Journal of Sciences, Islamic Republic of Iran 36(2): 105 - 110 (2025) http://jsciences.ut.ac.ir
University of Tehran, ISSN 1016-1104

Determination of Ethanol in Alcoholic Beverages Using Conventional Method and Gas Chromatography-Mass Spectroscopy

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Received: 25 June 2025 / Revised: 25 November 2025 / Accepted: 13 June 2026

Abstract

The objective of this study was to develop a straightforward and rapid analytical procedure for ethanol quantification. Specifically, a GC-MS method was compared to a conventional approach for detecting ethanol levels in alcoholic beverages. In the GC-MS system, the oven temperature rate and the mobile phase total flow rate were set at 10 °C/min and 104 mL/min, respectively. This method successfully detected ethanol in less than 2 minutes, demonstrating a determination coefficient (R2) of 0.994 within a 0.1–5% standard calibration range. It exhibited high selectivity, accuracy (100.8%), and precision (RSD of 3.01–3.25%). In contrast, the conventional method required 24 hours of analysis time, with a narrow standard curve range of 0–0.05%. However, this investigation demonstrated that GC-MS is superior to the traditional technique, which was found to be time-consuming, non-specific, inaccurate, and imprecise. Therefore, GC-MS is highly recommended for confirmatory testing of ethanol in alcoholic beverages, whereas the conventional method should only be utilized for preliminary screening.

Keywords: Conventional Method; Chromatography Technique; Ethanol Analysis; Confirmatory Test; Preliminary Test.

Introduction

In the last ten years, ethanol analysis in alcoholic beverages was an essential component in solving, breaking, and cracking a criminal and crime problems. However, alcohol intake has been associated with an increase in crime and human mortality. Alcohol-related death rates are rising by up to 5.9% globally (1). Alcohol intake can contribute to several health risks, including dementia, breast cancer, colorectal cancer, cirrhosis, and alcohol dependence (2). Therefore, Islam forbids individuals from drinking alcohol since it has harmful effects on the body.

Dangerous chemical components in the alcohol beverages created an undesirable effect in the human body. According to Jung et al. (3) and Wiśniewska et al. (4), vodka contains both volatile and non-volatile compounds, such as ethanol. Volatile chemicals present in alcoholic beverages include ethanol, ethyl acetate, methanol, i-propanol, n-propanol, i-butanol, n-butanol, i-amyl alcohol, and n-amyl alcohol (5). Another study found that Romanian home-made plum spirits contain volatile compounds such as methanol, 1-propanol, isobutanol, 1-butanol, 3-methyl butanol, acetone, and ethanol, with ethanol content ranging between 52-76% (3). Several quantitative techniques have been developed to ensure ethanol content, including amperometric

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biosensors (6), HPLC (7), spectrophotometry (8, 9), gas chromatography (3, 10), and infrared sensors (11).

Therefore, this study will use both GC-MS and conventional techniques to determine the ethanol level in alcoholic beverages. The usage of GC-MS is due to its effectiveness in detecting volatile chemicals in beverage products. GC is a chomatography-based approach that is thermally stable (12). Tiscione et al. (13) used GC-FID to evaluate ethanol at a concentration of 0.010 g/dL, with an accuracy of ± 8% and a precision of 3.1%. Ethanol analysis by GC has several advantages, including excellent sensitivity, selectivity, repeatability, robustness, linearity, and ease of use for regular alcohol analysis.

In addition, the MS detector is utilized to quickly identify unknown chemical compounds in beverage samples by comparing fragmentation to libraries. Furthermore, GC-MS can resolve the retention time overlap problem of methanol with other volatile chemicals, which can result in human deaths (14). Therefore, GC-MS is an appropriate qualitative confirmation approach for forensic laboratories (15, 16).

The Food and Drug Administration (FDA) (17) recommended using GC-MS to detect ethanol and isopropanol in hand sanitizers. For ethanol analysis, GC-MS found ethanol in fragrance product with a retention time of 2.659 minutes and proved linearity and repeatability (18). GC-MS is not only useful for ethanol analysis; it may also detect other volatile substances in food and drinks (19, 20). The effectiveness of GC-MS in detecting ethanol will be compared with conventional methods. The conventional approaches for detecting alcohol in beverages use the $K_2Cr_2O_7$ reagent. This method employs an oxidation-reduction process. The colorimetric technique has various benefits, including simplicity and ease of use (21, 22)

Materials and Methods

Development of Conventional Method for Alcohol Analysis

1) Time contact $K_2Cr_2O_7$ reagent with ethanol

Ethanol 5% was treated with the $K_2Cr_2O_7$ reagent at different times, including 1, 3, 6, and 24 hours. The optimum time to react ethanol with $K_2Cr_2O_7$ reagent is when the color change is stable. The reduction of the $K_2Cr_2O_7$ reagent produces a lovely blue color.

2) Determination of ethanol content in alcoholic beverages

The quantitative analysis for identifying ethanol involved creating multiple standard ethanol concentrations (0.01%, 0.02%, 0.03%, 0.04%, 0.05%, 0.1%, 1%, and 5%). This investigation was completed within the acceptable time of 24 hours. Colorimetric analysis was subsequently employed to determine the ethanol concentration in the samples directly.

Optimization of GC-MS Application for Detecting Ethanol Content in Alcoholic Beverages

1) Effect of GC-MS instrumental parameters

The GC-MS instrument has been optimized through modification of several GC-MS parameters. Several parameters were changed, such as the oven temperature, temperature rate, injection temperature, mobile phase flow rate, and hold conditions. The criteria chosen for best results include selectivity and short-term retention. Table 1 shows the GC parameters that were employed.

2) Alcohol analysis in beverages using GC-MS

The ethanol content of alcoholic beverages was determined using GC-MS (Model: Shimadzu-QP2010S), with an ethanol standard concentration range of 0.1-5%. The standard solutions used in the detection of ethanol level in alcoholic beverages are methanol, ethanol, and acetonitrile (internal standard), as described in Table 2. All standard solutions were injected into the GC-MS instrument. The last step, an unknown sample was determined using a similar procedure.

Table 1. Setting of GC-MS instrument to determine ethanol in alcoholic beverages.

Method	Column Oven Temperature (°C)	Injection Temperature (°C)	Oven Temperature Rate (°C/min)	Flow Rate of Mobile Phase (mL/min)	Hold (min)
1 (C)	40	100	10	104	5
2 (B)	40	100	8	31,8	5
3 (A)	40	100	4	31,8	5

Table 2. Standard solution mixtures for alcohol analysis in beverages

Ethanol concentration (%)	Methanol (µL)	Ethanol (µL)	Acetonitrile (µL)*
0.1	10	10	500

Statistics Analysis

The data was calculated using Tukey's test to show significant differences ($p<0.05$)

Results and Discussion

Development of a Conventional Method for Analysis of Ethanol Content in Alcoholic Beverages

Alcohol assays, particularly for ethanol, were carried out using a conventional analytical approach based on colorimetry. This method is straightforward and easy to use since it uses the oxidation-reduction reaction of $K_2Cr_2O_7$ or $KMnO_4$ reagents with ethanol in alcoholic beverages. The $K_2Cr_2O_7$ reagent was applied to detect alcohol because it may be reduced from Cr (VI) to Cr (III) by alcohol in acidic conditions, causing a color change from yellow to blue-green. Furthermore, $KMnO_4$ is dissolved in acetic acid, it forms the brown precipitate MnO_2. In this study, $K_2Cr_2O_7$ provides a more precise endpoint than $KMnO_4$. Figure 1 depicts the ethanol oxidation process that uses $KMnO_4$ and $K_2Cr_2O_7$ reagents.

However, this colorimetric approach is susceptible to interference from glucose concentrations, as glucose also reduces Cr(VI) to Cr(III) (Figure 2). To overcome this limitation, the analysis leverages the volatile nature of ethanol. The schematic design of the specialized analytical apparatus is depicted in Figure 3. The optimum incubation period for securing a stable response in alcoholic beverages was determined to be 24 hours, yielding a highly stable green-blue complex.

For quantitative analysis, the calibration curves for ethanol standard concentrations are 0-0.05. The color change of the $K_2Cr_2O_7$ reagent is difficult to identify when ethanol standard concentrations exceed 0.05% (Figure 4). Based on Figure 4, an unknown sample can be seen that is most similar to the ethanol standard of 0.05%.

The use of GC-MS for Determining Ethanol Content in Alcoholic Beverages

Alcohol analysis is influenced by several GC-MS parameters, including oven temperature, temperature rate, injection temperature, mobile phase flow rate, and hold time. The flow rate of the mobile phase and the oven temperature rate of the end-product chromatogram has been evaluated in this study. A previous study from Sirhan et al. (23) noted that the first oven temperature was set to 400°C. The results revealed that the oven temperature rate was increased by twofold, but there was no significant variation in time retention (Figures 5a and 5b). Changes in oven temperature rate and mobile phase flow rate by roughly 1.3 and 3.3 times, respectively, can

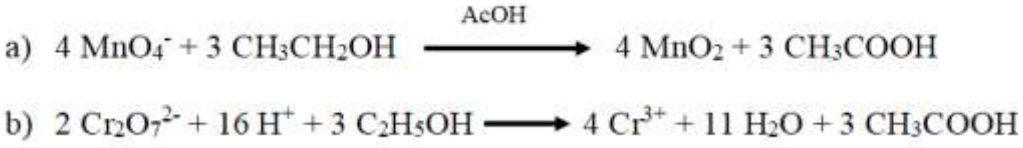

Figure 1. Oxidation/reduction reaction of ethanol: a) with $KMnO_4$; b) with $K_2Cr_2O_7$.

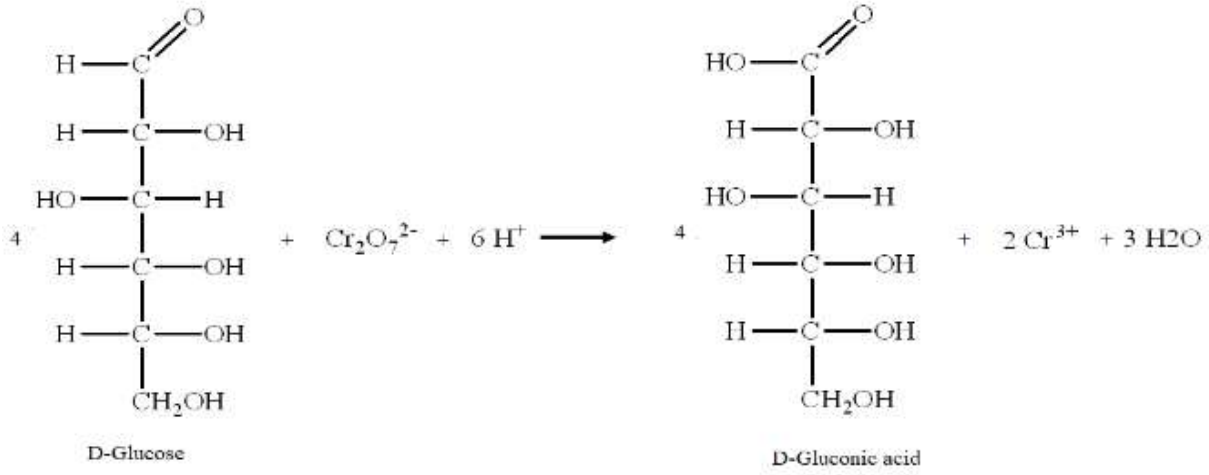

Figure 2. Reduction of $K_2Cr_2O_7$ reagent by glucose in beverages.

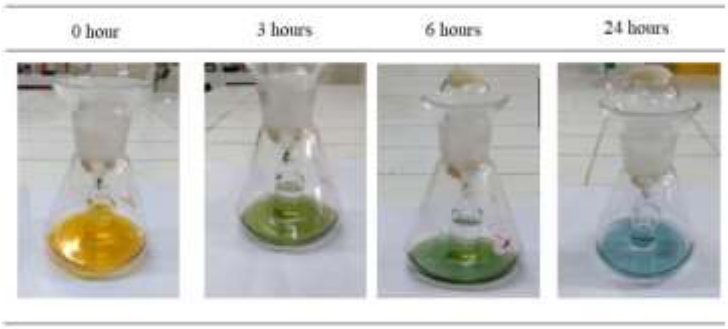

Figure 3. The color change of $K_2Cr_2O_7$ reagent by standard ethanol 5% in various times.

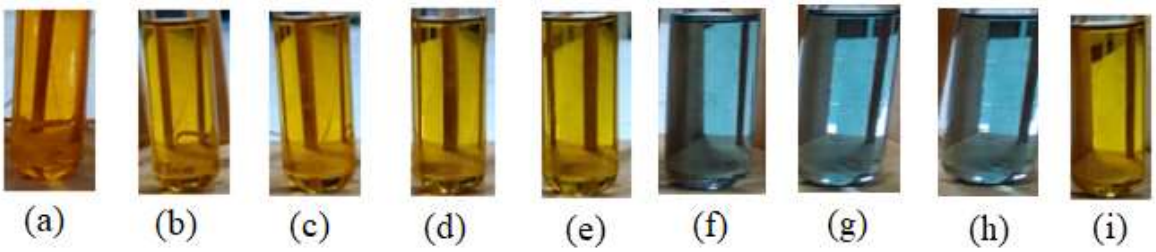

Figure 4. Various ethanol concentrations reacted by $K_2Cr_2O_7$ reagent for 24 hours: (a) 0%, (b) 0.02%, (c) 0.03%, (d) 0.04%, (e) 0.05%, (f) 0.1%, (g) 1%, (h) 5%, and (i) unknown sample.

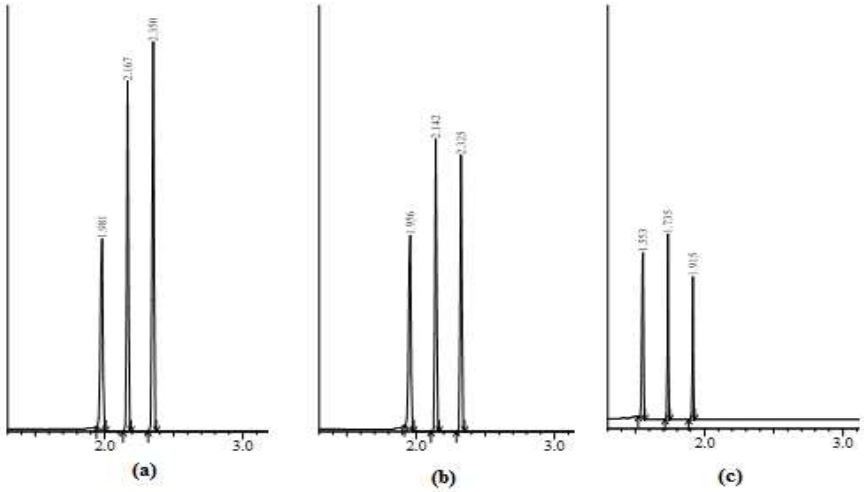

Figure 5. Chromatogram of methanol (peak 1st), ethanol (peak 2nd), and acetonitrile (peak 3rd) produced by GC-MS with different settings of oven temperature rate and total flow rate of mobile phase, (a) 4 ^{0}C and 31.8 ml/min, (b) 8 ^{0}C and 31.8 mL/min, (c) 10 ^{0}C and 104 mL/min.

have an impact on retention time results (Figure 5b and 5c).

The oven temperature and a mobile phase flow rate of 10 °C and 104 mL/min can be applied to create a shorter retention time for detecting ethanol. Ethanol is detectable at 1.735 minutes. Previous research determined that ethanol requires an elution time of 2.73 minutes or 2.659 minutes (10, 18). Furthermore, this method produces good resolution. The resolution of ethanol and methanol is 6, while the resolution of ethanol and acetonitrile is 7.2 (Table 2). Therefore, this method can separate all compounds and the criteria are acceptable because they are more than 1.5 (24). Wang et al. (10) reported that their research has a greater resolution of roughly 24. Therefore, this study outperforms from the reference from Wang et al. (10), due to this study takes less time.

Furthermore, alcohol analysis by GC-MS is carried out at a temperature of 10 °C and a mobile phase flow rate of 10^4 mL/min. Range linearity is approximately 0.1-5%, with a determination coefficient (R2) of 0.994. Based on these findings, it is possible to conclude that the work area in the analysis of alcohol using GC-MS in the

Table 3. GC-MS Performance for the Analysis of Ethanol in Alcoholic Beverages.

Parameters	Results	Limit
Specification	Rs: 6 and 7,2	Rs: 1,5
Linearity Range	0.1-5%	-
Determination Coefficient (R2)	0.994	0.99
Accuracy (%)*	100,8	97-103
Precision (%)*	RSD: 3.01	< 2.7

range of 0.1-5% is well correlated with the equation: y = 3,010,577.126 x + 11,292,979.971. The resulting determination coefficient (R2) is less than R2 of 0.999 (19). However, this work is acceptable because it maintains good linearity. GC-MS also achieved high accuracy (100.8%) but was less precise (Table 3). The good accuracy and precision for analytes ranging from 1 to 10% are 97-103% and 2.7%, respectively (25). According to the study from Ordell et al., (26) the regression curve for ethanol analysis has a determination coefficient (R2) of 0.99 in the range of 0.2-100 mg/L (0.02-10%), with limits of detection (LOD) and lower limits quantification (LOQ) of 0.1-0.2 mg/L and 0.5-1 mg/L, respectively. According to the literature, GC-MS is not precise, but it is still acceptable for ethanol analysis in alcoholic beverages because the precise value is not greater than 5%. To improve precision, this method can be combined with headspace (HS) equipment (27). This study successfully detected the ethanol content of an unknown sample, which was around 2.83±0.09%.

This study demonstrated that both methods (conventional and GC-MS) can be used for ethanol analysis in alcoholic beverages. GC-MS is more accurate than conventional methods for detecting ethanol, with a detection rate of 2.83±0.09% compared to 3% (triplicates). However, the conventional method detects all volatile compounds found in alcoholic beverages. Previous research from Wang et al., (10) demonstrated that the gas chromatography methods, oxidized $K_2Cr_2O_7$ method, and hydrometric method were not significantly different. Based on these findings, the conventional method for ethanol can be used because it is less expensive and simpler, but it does not distinguish between alcohol types in alcoholic beverages.

Conclusion

The conventional technique for measuring ethanol in alcoholic beverages requires 24 hours, whereas GC-MS takes only a few minutes. Furthermore, because the conventional technique does not detect ethanol specifically, it noticed all volatile chemicals. However, GC-MS for ethanol analysis is more costly than the conventional technique. According to the findings of this study, GC-MS can be used as a confirmatory test, whereas the conventional method is applied as a preliminary or screening test.

Acknowledgement

Authors thankful to LPPM Universitas Ahmad Dahlan for research funding through the Fundamental Scheme in 2017.

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