetoH}}{(m_{EtoH}/0.789 + m_{MetoH}/0.792)} \times 10^5$$

In Table 2, minimum ethyl alcohol concentrations and maximum methanol concentration limits set by Turkish Codex for Turkish Rakı, distilled gin, cider, perry spirits,

fruit brandy, distilled fruit residue beverage, distilled grape pulp beverage, brandy, wine distillate and vodka were given.

Table 2. The min. total alcohol concentration limits and max. methanol concentration limits of the different types alcoholic beverages which is set by Turkish Food Codex Communiqué on Distilled Alcoholic Beverages.

Deverages.											
				Cider, Perry							
Rakı		Distilled gin		Spirits							
Min alcohol (%)	Max MeOH ^a	Min alcohol (%)	Max MeOH ^a	Min alcohol (%)	Max MeOH ^a						
40	150	37.5	5	37.5	1000						
				Distilled Grape Pulp							
Fruit Brandy ^b		Distilled Fruit Residue Beverage		Beverage							
Min alcohol (%)	Max MeOH ^{a,b}	Min alcohol (%)	Max MeOH ^a	Min alcohol (%)	Max MeOH ^a						
37.5	1000	37.5	1500	37.5	1000						
Brandy		Wine Distillate		Vodka							
Min alcohol (%)	Max MeOH ^{a,b}	Min alcohol (%)	Max MeOH ^a	Min alcohol (%)	Max MeOH ^a						
36	200	37.5	200	37.5	10						

^a g methanol per hectoliter of 100% total alcohol

^b This value varies up to 1350 g depending on the type of fruit used.

Turkish Rakı

Rakı is a strong alcoholic beverage and is termed as Turkey's national drink. Pastis, ouzo, sambuca, arak and aguardiente are the other rakı-like aniseed-containing beverages around the world (1).

Turkish Food Codex (Communiqué no 2016/55) states that Rakı is a Turkish distilled alcoholic beverage by distilling suma only or suma plus agricultural ethyl alcohol mixture in 5000 L or smaller copper stills, along with Turkish aniseed (*Pimpinella anisum*) (12). As seen in Table 2, it is imperative that methanol content must not be greater than 150 gram per hectoliter of 100% total alcohol in rakı samples and total alcohol content must be at least 40% per volume.

Evaluation of Rakı-Based Results

In this study, 96 rakı samples were analyzed and the results were evaluated. All results of rakı samples are given in Table S1 (see the supplementary material). Both methanol and ethanol were detected in 16 samples, while ethanol was determined alone in the rest of Rakı samples (N = 80). Anethole was detected in all of these 96 samples. The first 16 results in Table S1 were organized by sorting by the methanol results in an increasing order. The first result had methanol in a very low concentration, but denaturant (tert-butanol) was present, and no higher alcohols (which were present in food alcohols) were found, therefore this sample does not conform to the Communiqué. The number eight raki sample showed n-propanol and ethyl acetate in the analysis, but since denaturant was present, this sample too does not conform to the Communiqué like sample #1. Among 16 rakı samples with methanol detected, only 7 of them

had higher alcohols, and they are suitable in terms of codex criteria.

Figure 1 exhibits the total ion chromatogram of the rakı sample #2, in Table S1, row 2, which conforms the Turkish Food Codex. Rakı samples in Table S1, rows 10 - 16 do not conform the criteria of methanol concentration limits, and even it is very clear that these samples are at very high concentration. It can be easily concluded that food ethanol was not used in these alcoholic beverages. The peaks of ethyl acetate and methanol seems to be very close each other in the Figure 1, but as seen in Figure S1 in the supplementary file no overlap is observed in the chromatograms if m/z 31 and m/z 43 are scanned. The mass library search results and definitions of the analytes presented in this sample are given in supplementary material, please see from Figure S2 to Figure S8.

Ethanol concentration range was 41.9 - 65.1% in the rest of 80 rakı samples, which did not contain methanol (See Table S1, from #17 to #96). All of these 80 samples had tert-butanol, which is a denaturant, so they do not comply with the criteria. A sample contained isobutanol and ethyl acetate whereas two samples only contained n-butanol. The TIC of a Rakı sample (Table S1, row 68) was given in Figure 2. Overlaid ion chromatograms are demonstrated in Figure S9 and the library definitions are given in Figure S10-12. Though the peaks of tertbutanol and ethanol appear allocated in Figure 1, the m/z 59 and m/z 45 ion chromatograms allow successful separation and analysis (see Figure S9).

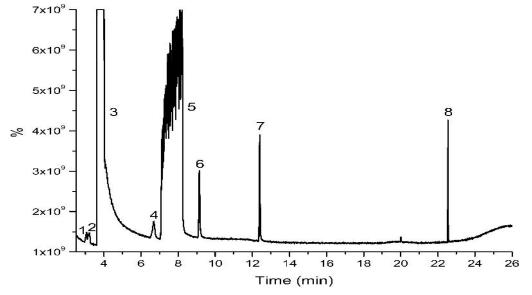


Figure 1. Total ion chromatogram of a rakı sample which is suitable to the Codex. 1) Ethyl acetate, 2) methanol (0.02% in the drink), 3) ethanol (43.9% in the drink), 4) n-propanol, 5) water peak, 6) 2-methyl-1-propanol, 7) 3-methyl-1-butanol, 8) anethole.

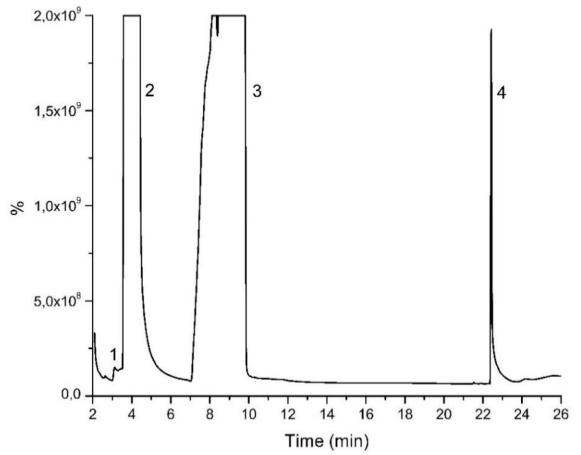


Figure 2. A total ion chromatogram of a sample (Table S1, row 68) containing denaturant, but no methanol. 1) Tert-butanol, 2) ethanol (51.5%), 3) water peak, 4) anethole.

In brief, a rakı sample must obey the following criteria: I) Food grade alcohol must be used and it must not contain any denaturant. II) It must have higher alcohols (fusel oil). III) Methanol concentration must not be greater than 150 g/hL for 100% total alcohol. IV) Even though a Rakı sample does not contain methanol, when a non-agricultural-originating ethanol was used for production, only ethanol, anethole and tert-butanol were detected in the sample.

The commercial and legal "Rakı" beverages (A traditional Turkish alcoholic beverage) are about five times more expensive than the homemade ones. In Turkey, methanol intoxication-related deaths were reported by consumption of homemade alcoholic beverages (1). Arslan et al. investigated 56 different types of raki samples called as "Bogma Rakı" were obtained from local informal manufacturers and 12 types of different rakı samples were bought from markets in Hatay (Mediterranean region in Turkey). Both raki types are produced with the same method, but the distillation procedure of commercial raki samples is more sensitive. They reported that the methanol concentration was 0.220% \pm 0.089 in the uncontrolled rakı samples (1). This level seems to be a bit more from the limits. However, methanol can be purposely added to some illegally produced drinks (see Table S1,

rows 10 - 16) and, unfortunately, this can lead to deaths.

Beer and Wine Results

In Table S2, analysis results of eight beer samples and one wine sample were demonstrated. The concentration range of ethanol in the beer samples was 4.2 - 5.2%, and no methanol was detected. Beer samples also had n-propanol, isoamyl alcohol, isobutanol, n-butanol, and ethyl acetate. Additionally, 11.3% ethanol and 0.023% methanol were determined in the wine sample, along with npropanol, isoamyl alcohol, isobutanol, and ethyl acetate. For wine, low methanol concentration (180 mg/L), presence of higher alcohols, and no denaturant shows that these comply with the Codex. In the literature, Hodson et al. carried out a research about methanol levels in wine samples. In red wine, methanol concentration range was reported to be 120 - 250 mg/L while in white wine, this range was lower and was 40 - 120 mg/L. In addition, depending on the type of grape, methanol concentration could be changed and in fact, it could reach to 364 mg/L (15).

Results of Strong Alcoholic Beverages

The results of 101 strong alcoholic beverages and 2 non-food-ethanol containing liquids

analyzed in our Institute are summarized in Table S3. First seventeen rows of the table contained methanol and sorted by increasing order. Drink samples collected in the areas of crime are sometimes sent in some other flasks than original bottles, therefore we preferred to use "strong alcoholic beverages" definition. However, the presence of a label on the flask or bottle helps determination and evaluation, because the criteria outlined in the Communiqué for the investigated beverage are considered. The key issues that need to be considered, when a sample is investigated, are classified step-bystep in Table S3.

Since the first four samples in Table S3 have very low methanol concentrations, but the presence of tert-butanol (denaturant) and no higher alcohols that should be at trace concentrations, lead us to conclude that they are not compliant with Turkish Food Codex criteria. Table S3, rows 5 – 7 demonstrate that the data about them are compliant to the Communiqué. However, if they are labeled and the label reads "vodka" or "gin", their methanol concentrations are not compliant to the Communiqué. Tulashie et al. conducted a research in Ghana and they reported that methanol concentration range in methanol-containing homemade and foreign beverages was 0.003 - 0.161% (10). Osobamiro reported that, after GC analyses of alcoholic beverages purchased from the supermarkets in Lagos - Nigeria, methanol was not detected in three spirit samples while other three types of spirits had 18, 22, and 176 mg/L methanol and the concentrations were within the allowed range (16).

High methanol concentrations and the absence of higher alcohols, which are given in Table S3, rows 8 - 11, are striking. In some strong alcoholic beverages (vice versa), it is allowed to contain 1000 - 1500 g methanol in hectoliter of 100% total alcohol, but for rows 8 - 11 in Table S3 the methanol results are 8400 - 12200 g/hL. Moreover, the Communiqué states that ethanol concentrations for samples containing high concentrations of methanol must be minimum 37.5%, but for samples in the rows 8 - 11 showed that the maximum ethanol concentration was 32.5%. One can easily conclude that these four samples are not compliant with the Communiqué with many aspects. The following rows, 12 - 16 had samples with isoamyl alcohol and isobutanol and it indicates that agricultural ethanol was used, but most probably the producer malevolently added methanol to decrease the production costs. The reason why the other higher alcohols were not detected, it could be claimed that methanol addition dilutes these ingredients. The samples in Table S3, rows 12 - 16 are apparently not suitable according to the Communiqué. Table S3, row 17 had the

highest methanol concentration in the series, ethanol concentration was 17.4% in the sample and no higher alcohols were detected. Sample 17 is definitely not suitable to the Communiqué. On the other hand, no methanol was detected in the samples with row no 18 - 93, so we sorted them by possessing denaturant tert-butanol. Some samples in Table S3, rows up to 53 had higher alcohols, either all or some of them. Table S3, rows 54 - 93 had no higher alcohols, only tert-butanol was present. Thus, Table S3, rows 18 - 93 are not suitable to the Communiqué and Regulations since denaturant was detected in them. Ethanol concentrations ofliquid samples stated in rows 102 and 103 in Table 4 were 80.3% and 99.3%, respectively, and only tert-butanol was detected in them. So, these liquids were concluded to be used in the production of liquors of 18 - 93 in the Table S3, as non-food alcohol. On the other hand, Table S3, rows 94 - 101 are suitable alcoholic beverages with all aspects of criteria.

CONCLUSION

In this paper, we point out the key points of evaluation with the HS-GC-FID and HS-GC-MS analysis results of the alcoholic beverages, which were collected as evidences by officers from the crime scenes or from clandestine laboratories, and their compliance to the Communiqué and Regulations released by the authorities. The alcoholic beverages containing high concentration of methanol were analyzed after the methanol poisoning cases or outbreak took place in the 2015 Autumn in Istanbul whereas some of the other samples, which are collected in police raids, were examined before the drinks were launched. In terms of evaluation of the results, addition of methanol and/or nonagricultural-origin ethanol gives rise to dilution of higher alcohols (fusel oil) that need to come from agricultural alcohol. Thus, utilizing the appropriate approach described in this paper, the methanol concentrations (g/hL) can be calculated by dividing methanol concentration to the sum of ethanol and methanol concentration values instead of calculating with amounts of the other minor ingredients. It could be said that 7 of 96 Rakı samples were suitable, while methanol concentration levels and denaturant contents make the remaining 89 samples non suitable to the Communiqué and Regulations. Beer and wine samples were all suitable. Only 11 of 101 strong alcoholic drinks were suitable to the Communiqué. Two liquid samples with very high concentrations of ethanol results were also reported, both of which contained tertbutanol, therefore, they are most probably used for production of illicit alcoholic beverages.

ACKNOWLEDGEMENT

The authors are grateful to the Council of Forensic Medicine (ATK) Chairmanship, TURKEY, for permitting, supporting and encouraging us to conduct this research.

REFERENCES

1. Arslan MM, Zeren C, Aydin Z, Akcan R, Dokuyucu R, Keten A, et al. Analysis of methanol and its derivatives in illegally produced alcoholic beverages. J Forensic Leg Med. 2015;33:56–60.

2. World Health Organization. WHO-Turkey [Internet]. 2010 [cited 2018 Sep 3]. Available from:

http://www.who.int/substance_abuse/publicatio ns/global_alcohol_report/profiles/tur.pdf?ua=1

3. World Health Organization. WHO Global Information System on Alcohol and Health (GISAH) [Internet]. [cited 2018 Sep 3]. Available from: http://apps.who.int/gho/data/node.main.GISAH

4. Ohimain EI. Methanol contamination in traditionally fermented alcoholic beverages: the microbial dimension. Springerplus. 2016;(5):1–10.

5. Akhgari M, Panahianpour MH, Bazmi E, Etemadi-Aleagha A, Mahdavi A, Nazari SH. Fatal methanol poisoning: features of liver histopathology. Toxicol Ind Health. 2013;29(2):136–41.

6. Lee X-P, Kumazawa T, Kondo K, Sato K, Suzuki O. Analysis of methanol or formic acid in body fluids by headspace solid-phase microextraction and capillary gas chromatography. J Chromatogr B Biomed Sci Appl. 1999;734:155–62.

7. Wallage HR, Watterson JH. Formic acid and methanol concentrations in death investigations. J Anal Toxicol. 2008;32:241–7.

8. Zakharov S, Nurieva O, Navratil T, Diblik P, Kuthan P, Pelclova D. Acute methanol poisonings: Folates administration and visual sequelae. J Appl Biomed. 2014;12:309–16.

9. Moffat AC. Clark's Isolation and Identification of Drugs. 2nd ed. London: Pharmaceutical Press; 1986. 744-745 p.

10. Tulashie SK, Appiah AP, Torku GD,

Darko AY, Wiredu A. Determination of methanol and ethanol concentrations in local and foreign alcoholic drinks and food products (Banku, Ga kenkey, Fante kenkey and Hausa koko) in Ghana. International Journal of Food Contamination; 2017;14(4):1–5.

11. European Commission. Regulation (EC) No 110/2008 of the European Parliament and of the Council of 15 January 2008 on the definition, description, presentation, labelling and the protection of geographical indications of spirit drinks and repealing Council Regulation (EEC) No. Off J Eur Union. 2008;L39(110):16–54.

12. Turkish Food Codex Communiqué of Distilled Alcoholic Beverages (2016/55) [Internet]. 2016 [cited 2018 Nov 14]. Available from: http://www.mevzuat.gov.tr/Metin.Aspx?Mevzuat Kod=9.5.23431&MevzuatIliski=0&sourceXmlSea rch=alkol

13. The Republic of Turkey, Tobacco and Alcohol Market Regulatory Authority, Regulation of the Production of Ethyl Alcohol and Methanol with Procedures and Principles about Domestic and Foreign Commerce [Internet]. 2011 [cited 2018 Oct 6]. Available from: http://www.mevzuat.gov.tr/Metin.Aspx?Mevzuat Kod=7.5.15462&MevzuatIliski=0&sourceXmlSea rch=#

14. Kingsbury JA, Delzer GC, Hopple JA. Anthropogenic Organic Compounds in Source Water of Nine Community Water System that Withdraw from Streams, 2002-05: U.S. Geological Survey Scientific Investigations Report 2008–5208. Virginia; 2008.

15. Hodson G, Wilkes E, Azevedo S, Battaglene T.Methanol in wine. In: 40th World Congress of Vine and Wine. 2017.

16. Osobamiro T. Analysis of Some Contaminants Commonly Found in Alcoholic Beverages. Am-Euras J Sci Res. 2013;8(1):53– 6.