1 Introduction

Catalytic conversion of renewable sources (biomass) to bio-fuels and other chemical products has received considerable attention because of economic, environmental, and political concerns [1]. Thermochromically, there are three different technical approaches for biomass conversion i.e., gasification, pyrolysis, and hydrolysis [1, 2]. Among these approaches, pyrolysis is probably the only industrially realized process for conversion of biomass [3]. In pyrolysis, the vapors formed during the process are condensed into a liquid product, which is commonly referred to as bio-oil. The bio-oil from the biomass pyrolysis process has been reported as the most abundant product (60-75 wt.% of biomass), while other products may be present at low abundance including char (10-20 wt.% of biomass) and non-condensable gases (10-25 wt.% of biomass) [1, 3, 4].

Biomass pyrolysis is known as the most innovative and promising technique for the production of bio-oil. Nowadays, biomass pyrolysis has been effectively developed and the process is able not only to produce high amounts of bio-oil but also to meet standard requirements. Practically, however, the physico-chemical characteristics of the bio-oil such as high acidity, corrosiveness, high water content, high viscosity, low heating value, thermalchemical instability, and poor ignition properties have limited the application. Thus, the idea of upgrading bio-oil has been explored, aiming to overcome the aforementioned problems. Two major routes have been investigated for the bio-oil upgrading process [5]. The first is catalytic cracking using acidic zeolite-based catalysts in the absence of H₂ [6]. The second is hydrodeoxygenation (HDO): catalytic hydro-processing under high pressure H₂ at elevated temperatures [7]. Between these two major routes, HDO is more advantageous compared with catalytic cracking because HDO gives a better quality of fuel products that are less energy dense, non-corrosive, and easily separated from water [8, 9]. HDO is therefore a highly sought-out technology for the upgrading of bio-oil into fuel and chemicals. In principle, HDO of bio-oil is a catalytic
hydrogenolysis process for removing oxygen from oxygen containing bio-oil compounds in the presence of H₂ under high-pressure conditions [9, 10]. The HDO process may occur as a set of complex reactions due to a wide span of reaction temperatures and the complexity of the biomass composition. Therefore it is difficult to understand the exact mechanism [9]. In an HDO process it is important to highlight that the products, in the form of liquid fuels (organic and aqueous phase) and gases (gaseous phase), are characterized by chemical compounds containing the elements C, H, and O[11]. The liquid fuels may contain numerous individual organic compounds [1, 8, 11, 12] and other components such as H₂, CO₂, CO and CH₄ might be found in the gaseous phase [12, 13].

From an analytical point of view, identification of individual components or classes of compounds using many dedicated analytical techniques has been intensively reported. The techniques may include gas chromatography equipped with mass spectroscopy [14,15], flame ionization [15, 16], or thermal conductivity detection [16]. In addition to gas chromatography, other techniques such as Fourier transform infra-red [17, 18], nuclear magnetic resonance [18], gel permeation chromatography [18], high performance liquid chromatography [19], and supercritical fluid chromatography [20] have also been reported. Among those reported techniques, gas chromatography is used almost exclusively for both qualitative and quantitative identification since this technique can achieve a complete separation of a wide variety of compounds.

The primary reason for the upgrading of bio-oil under high pressure and temperature is to yield useful bio-liquid fuels [9, 10, 12], implying that the component by-products in the gaseous phase such as H₂, CO₂, CO, and CH₄ may not be included as the main targets. In fact, published scientific reports on quantitative measurements of the component by-products in the gaseous phase are fewer than those of components in the liquid phase. The component by-products in the gaseous phase may be neglected when the amounts are low. However, under the pollution program perspective, tiny amounts of component by-products in the gaseous phase, mainly CO₂, CO, and CH₄, are regarded as undesirable. Those components are considered as pollutants when they are released into atmospheric environment and will particularly affect the urban atmosphere. Regardless of the released amount of those components into the atmospheric environment it is important to relate the fact that accurate and reliable data from any measurement is required because accurate and reliable measured data is commonly used as the basis for decision making associated with regulatory enforcement in any aspect of our daily lives [21, 22]. In the area of analytical chemistry, accurate and reliable data may only be achieved by validating the analytical method. Validation of an analytical measurement method is part of quality assurance to declare that a high quality of analytical result is provided by a testing laboratory [21, 23]. The validated method is a documented procedure used by a laboratory to ensure that the method performance for measurement of a particular substance meets the criteria as required [23-25].

In this study, the analytical method of gas chromatography equipped with a thermal conductivity detector (GC-TCD) for the measurement of the main component by-products in the gaseous phase (H₂, CO₂, CH₄, and CO) of the HDO of bio-oil is validated with the purpose of obtaining reliable measurement data. Evaluation of the method validation was carried out in accordance with the state of the art, particularly in accordance with definition of the ISO/IEC 17025 guidelines [26-28] by bearing in mind the following parameters such as selectivity, precision (repeatability and reproducibility), accuracy, linearity, limit of detection (LoD), limit of quantitation (LoQ), and robustness.

2 Experimental

2.1 Materials

Two standard gas mixtures (labeled as SGM-X and SGM-Y) were prepared in-house by the gravimetric method. The preparation procedure is described in the Preparation of SGMs section. The gaseous sample was obtained from the HDO bio-oil process using the procedure described in the HDO of bio-oil section. All gases used (H₂ with 99.999% purity, CO with 99.9% purity, CO₂ with 99.999% purity, CH₄ with 99.993% purity, He with 99.999% purity, and N₂ with 99.999% purity, Air Liquide Indonesia) were of ultra high purity grade and used without further treatment.

2.2 Methods

Catalyst Preparation and Characterization. The catalyst used for the HDO process was ruthenium (Ru) metal supported by an activated carbon catalyst (Ru/C catalyst). The Ru/C catalysts were prepared by the wet impregnation method. The amount of Ru metal loaded onto activated carbon was varied i.e., 1%, 3% and 5% based on the mass of activated carbon. The prepared Ru/C catalysts were then analyzed by a surface area analyzer, scanning
electron microscope (SEM), and X-ray diffraction (XRD). The detailed procedure can be found in [29].

Preparation of SGMs: Two standard gas mixtures (SGM-X and SGM-Y) containing \( \text{H}_2, \text{CO}_2, \text{CO} \), and \( \text{CH}_4 \) at various concentrations were prepared in-house using a gravimetric method in accordance with ISO Guide 6142 [30], which has been also used as an adopted procedure in our previous work [22, 23, 31, 32]. In a typical experiment, clean and well-evacuated aluminum compressed gas cylinders (Liaoning Alsafe Technology, China) with 2.5 L internal volume and a brass valve were used to prepare the SGM-X and SGM-Y. Each evacuated cylinder was first weighed and then with the filled \( \text{CO}_2 \) on an analytical balance (Mettler Toledo, 0.1 gr readability) and this step was repeated for \( \text{CO}, \text{CH}_4 \), and \( \text{H}_2 \), consecutively. Following the addition of the \( \text{CO}_2, \text{CO}, \text{CH}_4 \), and \( \text{H}_2 \), a certain amount (gr) of high purity \( \text{He} \) was added into the cylinder. The final concentrations of \( \text{CO}_2, \text{CO}, \text{CH}_4 \), and \( \text{H}_2 \) in SGM-X and SGM-Y are tabulated in Table 1. The SGMs were prepared by considering not only the maximum allowable level of target relative uncertainty (1.25%, \( k=2 \)), but also user safety issues. The target relative uncertainty of the SGM primarily depends on the weighing process of the gas during SGM preparation. If too small an amount of gas was added there was a correlation within creased relative uncertainty of the SGMs, while too high an amount of gas would increase the potential for explosion if there was cylinder leakage and the gasses were exposed to air because the SGMs consisted of some flammable gases such as \( \text{H}_2 \) and \( \text{CH}_4 \) (Table 1).

GC instrumentation systems and operating conditions: The separation of the target gas components (\( \text{CO}_2, \text{CO}, \text{CH}_4 \), and \( \text{H}_2 \)) from the mixture was conducted based on previous studies [31, 33]. Typically, a GC instrumentation system (Agilent Model 6890 series, CA, USA) was used under optimized analytical conditions (Table 2). A single stage dual-packed GC column was used. In such a dual-packed column, a packed J&W porapack Q column (6 feet x 1/8 inch o.d. x 2 mm, 80-100 mesh particle size) was connected in series to a packed J&W molsieve 5A column (9 feet x 1/8 inch o.d. x 2 mm, 80-100 mesh particle size).

Measurement of SGMs: Measurement of the target gas components in all SGMs was conducted as follows. A certain amount of gas sample was introduced into the column of the GC-TCD system at a constant flow rate (28 mL/min) under operating conditions as tabulated in Table 2. To introduce the gas sample into the GC system with a constant sample flow rate, a Brook Type 5800E mass flow controller was used. The mass flow device was installed just before the GC injection line. The gas sample was passed through the 2 mL sample loop at a rate of 100 mL=min. The temperature of the valve box was set at 100°C. The target gas components were detected using a TCD. Automated integration of the area under the resolved chromatogram profile and estimation of the chromatographic data was conducted by OpenLAB CDS Chemstation version A.2.3.57 installed on an HP computer (HP Pavilion Slimline 400 PC series) [31, 33].

Development and validation of analytical methods: The analytical method was first developed by optimization of the GC-TCD. The optimization process was conducted by injecting the sample while varying instrument conditions such as oven temperature, detector temperature, and flow rate of carrier gas. Subsequently, the optimized method was validated and the following validation parameters were taken into account including selectivity, precision (repeatability and reproducibility), accuracy, linearity, limit of detection (LoD), limit of quantitation (LoQ), and robustness. The calculation procedures for the determination of each parameter were adopted from the literature [23, 26, 30, 31, 33, 34] and can be briefly described as follows. The calculation for the selectivity of the method was conducted by performing seven replicate injections of the gas standard (SGM-X) and was evaluated in relation to its selectivity factor (\( \alpha \)). The precision of the GC-TCD method was evaluated based on method repeatability and reproducibility. Repeatability of the method was established by measuring the response of the SGM-X gas standard and expressed as percentage relative standard deviation (%RSD) of seven injection replicates over a short time interval. For the reproducibility evaluation, the procedure remained the same as the repeatability evaluation, but the injection of the SGM-X gas standard was conducted on different day. The accuracy of the GC-TCD method was evaluated by comparing the concentration of the SGM-X gas standard against another independent gas standard (SGM-Y). The linearity of the method was determined from a calibration curve evaluation. For the construction of the calibration curve, a high precision gas calibrator (MCZ Umwelttechnik, Germany) was used as a diluter system to introduce different amounts of the SGM standard into the GC instrument at a constant rate and then the peak area of each component in the SGM standards was plotted against the concentration. The LoD and LoQ of the method were established at a signal-to-noise ratio (S/N) of 3 and 10 respectively, of the chromatogram at the lowest concentration of each component used. The robustness of the method was evaluated by performing small changes in the GC-TCD operating conditions such as detector temperature, flow rate of carrier gas, and oven temperature.
Hydrodeoxygenation of bio-oil: For the hydrodeoxygenation (HDO) of bio-oil, a stainless steel autoclave type reactor having an internal volume of 68 ml was used. The HDO procedure was adopted from the literature [35]. In a typical experiment, a certain amount of bio-oil (10 g) and Ru/C-based catalyst (0.5 g) was fed into the reactor, followed by feeding H₂ gas into the reactor to give a pressure of 20 bars and then the reactor was immediately tightly closed. The HDO reaction was carried out at 250°C for 4 h. At the end of the HDO reaction, the reactor containing HDO products was allowed to reach room temperature. The gaseous HDO products were then collected into gas sampling bags for further GC-TCD measurement.

Measurement of HDO gaseous products: A certain amount of the gaseous of HDO products from the gas-sampling bag was injected into the GC-TCD and the measurements were conducted using the validated analytical method. In the case of large concentrations of components in the HDO gaseous sample, a dilution was performed so that the concentrations of target gas components in the gaseous sample were located in the concentration range used for constructing the calibration curve. This approach will not cause any problems in the validation method process even though the actual concentration of target component gas is much higher than the available concentration of components in the SGMs.

3 Results and Discussion

3.1 Catalyst Characterization

The characterization results showed that the surface area of 1%, 3%, and 5% carbon catalyst-loaded Ru was found to be 781, 775, and 745 m²/g, respectively, showing that an increase in the amount of loaded Ru led to a decrease in surface area. The decrease in surface area of catalyst from an increase in Ru was caused by the impregnation of Ru into the pores of the activated carbon, and the surface area of non-impregnated activated carbon was 789 m²/g. From SEM-EDX analysis, it was confirmed that the surface morphology was porous, and the existence of metallic Ru was not detected as the metal was impregnated into pores of the catalyst [36]. Moreover, according to XRD analysis it was found that the catalyst was amorphous which is characterized by a broadening of the 2θ at peak 20-30°. The detailed characteristics of the Ru/C catalyst can be found in [29].

3.2 Development of the Analytical Method

Method development is the initial step in every method validation process. In this study, the GC-TCD method development was carried out by optimizing the following GC parameters: detector temperature, flow rate of carrier gas, and oven temperature. These GC-TCD conditions are considered crucial for achieving good component separation [31, 37]. Practically, these three parameters were optimized separately using the SGM-X gas standard then optimized in combination.

In this investigation, it was observed from Figure 1 that all gas components were well-separated under the optimum conditions of the GC-TCD instrument (Table 2) with their retention times (tᵣ) listed in Table 3. From Table 3, it can be seen that the percentage relative standard deviation (%RSD) of the tᵣ is very low, indicating that the GC had good consistency from run to run. In addition, no other interference peaks can be found, implying that the GC-TCD method has been successfully developed [31, 32]. Thus, the method validation process can be continued.

3.3 Validation of Analytical Method

Selectivity: The selectivity or selectivity factor (α) of a GC is defined as the ability of the GC method to distinguish and quantify the response of target component in the presence of other components as interference in the mixture. Therefore α is considered to be crucial [23, 38]. The α refers to the relative retention time of two adjacent peaks. The α value is highly dependent on the change of the tᵣ of the

| Standard Gas Mixtures (SGMs) | Gas component |
|-----------------------------|---------------|
|                             | H₂  | CO₂ | CH₄ | CO  | He  |
| SGM-X (%mol/mol)            | 6.51| 15.58| 3.95| 8.94| Matrix |
| SGM-Y (%mol/mol)            | 1.74| 4.14 | 1.05| 2.38| Matrix |

Note: Relative uncertainty for both standards is 1.25% (k=2)
two corresponding target gas components. From Table 3, it can be observed that all $\alpha$ values between two adjacent peaks are found to be excellent having a value larger than 1.0 [39]. Thus, the GC-TCD used in this study shows its ability to discriminate each target gas component without any interference.

### Table 2. Optimized analytical conditions of the GC-TCD

| Apparatus                  | Agilent 7890 GC System |
|----------------------------|------------------------|
| Sample loop                | 2 mL, stainless steel  |
| Valve box temperature     | 100 °C                 |
| Oven/Column temperature    | Temperature program, 40°C (10 min), rate:60°C/min, 160°C (1 min) |
| Running time               | 13 min                 |
| Gas carrier                | Helium ultra-high purity grade (99.999%) |
| Carrier gas flow rate      | 28 mL/min              |
| Detector temperature: He   | 250 °C                 |
| Reference flow (He)        | 20 mL/min              |
| Make up flow (He)          | 7 mL/min               |

Figure 1. Representative GC-TCD chromatogram of the SGM-X at optimized conditions, showing a good peak separation of target gas components (For interpretation of the references to color in the figure, the reader is referred to the web version of the article).

### Table 3. Retention time and selectivity factor of the target gas component

| Gas Component | Parameter | Retention time ($t_R$, min) | Selectivity factor ($\alpha$) |
|---------------|-----------|-----------------------------|-----------------------------|
| H$_2$         |           | 1.59                        | 2.17                        |
| CO$_2$        |           | 3.46                        | 3.02                        |
| CH$_4$        |           | 10.44                       | 1.15                        |
| CO            |           | 12.01                       |                              |

Precision: In this study, repeatability and reproducibility parameters were used to evaluate the precision of the analytical method. The method reproducibility defines the closeness between measured values obtained from an independent measurement using the same instrument and under the same analytical conditions by the same operator and within a short time interval [23, 34, 40]. The repeatability was theoretically determined by the prediction of the relative standard deviation (%RSD) using the Horwitz function (Eq. 1) [41].

\[
CV_{\text{Horwitz}}(\%) = 2(1 - 0.5 \log c)
\]

where is the concentration of the target gas component stated in a decimal fraction.

Horwitz’s function requires that the %RSD for the repeatability must fall between 0.5 and 0.75 % of the CV – Horwitz. In a word, the repeatability of the method is categorized acceptable if the %RSD is less than 0.67 of the CV–Horwitz (0.67CV–Horwitz) [23, 42]. The calculated % of CV–Horwitz for each target gas component in SGM-X are listed in Table 4. It can be seen from Table 4 that each target gas component has a calculated %RSD that is less than 0.67 CV–Horwitz. These results imply that the analytical method used for the measurement of all target gas components is repeatable.

The reproducibility is an analytical parameter showing the variability of repeated measurement results obtained by using the same test method on a different measurement day [23, 24, 34]. Seven repeated injections on different days for the evaluation of method reproducibility were conducted and the results are listed in Table 4. It can be observed from Table 4 that the %RSD values for all target gas components are less than their corresponding CV–Horwitz, implying that the analytical method is reproducible for all measured components [25].

Additionally, controlling the quality of the measurement results is essential to make sure that the measurement reproducibility is fit for purpose [43]. In this regard, a control limit chart (warning and action limit) was created as it is the most common control program in the area of GC measurement. The control limit chart consists of five lines including one average line (AL), two warning limit (WL) lines, and two control limit (CL) lines. The AL represents the mean of the control measurement values. The values of the two WL lines represent the AL line ± two times standard deviation (AL ± 2SD) and the two CL lines represent the AL line ± three times standard deviation (AL ± 3SD) [23, 31, 44]. Figures 2a-d display the control limit charts for all measured components. The results indicate that the AL values for all component data (H$_2$, CO$_2$, CH$_4$ and
CO) are located inside the WL and CL lines, meaning that no error was found for the measurement reproducibility. This implies that the analytical method for the measurement of all target gas components is reproducible. On the other hand, a corrective action is required to identify and then remove the source of error when control data values fall outside the CL lines [23, 31, 43].

Accuracy: Accuracy of an analytical method can be defined as how close the values of the measured concentrations are to the accepted (true) concentration of the target components [34, 40]. The method accuracy is highly dependent on two analytical factors – bias and precision. The bias of the method indicates the difference between the measured concentration and the concentration of a certified reference standard. In this case the SGM-Y was used as the reference standard. The bias of the method was determined using the following equation (Eq. 2) [23-25, 31].

\[ C\Delta = \bar{A} - B \]  

where is the average measured concentration of the reference standard (SGM-Y), and is the value from the certified SGM-Y. To assess the method accuracy, the precision of the analytical method (σ) from repeatability and reproducibility was included. While an uncertainty value from the certified SGM-Y was also included for estimating the σ value. Thus, the value of (σ) was obtained by combining those three components using the following expression (Eq. 3) [23, 25, 31, 42].

\[ \sigma = \sqrt{SD_b^2 + SD_w^2 \cdot n + \mu_{RM}^2} \]  

Accuracy: Accuracy of an analytical method can be defined as how close the values of the measured concentrations are relative to the accepted (true) concentration of the target components [34, 40]. The method accuracy is highly dependent on two analytical factors – bias and precision. The bias of the method indicates the difference between the measured concentration and the concentration of a certified reference standard. In this case the SGM-Y was used as the reference standard. The bias of the method was determined using the following equation (Eq. 2) [23-25, 31].

\[ C\Delta = \bar{A} - B \]  

where is the average measured concentration of the reference standard (SGM-Y), and is the value from the certified SGM-Y. To assess the method accuracy, the precision of the analytical method (σ) from repeatability and reproducibility was included. While an uncertainty value from the certified SGM-Y was also included for estimating the σ value. Thus, the value of (σ) was obtained by combining those three components using the following expression (Eq. 3) [23, 25, 31, 42].

\[ \sigma = \sqrt{SD_b^2 + SD_w^2 \cdot n + \mu_{RM}^2} \]  

- Concentration in % mol/mol;  
- Repeatability (%RSD; n=7);  
- Reproducibility (%RSD; n=7)
where $SD_p$ is the standard deviation from the reproducibility evaluation, $SD_w$ is the standard deviation from the repeatability evaluation, and $\mu_{RM}$ is the uncertainty of standard SGM-Y. Based on the ISO Guide 33:2000 “Uses of certified reference materials” [45], the acceptance criterion for the method accuracy is the bias (Ca). No bias is occurred if the value falls within $\pm 2\sigma$ (Eq. 4) at a confidence level of 95%. From Table 5, it can be observed that the values of all measured components lie within $\pm 2\sigma$. This finding indicates that no bias of the analytical method can be found.

$$-2\sigma < \text{Ca} < 2\sigma$$ (4)

Linearity: In a GC measurement, the linearity of an analytical method is the ability of the GC, within the given concentration ranges, to obtain measurement results which are directly proportional to the concentration of analyte in the sample [23, 31, 34, 40]. The linearity of the GC-TCD method (Table 6) was evaluated by constructing a calibration curve using a series of CGM-X standards (CGM-X1 to CGM-X7). Each concentration level was analysed using GC-TCD in seven replications ($n = 7$). As it can be seen from Table 6, an excellent linearity was obtained for all gas components. The correlation coefficient ($R^2$) were found to be equal to or greater than 0.9956, implying that method may be fit for purpose for the determination of $H_2$, $CO_2$, $CH_4$ and $CO$ in a gaseous mixture.

LoD and LoQ: For every analytical measurement, determination of LoD and LoQ is essential. Practically, the LoD and LoQ assessments are equally important in comparison to other method validation parameters. The LoD of an analytical method refers to the lowest amount of analyte that can be detected which is not necessarily quantified as an exact value. Meanwhile, the LoQ is the lowest concentration of an analyte that can be quantitatively determined with appropriate precision [23, 24, 31, 34, 40]. Both LoD and LoQ were estimated based on the signal-to-noise ratio. The signal to noise of LoD and LoQ are 3:1 and 10:1, respectively. If the LoD and LoQ levels are higher than the measured values, it may contribute to a high measurement uncertainty, thus an unreliable measurement would occur. At a LoD level, only qualitative analysis is possible for the evaluation. While at a LoQ level, both quantitative and qualitative analyses are possible. However, at the LoQ level, the quantitative analysis performed may produce inaccuracy and imprecise results, leading to a high uncertainty contribution on the final analytical results [23, 25, 31]. The LoD and LoQ for all target gas components are listed in Table 6.

Robustness: Robustness is a common term representing the capacity of the analytical method to produce good measurement results, which remain unaffected by minor changes of the GC experimental conditions [23, 31, 40]. In this study, the robustness of the analytical method was assessed by observing the effects of slight changes in the GC operating conditions (such as detector temperature, flow rate of the carrier gas, and oven temperature) and the results are tabulated in Table 7. It can be observed in Table 7 that small changes in the GC operating condition have statistically no significant effect ($p > 0.05$) on the results of the analytical measurement for all measured components. Thus, it can be justified that the analytical method is quite stable under small changes of the GC conditions. However, a large change on the flow rate level of carrier gas has a

### Table 5. Accuracy data of the GC-TCD for the measurement of $H_2$, $CO_2$, $CH_4$, and $CO$ in their mixture

| Parameters               | $H_2$ | $CO_2$ | $CH_4$ | $CO$ |
|--------------------------|-------|--------|--------|------|
| Bias (% mol/mol)         | 0.04  | 0.05   | 0.27   | 0.02 |
| Precision of method, $\sigma$ (% mol/mol) | 0.09  | 0.12   | 0.23   | 0.14 |
| $+ \ 2\sigma$ (% mol/mol) | 0.18  | 0.24   | 0.46   | 0.28 |

### Table 6. Data indicating the linearity of the method for all gas components and their LoD and LoQ values.

| Gas component | SL  | INT  | LR       | n  | $R^2$ | LoD $^*$ | LoQ $^*$ |
|---------------|-----|------|----------|----|-------|----------|----------|
| $H_2$         | 16.66 | -1.64 | 0.30 - 6.51 | 7  | 0.9998 | 303.57  | 1011.89  |
| $CO_2$        | 462.14 | -133.60 | 0.72 - 15.58 | 7  | 0.9993 | 38.52   | 128.41   |
| $CH_4$        | 280.58 | -48.72 | 0.18 - 3.95  | 7  | 0.9956 | 182.32  | 607.72   |
| $CO$          | 392.93 | -43.67 | 0.41 - 8.94  | 7  | 0.9997 | 24.92   | 83.06    |

$SL =$ slope; $INT =$ intercept; $LR =$ linear range (in % mol/mol); $n =$ injection replications; $^* _a$ and $^* _b =$ in μmol/mol
significant effect on the measurement results [31].

Application of validated method: In upgrading of bio-oil, the Ru/C catalyst showed superior activity with a deoxygenation level of up to 90%, a high yield of oil, and the heating value of the upgraded bio-oil increased by a factor of two (up to 40 MJ kg⁻¹) [46]. Over the Ru/C catalyst, hydrogen consumption increased as a function of the degree of deoxygenation [35]. The Ru/C catalyst showed a high activity in upgrading bio-oil to produce polyols and alcohols [47]. Accordingly, the use of a Ru/C catalyst is important in upgrading bio-oil such as for HDO processes. The HDO is one method for upgrading bio-oil into high quality fuel with high carbon efficiency [48]. In a catalytic hydrotreatment process, several reactions may have occurred in parallel including hydrogenation, hydrodeoxygenation, decarboxylation, cracking/hydrocracking, and polymerization [46]. Analysis of gaseous products obtained from the HDO reaction is important because the data obtained can be used to calculate the mass balance of the reaction, to indentify the reaction mechanism, and to calculate hydrogen consumption. As mentioned above, however, this study was focused on the validation of an analytical method for quality assurance purposes to declare that a high quality analytical result is provided. For such a purpose, the gaseous product from the HDO process was used as a simple model for method validation investigation. Figure 3 depicts the GC-TCD chromatogram of a HDO gaseous sample obtained using carbon-based catalyst containing different concentration of Ru-metal. Previous studies reported that other gas components, classified as light hydrocarbon (C₂H₄, C₂H₆, and C₃H₈), may also be formed during the HDO process.

| GC Condition | Gas component | C | SD | p-V | C | SD | p-V | C | SD | p-V |
|--------------|---------------|---|----|-----|---|----|-----|---|----|-----|
| Detector temp. | H₂ | CO₂ | CH₄ | CO |
| 248°C | 6.58 | 0.032 | 15.56 | 0.013 | 4.76 | 0.013 | 8.87 | 0.009 |
| 250°C | 6.53 | 0.032 | 15.60 | 0.019 | 4.22 | 0.012 | 8.97 | 0.014 |
| 252°C | 6.58 | 0.043 | 15.61 | 0.020 | 5.11 | 0.043 | 8.89 | 0.027 |
| Flow rate of carrier gas | H₂ | CO₂ | CH₄ | CO |
| 26 mL/min | 6.41 | 0.042 | 15.60 | 0.026 | 4.42 | 0.037 | 8.93 | 0.023 |
| 28 mL/min | 6.53 | 0.043 | 15.62 | 0.030 | 4.23 | 0.037 | 8.96 | 0.017 |
| 30 mL/min | 6.60 | 0.040 | 15.56 | 0.021 | 4.49 | 0.021 | 8.85 | 0.019 |
| Oven temp. | H₂ | CO₂ | CH₄ | CO |
| 38°C (10 min), 58°C/ min, 198°C (1min) | 6.67 | 0.027 | 15.57 | 0.030 | 4.88 | 0.027 | 8.94 | 0.016 |
| 40°C (10 min), 60°C/ min, 200°C (1min) | 6.54 | 0.031 | 15.62 | 0.029 | 4.23 | 0.037 | 8.97 | 0.009 |
| 42°C (10 min), 62°C/ min, 202°C (1min) | 6.53 | 0.025 | 15.51 | 0.031 | 4.54 | 0.028 | 8.79 | 0.005 |

Note: **C** = CSD = standard deviation (n = 7 injections); p-V = statistically not significant for p>0.05.
Gaseous samples were found in high concentrations. Meanwhile, the CH₄ and CO were produced in low concentrations (see also Figure 3). A high concentration of CO₂ as the main component in the gaseous product was caused by decarboxylation and/or redox reactions among organic molecules. Water was also produced during the HDO experiment as by-product of the reaction (data not shown here) [29]. A previous study reported that water produced from the HDO process (prepared at 350°C, 200 bar, 5 wt% Ru/C, 4h of reaction time) was about 1.5% in comparison to that of the original fast pyrolysis process, where the water content was found at about 30% [35].

In this work, however, the C₂H₄, C₂H₆, and C₃H₈ components that may be present in the HDO gaseous sample were not determined since the validation method was only focused on the analysis of HDO gaseous sample using GC equipped with TCD. The GC-TCD is not suitable for measurement of C₂H₄, C₂H₆, and C₃H₈. A GC equipped with FID, on the other hand, could be used for the precise measurement of the C₂H₄, C₂H₆, and C₃H₈ in HDO gaseous samples.

Table 8 tabulates the measured target gas components in the gaseous samples. From Table 8, it can be observed that the target gas components of H₂ and CO₂ in the gaseous samples were found in high concentrations. Meanwhile, the CH₄ and CO were produced in low concentrations (see also Figure 3). A high concentration of CO₂ as the main component in the gaseous product was caused by decarboxylation and/or redox reactions among organic molecules. Water was also produced during the HDO experiment as by-product of the reaction (data not shown here) [29]. A previous study reported that water produced from the HDO process (prepared at 350°C, 200 bar, 5 wt% Ru/C, 4h of reaction time) was about 1.5% in comparison to that of the original fast pyrolysis process, where the water content was found at about 30% [35].
The HDO product with less water content is feasible for direct application in internal combustion engines and accomplishes the rigorous application for bio-fuels.

Additionally, from Table 8, it can also be seen that measurement by GC-TCD using the validated analytical method was consistent from run to run for triplicate injections which is indicated by very low %RSD value (<1.0%). This implies that the validated method for the measurement of \( \text{H}_2, \text{CO}_2, \text{CH}_4 \) and \( \text{CO} \) in HDO gaseous samples is acceptable and it can be applied for a routine analysis.

4 Conclusions

A simple GC-TCD method was developed for the measurement of gaseous product from HDO of bio-oil. The validation of the analytical method was conducted as defined by the ISO/IEC 17025 and all parameters met the criteria acceptable for routine analysis. The proposed method is selective, precise, accurate, robust, and is able to separate the target component \( \text{H}_2, \text{CO}_2, \text{CH}_4 \) and \( \text{CO} \) from their mixtures without any special sample treatment. In addition, there was no observed interference from other possible components in the HDO gas. It can be noted from this study that the application of the validated analytical method for measurement of the \( \text{H}_2, \text{CO}_2, \text{CH}_4 \) and \( \text{CO} \) in HDO gas may provide a reliable data; thus, such a validated analytical method can be used for the routine measurement of \( \text{H}_2, \text{CO}_2, \text{CH}_4 \) and \( \text{CO} \) in a HDO gaseous sample. In addition, the validation process of the analytical method can be easily and conveniently adopted for the routine analysis of \( \text{H}_2, \text{CO}_2, \text{CH}_4 \) and \( \text{CO} \) components in the gaseous phase of HDO of bio-oil.

Acknowledgement: The authors greatly appreciate financial support from the Indonesian Government within the scope of project “Strengthening Chemical Metrology Competency and Infrastructure” and project “Catalytic Conversion of Palm Oil Waste into Bio-Oil and Fine Chemicals” of the LIPI. Authors also recognize financial support from the Indonesian Government within “Beasiswa Competency and Infrastructure” and project “Catalytic Conversion of Palm Oil Waste into Bio-Oil and Fine Chemicals” of the LIPI. Authors also recognize financial support from the Ministry of the Research, Technology and Higher Education of Indonesia through “Beasiswa Program Pendukung 2017”.

References

[1] Huber, G.W., Iborra, S., Corma, A., Synthesis of transportation fuels from biomass: chemistry, catalysts, and engineering. Chem. Rev., 2006, 106, 4044-4098.
