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Halal Authentication Analysis / GC-2010 Plus

Quantitative Determination of Ethanol in Beverages and Oral Rinses by GC-FID Method

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❑ Introduction

Halal (حلال) is an Arabic word often used to describe foods and drinks that Muslims are permitted to consume, according to Qur'an, the holy book of Islam. In the recent years, there is a fueling demand for Halal diet due to the rise in Muslim population. The Halal food market is one of the fastest growing consumer segments with its value estimated at US$560 billion[1,2]. Yet, the requirements remain stringent. Companies seeking opportunities in the Halal food market have to comply with the religious requirements imposed by the various export destination countries, and only when the requirements are met do the Islamic statutory body award a Halal certification to endorse the product is suitable for Muslims' consumption.

One of the most prominent prohibitions by the Islamic laws is alcohol, specifically ethanol. Muslims are forbidden to ingest alcoholic content. In this study, we developed an easy and fast GC method to detect the presence and determine amount of ethanol in beverages and oral rinses. The thresholds of ethanol for Halal consumption used in this study were based on Singapore's Halal guidelines[3]. Various liquid samples were treated with minimal preparation and thereupon injected to the GC for analysis. The method was demonstrated to be sturdy, swift and simple for detection and determination of ethanol.

❑ Experimental

Instrumental and analytical conditions

A gas chromatograph GC-2010 Plus coupled with flame ionization detector FID-2010 (Shimadzu Corporation, Japan) was employed in this study. The detailed conditions are shown in Table 1.

Table 1: GC analytical conditions for ethanol analysis.

Item	Description
GC model	GC-2010 Plus with AOC-20i
Column	SH-Rxi-5Sil MS, 30m x 0.25mm x 0.25µm
Injection Condition	250°C, split mode, split ratio 50:1
Injection Volume	0.20µL
Carrier Gas	Helium, 99.9997% purity
Gas Flow Condition	Constant linear velocity mode, linear velocity 31.7cm/s, purge 3mL/min
Oven Temperature Programming	30°C (2min) → 40°C/min to 250°C (2.5min)
Detector	FID-2010
FID Temperature	250°C
Gas Flow Condition	Hydrogen flow 30mL/min Air flow 400mL/min Makeup gas flow (N2) 40mL/min

Samples and chemicals

GC-grade ethanol and isopropyl alcohol (IPA, used as internal standard) with purity higher than 99.5% were obtained from Kanto Chemical. Stock solutions used for making calibration curves were prepared using Milli-Q water as diluent to resemble the authenticity of beverage and oral rinse samples.

A calibration curve with seven calibration levels was developed. The calibration curve encompasses ethanol content from 10, 25, 50, 100, 250, 500 to 1000 µg/mL, spiked with 100 µg/mL IPA at each level.

The samples used in this study were purchased directly from local stores. Seven fermented products and seven non-fermented products were analysed. Internal standard was added to the samples to reach a final concentration of 100µg/mL, and then directly injected to GC for analysis. For samples with alcohol content higher than the calibration range, as well as highly viscous samples, a 10-times dilution was performed before the addition of internal standard and GC analysis. Further dilution was performed if necessary.

❑ Results and Discussion

Method Development

Injection of aqueous samples could be very damaging to GC capillary columns with non-bonded phases, partially bonded phases, or high-polarity phases. Therefore, the fully bonded SH-Rxi™ column was employed in this study (P/N: 221-75954-30). The Crossbond™ polymer stationary phase in this column is highly inert, even towards aqueous analyses.

Backflash is another concern for aqueous sample injection as water expands notably to more than 1000 times its original volume when vaporised. With the vaporised volume larger than the capacity of the glass insert, sample will burst back to the top of injector port and even to the carrier gas line, causing a range of problems from "ghost peaks" to poor repeatability[4]. Hence, it is advised to inject the smallest volume possible into the largest internal diameter glass insert available. In order to maintain stable injection volume as small as 0.2µL, injections are handled by a liquid syringe of 0.5µL capacity (P/N: 227-35002-01). Meanwhile, a high split ratio is utilized to further minimize any backflash contamination.

In high split ratio analysis, sample passes through the glass insert rapidly. Water, being a compound with high latent heat of vaporization, will not be able to vaporise instantaneously unless it is subjected to proper heat exchange in the injector chamber. As such, it is crucial to insert additional silica wool into the glass insert. Not only will this increase the vaporisation efficiency, it also helps to mix the vaporised sample with carrier gas evenly, producing good result repeatability.

A 10mg of silica wool was already supplied in the split/splitless dual purpose glass insert for general use (P/N: 221-75193). For an aqueous analysis, an additional 20mg of silica wool (P/N: APARES24324, 10g/bottle) is inserted manually using a wool pick-up jig (P/N: 221-37391-91). The tools are shown in Figure 1 and the difference in amount of wool is illustrated in Figure 2. It is worth noting that with a larger amount of wool, the tip of syringe will penetrate into the wool during injections. This ensures reproducible discharge and thorough heating of sample upon every injection[5].

Method Performance Evaluation

Linear calibration curve was obtained with correlation coefficient (R^2) of 0.999 across the range of 10µg/mL to 1000µg/mL, while each level consisted of 3 repeat data. The calibration range was derived based on Singapore's Halal guidelines, in which 0.1% (i.e. 1000µg/mL) is the maximum allowable industrial alcohol in final products (0.5% in additives)[3]. For expected ethanol content higher than 0.1%, samples should be diluted for accurate quantitation. The calibration curve is shown in Figure 3.

The repeatability of the method was evaluated using 10 standard runs at the lowest, middle and highest levels. The relative standard deviation (%RSD) at the low concentration (10µg/mL) were below 5%. The concentration %RSD of ethanol were found to be between 0.9% to 1.2% for higher concentrations at 100µg/mL and 1000µg/mL.

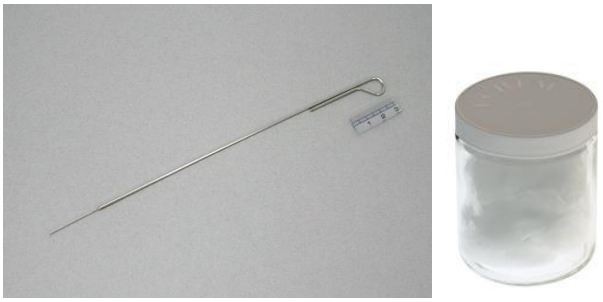

Figure 1: Wool pick-up jig (left) and silica wool in a bottle (right).

Figure 2: Positioning of additional silica wool in glass insert for aqueous analysis. The additional wool was packed more loosely than the originally supplied portion, as overly compact wool might create unnecessarily high pressure within the injector port during use.

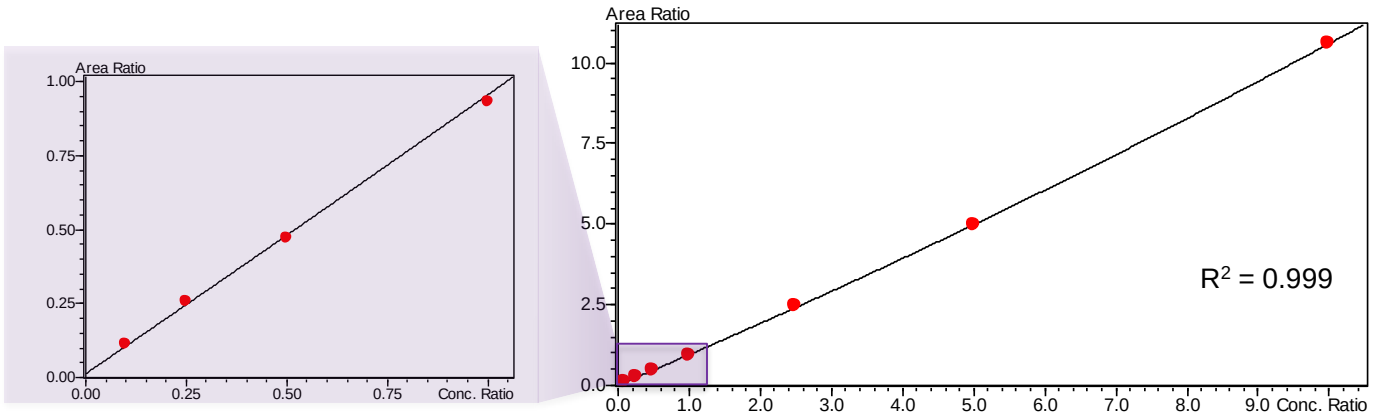

Figure 3: Zoomed portion of the lowest levels (left) and full calibration curve of ethanol (right) from 10 to 1000µg/mL.

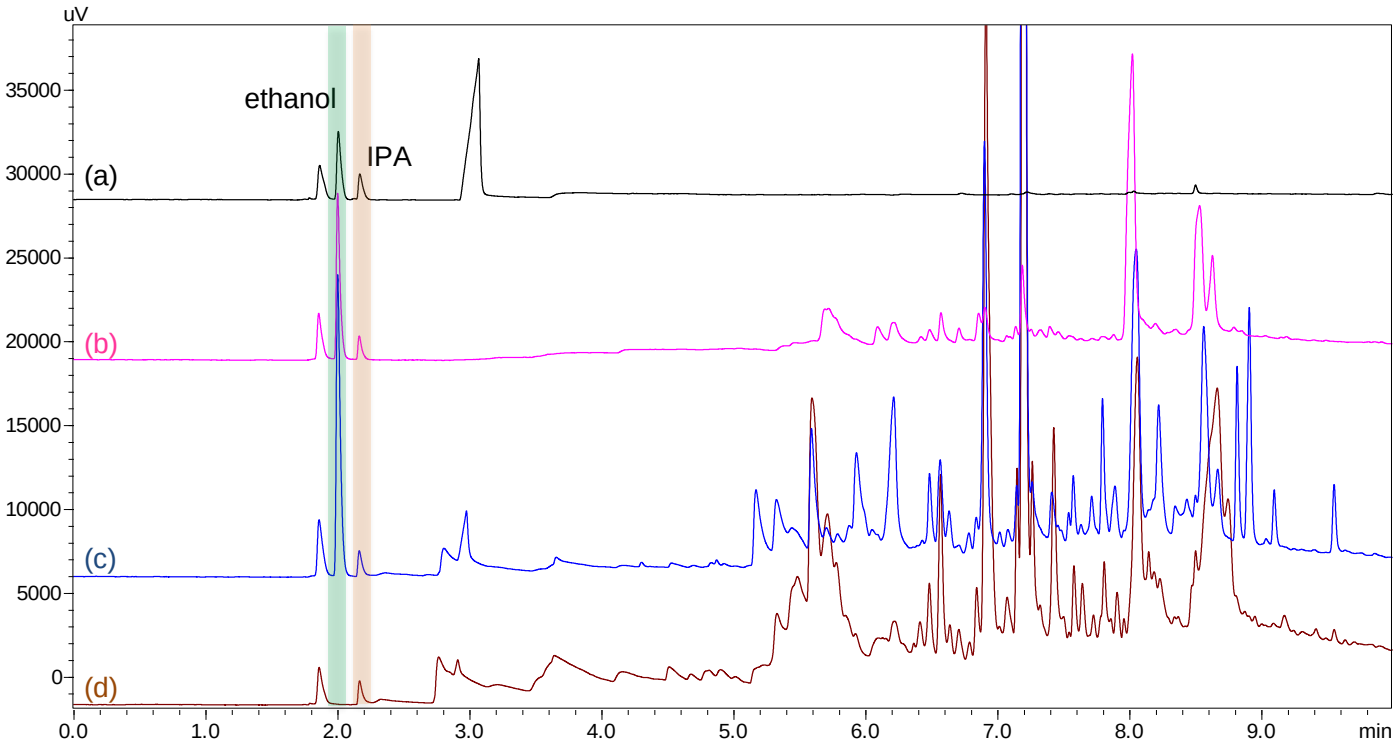

Figure 4: Chromatograms of select samples spiked with isopropyl alcohol as the internal standard: vinegar chilli (10x diluted) (a), light soy sauce (b), energy drink 2 (c) and sweetened carbonated drink (d).

Table 2: Mean concentrations (conc.) of ethanol detected in select samples with duplicate injection using the method developed.

Non-fermented Products			Fermented Products		
No.	Sample type	Mean conc. (µg/mL)	No.	Sample type	Mean conc. (µg/mL)
1	Carbonated drink	not detected	1	Soy sauce	not detected
2	Sweetened carbonated drink	not detected	2	Vinegar	16.3
3	Mouthwash 1	15.1	3	Fermented drink 1	39.3
4	Energy drink 1	17.4	4	Fermented drink 2	35.5
5	Non-alcoholic mouthwash	21.7	5	Vinegar chilli (10x dilution)	248*
6	Energy drink 2	1080	6	Light soy sauce	628
7	Mouthwash 2 (100x dilution)	1500†	7	Fruit vinegar	792

†concentration of ethanol after 100x dilution

*concentration of ethanol after 10x dilution

Table 3: Ruggedness test results of 100µg/mL ethanol spiked in sweetened carbonated drink injected over 100 repeated runs.

Runs	1-10	11-20	21-30	31-40	41-50	51-60	61-70	71-80	81-90	91-100	**1-100**
Area %RSD	1.15	0.996	3.24	2.69	2.31	2.28	1.13	2.10	1.26	1.86	**2.67**
Conc %RSD	0.806	1.23	0.958	1.51	0.766	1.37	0.833	0.958	1.30	1.22	**1.18**

Analysis of ethanol in actual samples

Fourteen samples were analysed using the established method. Seven of the samples contain non-fermented products while the other seven fermented. Select chromatograms are shown in Figure 4. The mean concentrations of duplicate sample runs are summarized in Table 2.

Fermented products are expected to contain a higher content of ethanol. There is however no specified guideline by Singapore on the maximum amount of naturally-produced ethanol. As such, these samples shall be subjected to the 0.1% ethanol limit in final products and 0.5% limit in additives, which is the guideline for industrial alcohol.

By applying the calibration curve ranging from 10µg/mL to 1000µg/mL, the amount of ethanol present in samples could be found out directly. For highly viscous samples and samples expected to contain high amount of ethanol, dilution had to be performed before analysis. This is illustrated by the mouthwash 2 (100x dilution) and vinegar chilli (10x dilution). The dilution factor was multiplied by the calibrated value to evaluate the actual amount of ethanol in these diluted samples. For instance in mouthwash 2, the amount of ethanol was around 1500 x 100 = 150000 µg/mL, i.e. 15%.

Amongst the non-fermented food products, energy drink 2 contained around 1080µg/mL of ethanol. That is more than the required amount of 0.1% in final products. Meanwhile, the analysis of vinegar chilli revealed its content of around 248 x 10 = 2480µg/mL, i.e. 0.25%. This also exceeds the amount stated in the Halal guideline for final products.

As noticed from the chromatograms (see Figure 4), the target components eluted within the first three minutes of the analysis. The actual run time, however, was developed to be 10-minute long, with a final holding time of 2.5 minutes at 250°C. This is crucial as most samples contained large amount of matrix, and the extended run time allows complete purging. Without purging, it is likely that the matrix will remain in the column, and contaminate the subsequent analyses in the form of "ghost peaks".

Method Ruggedness

As shown in Figure 4d, the sweetened carbonated drink contained a highly complicated sample matrix, high sugar content (deduced from the sweetened taste) but virtually no ethanol. It had since been selected as the sample matrix to test the ruggedness of the method developed.

100 µg/mL of ethanol and IPA were respectively spiked into the sweetened carbonated drink sample, then directly injected into the GC. This is to simulate the condition in the food industry where sample preparation is preferably minimal and large number of samples need to be analysed.

As illustrated in Table 3, the result is very consistent even up to 100 repeated injections, with both the peak area and concentration %RSD being less than 3%. It is recommended that after a certain number of analyses (for example 10), a control sample with known amount of ethanol in water is injected to rinse the column. Consequently, if the concentration of the control sample is within 70-130% of actual value, the calibration curve is considered valid for further use[6].

❑ Conclusions

A GC method for the analysis of ethanol in liquid samples was established using Shimadzu GC-2010 Plus with minimal sample preparation. The method performance with sensitivity, linearity, repeatability and ruggedness was evaluated. The results proved that the method is feasible and reliable for easy and fast determination of ethanol in beverages and oral rinses to ensure Halal food integrity.

❑ References

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