

## Research

# Gravimetric and Sim-Headspace GC-MS For Residual Organic Solvents Detection in Halal and Wholesomeness Food Analysis

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## ABSTRACT

The demand for halal foods and beverages is increasing globally. While most halal analysis focuses on porcine, this study focuses on assessing residual organic solvents to ensure their halal compliance and wholesomeness, following several Malaysian standards and guidelines. A significant challenge in this study was the volatility of the residual solvents during the preparation of standards and quality control. To address this issue, a gravimetric technique was employed and effectively minimized the difference between theoretical (1,000 ppm) and actual (710 – 892 ppm) concentrations of the residual organic standard stock solution, except for acetone (588 ppm). The aim of this study was to establish a validated, reliable, and accurate method using SIM-headspace GC-MS to identify and quantify residual organic solvents for halal and wholesomeness analysis. Confirmation of each residual organic solvent was achieved by comparing the obtained spectra with the NIST 11 spectral database, containing 70,832 compounds, with similarity ranging from 80.9% to 96.6%, except for acetonitrile at 52.2%. The validation parameters were carried out according to ISO 17025:2017, the Center for Drug Evaluation and Research, and the European Guidelines. The parameters included recovery ranging from 95.65% to 95.68%, precision from 10.08% to 19.65% RSD, linearity between 0.996 to 0.999, limit of detection from 0.01 to 0.08 ppm, and limit of quantification from 0.02 to 0.24 ppm. Uncertainty considerations were limited to recovery, precision, and linearity, as other uncertainties were negligible based on the bottom-up approach using in-house validation data. This combination of gravimetric and SIM-headspace GC-MS techniques has provided valuable insights for discussions and collaborations among halal authorities worldwide to establish a consensus analytical methodology for halal and wholesomeness assessment.

**Key words:** Food safety, GC-MS, halal, method validation, residual organic solvent, wholesomeness

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## INTRODUCTION

The global halal market is expected to reach USD 3.2 trillion by 2024, contributed by six economic sectors: food and beverage, tourism, media and recreation, fashion, pharmaceuticals, and cosmetics (Anonymous, 2019). By 2025, the food and beverage sector is expected to make the largest contribution, with USD 740 billion (Anonymous, 2018). This expectation arises from significant demand from global Muslim consumers, a consequence of the projected growth of the global Muslim population from about 1.9 billion to approximately 2.2 billion from 2020 to 2030 based on global population projection (Grim & Karim, 2011).

Halal is related to kosher, which is specifically for Jewish consumers due to the similarity of concepts between halal and kosher (Azam & Abdullah, 2020). In the USA, for instance, the Muslim population spends approximately USD 16 billion per year on kosher products due to limited access

to halal food and beverage options (Azam & Abdullah, 2020). Furthermore, certain non-Muslims also prefer halal food and beverage products as certified halal products adhere to Good Hygiene Practices (GHP), Good Manufacturing Practices (GMP), and Hazard Analysis Critical Control Point (HACCP) standards (Krishnan *et al.*, 2017).

Ensuring that food and beverages are halal involves more than just considering the absence of prohibited animals according to Islamic law; it also involves addressing the issue of wholesomeness (Omar *et al.*, 2011). Halal, a well-known Arabic term meaning “permissible” is always associated with wholesomeness, known as *halalan-tayyiban* (Shafiee *et al.*, 2018). The fundamentals of *halalan-tayyiban* are briefly explained in the Malaysia Halal Certification Procedure Manual (MPPHM, 2020) and Malaysian Halal Standard (MS 1500:2019, 2019). In general, beverages are considered halal and wholesome, except for those that are poisonous, hazardous, or intoxicating to health.

However, current trends in halal research show that most established analytical methods focus on halal authentication (Yuswan *et al.*, 2018, 2019, 2021; Amir *et al.*, 2021), while specific analytical methods addressing the issue of wholesomeness are scarce. According to the Malaysian Halal Management System (MHMS, 2020), residual organic solvents can be considered a halal critical point for the issue of wholesomeness. Organic solvents can be classified into several categories based on their functional groups. A crucial solvent for halal evaluation is alcohol, specifically ethanol. Ethanol is permissible if its content is less than 1% for beverages and 0.5% for food additives, with the right intention and processing according to the Malaysian halal authority (Baharuddin *et al.*, 2022). Halal authorities from Brunei, Indonesia, and Singapore have slightly different permissible limits for ethanol, ranging from 0.1 to 2% depending on their respective regulations (Pauzi *et al.*, 2019). The usage of ethanol as a food flavor during cooking is also a controversial issue in terms of halal and wholesomeness due to the residual ethanol after cooking (Mateus *et al.*, 2011).

Other alcohols such as methanol and 2-propanol have not yet been discussed from the perspective of halal and wholesomeness. These alcohols are also detected as a minor by-product of fermentation that can cause death when their levels exceed permissible limits. According to the World Health Organization (WHO, 2014), several death cases have been reported due to methanol contamination in Cambodia, the Czech Republic, Ecuador, Estonia, India, Indonesia, Kenya, Libya, Nicaragua, Norway, Pakistan, Turkey, and Uganda. In Malaysia, 19 cases of methanol contamination were reported in June 2019, resulting in eight deaths and involving Malaysian and foreign workers from Bangladesh, India, Myanmar, Nepal, and Pakistan (Md Noor *et al.*, 2020). The permissible limit of methanol in beverages is between 0.2 – 1.5% according to the UE analytical method, while the Food and Drug Administration (FDA) analytical method sets the limit at less than 0.1% (Ohimain, 2016). Furthermore, other organic solvents such as acetone and acetonitrile, widely used in various industries such as food packaging and pharmaceuticals have also not been addressed by halal authorities. In general, most residual organic solvents are toxic.

The current standard analytical methods of the Food and Agricultural Organization (FAO, 2000) and the AOAC Official Method 972.10 (AOAC, 2005) require a gas chromatography-flame ionization detector (GC-FID) to identify and quantify volatile compounds. However, none of these official methods addresses the issue of halal and wholesomeness, as their analysis is conducted at a high level, while analysis at the residual level is required for halal and wholesomeness evaluation. GC-FID is selected due to its robustness in retention time, high sensitivity, ease of use, and wide linear range of detection (Diekmann *et al.*, 2020). However, GC-FID is unable to directly identify compounds because it relies solely on retention time and requires full compound speciation. It also has an unresolved issue of co-elution and matrix effect (Diekmann *et al.*, 2020).

Therefore, this study presents a validated analytical method using selected ion monitoring-headspace gas chromatography-mass spectrometry (SIM-headspace GC-MS) acquisition to identify and quantify residual organic solvents, including ethanol, methanol, 2-propanol, acetone, and acetonitrile for halal and wholesomeness analysis. This technique eliminates the matrix effect by trapping only volatile compounds and resolves the co-elution issue by choosing specified quantifiers as well as qualifier ions, along with assisted database identification for selectivity. The proposed technique is expected to be beneficial in the halal authority community for the evaluation of halal and wholesomeness from the perspective of residual organic solvents.

## MATERIALS AND METHODS

### Materials

Both liquid chromatography-grade acetonitrile (assay  $\geq 99.9\%$ ) and 1-propanol (assay  $\geq 99.8\%$ ) were purchased from Merk KGaA, Darmstadt, Germany, and EMD Millipore Corporation, Massachusetts,

United States, respectively. Both High-Performance Liquid Chromatography (HPLC) grade methanol (assay 99.9%) and 2-propanol (assay 99.97%) were purchased from Fisher Scientific, Seoul, Korea, and Fisher Scientific, Loughborough, United Kingdom, respectively. Pharmacopoeia standard grade absolute ethanol (99.4% v/v) was purchased from Fisher Scientific, Selangor, Malaysia. Analytical reagents grade acetone (assay 99.5%) was purchased from Friendemann Schmidt, Australia. Water (conductivity  $\leq 18$  M $\Omega$  cm) was obtained from an ultrapure water system (Arium®611VF, Sartorius Stedim, Goettingen, Germany). Commercial food and beverage products were randomly purchased from a local market in the nearby area of Serdang, Selangor, Malaysia. All samples were grouped based on the Malaysia Food Act 1983 (Act 281) and Food Regulation 1985, then determined to be either halal or unknown based on the halal certification on the product's label. Details of commercial food and beverage products were provided in Supplementary 1.

### Standard and quality control preparation

A 1,000-ppm standard stock solution was gravimetrically prepared in a 100 mL DURAN® volumetric flask (class A) by adding 0.13 mL for ethanol, methanol, 2-propanol, acetonitrile, and acetone, respectively. Then, in a separate 100 mL DURAN® volumetric flask (class A), a 50,000-ppm internal standard stock solution was prepared gravimetrically by adding 6.12 mL of 1-propanol. Subsequently, 10 replicates of five levels of working standard solution (L1: 1 ppm, L2: 10 ppm, L3: 100 ppm, L4: 250 ppm, & L5: 500 ppm) were constructed from different individual 1,000-ppm standard stock solutions. Each level of working standard solution and sample was spiked with 10  $\mu$ L of the 50,000-ppm internal standard stock solution. The actual concentration for both the standard and internal standard stock solutions was determined based on the gravimetric records of each organic solvent, using Equation 1 (Harris, 2010):

$$\text{Actual concentration of standard (ppm)} = \text{Gravimetric record (g)} \div 100 \text{ mL} \times 1,000,000$$

### Sample preparation

All samples were prepared according to the AOAC Standard Method Performance Requirements 2016.001 (AOAC SMPR, 2016) with some modifications. For solid samples, 10 g of the sample was weighed into a 100-mL DURAN® volumetric flask (Class A). As for semi-solid and liquid samples (Supplementary 1), 5 mL of the respective samples were weighed into individual 100-mL DURAN® volumetric flasks (Class A). Next, 5 mL of each sample was transferred to a flat bottom 20-mL Agilent headspace (HS) vial. A 100-ppm internal standard solution was added to each sample by adding 10  $\mu$ L of the 50,000-ppm internal standard stock solution. Each Agilent HS vial was then sealed with 20-mm Agilent PTFE silicon septa using a crimping tool. All samples were thoroughly mixed by vortexing. Each sample was prepared in triplicate before SIM-headspace GC-MS acquisition. A recovery factor (RF) of the assay was determined from a spiked internal standard solution according to Equation 2 (Harris, 2010):

$$\text{RF} = \text{Detected internal standard (ppm)} / 100 \text{ ppm}$$

Then, a determined concentration of each residual organic solvent was proportioned to RF according to Equation 3 (Harris, 2010):

$$\text{Determined solvent (ppm)} = \text{Detected solvent (ppm)} \times \text{RF}$$

The actual concentration of each residual organic solvent was determined according to Equation 4 (Harris, 2010):

$$\text{Actual solvent (\% w/w)} = \text{Determined solvent (ppm)} / \text{Weight of sample} \times 100 \text{ mL} \times 0.0001$$

### SIM-headspace GC-MS acquisition

The analysis of organic solvents was conducted using an Agilent Technologies G1888 network headspace sampler (Milan, Italy) for sample introduction, an Agilent Technologies 7890A gas chromatograph (Shanghai, China) for chromatographic separation, and an Agilent Technologies 5975C inert MSD (Santa Clara, CA, USA) for mass spectra detection. Initially, each headspace (HS) vial was equilibrated and pressurized on the network headspace sampler with the following settings: oven temperature of 75°C, loop temperature of 90°C, transfer line temperatures of 95°C, vial pressure of 15 psi, carrier gas pressure of 9.1 psi, vial equilibration for 10 min, vial pressurization for 0.2 min, loop

fill for 0.1 min, loop equilibration for 0.05 min, and injection for 1 min. Then, 1  $\mu\text{L}$  of the sample was injected into a Zebron ZB-624plus capillary GC column (30 m  $\times$  0.25 mm  $\times$  1.4  $\mu\text{m}$ ) (phenomenex®, USA), at a flow rate of 1 mL/min using helium as a carrier gas. The GC conditions were as follows: inlet heater temperature of 220°C, inlet pressure of 9.5 psi, septum purge flow of 3 mL/min, split mode ratio of 10:1, column temperature started at 40°C and held for 6 min, followed by an increase to 150°C at a rate of 80°C/min. The mass spectrometer (MS) operated in selected ion monitoring (SIM) mode with an electron multiplier voltage (EMV) of 1812 V (1 gain factor). The retention time and mass over charge (m/z) values for SIM analysis of ethanol, methanol, 2-propanol, acetonitrile, acetone, and 1-propanol were determined using the National Institute of Standards and Technology Mass Spectral Library (NIST 11).

### Method validation

The analytical method was validated according to the criteria outlined in ISO 17025:2017 (2017), the Center for Drug Evaluation and Research (CDER, 1994), and the European Guidelines (European Commission, 2017). The validation included the assessment of various parameters, namely specificity, linearity, recovery, precision, limit of detection (LOD), and limit of quantification (LOQ). For the limit measurement, both the limit of detection (LOD) and the limit of quantification (LOQ) were determined by Equation 5 (Shrivastava & Gupta, 2011):

$$\text{LOD/LOQ} = a \times \text{SDL} \div m$$

where  $a$  is equal to 3.3 and 10 for LOD and LOQ, respectively; SDL is the standard residual variance; and  $m$  is the slope of the linearity. The estimation of the measurement uncertainty ( $U$ ) was determined following the EURACHEM/CITAC Guide (EURACHEM, 2012).

### Data analysis

Actual concentrations for both standard and internal standard stock solutions were statistically analyzed by a one-sample  $t$ -test at a  $P$ -value of 0.05. The null hypothesis ( $H_0$ ): the actual concentration is equal to the hypothetical concentration, whereas the alternative hypothesis ( $H_a$ ): the actual concentration is not equal to the hypothetical concentration. The hypothetical concentrations were 1,000 and 50,000 ppm for standard and internal standard stock solutions, respectively. All raw data and data analysis were executed using the RStudio Team (Version 1.4.1717): Integrated Development Environment for R, 2015.

## RESULTS AND DISCUSSION

### Issues on standard and quality control for halal and wholesomeness analysis

Theoretically, 0.13 mL of a standard stock solution consisting of methanol, ethanol, acetone, 2-propanol, and acetonitrile will be given 0.1 g for respective gravimetric records to achieve a theoretical concentration of 1,000 ppm. Additionally, a volume of 6.12 mL of 1-propanol internal standard stock solution will be given 4.9 g for its gravimetric record to obtain a theoretical concentration of 50,000 ppm (as required in Section 2.2). Therefore, a one-sample  $t$ -test was conducted to verify this theory by comparing the actual values with the hypothetical value. Table 1 shows the results of the one-sample  $t$ -test for the actual concentration of the stock solution for each residual organic solvent, based on the respective actual gravimetric record. Although each actual gravimetric record showed a value close to the hypothetical value, there was a significant difference between the actual and hypothetical concentrations for each residual organic (Table 1). This difference can be attributed to the volatility characteristics of residual organic solvents (Chung *et al.*, 2015), which pose challenges for halal and wholesomeness analysis. As a result, the concentration of methanol, ethanol, acetone, 2-propanol, and acetonitrile was proportionated to 1-propanol as the internal standard to standardize and minimize errors. The selection of 1-propanol as the internal standard was based on its similar chemical characteristics, except for acetone and acetonitrile. Additionally, 1-propanol can be detected at low concentrations, as reported in a previous study (Prenesti *et al.*, 2019). Thus, the stability of 1-propanol is crucial. In this study, the concentration of 1-propanol showed a small difference, with the actual concentration ( $M = 47,830$ ,  $SD = 228.42$ ) being lower than the hypothetical concentration,  $t(9) = 30.04$ ,  $p = 2.454e^{-10}$ . Furthermore, attention should also be given to acetone in halal and wholesomeness analysis, as it exhibited the lowest level of actual concentration ( $M = 588$ ,  $SD = 84.29$ ) compared to the hypothetical concentration,  $t(9) = -15.46$ ,  $p = 8.70e^{-8}$ . In this case, the actual concentration of acetone was half of its theoretical concentration.

**Table 1.** One-sample t-test for an actual concentration of stock solution for each residual organic solvent based on the respective actual gravimetric record

Residual organic solvent	Type of solution	Actual gravimetric record <sup>1</sup> (g)	Actual concentration, $M^2$ (ppm)	$SD$	$df$	t-statistic	P-value
Methanol	Standard	$0.07 \pm 0.006$	710	61.64	9	-14.88	$1.21e^{-7}$
Ethanol	Standard	$0.09 \pm 0.006$	863	59.45	9	-7.29	$4.63e^{-5}$
Acetone	Standard	$0.06 \pm 0.008$	588	84.29	9	-15.46	$8.70e^{-8}$
2-propanol	Standard	$0.09 \pm 0.003$	892	29.36	9	-11.63	$1.00e^{-6}$
Acetonitrile	Standard	$0.09 \pm 0.004$	858	35.84	9	-12.53	$5.33e^{-7}$
1-propanol	Internal Standard	$4.78 \pm 0.023$	47,830	228.42	9	-30.04	$2.45e^{-10}$

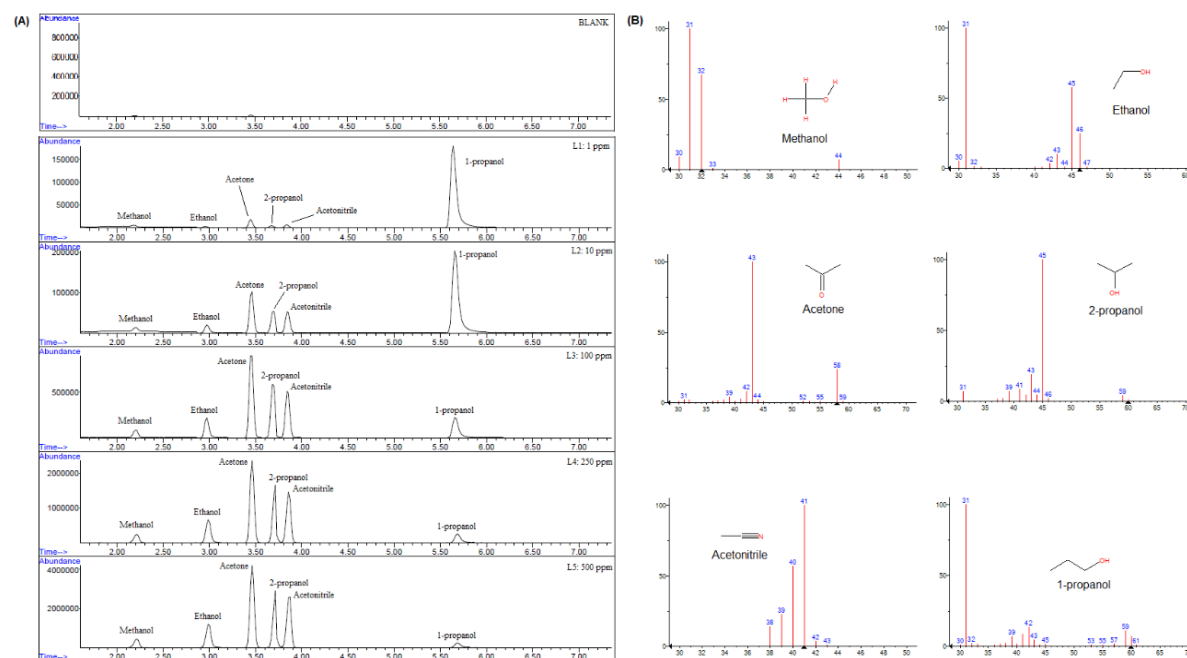
<sup>1</sup>The value is the mean with a standard deviation that was determined from 10 replicates. Hypothetical gravimetric records are 0.1 and 4.9 g for standard and internal standard stock solutions, respectively

<sup>2</sup>The value is the mean of 10 replicates. Null hypothesis ( $H_0$ ): actual concentration is equal to hypothetical concentration. Alternative hypothesis ( $H_a$ ): actual concentration is not equal to hypothetical concentration. Hypothetical concentrations are 1,000 and 50,000 ppm for standard and internal standard stock solutions, respectively

g = gram, ppm = part per million, M = mean,  $SD$  = standard deviation, and  $df$  = degree of freedom.

### Characterization of residual organic solvents and their selectivity

Interferences can cause incorrect detection and measurement for the halal and wholesomeness analysis. During method validation, establishing selectivity is essential to ensure no interferences such as impurities, co-eluents, and matrix effects. In the analysis of halal and wholesomeness, residual organic solvents including methanol, ethanol, acetone, 2-propanol, and acetonitrile were profiled through gas chromatographic separation. Subsequently, quantifier and qualifier ions for each residual organic solvent were detected by selected ion monitoring mass spectrometry (SIM-MS) acquisition with assistance from the NIST 11 spectral database, and proportions were adjusted using an internal standard of 1-propanol. Figure 1 displays complete chromatogram profiles and spectra of methanol, ethanol, acetone, 2-propanol, acetonitrile, and 1-propanol. No other chromatograms were detected in the blank sample. At each standard solution level, all chromatograms show high resolution ( $> 2.0$ ) with different retention times, as recommended by CDER (1994). A high-resolution chromatogram with a minimum value of 2.0 is crucial for an MS detector due to its limitations in identifying isobaric compounds (Diekmann et al., 2020).



**Fig. 1.** (A) Complete chromatogram profiles of methanol, ethanol, acetone, 2-propanol, and acetonitrile at five levels (L1: 1 ppm, L2: 10 ppm, L3: 100 ppm, L4: 250 ppm, and L5: 500 ppm). 1-propanol is a 100-ppm internal standard solution at all levels. No interferences are detected in a blank sample. (B) A spectrum of methanol, ethanol, acetone, 2-propanol, acetonitrile, and 1-propanol based on the NIST 11 spectral database

Details regarding retention times and  $m/z$  values of SIM-MS acquisition are presented in Table 2. The eluting time ranged from 1.5 to 7.4 min. This analytical method is currently the fastest acquisition method for halal and wholesomeness analysis compared to other organic solvent analytical methods (Mateus *et al.*, 2011; Destanoglu & Ates, 2019; Diekmann *et al.*, 2020). The shortest and longest retention times observed were 2.20 and 5.56 min, corresponding to methanol and internal standard 1-propanol, respectively. Sequentially, the other residual organic solvents were retained based on their structural complexity (Figure 1B), with ethanol eluting at 3.03 min, acetone at 3.45 min, 2-propanol at 3.69 min, and acetonitrile at 3.85 min. Previous studies have shown slightly different retention times for each residual organic solvent (Diekmann *et al.*, 2020); however, the sequential pattern of retention time remains consistent for all residual organic solvents in halal and wholesomeness analysis.

**Table 2.** Retention time and  $m/z$  values for each residual organic solvent for the halal and wholesomeness analysis

Residual organic solvent	Retention time <sup>1</sup> (min)	Mass over charge value <sup>2</sup> ( $m/z$ )		NIST 11 spectral database <sup>3</sup> (%)
		Quantifier ion	Qualifier ion	
Methanol	2.20 ± 0.009	31	32, 44	80.9
Ethanol	3.03 ± 0.241	31	45, 46	96.6
Acetone	3.45 ± 0.009	43	42, 58	92.5
2-propanol	3.69 ± 0.014	45	41, 43	85.2
Acetonitrile	3.85 ± 0.010	41	39, 40	52.2
1-propanol	5.66 ± 0.019	31	42, 59	95.5

<sup>1</sup>Value of retention time is the mean with a standard deviation of 50 injections

<sup>2</sup>Both quantifier and qualifier ions are determined from the NIST 11 spectral database

<sup>3</sup>Result is a percentage of similarity between each residual organic solvent spectrum and the NIST 11 spectral database, which contained 95,409 spectra and 70,835 compounds for MS-SIM acquisition

Furthermore, establishing both quantifier and qualifier ions determined from the NIST 11 spectral database for SIM-MS acquisition also assists in achieving selectivity. SIM-MS acquisition can eliminate potential co-eluent and matrix effects in the chromatogram. According to the European Commission (2017), a minimum of three  $m/z$  ions is required for SIM-MS acquisition (Table 2). In this study, all alcohols exhibited a quantifier ion of 31  $m/z$  (except for 2-propanol, which had a quantifier ion of 45  $m/z$ ) with an ID confirmation of 80.9 – 96.6% based on the NIST 11 spectral database. The quantifier ion of 31  $m/z$  is a characteristic of primary alcohols (Jalbert *et al.*, 2012), which explains the different quantifier ion values for 2-propanol as a secondary alcohol. Using a quantifier ion of 31  $m/z$  for ethanol in halal and wholesomeness analysis aligns with previous studies (Mateus *et al.*, 2011; Heit *et al.*, 2016). Although methanol, ethanol, and 1-propanol have a similar quantifier ion of 31  $m/z$ , the qualifier ion values are unique for each of them (Table 2). Additionally, it is necessary to evaluate methanol and 1-propanol for halal and wholesomeness status, as some fermenting microorganisms may produce volatile congeners, including acetone in alcoholic beverages (Ohimain, 2016). Previous studies reported a quantifier ion of 58  $m/z$  for acetone (Shah *et al.*, 2010), but in this study, 58  $m/z$  was used as a qualifier ion for acetone in halal and wholesomeness analysis, with an identification confirmation of 92.5% based on the NIST 11 spectral database. Another study reported the presence of ethanol, methanol, and acetone in pharmaceutical samples (Hashimoto *et al.*, 2001). Regarding acetonitrile, a quantifier ion of 41  $m/z$  was used for halal and wholesomeness analysis, although another study reported dominance at 42  $m/z$  (Dunne *et al.*, 2012). Therefore, the ID confirmation for acetonitrile in this study is only 52.2%, even though the 41  $m/z$  ion is the most intense for acetonitrile. Acetonitrile is extensively used in the chemical extraction process and the production of polymers, plastics, dyes, and medicines. This organic solvent is easily absorbed into the gastrointestinal tract and lungs, and then distributed throughout the body as cyanide, formaldehyde, and formate with a long half-life (De Capitani *et al.*, 2017).

### Performance of SIM-headspace GC-MS acquisition on residual organic solvents for halal and wholesomeness analysis

Several validation parameters were assessed to ensure the developed analytical method for residual organic solvents in halal and wholesomeness analysis is suitable for its intended purpose. Table 3 provides a summary of the validation parameters, including accuracy, linearity, and the limitation of residual organic solvents in halal and wholesomeness analysis. The observed concentration is lower than the actual concentration, which can be attributed to deviations in the GC-MS system (Table 3).

To correct the system deviations, adjustments were made based on the recovery factor of the spiked internal standard. The accuracy of each residual organic solvent was estimated in terms of recovery and precision. Overall, all residual organic solvents exhibited a recovery rate of 96%, while the precision ranged from 11 – 21% relative standard deviation (RSD). Both recovery and precision were determined based on 10 replicates of a spiked internal standard 1-propanol solution at 100 ppm, following the guidelines provided by the European Commission (2017). Regarding linearity, the coefficient of regression ( $R^2$ ), slope, intercept, and residual standard error were considered crucial. In most cases, an  $R^2$  value of  $\geq 0.999$ , derived from a minimum of five levels is recommended (CDER, 1994; European Commission, 2017). For the halal and wholesomeness analysis, all residual organic solvents exhibited an  $R^2$  value of  $\geq 0.999$ , except for acetone and 2-propanol which had values of 0.998 and 0.996, respectively. A previous study on residual organic solvents in the blood matrix found  $R^2$  values of 0.981, 0.997, 0.996, 0.998, and 0.998 for methanol, ethanol, acetone, 2-propanol, and acetonitrile, respectively (Diekmann *et al.*, 2020). This discrepancy may be attributed to different solvent evaporation rates, which significantly affect the  $R^2$  value (Gray, 1974). Furthermore, the slopes with varying and high values (Table 3) indicate the sensitivity and selectivity of each residual organic solvent as each slope value is dissimilar (Moosavi & Ghassabian, 2018). For the limit measurement, both the limit of detection (LOD) and the limit of quantification (LOQ) were calculated using a five-level serial standard solution with 10 replicates per level. In this study, the LOD ranged from 0.01 – 0.08 ppm, while the LOQ ranged from 0.02 – 0.25 ppm (Table 3). The lowest and highest LOD or LOQ values were associated with acetone and methanol, respectively. This observation can be attributed to the physicochemical properties of residual organic solvents such as molecular weight, boiling point, melting point, flash point, density, and solubility (Joshi & Adhikari, 2019).

However, the method validation is considered incomplete without proper determination of measurement uncertainty (U) (EURACHEM, 2012). Measurement uncertainty refers to the dispersion value that could be attributed to the concentration of residual organic solvent. Table 4 presents the measurement of uncertainty for residual organic solvents in halal and wholesomeness analysis. The same approach was adopted as in a previous study (Burr *et al.*, 2021), where all uncertainty contributions were neglected except those associated with recovery (Urec), precision (Urep), and linearity (Ur). In this study, the highest uncertainty contributors were 2-propanol (Urec = 0.19 ppm & Urep = 0.23 ppm) except for Ur, where methanol had the highest value (Ur = 0.07 ppm). Subsequently, a combined uncertainty (Uc) was determined before calculating the expanded uncertainty (Ue) using an appropriate coverage factor (k) of 2 for a 95% confidence interval. The range of Uc and Ue was between 0.15 – 0.31 and 0.31 – 0.61 ppm, respectively, with the highest value associated with 2-propanol. The selection of uncertainty contributors was based on a bottom-up approach, wherein each potential uncertainty was identified, quantified, and combined using in-house validation data (Burr *et al.*, 2021)

### **Assessment of halal and wholesomeness status by the validated SIM-headspace GC-MS method**

An assessment was conducted to ensure that the developed and validated analytical methods can verify and evaluate the halal status, as well as the unknown halal status, of 13 different sample types of commercial foods and beverages (Supplement 1). The halal and wholesomeness assessments were conducted by the Malaysian halal requirements. Among the 13 different sample types, two samples were commercial alcoholic beverages. However, excluding the commercial alcoholic drinks, all the analyzed samples including those with unknown halal status complied with the proposed limit of < 1% for ethanol set by the Malaysian halal authority. Even though the label for the commercial alcoholic drinks stated, "Contain 5% of alcohol v/v", the ethanol concentration was slightly more than 5%. In addition, methanol and 2-propanol were below the LOD and LOQ. Regarding wholesomeness evaluation, other residual organic solvents such as acetone, 2-propanol, and acetonitrile were not detected in any of the different sample types except for commercial alcoholic drinks. However, these residual organic solvents (acetone, 2-propanol, & acetonitrile) were also below the LOD and LOQ limits.

### **CONCLUSION**

In conclusion, the combination of gravimetric technique and SIM-headspace GC-MS acquisition can be considered a successful strategy for addressing analytical challenges in halal and wholesomeness assessment. This technique minimizes errors caused by volatility, matrix effects, and co-elution.

**Table 3.** Summary of validation parameters for accuracy, linearity, and limitation of residual organic solvents for the halal and wholesomeness analysis

Residual organic solvent	Concentration <sup>1</sup> (ppm)		Accuracy <sup>2</sup> (%)		Linearity <sup>3</sup>			Limitation <sup>4</sup> (ppm)		
	Observed	Actual	Recovery	Precision (RSD)	R <sup>2</sup>	Slope	Intercept	Residual standard error	LOD	LOQ
Methanol	101.23 ± 14.19	105.79 ± 14.71	95.671	14.660	0.999	1517.71	486.90	39.31	0.08	0.24
Ethanol	100.98 ± 11.27	105.55 ± 11.71	95.670	11.697	0.999	3999.54	-4534.06	39.32	0.03	0.09
Acetone	108.39 ± 15.17	113.33 ± 15.98	95.669	14.863	0.998	19516.10	135474.80	34.21	0.01	0.02
2-propanol	102.44 ± 20.13	107.08 ± 20.97	95.671	20.648	0.996	11026.30	72160.60	51.22	0.01	0.04
Acetonitrile	104.47 ± 10.53	109.19 ± 10.89	95.668	10.524	0.999	9055.07	25772.82	25.99	0.01	0.03

<sup>1</sup>Value is a mean with a standard deviation from 10 replicates at the 100 ppm level. The observed and actual concentrations are determined by experimental data and the recovery factor of spiked internal standard 1-propanol solution at 100 ppm, respectively

<sup>2</sup>Both recovery and precision are determined from 10 replicates proportionate to the spiked internal standard 1-propanol solution at 100 ppm. RSD = relative standard deviation

<sup>3</sup>Linearity is calculated from five levels of serial standard solution (L1: 1 ppm; L2: 10 ppm; L3: 100 ppm; L4: 250 ppm; L5: 500 ppm) with 10 replicates for each level.

<sup>4</sup>LOD = limit of detection; the calculation is based on 3.3 x residual standard deviation ÷ slope. LOQ = limit of quantification; the calculation is based on 10 x residual standard deviation ÷ slope

**Table 4.** Measurement of uncertainty for residual organic solvents for halal and wholesomeness analysis

Residual organic solvent	Uncertainty measurement (ppm)				
	$U_{rec}^1$	$U_{rep}^2$	$U_r^3$	$U_c^3$	$U_e^4$
Methanol	0.14	0.15	0.07	0.22	0.44
Ethanol	0.11	0.15	0.06	0.19	0.39
Acetone	0.14	0.17	0.05	0.23	0.46
2-propanol	0.19	0.23	0.03	0.31	0.61
Acetonitrile	0.10	0.09	0.06	0.15	0.31

<sup>1</sup> $U_{rec}$  is the uncertainty of recovery at 100 ppm based on the recovery factor of the internal standard 1-propanol solution from 10 replicates

<sup>2</sup> $U_{rep}$  is the uncertainty of precision from 10 replicates of linearity (pooled RSD)

<sup>3</sup> $U_r$  is the uncertainty of the calibration straight line with 10 replicates

<sup>4</sup> $U_e$  is combined uncertainty with contributions from  $U_{rec}$ ,  $U_{rep}$ , and  $U_r$

<sup>5</sup> $U_e$  is expanded uncertainty with an appropriate coverage factor (k) of 2 for a 95% confidence interval



Validation parameters, including accuracy, precision, and limitation adhere to international standards' criteria. Therefore, it is recommended that halal authorities worldwide consider this method for assessing the halal and wholesomeness of food and beverages from the perspective of residual organic solvents.

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### ETHICAL STATEMENT

Not applicable.

### CONFLICT OF INTEREST

The authors declare no conflict of interest.

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