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Evaluation of methanol content of beverages using an easy modified chromotropic acid method

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1. Introduction

Naturally, all alcoholic beverages contain trivial amounts of methanol (Paula et al., 2003; Sefidbakht et al., 2007; Arora et al., 2007). Legislation against production and consumption of alcoholic beverages in some countries as well as the high expressivity and low availability of standard commercial spirits in some others results in manufacturing homemade alcoholic beverages from fermented sweet fruits with no standard chemical analysis to determine its content of methanol (Croitoru et al., 2013; Vaskova. 2014). This increases the risk of methanol intoxication with an illegal and non-standard liquor (Hassanian-Moghaddam and Zamani, 2016; Hassanian-Moghaddam et al., 2015) that usually contains a methanol content of more than the European (EU) standards (up to 4000 mg/L methanol in spirits with 40% v/v ethanol concentration) (Croitoru et al., 2013; Vaskova. 2014; Paine and Davan, 2001). On the other hand, methanol is cheaper and easily available and may therefore be intentionally added to gain more profit while it is potentially toxic after ingestion and causes serious neurological symptoms and death (Paula et al., 2003; Arora et al., 2007; Hassanian-Moghaddam et al., 2007, 2017; Moghaddami et al., 2008; Sanaei-Zadeh et al., 2011; Pajoumand et al., 2017; Sanei Taheri et al., 2010; Paasma et al., 2012).

Currently, methods based on high performance liquid chromatography (HPLC), gas chromatography (GC), and GC–MS (usually GC) are used to detect methanol content of the beverages (Garcia de Maria et al., 1995; Quanmin and Huanhuan, 2008; Savary and Nu˜nez, 2003; Wu et al., 2007; Ashraf et al., 2008; Wang et al., 2004). However, application of these techniques needs very expensive devices and high technical knowledge that make them inapplicable in common laboratories (Savary and Nu˜nez, 2003). This is while association of official analytical chemists (AOAC) has recommended chromotropic acid (CA) method as a standard technique for determination of methanol content in beverages (Savary and Nu˜nez, 2003; Wu et al., 2007). This reference method requires long operation time and has a painstaking process (Wang et al., 2004) although its major drawback is consumption of large volume of hot concentrated sulfuric acid which is potentially hazardous and corrosive (Fagnani et al., 2003). Therefore, having access to a safe and easy CA method is a great advantage. The main goal of the current study was to investigate the efficacy of a new kit working based on modified CA method in determining the methanol content of some self-made aqueous alcoholic solutions (as beverage samples) with pre-determined methanol content using add found technique. This technique is an uncommon reference method (Dean, 1995; Alfassi, 1998) with no need to advanced equipment and from this point of view, it can be applied and helpful in poor and developing countries. Secondly, we aimed to determine the methanol content of some in-market alcoholic beverages and compare it with the EU standards.

2. Materials and methods

In this study, 63 self-made (prepared by add found technique) and 30 real (different beverages) samples were used to do tests. After approving the kit function (Tables 1 and 2), the methanol content of self-made samples was checked using the designed kit (Table 3). Then, the methanol content of the real alcoholic beverages was determined (Table 4).

2.1. Chemicals and preparation

Sixty-three self-made samples (in seven 9-sample groups) were produced from aqueous ethanol solutions (10–70% v/v ethanol) contaminated by different amounts of methanol (1000–20000 mg/L) using add found technique (Table 3; Dean, 1995; Alfassi, 1998). To prepare the (all self-made and real) samples, 100 µl of each self-made sample was diluted with 99 vol (9.9 ml) of D. W to reach a dilution of 1:100.

The final methanol content of these self-made samples was

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Table 1
Parameters of validations.

Analyte	Calibration curve	R^2	LOD[a] (mgL^{-1})	LOQ[b] (mgL^{-1})
Methanol	Y = 0.0042x + 0.0224	0.9994	700	1250

[a] LOD: Limit of Detection.
[b] LOQ: Limit of Quantification.

Table 2
Precision and accuracy data.

Methanol	Intraday (n = 5)		Interday (n = 5)	
Concentration (mgL^{-1})	RME%	RSD%	RME%	RSD%
1250	3.1%	2.4%	3.4%	4.9%
10000	0.6%	0.8%	1.9%	2.6%
20000	0.3%	0.5%	1.1%	2.9%

RME = Relative Median Error, RSD = Relative Standard Deviation.

determined using add found technique (USDA Nutrient Database for Standard Reference, 1999; Carughi, 2008). They were considered as the control group (Table 1). Thirty samples were taken from the in-market alcoholic beverages as the real samples (cases, Table 2). At first step, the methanol content of self-made samples was checked using the new modified CA method and compared with the results drawn by add found technique. Then, the methanol content of the real alcoholic beverages was determined and compared with the EU standards. Statistical analysis was performed using statistical package for social sciences (SPSS) version 24.

2.2. Instrumentation

A Jenway (model 6405, England) spectrophotometer (UV/VIS) was used to measure the methanol level of the samples. Also, a calibrated alcohol densitometer (alcoholmeter) was used to determine ethanol level in some suspected samples with low amounts of methanol to determine the quality of distillation process.

2.3. Chemicals

Methanol and ethanol for preparation of self-made samples were purchased from Merck Company in Iran. Also, a newly designed specific kit produced by Arya Mabna Tashkhis Co., Tehran, Iran, was used to determine methanol content of the samples. This kit contains five reactants (A, B, C, D and E), five standards with 0, 12.5, 25, 50, and 100 mg/L concentrations of methanol, and an instruction brochure. Thirty samples of different beverages purchased from the black market with valid (10 samples) and invalid (20 samples) trademarks were used as actual samples. Those with valid trademarks included three samples of white and red wine, four samples of scotch, two samples of brandy, and one sample of champagne. The other 20 samples manufactured by illegal local producers included four samples of white and red wine, eleven samples of raisin distillate, two samples of apple distillate, and three samples of liquor with unknown source.

2.4. Procedure

According to the brochure, 50 µl of each standard and all diluted samples (1:100) was poured into separated previously labeled test tubes with 50 µl A and 100 µl B reactants (sulfuric acid and potassium permanganate solutions), and shaken. Fifteen minutes later, 50 µl of C reactant (sodium hydrogen sulfite solution) was added to the tube and shaken hardly to become a colorless solution. Fifty µl of D reactant (chromotropic acid solution) and 1 ml of E reactant (concentrated sulfuric acid) were then added to the test tubes and shaken. Absorbance of each test tube was read at 575 nm 5 min later (after cooling down in room temperature) and then, the methanol content of each sample was computed in comparison with the standard curve by multiplying the result by the dilution factor (100).

2.5. Statistical analysis

In control samples, mean results in each row was compared with its related real methanol concentration. Relative standard deviation (RSD) and relative mean error (RME) were also calculated. Finally, paired *t*-test was applied to investigate the ethanol concentration effect on the purposed method function. A P value less than 0.05 was considered to be statistically significant.

3. Results

Tables 1 and 2 show the results on accuracy of the kit (Guidance for Industry Bioanalytical Method Validation, 2017). Analytical quality assurance of the method indicated a good linearity with high coefficient of correlation (more than 0.99). Also, as observed in Table 2, the suggested kit is accurate and precise enough to detect a content of 1250 mg/L which lies below the permitted dose and safe content of methanol in beverages regulated by the European parliament and the council (0.4% or 4000 mg/L of methanol in alcoholic drinks with 40% v/v ethanol).

Data pooled out of 63 examined self-made samples, their real values of methanol, RME, and RSD are shown in Table 3. As depicted, methanol content of the samples varied from 1000 mg/L to 20000 mg/L in 10–70% v/v concentrations of ethanol. As observed in Table 3, the results with 1000 mg/L methanol are shown as ND (Not Detectable), but the solutions containing methanol more than 1250 mg/L were successfully determined. Comparison of the pooled results with previous definite methanol concentrations shows similar results, as the similarity of means and real amounts of methanol concentrations in the related columns (7562.50 and 7594.04 mg/L, respectively; Table 3) and very little difference between them (31.54 mg/L) confirmed this. Likewise, these findings confirmed that ethanol concentration did not affect the method function because the sensitivity of our method was not affected by ethanol concentration and methanol content was independently determined in aqueous media containing ethanol. Therefore, our chemical method had enough validity and could be applied in similar examinations.

On the other hand, as shown in Table 4, most of the industrial samples (except two cases with concentrations of 3309 and 2116 mg/L) had methanol content less than LOD or 1500 mg/L making the qualitative methods of methanol determination impossible. This is while most of the nonindustrial beverages prepared by homemade method had a methanol content between 1824 and 14320 mg/L with a mean content of 7182.2 mg/L.

4. Discussion

Small amounts of methanol are usually present in alcoholic beverages. Determination of methanol content of these beverages is important due to the hazardous effects of methanol on human body. This is a major step in quality control process of the factories that produce these beverages. However, there is no such control on the process of manufacturing of homemade alcoholic drinks. On the other hand, since determination of methanol content of beverages needs application of expensive and complicated methods and devices, having access to easier and less complicated techniques is warranted.

As shown in Table 3, differences between the mean measured amount of methanol and their real means are 1, 12.3, 29.3, 31.3, 88.9, 64.4, 75.6, and 165.3 mg/L (range; 1–165.3 mg/L), respectively. However, samples with 1000 mg/L methanol were reported as ND. Also, the mean methanol content of all self-made samples was near