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Assessment of uncertainty estimation for measurement of ethanol concentration in COVID-19 hand sanitizer using FTIR spectroscopy

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Abstract: Analysis of uncertainty estimation for measurement of type and concentration of alcohol in hand sanitisers is a matter of urgency in the COVID-19 situation. FTIR spectroscopy was used to investigate hand sanitisers made in our laboratory and commercial products. An internal standard addition method was used to control the measurement quality. The absorption spectra of ethanol were found to be at 1086 and 1044 cm^{-1}, corresponding to C-O stretching. The area under the C-O adsorptions is used to create a calibration curve, which is then used to calculate the ethanol percentage. Additional standard sample and quality control sample showed calibration curves with slopes of 0.1267 and 0.1285, respectively. The regression coefficients and residual variance of 0.0057 showed a 'best fit' with the predicted value. These parameters were used to estimate the uncertainty of six commercial products. The ethanol concentration of commercial products is measured between 71.38 and 81.54% v/v, with an estimated uncertainty of 1.14% v/v. The results showed that the ethanol content of all products differed from the label but could be used to kill bacteria and viruses. This entire process was established as a SOP for measuring alcohol concentration in hand sanitizer.

Keywords: COVID-19 pandemic; alcohol concentration; hand Sanitiser; ISO/IEC 17025; uncertainty estimation; FTIR spectroscopy.

1. Introduction

As evidence of the novel SARS-CoV-2 continues to emerge, the World Health Organization (WHO) has cautioned civilians to stay at home (work from home), wear masks when going out in public,

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and wash their hands frequently to avoid infection from the coronavirus disease 2019 (COVID-19) [1]. As a result, alcohol-based hand sanitisers have exploded in popularity. These products are in short supply on the market [2]. In response to this shortage, Thailand's regulatory agency, Food and Drug Administration (FDA), Ministry of Public Health, has issued recommendations for the temporary production of these sanitisers by specific enterprises and pharmacies to increase supplies during this public health emergency. Consumer safety, on the other hand, must come first. Methanol has been discovered in hand sanitisers, and the hand gel's alcohol concentration is not standardized [3]. These circumstances were detrimental to the customer. For the reasons stated above, measuring both alcohol concentration and the type and combination of hand sanitisers is critical and very urgent in the COVID-19 pandemic. Isopropanol, ethanol, n-propanol, or a combination of two of these alcohols make up most alcohol-based hand sanitisers. Alcohol's capacity to denature proteins is thought to be responsible for their antibacterial action. Ethanol (60–85%) and isopropanol (60–80%) solutions have the highest antibacterial effectiveness [4]. Because proteins do not denature easily in the absence of water, products with higher alcohol content are less effective, but solutions containing less than 60% alcohol may only slow the growth of germs rather than kill them [5].

The various methods for measuring the alcohol concentration in hand sanitizers have been investigated. Chromatography has been employed in the laboratory as a versatile technique for quantifying components in mixtures of organic substances. However, gas chromatography (GC) and high-performance liquid chromatography (HPLC) take a long time, are expensive, and can only be done by a skilled individual [6]. Vibrational spectroscopic techniques are becoming more popular and important in the pharmaceutical industry because they are nondestructive and can provide quick and convenient solutions to routine analytical problems. Fourier transform infrared (FTIR) spectroscopic analysis can provide information about the molecular structure of complex mixtures. Because of the ease of sample preparation and operation, as well as the nondestructive nature of the analyses, FTIR is appealing as a characterisation technique for analysing gel formulations [7, 8].

Many researchers have published their findings from alcohol analysis using the FTIR technique. Mohammed T. Islam and his co-worker used the FTIR with attenuated total reflection (ATR) technique to investigate the hydrogen-bond interaction between Carbopol-neutraliser and Carbopol-surfactant in topical gel formulations. Gels were created using three different solvent systems: aqueous, alcoholic (anhydrous), and hydroalcoholic. They employed FTIR as an analytical method to assess hydrogen bond and electrostatic interactions in fully hydrated and dried Carbopol gel formulations [9]. Kenneth Mineart et al. applied the FTIR (mid-infrared region) technique to quantify the diffusivity of two distinct diffusion probes in organogels [10]. Celio Pasquini and his colleague in Brazil used near-infrared spectroscopy (NIRS) to monitor the quality of ethanol-based hand sanitisers. They demonstrated that the NIRS is an effective and practical tool for estimating the efficacy of various ethanol-based sanitiser formulations in volatilisation studies [11]. Fernando Fonseca Jr. et al. presented partial least squares (PLS) regression models based on MIR and NIR spectra developed to determine the ethanol content of alcohol-based hand gel sanitisers. They believe the methods are critical for Brazil's police and regulatory agencies to ensure product quality. This method can also be used in industry to ensure quality control. This result emphasises the need for authorities to maintain constant vigilance to ensure that the products meet the required specifications [12]. The National Institute of Standards and Technology (NIST, U.S. Department of Commerce) developed and tested four instrumental measurement approaches for measuring ethanol and impurities in 72 different brands and formulations of hand sanitisers. Gas chromatography with flame ionisation detection (GC-FID, liquid chromatography with ultra-violet (LC-UV) detection, quantitative nuclear magnetic resonance spectroscopy (qNMR), and ATR-FTIR were among the techniques used. All four instrumental methods can detect and compare ethanol. All methods can also confirm the presence of other alcohols that may be present in significant amounts. This study found that the ATR-FTIR approach was quick, simple, and applicable to a wide range of hand sanitisers, regardless of sample complexity [6]. A hand sanitiser sample containing a high amount of methanol is one prominent example where FTIR results did not correlate well with the results of other techniques. The presence of methanol was easily apparent in the sample analysed with ATR-FTIR. These recent studies have begun to gain insight on how to use a measurement technique and report the type and alcohol concentration. One limitation of previous studies is that they concentrated on the type and percentage of alcohol concentration. This means that

researchers currently know very little about uncertainty of measurement. If the researchers want a better understanding of the measurement result, alcohol concentration's acceptable range, and measurement quality control, then reporting the uncertainty and measurement quality control are critical.

As mentioned above, the measuring result must be standardised and accepted worldwide. Testing laboratories must ensure that test results are valid and comparable worldwide according to global standards. As a standard, ISO/IEC 17025 can be used to develop a quality system for a laboratory and evaluations by laboratory clients or third parties [13]. The standard operating procedures (SOP) and the uncertainty of measurement play an important role in the quality systems. The Joint Committee for Guides in Metrology JCGM 100:2008 standard (also known as the *Guide to the Expression of Uncertainty in Measurement*, GUM) for evaluating uncertainty is based on the law of uncertainty propagation (LPU) [14]. Another document worth mentioning in the field of analytical chemistry is the *Quantifying Uncertainty in Analytical Measurement* guide, produced by a joint 10 Metrology EURACHEM/CITAC Measurement Uncertainty Working Group [15]. This document primarily outlines the uncertainty evaluation process in accordance with the GUM's recommendations, and it includes several examples from the analytical chemistry field.

In practice, the uncertainty of the result can arise from a variety of sources, including incomplete measurand definition, sampling, matrix effects and interferences, environmental conditions, uncertainties of masses and volumetric equipment, reference values, approximations and assumptions incorporated in the measurement method and procedure, and random variation [16]. One of the most valuable tools for the testing laboratory is the calibration curve. It is widely used in measurement systems where the property value to be measured cannot be obtained directly [17]. Instead, the system's response is measured. In this circumstance, a calibration curve is used to correlate the system's response with well-known property values, typically calibration standards. The property value for a new unknown example can be determined by the mean of a calibration curve in the main, which is normally adjusted through a linear regression using the equations for the fitted curve [18,19]. However, the calibration curve contains errors that lead to uncertainty because of the lack of fit to experimental data. For ensuring the validity of results, the internal standard addition method was frequently used as quality control samples. The standard addition method (SAM) is frequently used to overcome matrix effects and to control the quality of measurement method [20]. The SAM is the addition of known concentration into the same analyte sample. Details, background, and history review of SAM were reported by Thorburn Burns and Michael J. Walker [21].

This work aims to precisely determine the percentage of alcohol in hand sanitiser sold in drug stores and establish the SOP for ethanol content determination according to the ISO/IEC 17025 requirements. Uncertainty and quality control of measurement were also clearly reported by the calibration curve as well.

2. Experimental

2.1. Hand Sanitiser Preparation, Internal Addition Standard Sample and Commercial Products

The 500.0 mL samples of hand gel sanitiser with varying ethanol concentrations were prepared in accordance with WHO guidelines [22]. The 95% v/v of ethyl alcohol (or ethanol, purchased from Sigma-Aldrich Pte. Ltd., Pharmaceutical Secondary Standard; Certified Reference Material), an acrylates/C10-30 alkyl acrylate crosspolymer (or Carbopol 940, purchased from Value Industrial Product Co., Ltd., Thailand), a triethanolamine 99% (or TEA, purchased from Sigma-Aldrich Pte. Ltd.) and a 1,2,3-propanetriol (or glycerin, purchased from Krungthepchemi Co., Ltd., Thailand) were the starting materials of the gel sanitizer The 66.5% v/v ethanol sanitiser was prepared by following this sequence. First, Carbopol 940 (2.5 g) was gradually poured into 142.7 mL of hot distilled water. Second, to adjust the pH of the solution, 1.75 g of TEA was added to the gel. Next, 3.0 g of glycerin was added to boost skin moisture. The solution was stirred until it was completely mixed. Finally, the solution was adjusted to 150 mL by volume. This solution was then gentle mixed with 350.0 mL of 95% ethanol. The portion of starting materials was varied to produce a standard ethanol calibration curve, as shown in Table 1. The standard ethanol gel sanitiser samples namely, HGS_0%, HGS_15%, HGS_30%, HGS_50%, HGS_60%, HGS_66.5%, HGS_70, HGS_75%, and HGS_80% contained ethanol levels of 0%, 15%, 25%, 50%, 60%,

66.5%, 70%, 75%, and 80%, respectively. To study the effect of SAM, 10.0 mL of 95% ethanol were added to standard samples HGS_50%, HGS_60% HGS_66.5% and HGS_70% to shift the ethanol concentration by approximately 2% v/v. The SAMs were named as SAM_52%, SAM_62%, SAM_68.5%, and SAM_72%, as the ethanol concentration of 52.0%, 62.0%, 68.5%, and 72.0%, respectively. Six commercial hand sanitisers were purchased from medical stores in Chonburi province, Thailand. Table 2 shows the details of the commercial products.

Table 1. The portion of starting materials for hand sanitizer preparation.

Sample No.	Desired ethanol (%)	(1) Distilled water (mL)	(2) Carbopol, TEA and Glycerin dissolved in (1)	(1+2) Adjusted solution (mL)	Ethanol 95% (mL)
HGS_0%	0.0	492.75	7.25 g	500.00	0.0
HGS_15%	15.0	413.85	7.25 g	421.10	78.9
HGS_30%	30.0	334.45	7.25 g	341.70	158.3
HGS_50%	50.0	229.55	7.25 g	263.80	263.2
SAM_52%	52.0	229.55	7.25 g	263.80	273.2
HGS_60%	60.0	176.95	7.25 g	184.20	315.8
SAM_62%	62.0	176.95	7.25 g	184.20	325.8
HGS_66.5%	66.5	142.75	7.25 g	150.00	350.0
SAM_68.5%	68.5	142.75	7.25 g	150.00	360.0
HGS_70%	70.0	124.25	7.25 g	131.50	368.5
SAM_72%	72.0	124.25	7.25 g	131.50	378.5
HGS_75%	75.0	98.05	7.25 g	105.30	394.7
HGS_80%	80.0	71.75	7.25 g	79.00	421.0

Table 2. Ingredients and ethanol percentage of six commercial hand gel sanitizers collected from the drug stores

Sample No.	Ethanol (%v/v)	Expire date	Ingredients (as label)
Com1_70%	70.0	08.05.2022	Alcohol, Aqua, Glycerin, Acrylates/C10-30 Alkyl Acrylate Crosspolymer, Triethanolamine, Tocopheryl Acetate, Disodium EDTA, Aloe Barbadensis Leaf Juice, Citric Acid, Sodium Benzoate
Com2_72.4%	72.4	21.03.2022	Alcohol, Aqua, Propylene Glycol, Carbomer, Triethanolamine, Fragrance, Allantoin, Butylene Glycol, Glycerin, Aloe Barbadensis Leaf Juice, etc.
Com3_75%	75.0	13.03.2022	Ethyl Alcohol, Aqua, Propylene, Glycerin, Carbomer, Tocopherol, Fragrance, etc.
Com4_75%	75.0	09.04.2022	Ethanol, Aqua, Propylene Glycol, Acrylates/C10-30 alkyl acrylate crosspolymer, Glycerin, Methylparaben, Propylparaben, Mentha piperita oil, Triethanolamine
Com5_75%	75.0	24.04.2022	Ethyl Alcohol, Aqua, Acrylates/C10-30 Alkyl Methacrylates copolymer, Triethanolamine, Aloe Barbadensis Leaf Extract, Glycerin, Fragrance Free
Com6_76%	76.0	08.04.2023	Ethanol, Water, Carbomer, Triethanolamine, Propylene Glycol, Fragrance, CI 420-90

2.2. Measurement Method via FTIR Spectroscopy

A Bruker (Model INVENIO R equipped with the OPUS software) FTIR spectrometer was used to determine the concentrations of ethanol and isopropyl alcohol (if present) in hand sanitizer The attenuated total reflectance accessory was used to measure all samples (Single Reflection Diamond ATR). The absorption/transmission spectrum of the samples was determined using the OPUS software (ver. 8.5

SP1, Bruker Optik, GmbH, 2020). The spectra were recorded in the frequency range of 4000–400 cm^{-1} at a resolution of 4 cm^{-1} with a total of 32 scans. The relative humidity and ambient temperature were kept below 20% and between 18–25 °C, respectively. At a controlled ambient temperature, approximately 1.0 mL of the sample was dropped onto the crystal plate. A repeatability test was performed by measuring the same sample three times after 30 minutes had passed, whereas the reproducibility of ATR-FTIR profiles was investigated by analysing samples from three different aliquots of the same brand.

According to Beer–Lambert's law, the amount of light absorbed by a material dissolved in a completely transmitting solvent is directly proportional to the substance's concentration and the light's path length through the solution [8,23]. We can use the absorbances to calculate the concentration of a solution, or we can plot a graph of various concentrations [24]. To demonstrate this linear relationship, eight standard ethanol sanitiser samples (HGS_0%-75%) were measured and collected using FTIR spectroscopy. Before beginning any experiments, the interferogram signal was checked, and the peak position was recorded. The background signal spectrum was then measured. The defined regions for each peak, as well as the software's baseline correction, were kept consistent across all standards and samples measured. The quality control samples by SAM (SAM_52%-72%) were also evaluated and then compared to those of standard samples. Plots of different specific areas versus concentrations were used for the calibration curve. A linear calibration curve of absorbance vs concentration was produced from these absorption spectra. The concentration of commercial hand sanitiser was determined by the calibration plot of the standard samples, as indicated in the preceding paragraphs.

2.3. Estimation of Uncertainty Measurement and Quality Control

In the case of evaluating the uncertainty of ethanol concentration, based on the LPU and correlation terms [18], the uncertainty of the predicted value of x_i (ethanol concentration) and the corresponding observation value y_i (peak area) were applied to the linear regression model in the form of Equation (1).

$$x_i = \frac{y_i - a}{b} \quad \text{or} \quad y_i = a + bx_i \tag{1}$$

$$S_e^2 = \frac{\sum(y_i - \hat{y}_i)^2}{n-2} \tag{2}$$

where a and b are the intercept and the slope parameters of the linear regression,
n denotes the number of points used to create the curve,
y_i denotes the values for the independent variable of the linear equation for each x_i, and
S_e^2 denotes the residual variance of the fitted curve, as calculated by Equation (1) where $\hat{x}_i = \frac{y_i - a}{b}$ are the interpolated values for each y_i in the fitted curve. The standard uncertainty of the predicted u_{xi} value is frequently used expression in Equation (3).

$$u_{xi} = \frac{S_e}{b}\sqrt{\frac{1}{m} + \frac{1}{n} + \frac{(\bar{y}_i - \bar{y})^2}{b^2 \sum(x_i - \bar{x})^2}} \tag{3}$$

where S_e is the residual standard deviation of the fitted line,
m is the number of observations of y_i,
n is the number of points composing the calibration curve, and
$\bar{y}_i$ is the average value obtained from the observation of y_i.

The following equation is used to calculate the uncertainty component due to the observation of y_0:

$$u_{yi} = \frac{S_e}{\sqrt{m}} \tag{4}$$

3. Results and Discussion

Figure 1 shows the IR spectra of prepared hand sanitiser samples. The characteristic bands of ethanol and water in the MIR region are associated with O-H intermolecular hydrogen bond stretching (3400–3200 cm^{-1}), C-H stretching (2979 and 2902 cm^{-1}), H_2O bending (1643 cm^{-1}), C-H bending (1383 and 1452 cm^{-1}), and C-O stretching (1086, 1044 and 878 cm^{-1}). The brown line in Figure 1 shows 0% of ethanol concentration; the sample only contains water, TEA, Carbopol and glycerin, and there is no absorbance in the wavenumber of 2978 cm^{-1}, 2901 cm^{-1}, 1452–1275 cm^{-1}, 1088 cm^{-1},1044 cm^{-1}, and 878 cm^{-1}, which correspond to the absorbance of C-H and C-H, respectively. The IR spectra show the identities of ethanol peaks as described in Figure 1. In particular, the C-H stretching at 2979 and 2902 cm^{-1} disappeared for the HGS_0% sample. Figure 2 shows the identity peaks of ethanol for standard samples at the wavenumber range of 1140 to 1000 cm^{-1}. These two peaks in Fig.2 were used to calculate the ethanol concentration using the peak area.

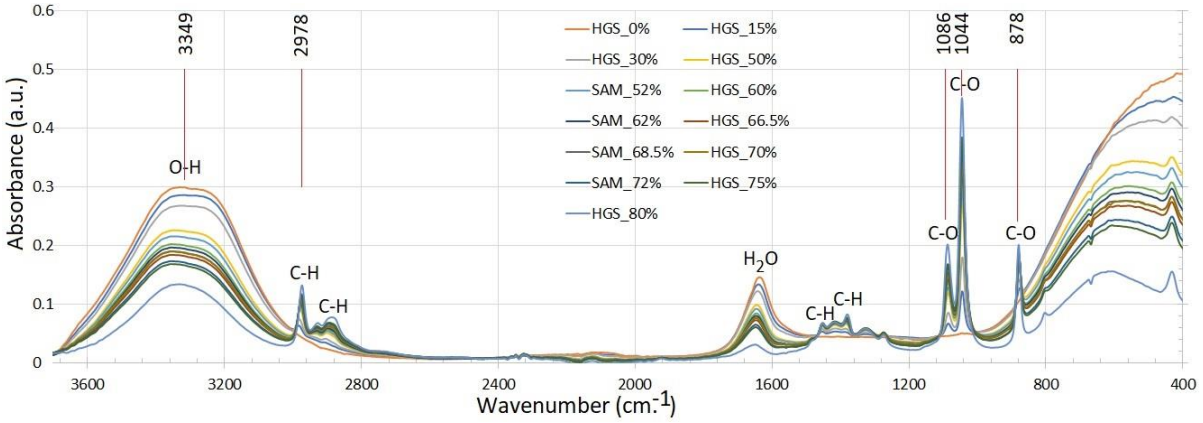

Figure 1. IR spectra of hand sanitiser at various ethanol concentration prepared in the laboratory

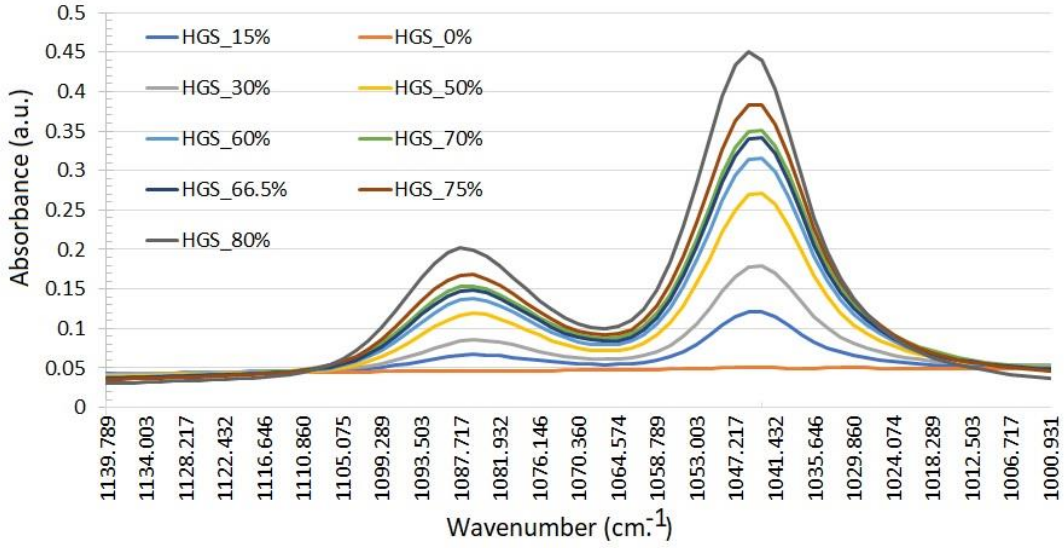

Figure 2. The IR spectra area of hand sanitisers (HGS_0-80%) used for the calibration curve

In the case of isopropanol-based hand sanitiser, the absorbances were reported at the wavenumbers of 1160 cm^{-1}, 1128 cm^{-1}, and 1105 cm^{-1}, which correspond to the absorbance of C-H bending and C-O stretching. The absorption of C-H stretching vibrations occurs at 2902 cm^{-1}, which is a common wavenumber for any molecule with alkyl groups [24]. C-C-C skeletal vibrations in isopropanol exhibit absorptions at wavenumbers 1175 to 1140 cm^{-1} and 840 to 790 cm^{-1} for a -$C(CH_3)_2$ grouping. In aliphatic alcohols such as isopropanol and ethanol, C-O stretching vibration and C-H deformation vibration absorption bands with wavenumbers ranging from 1350 to 1030 cm^{-1} are observed (Figure 1). The broad O-H stretching vibration peaking at 3350 cm^{-1} is a distinguishing feature of absorption that is only present in the infrared spectra of alcohols and is not present in ethers. Quality control of these measurements was done using the SAMs. Figure 3 shows the IR spectra of SAM_52–72%, and also shows the peak area of the SAMs. In comparison, the HGS_15–75% and SAM_52–72% samples revealed a high degree of similarity in terms of peak position and concentrations.

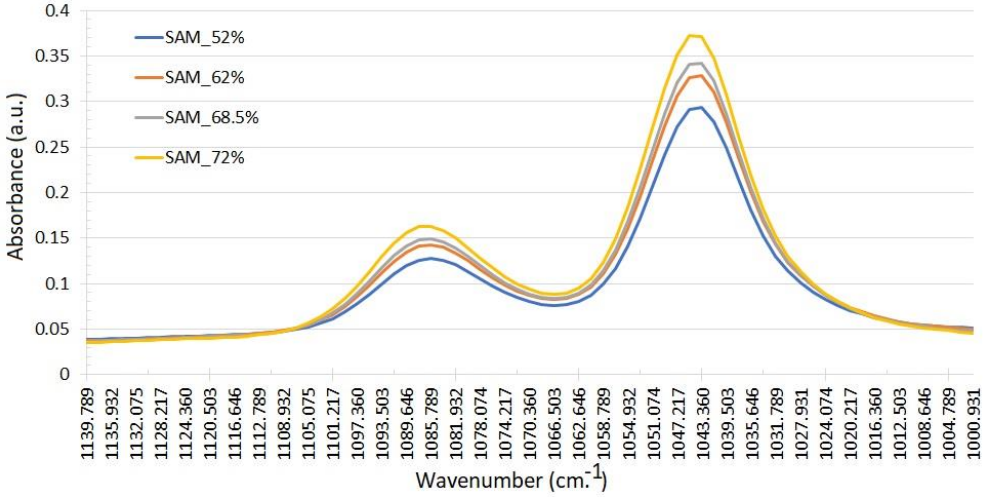

Figure 3. The IR spectra of hand sanitisers (SAM_52-72%) used for the quality control purpose

The mode of drawing the baseline is critical for quantitative measurement in FTIR spectroscopy, as it frequently determines the magnitude of the error. In this study, baseline correction was employed in the peak area calculation. This process was simplified by OPUS software. The interference peak's contribution to the area was eliminated, and no errors were discovered in the result during the subtraction procedure. The overlapped peak-decomposition method is typically used with a fitting procedure (Gaussian or Lorentz fitting), and it is critical to select the fitting parameters and appropriate baseline corrections to obtain correct results. As a result, measuring the peak area is simple in many commercial instruments.

3.1. Calibration Curves from Standard Samples and Quality Control Samples

The calibration curve was developed using IR spectra derived from the area of peaks at the wavelength of 1110.5 cm^{-1} to 1007.0 cm^{-1}, which correspond to the C-O stretch in a primary alcohol (Figure 2). The peak area was measured against ethanol concentrations using OPUS software, and a linear calibration curve was created. Figure 4 shows the relationship between the area of the curve and its concentration. The area of identified peak was represented by **y**, whereas the percentage of ethanol concentration was represented by **x**. The slope value of the ethanol standard sample (HGS_0–75%) was 0.1267, whereas the slope value of the quality control sample (SAM_52–72%) was 0.1285. These two slopes appeared to have a high correlation coefficient (r) greater than 0.99. Because they are in the same matrix, the slopes of the HGS_0–75% and SAM_52–72% samples are very similar.

SAM_52–72% results are consistent with those of HGS_0–75%. The alcohol concentration could be shifted 2% by the addition of 10.0 mL of 95% ethanol. The ethanol concentration of all samples was increased to the expected level. Following validation of the SAM's linearity for ethanol concentration, a single point of SAM can be substituted for the developed multiple standard addition points. Only one addition of 2% ethanol to the standard sample must be quantitatively prepared to measure the ethanol concentration. The single-point method gives the same result as the multiple-point standard addition method, and it can be used to accurately determine the ethanol concentration in hand sanitizer In summary, the SAM can be used to control the measurement quality. Only one standard addition sample is required for the conventional analysis of ethanol concentration. However, more attention should be paid to the preparation of hand sanitisers containing more than 75% ethanol due to the ethanol evaporating during the mixing and stirring process [25].

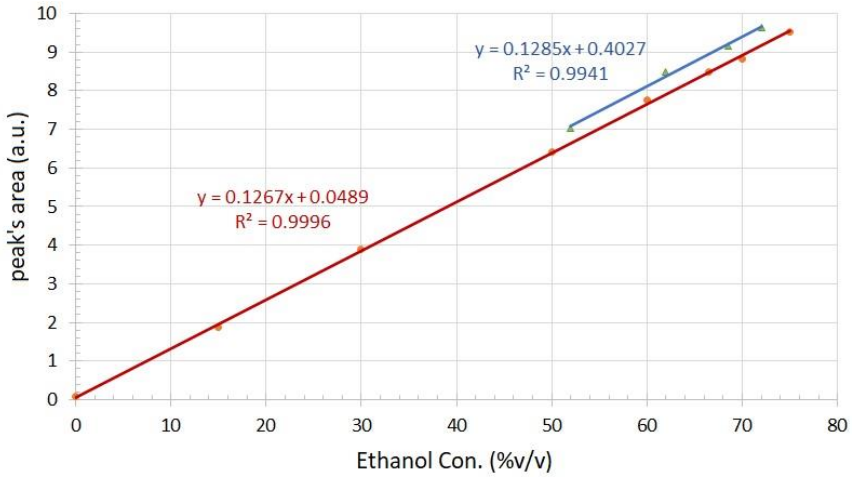

Figure 4. Peaks area vs ethanol concentration of hand sanitiser: the calibration curves and its quality control by SAM

The correlation coefficient, r (square root of R^2 in Figure 4), measures the degree of correlation between the **y** and **x** values. These R^2 are described as 'Multiple R' in the MS Excel output. The correlation coefficients are 0.9997 and 0.9970, respectively. One of the statistics frequently used in analytical measurement is the correlation coefficient r. Surprisingly, it is easily misunderstood because correlation and linearity are only tangentially related. The coefficient r measures correlation rather than linearity. It is relatively simple to generate data that appear to have a strong correlation. A plot of the data, on the other hand, may reveal that the data are unsuitable for calibration. In our experiment, as shown in Figures 4, the goal of presenting R^2 is only to compare the slope of the two plots. We can only say that adding 10.0 mL of ethanol shifts the ethanol concentration in hand gel sanitiser by 2%. To have small uncertainties in the calibration curve predictions, r must be very close to 1. However, it was uncommon in practical analysis. Barwick shows the value of r that would indicate a statistically significant correlation for a different number of data points [18]. In our case, with eight data points, a value of r = 0.7 would be statistically significant at the 95% confidence level. However, a calibration curve with a correlation coefficient of 0.7 is unlikely to be useful because the uncertainties associated with predicted values obtained from such a line would be prohibitively large. As described in the previous section, the LPU was the best fit for predicting ethanol concentrations and their uncertainties in this case.

3.2. Regression Analysis and Residual Plot

In this investigation, we discovered the effect of individual outliers. The peak areas of HGS_80% sample shifted the intercept of the fitted line. These individual outliers were removed from the calibration curve. From the previous results, the calibration curve of HGS_0–75% sample was employed to calculate the ethanol concentration of the commercial products. The linear relationship between peak area (**y**) and

concentration level (**x**) was established by the linear regression equation. This relationship is described by Equation 5.

$$\hat{y}_i = 0.1267x_i - 0.0489 \quad (5)$$

A residual is the difference between an observed y_i value and the $\hat{y}_i$ value calculated using the equation of the fitted line (see Figure 4 and Equation 1). The residual plot of the calibration curve is shown in Figure 5. These residuals are distributed roughly randomly around zero, and there is no correlation between residual spread and concentration. In Figure 5, the sum of squared residuals shows a well-fitting line. This line with the smallest sum of squared residuals is the best representation of the linear relationship between the **x** and **y** variables. According to Equation 3, the regression coefficients and residual standard deviation are required. Table 3 lists the necessary parameters used for the uncertainty estimation process.

Using partial least squares regression (PLS), the concentration of ethanol in hand sanitiser was predicted based on peak areas and intensities [12]. FTIR with PLS offers significant advantages compared to traditional methods. The FTIR method can be used to determine both the methanol and ethanol content of extract-free samples and the ratio of these two volatile components in brandies. The significance of the methods is justified by the fact that they are a quick, efficient, and non-destructive tool for screening alcoholic beverages [26].

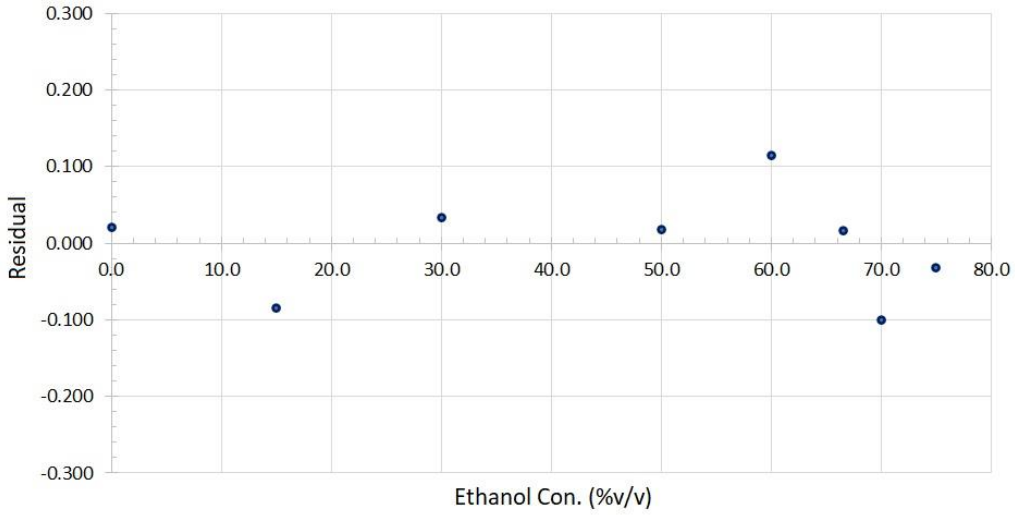

Figure 5. The residual plot of calibration data

Table 3. Statistic results of the regression and residual analysis

Sample No.	True (x_i)	$(x_i - \bar{x})^2$	Abs. peak's avg. area (y_i) m=3	Predicted ($\hat{y}$)	Residuals $y_i - \hat{y}$	(Residuals)^2 $(y_i - \hat{y})^2$
HGS_0%	0.0	2098.785	0.069	0.0489	0.020	0.000
HGS_15%	15.0	949.410	1.865	1.9494	-0.084	0.007
HGS_30%	30.0	250.035	3.884	3.8499	0.034	0.001
HGS_50%	50.0	17.535	6.402	6.3839	0.018	0.000
HGS_60%	60.0	201.285	7.766	7.6509	0.115	0.013
HGS_66.5	66.5	427.973	8.491	8.4745	0.017	0.000
HGS_70%	70.0	585.035	8.817	8.9179	-0.101	0.010
HGS_75%	75.0	851.910	9.520	9.5514	-0.031	0.001
n=8	$\bar{x}$	$\sum(x_i$	$\bar{y}$			$\sum(y_i - \hat{y})^2$
m=3	45.812	5381.968	5.851			0.034

3.3. Predicted Ethanol Concentration with Uncertainty of Measurement

The commercial hand sanitisers were obtained from a medical drug store in Chonburi, Thailand. These products may contain up to 70% v/v of ethanol as labelled. FTIR spectra of these samples were determined, and the area of C-O adsorptions was recorded (as seen in Figure 6). Other absorption peaks were also observed at different wavelengths because commercial products have different compositions, and different vibrational absorptions resulted from those of various molecular types. In the literatures, methanol was frequently found at the wavelength of 1020 cm^{-1} in samples [27,28]. Infrared spectra of methanol, ethanol and n-propanol were reported to demonstrate the effect of methanol in the absorbance IR spectrum [27]. Because the methanol and ethanol absorbance peak positions are so close, the low limit of methanol detection was reported by Coldea et al. A mixed solution of ethanol: methanol was added with increasing concentrations of pure methanol 2.5%, 5%, 9%, 20%, 33%, 43%, and 50% v/v, and the intensity frequencies at 1112 cm^{-1} and 1020 cm^{-1} gradually increased. The gradual evolution of methanol and ethanol absorption signals at 1047 cm^{-1}, 1087 cm^{-1}, 1020 cm^{-1}, and 1112 cm^{-1} were clearly observed [26]. This study also looked at the presence of methanol in commercial products. Figures 1, 2, 3 and 6 show no methanol absorption peak in any of the sampled products. As a result, the FTIR technique was useful for both identifying and quantifying ethanol and for detecting the presence of methanol and determining the ethanol-to-methanol ratio. Based on the LPU, the uncertainty of each measurement on the hand sanitisers are shown in Table 4. The residual variance is calculated using Equation (2) and Table 3:

$$S_e^2 = \frac{\sum(y_i - \hat{y}_i)^2}{n-2} = \frac{0.034}{8-2} = 0.0057 \quad (6)$$

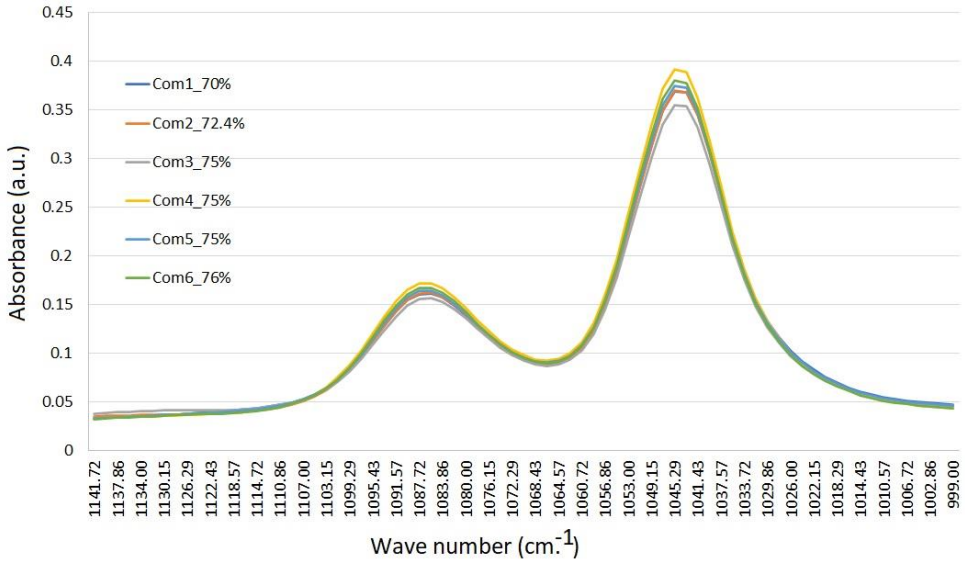

Figure 6. The IR spectra of commercial hand sanitiser products

From Equation (6), the residual standard deviation is S_e = 0.075. Note that three measurements are made on each sample, so m = 3. Applying Equation (3), the prediction interval for a sample that gives an instrument response of COM1_70% sample, y_0= 9.318, is as follows:

$$u_{x1} = \frac{S_e}{b}\sqrt{\frac{1}{m}+\frac{1}{n}+\frac{(\bar{y}_1-\bar{y})^2}{b^2\sum(x_i-\bar{x})^2}}$$

$$u_{x1} = \frac{0.075}{0.1267}\sqrt{\frac{1}{3}+\frac{1}{8}+\frac{(9.318-5.851)^2}{0.1267^2(5381.968)}}$$

$$u_{x1} = 0.457$$

The predicted value of x_0 is as follows:

$$x_{pred} = \frac{9.318-(0.0489)}{0.1267} = 73.157 \quad (7)$$

The 2-tailed Student-t value for n = 8 degrees of freedom at the 95% confidence level is 2.36. The 95% confidence interval for x_{pred} is 0.457 × 2.36 = ±1.078% v/v. By the subsequent calculations from Equations 6–7, which are the prediction intervals, the measurement uncertainty is shown in Table 4. The ethanol concentration of commercial hand sanitiser is around 71.36–81.54% v/v (Table 4). According to the WHO guide, all commercial products can be used to kill bacteria and viruses on organic surfaces. The COM4_75% sample is 8.72% different from its label, whereas sample number COM5_75% is very close. When the 95% confidence interval is used, the predicted values retain their associated measurement uncertainties, as shown in the last column of Table 4.

Table 4. Quantitative results of %ethanol and its uncertainty exist in the commercial hand gel sanitizer products

Sample No.	**Label (% v/v)**	**Abs. peak's avg. area (y_i) n=3**	**predicted value (x_{pred})**	**u_{xi} (% v/v)**	**u_{ix} at 95% of confidence**	**Report Value (% v/v)**	**% Different from label**
Com1_70.0	70.0	9.318	**73.157**	0.457	1.057	**73.52**±1.06	4.51
Com2_72.4	72.4	9.618	75.526	0.467	1.079	75.53±1.08	4.32
Com3_75.0	75.0	9.091	**71.366**	0.451	1.041	**71.38**±1.04	4.85

Com4_75.0	75.0	10.380	81.540	0.494	1.140	81.54±1.14	8.72
Com5_75.0	75.0	9.480	74.436	0.463	1.068	74.44±1.07	0.75
Com6_76.0	76.0	10.060	79.014	0.482	1.114	79.01±1.11	3.97

The estimated uncertainty for the entire report is around 1.14% v/v. When releasing the report, however, the measurement uncertainty should be minimised. The preparation of the standard concentration process and a number of replicate measurements (m) were the two main sources of uncertainty in this measurement. A 'best-fitted' calibration curve is produced by a well-prepared standard sample and a precise volumetric of starting materials, resulting in a small residual variance. The standard uncertainty decreases as the number of measurements increases (see Equation 3). In this experiment, the ethanol standard concentrations of 80% v/v were removed from the calibration curve because of their residual value. Furthermore, ethanol concentrations of 80 and 95% v/v evaporate easily in ambient air, making preparation and measurement difficult. We believe that most hand sanitisers contain 60–75% v/v ethanol. The standard ethanol concentration used in this study, 0–75% v/v, covered the working interval of calibration. The uncertainties of measurements, estimated at approximately ± 1.14% v/v at the 95% confidence interval, is satisfactory for the testing laboratory.

The ultimate purpose of this research project is to develop standard operating procedures (SOP) for alcohol concentration testing in accordance with ISO/IEC 17025 requirements. The calibration standards concentrations should be evenly spaced across the calibration range and should adequately cover the range of concentrations encountered for test samples. The validation of methods, quality control of testing results, and decision rule will be well established as a result of these investigations. The degradation of the hand sanitizer after the first usage will be the focus of future research. The effect of the matrix in ethanol gel on the calibration curve will be of great interest. The ethanol concentration will be measured quantitatively using gas chromatography and compared to the concentrations measured using FTIR spectroscopy.

4. Conclusions

Ethanol concentrations, with uncertainty estimations, in hand sanitisers were successfully measured by the ATR-FTIR spectroscopy. Infrared absorption spectra of ethanol were discovered to be at 1086 cm^{-1} and 1044 cm^{-1}, corresponding to C-O stretching. The area under the C-O adsorptions is used to create a calibration curve, which is then used to calculate the percentage of ethanol. No methanol absorption peak was observed in any of the samples. The linear relationship between the peak area of standard samples and their concentration level was also successfully demonstrated by the linear regression equation. Standard samples produced an excellent calibration curve with a slope of 0.1267. Quality control was done by the standard addition method. The alcohol concentration of each standard sample could be shifted 2% by the addition of 10.0 mL of 95% ethanol. These results indicated that the calibration curve and addition method are promising for measuring the concentration of alcohol. The regression coefficients and residual variance showed the best fit with the prediction value. These parameters were used to estimate the uncertainty of the measurement process. The concentrations of ethanol measured from commercial hand sanitiser products were slightly different from the label values. This could be because of the manufactured process or evaporation during packaging and measuring. However, commercial products can effectively kill bacteria and viruses on organic surfaces. The uncertainties of ethanol concentration measurement were also estimated using the regression coefficients and residual variance. The uncertainties estimation was approximately ±1.14% v/v with a 95% confidence level. The preparation of ethanol standard samples, standard addition method, regression analysis, and uncertainty estimation were all significant processes in establishing standard operating procedures for measuring alcohol concentration in hand sanitizer This result emphasises the importance of government authorities maintaining constant vigilance to ensure that the products meet the required specifications. This process can also be used in industries to ensure quality control.

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