VALIDATION OF ANALYTICAL METHOD FOR DETERMINATION OF HIGH LEVEL CARBON DIOXIDE (CO_2) IN NITROGEN GAS (N_2) MATRIX USING GAS CHROMATOGRAPHY THERMAL CONDUCTIVITY DETECTOR

VALIDAÇÃO DE MÉTODO ANALÍTICO PARA DETERMINAÇÃO DE NÍVEIS ELEVADOS DE DIÓXIDO DE CARBONO (CO_2) EM MATRIZ DE GÁS NITROGÊNIO (N_2) UTILIZANDO CROMATOGRAFIA GASOSA COM DETECTOR DE CONDUTIVIDADE TÉRMICA

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RESUMO

Resultados precisos da medição de dióxido de carbono (CO_2) são de grande importância uma vez que os resultados (dados) são utilizados como base para tomada de decisões relacionadas a regulamentação do programa de monitoramento e aplicações da leis regulamentadas. Portanto, no presente estudo, um método para medição de grandes quantidades de dióxido de carbono em uma matriz de nitrogênio (N2) utilizando cromatografia gasosa com detector de condutividade térmica (CG-DCT) foi validado para atingir o desempenho ótimo do método. Assim sendo, foram avaliadas a confirmação de identidade, seletividade, limite de detecção (LD), limite de quantificação (LQ), repetibilidade, reprodutibilidade, precisão e linearidade do método. Os resultados mostram que o método CG-DCT tem boa precisão em termos de reprodutibilidade e repetibilidade tendo valores de 0,07 e 0,37%, respectivamente. Nenhum viés do método pode ser encontrado e uma excelente linearidade do método foi obtida na faixa de 2 – 13.97% mol/mol.. Deste modo, com base na avaliação dos resultados sob a critérios dados deste estudo, pode ser concluído que o método GC-TCD é confiável e adequado para a determinação de níveis elevados de CO_2 em matriz N_2 .

Palavras-chave: dióxido de carbono, de validação de método, de cromatografia gasosa, detector de condutividade térmica (CG-DCT).

ABSTRACT

High accurate result of carbon dioxide (CO_2) measurement is of great importance since the result (data) is used as the foundation for decision making related to regulated monitoring program and law enforcement. In this study, therefore, method for measurement of high level of carbon dioxide (CO_2) in nitrogen (N_2) matrix using gas chromatography thermal conductivity detector (GC-TCD) was validated to achieve the optimum performance of the method. For this purposes, identity confirmation, selectivity, limit of detection (LoD), limit of quantitation (LoQ), repeatability, reproducibility, accuracy, and linearity of the method were evaluated. The result shows that the GC-TCD method has good precision in term of repeatability and reproducibility having values of 0.07 and 0.37%, respectively. No bias of the method can be found and an excellent linearity of the method was obtained in the range of 2 - 13.97% mol/mol. Thus, based on the result evaluation under given criteria of this study, it can be concluded that the GC-TCD method is reliable and suitable for determination of high level of CO_2 in N_2 matrix.

Keywords: carbon dioxide, method validation, gas chromatography thermal conductivity detector (GC-TCD).

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INTRODUCTION

Anthropogenic CO_2 is the primary greenhouse gas that contributes to air pollution and global warming (Lindenmaier *et al.*, 2014). The anthropogenic CO_2 is mainly resulted from the combustion of fossil fuels in power generation, industrial, residential, and transport sector (Lindenmaier *et al.*, 2014; Metz *et al.*, 2005; Miguel Olaizola 2003; M Olaizola *et al.*, 2002). In addition to CO_2, other gases are also generated during such combustion process including N_2, O_2, CO, H_2O, SO_x and No_x (Metz *et al.*, 2005). In a combustion system, the stack gas may contain CO_2 gas at various levels, depending on the type of fuel used. For example, 3-4% by volume of CO_2 concentration can be generated from a power plant processing operated by using natural gas fuel while up to 15% when coal is used (Metz *et al.*, 2005).

The increasing of atmospheric CO_2 level from time to time due to the emission from anthropogenic source has devised the more stringent of regulations with the purpose to keep the concentration at allowable levels. Although the more stringent discharge regulated limit of pollutant has improved for meeting the air quality standards; it is also predictable that this action alone might not enough to ensure an adequate protection of atmospheric environment. In order to evaluate the adequacy of the regulation for protecting the atmospheric environment, an efficient control via regulatory monitoring programs and its enforcement are essential. Therefore, to provide the CO_2 data with high level of validity, a reliable measurement of CO_2 gas is extremely required. Owing to the fact that valid and reliable of CO_2 analytical data is used as the basis for regulatory decision making related to control of air pollution and to implement the regulation of CO_2 emission.

There are numbers of analytical method are accessible and have been used for analyzing of CO_2 including gas chromatography (Zuas *et. al.*, 2014), non-dispersive infrared spectroscopy (Pandey & Kim, 2000), Fourier transform infrared spectroscopy (Esler *et al.*, 2000), cavity ring down spectroscopy (Budiman, Deullae *et al.*, 2009). Among others, the chromatography-based technique (GC) is one of the most preferred techniques over the last century due to its inexpensive of operational cost (Barwick, 1999). As nowadays, the GC is easily available worldwide to both government and private sector including university, research institute and industry. In spite of the progress that has been made, there is still effort remaining that can be directed toward validation of the GC method for a specific purpose.

According to ISO 17025, validation of a analytical method is required to obtain a reliable result before the method is used. In testing laboratory, the method validation is a part of quality assurances to ensure that a high quality of analytical result can be achieved (Budiman, Krismastuti, *et al.*, 2009; Taverniers, Bockstaele, *et al.*, 2004; Taverniers, Loose, *et al.*, 2004; Thompson *et al.*, 2002). In general, method validation means a procedure used by laboratory to investigate whether the method performance for determination of a particular analyte meets required criteria (Taverniers, Bockstaele, *et al.*, 2004; Taverniers, Loose, *et al.*, 2004; EURACHEM Guide, 1998). This article details the validation of GC-TCD method for measurement of CO_2 in the gas mixture. The evaluation was focused on the following validation parameters including confirmation of identity and selectivity, limit of detection, limit of quantitation, precision, accuracy, and linearity. An optimum condition and the limitation of the analytical system are discussed.

EXPERIMENTAL

2.1. Materials

Certified and working standard gas mixtures (SGM in short) having composition as listed in Table 1 were utilized in all experiment runs. Both certified and working SGM were purchased from a specialty gas company (MESA, USA). The concentration values of gas components in the certified SGM are traceable to National Institute of Standards and Technology (NIST), USA.

2.2. Instruments

A GC Model 6890 equipped with a TCD detection (Hewlett Packard Agilent, CA, USA) was used for measurement of high level of CO_2 in N_2 matrix. The gas sample from cylinder was introduced to the GC system through mass flow controller type 5890E (Brooks Instrument, Hatfield, USA) in order to maintain the gas sample at constant flow. The mass flow controller was installed just before the sample loop of the GC system.

Calibration system model CMK (MCZ Gmbh, Dieseltrasse, Germany) was utilized for diluting the SGM using zero gas to produce different concentration of the SGM. The concentration of gas components in the SGM depends on both control ranges of the mass flow controller and concentration of gas component in SGM cylinder. This CMK instrument was only used in the work to investigate the linearity of the methods.

Table 1. *Composition of gas components in the mixture of certified and working SGMs.*

Gas Components	Concentration (% mol/mol)	
	Certified SGM	**Working SGM**
CO_2	13.97	2.12
CO	3.18	3.44
C_3H_8	2.18	1.81
N_2	80.67	92.63
Relatives Uncertainty	±2% from reported concentration	No uncertainty stated

2.3. Procedure

All parameters of method validation including confirmation of identity, selectivity, LoD, LoQ, repeatability, reproducibility, linearity, accuracy, and roughness were determined by adopting the procedures as detailed in EURACHEM Guide (EURACHEM Guide, 1998) using GC-TCD with optimum operating conditions as shown in Table 2. The injection of CO_2 containing SGM sample to the GC-TCD system was carried out at least seven replications in entire stages of method validation to obtain the reliable data.

RESULTS AND DISCUSSION:

3.1. Confirmation of identity and selectivity

Similar to other GC experiments, in this GC analysis, the response identity of each gas component in the SGM have to be clearly identified before a quantitative analysis is carried out. The optimization of the GC-TCD was performed to obtain a good separation of CO_2 from other gas components presence in the SGM. Figure 1 presents a typical chromatogram of SGM, showing that peak of CO_2 was detected at retention time of 6.21 min.

Table 2. *Optimum operating conditions of the GC-TCD system used in this study.*

Apparatus	**Agilent 6890 GC System**
Injection	
Flow rate sample	100 mL/min
Loop volume	2 mL
Valve box temperature	100°C
Column	
Column	Porapak Q, packed column stainless steel, 1/8inch OD, 6 feet, 80-100 mesh
Oven temperature	Program temperature, 40°C (3.5 min), 60°C/min, 200°C (2 min)
Gas carrier & flow rate	Helium with ultra-high purity grade (99.999%), 40 psi (28.5 mL/min)
Detector	
Temperature	250°C
Gas reference & flow rate	Helium, 20 mL/min
Gas make up & flow rate	Helium, 7 mL/min

Selectivity is the capability of method to discriminate and quantify the response of target component with the presence of other components as interference (NATA, 2013; EURACHEM Guide, 1998; Huber, 2010). Figure 1 illustrates good separation of CO_2 relative to other gas components and no interference nearby the CO_2 peak was found, indicating that separation of each gas component was clearly obtained. It is indicated by the resolution (R) with value larger than 1.5 and its high selectivity factor (α) (Taylor, Kanfer, Walker, & Patnala, 2013), as listed in Table 3.

Considering the general application of the

GC-TCD for measuring the CO_2 in a real sample, the presence of some following impurities including CO, NO, NO_2, N_2O, O_2 and hydrocarbons (such as CH_4 and C_3H_8) may affect to the GC separation (resolution) process. It would be a widely acceptable idea that optimization of the GC parameters and conditions for obtaining a good separation is very crucial. In this study, a GC- TCD equipped with Porapak Q column was used under an optimized condition, where the most possible detectable components among those impurities are CO and hydrocarbons (Sarkar & Haselden, 1975), while other impurities such as NO, NO_2, N_2O, and O_2 would be undetectable (Yinghong & Yuesi, 2010; Thijsse, 1978; Lamb *et al.*, 2012). Moreover, although the CO and C_3H_8 hydrocarbon are detectable using the present GC-TCD system, but they can be well-separated as shown in Fig. 1. Thus, it can be argued that not all those impurities would interfere the CO_2 measurement under the optimized instrument conditions of this study.

3.2. Limit of detection (LoD) and Limit of quantitation (LoQ)

Limit of detection (LoD) is defined as the lowest amount of analyte that can be detected which is not necessarily quantified as an exact value (NATA, 2013; CDER, 1994; EURACHEM Guide, 1998; Huber, 2010; Kaiser & Ritts, 2006). Meanwhile, limit of quantitation (LoQ) is the lowest concentration of an analyte that can be quantitatively determined with appropriate precision (repeatability) and accuracy (Huber, 2010; EURACHEM Guide, 1998). The LoD and LoQ assessment are equally important with the investigation of other method validation parameters (Huber, 2010). At the LoQ level, the quantitative analysis is able to be carried out however it produces the inaccuracy and imprecise result since the uncertainty contribution at this lower level is larger than the measurement result itself. Only qualitative analysis is possible at the concentration of LoD level (Huber, 2010; Taverniers, Loose, et al., 2004). The LoQ evaluation establishes the minimum concentration of analyte that can be reliably determined. When the quantification produces a value below the LoQ level, it could make the quite high uncertainty which associated with the measurement. Consequently, the unreliable measurement would occur.

For the determination of LoD and LoQ of CO_2 target component, a signal to noise (S/N) ratio method as proposed by Mcnair & Miller (1998) was used. Based on this method the ratio values of LoD and LoQ are 3:1 and 10:1, respectively. The S/N ratio method is suitable for application under which the concentration of the component in the SGM used is very close to the LoD of the component to be determined. In this study, the CO_2 in the SGM was diluted using CMK gas calibration system in order to close its concentration to the LoD of CO_2 as the target component. In addition to S/N ratio, a regression curve is another method that can also be used to estimate the LoD and LoQ of the target component in a GC analysis. However, the regression curve method can be applied for determining the LoD and LoQ when the concentration of the component in an SGM is not close to LoD of the component to be determined (NATA, 2013; EURACHEM Guide, 1998). Table 3 lists the calculated of LoD and LoQ values of CO_2 as target component, which were obtained using S/N ratio method.

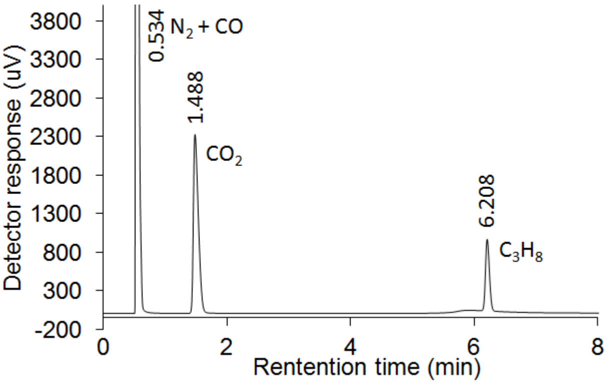

Figure 1. *A typical chromatogram of gas component in the SGM.*

3.3. Repeatability

Investigation of repeatability precision was carried out to evaluate the closeness between measured values in number of measurements under the same analytical condition during a short period of time (NATA, 2013; AOAC International, 2007). For examining the repeatability of the method, at least seven replication of measurement of certified SGM at same day were carried out and its relative standard deviation (%RSD) was calculated, and the results are presented in Table 3.

Table 3. *Results of method validation for the CO_2 measurement in N_2 matrix using GC -TCD*

Parameters	Result
Selectivity (α)	2.80[a] 4.18[b]
Resolution (R)	15.32[a] 63.11[b]
LoD, µmol/mol	3.13
LoQ, µmol/mol	10.47
Precision	
Repeatability relative standard deviation, %	0.07
Reproducibility relative standard deviation (RSD), %	0.37
CV Hortwitz, %	2.69
0.67 CV Hortwitz, %	1.80
Accuracy	
Bias, % mol/mol	0.002
Precision of method (σ), % mol/mol	0.15
±2σ, % mol/mol	0.30

[a] calculated by comparison with peak of N_2 and CO

[b] calculated by comparison with peak C_3H_8

The calculated %RSD can be compared with the precision data from existing reference/standard method. Since no information of precision from reference/standard methods are available for a comparison with the precision parameter of this work, therefore, the precision comparison was made by using the prediction of relative standard deviation of precision that is theoretically calculated using Hortwitz function in (Eq. 1) (Jorhem & Engman, 2001; Linsinger & Josephs, 2006; Taverniers, Loose, *et al.*, 2004; Walker & Lumley, 1999).

$$CVHortwitz()=2^{(1-0.5\log c)}(Eq.1)$$

Where c is the concentration of analyte stated in decimal fraction. The requirement of %RSD for repeatability is between half and two-third times of a theoretical values determined by Hortwitz function (Taverniers, Loose, *et al.*, 2004). The %RSD of CO_2 for repeatability precision was found to be 0.07, as listed in Table 3. The repeatability of the method is categorized acceptable since the %RSD is less than 0.67 of coefficient of variability Hortwitz (CV Hortwitz) (Table 3). It means that our laboratory performed the method with sufficient precision. Therefore, repeating the set of measurement is not necessarily to be carried out further to obtain a lower relative standard deviation of repeatability (Walker & Lumley, 1999).

3.4. Intermediate precision

Seven repeated measurements at different days during three months period of time were conducted to check intra-laboratory reproducibility (intermediate precision) of method and results were shown in Fig 2. Two standard deviations from the average value of measurements were set as warning limit while three standard deviations were used as control limit. The method would have an analytical system under control if no higher than 5% of the measured values is surpassing warning limit(Taverniers, Loose, *et al.*, 2004). If the measured values are higher than 5%, the analytical system of the method is out of control. Consequently, the action should be taken by re-conducting the measurement such as repeating the set of measurement, re-optimizing the analytical instrument, controlling the environmental condition, and re-calibrating the instrument.

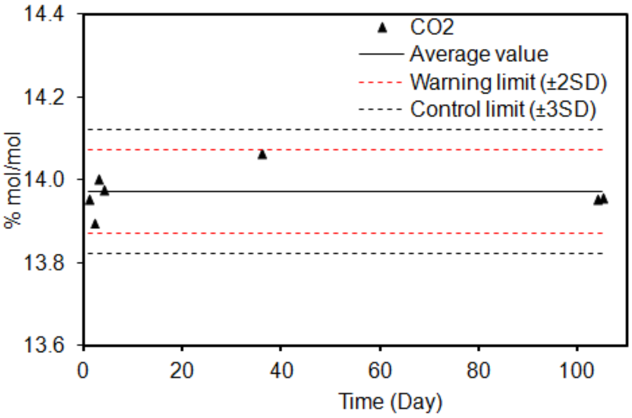

Figure 2. *Reproducibility for determination of CO_2 in certified SGM during period of three months.*

Figure 2 shows that the entire measured values fall between the warning limit. As the repeatability precision, CV Hortwitz is also used for the basis of acceptable criteria for the intermediate precision. The relative standard deviation of intermediate precision should require

0.5 - 2 times of CV Hortwitz (Taverniers, Loose, *et al.*, 2004). Table 3 presents that %RSD of CO_2 for intermediate precision is 0.37 %. This value indicates shows that the intermediate precision of method was acceptable because the relative standard deviation of intermediate precision fulfills the requirements of %RSD ≤ CV Hortwitz (Table 3).

3.5. Accuracy

Accuracy of analytical results is defined as the closeness of agreement between measured and accepted (true) value of target analyte. In this work, the accuracy of method was investigated by measuring the concentration of gas CO_2 in certified SGM using GC-TCD and comparing the result with concentration of CO_2 stated in the certificate. The accuracy of analytical data is dependent on two factors i.e., the bias and precision of analytical method (AOAC International, 2007; EURACHEM Guide, 1998; Pintar, 2013; Taverniers, Loose, *et al.*, 2004; Walker & Lumley, 1999). The bias of method is the difference between measured value and the value stated in the certificate of the certified SGM, and it can be calculated by using (Eq. 2). The bias of analytical method for CO_2 measurement is presented in Table 3.

$$\Delta = X,^{-} - Y \qquad (\mathrm{Eq.}\,2)$$

Where X^- is the average of measured value of target gas component in the SGM, Y^- is the value stated in certificate SGM. In laboratories validation of method, the use of an internationally traceable standard or also called certified reference materials (CRMs in short) is very important, because CRMs could give useful information for elucidating all aspects of bias including method, laboratory and run bias (Taverniers, Bockstaele, *et al.*, 2004; Taverniers, Loose, *et al.*, 2004).

As mentioned before, precision of analytical method (σ) is also affect the accuracy of analytical data. The assessment of precision of analytical method value includes three different data sources i.e., repeatability, reproducibility, and uncertainty value from certificate of certified SGM where the following equation is used (Eq. 3) (Walker & Lumley, 1999).

$$\sigma = \sqrt{S_b^2 + \frac{S_w^2}{n} + \mu_{RM}^2}\,(Eq.3)$$

Where S_b is standard deviation from reproducibility, S_w is standard deviation from repeatability and μ_{RM} is the uncertainty stated in SGM certificate. In Table 3 the obtained value of precision of analytical method (σ) is listed as 0.15%. According to ISO Guide 33:2000 "Uses of certified reference materials" (ISO GUIDE 33, 2000), the method of laboratory is found no presence of bias if the observed bias of method falls within ±2σ at confidence level 95% (Eq. 4).

$$-2\sigma < X,^{-} - \mu < 2\sigma \qquad (Eq.4)$$

From Table 3, it can be seen that the obtained value of bias of analytical method for measurement of CO_2 are 0.002 % mol/mol. Since the values of bias (0.002 % mol/mol) falls within ±2σ (0.30% mol/mol); thus, it can be concluded that there is no evidence of bias can be found in the analytical method used.

3.6. Linearity

Linearity is the ability of the method to demonstrate that the test results is proportional to the concentration of sample (Taverniers, Loose, *et al.*, 2004; Huber 2010). In this work, to investigate the linearity range of method, a calibration curve was generated from eight different concentrations of SGM ranging from 1% mol/mol to 13.97% mol/mol and the results are listed in Fig 3. The 1 - 4% mol/mol of CO_2 concentration (≈1, 1.5, 2, 2.5, 3, 3.5, and 4.0) were obtained by diluting the 13.97 % of CO_2 SGM using ultra-high purity N_2 gas. Each concentration level was analyzed in two replications and the results are shown in Fig. 3. The data in Table 3 suggests that the linearity criteria of the method was obviously attained with %RSD < 2% for each concentration level, while the determination coefficient (R^2) was found to be >0.9999. In this method validation study, the acceptance criterion for the linearity was set more stringent than for a routine work (%RSD<5%, R^2>0.995), because the method will be used for confirming the concentration of the components in the primary SGM, which has relative uncertainty of about 0.1%.

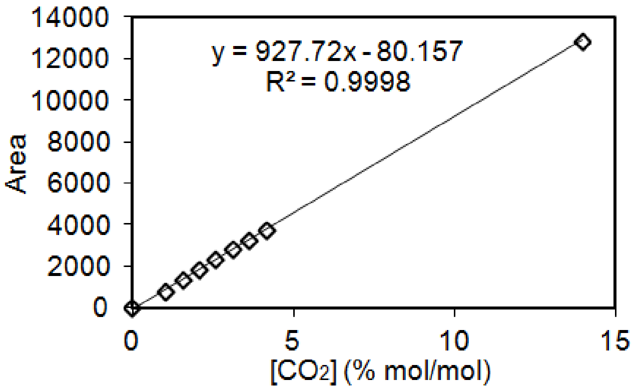

***Figure 3.** Calibration curve showing all data points for CO_2 measurement using GC-TCD method.*

Figure 3 shows a calibration curve for all data points from CO_2 measurement in the SGM. From Fig 3, it can be clearly observed that the two lowest data point i.e., 1 and 1.5% mol/mol are preferably being deviated from linearity. It is proven by establishment of the graphic of relative response or sensitivity as a function of concentration, as described in Fig 4. Relative response is calculated by dividing the area response with the respective concentration. The linearity evaluation of method was carried out by examining each data point and it should lie in the range value of 95% and 105% of constant response (R_c) (Kaiser & Ritts, 2006; Taverniers, Loose, *et al*., 2004). The lowest concentration of 1% mol/mol is presence below 95% of the constant response line that are reflected to be outside of the linear range, as described in Fig 4. while the second of low data point, 1.5% mol/mol, was nearly in the line of 95% range value. Consequently, the first two data points i.e., data 1 and 1.5% mol/mol were eliminated from data set.

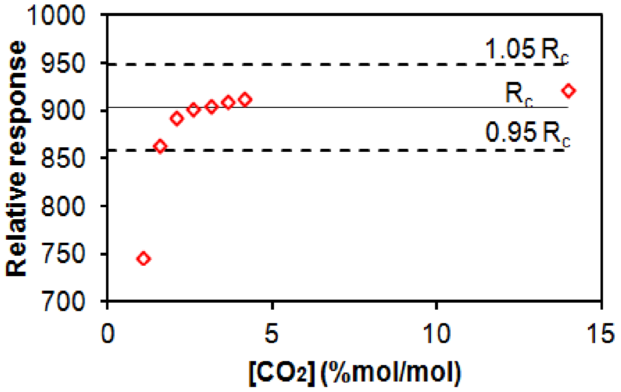

***Figure 4.** Graph of relative response versus CO_2 concentration for linearity evaluation*

***Table 4.** Data used for linearity evaluation*

$[CO_2]$ %mol/mol	Area	SD	%RSD
0	0	-	-
1.03	771.00	139.68	18.12
1.55	1339.65	39.00	2.91
2.07	1845.03	10.54	0.57
2.59	2330.36	0.72	0.03
3.10	2805.37	9.91	0.35
3.62	3293.12	1.31	0.04
4.14	3776.40	6.97	0.18
13.97	12878.10	16.69	0.13

Moreover, examination of %RSD value of each data point is another approach to determine the linearity of an analytical method (Kaiser & Ritts, 2006). In this basis, it can be seen from Table 4 that data point 1 and 1.5% mol/mol had %RSD of 18 and 3, respectively. These two data point are obviously greater than 2%RSD of acceptable criterion. Thus, the data from the first two lowest concentrations were eliminated from the curve. This finding is in good agreement with the result from linearity assessment using relative response plot criterion as previous described.

Fig 5 shows the graph after elimination of the first two data of concentration level (1 and 1.5% mol/mol). As can be obviously seen that the value of R^2 has increased to a better value of 1, indicating that method evaluation have fulfilled the required regression criterion with R_2>0.9999.

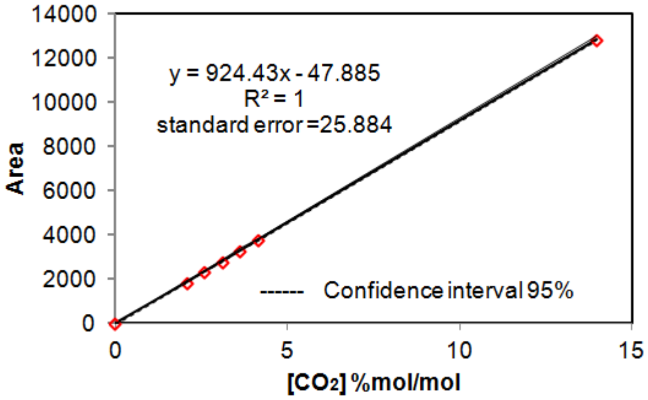

***Figure 5.** Calibration curve using data points except the two of very low concentration data of analysis CO_2 in N_2 matrix using GC-TCD*

In addition, the linearity assessment of calibration curve was also carried out by means of a method called the “lack of fit test”. The lack of fit test is used to determine whether the regression model adequately fits the data and follows the linear model (Miller, 1991; Van Locco, Elskens, Croux, & Beernaert, 2002). Normally, the test values for lack of fit test follows the F-distribution from which the significance of test value can be assessed. Under the F-distribution The critical value of $F_{(x,y)}$ are taken from the reference table with degree of freedom x= n-2, y= n(p-1) at 95% confidence level where n is the amount of standard and p is the number of measurements for each standard. The analyses of variance for residual, pure error, and lack of fit gave the values as listed in Table 5. The sum and mean squares were calculated to test for lack of fit. The calculated significance of F value (1.4) as listed in Table 5, was found to be smaller than the critical value of $F_{5,7}$ (3.97) at 95% probability. Therefore, it can be concluded from the test that there is no significance lack of fit to the linear model. In other words, the calibration curve of analysis CO_2 in N_2 matrix using GC-TCD fits to the linear model. Based on the result of linearity assessment, the linearity of the method has been established ranging from 2 - 13.97 %mol/mol.

Table 5. *The analysis of residual variance*

Source of variation	Sum of squares	Degree of freedom	Mean square	F
Residual	7238.628	12		
Pure error	538.593	7	957.148	1.4
Lack of fit	6700.035	5	1340.007	

3.7. Roughness

The roughness of an analytical method is a measure of method capacity which remains unaffected by minor changes of the experimental conditions during analysis (EURACHEM Guide; 1998). In this study, the roughness of method is examined by measuring the effect of small changes in instrument conditions including flow rate, oven temperature and detector temperature of GC-TCD. For the roughness evaluation, effect of changes of instrument conditions on the mean values of the CO_2 measurement was investigated (NATA, 2013; EURACHEM Guide, 1998; Taverniers, Loose, et al., 2004). The degree of roughness was statistically evaluated to indicate the difference among the mean values of the CO_2 measurement using the least significant difference test in one way analysis of variance (ANOVA). A 95% confidence limit ($p < 0.05$) was applied for the indication the possible significant difference resulted from the small changes of the instrument conditions and the results are listed in Table 6. According to the results of statistical analysis as shown in Table 6, it can be observed that the small changes in flow rate, oven temperature and detector temperature of GC-TCD for analysis of CO_2 have no significant effect ($p>0.05$) on the results of analytical measurement.

Table 6. *Roughness test of analysis of CO_2 using GC-TCD*

Intrument condisions	Mean values (%mol/mol)	*s*	*p*-values
Flow rate			
28 mL/min	13.948	0.03	0.511
28.5 mL/min*	13.971	0.05	
29 mL/min	13.955	0.02	
Oven temperature			
39^{o}C (3.5 min), 59^{o}C/min,	13.943	0.02	0.388
40^{o}C (3.5 min), 60^{o}C/min,	13.971	0.05	
41^{o}C (3.5 min), 61^{o}C/min,	13.942	0.03	
Detector temperature			
249^{o}C	13.933	0.01	0.12
250^{o}C*	13.971	0.05	
251^{o}C	13.955	0.02	

*optimum condition applied in the method validation

CONCLUSIONS:

From the above discussion, it can be concluded that validation is an important step to be started before an instrument is continuously used for long period after method development. All results for all assessed parameters of validation provide a sufficient evidence for proving the reliability of the GC-TCD method and the method is fit for determination of CO_2 in N_2 matrix. The use of validated method would have enormous advantage not only for the method developer but also to the user because it can build a degree of confidence for data generated using such validated method.

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