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A simple and low-cost method for determination of methanol in alcoholic solutions

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ABSTRACT

Methanol poisoning can occur through consumption of methanol-containing alcohols, especially in areas where production, distribution, sale and consumption of alcohol is lawfully prohibited. Due to its toxic potency, determination of methanol in alcoholic solutions is important. The aim of the present study was to develop a rapid, simple and inexpensive method for quantification of methanol in alcoholic solutions that uses minimal equipment available in most laboratories. The method developed is based microdistillation and chromotropic acid, which can be conducted without sophisticated instruments or personal. The system consists of a micro-tube suspended in a falcon tube to function as a collector. Methanol is separated from wine by microdistillation at 90°C in water bath and converted to formaldehyde in the collector. The collector contains an acidic permanganate solution that converts methanol to formaldehyde. Formaldehyde was then quantified by use of chromotropic acid in concentrated sulfuric acid. Experimental variables were optimized by using central composite design (CCD). Method detection and quantification limits were 183 mg L^{-1} and 584 mg L^{-1}, respectively. The percent relative standard deviation (RSD%) were between 6.4 and 7.9. Accuracies were between 89.6 % and 92.4 %. Concentrations of methanol in five alcoholic solutions were between 2.9×10^4 and 3.0×10^4, mg/L, v/v (ppm). Due to its simplicity and cost effectiveness, this method can be used for routine, real-time determination of methanol in alcoholic solutions.

1. Introduction

Wines are alcoholic solutions that produced from natural fermentation of fruit juices [1]. They consist mainly of water, sugars, acids, pigments alcohols, aldehydes, ketones and various aromatic compounds [2]. During production of alcoholic solutions, methanol (CAS 67–56–1) [3] is a byproduct generated by degradation of pectins [4].

Methanol intoxication is observed in consumers of illegal alcoholic beverages. Mass methanol poisonings, due to consumption of illicit alcohol containing large amounts of methanol continues to be a public health challenge that contributes to severe long-term health impairment in survivors, and mortalities ranging from 18 % to 44 % and [5,6]. Due to rapid absorption by the mucosa and several parts of the gastrointestinal system, methanol reaches maximum plasma concentrations after 30–90 min. Methanol, also called methyl alcohol and wood spirit, among other regional common names, is an organic chemical and the simplest aliphatic alcohol, with the formula CH_3OH (molecular mass 32.04 g mol^{-1}) is metabolized to formaldehyde and formic acid by enzymes in the liver [5]. Formic acid causes toxicity by preventing oxidative metabolism in mitochondria by inhibition of cytochrome C oxidase activity [7]. Ingesting as little as 10 mL of methanol can cause permanent blindness by destruction of the optic nerve and ingestion of 30 mL is potentially fatal [8]. The median lethal dose is 100 mL or 1–2 mL/kg body mass. Toxic effects begin hours after ingestion, and antidotes can often prevent permanent damage. Because of its similarities in appearance and odor to ethanol it is impossible for consumers to detect in alcoholic solutions [9].

In recent years, various methods have been described for

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quantification of methanol in alcoholic solutions [10-12], including gas chromatography (GC) and quantitative ^{1}H NMR spectroscopy (qNMR) [2,10]. However, GC and NMR require complicated and expensive devices and trained operators that are often not available, especially in developing countries or smaller production facilities [13]. Previously, more simple methods such as titrimetric [2] and enzymatic [14] were introduced for quantification of methanol in alcoholic solutions, these have poor accuracy and precision [2]. The standard method for assaying methanol in alcoholic solutions is based on a methanol/potassium permanganate/$H_2C_2O_4$/fuchsin sodium sulfite (FSS) reaction system [15]. However, it is difficult to control the temperature of the reaction product and intensity of color in this assay. Thus, due to generation of CO and CO_2 in the reaction, that method has poor repeatability [15]. The Association of official analytical chemists (AOAC) has recommended the chromotropic acid (CA) method as a standard technique for quantification of methanol in beverages [16,17], but use of a large volume of concentrated sulfuric acid is a limitation [16]. It was reported that 2, 4-Dichlorophenoxy acetic acid (2,4-D), which has been used extensively for control of weeds in agriculture, such as grapes [18], is an important interference, when determination of methanol relied on chromotropic acid (Hassanian-Moghaddam et al., 2018).

Microdistillation provides separation of volatile compounds, such as methanol and reduces effects of other component of sample matrix on test results [19]. A simple, low-cost approach has been developed for determination of methanol in biodiesel, based on microdistillation [19]. In the proposed method, in a falcon tube methanol was evaporated and captured in a second tube containing potassium permanganate in sulfuric acid medium. Analytical measurement was based on discoloration of the permanganate solution [19].

Currently, during development of analytical methods an overall experimental design is used for optimization instead of traditional one-variable-at-a-time (OVAT) approach. Unlike conventional OVAT optimization, the experimental design procedure (DOE) allows one to take interaction parameters into consideration. Besides, the DOE procedure needs a minimum number of experiments to reach an optimum point [20]. Response surface methodology (RSM) is one of the most commonly used experimental design techniques. The main objective of RSM is optimization of response surfaces that are influenced by various process parameters. CCD is commonly used as the design to perform RSM. This method gives almost as much information as a multi-level factorial and requires much fewer experiments than a full factorial [20,21].

Since all previously mentioned methods had some limitations, precise and simple method for the determination of methanol in alcoholic solutions especially in the methanol poisoning outbreak is necessary, Therefore, the goal of this study was to develop a novel, low-cost analytical method, based on merits of microdistillation and chromotropic acid methods, for colorimetric determination of methanol in alcoholic solutions. RSM was employed to optimize analytical responses.

2. Methods and materials

2.1. Reagents and solutions

All chemicals and reagents were of analytical grade. Potassium permanganate, sulfuric acid (95–98 %) and methanol were purchased from Merck Chemical Company (Darmstadt, Germany). Chromotropic acid (1,8-dihydroxynaphthalene-3,6-disulphonic acid) was obtained from Sigma-Aldrich (Merck). Potassium permanganate and chromotropic acids solutions were prepared in deionized water (resistivity > 18 MΩ cm). A 3.6 mol L^{-1} sulfuric acid solution was prepared by dilution of 2 mL of the concentrated acid in 10 mL of water. Alcoholic solutions were provided from black markets.

2.2. Apparatus

Microdistillation method was similar to the previous published study [19]. The apparatus consists of a 1.5 mL micro-tube containing the collector solution, which was suspended inside a 15 mL Falcon tube capped tube containing the alcoholic solutions sample. The collector containing of permanganate solution (various volume) and sulfuric acid (500 μL, 3.6 mol L^{-1}). In the next step the micro tube was suspended inside the Falcon tube containing various volumes of sample [19]. Microdistillation was performed in a water bath (Grant instruments, model SS40) at 90°C. After 30 minutes, 150 μL of each collector solution were transferred to the next new tube and mixed with 300 μL concentrated sulfuric acid and 50 μL chromotropic acid (different concentration). By means of UV–visible spectrophotometer (SPECORD 210 PLUS, Germany), absorbance of each test tube was read at 575 nm (Hassanian-Moghaddam et al., 2018).

2.3. Experimental design and statistical analysis

The current study used Design Expert 12 Software to reduce the number of experiments, and to examine effects of variables on responses, and optimize generation of color. For this purpose, a CCD was used for regression and mathematical modeling of the experimental data. Based on a literature survey, methanol concentration (A), potassium permanganate volume (B), potassium permanganate concentration (C), chromotropic acid concentration (D) and sample volume (E) were selected as effective factors to be optimized in this study. According to the experimental design matrix, 53 different experimental sets were performed in the predetermined range of values: A (1.4–27.5 % (v/v)); B (146–503 μL); C (1.2–10.7 mM); D (19.3–90.6 mM); and E (155–1344 μL) (Table 1). Results were obtained from the statistical experimental runs proposed by the face-centered CCD (FCCD) with $\alpha = 1$. Optimal conditions, according to the models were achieved by Nelder Mead simplex method with the help of the Design-Expert 12 software, running the optimizer for each system. The optimizer uses a Nelder Mead simplex method with the fitted response functions to optimize an overall desirability function combining the individual desirability of each response.

3. Results and discussion

3.1. Experimental design and statistical analyses

Effect of five canonical factors were evaluated with help of CCD of RSM. Depending on these five factors, the CCD design required 53 runs including 16 and 8 and 7 runs as factorial, axial and center points, respectively. Values in coded and actual values of these variables are given (Tables 2 and 3). Analysis of variances (ANOVA) was used to assess the suitable response surface model and its significances (Table 2). Prior to use of the ANOVA, it was determined that the data were adequately described by the normal probability function.

Effects of various parameters and the final model were predicted based on the data presented in Table 2. P values less than 0.05 were used to select more significant parameters. The p value for the lack of fit implies that it is not significant relative to experimental error and confirms the validity of the model. R squares values and other statistical parameters of the models were all acceptable. The final empirical model for optimization of quantification of methanol was determined (Eq. 1).

$$R = 0.2304 - 0.024A + 0.009B + 0.005C + 0.013D + 0.008E + 0.017AB + 0.011AD - 0.012AE - 0.004BD - 0.006BE - 0.012CD + 0.015CE + 0.023A^2 - 0.013C^2 + 0.09D^2 + 0.036E^2 \quad (1)$$

Where R is the desirability value and the other terms are corresponding to the parameters defined in Table 1.

Adequacies of models were further evaluated through interpretation of results of the ANOVA. Probabilities of F less than 0.05 imply that the model terms were significant. Prob > F indicates the probability that all variability in results is caused by random error and not due to

Table 1
CCD Experimental Design for optimization of quantification of methanol in alcoholic solutions.

	Methanol percent (A)	Potassium permanganate volume (μL), (B)	Potassium permanganate concentration, (mmol/L) (C)	Chromotropic acid concentration (mmol/L), (D)	Sample volume (μL), (E)	Response (R)
1	14.5	325	6	55	750	0.238
2	14.5	325	6	55	750	0.222
3	9	400	8	40	500	0.192
4	20	250	4	70	500	0.173
5	9	400	4	40	1000	0.178
6	20	250	8	70	1000	0.163
7	14.5	325	6	55	700	0.232
8	9	400	4	70	500	0.197
9	20	400	4	40	500	0.161
10	9	250	4	40	500	0.185
11	9	250	4	70	1000	0.215
12	9	250	8	70	500	0.179
13	9	250	8	40	1000	0.29
14	20	400	4	70	1000	0.189
15	9	400	8	70	1000	0.218
16	20	400	8	70	500	0.206
17	14.5	325	6	55	750	0.252
18	20	400	8	40	1000	0.185
19	20	250	8	40	500	0.345
20	20	250	4	40	1000	0.069
21	20	400	4	70	500	0.254
22	20	250	4	70	1000	0.175
23	20	400	4	40	1000	0.149
24	9	250	8	70	1000	0.274
25	20	400	8	70	1000	0.192
26	14.5	325	6	55	750	0.221
27	9	250	4	40	1000	0.196
28	9	400	8	70	500	0.222
29	9	400	4	70	1000	0.213
30	9	250	8	40	500	0.172
31	9	400	8	40	1000	0.225
32	14.5	325	6	55	750	0.245
33	14.5	325	6	55	750	0.224
34	9	250	4	70	500	0.235
35	20	400	8	40	500	0.492
36	14.5	325	6	55	750	0.228
37	9	400	4	40	500	0.188
38	20	250	4	40	500	0.1
39	20	250	8	40	1000	0.154
40	20	250	8	70	500	0.154
41	14.5	325	6	90	750	0.028
42	14.5	325	10	55	750	0.163
43	1	325	6	55	750	0.427
44	14.5	325	6	55	750	0.24
45	14.5	325	6	55	750	0.238
46	14.5	325	6	55	750	0.2206
47	27	325	6	55	750	0.295
48	14.5	503	6	55	750	0.253
49	14.5	146	6	55	750	0.196
50	14.5	325	6	55	1344	0.462
51	14.5	325	1	55	750	0.146
52	14.5	325	6	55	155	0.414
53	14.5	325	6	19	750	0.124

relationships between the specific parameters. The residual lack-of-fit was not significant, and the model F value of 63.04 implies that the model was significant with a p-value < 0.0001. Values (<0.0001) observed for the responses indicate that obtained data are not random and the model is significant. Results of the ANOVA, and the sum of squares, lack of fit and R^2 tests revealed that a reduced quadratic was the most adequate model (Table 2).

Concentrations of methanol and chromotropic acid had significant effects. The quadratic effects of concentrations of methanol and chromotropic acid and potassium permanganate and sample volume were significant effects. In addition, interaction terms AB, AD, AE, BD, BE, CD and CE were significant model factors.

3.2. Verification of regression models on diagnostic plot

The normal probability plot of the residuals gives useful information about normality of distribution of errors and their independence from each other (Fig. 1). Plotted points are scattered around the regression line, which implies that the data set is approximately normally distributed. Based on visual examination of the data, it is possible to conclude that residuals have a normal distribution. This behavior indicates the adequacy of obtained model for the observed data. This information also supports the use of the parametric statistical procedures.

3.3. Three-dimensional response surface plots

The analytical signal is increased was proportional to concentration of methanol but the volume of potassium permanganate had little effect on the response (Fig. 2a).

Analytical signal was proportional to concentration of chromotropic acid in the range of 20–60 mmol L^{-1} (Fig. 2b). However, greater concentrations of chromotropic acid resulted in lesser analytical signal. At

Table 2
ANOVA table for a quadratic response surface model.

Source	Sum of Squares	df	Mean Square	F-value	p-value		R^2	Adjusted R^2
Model	0.2361	20	0.0118	63.04	< 0.0001	significant	0.98	0.96
A-methanol percent	0.0223	1	0.0223	119.14	< 0.0001			
B-potassium permanganate volume	0.0036	1	0.0036	19.02	0.0002			
C- potassium permanganate concentration	0.0010	1	0.0010	5.31	0.02			
D-chromotropic acid concentration	0.0054	1	0.0054	28.84	< 0.0001			
E-sample volume	0.0032	1	0.0032	17.31	0.0003			
AB	0.0079	1	0.0079	42.19	< 0.0001			
AC	0.0003	1	0.0003	1.51	0.23			
AD	0.0037	1	0.0037	19.96	0.0001			
AE	0.0029	1	0.0029	15.64	0.0006			
BC	0.0001	1	0.0001	0.7449	0.39			
BD	0.0005	1	0.0005	2.89	0.10			
BE	0.0010	1	0.0010	5.19	0.03			
CD	0.0033	1	0.0033	17.86	0.0003			
CE	0.0065	1	0.0065	34.57	< 0.0001			
DE	0.0006	1	0.0006	3.11	0.08			
A^2	0.0279	1	0.0279	148.96	< 0.0001			
B^2	0.0001	1	0.0001	0.4490	0.50			
C^2	0.0099	1	0.0099	52.89	< 0.0001			
D^2	0.0742	1	0.0742	396.32	< 0.0001			
E^2	0.0709	1	0.0709	378.58	< 0.0001			
Residual	0.0047	25	0.0002					
Lack of Fit	0.0037	17	0.0002	1.81	0.19			
Pure Error	0.0010	8	0.0001					
Correlation Total	0.2408	45						

Table 3
Optimized values of parameters for quantification of methanol in alcoholic solutions.

Methanol percent (A)	potassium permanganate volume (B)	potassium permanganate concentration (C)	Chromotropic acid concentration (D)	Sample volume (E)	Response	
					Predicted	Experimental
20	400	4.6	58	500	0.369	0.373 (±0.029)

greater concentrations of chromotropic acid it acts as an oxidant [22] and converts formaldehyde to formic acid or further metabolizes it to CO_2 and H_2O. The greatest analytical signal was obtained when volume of sample or concentration of methanol is maximum (Fig. 2c), which is intuitive because with more methanol present more formaldehyde is formed obtained. Greater volumes of sample also resulted in proportionally greater analytical signal (Fig. 2d). The greatest analytical response was obtained when the volume permanganate solution was greater. The greater volume of acidic permanganate increases the chance of methanol being trapped and converted in the collector. Greater concentrations of permanganate did not reveal significant effect (Fig. 2e). Also, greater concentrations of chromotropic acid also resulted in greater analytical signal. The best results were obtained when a large volume of sample was used (Fig. 2f), but greater concentrations of permanganate did not result in greater analytical response.

Methanol is oxidized by potassium permanganate to formaldehyde, formic acid or carbon dioxide all of which is dependent on pH [19]. The optimal concentration of sulfuric acid has been shown previously to be 3.6 mol L^{-1} [19]. Greater concentrations of H_2SO_4 (7.0 mol L^{-1}) gives optimal sensitivity, but linearity of the external standard curve was poor. Based on these findings in the study results of which are presented here a concentration of 3.6 mol L^{-1} sulfuric acid was used.

Greater concentrations of potassium permanganate results in slightly more conversion of formaldehyde to formic acid or carbon dioxide [19]. When temperature of reaction was increased from 60 to 90°C, analytical response was also increased. Since the distillation was conducted in a water bath and the boiling point of water is 100°C, higher temperatures were not investigated. Hence, 90°C was selected as the optimal temperature for the method.

Optimized values of the methanol percent, volume and concentration of potassium permanganate and concentration of chromotropic acid and volume of sample were 20 %, 400 μL, 4.6 mmol L^{-1}, 58 mmol L^{-1} and 500 μL, respectively.

3.4. Confirmation test

In order to confirm the adequacy of the proposed method, three commercial samples of alcoholic solutions were analyzed under optimized conditions. Samples were spiked with methanol at 20 % (v/v). Based on Table 2, the predicted values were close to the obtained values. The good responses were obtained in real samples which ranged from 0.34 to 0.398. These results demonstrated adequate reliability, accuracy and precision of the proposed method, so that matrix constituents of the samples particularly carbohydrate compounds had no significant effects on quantification of methanol.

3.5. Analytical performance

In order to assess the applicability of the suggested method, the analytical characteristics including limit of detection (LOD), limit of quantification (LOQ), linearity, repeatability and accuracies were determined under optimal conditions. These parameters were determined by a series of experiments with samples of alcoholic solutions spiked with methanol. The LOD as well as LOQ were calculated (Eqs. 2 and 3).

$$C_{LOD} = 3S_b/m \quad (2)$$

$$C_{LOQ} = 10S_b/m \quad (3)$$

Where S_b is the standard deviation of blank and m is the slop of the calibration curve. Accuracy and precision were evaluated by spiking samples at two concentration levels, including 0.5, 5 mg L^{-1} with three

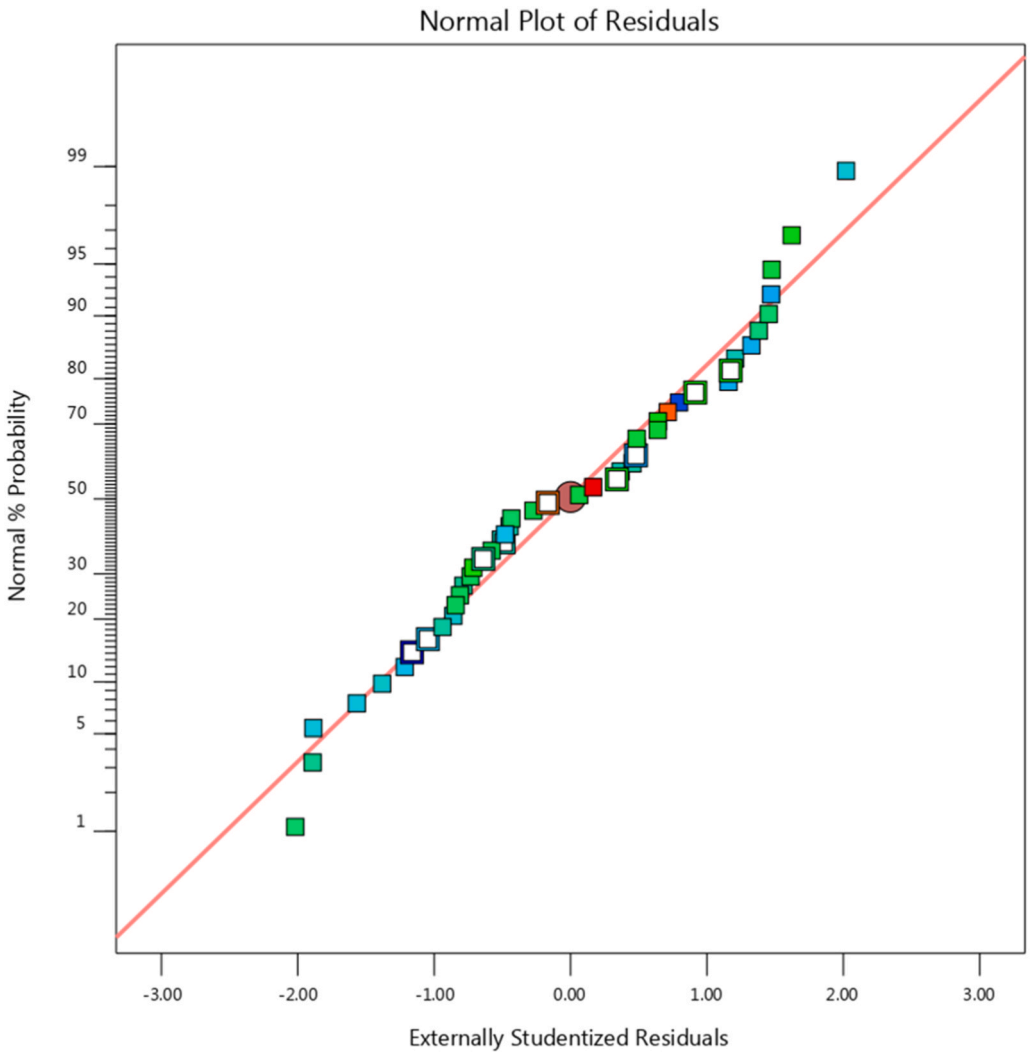

Fig. 1. Normal probability plot of residuals.

replicates. The LOD and LOQ were 183 and 584 mg L^{-1}. Percent relative standard deviations (RSD%) at a concentration of 0.5 and 5 mg L^{-1} were between 6.4 and 7.9. Accuracies were between 89.6 % and 92.4 %. The proposed method has excellent linearity in the range of 189–1582 and 1582–185000 mg L^{-1} with coefficients of determination of essentially 1.0.

3.6. Comparative study

In Soares and Rocha method, quantifications of methanol were based on discoloration of an acidic potassium permanganate solution, caused by oxidation of methanol to formaldehyde (Eq. 4) and consequent reduction to Mn (II) [19].

$$5CH_3OH + 2MnO_4^- + 6H^+ \rightarrow 5CH_2O + 2Mn^{2+} + 8H_2O \quad (4)$$

Due to lack of selectivity, the proposed system gives similar results with ethanol. Whereas alcoholic solutions contain different level of ethanol, the proposed method cannot be applied directly for determination of methanol in alcoholic solutionss. Therefore, in the present study, so that the method could be applied selectively to quantify methanol in alcoholic solutions, sulfuric acid and chromotropic acid reagent were added to determine concentrations of formaldehyde in the acidic permanganate solution [19].

An international reference method for determination of methanol in alcoholic solutions was described by the OIV-MA-AS312–03B IV (A 41 revised by 377/2009) [23]. In spite of valuable merits, such as low limit of detection and simplicity, consumption of 5 mL of H_2SO_4 for each test was a major limitation (OIV, 2018). Since H_2SO_4 is a hazardous and corrosive solvent the volume used in the present study was reduced to only 300 μL for reaction of formaldehyde with chromotropic acid [24]. In addition, the OIV method requires sodium sulfite to decolorize the permanganate. But, in the present study, after reaction of methanol with permanganate, the pink color of collector solution disappears (Eq. 1).

The widely used herbicide, 2,4-D is an important interference, when quantification of methanol relies on chromotropic acid (Hassanian-Moghaddam et al., 2018). In fact, one colorimetric method for quantification of 2,4-D relies on chromotropic acid [25]. In this study it was found that 2,4-D did not affect results, when the microdistillation system was used to separate methanol from more polar and less volatile compounds like 2,4-D. Microdistillation was operated at 90°C, which is less that boiling point of 2,4-D of 160°C at 4 mm Hg [26]. Concentrations of 100, 500 and 1000 ppm of 2,4-D did not produce any color so could not interfere in the collector solution.

It was reported that inorganic ions can react with chromotropic acid in concentrated sulfuric acid [27]. Nitrate, nitrite, chromate, and dichromate react with chromotropic acid to give colors in the visible range under the conditions of the test [27]. Presence of these chemical substance may interfere with direct determination of methanol in alcoholic solution. In previous methods like that introduced by OIV, colored substance in matrix may affect the results [2]. Hence, microdistillation

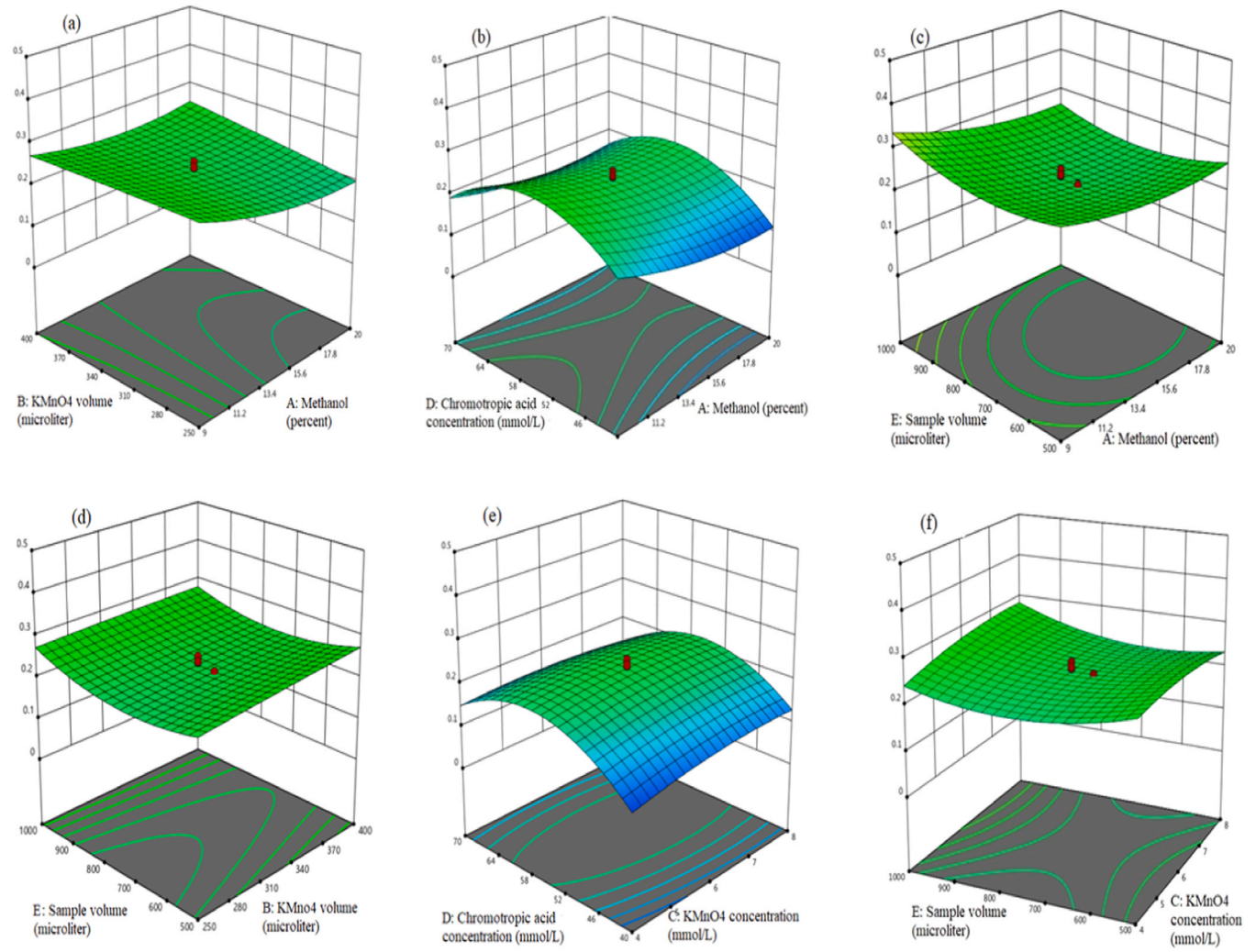

Fig. 2. (a-f): Response surface plots showing the effect of the methanol percent, potassium permanganate volume, potassium permanganate concentration, chromotropic acid concentration and sample volume on analytical response.

provide a chance to remove the effects of such interferences.

3.7. Analysis of real samples

Analysis of methanol in five homemade alcoholic solutions sample was presented in Table 4.

Results revealed that all the samples were contaminated with methanol. Except one sample, other ones had methanol concentration higher that permissive levels (4000 mg L^{-1}). Similar results were reported by Hassanian-Moghaddam and co-workers. They showed that homemade alcoholic solutions had high methanol content ranging from 1800 mg/L to 14320 mg/L (Hassanian-Moghaddam et al., 2018).

Methanol is produced from methoxyl groups of pectin by enzymatic hydrolysis in fermentation process. The maximum methanol formation occurs in 25 °C after seven days of fermentation. Therefore, methanol concentrations more than 1000 mg/L are usually reported in alcoholic solutions (Murghagh, 1999), a finding compatible with our results. Consumption of alcohol in Islamic countries is illegal [28]. Hence, there is no accurate control on the process of production of homemade alcoholic solutions. Since determination of methanol content of alcoholic solutions requires expensive and complicated devices, having access to low-cost and simple methods is warranted.

Table 4
The concentration of methanol in five homemade alcoholic solutions sample.

Sample number	Alcoholic solutions type	Result (mg/L)
1	pomegranate	29768
2	grape	20412
3	grape	2948
4	grape	28946
5	grape	11588

4. Conclusions

An analytical method was introduced for determination of methanol in alcoholic solutions samples based on merits of microdistillation and chromotropic acid. The detection system was consisting of a collector containing acidic permanganate solution that suspended in a falcon. Herein, methanol was separated form alcoholic solutions by microdistillation method and converted to formaldehyde in collector solution. Determination of formaldehyde was based on chromotropic acid reaction. In comparison with previous methods, this system uses less hazardous sulfuric acid and results cannot be affected by interference such as 2,4-D. The effects of the experimental variables on analytical response were optimized by using central composite design (CCD). Method detection and quantification limits were calculated to be 183 mg L^{-1} and 584 mg L^{-1}, respectively. The proposed method can be used for determination of methanol in alcoholic solutions samples, due to its low-cost and simplicity.

CRediT authorship contribution statement

Nastaran Eizadi-Mood: Writing – review & editing, Supervision, Investigation. **Hasan Badibostan:** Writing – review & editing, Visualization, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization. **Mohammad Khodadadi:** Methodology, Investigation, Formal analysis, Data curation. **John P. Giesy:** Writing – review & editing, Methodology, Investigation. **Rokhsareh Meamar:** Writing – original draft, Investigation. **Ali Mohammad Sabzghabaee:** Writing – review & editing, Investigation. **Azadeh Khosravi Neisiani:** Investigation, Formal analysis.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

The data used to support the findings of this study are available from the corresponding author upon reasonable request.

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