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TRACE MEASUREMENT OF CO, CH₄, AND CO₂ IN HIGH PURITY GASES BY GC-FID-METHANIZER: METHOD VALIDATION AND UNCERTAINTY ESTIMATION

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Abstract: In this study, method validation and uncertainty estimation for the measurement of trace amounts gas impurities such as carbon monoxide (CO), methane (CH₄), and carbon dioxide (CO₂) using gas chromatography flame ionization detector with methanizer (GC-FID-methanizer) are reported. The method validation was performed by investigating the following performance parameters such as selectivity, limit of detection (LOD), limit of quantification (LOQ), precision, linearity, accuracy, and robustness. The measurement uncertainty to indicate the degree of confidence of the analytical results was estimated by using a bottom up approach. The results reveals that the method possess good repeatability (% relative standard deviation RSD < 1 %) and intermediate precision (RSD % < 5 %) properties for the measurement of trace level CO, CH₄, and CO₂. No bias was found for the validated method. The linearity of the method was found to be remarkable with correlation coefficient (R²) higher than 0.995 for all target analytes. In addition, the measurement uncertainty of the CO, and CO₂ in high purity helium (He) gas sample measured using the validated method were found to be 0.08 μmol·mol⁻¹, and 0.11 μmol·mol⁻¹, respectively, at 95 % of confidence level. No measurement uncertainty was obtained for CH₄ in high purity gas sample due to its concentration was below the GC-FID-methanizer detection level. In conclusion, the GC-FID-methanizer under experimental condition of this study is reliable and fit for the measurement of trace levels of CO, CH₄ and CO₂ in high purity gas samples.

Keywords: *gas impurities, industrial gases, measurement uncertainty, method validation, precision, trace concentration*

INTRODUCTION

Measurement of trace levels of gaseous impurities in high purity gases for many applications including specialty gases, semiconductor, hydrocarbon processing, food, and the medical industries has become an essential aspect [1, 2]. The identification and quantification of the trace amount of gaseous impurities is not only affect the price of high purity gases itself but also the processes and quality of the end products [1 – 3]. In this regards the developments of accurate and reliable analytical methods for the measurement of gaseous impurities at trace levels (ppm and sub-ppm) are tremendously needed.

Various analytical methods for the measurement of trace level gaseous impurities are available and have been commonly employed such as electrochemical sensor [4], gas chromatography (GC) thermal conductivity detector (TCD), and flame ionization detector with methanizer (FID-methanizer) [2, 5], gas chromatography pulse discharge helium ionization detector (GC-PDHID) [3], Fourier transform infrared spectroscopy (FTIR) [6], and gas chromatography mass spectrometry (GC-MS) [7]. Dragger and Luebeck reported the analysis of trace levels of carbon monoxide (CO) in the air by using electrochemical sensor [4]. A GC equipped with TCD and FID-methanizer combination with a pre-concentration technique to determine trace amounts of oxygen and argon (O_2+Ar), nitrogen (N_2), carbon monoxide (CO), methane (CH_4), and carbon dioxide (CO_2) in high purity hydrogen has been reported by Kamisnky et al [2]. Weijun described the development of method for the measurement of trace level impurities such as H_2 , O_2 , Ar, N_2 , helium (He), methane (CH_4), ethylene (C_2H_4) and propylene (C_3H_6) in bulk gases by using GC-PDHID [3]. Additionally, FTIR has been applied by Esler et al [6] for simultaneously measurement of trace levels of CO, CO_2 , CH_4 and other compounds in the air sample. Etienne and Mettes reported the measurement of impurities in gas silane by GC-MS [7]. From all these published literatures, it was noticed that the works are closely related to method validation. Validation of analytical method is required for any new or modified method with the purpose to assure the reliability and accuracy of the measurement results [8].

In a practical experiment, a validated analytical method with full uncertainty evaluation is the basis for a reliable and accurate measurement result. According to the ISO/IEC 17025, method validation is a confirmation of method by the evaluation and determination of objective proof that the particular requirements of a specified intended use are fulfilled [9]. Thus, method validation is required to investigate whether or not an analytical method is applicable to a specific type and concentration of analyte, and fit for a particular analytical purpose. The analytical purpose of the method is the accomplishments of high quality analytical results with an acceptable of measurement uncertainty level [8, 10]. This estimation of measurement uncertainty should be applied by the laboratory because of its importance for a sufficient interpretation of the result and a necessary tool for supporting decision making [11, 12].

In this paper, method validation for the measurement of trace amount of CO, CH_4 , and CO_2 in high purity gas sample using GC-FID-methanizer was carried out by investigating several method performance characteristics, such as selectivity, limit of detection (LOD), limit of quantification (LOQ), precision, linearity, accuracy, and robustness. In addition to method performance characteristic investigation, the measurement uncertainty for the quantification of trace amount of CO, CH_4 , and CO_2 in

high purity helium (He) gas sample was also estimated to measure the quality of analytical result by employing the bottom-up approach according to “guide to the expression of uncertainty in measurement” (GUM) [12 - 16].

MATERIALS AND METHOD

Materials

The certified standard gas mixture (CSGM) containing CO, CH₄, and CO₂ in helium (He) balance was purchased from Scott Company, Plumsteadville, PA, USA and used in all experimental runs. The working standard gas mixture (WSGM) was prepared by the dilution of the CSGM with ultra-high purity of helium gas (99.999 % mol / mol, Air Liquide Company, Indonesia). The gravimetric method in accordance with ISO 6142 was applied for the preparation of the WSGM. The composition of CSGM and WSGM are presented in Table 1.

Table 1. Composition of standard gas mixture used in this study

Standard gas mixture	Concentration [μmol·mol ⁻¹]				Relative Uncertainty (from reported concentration)
	CO	CH ₄	CO ₂	He	
CSGM	7.80	7.80	7.70	Balance	±5.00 %
WSGM-A	5.94	6.02	6.02	Balance	±8.19 % (k = 2)
WSGM-1	2.03	2.06	2.06	Balance	
WSGM-2	2.93	2.97	2.97	Balance	
WSGM-3	3.95	4.01	4.01	Balance	
WSGM-4	5.86	5.94	5.94	Balance	

k - coverage factor at confidence level 95 % to express expanded uncertainty

Equipment

A GC (7890B series, Agilent Hewlett Packard, USA) equipped with FID-methanizer was used for the measurement of the CO, CH₄, and CO₂ concentration in gas mixtures. The analytical measurement procedure and operating conditions of the GC-FID-methanizer the measurement of the CO, CH₄, and CO₂ are as follows. The gas mixture sample was introduced into GC system through a Brooks Type 5800E mass flow controller (MFC) in order to maintenance a constant of the sample flow rate. The MFC was installed before the injection line of GC system. The flow rate of samples was set 100 mL·min⁻¹. Sample of gas mixture from injection line was flown through the 2 mL of sample loop. The valve box temperature was maintained at 100 °C. A stainless steel packed column (6 feet, 1/8” outer diameter), Porapak Q was used for separating the component in gas mixtures. The column oven was set to isothermal temperature at 40 °C. The ultrahigh purity of helium (99.999 %) at a flow rate 28 mL·min⁻¹ was employed as carrier gas. The methanizer and detector temperature were kept at 375 °C and 250 °C, respectively. The flow rates of H₂ and air for FID gases were 50 and 400 mL·min⁻¹, respectively.

For the gravimetric preparation of the WSGM, a mass comparator type XP10003S (10 kg of maximum capacity and 1 mg of readability) manufactured by Mettler Toledo, Switzerland was used. In a typical experiment, the mass comparator was placed on the granite stone and installed in the isolated chamber to minimize the vibration and perturbation by air flow. Transferring of the gas from the CSGM and diluting it with the high purity He was carried out by the gas filling station. The gas filling station was constructed by several components such as diaphragm valves, ball valves, electronic pressure gauge, stainless steel tubing (1/8" and 1/4" outer diameter), and scroll pump. All connections of gas filling station were set with VCR connection for leakage prevention. The gases were transferred into 1 L of high pressure aluminum cylinder having 150 bar of working pressure. Before the filling process, the cylinder was cleaned by evacuating process using a vacuum system model Hi-Cube 300 classic, Pfeiffer, Germany.

Method validation

Several method validation parameters such as selectivity, LOD, LOQ, precision, linearity, accuracy, and robustness were investigated.

Selectivity was examined by analyzing the chromatographic parameters from component of gas mixture CSGM injected to GC system. The following chromatographic parameters were evaluated for the investigation of the method selectivity such as retention time, selectivity factor, and resolution.

Signal to noise ratio from analysis of known lowest concentration of gas mixture was calculated to determine the LOD and LOQ. A typical signal to noise ratio for LOD and LOQ are 3:1 and 10:1, respectively.

To study the precision of method, repeatability (intra-day precision) and intermediate precision (inter-day precision) of the method were assessed. The repeatability was determined by measuring the CSGM at least seven replicates of sample injection under the same operating condition in the same day. To evaluate the intermediate precision, seven replications of injection measurements of the CSGM at different days during two months period of time were carried out.

Linearity was evaluated by evaluating the calibration curve of standard gas mixture. Five points of different concentration level of standard gas mixture were gravimetrically prepared to produce the calibration curve. The linearity of the calibration curves were examined by using a least squares method.

The accuracy of method was assessed by comparing between the value of bias and precision of method (σ). The bias (Δ) of the method was determined by calculating the difference between measured value and certified value of the CSGM using the equation (1):

$$\Delta = \bar{X} - Y \quad (1)$$

where \bar{X} is the average of measured value of target gas component in the CSGM, Y is the value stated in certificate CSGM. Subsequently, the precision value of method (σ) was obtained from the combined calculation of standard deviation of repeatability, standard deviation of intermediate precision, and uncertainty from CSGM using the equation (2):

$$\sigma = \sqrt{S_b^2 + \frac{S_w^2}{n} + \mu_{RM}^2} \quad (2)$$

where S_b is standard deviation from reproducibility, S_w is standard deviation from repeatability, n is the amount of replications and μ_{RM} is the uncertainty stated in the certificate of CSGM certificate.

Evaluation of the method robustness was carried out by the evaluation of the effect of slight changes of instrument condition such as oven temperature, flow rate, and detector temperature. Afterward, the statistical evaluations were performed to examine the significance difference ($p < 0.05$) among the mean of measurement results using one way ANOVA at 95 % of probability level. In addition, the Fischer F-statistic at 95 % confidence level was employed to evaluate the significance difference ($p < 0.05$) between standard deviation.

Preparation of working standard gas mixtures

The gravimetric method in compliance with ISO 6142 was applied to prepare the WSGM. The preparation of the WSGM was carried out by filling the cleaned cylinder with a certain amount (in g unit) of the CSGM followed by diluting with high purity of He as gas matrix. The weight difference on the mass-comparator between sample cylinder (empty, filled with CSGM, and filled with He gas) and tare cylinder were recorded to obtain a high accuracy of gas mass. After weighing, gas mixture was homogenized by rotating the cylinder using a homogenization system. The compositions of prepared WSGM are shown in Table 1. The WSGM-1 through WSGM-4 were used to generate the calibration curve for method linearity evaluation. Meanwhile, the WSGM-A was only used to investigate the accuracy of method.

RESULTS AND DISCUSSION

Selectivity

The measurement of trace amounts of CO, CH₄, and CO₂ using GC-FID can be problematic. Even though those components can be separated well in Porapak Q column, the CO and CO₂ component are not able detected by FID. This occurs because the FID is a type of detector that is commonly used for the measurement of organic compound containing carbon atoms, for instance hydrocarbon compound [13]. The CO and CO₂ components are not burned in FID since no organic carbon (consists of carbon and hydrogen atoms) is contained in the components. Therefore, the use of methanizer conjunction with FID is required to detect trace levels of CO and CO₂. The CO and CO₂ are catalytically converted to CH₄ before detection in FID by flushing those gases with hydrogen through hot nickel powder catalyst filled in methanizer [2, 4, 15]. The methanization is occurred based on the following reaction scheme, as shown in equation (3):

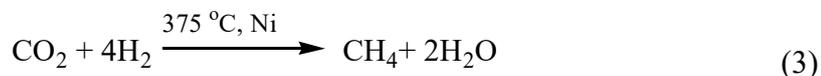


Figure 1 displays the typical chromatogram of components separation from measurement of trace level concentration of CO, CH₄ and CO₂ in CSGM by GC-methanizer-FID.

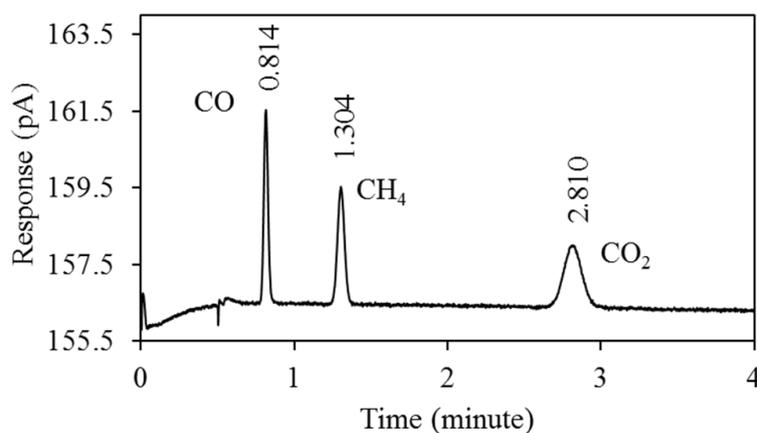


Figure 1. Chromatogram separation of components gaseous in CSGM analyzed by GC-methanizer-FID

Selectivity is the capability of the method to distinguish and measure the analyte in the existence of other components as interference [8, 6, 17]. As can be shown in Figure 1, the components of CO, CH₄ and CO₂ can be discriminated by GC-FID-methanizer under optimum operating condition at retention time 0.814 min, 1.304 min, and 2.810 min, respectively. In addition, it can be seen from Figure 1 that no co-eluting peaks near by the retention time of those target components are observed, indicating that the GC-FID-methanizer is capable to individually detect and separate those components. A good separation of the components in chromatography can be determined by evaluating the resolution and the selectivity factor. The complete separation between two peaks is achieved when the resolution value is larger than 1.5 [18]. Meanwhile, high selectivity method is indicated by the factor selectivity larger than 1. Good resolution ($R > 1.5$) and high selectivity ($\alpha > 1$) of the method (Table 2) for the trace level measurement of CO, CH₄ and CO₂ in SGM were obtained by GC-FID-methanizer method.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

LOD is the smallest concentration of an analyte that can be detected, however, it is not essentially quantified as an precise value. Meanwhile, LOQ is the lowest amount of an analyte that can be quantitatively determined with proper precision (repeatability) and accuracy. In this study, the LOD and LOQ are determined based on signal to noise ratio approach. Signal to noise ratio 3:1 and 10:1 were applied to estimate the LOD and LOQ, respectively. In this proposed method, determination of the signal to noise ratio was carried out by comparison the height of background noise from the known lowest concentration of components in gas mixtures ($2 \mu\text{mol}\cdot\text{mol}^{-1}$ of CO, CH₄ and CO₂ in He balance) with the height of peak of those known lowest concentration. The calculated LOD and LOQ of CO, CH₄ and CO₂ under this study is tabulated in Table 2. The LOD and LOQ evaluation are similarly essential with the assesment of other method

validation parameters [19]. At the concentration of LOD level, it is only possible to carry out the qualitative analysis. Nevertheless, the quantitative analysis performed at this level will produce inaccurate and imprecise result. When the quantification produces a value below the LOQ level, it can result the fairly high uncertainty which associated with the measurement result. Consequently, the unreliable measurement will occur [8].

Linearity

Linearity is defined as the ability of analytical procedure to acquire test result that are directly proportionate to the concentration of analyte in the sample [8, 19]. In order to investigate the linearity range of the GC-FID-methanizer to analysis trace concentration of CO, CH₄, and CO₂, the calibration curves were generated from five different concentrations of standard gas mixtures ranging from 2.5 $\mu\text{mol}\cdot\text{mol}^{-1}$ to 7.8 $\mu\text{mol}\cdot\text{mol}^{-1}$ (Table 2). Each concentration level was analyzed in seven replicates. The calculated of % relative standard deviation (RSD %) from each concentration level and the determination coefficient (R^2) is then compared with the acceptance criteria established for the linearity in this study (RSD % < 2 %, and R^2 > 0.995). It can be observed in Table 2 that the linear ranges were satisfactory for all gas components (CO, CH₄, and CO₂) by which the RSD % values were less than 1.5 % for each concentration level. Additionally, the values of R^2 for all gas component were found to be higher than 0.995, showing a good linearity property of the proposed method.

Table 2. Validation parameters of selectivity, resolution, LOD, LOQ, and Linearity

Parameters	CO	CH ₄	CO ₂
<i>Selectivity (n = 7)</i>			
$\alpha \pm \text{SD}; \text{RSD} (\%)$	1.60 \pm (4 \cdot 10 ⁻⁴); 0.03	1.60 \pm (4 \cdot 10 ⁻⁴); 0.03 ^a 2.16 \pm (1.9 \cdot 10 ⁻³); 0.09 ^b	2.16 \pm (1.9 \cdot 10 ⁻³); 0.09
<i>Resolution (n = 7)</i>			
R \pm SD; RSD [%]	7.07 \pm 0.04; 0.49	7.07 \pm 0.04; 0.49 ^a 9.59 \pm 0.13; 1.37 ^b	9.59 \pm 0.13; 1.37
<i>LOD, LOQ (n = 7)</i>			
LOD [$\mu\text{mol}\cdot\text{mol}^{-1}$]	0.08	0.15	0.30
LOQ [$\mu\text{mol}\cdot\text{mol}^{-1}$]	0.29	0.51	0.99
<i>Linearity (n = 7)</i>			
LRE	y = 1.61x + 0.08	y = 1.58x + 0.15	y = 1.48x + 1.17
R^2	0.9998	0.9997	0.9987
p	5	5	5
Range RSD [%]	0.13 – 0.82	0.22 – 0.98	0.56 – 1.88

α : selectivity factor; R: resolution (^a: compared with peak of CO; ^b: compared with peak of CO₂); LOD: limit of detection; LOQ: limit of Quantification; LRE: linear regression equation expressed by formula $y = mx + c$ where x is the concentration of analyte in $\mu\text{mol}\cdot\text{mol}^{-1}$ and y is the peak area; p: number of points of each calibration curve; Range RSD: range of relative standard deviation obtained by each point in calibration curve.

Precision

Repeatability and intermediate precision were investigated to evaluate the precision of the method. Repeatability is the closeness between measured values in number of measurements under the same analytical condition during a short period of time

[20, 21]. In order to examine the repeatability of the method, at least seven replication of measurement of WSGM at same day were conducted. The result for repeatability, expressed as RSD %, are presented in Table 3. Subsequently, the calculated RSD % was compared with the RSD % value of precision that is theoretically predicted by Hortwitz function in equation (4):

$$CV_{Hortwitz}(\%) = 2^{(1-0.5 \log c)} \quad (4)$$

where c corresponds to the analyte concentration indicated in decimal fraction. The repeatability of the method is satisfactory when the RSD % value is between half and two-third times theoretical values calculated by Hortwitz formula [8]. From the Table 3, the RSD % of CO, CH₄, and CO₂ for repeatability are 0.32 %, 0.21 %, 0.64 %, respectively. The RSD % values are less than 0.67 of coefficient of variability Hortwitz (CV Hortwitz), indicating an adequate method repeatability.

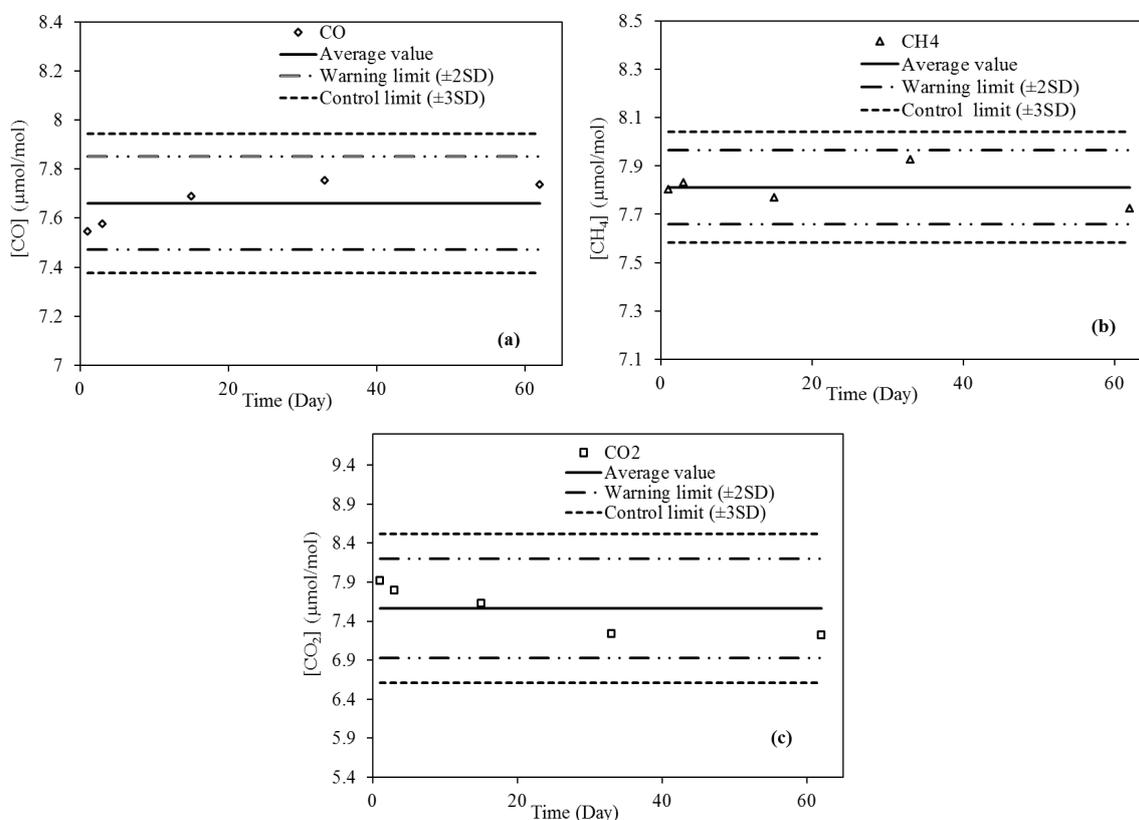


Figure 2. Intermediate precision for measurement of trace amounts of a) CO, b) CH₄, c) CO₂ in CSGM during two months period of time

Furthermore, the intermediate precision was investigated by analyzing the WSGM at least seven replication measurements at different days during two months period of time. For the repeatability of method, the comparison between the calculated RSD % and CV Hortwitz is conducted to evaluate the acceptance of the intermediate precision. The RSD % of the intermediate precision should fulfill 0.5 - 2 times of CV Hortwitz [8]. In Table 3, it can be seen that the RSD % of CO, CH₄, and CO₂ for intermediate precision are found to be 1.23 %, 0.98 %, 4.21 %, respectively. Those values indicate that the intermediate precision of method was acceptable because the RSDs % of

intermediate precision achieve the requirements *i.e.*, the $RSD \% \leq CV$ Hortwitz. The evaluation of intermediate precision of the method was also conducted by making Shewhart control chart [12], as presented in Figure 2. In the Figure 2, the three different lines in the chart are corresponding to the average value (solid line), two standard deviations from the average value as warning limit (dashed and dotted line), and three standard deviations from the average value as control limit (dotted line). The analytical system of the method is considered as under control if the measured values is lower than 5% (below the warning limit line). While if the measured values are higher than 5%, the analytical system of the method is out of control [12].

Accuracy

Accuracy of a measurement results is described as the closeness of agreement between measured and known reference value (true value) of target analyte. Two factors are contributing to the accuracy of a measurement result such as bias and and precision.

In this work, the bias of the method was determined by measuring the concentration of gas CO, CH₄, CO₂ in CSGM using GC-FID-methanizer and calculating its difference - using equation (1) - with the concentration of components stated in certificate of CSGM. The calculated bias of CO, CH₄, CO₂ (Table 3) are found to be 0.01, 0.02, 0.14 $\mu\text{mol}\cdot\text{mol}^{-1}$, respectively.

Table 3. Results for precision and accuracy

Precision (n = 7)	R _t [minute]	Concent [$\mu\text{mol}\cdot\text{mol}^{-1}$]	RSD [%]	CV Hortwitz [%]	0.67 X CV Hortwitz [%]
<i>Repeatability</i>		$\pm S_w$			
CO	0.81±0	7.55±0.02	0.32	11.80	7.91
CH ₄	1.30±(5·10 ⁻⁴)	7.80±0.02	0.21	11.74	7.87
CO ₂	2.81±(3.7·10 ⁻³)	7.92±0.05	0.64	11.72	7.86
<i>Intermediate precision</i>		$\pm S_b$			
CO	0.81±(1.5·10 ⁻³)	7.66±0.09	1.23	11.78	-
CH ₄	1.30±(2.4·10 ⁻³)	7.81±0.08	0.98	11.74	-
CO ₂	2.81±(6.4·10 ⁻³)	7.56±0.32	4.21	11.80	-
Accuracy (n = 7)	Bias [$\mu\text{mol}\cdot\text{mol}^{-1}$]	Uncertainty [$\mu\text{mol}\cdot\text{mol}^{-1}$]	μC_{CRM} [$\mu\text{mol}\cdot\text{mol}^{-1}$]	σ [$\mu\text{mol}\cdot\text{mol}^{-1}$]	2 σ [$\mu\text{mol}\cdot\text{mol}^{-1}$]
CO	-0.01	0.39	0.22	0.24	0.48
CH ₄	-0.02	0.39	0.23	0.24	0.48
CO ₂	-0.14	0.39	0.23	0.39	0.78

R_t: retention time; Concent: concentration of analyte; CV Hortwitz: Coefficient Variability of Hortwitz; Uncertainty: relative uncertainty stated in certificate multiplied by the concentration of analyte; μC_{CRM} : uncertainty value divided by squared root three (rectangular distribution assumption); σ : precision of method; S_w: standard deviation within (repeatability); S_b: standard deviation between (intermediate precision)

In addition to bias, precision is another factor taken into consideration in the evaluation of accuracy of method. Three different paramaters of measurement affect to the precision of method including repeatability, intermediate precision, and uncertainty value from certificate of WSGM. Thus, the precision of the method is calculated by

combining the three parameters using equation (2). From the calculation, the obtained values of precision (σ) of GC-FID-methanizer method for determining of the CO, CH₄, CO₂ (Table 3) are found to be 0.24, 0.24, 0.39, respectively. Based on the ISO Guide 33:2000 "Uses of certified reference materials", there is no evidence for the presence of bias in the method applied by laboratory if the experimental bias of method lies between $\pm 2\sigma$ at confidence level 95 % [22]. From the Table 3, the calculated value of bias of the GC-FID-methanizer method for determining CO, CH₄, CO₂ fall within $\pm 2\sigma$. These result indicates that no bias of method was found in the application of the GC-FID-methanizer for trace level measurement of CO, CH₄, CO₂ in SGM.

Robustness

The robustness of an analytical method is a method capability to unaffected by slight modifications of the method parameter conditions during analysis [16, 20]. In this work, the robustness of method is examined by measuring the effect of small changes of instrument conditions including oven temperature, flow rate, and detector temperature of GC-FID-methanizer. Effect of changes to precision and mean value of the method are investigated to assess the robustness [8, 16, 20]. The statistical significance test is conducted to evaluate the degree of robustness. One way ANOVA at the 95 % of confidential level was performed to examine the difference among mean results of CO, CH₄, CO₂ measurement using GC-FID-methanizer. As can be seen in Table 4, the statistical analysis using one way ANOVA shows that the obtained values of probability (p -value) are larger than 0.05. Therefore, it can be decided that no significance differences are remarked to the analytical result due to the small changes in flow rate, oven temperature and detector temperature of GC-FID-methanizer to measure trace amount of CO, CH₄ and CO₂. In addition, the significance difference between precision from the optimum condition and from the minor changes of the condition were compared by using the Fisher F-statistic at 95 % probability. From the p -value of F-test in Table 4, it can be argued that there is no significance difference in precision (p larger than 0.05) between the optimum condition and minor changes condition.

Measurement uncertainty evaluation

The two methods have been proposed for the uncertainty estimation related to the measurement result such as bottom up and top down methods. A bottom up approach was applied for the estimation of uncertainty for the measurement of trace amounts CO, CH₄, and CO₂ in high purity He gas sample using GC-FID-methanizer. The evaluation of uncertainty was conducted by using bottom up approach according to "guide to the expression of uncertainty in measurement" (GUM) [12, 16]. The bottom-up approach comprises the four main steps of the analysis, including specification of measurand, identification of uncertainty sources, quantification of the relevant uncertainty sources, and combination of individual uncertainty sources. The method evaluates the measurement uncertainty based on the mathematical model of the measurement and the law of error-propagation of the uncertainty. The error-propagation approach requires the identification and quantification of all uncertainty contribution, which has to be performed separately for each analytical method [12].

Table 4. Results of the robustness test of the method

Condition	Mean value ± S [μmol·mol ⁻¹]			P-Value					
				ANOVA			F-test		
	CO	CH ₄	CO ₂	CO	CH ₄	CO ₂	CO	CH ₄	CO ₂
<i>Oven Temperature</i>									
38 °C	7.57 ±0.04	7.76 ±0.05	7.52 ±0.05	0.47	0.09	0.77	0.44	0.32	0.29
40 °C	7.58 ±0.03	7.82 ±0.05	7.80 ±0.13						
42 °C	7.57 ±0.06	7.75 ±0.06	7.77 ±0.06				0.19	0.47	0.25
<i>Flow Rate</i>									
26 mL·min ⁻¹	7.58 ±0.05	7.77 ±0.04	7.52 ±0.05	0.06	0.06	0.87	0.05	0.29	0.43
28 mL·min ⁻¹	7.58 ±0.03	7.83 ±0.05	7.72 ±0.07						
30 mL·min ⁻¹	7.62 ±0.03	7.84 ±0.03	7.73 ±0.04				0.37	0.25	0.17
<i>Detector Temperature</i>									
248 °C	7.59 ±0.03	7.78 ±0.06	7.72 ±0.12	0.17	0.19	0.24	0.34	0.37	0.27
250 °C	7.58 ±0.03	7.83 ±0.05	7.74 ±0.08						
252 °C	7.56 ±0.03	7.83 ±0.06	7.64 ±0.11				0.42	0.35	0.27

S: the standard deviations of measurement from 7 replications

Specification of measurand

The mathematical model, expressed in equation (5) was applied for the calculation of analyte concentration from the gas mixtures sample.

$$C_{\text{sample}} = C_{GC} \times \frac{1}{Rec} \quad (5)$$

where C_{GC} is the analyte concentration obtained from the multi-point calibration curve that follows simple linear regression by ordinary least squares (OLS); Rec is the recovery that indicates the deviation from true value. From the mathematical model in equation (4), the measurand can be determined as the concentration of CO, CO₂, CH₄ in the gas mixtures sample (C_{sample}), which depend on the concentration of analyte found from the multi-point calibration and the recovery of method.

Identification of sources uncertainty

All of the possible uncertainty sources in measurement of trace amount CO, CH₄, and CO₂ by GC-FID-methanizer were identified and determined. In order to describe the influence of uncertainty sources from each parameter to the value of measurand, the cause-effect diagram (Ishikawa fishbone diagram) was constructed, as shown in Figure 3. As illustrated in Figure 3, it can be seen that the results of gas concentration measurement were primarily affected by the following uncertainty sources i.e., the uncertainty of the concentration of gas from GC resulted from calibration curve, the uncertainty of recovery of method, and the uncertainty of reproducibility.

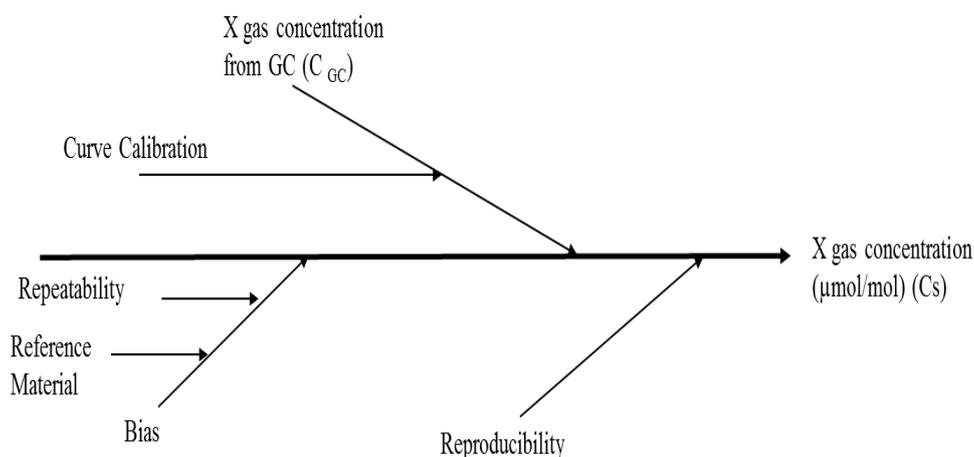


Figure 3. The uncertainty sources for the measurement of trace amounts of CO , CH_4 , CO_2 in gas mixtures using GC-FID-methanizer

Quantification of Uncertainty

Uncertainty of the multi-point calibration

As mentioned before, the analyte concentration (C_{GC}) was obtained from the calculation using the multi-point calibration curve. In this study, the calibration curve follows simple linear regression by ordinary least squares (OLS) that is expressed using the relationship in equation (6).

$$y = mx + b \quad (6)$$

In equation (6), x corresponds to the concentration of analyte in the gas mixtures sample, and y corresponds to the peak area of analyte from the GC measurement. In addition, m and b parameters are corresponding to the slope and the interception of linear regression curve, respectively. Then, the determination of analyte concentration (C_{GC}) is calculated using the equation (7):

$$x = \frac{y - b}{m} \quad (7)$$

The uncertainty of the analyte concentration can be estimated using equation (8) that is derived from the simple linear regression formula. This approach of uncertainty estimation has been reported elsewhere [16, 23].

$$s_x = \frac{s_{y/x}}{m} \sqrt{\frac{1}{N} + \frac{1}{n} + \frac{(\bar{y}_o - \bar{y})^2}{m^2 \sum_{i=1}^n (x_i - \bar{x})^2}} \quad (8)$$

where $s_{y/x}$ is the residual standard deviation, m is the calibration curve slope, n is the number of data point used to create the calibration curve, N is the number of replicate analysis for each data point, \bar{y}_o is the experimental analytical response of each standard, \bar{y} is the response estimated from calibration curve for each standard, x_i is the value of concentration on the x-axis, and \bar{x} is the mean value of the x_i value.

Uncertainty of the recovery (accuracy study)

The recovery (accuracy) of the method was investigated by measuring the CSGM and comparing the measurement result of analyte concentration (measured value) with the value of analyte concentration stated in certificate (certified value). The investigation of accuracy of method concludes that no bias was present in the application of method since the observed of bias lies within $\pm 2\sigma$, as shown in Table 3. Therefore, the correction was not applied to the method due to the presence of bias. In other word, the recovery value of the method (R_m) is 1. However, the uncertainty due to systematic errors component should be taken into consideration in the evaluation of overall uncertainty. As reported by Styarini et al. [23] the standard uncertainty associated with recovery (accuracy study) can be calculated using the equation (9):

$$\mu(R_m) = R_m \times \sqrt{\left(\frac{s_{obs}^2}{n \times C_{obs}^2}\right) + \left(\frac{\mu(C_{CSGM})}{C_{CSGM}}\right)^2} \quad (9)$$

where s_{obs} is the standard deviation of the results from the replicate analyses of the CSGM, n is the number of replication, C_{obs} is the concentration of analyte in CSGM measured by the method, C_{CSGM} is the concentration of analyte stated in certificate of CSGM, $\mu(C_{CSGM})$ is the standard uncertainty of the certified value of CSGM.

Uncertainty of the intermediate precision

The method validation result in Table 3 shows that the intermediate precision of the method for analysis of CO, CH₄ and CO₂ were found to be 0.01, 0.008, and 0.03 (as RSD), respectively. This value was used directly for the calculation of the combined uncertainty associated with the different intermediate precision terms.

Combined standard uncertainty

The value of parameters for calculation of x component gas concentration in high purity gas sample, their standard uncertainties and their relative standard uncertainties are summarized in Table 5. Based on the values given in Table 5, the concentration of analyte in gas mixtures can be calculated using equation (7). Table 5 shows that the concentration of CO and CO₂ in gas mixture sample were 1.13 $\mu\text{mol}\cdot\text{mol}^{-1}$ and 0.68 $\mu\text{mol}\cdot\text{mol}^{-1}$, respectively. Subsequently, the combined standard uncertainty from this quantification was determined by the equation (10). The calculation of combined standard uncertainties for CO and CO₂ measurement resulted 0.04 $\mu\text{mol}\cdot\text{mol}^{-1}$ and 0.06 $\mu\text{mol}\cdot\text{mol}^{-1}$, respectively, as presented in Table 5. Since CH₄ was not detected in the high purity gas sample by GC-FID-methanizer, uncertainty measurement for CH₄ was not estimated in this study. In this case, the LOD of CH₄ from the method validation of GC-FID-methanizer can be set as value for reporting the concentration of CH₄ in the high purity gas. Thus, it can be said that the concentration of CH₄ in the high purity gas is below the LOD of the method for the determination of CH₄.

$$\mu(C_{sample}) = C_{sample} \times \sqrt{\frac{\mu(C_{GC})^2}{C_{GC}^2} + \frac{\mu(Rec)^2}{Rec^2} + \frac{\mu(Rep)^2}{Rep^2}} \quad (10)$$

Table 5. Uncertainty in measurement of trace amount of CO and CO₂ in high purity He gas sample

Symbol	CO		CO ₂		Unit	Type A/B
	Value (X)	Standard Uncertainty (μ_x)	Value (X)	Standard Uncertainty (μ_x)		
C _{GC}	1.13	0.01	0.68	0.04	$\mu\text{mol}\cdot\text{mol}^{-1}$	A
Rec	1	0.03	1	0.03	-	A
Reproducibility (Rep)	1	0.01	1	0.04	-	A
C _{sample} and combined standard uncertainty	1.13	0.04	0.68	0.06	$\mu\text{mol}\cdot\text{mol}^{-1}$	
Expanded uncertainty U(C _{sample}) at k = 2		0.08		0.11	$\mu\text{mol}\cdot\text{mol}^{-1}$	

Expanded uncertainty

From Table 5, the combined standard uncertainties are multiplied by a coverage factor 2 (at confidence level 95 %) to obtain expanded uncertainty of the measurement. The expanded uncertainty of measurement CO and CO₂ in high purity He gas sample are obtained 0.08 and 0.11 $\mu\text{mol}\cdot\text{mol}^{-1}$, respectively.

The contribution uncertainties of different parameters are illustrated in Figure 4. The largest contribution of uncertainty arises from the determination of analyte (C_{GC}) concentration using calibration curve for CO₂ measurement and the bias evaluation for CO measurement.

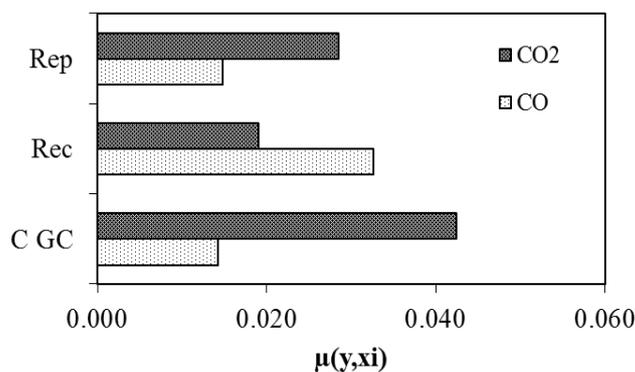


Figure 4. Uncertainty contributions for the measurement of trace amounts of CO and CO₂ in high purity He gas sample

CONCLUSION

From the study above, GC-FID-methanizer demonstrates the measurement of trace amounts of CO, CH₄, and CO₂ in high purity gas sample with excellent degree of accuracy and reliability. The experimental result with respect to selectivity, LOD, LOQ, precision, linearity, and robustness in validation assessment provided adequate evidence

that the method is reliable and proper for measurement of trace amounts of CO, CH₄, and CO₂ in high purity gas sample.

The uncertainty estimations of measurement of trace amounts of CO, CO₂ in high purity He gas sample using the proposed method are obtained 0.08 $\mu\text{mol}\cdot\text{mol}^{-1}$, and 0.11 $\mu\text{mol}\cdot\text{mol}^{-1}$, respectively, at 95 % of confidence level. The uncertainty estimation for CH₄ in high purity gas was not carried out due to undetected of CH₄ in GC-FID-methanizer. Furthermore, the uncertainty estimations expose that the bias evaluation and calibration curve were the major contribution to the uncertainty of the final results of CO and CO₂ content, respectively.

Thus, the overall study confirm that it is crucial to thoroughly determine the characteristics of method performance and estimate the all of uncertainties contributing to the measurement result in order to enhance the degree of confidence on the analytical result obtained using the proposed method.

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REFERENCES

1. Miricioiu, M., Niculescu, V., Zgavarogea, R., Petrenau, I., Nechifor, G.: Critical aspects in gas chromatography: low level detection of gas impurities, in *15th International Multidisciplinary Scientific GeoConference SGEM 2015*, **2015**, 126-130;
2. Kaminski, M., Jastrze, D., Kaminski, M.M.: Determination of carbon monoxide, methane and carbon dioxide in refinery hydrogen gases and air by gas chromatography, *Journal of Chromatography A*, **2003**, 989, 277-283;
3. Weijun, Y.: Trace analysis of impurities in bulk gases by gas chromatography-pulsed discharge helium ionization detection with ‘heart-cutting’ technique, *Journal of Chromatography A*, **2007**, 1167, 225-230;
4. Drager and Lubeck, Pac III Gas Detection Instruments, **1997**;
5. Xiuqi, L., Huannan, H., Jianying, Z., Bohua, Y., Pingtian, M.: Determination of trace amounts of permanent gases in ultra pure hydrogen by gas chromatography, *Fresenius Zeitschrift Analytische Chemie*, **1988**, 520-524;
6. Esler, M.B.D., Griffith, W.T., Wilson, S.R., Steele, L.P.: Precision Trace Gas Analysis by FT-IR Spectroscopy: Simultaneous Analysis of CO₂, CH₄, N₂O, and CO in Air, *Analytical Chemistry*, **2000**, 72 (1), 206-215;
7. De Saint Etienne, C., Mettes, J.: Gas phase impurities in silane determined by gas chromatography mass spectrometry, *Analyst*, **1989**, 114, 1649-1653;
8. Taverniers, I., De Loose, M., Van Bockstaele, E.: Trends in quality in the analytical laboratory. II. Analytical method validation and quality assurance, *Trends in Analytical Chemistry*, **2004**, 23 (8), 535-552;
9. International Standard ISO/IEC 17025:2005, General requirements for the competence of testing and calibration laboratories, 2nd edition, **2005**;
10. Taverniers, I., Van Bockstaele, E., De Loose, M. Trends in quality in the analytical laboratory. I. Traceability and measurement uncertainty of analytical results, *Trends in Analytical Chemistry*, **2004**, 23 (7), 480-490;

11. Stepan, R., Hajšlová, J., Tichá, J.: Uncertainties of gas chromatographic measurement of troublesome pesticide residues in apples employing conventional and mass spectrometric detectors, *Analytica Chimica Acta*, **2004**, 520, 245-255;
12. Hund, E., Massart, D. L., Smeyers-Verbeke, J.: Comparison of different approaches to estimate the uncertainty of a liquid chromatographic assay, *Analytica Chimica Acta*, **2003**, 480, 39-52;
13. Budiman, H., Hamim, N., Zuas, O.: Comparison between GC-TCD and GC-FID for the determination of propane in gas mixture, *Procedia Chemistry*, **2015**, 16, 465-472;
14. Min, D., Kang, N., Moon, D.M., Lee, J.B., Lee, D.S., Kim, J.S.: Effect of variation in argon content of calibration gases on determination of atmospheric carbon dioxide, *Talanta*, **2009**, 80 (2), 422-427;
15. Prokopowicz, M., Luboch, E., Namiesnik, J., Przyjazny, A.: Utilization of standards generated in the process of thermal decomposition chemically modified silica gel for a single point calibration of a GC/FID system, *Talanta*, **1997**, 44, 1551-1561;
16. Magnusson, B., Ornermark, U.: *Eurachem Guide: The Fitness for Purpose of Analytical Methods - A Laboratory Guide to Method Validation and Related Topics*, Second Edition, **2014**;
17. Kaiser, H.J., Ritts, B.: Validation of analytical methods used in cleaning validation, *Analytical Methods Validation*, Institute of Validation Technology, **2006**, 15-30;
18. Taylor, P., Kanfer, I., Walker, R.B., Patnala, S.: *Encyclopedia of Pharmaceutical Science and Technology*, Fourth Edition, Chromatographic Methods of Analysis: Gas Chromatography Chromatographic Methods of Analysis: Gas Chromatography, **2013**, 37-41;
19. Huber, L.: *Validation of Analytical Methods*, vol. 2, Agilent Technologies, **2010**;
20. *Guidelines for the validation and verification of quantitative and qualitative test methods*, National Association of Testing Authorities (NATA), **2013**;
21. *How to Meet ISO 17025 Requirements for Method Verification*, The Analytical Laboratory Accreditation Criteria Committee (ALACC), AOAC International, **2007**;
22. International Standard ISO Guide 33:2000, Uses of certified reference materials, **2000**;
23. Styarini, D., Zuas, O., Hamim, N.: Validation and Uncertainty Estimation of Analytical Method for Determination of Benzene in Beverages, *Eurasian Journal of Analytical Chemistry*, **2011**, 6 (3), 159-172.