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Halal Authentication Analysis / GC-2014 & HS-10

Quantitative Determination of Ethanol in Liquid Condiments and Beverages using Headspace GC

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❑ Introduction

The Arabic word Halal (حلال) means 'permissible' or 'lawful'. It refers to any substance, object or action that is allowed to be used or taken part in accordance to the Islamic Law. In the food industry, industrial ethanol is widely used as a solvent for flavour extraction, as a disinfectant or a washing agent for machinery, or added as a flavouring or preservative. Ethanol may also be produced in the food product naturally by fermentation. Most Islamic statutory bodies in Southeast Asia allow the presence of small amounts of alcohol in food products. However, the maximum allowable alcohol limit differs between countries[1]. The headspace (HS) sampler coupled with gas chromatograph (GC) is employed to extract the volatile ethanol in traces for easy detection. The methods are developed based on minimal sample preparation and application on a wide variety of liquid samples, aimed for routine use in the food industry.

❑ Experimental

Instrumental and analytical conditions

A gas chromatograph GC-2014 coupled with flame ionization detector (FID) was connected to a headspace sampler HS-10 (Shimadzu Corporation, Japan) in this study. Detailed conditions are shown in Table 1.

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 simulate the authenticity of actual condiment and beverage samples.

In this study, 16 liquid samples labelled Halal were purchased directly from local stores and analysed. A wide range of samples were tested. These include beverages, dairy products, vinegars and sauces.

An internal standard method was established in this study, with eleven calibration levels from 1, 2.5, 5, 10, 25, 50, 100, 250, 500 to 1000µg/mL ethanol, spiked with 25µg/mL IPA at each level. Internal standard was added to each sample to reach a final concentration of 25µg/mL. Each vial containing 1mL of liquid was then sent for HS-GC analysis. For the accuracy of this study, each sample was prepared and analysed in triplicates.

Table 1: HS analytical conditions for ethanol headspace analysis

Headspace Sampler	**HS-10**
Oven Temperature	80°C
Sample Line Temperature	100°C
Transfer Line Temperature	110°C
Pressurize Gas Pressure	60kPa
Equilibration Time	15min
Pressurizing Time	2min
Pressure Equilibration Time	0.25min
Load Time	2min
Load Equilibration Time	0.1min

Table 2: GC analytical conditions for ethanol headspace analysis

Gas Chromatograph	**GC-2014**
Column	SH-Rxi-5Sil MS, 30m x 0.25mm x 0.25µm
Injection Condition	250°C, split mode, split ratio 50:1
Carrier Gas	Helium, 99.9997% purity
Gas Flow Condition	Constant linear velocity 17cm/s, purge flow 3mL/min
Oven Temperature Programming	30°C (4min) → 40°C/min to 250°C (2.5min)
Detector	**FID-2014**
FID Temperature	250°C
Gas Flow Condition	Hydrogen flow 30mL/min Air flow 400mL/min Makeup gas flow (nitrogen) 40mL/min

❑ Results and Discussion

Halal Guidelines of Ethanol

The Halal guidelines set by MUIS (Islamic Religious Council of Singapore) state that the content of industrial ethanol in food additives should not exceed 0.5%, while the content in the final food product should not exceed 0.1%. Limitations for naturally-produced ethanol is unspecified under MUIS[2]. It is thus assumed to follow that of industrial ethanol in this study.

A summary of religious requirements by the administrative bodies in some other Southeast Asian countries are compiled in Table 3. These include JAKIM (Department of Islamic Development Malaysia), MUI (Indonesian Ulama Council) and BIRC (Brunei Islamic Religious Council).

JAKIM states that the content of industrial ethanol in the final food product should not exceed 0.5%, while the content of naturally-produced ethanol should not exceed 1% in the final food product[3]. MUI specifies that industrial ethanol can be used in food processing, but the content in the final food product should be zero. The content of naturally-produced ethanol should not exceed 1%[4]. BIRC states that naturally-produced ethanol should not exceed 2% while industrial ethanol is completely not allowed[5].

Headspace Sampling Technique

Headspace sampling is an excellent technique to access volatile analytes of interest which can be efficiently partitioned from the sample matrix, be it solid or liquid, into the headspace gas volume. Higher boiling volatiles and semi-volatiles are less sensitive towards detection with this technique due to their low partition in the headspace. As such, it is typically applied on dirty samples and complex matrices for trace analyses of volatiles.

Water-based samples are also great for headspace sampling. While modern GC capillary columns are quite tolerant towards aqueous analyses, much precautions are needed to prevent backflash which leads to carryover and repeatability issues[6]. With the headspace technique, injection of water is greatly reduced and backflash can be circumvented.

Other ideal candidates include food sample matrices, which can be difficult to analyse directly or would otherwise require sample extraction or preparation. The food samples can be placed directly in a headspace vial with little or no preparation, resulting in time and cost effective analyses. The analysis of water-based food samples is illustrated in this study.

Table 3: Maximum allowable content of ethanol according to various Halal administrative bodies in Southeast Asia

Islamic Statutory Body	Maximum allowable industrial ethanol	Maximum allowable naturally-produced ethanol
MUIS[2] (Singapore)	0.5% in additives 0.1% in final product	Unspecified
JAKIM[3] (Malaysia)	0.5% in final product	1%
MUI[4] (Indonesia)	Can be used, but has to be 0% in final product	1%
BIRC[5] (Brunei)	prohibited	2%

Headspace Method Development

The HS-10 headspace sampler utilized in this work is highly cost efficient, and it is equipped with advanced features such as a mixing function and the ability to heat-ahead sample vials waiting for analysis. With the mixing function, each vial can be shaken during incubation, allowing the headspace contents within each sample to come to equilibrium sooner, ultimately reducing time and increasing analysis throughput.

The sampler thermostating oven temperature has a direct influence on the partition coefficient of sample components in gas phase. As water is the main component in most samples, the oven was set to 80°C to reduce the partitioning of water and its tendency to overshadow the target analytes. The sample line and transfer line temperatures have been set higher to prevent any carryover in the process of sample transfer between the HS and GC instruments.

Equilibration time is crucial to ensure repeatable results. While a longer equilibration time can ensure this, it is neither practical nor cost effective. An equilibration time study (Figure 1) reveals that 15min is the shortest time needed for complete equilibration between the liquid and headspace partitioning. This HS sampler equilibration time of 15min is almost as long as the time the GC completes one analysis (12min runtime with 3.4min cooldown). Using the heat-ahead function, up to 6 vials can be incubated at the same time and be ready for injection before the previous analysis cycle has ended. This greatly shortens the total analysis time required.

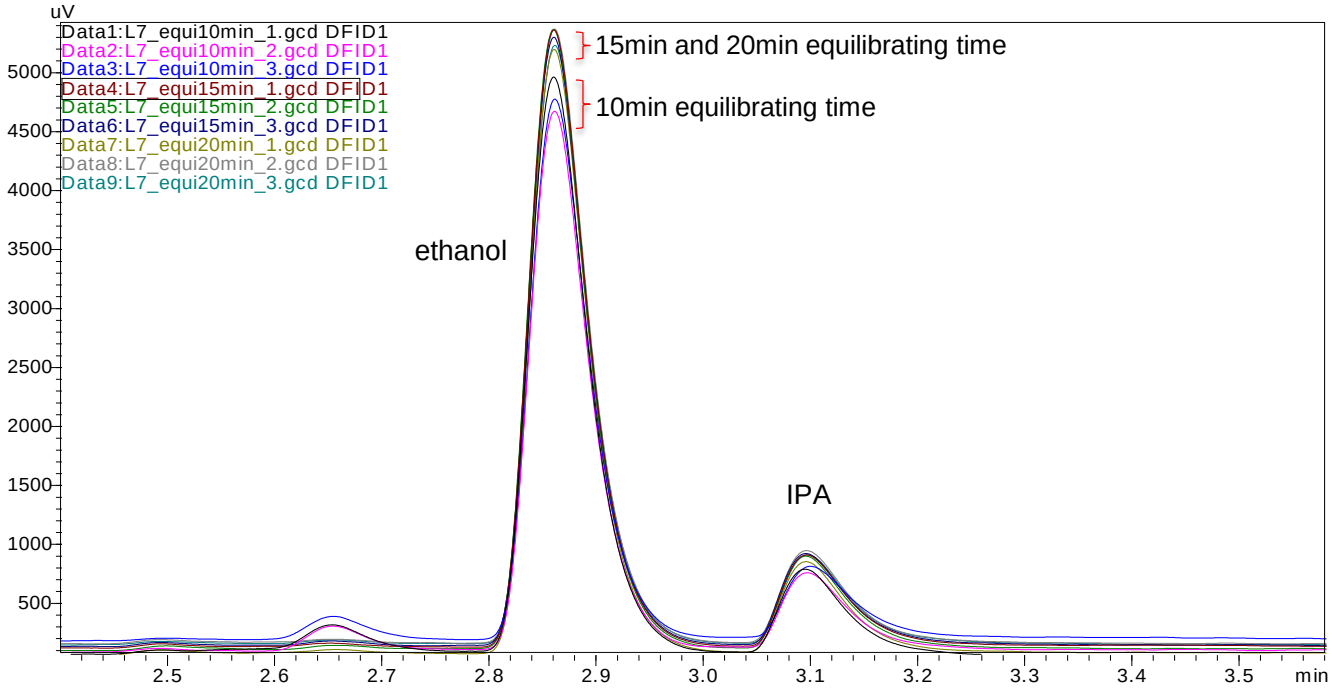

Figure 1: Equilibrating time study shows that 15min and 20min give consistent peak area results, while 10min is still short from complete equilibration.

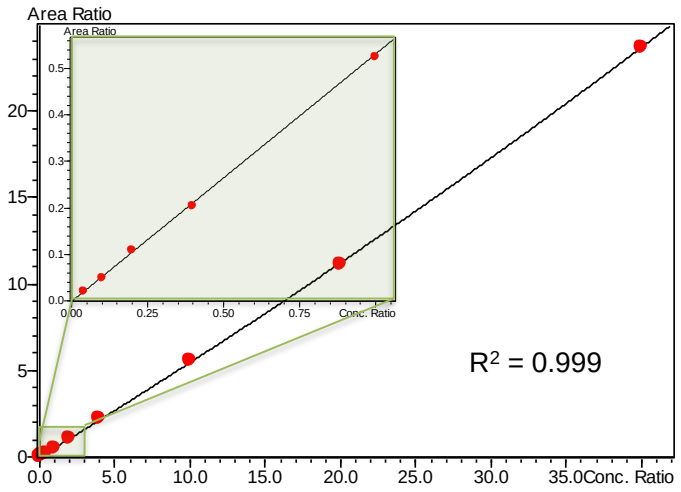

Figure 2: Zoomed portion of the lowest levels (insert) and full internal standard calibration curve of ethanol from 1µg/mL to 1000µg/mL for liquid samples.

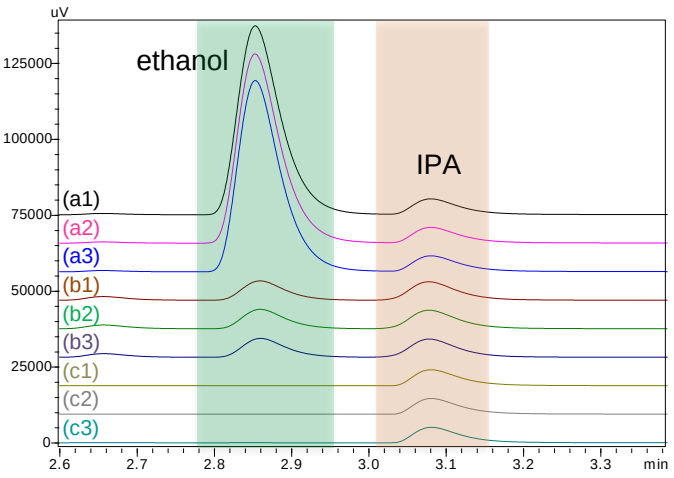

Figure 3: Triplicate chromatograms of select liquid samples: energy drink (a), soy bean milk (b) and ginger ale (c).

Table 4: Mean concentration of ethanol detected in Halal samples with triplicate injection using the method developed.

S/N	Sample type	Mean concentration (µg/mL)	S/N	Sample type	Mean concentration (µg/mL)
1	Carbonated drink	not detected	9	Vinegar 1	not detected
2	Ginger ale	not detected	10	Wintermelon tea	1.4
3	Green tea	not detected	11	Dipping sauce	4.1
4	Isotonic drink	not detected	12	Honey drink	4.9
5	Milk	not detected	13	Vinegar 2	10.9
6	Probiotic dairy drink	not detected	14	Grape juice	37.1
7	Sparkling water	not detected	15	Soy bean milk	43.7
8	Sweetened drink	not detected	16	Energy drink	497.8

Internal Standard Method

Linear calibration curve was obtained with correlation coefficient (R^2) of 0.999 across the range of 1µg/mL to 1000µg/mL. The relative standard deviation (RSD) for each level was less than 2% over 3 repeat data except the lowest concentration (1µg/mL, RSD 6%). The calibration curve is shown in Figure 2, depicting excellent linearity.

The chromatograms achieved from this method were satisfactory, as shown in Figures 1 and 3. The ethanol and IPA peaks were able to separate well with excellent peak shapes. The retention times for ethanol and IPA were found to be 2.85min and 3.08min respectively.

Analysis of ethanol in actual samples

Sixteen samples each was analysed using the established method. Select chromatograms are shown in Figure 3. The mean concentration of triplicate sample runs are summarized in Table 4. The lowest calibration level of this method was 1µg/mL. As such, values lower than this are recorded as "not detected" in the summary table.

All the samples analysed in this project were labelled Halal, and from the experiment, the concentrations of ethanol in samples were found to be less than 0.05%. These samples indeed met the Halal guidelines in Singapore and Malaysia[2,3].

With regards to Indonesia and Brunei's guidelines, ethanol is strictly prohibited in the final food product[4,5]. Food samples tested with non-zero values, however, may be held questionable by the Halal administrative bodies in these countries.

❑ Conclusions

A headspace-gas chromatographic (HS-GC) method for quantitative analysis of ethanol in Halal testing for liquid condiments and beverages was established using Shimadzu HS-10 and GC-2014 with minimal sample preparation. The R^2 value for the method was more than 0.999, and triplicate runs of each sample also show that the methods were repeatable. The results presented in this study prove that the method is feasible for easy and fast determination of ethanol in a wide variety of liquid condiments and beverage samples to ensure Halal food integrity.

❑ References

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