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Introducing a New Kit based on Modified Chromotropic Acid Method for Easy Determination of Methanol

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Abstract: Methyl alcohol poisoning usually happens by use of contaminated ethanol. So, development of an easy and cost-effective method to determine methanol in these products can be helpful to diagnosemethanolintoxication. The aim of this paper is introducing a new kit based on chromotropic acid method for measuring the methanol content of alcoholic beverages. In this study, a new modified chromotropic acid method (as a kit) was used to determine of methanol in 700 self-made samples with diverse ethanol concentration prepared by "add found" technique. Briefly, in the proposed method, produced formaldehyde by methanol oxidation is reacted with chromotropic acid in a high acidic media. The quantification limit of this kit lies below the permitted dose and safe amount of methanol in the beverages regulated by the European Parliament and the Council. The attained results indicate Limit of Quantification of the method is1250 mg/Land all examined samples with more methanol amounts are easily determined with good accuracy and precision. As for the European standard about permitted dose of methanol in beverages (up to 4000 mg/lin 40% v/v of alcohol strength) and gained results, it seems, this proposed method practically enables rapid and easy quantitative determination of methanol in all kind of alcoholic strength with suitable accuracy and precision. However, conclusive conclusions in this area will require further examination in actual samples of alcoholic beverages. But, to the best of our knowledge, there isn't any report about such easy method.

Keywords: Alcohol, Chromotropic Acid Method, Kit, Methanol, Poisoning

Introduction

Drinking of nonstandard alcoholic beverages contaminated with methanol may cause methanol intoxication (Shadnia *et al*., 2013). Methanol metabolites in human body can cause severe metabolic and neurological side effects that may lead to coma and even death (Rafizadeh *et al*., 2010; Brent, 2009; Paasma *et al*., 2012; Rostrup *et al*., 2016). Most alcoholic drinks contain methanol in low levels that does not cause side effects (Croitoru *et al*., 2013; Lachenmeier *et al*., 2006; Paine and Davan, 2001). In addition, there are regulations that control harmful contents of alcoholic drinks (Lachenmeier *et al*., 2011). Many authorities have set tolerable methanol limits in alcoholic drinks for example European Union Commission accepts up to 0.4% (V/V) methanol in an alcoholic drink with 40% ethanol (Croitoru *et al*., 2013; Paine and Davan, 2001). However, these limits and regulations are applied on recorded alcoholic manufactures and homemade drinks are not covered by these regulations (Rafizadeh *et al*., 2010; Lachenmeier *et al*., 2011).

Methanol toxicities may be a result of accidental ingestion of non-drinkable alcohols (Salek *et al*., 2014) or consumption of contaminated alcoholic preparations (Rostrup *et al*., 2016). In countries where alcohol drink and distribution are banned, smuggling of homemade and unsupervised alcoholic preparations is a profitable trade (Shadnia *et al*., 2013; Rafizadeh *et al*., 2010; Rostrup *et al*., 2016). Homemade Alcoholic beverages are sometimes

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intentionally contaminated with methanol for financial benefits (Rostrup *et al*., 2016) and cause methanol toxicity outbreaks (Hassanian-Moghaddam *et al*., 2015).

Chromotropic Acid (CA) colorimetric method for methanol detection in spirits has been recommended by Association of Official Analytical Chemists (AOAC) (Rafizadeh *et al*., 2010; Vaskova, 2014; Hassanian-Moghaddam *et al*., 2018). This reference method has some limitations. It is time consuming and needs large volume of hot concentrated sulfuric acid which is potentially hazardous and corrosive (Fagnani *et al*., 2003). Unfortunately, we face outbreaks of methanol toxicity due to smuggling of methanol contaminated alcoholic beverages in Iran (Hassanian-Moghaddam *et al*., 2015) and existence of a reliable method for easy identification and quantification of methanol in such situations is a great advantage. In this paper a new self-made kit based on modified CA method for easy determination of methanol in beverages is introduced which is much easier to use than the reference method. Precision and accuracy of this kit are determined by its comparison with pretreatment methanol amounts (add found method) (Alfassi, 1998; Dean, 1995) as gold standard. Also, the study of different concentrations of ethanol on possible results is another aim of our study.

Materials and Methods

In this study, methanol contents of 700 self-made alcoholic samples (resembling unrecorded beverages) prepared by add found technique in distillated water (D.W) were accidentally examined. The examiners of samples were blind about the real pre-determined concentration of methanol and finally, attained results by the kit were compared with initial contents of samples' methanol. Analysis was performed by Excel 2012 software.

Instrumentation

A single beam spectrophotometer-UV/VIS (Jenway 6405, England) was used to determine methanol content in samples.

Chemicals

The needed methanol and ethanol for preparation of samples were purchased from Merck Company in Iran. Our 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 of methanol with concentrations of 0, 12.5, 25, 50, and 100 mg/l, and an instruction brochure. Furthermore, a high quality of de-ionic distillated water (D.W) was used for preparation and dilution of samples.

Preparation

In this study, different amounts of ethanol were added to D.W to prepare samples with diverse concentrations (20-72%) of it and then, they were again contaminated by methanol with concentrations of 1564.2-19552.5 mg/l. Finally, we had 70 groups of preparations with 10 in each group with clear methanol contents before using new kit. Three control solutions with 1250, 10000 and 20000 mg/lof methanol in alcohol 40% v/v were also prepared with the same method and tested to define Limit of Quantification (LOQ) of the method. To perform the test, each sample was diluted (1:100 ratio) with D.W as triplicate and examined by proposed kit method.

Procedure

Based on the kit brochure, 50 µl of each standard and all diluted (1:100) samples were 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 test tubes and they were shaken hardly to fade the color. Fifty µl of D reactant (CA solution) and 1 ml of E reactant (concentrated sulfuric acid) were then added to the test tubes and they were shaken. After spontaneous cooling of test tubes at room temperature, the absorbance of each test tube was read at 575 nm 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).

Statistical Analysis

At first, the means and standard deviations of kit results were compared with pre-determined values of methanol level (as gold standard). Then, the results were discussed as Relative Standard Deviation (RSD) and Relative Mean Error (RME) were calculated to determine quality assurance of the kit.

Results

The accuracy and precision were used to confirm the proposed kit efficacy (Bioanalytical Method Validation Guidance for Industry, 2017). The Table 1 shows the used kitanalytical quality assurance in ethanol-based solutions. As it is visiblein Fig. 1, the kit standard curve has good linearity with high coefficient of correlation (more than 0.99). Also, the kit has a good accuracy and precision to determine methanol content of the samples (Table 1).

To measure accuracy of the kit, test was applied on 700 self-made aqueous ethanol solutions. The methanol concentrations of preparations increased respectively from 1564.2 in the first group of samples to 19552.5 mg/lin the 70th group. The ethanol concentrations of samples in each group (from 1th to 10th sample) varied from 20% V/V to 72% V/V. All real and determined methanol concentrations (mean of methanol content of 10 samples in each group obtained by our new kit) are shown in Table 2.

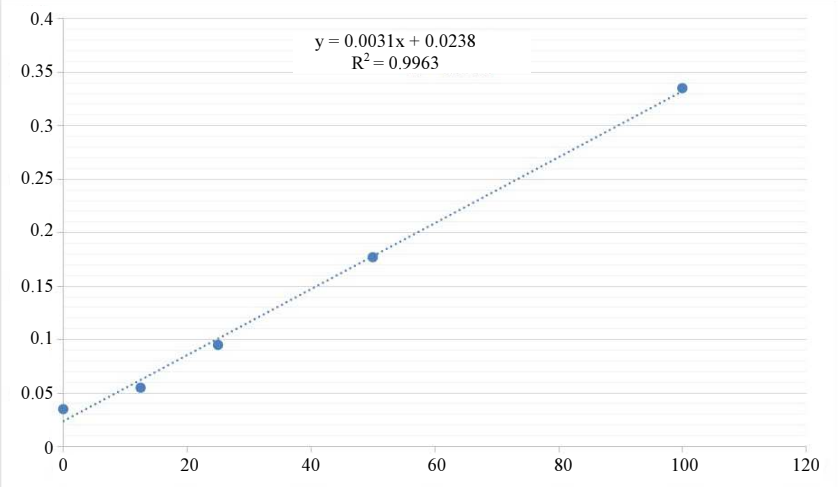

Fig. 1: Methanol calibration curve

Table 1: Precision of proposed method

Methanol Concentration (mg/L)	Intraday (n=5) RME%	Intraday (n=5) RSD%	Inter-day (n=5) RME%	Inter-day (n=5) 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%

LOD: Limit of Detection = 700 mg/l
LOQ: Limit of Quantification = 1250 mg/l

Table 2: Results of analysis of methanol in 70 groups of samples with our kit

Group	Methanol concentration: True values	Methanol concentration: New kit	RME (%)	RSD (%)
1	1564.2	1338.1	-14.5	4.7
2	1759.7	1644.7	-6.5	3.8
3	1955.3	2003.7	2.5	3.2
4	2737.4	3172.0	15.9	1.9
5	3323.9	3774.8	13.6	4.5
6	2541.8	3218.9	26.6	1.2
7	2346.3	3173.0	35.2	3.7
8	2150.8	2608.1	21.3	6.2
9	3715.0	4370.9	17.7	5.3
10	3519.5	4047.3	15.0	2.1
11	3128.4	3828.1	22.4	3.2
12	2932.9	3676.5	25.4	1.9
13	5132.5	4885.0	-4.8	3.2
14	4154.9	4465.3	7.5	3.0
15	4399.3	4578.5	4.1	2.9
16	3910.5	4482.0	14.6	4.4
17	7332.2	7102.5	-3.1	2.1
18	4643.7	4530.5	-2.4	4.0
19	4888.1	4657.1	-4.7	2.1
20	5621.3	4934.7	-12.2	4.4
21	5376.9	4766.0	-11.4	3.3
22	5865.8	5915.3	0.8	1.0
23	6110.2	5396.8	-11.7	1.7
24	6354.6	5565.6	-12.4	3.5
25	6599.0	5876.9	-10.9	1.4

Table 2: Continue

26	8798.6	8973.9	2.0	1.3
27	7576.6	8027.4	5.9	2.0
28	8065.4	8223.1	2.0	3.3
29	7821.0	8148.1	4.2	3.8
30	9043.0	9287.8	2.7	1.4
31	9776.3	10020.5	2.5	1.6
32	8309.8	8833.5	6.3	2.7
33	8554.2	8870.8	3.7	0.7
34	9287.4	9848.3	6.0	2.0
35	10753.9	10356.8	-3.7	1.1
36	6843.4	7454.4	8.9	0.7
37	11144.9	10509.3	-5.7	1.3
38	7087.8	6984.0	-1.5	3.6
39	9971.8	10033.3	0.6	2.4
40	11927.0	11020.2	-7.6	3.9
41	10362.8	10296.7	-0.6	0.7
42	11536.0	10726.6	-7.0	2.5
43	13100.2	13504.8	3.1	1.2
44	12318.1	12191.1	-1.0	2.3
45	15642.0	15713.0	0.5	1.1
46	12709.1	12316.5	-3.1	2.1
47	14664.4	14890.0	1.5	0.7
48	13491.2	13545.7	0.4	2.6
49	15837.5	15799.8	-0.2	0.8
50	13882.3	14216.5	2.4	0.6
51	16815.2	16067.8	-4.4	4.0
52	16424.1	16033.2	-2.4	2.3
53	14468.9	14858.4	2.7	1.6
54	14859.9	15033.2	1.2	3.1
55	14273.3	14635.8	2.5	0.4
56	15055.4	15224.8	1.1	4.2
57	15446.5	15477.4	0.2	0.9
58	16228.6	15870.7	-2.2	3.5
59	16619.6	16390.9	-1.4	1.0
60	15251.0	15380.0	0.8	2.4
61	17010.7	16539.0	-2.8	0.6
62	16033.1	15800.0	-1.5	1.5
63	17597.3	18295.6	4.0	1.2
64	17206.2	16643.4	-3.3	0.5
65	18086.1	18444.5	2.0	0.9
66	17401.7	16993.4	-2.3	1.4
67	18574.9	18613.9	0.2	0.7
68	17792.8	19090.0	7.3	1.2
69	19063.7	18159.4	-4.7	0.6
70	19552.5	22593.4	15.6	0.9

RME: Relative Mean Error
RSD: Relative Standard Deviation
Notice: all of concentrations are based on mg/l

The comparison of methanol concentrations obtained by new kit with previous definite methanol levels (gold standard) (Alfassi, 1998; Dean, 1995) shows the acceptability of the new method. Because, the means of real and determined methanol levels in 70 series of examined samples were similar (10060 and 10144 mg/l respectively). Mean of RSDs of examined groups was 2.3% that is indicative of high precision. This calculation is reinforced by observation of just two (2.86%) RSDs more than 5% in 70 groups of examined samples which demonstrates 97.14% of examined samples have acceptable RSD and henceit is deductible, the purposed kit has high precision. Also, mean of RMEs of examined groups was 6.6% and 27 (38.6%) REs were more than 5%.

Although this finding requires more investigation, it shows, 61.4% of examined groups have acceptable RE and hence it seems, the kit has a relatively suitable accuracy.

Discussion

High levels of methanol in alcoholic drinks can lead to methanol toxicity that is accompanied by severe symptoms, different organ failures (especially, blindness) and death. Determination of methanol content in alcoholic products is important in quality control of formal alcoholic beverages. In contrast, homemade or traditionally produced alcoholic drinks are not monitored for methanol. Common methods used for determination of methanol in alcoholic drinks such as High Performance Liquid Chromatography (HPLC), gas Chromatography (GC) and GC–MS (usually GC) are not easily applicable in developing countries with limited financial and expert resources (Hassanian-Moghaddam *et al*., 2018). Introduction and use of a feasible method for quantification of methanol in developing countries is an advantage. We could not find many report similar to our study but Hassanian-Moghaddam *et al.* (2018) were evaluated the same kit in real samples (alcoholic beverages) (Hassanian-Moghaddam *et al*., 2018). Unfortunately, we were not able to compare the present results with more previous studies.

Methanol concentrations of all self-made samples determined by our new kit were close to their real levels and our method had acceptable precision in every concentration of ethanol. Also, the gained results were shown, the sensitivity of proposed method is not affected by ethanol concentration and methanol content is independently determined in aqueous media containing different concentrations of ethanol. So, from this point of view, the proposed kit has enough credit and can be applied in similar cases. However, the accuracy of the kit was not ideal (total RME= 6.6%) that can be due to some errors and needs more investigation.

In reference colorimetric CA method which is recommended as the standard method for determination of methanol in alcoholic drinks by AOAC (Rafizadeh *et al*., 2010; Vaskova, 2014; Hassanian-Moghaddam *et al*., 2018), methanol is changed to formaldehyde (HCHO) and this compound determined indirectly by its reaction with CA in hot concentrated sulfuric acid media (Fagnani *et al*., 2003; Mohammed *et al*., 2008). Thus, the formaldehyde and formic acid contents of the sample always influence the amount of determined methanol level if they simultaneously exist in the sample. Therefore, to obtain higher levels of methanol in real samples (alcoholic beverages) by this kit than the other current methods are possible, however, confirmation of it needs more investigation. Perhaps, this feature reduces the specificity of the CA method, but given the fact that formaldehyde and formic acid are the main toxic metabolites of methanol in human body and cause methanol poisoning symptoms, it looks like an advantage. On the other hand, certainly, AOAC with full knowledge of mentioned points has recommended this method as a reference technique for determination of methanol in alcoholic beverages (16).

All above mentioned points demonstrate that the proposed method (kit) in this study has enough credit and ability to determine methanol in alcoholic beverages with different concentrations of ethanol and can be used as a trustable alternative tool instead of the other current for routine determination of methanol in these products by different users with limited facilities and minimum laboratory equipment. However, it should be to mention that we did not check the methanol content of the samples by advanced methods (like GC) and this is probably the major limitation of the current study. Also, useless of real alcoholic beverages as sample was the second major limitation of our study that must be done in future. But, introducing a non-common reference method (add found technique) for evaluation of a new chemical method seems to be one of the most important feature of this study.

Conclusion

Our study was shown; the new used kit has acceptable sensitivity and accuracy for easy quantification of methanol in alcoholic drinks with no need to advanced laboratory equipment, professional knowledge and financial resources. However, the final judgment about its feasibility needs more investigations on real samples in comparison with other gold standards like GC.

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Author's Contributions

Mohammad Reza Ghadirzadeh: Contributed in writing proposals, final report and also, representation of scientific comments.

Ali Rafizadeh: Contributed in the preparation of proposals, final report and also, performance of experiments.

Akbar Fattahi: Contributed in pretreatment and making of self-made samples.

Seyed Davood Mirtorabi: Contributed in writing proposals, final report and also, representation of scientific comments.

Hajar Nazari: Contributed in performance of statistical analysis of data.

Melika Rafizadeh: Contributed in preparing of samples and controls to performance of tests.

Ethics

This article does not contain any studies with human participants or animals performed by any of the authors.

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