

Simultaneous Gas Chromatographic Quantitation Of Ethanol And Methanol From Beer

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Abstract: Beer is the most widely consumed alcoholic beverage in South Korea. The ethanol content on the label of alcoholic beverages must be within 0.5% of the actual level and the methanol content limit in beer is 500 mg/L (0.05%) in Korea. Therefore, regular testing of ethanol and methanol is a legal requirement. In this research, we devised a GC-FID analytic method for determining ethanol and methanol levels in beer, based on the use of a DB-624 capillary column combined to the direct aliquot injection. C-18 Cartridge purification proved inappropriate for the analysis of ethanol and the use of ethyl acetate as an internal standard was found to overestimate methanol content. The devised method was successfully applied to thirteen kinds of domestic and imported beers in the market. The labelled ethanol percentages in beer samples were within 0.5% of measured levels, and methanol levels in all beer samples were under 500 mg/L ($\mu\text{g/mL}$). Therefore, all beer samples analyzed met legal requirements regarding ethanol and methanol levels.

Index Terms: Beer, C-18 cartridge, Ethanol, Methanol, Simultaneous analysis, Gas Chromatography, Quantitation.

1 INTRODUCTION

Beer is the most popular alcoholic beverage in South Korea. The consumption of beer in Korea took off in the eighties in line with increases in disposable income, and soon beer established a dominant position in the alcoholic beverage market. The history of beer in Korea started with an influx of Japanese residents triggered by the Japan-Korea Treaty of 1876. Along with the enactment of the State Tax Ordinance on taxed alcoholic beverages by Japan in Korea, beer consumption began to increase markedly in 1905 when the Girin beer agency (a Japanese concern) opened in Seoul. In 1933, Japan established Korea's first beer company, the Chosun Beer company, and this was followed by the establishment of the Sohwa Girin Beer company in December of the same year. These two major beer factories transformed to Chosun Beer and Dongyang Beer in 1952 after a change from governmental to private ownership after Korean independence in 1945 [1]. Another massive change in Korean beer history occurred more recently due to the emergence of small breweries. The craft beer phenomenon began in the early 2010s in Korea and was engendered by revision of the Liquor Tax Law in 2014, which enabled small breweries to produce and distribute beers. In 2017, the number of domestic beer manufacturing licenses granted had exploded to around 130 and overall sales stood at ~40 billion Korean won [2]. In addition to the rapid growth of domestic craft beer market, imported beer brands have made inroads into the domestic beer market, and in 2018 beer imports stood at KRW 37.2 billion, which represented a 18 % increase from 2017 [3]. Furthermore, market share of imported beer in the total Korean beer market (KRW 4.2 trillion) increased from 4.9% in 2013 to 16.7% in 2017. On the other hand, domestic craft beer market share only accounted for 40 billion Korean Won in 2017, that is, at less than 1% of the total market, which is attributed to a the high liquor tax (72%), imposed to the higher costs of small-scale brewing [3], [4].

Accordingly, liquor taxes have largely structured the Korean beer market. The liquor tax on beer has fallen from 150% in 1996 to 72% today, but is still one the most taxed alcoholic drinks. According to Korean Food safety standards for beer as detailed in the Korea Food Code, alcohol (ethanol) content should be $\leq 25\%$ (v/v), and the ethanol content stated on the container label must be within 0.5% of the actual level [5]. Thus, if the labelled ethanol content of a beer is 5%, legally the real ethanol must lie between 4.5% to 5.5%. Of the various alcohols present in beer, methanol is the simplest and contains only one carbon atom. However, methanol is toxic and has been reported to cause hangovers due to its conversion to formaldehyde by alcohol dehydrogenase (ADH) in liver [6]. Methanol poisoning, which typically manifests as a visual disorder, can occur when alcoholic drinks are improperly fermented and distilled. Nevertheless, methanol is present at low concentrations in various plant-based products and processed food materials, such as fruit, aspartame, and dimethyl dicarbonate (DMDC). Pectin consists of a large number of methoxy polygalacturonic acids and is believed to be the source of methanol in plant-based products. During fermentation, enzymatic hydrolysis of these methoxy esters generates free methanol and this can be concentrated by subsequent distillation process. According to published information on alcoholic fermentation congeners detected in various alcoholic beverages, methanol levels are as follows; beer (1-27 mg/L), wine (8-151 mg/L), fortified wine (125-329 mg/L), brandy (176-4766 mg/L), whiskey (6-328 mg/L), rum (6-131 mg/L), and vodka (0-170 mg/L) [7]. In some countries, standards have been set for maximum methanol concentrations in beverages, for example, Brazil (0.5%), EU (0.2-1.5%), Czech Republic (1.2%), South Korea (0.015-0.1%), Thailand (0.024%), Australia/New Zealand (0.8%), USA (0.1%), Vietnam (0.3%) and Nigeria (0.0005%) [8], [9]. However, in a recent study conducted in Jecheon, South Korea, methanol exposure due to the consumption of alcoholic beverages was not at a hazardous level. In this study, no beer sample was found to contain more methanol than the limit of quantification (LOQ; 5 mg/L) [10]. Nevertheless, in South Korea, the regulated limit for methanol in beer is 500 mg/L (0.05%) [5], [9], and thus, regular analysis of methanol is obligatory for beer produced in South Korea. The official methods used to analyze ethanol and methanol in alcoholic beverages are detailed in the "Alcohol analysis regulations" issued by the Korean National Tax Service (NTS) [11]. Actually, the NTS details four and two official methods for the analysis of ethanol

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and methanol in beer, respectively. For ethanol, the alcohol hydrometer method and vibration type density meter method are used for general measurements, whereas an oxidation-based method and a gas chromatography (GC) based method are recommended for beer samples with ethanol contents of < 2%. For methanol, two methods are detailed, that is, the fuchsin-sulfurous acid method and a GC method. However, these two methods are only detailed in the Ministry of Food and Drug Safety (MFDS) official analysis method for makgeolli (rice wine) [12] and fermented rectified ethanol [13]. Furthermore, only GC-based methods are suitable for the simultaneous analysis of ethanol and methanol. GC was developed by James and Martin in 1952, and is used to analyze volatile chemicals [14]. Beer contains literally hundreds of volatiles, such as alcohols, short-chain aldehydes, and many other flavoring components, and GC has sufficient resolution capacity to separate these compounds. Ethanol and methanol are thermally stable volatiles with low boiling points, and thus, are amenable to GC analysis. Traditionally, packed column GC has been used for methanol analysis, and because of the highly polar characters of ethanol and methanol, polar stationary phase materials are used as adsorbents [15]. Even after capillary GC became dominant, polar phase coated capillary columns continued to be used for alcohol analysis. In the present study, a simple quantitative method with minimal pretreatment was developed to measure ethanol and methanol concentrations. Considering beers are rich in volatile flavoring compounds, we used a DB-624 capillary column, which provided sufficient separation of large ethanol and small methanol peaks from other volatiles. The devised method could be adopted as a standard means of determining beer ethanol and methanol contents with minimum effort and expense.

2 MATERIAL AND METHODS

2.1 Materials and reagents

Thirteen tested beers are listed in Table 1. The canned beers of B-1 ~ B-10 were purchased in a local market in Jecheon, Chungbuk province, South Korea. The bottled craft beers of B-11 ~ B-13 were acquired in "Bank Creek Brewery" located in Bongyang-up, Jecheon. All samples were kept in refrigerator at 4 °C before analysis. Beer samples were kept at room temperature just before sample preparation. Standard ethanol (≥99.8%), methanol (≥99.9%), acetonitrile (HPLC grade), ethyl acetate (HPLC grade), 1-butanol (≥99.7%), water (HPLC grade) and all other solvents & reagents were acquired from Sigma-Aldrich (St. Louis, MO, USA). For preparing all stock and standard mixture samples, HPLC grade water and 100 mL size volumetric flask were used.

Table 1: Tested beers in South Korean market

Company Name	Beer brand	ID
Oriental Brewery Co., S. Korea	Max	B-01
	Cass	B-02
	OB Premier	B-03
	Hoegarden	B-04
Lotte Chilsung Beverage Co. Ltd., S. Korea	Cloud	B-05
Hite Jinro Co. Ltd. Heit Jinro Co. Ltd., S. Korea	Black Beer Stout	B-06

Paulaner Brewery, Germany	Hefe Weissbier	B-07
Heineken International, The Netherlands	Heineken	B-08
Darguner Brauerei, The Netherlands	L7 Helles Weigen	B-09
Schofferhofer, Germany	Schöffhofer Grapefruit	B-10
Bank Creek Brewing. S. Korea	Soliti original blonde	B-11
	Soliti original brune	B-12
	Soliti Belgian pale ale	B-13

Table 2: Standard chemical composition in each standard mix for standard curve

No.	Ethanol (% v/v)	Methanol (mg/L)	Acetonitrile (% w/v)	Ethyl acetate or 1-Butanol (mg/L)
1	0.5	25	5.0	100
2	1.0	100	5.0	100
3	2.0	200	5.0	100
4	5.0	500	5.0	100
5	8.0	800	5.0	100

Ethanol stock solution (50%, v/v) was prepared by adding water up to 100 mL after transferring 50 mL of ethanol in a volumetric flask. Methanol stock solution (5000 mg/L) was made by transferring 0.5 g of methanol to the volumetric flask followed by adding water up to 100 mL. Internal standard (IS) for ethanol, acetonitrile stock solution was prepared by transferring 50 g of acetonitrile followed by adding water up to 100 mL in a volumetric flask. And stepwise dilution was proceeded to prepare five standard mixture samples for standard curve as described in Table 2.

2.2 Standard curve and sample preparation

Standard curve was prepared by internal standard method. Standard curves for ethanol and methanol, acetonitrile and ethyl acetate or 1-butanol were used as IS, respectively. Each point of five ethanol concentration was divided by the concentration of IS, acetonitrile. The ethanol concentration ratios (0.1-1.6) were plotted in x-axis versus each ethanol peak area ratio (ethanol peak area / acetonitrile peak area) in y-axis. For methanol standard curve, methanol concentration ratios (0.25-8.0) also acquired by dividing each methanol concentration by ethyl acetate or 1-butanol concentration. In y-axis, each methanol peak area ratio, by ethyl acetate peak area, were plotted. All beer samples were filtered by Whatman qualitative filter paper, Grade 597 circles (185 mm diameter, Sigma-Aldrich) to eliminate any foreign material and CO₂. This filtering paper is described as good to be used for fat analysis in food testing and elimination of CO₂ & turbidity from beverages such as beers [17]. A typical hydrophobic sorbent C-18 cartridge column (Strata C18-E, 55 µm, 70 Å, 1 g/ 6 mL tube, Phenomenex, Torrance, CA, USA) filtration was tried to remove any disturbing materials from beer sample. After taking out beer samples from refrigerator, they opened and capped, followed by letting them standing in room table until their temperature is reached to ambient temperature (25 °C). After taking out beer samples from refrigerator, they opened and filtered, followed by letting them standing in room temperature (25 °C) until their temperature is reached it. The room temperature achieved samples were purified by PTFE syringe

filter (0.45 μm , 13 mm, Advantec, Dublin, CA, USA) and transferred to 2 mL size screw cap autosampler vial (Agilent, Santa Clara, CA, USA) before GC analysis.

2.3 GC analysis

Agilent 7890 GC equipped with flame ionization detector (FID) and autosampler was used. At the initial stage of experiments, mobile phase nitrogen was used and it was replaced by helium in main experiments. All analysis was performed with DB-624 capillary column (60 m \times 0.25 mm I.D., 1.4 μm d_f , Agilent, Santa Clara, CA, USA). The nitrogen or helium mobile phase flow was set as 1 mL/min at constant flow mode. Temperatures of the split injector and FID were 200 and 240 $^{\circ}\text{C}$, respectively. Oven temperature was programmed from 40 $^{\circ}\text{C}$ (held 2 min) and ramped to 120 $^{\circ}\text{C}$ by 10 $^{\circ}\text{C}/\text{min}$. And it was rapidly ramped to 240 $^{\circ}\text{C}$ by 20 $^{\circ}\text{C}/\text{min}$ and held until no more peaks came out. The sample size was 1 μL and split ratio of injector was 30:1. The single tapered 4 mm ID glass liner (loosely filled with deactivated glass wool) was used (Agilent P/N 5062-3587, Mulgrave, VIC, Australia). All GC analyses were replicated three times.

2.4 Method validation

Method validation was performed with 5% ethanol solution containing 100 $\mu\text{g}/\text{mL}$ of methanol. Precision was estimated by 6 replicate analyses. Recoveries acquired by the percent ratio of the measured concentration by the added concentration for ethanol and methanol. For the estimation of C-18 cartridge column efficiency, any loss of target analyte was observed. Ethanol percent of the samples, C-18 cartridge column treated, and not-treated samples were compared. Limit of detection (LOD) of methanol was acquired by analysis of the methanol samples with gradual concentration reduction. Methanol concentration at the signal to noise value 3 of the methanol peak was designated as LOD. Limit of quantitation (LOQ) of methanol was estimated by multiplication 3 to LOD. LOD and LOQ of ethanol was not tested due to the too high target concentration range.

2.5 Statistical analysis

Mean, percent relative standard deviation (%RSD), and nonparametric t-test were calculated by "Sigma Plot (ver. 13., Systat Software Inc., San Jose, CA, USA)".

3 RESULTS AND DISCUSSION

3.1 Statistical analysis

Linearity of standard curve is expressed as the correlation coefficient (R^2) that was 0.999 for ethanol. The formula was $y = 1.0121x + 0.0028$. The precisions of 5% ethanol level were 0.9 and 1.5% for intra-day and inter-day, respectively. The recovery of ethanol (at the level of 5%) was observed as 100.5% without C-18 cartridge purification step.

Table 3: Ethanol analysis results in beer samples pretreated with/without C-18 SPE

Beer	Labelled EtOH %	With C-18 SPE		Without C-18 SPE	
		EtOH %	Recovery %	EtOH %	Recovery %
B-01	4.5	3.1	69.6%	4.4	98.8%
B-02	4.5	3.8	85.2%	4.5	99.2%

B-03	5.2	4.1	78.1%	5.1	98.8%
B-04	4.9	3.5	71.7%	4.8	98.8%
B-05	5	3.9	78.7%	5.0	99.2%
B-06	5	3.6	72.5%	5.0	99.7%
B-07	5	3.0	60.8%	4.9	97.4%
B-08	5	3.6	71.1%	4.9	98.8%
B-09	5.1	3.6	71.2%	5.0	98.1%
B-10	2.5	1.5	58.3%	2.4	95.8%
B-11	6.1	4.9	80.0%	6.3	103.8%
B-12	6.1	4.8	79.3%	6.2	102.0%
B-13	5	4.2	84.5%	5.5	109.2%
Mean recovery%		73.9 \pm 5.3%		100.0 \pm 1.7%	

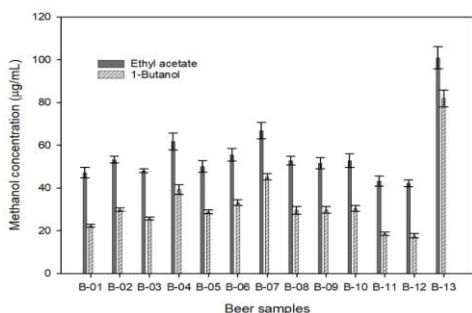
3.2 Ethanol loss due to C-18 cartridge column filtration

As a sample purification step, solid phase extraction (SPE) using a C-18 cartridge was investigated. To verify ethanol recovery, 5% ethanol solution was prepared and purified using a C-18 cartridge. Average ethanol recovery of the three 5% ethanol solutions was 75.0% with an RSD% of 2.2%, after C-18 cartridge purification, indicating some ethanol loss during SPE. This loss was verified by an average recovery of 100.2% (RSD%, 1.1%) was obtained without C-18 cartridge purification. This finding was confirmed by analyzing beer samples (Table 3), which returned mean ethanol recovery results of 73.9% and 100% with or without C-18 cartridge treatment. Recovery was calculated by comparing measured values to labelled ethanol percentages on each of the 13 beers analyzed. Theoretically, C-18 cartridge purification should not reduce ethanol recovery and this unexpected recovery shortfall was possibly due to a C-18 cartridge problem; the "C-18" is derived from the C-18 hydrocarbon chemically bonded to the adsorbent. However, some -OH sites of the adsorbent may not have been well covered by C-18 chain, and thus ethanol in samples might have interacted with uncovered polar site caused these poor recoveries. As a result of this observed recovery loss, subsequent analyses were performed without C-18 cartridge pretreatment.

3.3 Linearity, LOD, LOQ, precision and accuracy of methanol analysis

The correlation coefficient (R^2) was 0.999 for the methanol standard curve. Two different formulas were obtained when ethyl acetate or butanol were used as internal standards, that is, $y = 1.4401x - 0.5605$ and $y = 0.6661x - 0.0941$, respectively. The LOD and LOQ of methanol in 5% ethanol solution was 1.0 and 3.0 $\mu\text{g}/\text{mL}$, respectively. Intra-day and inter-day precision of 200 $\mu\text{g}/\text{mL}$ methanol in 5% ethanol solution using ethyl acetate as the IS were 3.8 and 5.4% of RSD%, respectively. For 1-butanol, those values were 2.9 and 4.9%, respectively. Methanol (at 200 $\mu\text{g}/\text{mL}$ in 5% ethanol solution) recoveries were 97.8 and 92.5% when ethyl acetate or 1-butanol, respectively, were used as ISs.

Fig. 1: Methanol concentration of the beer samples quantitated with internal standards, ethyl acetate or 1-butanol



3.4 Methanol analysis results of the two ISs

Methanol concentration results obtained when ethyl acetate or 1-butanol were used as ISs are summarized in Fig. 1. Results were statistically analyzed using "Wilcoxon's Rank Sum Test" (a typical nonparametric test) to investigate differences between the two data sets. In the event, methanol concentrations acquired using 1-butanol were significantly lower than those obtained using ethyl acetate ($p < 0.001$). However, the accuracies of the techniques could not be determined due to the lack of an authentic methanol in beer standard. Therefore, methanol recovery was investigated using a lab made sample of 5% ethanol containing 500 µg/mL of methanol. The results obtained showed an average recovery percent of 96.9% for 1-butanol and 130.7% for ethyl acetate. These results showed the use of ethyl acetate as the IS might lead to over-estimates of methanol concentration in beer.

3.5 The level of ethanol and methanol measured in the beer samples

The labelled ethanol percentages in beer samples were within 0.5% of measured levels, and that all beer samples contained < 500 mg/L of methanol. Accordingly, all beers tested met legal requirements for ethanol and methanol contents.

4 CONCLUSION

Direct GC-FID analysis using a DB-624 capillary column was used to determine the concentrations of ethanol and methanol in beer. SPE pretreatment using a C-18 cartridge caused ethanol recovery losses, and the use of ethyl acetate as the IS resulted in over-estimations of methanol content. Thus, 1-butanol was found to be the more appropriate IS for methanol analysis. All beer samples examined in the present study met legal requirements regarding ethanol contents stated on labels and maximum permitted methanol content.

5 CONFLICTS OF INTEREST

The author declare that there are no conflicts of interest regarding the publication of this article.

6 ACKNOWLEDGEMENT

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