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A Rapid Method for Determination of Ethanol in Alcoholic Beverages Using Capillary Gas Chromatography

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ABSTRACT

A simple and rapid method was developed to determine ethanol content in alcoholic beverages using megapore polar column (CP-Wax 58 CB, 30 m × 0.53 mm) with direct injection gas chromatography. Contrary to packed GLC method, distillation and/or stepwise dilution of samples were not necessary by the method developed in this study. Ethanol in sample was injected directly into GC for analysis, after adding suitable amount of internal standard, acetonitrile solution. Using this method, less than 8 min was required to obtain result since sample preparation, and the limit of quantitation (LOQ) was about 0.5 μg/mL. Recovery studies were performed using 0.5 mL of red wine and whisky. Each was spiked with ethanol at 50 and 100 mg, respectively. The recoveries were found in the range of 99~104% and 99~101%, respectively. The coefficients of variation were less than 3.4%. Comparisons of the AOAC method (AOAC 969.12 and 920.57) with current method showed no significant difference. These results suggested that precision of direct injection GC method was higher than that of AOAC methods. Several commercial alcohol beverages, including distilled and non-distilled spirits, were analyzed by the current method. The ethanol content of distilled and non-distilled spirit were found as: 165.2 ± 4.9~415.7 ± 17.6 and 28.2 ± 0.8~141.2 ± 4.9 mg/mL, respectively. Using this GC method, we could change the concentration in gravimetric percentage (%, w/v) to volumetric percentage (%, v/v) by the equation (%, w/v) = 0.814 (%, v/v) with linear coefficient R^2, higher than 0.999.

Key words: alcoholic beverages, ethanol, direct injection gas chromatography, quantitative analyses.

INTRODUCTION

Ethanol content is very important for the mouth-feel and flavor of alcoholic beverages. Ethanol contents of wine, liqueur, and beer range from 7~21%(v/v), 20~50%(v/v), and 3~6%(v/v), respectively[1]. In general, ethanol contents serve as the quality index and taxation factor for alcoholic beverages[2]. After entering WTO, alcoholic beverages in Taiwan are taxed according to the ethanol contents, like the taxation system in United States[3]. The higher the ethanol content in an alcoholic beverage, the higher the tax. A simple, accurate, and quantitative analysis method for the determination of different levels (0~50%, v/v) of ethanol content is needed as the standard method of quality control for beverage manufacturers and as a guide for government-related sanitary agencies.

Currently, ethanol determination in alcoholic beverages can be performed in several ways: (1) boiling point depression of the ethanol solution relative to water,[1] (2) densimetric analysis[4], (3) refractive index method[1,4], (4) oxidation of the distillate[1,4-5], (5) dichromate oxidation spectrophotometry[1,4,6], (6) enzymatic method[7-9], (7) biosensor[10], (8) potentiometry[11], (9) gas chromatography (GC)[1,4,12-21], (10) capillary electrophoresis[2], (11) high performance liquid chromatography (HPLC)[22-23], (12) modular Raman spectrometry[24], (13) near-infrared (NIR) spectroscopy[25], (14) beer analyzer[4], and (15) flow injection analysis[26-27]. Due to complicated pretreatment procedures (e.g. sample distillation and accurate weighing process) and large sample volume required, the two most popular methods, picnometry and densimetric analysis, are not applicable for samples with small amount. As for oxidation of the distillate and dichromate oxidation spectrophotometry, more than 5 mL of sample volume is required for analysis. Besides, the reagents used are highly toxic and classified. Low stability, low reproducibility and low accuracy are the disadvantages for enzymatic method, biosensor, and potentiometry[9]. Raman spectrometry and capillary electrophoresis, are not popular due to the expensive instruments required. Since sample distillation and accurate weighing process are still required as the pretreatment procedures, and only spectrophotometry is used for analysis, HPLC method obtains a comparatively low sensitivity[22]. Recently developed NIR spectroscopy and beer analyzer are time consuming in establishing calibration curves and have low accuracy as they can be interfered by

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other alcohols in alcoholic beverages. In conclusion, GC method is the most appropriate and rapid method for determination of ethanol contents in alcoholic beverages with complicated alcohol contents and small sample amount.

Bouthilet et al. had developed a packed GC method to analyze ethanol contents in alcoholic beverages[14]. However, the method required at least 100 mL of sample and distillation as the pretreatment process. The packed GLC[4,12-13,15-16] and capillary GC[17-21], which required only small sample amount, were then developed. The GC methods currently available for determining ethanol contents in alcoholic beverages are US official methods, AOAC 968.09, 984.14, and 986.12[4], and Taiwan official method, CNS N6181[15]. These official GC methods adapt different sample preparation procedures when dealing with different sort of samples, and these samples require distillation or dilution as the pretreatment process. Since packed column is used, lower resolution and interference by other alcohols in alcoholic beverages still exist[12,21]. Besides, durability of packed column and reproducibility of the retention time are relatively low[12]. When capillary GC is used in determining ethanol contents in alcoholic beverages, time-consuming pretreatment procedures, e.g. solid-phase extraction[17], head space balancing[18], solvent extraction[19] and distillation[20-21], are still required. The capillary GC method is one of the most important modern analytical techniques because of its advantage of high resolution and sensitivity. Owing to years of research using GC, we found that insertion of a ball of glass wool into a liner of GC injector could effectively prevent the non-volatile compounds form getting into the analytical column and thus, moderate the interference from the contaminants. We find that commercially available megapore capillary GC column is superior in water resistance. Even when an aqueous solution sample is injected directly into the column, the resolution and reproducibility of retention time are almost the same as a new column[28-30]. In this study, we will prepare and inject the alcoholic beverages with adequate amount of internal standard solution directly into a GC analyzer without any pretreatment process,. Together with the application of appropriate column and GC conditions, we plan to develop a simple, rapid and accurate method in determining ethanol contents in alcoholic beverages. Meanwhile, the AOAC methods will be compared and assessed in recovery and standard deviations of intraday/ interday analysis in order to evaluate the accuracy and precision of the study method.

MATERIALS AND METHODS

I. *Materials*

Twenty-six alcoholic beverages, including 12 distilled spirits (whisky, brandy, kaoliang wine, rice wine, Mizhiu Tou, and medicine wine) and 14 non-distilled spirits (millet wine, grape wine, fruit wine, Shaohsing wine, and beer), were purchased from supermarkets in Tainan, Taiwan. LC grade (purity > 99.5%) ethanol, acetonitrile, 1-propanol, isopropanol, acetone, 1-butanol and t-butanol were obtained from ALPS (Taiwan).

II. *Methods*

(I) *Preparation of standard solution and internal standard solution*

Ten grams of ethanol and acetonitrile were dispensed into 1000 mL volumetric bottle and then distilled water was added to the 1000-mL mark. This was the 1% (w/v) ethanol standard solution or internal (acetonitrile) standard solution.

(II) *Relative response factor (RRF) of ethanol to acetonitrile*

Ethanol, 1% (w/v), was mixed with 1% (w/v) acetonitrile in various ratios (ethanol:acetonitrile = 15:1, 10:1, 5:1, 2:1, 1:1, 1:2, 1:5, 1:10, and 1:15). A linear regression line was generated with the GC peak area-under-curve (AUC) ratio of ethanol to acetonitrile (Y-axis) against the concentration ratio of ethanol to acetonitrile (X-axis). Relative response factor (RRF) is the slope of the regression line, as in the Equation 1: $RRF = (A_S/W_S) \div (A_{IS}/W_{IS})$; in which, A_S = ethanol AUC, A_{IS} = acetonitrile AUC, W_S = ethanol weight (mg), W_{IS} = acetonitrile weight (mg).

(III) *Quantitative ethanol determination*

(1) Direct injected GC method

Beverage sample solution (0.5 mL) was dispensed into an l-mL caped sample vial, and then 5 mL of 1% internal standard solution (equivalent to 50 mg) was added. After mixing, 0.1 μL of the sample solution was injected directly into a GC with syringe. Ethanol content was calculated according to the Equation 2: $\text{Ethanol (mg/mL)} = (A_S/A_{IS}) \times (W_{IS}/RRF) \times 1/V$; in which, V = sample volume (mL).

(2) Dichromate oxidation method[1, 4]

Beverage sample solution (1~5 mL) was steam distillated to obtain alcoholic eluate (> 50 mL), and then oxidized with acidified dichromate. The excessive potassium dichromate was then titrated with ferric oxide. The ethanol content in beverage sample could be obtained by calculating the volume difference of potassium dichromate consumption between sample solution and control solution.

(3) Distillation-hydrometric method[1, 4]

Alcoholic volatile compounds in beverage samples were separated by distillation, and the gravity of the distillate was measured by hydrometer. The ethanol content was

Journal of Food and Drug Analysis, Vol. 11, No. 2, 2003

then converted.

(IV) *The limit of quantitation (LOQ) of ethanol by GC-FID*

Ethanol standard solution (10 mg/mL) was initially diluted with distilled water to make concentrations of 50, 25, 10, 5, 2, ,1, 0.5, 0.1, and 0.05 μg/mL, and then the internal standard solution was added to each solution. Solution was injected directly into a GC, which was equipped with an FID detector. The FID signal was set at range = 1, and attenuation = 1. The coefficient of variation (CV, %) of ethanol recovery was set at 15%. This was the LOQ of ethanol[28, 31]. Each analysis was carried out in triplicate.

(V) *Recovery*

5 and 10 mL of 1% (w/v) ethanol standard solution (equivalent to 50 mg, and 100 mg, respectively) was added into 0.5 mL of red wine and whisky (in 20-mL vial), respectively; and then 5 mL of 1% (w/v) internal standard solution was added. After gentle mixing, 0.1 μL of sample solution was injected into a GC with syringe for the determination of the ethanol content. Each analysis was carried out in triplicate. Blank sample was analyzed at the same time.

(VI) *Validation of the analysis method*

Ethanol standard solution (500 mg/mL) was diluted with distilled water to make concentrations of in 500, 250, 100, 50, 20, and 10 mg/mL. Each solution (0.5 mL) was dispensed into a 20-mL caped vial. After gentle mixing with 5 mL of 1% (w/v) internal standard solution (equivalent to 50 mg), 0.1 μL of the mixture solution was injected into a GC with syringe. Each concentration of ethanol standard solution was measured in triplicate in 1 day (intraday) or in 3 successive days (interday). Standard deviation (SD) and coefficient of variation (CV, %) were measured to evaluate precision of the method. The relative error of the mean (REM) was measured to evaluate accuracy of the method. REM was calculated according to the equation: REM (%) = [(measured value - true value) ÷ (true value) × 100%].

(VII) *GC conditions*

This study used Trace GC 2000 (TermoQuest, Milan, Italy), which was equipped with computer integrator software (Chrom-Card version 1.06 for Trace GC, TermoQuest, Milan, Italy) and an FID detector. The flow rates of H_2 and air were set at 30 and 300 mL/min, respectively. The temperature of the FID detector and the injection port was set at 285°C, and 225°C, respectively. Nitrogen (N_2) in the flow rate of 2 mL/min was used as the carrier gas. The CP-Wax 58 CB separation column (30 m × 0.53 mm, Chrompack, Netherlands) was used. Oven temperature was set initially at 45°C for 2 min, and then increased to the final temperature of 245°C in 1 min at the rate of 45°C/min. Injection volume was limited to 0.1 μL. Splittless injection mode was selected.

RESULTS AND DISCUSSION

I. *Analysis of GC Conditions*

During the selection of GC column, we had compared the polar and mid-polar megapore capillary columns in the determination of ethanol contents in alcoholic beverages. Because there was no sample pretreatment procedures required, and the sample could be injected directly into a GC for analysis, polar CP-Wax 58 CB column was the most appropriate column for the determination of ethanol contents in alcoholic beverages. When applying the GC conditions described in Materials and Methods, the retention time of ethanol standard solution was 2.73 min (Figure 1A, 1B, and 1C). The resolution of megapore capillary column (Rs = 5.8) was better than the packed GLC method (Rs = 1.4~1.9), as described in AOAC[4] and CNS[15]. The GC peaks of the other minute components in alcoholic beverages could be separated effectively with megapore capillary GC (data not shown).

In the selection of internal standard, distilled spirits (e.g. whisky) and non-distilled spirits (e.g. red wine) were

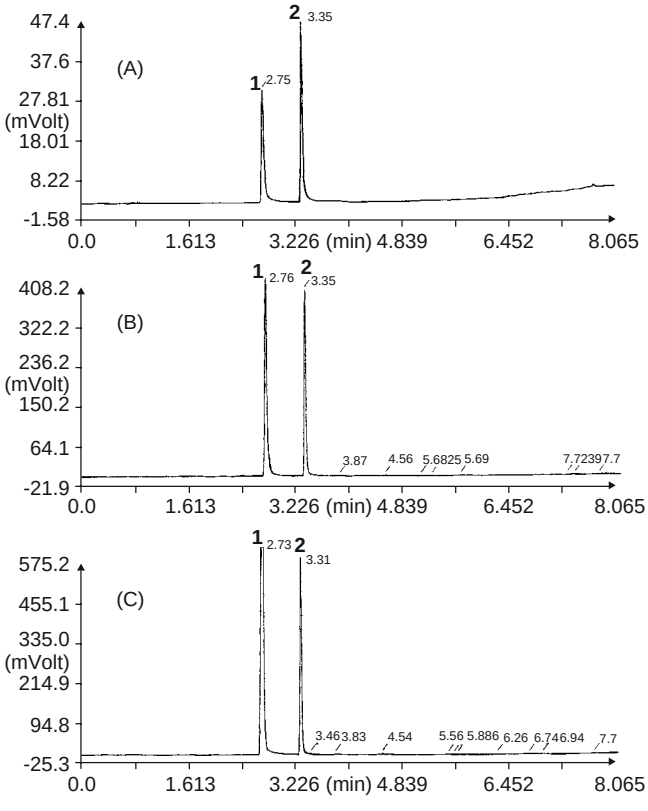

Figure 1. Gas chromatograms of (A) ethanol and acetonitrile authentic compound; (B) ethanol in non-distilled spirit (red wine) and (C) ethanol in distilled spirit (whisky) by splittless injection method. Peaks: 1 = ethanol, 2 = acetonitrile (IS).

added with minute quantity of 1-propanol, 2-propanol, acetonitrile, acetone, 1-butanol, and t-butanol. The ethanol contents were then analyzed according to the above-mentioned method. The results had shown that retention times of the 6 standard solutions were 4.43, 4.37, 3.32, 4.06, 5.96, and 5.72 min, respectively (data not showed). When GC chromatograms of distilled spirits were compared with non-distilled spirits, no overlapping of acetonitrile GC peaks was observed. Meanwhile, the GC peaks of acetonitrile and ethanol were closer to each other than the other compounds. Acetonitrile was then selected as the internal standard. Due to the possibility of its existence in some sort of alcoholic beverages[1,12], 1-propanol, acetone, t-butanol, or 1-butanol, which acted as the internal standards GLC methods (AOAC[4], CNS[15], and other researches[12, 16]), are not appropriate as the internal standard for the determination of ethanol contents in alcoholic beverages. Acetonitrile is the most appropriate internal standard for the determination of ethanol contents in alcoholic beverages, because no acetonitrile could possibly exist in alcoholic beverages.

For selection of the GC conditions, we initially selected low temperature, 45°C for 2 min, and then increased rapidly (at the rate of 45°C/min) to 245°C in 6 min. Ethanol and acetonitrile were eluted at 80°C and 100°C, respectively. Through this process, sample components will be eluted very rapidly and it takes 7-8 min to complete a sample analysis.

II. *Relative Response Factor of Ethanol to Iinternal Standard*

In this study, we selected acetonitrile as the internal standard for quantitative determination of ethanol contents in alcoholic beverages. In order to obtain an accurate quantification, the RRF value of ethanol against acetonitrile needed to be specified, then the ethanol contents could be calculated according to Equation 2. When the AUC ratio of ethanol to acetonitrile (Y-axis) was plotted against the concentration ratio of ethanol to acetonitrile (X-axis), a linear regression equation (Y = 0.952 X) was generated ($R^2 \geqq 0.999$). The slope of the linear regression line, 0.952, is the RRF of ethanol to the internal standard. The linearity of the adjusted regression line is in the range of 0-500 mg/mL.

III. *The Effect of Internal Standard Volume to the Accuracy of Ethanol Content Quantification for Various Alcoholic Beverages*

Ethanol contents in commercial alcoholic beverages vary a lot, even for the same sort of beverages, e.g. 3~6% (w/v) for beers, 7~21% (w/v) for fermented wines, and 20~50% (w/v) for distilled spirits.[1] Therefore, different pretreatment procedures are required when using the current official packed GLC methods for the determination of ethanol contents in various alcoholic beverages. In this study, a rapid capillary GC method, which requires smaller sample amount and simple pretreatment procedures, is developed and can be adapted in different sort of alcoholic beverages with ethanol contents between 0~500 mg/mL.

Ethanol standard solutions in the concentrations of 5, 10, 20, 30, 40, and 50% (w/v) were prepared, and then 0.5 mL of each solution was dispensed into a 20-mL caped vial. After adding and gentle mixing with 0.5, 1.0, 2.0, 2.5, 3.0, 4.0, 5.0, 6.0, 7.0, and 8.0 mL of 1% (w/v) internal standard solutions, respectively 0.1 μL of each solution was then injected directly into a GC for analysis. In order to achieve better accuracy, samples with high contents of ethanol needed larger amount of 1% (w/v) internal standard solution (Figure 2). For different beverage samples with ethanol contents of 5, 10, 20, 30, 40, and 50% (w/v), the ratios of 1% (w/v) internal standard solution required were 2, 4, 6, 8, 10, and 12, respectively. In order to obtain results with high consistency, 10x amount of the internal standard solution was added when ethanol contents in alcoholic beverages were determined in this study.

In conclusion, we have developed a method which requires only 0.5 mL, even 0.1 mL (data not shown), of beverage sample amount mixed with adequate amount (10x) of 1% internal standard solution. This sample solution is suitable for injecting directly into a GC for determination of ethanol content. No pretreatment procedure is required. This study method, is more simplified than official methods AOAC[4] and CNS[15], which require dilution or distillation as the pretreatment procedures. This study method is also easier in operation compared with methods developed by Collins *et al.*[2] (CE method) and by Antonelli[12] (packed GLC method), which require ultra-filtration (0.45 μm filter) and dilution as pre-

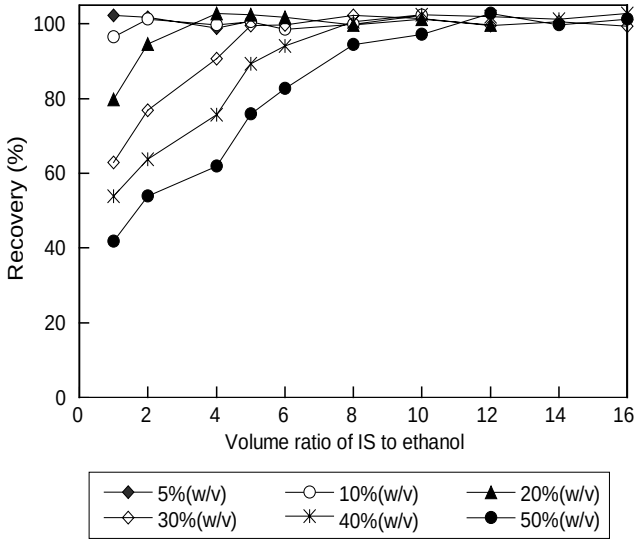

Figure 2. Effect of the volume ratio of 1% internal standard (IS) solution to various concentration of standard ethanol solution[1] on the quantitative determination[2] of ethanol content.

(1) The volume ratio of IS solution to standard ethanol solution, v/v, 0.5 mL of standard ethanol solution was used in each analysis.

(2) Recovery(%) = (level of ethanol detected)/(ethanol level of the standard solution) $\times$100%.

Journal of Food and Drug Analysis, Vol. 11, No. 2, 2003

treatment procedures.

IV. *The Limit of Quantitation (LOQ) of Ethanol by GC-FID*

In this study, 1% (w/v) ethanol standard solution was successively diluted, and then mixed with adequate amount of internal standard. The mixture solutions were then injected directly into a GC, equipped with an FID detector. The FID signal was set at range = 1, and attenuation = 1. The coefficient of variation (CV, %) of ethanol recovery was set at 15%. This was the LOQ of ethanol.[28, 31] The results showed that the LOQ of ethanol was around 0.5 μg/mL (Table 1).

V. *Validation of the Analysis Method*

The recoveries of ethanol standard solutions, which spiked into a non-distilled spirit (red wine) and a distilled spirit (whisky), were found in Table 2. The results show that the recoveries of ethanol with 50 and 100 mg fortification level into 0.5 mL of red wine and whisky were in the range of 99~104%, and 99~101%, respectively, with coefficient of variable (CV) less than 3.4%. When 6 ethanol samples with known concentrations (10~500 mg/mL) were analyzed using the current method, the coefficient of variant (CV, %) for intraday (1 day) and interday (successive 3-day) analyses was 1.0~4.7%, and 1.5~3.6%, respectively. These results indicate that the precision of the study method is very high. In addition, the REM of intraday and interday analyses was -3.5~4.2% and -1.0~3.0%, respectively. It indicates that the study method possesses very good accuracy (Table 3). Using the study method, after beverage samples are mixed with adequate amount of internal standard solution, they are ready for injecting directly into a GC for the determination of ethanol contents. It takes 7~8 min to complete an analysis. The GC chromatograms were illustrated in Figure 1A, 1B, and 1C. This study method is one of the most simple, rapid, and accurate methods published for quantitative determination of ethanol contents in alcoholic beverages. It also can be proposed as the reference method for routine analysis.

Table 1. Limit of quantitation, LOQ, of ethanol using gas chromatography with FID detector by direct injection method

Ethanol content (mg/mL)[a]	Recovery (%)[b]	CV (%)[c]
50.0	105.4 ± 0 2.4	2.3
25.0	97.5 ± 0 4.4	4.5
10.0	98.4 ± 0 6.6	6.7
05.0	104.7 ± 0 9.2	8.8
02.0	106.7 ± 0 5.4	5.1
01.0	095.7 ± 0 8.9	9.3
00.5	105.3 ± 13.3	12.6
00.1	107.2 ± 22.3	20.8
00.05	138.4 ± 41.1	29.7

a: FID range = 1, Attenuation = 1.
b: Average of triplicate analyses.
c: Coefficient of variation (cv %).

VI. *Comparison of Study Method with Dichromate Oxidation Method and Distillation-Hydrometric Method*

The study method was compared with dichromate oxidation method (AOAC 969.12) and distillation-hydrometric method (AOAC 920.57) for the determination of ethanol contents in red wine, rice wine, and whisky (Table 4). No statistically significant difference between these 3 methods ($p < 0.05$, $n = 3$) was observed, which indicates

Table 2. Recoveries of the ethanol in spiked commercial alcoholic beverage by direct injection gas chromatographic method

Sample	Blank[a] (mg) (A)	Amount added (mg)(B)	Amount found[b] (mg) (C)	Recovery(%)[c]	CV(%)[d]
Red wine	90.5 ± 2.7	50	142.7 ± 4.0	104.0	2.8
		100	189.1 ± 3.9	98.6	2.1
Whisky	317.9 ± 11.6	50	367.3 ± 10.3	99.0	2.8
		100	418.5 ± 14.4	100.6	3.4

a: Ethanol in 0.5 mL alcoholic beverage.
b: Average of triplicate analyses.
c: Recovery(%) = (C–A) / B × 100%.
d: Coefficient of variation (cv %).

Table 3. Precision and accuracy of intraday and interday validation for concentration range from 10 to 500 mg/mL

Concentration (mg/mL)	Intraday (n = 3)[a]		Interday (n = 9)[b]	
	Precision Mean ± SD (CV %)	Accuracy REM(%)[c]	Precision Mean ± SD (CV %)	Accuracy REM(%)
10	9.9 ± 0.1 (1.0)	-1.0	10.2 ± 0.2 (2.0)	2.0
20	19.7 ± 0.4 (2.0)	-1.5	19.8 ± 0.3 (1.5)	-1.0
50	52.1 ± 1.9 (3.6)	4.2	50.7 ± 0.8 (1.6)	1.4
100	96.5 ± 3.1 (3.2)	-3.5	102.4 ± 2.7 (2.6)	2.4
250	259.3 ± 8.5 (3.3)	3.7	255.6 ± 7.1 (2.8)	2.2
500	487.8 ± 23.1 (4.7)	-2.4	515.2 ± 18.7 (3.6)	3.0

a: n = 3, Repeated injection three times on the same day.
b: n = 9, Repeated injection three times each day and a successive three-day.
c: REM = relative error of the mean.

Table 4. Comparison of AOAC methods and proposed method for the determining of ethanol content in alcoholic beverages.

Sample	Proposed method[a]		Dichromate oxidation method[b]		Distisllation hydrometric method[c]	
	Ethanol (mg/mL)	CV (%)[d]	Ethanol (mg/mL)	CV (%)[d]	Ethanol (mg/mL)	CV (%)[d]
Red wine	107.4 ± 2.9	2.7	110.6 ± 15.7	14.2	114.1 ± 13.8	12.1
Rice wine	158.2 ± 6.0	3.8	151.4 ± 16.8	11.1	153.2 ± 14.7	9.6
Whisky	326.3 ± 7.8	2.4	311.3 ± 37.7	12.1	307.6 ± 40.5	13.2

a: Direct injection GC method in this study.
b: AOAC 969.12 method.
c: AOAC 920.57 method.
d: Average of triplicate analyses, coefficient of variation (cv%).

Table 5. Ethanol levels determined in alcoholic beverages.

Sample	Ethanol Content	
Distilled spirit	mg/mL	%, v/v
Whisky 1	327.6 ± 11.8	40.3 ± 1.4
Whisky 2	317.8 ± 12.4	39.0 ± 1.5
Whisky 3	331.3 ± 13.2	40.7 ± 1.6
White liquor 1	328.9 ± 09.5	40.4 ± 1.2
White liquor 2	413.6 ± 19.7	50.8 ± 1.8
Kaoliang1	327.5 ± 12.4	40.2 ± 1.5
Kaoliang2	415.7 ± 17.6	51.1 ± 1.7
XO	313.0 ± 13.6	38.5 ± 1.7
VSOP	289.7 ± 08.2	35.6 ± 1.0
Rice wine	165.2 ± 04.9	20.3 ± 0.6
Mizhiu Tou	324.3 ± 10.3	39.8 ± 1.3
Medecine wine	197.7 ±05.3	24.3 ± 0.7
Non-distilled spirit		
Millet wine1	77.2 ± 02.9	9.5 ± 0.4
Millet wine2	81.3 ± 01.4	9.9 ± 0.5
Red wine 1	90.1 ± 03.7	11.1 ± 0.5
Red wine 2	106.5 ± 04.5	13.1 ± 0.5
Red rose 1	128.4 ± 05.5	15.8 ± 0.7
Red rose 2	117.1 ± 03.7	14.4 ± 0.5
Fruit wine1	91.2 ± 02.6	11.2 ± 0.3
Fruit wine2	112.5 ± 03.4	13.8 ± 0.5
White wine 1	100.4 ± 03.1	12.3 ± 0.4
White wine 2	103.9 ± 03.7	12.8 ± 0.5
Shao-Hsing wine 1	141.2 ± 04.9	17.3 ± 0.6
Shao-Hsing wine 2	138.6 ± 05.5	17.0 ± 0.7
Beer 1	28.2 ± 00.8	3.5 ± 0.1
Beer 2	29.8 ± 00.6	3.7 ± 0.1

a: Average of triplicate analyses.

the study method we proposed obtains very high accuracy. The ethanol contents determined by the study method were: 107.4 ± 2.9 mg/mL in red wine, 158.2 ± 6.0 mg/mL in rice wine, and 326.3 ± 7.8 mg/mL in whisky (CV = 2.4~3.8%). The ethanol contents determined by dichromate oxidation method were: 110.6 ± 14.2 mg/mL in red wine, 151.4 ± 16.8 mg/mL in rice wine, and 311.3 ± 37.7 mg/mL in whisky (CV = 11.1~14.2%). The ethanol contents determined by distillation-hydrometric method were: 114.1 ± 13.8 mg/mL in red wine, 153.2 ± 14.7 mg/mL in rice wine, and 307.6 ± 40.5 mg/mL in whisky (CV = 9.6~13.2%). These results also indicate that the study method has better precision than the AOAC methods. Both AOAC methods require dilution and distillation as the pretreatment procedures. High deviation may be found if the connection tubes are not tightly secured, condensation efficiency is low, or the sample contains high contents of volatile compounds (e.g. acetic acid or sulfur dioxide) in distillation process[1]. Also, highly toxic reagents used in dichromate oxidation method make the operation and waste disposition troublesome and dangerous.

In the application point of view, the simple, rapid, and direct-injected capillary megapore column GC method developed in this study has shown more advantages and values compared with the AOAC[4] and CNS official methods[15]. Currently, different beverage samples require unique sample preparation procedures when the official AOAC and CNS packed GLC methods are applied, and distillation or dilution is required as the pretreatment procedure. Since the resolution is low for packed GLC methods[12], the accuracy for quantitative analysis and reproducibility of ethanol contents will be interfered for those beverages with high and complicated contents of volatile compounds (e.g. fruit and medicine wines)[21]. In addition, packed GLC column has shorter shelf life and lower durability. Besides, currently adapted capillary GC methods require time-consuming sample pretreatment procedures, such as solid-phase extraction[17], headspace balancing[18], solvent extraction[19], and distillation[20-21]. The study method we developed is a simple and rapid method, without any sample pretreatment procedure required. After beverage samples mixed with adequate amount of the internal standard solution, the samples are ready to be injected into a GC for determination of ethanol contents.

VII. *The Ethanol Contents of Commercial Alcoholic Beverages*

The ethanol contents of commercial alcoholic beverages are usually labeled in volume percent (%, v/v). Sometimes, it is convenient to be labeled in weight-volume percent (%, w/v). In this study, the ethanol standard solutions, 10%, 20%, 30%, and 40%, were prepared both in weight-volume percent (%, w/v), and in volume percent (%, v/v). After beverage sample were mixed with adequate amount of internal standard and injected into a GC for the determination of ethanol content, the relative response factor (RRF) was then calculated according to the above mentioned equation. The linear regression line of ethanol weight-volume percent (%, w/v, Y-axis) against ethanol volume percent (%, v/v, X-axis) was: $Y = 0.814\ X$, with $R^2 = 0.999$ (Figure 5).

Journal of Food and Drug Analysis, Vol. 11, No. 2, 2003

The ethanol contents of the 26 commercial alcoholic beverages (12 distilled spirits and 14 non-distilled spirits) were analyzed with the study method. As shown in Table 5, the ethanol contents of 12 distilled spirits were: 317.8~331.3 mg/mL (39.0~40.7%, v/v) in whisky, 328.9~413.6 mg/mL (40.4~50.8%, v/v) in white liquor, 327.5~415.7 mg/mL (40.2~51.1%, v/v) in kaoliang wine, 313.0 mg/mL (38.5%, v/v) in XO, 289.7 mg/mL (35.6%, v/v) in VSOP, 165.2 mg/mL (20.3%, v/v) in rice wine, 324.3 mg/mL (39.8%, v/v) in Mizhiu Tou, and 197.7 mg/mL (24.3%, v/v) in medicine wine. The ethanol contents of the 14 non-distilled spirits were: 77.2~81.3 mg/mL (9.5~9.9%, v/v) in millet wine, 90.1~128.4 mg/mL (11.7~15.8%, v/v) in grape wine, 91.2~112.5 mg/mL (11.2~13.8%, v/v) in fruit wine, 100.4~103.9 mg/mL (12.3~12.8%, v/v) in white wine, 138.6~141.2 mg/mL (17.0~17.3%, v/v) in Shaohsing wine, and 28.2~29.8 mg/mL (3.5~3.7%, v/v) in beer. The ethanol contents of these alcoholic beverages were consistent with their labels, without any fraud found.

CONCLUSIONS

In this study, it takes only 7~8 min to complete a sample analysis for the determination of ethanol content in a beverage sample. A sample solution (0.5 mL) is mixed with adequate amount (5 mL) of 1% (w/v) internal standard solution (acetonitrile, equivalent to 50 mg), and injected into a capillary GC. The study method we developed can be applied to alcoholic beverages with different alcoholic contents, and with the advantages of simple sample pretreatment procedures, rapidity and accuracy, and maybe a routine analysis method in substitution of current AOAC and CNS methods.

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