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A case study for in-house method validation of gas chromatography technique using class-1 calibration gas mixtures for greenhouse gases monitoring

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Abstract

In this study, analytical method validation has been done for the measurement of carbon dioxide/nitrogen (CO_2/N_2) and methane/nitrogen (CH_4/N_2) calibration gas mixtures using gas chromatography with flame ionization detector (GC-FID). Class-I calibration gas mixtures (CGMs) of CO_2 (500 µmol mol^{-1} to 1100 µmol mol^{-1}) and CH_4 (2 µmol mol^{-1} to 130 µmol mol^{-1}) used in method validation process has been prepared gravimetrically following ISO 6142-1. All prepared gas mixtures have expanded uncertainty 1 % at coverage factor (k) of 2 with 95 % confidence. The following parameters are chosen for this case study which include selectivity, accuracy, precision, linearity, limit of detection (LOD), limit of quantification (LOQ), robustness, stability, and uncertainty. Different statistical approaches are taken into consideration for each parameter assessment. The results indicate that GC-FID is selective for CO_2 and CH_4. CGMs represent good repeatability and reproducibility having percentage relative deviation < 1 % among measurements. A good linear behaviour was observed for CGMs of CH_4 and CO_2 on basis of least square regression with R^2 value 0.9995 and 1, respectively. LOD and LOQ for CH_4 are calculated 0.47 and 1.59 µmol mol^{-1} based on signal-to-noise ratio by taking its lowest concentration of 2.9 µmol mol^{-1}. The in-house validated method for GC-FID using CGMs for the measurement of greenhouse gases (CO_2 and CH_4) is found to be precise, accurate and fit for purpose.

Keywords Greenhouse gases · GC-FID · Method validation · Calibration gas mixture

Introduction

Carbon dioxide (CO_2) and methane (CH_4) are major greenhouse gases (GHGs) which contribute to climate change and major risk for biodiversity. These GHGs absorb the reflected solar radiation from the earth and results in elevated temperature in the atmosphere which are the major driving force of global warming. Major repercussion of global warming are the rising sea levels, change in precipitation pattern, heat waves, floods, droughts, hurricanes, etc. [1, 2]. Combustion of fossil fuel in many sectors like transport, power plants and manufacturing industries are major contributors for the increase in carbon footprint and other pollutants such as carbon monoxide (CO), sulphur oxides (SO_x), nitrogen oxides (NO_x) and particulate matter globally [3–5]. To control the unrestricted increment of carbon emission in the environment, strict action in regulation laws should be implemented. These regulation laws require robust monitoring programme to track the emissions of various pollutants emerging from different sectors. Accuracy in monitoring data ensures reliable measurement of pollutants. Many instrumental techniques were developed by times for measurement of greenhouse gases such as gas chromatography equipped with flame ionization detector (GC-FID), Fourier transform infrared spectroscopy (FTIR), non-dispersive infrared spectroscopy (NDIR), wavelength scanned cavity ring down spectroscopy (WS-CRDS) and various cavity-enhanced absorption spectroscopy [6–11]. Gas chromatography technique has high degree of resolution, more sensitive, separates complex mixture and versatile for micro and macro size samples [12]. Calibration of analytical instruments for the detection of various pollutants requires calibration gas

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mixtures (CGMs) which should have higher metrological order with low measurement uncertainty to magnify quality assurance. Uncertainties in atmospheric measurements can be minimized using accurate and precise CGMs with long-term stability.

Analytical method development and validation play a significant role in evaluation of scientific studies. Owing its importance, several guidelines are generated by notified international organizations for evaluation of validation parameters [13–16]. A validation must guarantee, through experimental studies, that the method meets the requirements of the analytical applications, ensuring the reliability of results. An accurate and calibrated measurement results define quality assurance and acceptability of a method that is fit for purpose [17].

In this study, class-1 binary calibration gas mixtures (CGMs) of CO_2 (500 µmol mol^{-1} to 1100 µmol mol^{-1}) and CH_4 (2 µmol mol^{-1} to 130 µmol mol^{-1}) of target amount were prepared gravimetrically, traceable to SI unit 'mole' [18, 19]. This is the primary method for preparation of CGMs having minimal uncertainty which helps in achieving accuracy in various analytical results. These gas mixtures are cost effective and can be prepared over a wide calibration range based on requirements. The prepared CGMs are used to validate a method for gas chromatography- flame ionization detector (GC-FID) to monitor greenhouse gases at ambient range. Several parameters like selectivity, calibration model, accuracy, precision (repeatability, intermediate precision), limit of quantification (LOQ), limit of detection (LOD) and robustness are considered for the method validation which will ensure to have accurate, precise, and reliable results in measurements [20]. The validated method is used for the detection of trace gases CO_2, CH_4 in air or for the calibration of different analytical instrument with higher accuracy and low uncertainty.

Materials and method

Calibration gas mixture

Calibration gas mixtures of CO_2/N_2 and CH_4/N_2 have been prepared individually using gravimetric method by following ISO 6142-1 (component-1 is CO_2/CH_4 and diluent N_2). Four CGMs of CO_2 in N_2 were prepared at nominal ambient level of 500 µmol mol^{-1} to 1100 µmol mol^{-1} and CH_4 in N_2 in the range of 2 µmol mol^{-1} to 130 µmol mol^{-1}. Preparation procedure and uncertainty estimation of all CGMs of CO_2 and CH_4 during entire scheme was carried out according to ISO 6142-1 and elucidated in a previous study with propane as example [21]. These amounts of gas mixtures were not prepared with a single step but by following 'cascading' dilution. A higher amount fraction series (generally 3 to 4)

was prepared at initial. From this, a further dilution has been done to achieve the required lowest amount fraction. The general method of preparation for CGMs is described as follows: A 10-L aluminium cylinder was evacuated up to 10^{-3} mbar while simultaneously heating it. Target mass of component and diluent gas was calculated according to ideal gas law equation. Gases were transferred into aluminium cylinder sequentially and weighed accurately using equal arm gas balance (Raymor HCE-25 G) having sensitivity of 1 mg. The difference in the weight of sample and reference cylinder gives the amount of component and diluent gas. Temperature and humidity were maintained during the entire weighing process (23 ± 2) °C and (45 ± 10) %, respectively. After the final weighing, amount fraction equation gives the molar composition (gravimetric amount fraction). Uncertainty during the entire preparation scheme was calculated by following ISO 6142-1 and GUM (Guide to the expression of uncertainty in measurement—2008) [22]. The prepared standards are traceable to SI unit of mass (kg) and amount of substance ('mole') and their traceability is also ensured by participation in international multilaboratory key comparisons (CCQM-K120, CO_2 in synthetic air) & (APMP.QM S7-1, CH_4 in N_2) [23, 24]. The gravimetrically prepared CGMs of CO_2/N_2 are 508 µmol mol^{-1}, 516 µmol mol^{-1}, 836 µmol mol^{-1} and 1100 µmol mol^{-1}, while CH_4/N_2 are 2.4 µmol mol^{-1}, 2.9 µmol mol^{-1}, 103.1 µmol mol^{-1} and 124.8 µmol mol^{-1} with 1 % relative expanded uncertainty at coverage factor (k) of 2 with 95 % confidence.

Gas chromatography

Gas chromatography (Agilent technology 6890N, Sr. No. US10723001) equipped with flame ionization detector (GC-FID) was used for the analysis of CO_2/N_2 and CH_4/N_2 CGMs. GC is incorporated with following parts: Injection source (for sample introduction), oven (having column for separation of gas mixture based on their physical properties) and detector. FID detectors contain H_2/air flame which burns organic compounds and converted them into ions. The generated ions are proportional to the concentration of the compounds present in sample gas stream. The ions are collected to the electrodes having potential difference, this generate an electrical signal and compound is detected. GC-FID detector is sensitive for hydrocarbons such as methane, ethane, propane etc. For the detection of inorganic species such as oxides of carbon, a methanizer is used with the FID detector. The methanizer contains a nickel catalyst which reduces the CO_2 and CO equivalent to CH_4. The efficiency of methanizer in GC-FID is also ensured by getting almost similar response for equivalent amount fraction of CO and CH_4 primary standards. A packed porapak Q (ethyl vinyl benzene-divinyl benzene polymer) column was used for the qualitative analysis of CGMs of CO_2/N_2 and CH_4/N_2. The

CGMs were introduced into GC through the injection source into the inlet where carrier gas (a mobile phase) takes the sample into the column which is enclosed in an oven and after separation, the sample enters the detector for identification. Table 1 shows the optimized conditions for GC-FID used in this study. A complete Schematic diagram of working of GC is represented in Fig. 1. Stream selection valve (SSV) is a multiport attached with GC which acts as an auto sampler for gases sequence run of all the gas mixtures. Outlet of SSV is connected to the mass flow controller (MFC;

Table 1 Operating conditions for GC-FID

GC parameters	Operating condition (CO_2 and CH_4)
Column	Porapak Q length 10′, mesh range (80/100), diameter 1/8″
Loop volume	2 mL
Inlet (temperature and Pressure)	150 °C, 24.37 Psi
Inlet flow rate	30 mL/min
Carrier Gas	He (25 mL/min)
Oven temperature	80 °C (isothermal)
Detector	FID, (250 °C)
H_2 flow rate	35 mL/min
Air Flow	400 mL/min
Thermal Aux# 1	115 °C
Thermal Aux#2 (Ni catalyst)	350 °C
Mass flow controller	40 mL/min

Make Alicat) which controls the flow of the sample into the gas sampling valve (GSV) with a sample loop attached to GSV a 10-port valve. When GC valve is 'OFF', the sample is loaded to the loop, while in 'ON' position, the loaded sample loop is connected with the column where the carrier gas takes the sample through column to detector. Figure 1 represents all the connection of SSV, MFC and GC valve.

Analytical method validation

Analytical method provides reliable results which describe importance of quality and potency in the analysis. Prior to implementation of analytical method, it must be ensured that it is acceptable for deliberate purpose. The main aim of validation of analytical method is that measurement done by this method gives the true values of the content in the samples. For method validation following parameters is taken into consideration: selectivity, linearity, accuracy, precision, limit of detection, limit of quantification, robustness, uncertainty evaluation and stability study [13, 14, 25].

Result and discussion

Selectivity

Selectivity is the potential to differentiate and assess target analyte present in mixture without the intrusion of

Fig. 1 Schematic setup of gas chromatography. 'OFF' position represents filling of gas sampling loop, and 'ON' position represents connection of loop with the column
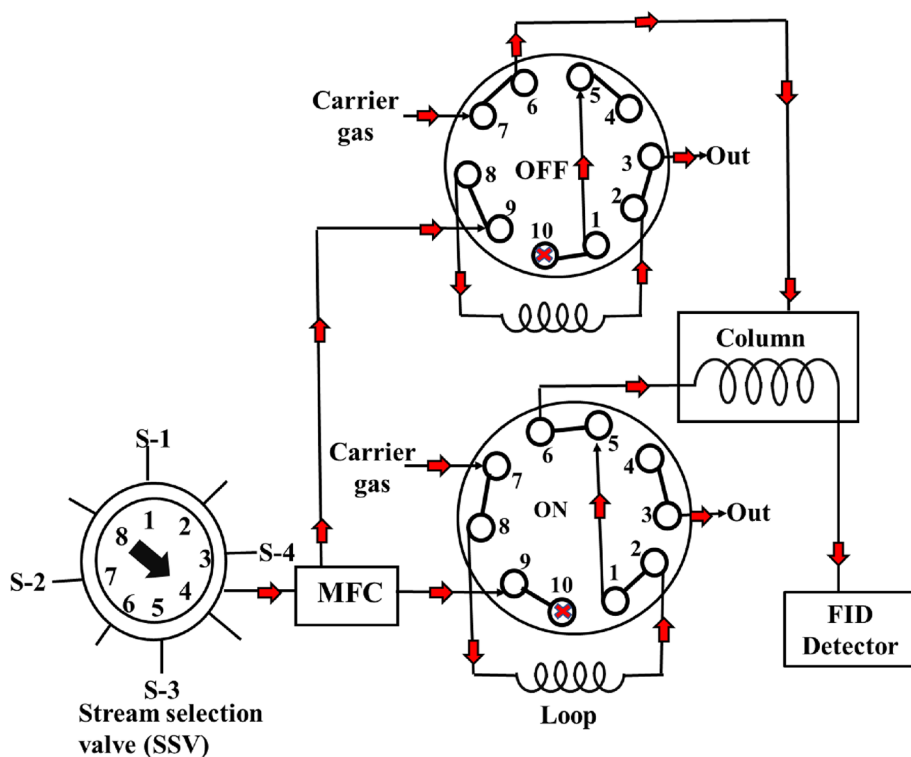
other components [26]. Selectivity can be determined by comparing the individual standard of interest in analytical instrument [13–15, 25]. In gas chromatography, selectivity is the relationship between adjacent peaks having different retention time. GC represents good tendency to separate most of organic compounds such as hydrocarbons, CO and CO_2 based on their physical properties [27]. CGMs of CH_4 and CO_2 were introduced into GC and their peaks were observed with retention time of 1.8 and 2.6 min, respectively, as shown in Fig. 2. Selectivity factor (α) was calculated to be 1.4 for CH_4 gas standard by comparing the retention time of CO_2 peak using Eq. (1) (Condition: $\alpha > 1$).

$$\alpha = \frac{t_{R2}}{t_{R1}} \tag{1}$$

t_{R2}, t_{R1} are the retention time of two adjacent peaks.

Resolution (R_s) is the degree of separation between two vicinal peaks in any mixtures; it is the ratio between difference in retention time and width of adjacent peaks and calculated as Eq. (2) [28]. As chromatography signals are in Gaussian distribution, applicable condition for resolution

factor is $1.5 > R_s > 1$. R_s for CH_4 gas standard peak was calculated as 6.29 with comparison of CO_2.

$$R_s = 2 \times \left(\frac{t_{R2} - t_{R1}}{w_{b2} - w_{b1}} \right) \tag{2}$$

w_{b2}, w_{b1} peak width of two adjacent peaks.

Calibration model

For the reliable quantification in any analytical method, relationship between the amount fraction of analyte in the sample and its corresponding response must be determined. Linearity is the relationship between the analyte amount fraction and the instrument response. For linearity evaluation in the present study, four different ranges in the amount fraction of CH_4; (2 μmol mol^{-1} to 124 μmol mol^{-1}) and CO_2; (500 μmol mol^{-1} to 1100 μmol mol^{-1}) were assumed. In case of CH_4, working ranges were 2.4 μmol mol^{-1}, 2.9 μmol mol^{-1}, 100 μmol mol^{-1}, 124 μmol mol^{-1} and for CO_2 were 508 μmol mol^{-1}, 516 μmol mol^{-1}, 836 μmol mol^{-1}, 1100 μmol mol^{-1}. Linearity was investigated by applying linear regression model. All calibration points of both CGMs (CO_2 and CH_4) were plotted against the instrument response (obtained in the form of peak area) with six repeatable measurements of each range. Calibration curve was obtained with R^2 (coefficient of determination) 0.9995 and 1 for CH_4 and CO_2, respectively, which indicates a good linear relationship. Linear equation obtained for both analytes is represented in Table 2, which is $y = mx + c$, where y represents (instrumental response), m (slope), x (concentration) and c (intercept). Linearity was evaluated with graphical approach which include response factor and residual plots [29]. A response factor plot shows the relationship between sensitivity (analyte peak area/corresponding concentration) and concentration. This type of plots is considered as suitable for linearity evaluation in gas chromatography technique [30]. Figure 3a and b represents response factor plot of selected range of amount fraction of CH_4 and CO_2 CGMs. It is evident from
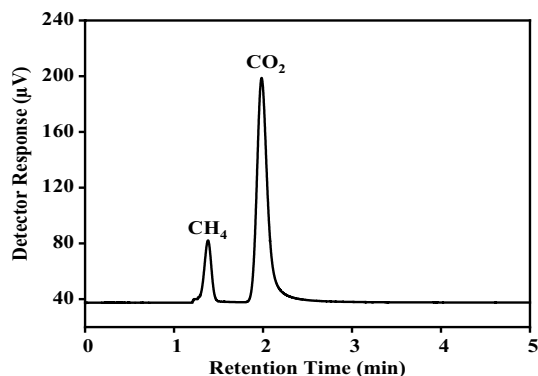
Fig. 2 A chromatogram showing separation of CH_4 and CO_2

Table 2 Evaluation of linearity in CGMs of CH_4 and CO_2 by GC-FID

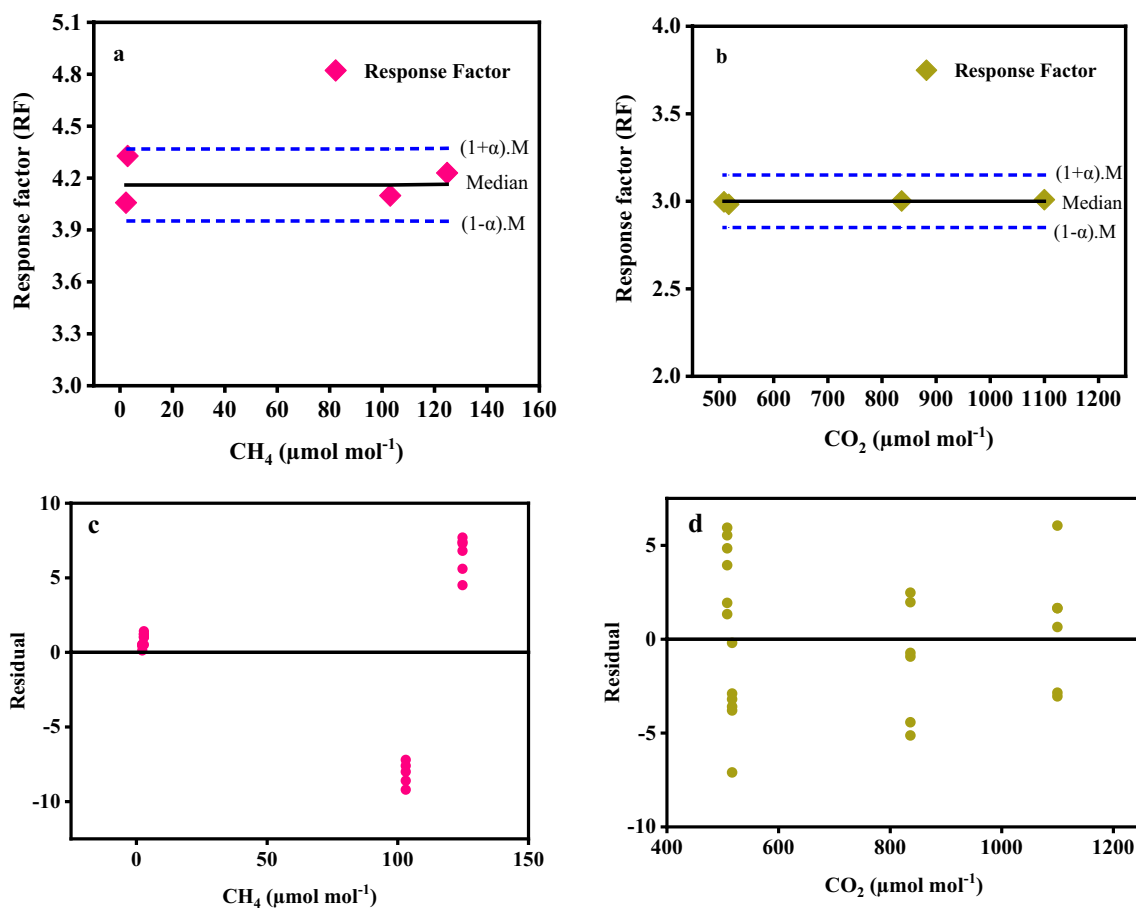| Linearity test	Acceptance criteria*	CH_4	CO_2	References*
Graphical mode				
Linearity plot	≤5 % tolerance	Within limit	Within limit	[35]
Residual plot	No trend	No trend	No trend	
Statistical significance test				
Mandel	H_o: $F_{exp} < F_{crit}$	83.14 > 161	0.45 > 161	[31]
Numerical parameters				
R^2	None	0.9995, ($y = 4.18x - 0.64$)	1, ($y = 3.02x - 18.5$)	[36]
RSD of slope	<2 %	1.56 %	0.25 %	[34]
% RE$_{max}$	< 15 to 20 %	1.25 %	0.021 %	[33]

Fig. 3 **a** and **c** Response factor and Residual plots of CH_4 and **b** and **d** Response factor and residual plots of CO_2, respectively, for linearity assessment in GC–FID. In response factor plot, the central black line represents median (M) of all relative response and dotted blue lines represent upper and lower tolerance limits [$(1 \pm \alpha) \cdot M$ where $\alpha = 0.05$]

graph that all computed response factor lies within lower and upper tolerance limit with no larger deviations which confirmed that linearity can be assured over this calibration range. Lower and upper tolerance limit was calculated as $(1 \pm \alpha) \cdot M$, where M is the median of all responses taken as central line along the calibration range and α is 0.05 with ± 5 % confidence level. Linear range of analyte can describe informally by residual plot, which is produced from linear regression curve of selected calibration points. The graph is drawn between residuals (measured–predicted value) of instrument response and independent variable that is amount fraction. Influence points, unequal variance, outliers from data set of measurements can be detected by this graph [30]. A chaotic pattern (sign sequence) in plot represents good linearity for selected ranges. Figure 3c and d represents a residual plot of CH_4 and CO_2 calibration gas mixtures of four calibration points. Each amount fraction range of gas mixtures is measured in six replications. The residuals are randomly scattered around zero (not making any systematic pattern) which confirms the linear model is

appropriate for the gas mixtures of CH_4 and CO_2. The only disadvantage of this type of plot is that it does not have any statistical significance.

Linearity of selected calibration points for both CGMs (CO_2 and CH_4) was performed statistically through Mandel test [31]. This type of test detects whether a straight line or quadratic curve fits the calibration data better. This test compares relative standard error (RSE) of both models, i.e. linear and quadratic model as given in Eq. (3). RSE for both models was calculated by applying Excel tool "LINEST". This tool calculates the regression statistics for a line using "least squares method" that fits data points. While applying Eq. (3), F_{Mandel} was calculated as 83.14 and 0.45 for CH_4 and CO_2 for the selected range of amount fraction. F_{crit} was calculated as 161 at 95 % confidence interval with $1/n–3$ degree of freedom, where n is number of calibration point. In working range of both CGMs, F_{Mandel} is observed lower than F_{crit} which gave the conclusion of acceptance of null hypothesis (value represented in Table 2). Therefore, in this case it can be assured

that quadratic term or second-degree polynomial does not have any significance to fit in data, but a linear model is appropriate.

$$F_{\text{Mandel}} = \left[\frac{\left(S_{y/x}\right)^2_{LIN} - \left(S_{y/x}\right)^2_{\text{NON - LIN}}}{\left(S_{y/x}\right)^2_{\text{NON - LIN}}} \right] \qquad (3)$$

where $S_{y/x}$ represent relative standard error of linear and quadratic model.

Numerically, there are two approaches taken for linearity evaluation: relative standard deviation of the slope (RSD_{slope}) and percent relative error (RE) [32, 33]. The RSD_{slope} test is the mathematical measurement to check goodness of fit (GOF) and dispersion of experimental data (obtained through analysis) around the regression line [34]. For an appropriated GOF, the tolerance level should be 2 % for the chromatographic techniques. Equation (4) was used to calculate the percentage relative standard deviation of the slope of regression line obtained from detector response and CGMs amount fractions. Standard error of slope (SE_{b1}) and b_1 represented in Eq. (4) was calculated by excel tool "LINEST" while performing regression analysis. RSD_{slope} for CO_2 and CH_4 CGMs was calculated 1.56 % and 0.25 %, respectively, which is < 2 %, indicates that selected calibration range are linear.

$$RSD_{\text{SLOPE}} = \left(\frac{SE_{b1}}{b1} \right) \times 100 \qquad (4)$$

where SE_{b1} represent standard error of slope and b_1 is slope of regression line.

The relative error (RE) method was used for comparing the analytical concentration (obtained by calibration curve) from the nominal or assigned concentration to detect any error contribution for the whole regression range. Relative error (RE) can be calculated as shown in Eq. (5) in which x_{meas} was calculated by linear equation $y = mx + c$. For each calibration point, a negative and positive deviation can be obtained. Acceptable criteria for the RE should be less than 15 % to 20 %. Both CGMs of CO_2 (1100 μmol mol^{-1}) and CH_4 (124 μmol mol^{-1}) come in the specific range of acceptable criteria as shown in Table 2.

$$RE = \left[\frac{\left(x_{\text{meas}} - x_{\text{theo}}\right)}{x_{\text{theo}}} \right] \times 100 \qquad (5)$$

where x_{meas} and x_{theo} define analytical and assigned concentration of analyte. Various linearity assessment test proves that selected range of CGMs are linear and compatible for various environmental monitoring programmes.

Accuracy (bias)

Accuracy (bias) represents variance between assigned value of component to measured value [37]. Accuracy is calculated using Eq. (6).

$$\Delta = \overline{X} - Y \qquad (6)$$

where $\overline{X}$ is the measured amount fraction of gas component obtained through instrument and Y is the assigned amount fraction. In case of in-house prepared gas mixtures, gravimetrically calculated amount fraction is taken as the certified value in which consistency in amount fraction was verified as per ISO 6143 [38]. Table 3 represents bias in CGMs of CH_4 (124.8 μmol mol^{-1}) and CO_2 (1099.8 μmol mol^{-1}) was 1.69 μmol mol^{-1} and 1.99 μmol mol^{-1}, respectively. Analytical amount fraction of CGMs was calculated by calibration curve while linearity evaluation in "Result and discussion" section where regression coefficient for CH_4 and CO_2 were calculated 0.995 and 1, respectively. The percentage relative deviation in amount fraction of CH_4 and CO_2 due to bias is calculated as 1.35 % and 0.18 %, respectively (condition: bias should in between 2 % and 6 %).

Accuracy of the gas mixtures was also influenced by precision of the method when there is larger deviation from the actual amount fraction. Precision (σ) is mainly dependent on repeatability (r), reproducibility (R) and uncertainty of the reference mixture (u_{CGM}) as shown in Eq. (7)

$$\sigma = \sqrt{s_R^2 + \frac{S_r^2}{n} + u_{\text{CGM}}^2} \qquad (7)$$

Table 3 Assessment of accuracy, precision parameters in method validation

Parameters	CH$_4$	CO$_2$
Gravimetric value (μmol mol^{-1})	124.8	1099.8
Analytical value (μmol mol^{-1})	126.5	1101.8
Accuracy		
Bias (Δ), (μmol mol^{-1})	1.69	1.99
Precision of the method (σ)*	1.31	1.59
±2σ, Condition: −2σ < bias < +2σ	2.63	3.18
Precision		
RSD$_r$ (% repeatability relative standard deviation) (exp)	0.065	0.088
RSD$_R$ (% Reproducibility standard deviation)	0.31	0.093
RSD$_R$ (Theo)	1.9	1.17
0.67% RSD$_R$ (Theo); Condition (RSD$_R$ (exp) < 0.67% RSD$_R$ (Theo)	1.27	0.79
Horrat value, Condition; Horrat value < 1.3	0.16	0.078

*Calculated using Eq. (7)

S_R is the standard deviation from reproducibility (measurement done 3 days in a row), S_r is the standard deviation from repeatability (number of replications in measurement on the same day), n is the number of replicate measurements and u_{CGM} is the stated (certified) uncertainty of the gas mixtures. Accuracy of the method should lie within the range of $\pm 2\sigma$. Table 3 shows the accuracy (Δ) and precision (σ) data of CGMs of CO_2 and CH_4 which confirms that accuracy of this method for both CGMs lies within the range of $\pm 2\sigma$.

Precision

This term represents the closeness of the value, through sequence of measurement (repeatability) derived from multiple analysis of the gas mixtures. Precision in a method can be described in two ways: repeatability (measurements within a day), intermediate precision (measurements within many days) [39]. The intermediate precision can be obtained by analysis of samples in 6 to 8 replications over several months and represented by control chart in Fig. 4. This graph represents the mean analytical value (solid line) of CGMs during an interval of 120 days and control limit (dash line). Control limit ($\pm 3SD$) is three times of the standard deviation obtained through change in amount fraction during this interval of time. In Fig. 4, it is shown that amount fraction of CGMs of CH_4 (124.8 µmol mol^{-1}) and CO_2 (1099.8 µmol mol^{-1}) were found within the limit in the period of four months. There are several conditions for reliability in precision study results such as experimentally determined percentage relative standard deviation of repeatability (RSD_r) and reproducibility (RSD_R) can be compared with theoretically calculated relative standard deviation of reproducibility (RSD_R theo) by Horwitz theory represented in Eq. (8) [40].

$$RSD_R(\text{theo}) = 2^{(1-0.5 \log C)} \tag{8}$$

RSD_r determined experimentally should be less than RSD_R (theoretically) and this condition is achieved; results are represented in Table 3 for both the calibration gas mixtures CH_4 (124.8 µmol mol^{-1}) and CO_2 (1099.8 µmol mol^{-1}). RSD_R calculated experimentally should be less than 0.67 % RSD_R (theoretically), this result is also achieved and shown in Table 3 for both calibration gas mixtures. To check reproducibility precision, Horrat equation is also applied which is fraction of RSD_R (experiment) and RSD_R (theoretical) [41]. Horrat value should be less than 1.3, so precision of the method is in the acceptable range (value of Horrat for both CGMs represented in Table 3).

Limit of detection (LOD) and limit of quantification (LOQ)

LOD is the lowest concentration of analyte that can be determined by a signal and LOQ is the lowest concentration which can be quantified with acceptable precision and accuracy. LOD can be calculated by three methods: signal-to-noise ratio, linear regression and through blank determination [42]. Signal-to-noise ratio is the best method for analytical procedure for evaluation of LOD [43]. Signal-to-noise ratio can be calculated as Eq. (9).

$$\frac{S}{N} = \frac{2H}{h} \tag{9}$$

where S/N is the ratio of signal to noise and H and h are the height of the peak for lowest possible concentration and noise. The acceptable criteria for LOD determination through S/N ratio are $2 \geq S/N \leq 3$. LOD and LOQ require S/N ratio of 3:1 and 10:1, respectively. Taking the lowest concentration of CH_4 gas standard (2.9 µmol mol^{-1}), LOD and LOQ were calculated 0.47 and 1.59 µmol mol^{-1} and a visual representation for this method (signal/noise) is shown in Fig. 5. LOD and LOQ for CO_2 were calculated as 0.73 and 2.45 µmol mol^{-1}, respectively.

Fig. 4 Precision study of CH_4 and CO_2 during four months of period
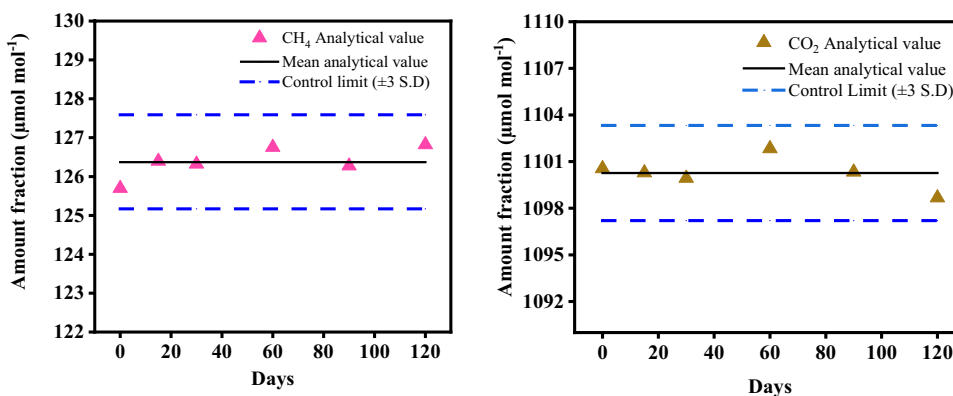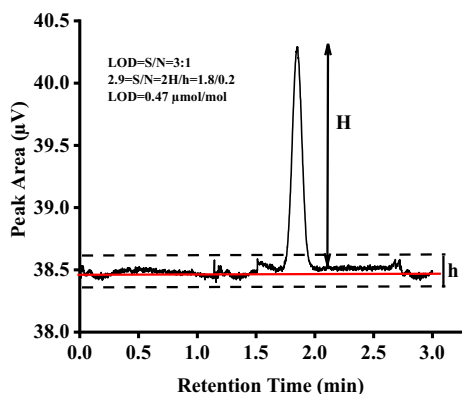
Fig. 5 A chromatogram of CH_4 (2.9 µmol mol^{-1}) CGM showing signal to noise

Robustness

Robustness is the measurement of susceptibility of method which indicates that the minor changes in the analytical condition do not impact on it. In this study, impact on analyte amount fraction (CH_4 and CO_2) changes were observed by varying the instrumental condition such as carrier gas flow rate, oven temperature, MFC flow rate. Standard operating conditions were described previously in methodology section and are followed by the oven temperature (80 °C), column flow rate (25 mL/min), MFC (40 mL/min). CH_4 (124.8 µmol mol^{-1}) and CO_2 (1099.8 µmol mol^{-1}) gas standards were taken to evaluate robustness. At operating condition, analytical amount fraction is represented in Table 4. Variation in amount fraction was noted by changing the operating conditions such as oven temperature (± 10 °C), MFC (± 10 mL/min) and column flow rate (± 5 mL/min) and extent of robustness was also evaluated by % relative standard deviation as shown in Table 4. From the measurement, it was observed that small changes in the operating conditions do not impact the resultant amount fraction of the gas standard.

Uncertainty estimation

Measurement uncertainty (MU) is a parameter, associated with the result of a measurement that represents the dispersion in the values of measurand (the quantity being measured) [44]. There are several points in method validation procedure which gives values of uncertainty such as accuracy, precision, linearity, and limit of detection as represented in Fig. 6. The process of quantifying measurement uncertainty, following the proposed ''bottom-up'' approach of guide of

Table 4 Robustness study for analysis of CO_2 and CH_4 using GC-FID

Gases	Operating condition								
	MFC (mL/min)			Oven temp. (°C)			Column flow rate (mL/min)		
	40*	30	20	80*	70	60	25*	20	30
CH_4									
A. F#	126.3	126.4	126.4	126.3	126.4	126.5	126.3	126.3	126.4
% RSD		0.04	0.06		0.07	0.08		0.01	0.03
CO_2									
A. F #	1099.9	1099.6	1101.7	1099.9	1099.5	1100.4	1099.9	1098.1	1099.8
% RSD		0.01	0.1		0.02	0.03		0.1	0.001

*Optimized condition used in analytical method,

A. F is the mean concentration (µmol mol^{-1}) with $n = 6$ replicates, RSD is the percentage relative standard deviation

Fig. 6 Fish bone diagram representing uncertainty parameters associated with measurement of CO_2 and CH_4
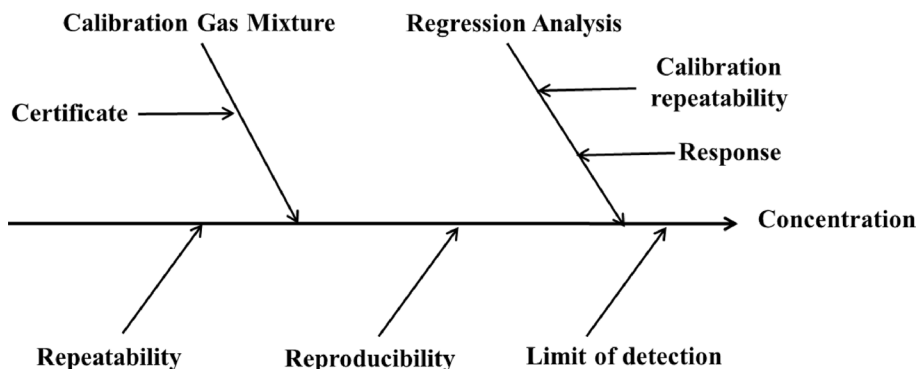
uncertainty measurement (GUM), involves mainly four stages: (a) creation of the model equation for the measurand; (b) identifying all significant sources of uncertainty in a method; (c) estimating their magnitude from experimental data; and (d) combining the individual uncertainties of each source to give the uncertainty in the reported value [22]. The main sources of uncertainty in the analytical procedure are gravimetrically prepared calibration gas mixture, linearity assessment, precision (repeatability and reproducibility) and limit of detection [45]. Uncertainty associated with calibration gas mixture (u_{CGM}) are taken from preparation scheme. Uncertainty due to repeatability (u_r) can be obtained by repeated number of measurement (6 measurements) and reproducibility (u_R) uncertainty are taken from measurement done at different time interval (mainly 3 days). Uncertainty due to calibration curve can be estimated by Eq. (10).

$$s_x = \frac{s_y}{m}\sqrt{\frac{1}{k} + \frac{1}{n} + \frac{(y_i - \bar{y})^2}{m^2 \sum_{i=1}^{n}(x_i - \bar{x})^2}} \qquad (10)$$

where s_x is uncertainty in concentration due to calibration curve, s_y standard deviation of response, m slope of the calibration curve, k no. of replicate measurement of sample, n no. of calibration points, y_i response of standard, $\bar{y}$ Average response of standard, x_i individual concentration of standard, $\bar{x}$ mean concentration of standards.

Uncertainty associated with limit of detection (u_{LOD}) is calculated in the form of relative, as LOD divided by represented concentration. Table 5 represents the uncertainty sources, their distribution type, related degree of freedom and relative standard uncertainty associated with each

Table 5 Different uncertainty sources, their distribution, standard uncertainty, and degree of freedom in the analysis of CH_4 and CO_2

Uncertainty Sources	Distribution/type A and B/divisor	Standard uncertainty $u(x)$		Degree of freedom (v_i)	Relative standard uncertainty ($u(x)/x^*$)	
		CH_4	CO_2		CH_4	CO_2
Repeatability (u_r)	Type A($\sigma/\sqrt{n}$)	$0.11/\sqrt{6}=0.042$	$0.78/\sqrt{6}=0.32$	6	$0.042/126.4=0.000339$	$0.32/1100=0.000292$
Reproducibility (u_R)	Type A($\sigma/\sqrt{n}$)	$0.40/\sqrt{3}=0.23$	$0.4/\sqrt{3}=0.23$	3	$0.23/126.17=0.001866$	$0.23/1100=0.0004$
Calibration (u_{cal})	Regression statistics	0.64	1.25	2	$0.64/126.41=0.005063$	$1.25/1100=0.001136$
Limit Of detection (u_{LOD})	Type A ($\sigma/\sqrt{n}$)	0.47	0.73	∞	$0.47/126.40=0.0037$	$0.73/1100=0.00067$
Calibration gas mixture (u_{CGM})	Type B ($\sigma/\sqrt{3}$)	0.624**	5.5**	∞	$0.624/124.8=0.003884$	$5.5/1099.8=0.005$
				u_c	1.02	5.70
				U	2.21 ($k=2.16$)	11.19 ($k=1.96$)

*Amount fraction related to uncertainty sources. **Standard uncertainty already mentioned before
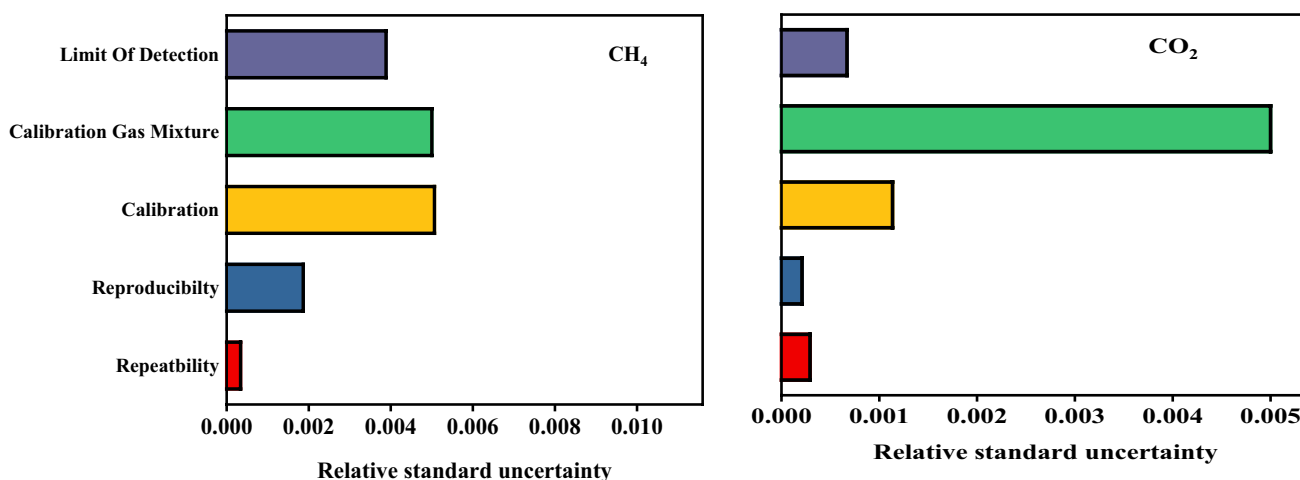

Fig. 7 Relative uncertainty contribution of each parameter during analysis of CH_4 and CO_2

parameter, and Fig. 7 represents the relative uncertainty contribution of each factor of CH_4 (124.8 μmol mol^{-1}) and CO_2 (1099.8 μmol mol^{-1}) calibration gas mixtures. The relative combined standard uncertainty (u_c) including all the five parameters can be calculated by following Eq. (11), and results are represented in Table 5.

$$u_c = \sqrt{u_r^2 + u_R^2 + u_{LOD}^2 + u_{Cal}^2 + u_{CGM}^2} \qquad (11)$$

Expanded uncertainty (U) can be calculated as Eq. (12).

$$U = k \times u_c \qquad (12)$$

Expanded uncertainty of CH_4 and CO_2 calibration gas mixture were calculated 2.21 and 11.19 μmol/mol at 95 % confidence interval with coverage factor $k = 2.36$ and 1.96, respectively. The coverage factor is calculated with respect to effective degree of freedom (v). v is calculated using Welch–Satterthwaite approximation formula represented in Eq. (13) [46, 47].

$$v_{eff} = \frac{u_c^4}{\sum_{i=1}^{n} \frac{u_i^4}{v_i}} \qquad (13)$$

where u_c is combined standard uncertainty and u_i is uncertainty associated with each parameter and v_i is degree of freedom of corresponding ith parameter. Major relative contribution of uncertainty in whole procedure is likely to obtained from regression statistics and certificate value. Relative uncertainty due to calibration curve (u_{cal}) for CH_4 and CO_2 gas mixture is 5×10^{-3} and 1×10^{-3}, respectively, and from amount fraction of calibration gas mixture (u_{CGM}) are 3×10^{-3} and 5×10^{-3}, respectively. Repeatability (u_r) and reproducibility (u_R) relative uncertainty contribution are relatively low.

Stability study

Stability is an important parameter in calibration gas mixtures and as one of the main criteria in method validation process. Stability study is categorized mainly in two types: (a) long-term stability under storage condition and (b) short-term stability which is used for transport facility [48]. This type of stability study mainly incorporated for the consistency in calibration gas mixtures. Although these CGMs are very stable in nature, a six-month stability study of calibration gas mixture of CH_4 and CO_2 was performed, and uncertainty associated with this long-term storage was calculated. According to ISO Guide 35, uncertainty related to long term stability of calibration standard gas mixture can be obtained Eq. (14).

$$u_{CGM} = \sqrt{u_{grav}^2 + u_{ver}^2 + u_{bb}^2 + u_{lts}^2} \qquad (14)$$

where u_{grav} is the gravimetric uncertainty, u_{ver} is the uncertainty due to verification, u_{bb} is the uncertainty due to homogeneity study carried out in between gas cylinder variation which is neglected in case of calibration gas mixture and u_{lts} is the uncertainty which is calculated during long-term storage. This Eq. (14) is originated from the assumption that there is not so much degradation in material over the period of time. u_{grav} and u_{ver} is the uncertainty associated with preparation procedure and verification analysis and their respective results are represented in Table 6. Uncertainty due to long-term storage will be $x.u_b$, where x defines the shelf life and u_b is the uncertainty due to slope which can be calculated by linear regression Eq. (15) with the analysis of 6 month of period of calibration gas mixture of CH_4 (124.8 μmol mol^{-1}) and CO_2 (1099.8 μmol mol.$^{-1}$)

$$y = bx + a \qquad (15)$$

Table 6 Uncertainty calculation due to long-term storage of CH_4 and CO_2 gas mixture

	Equations	CH$_4$	CO$_2$
u_{grav}	–	0.62	5.5
u_{ver}	–	0.03	0.39
Slope (b)	$b = \frac{\sum_{i=1}^{n}(X_i - \bar{X})(y_i - \bar{y})}{\sum_{i=1}^{n}(X_i - \bar{X})^2}$ (16) *	0.00011	−0.00882
Standard error of slope (u_b)	$s(t) = \frac{s}{\sqrt{\sum_{i=1}^{n}(X_i - \bar{X})^2}}$ (17) $s^2 = \frac{\sum_{i=1}^{n}(Y_i - b_0 - b_1 X_i)^2}{(n-2)}$ (18) This s is the points among line	0.00253	0.0048
u_{lts} (long-term storage)	$x \cdot u_b$	0.00126	0.00243

*where X_i is the gravimetric amount fraction of analyte, $\bar{X}$ is the average of all standards taken, y_i is the response value and $\bar{y}$ is the average of all responses

where b is slope and a is intercept, y is the concentration of CGMs, and x is the time period. Slope (b), standard deviation of slope (s) and uncertainty due to long-term storage of CGMs can be calculated by given Eqs. (16), (17) and (18) represented in Table 6. Combined uncertainty due to long-term storage (6 month of period) of CGMs is calculated according to Eq. (14) were 0.624 μmol mol^{-1} and 5.5 μmol mol^{-1} for CH_4 and CO_2, respectively. From the study it was calculated that relative contribution of uncertainty parameter of long-term storage were observed as 1×10^{-5} and 2.2×10^{-6} for CH_4 and CO_2, respectively, which were negligible. Therefore, there is no effect on the amount fraction of CGMs; hence, material can be stable over a long period of time. This stability study in method validation is a prime requirement for analyte so that it can be used for intended purpose.

Conclusion

In this study, a strategy is enacted for method validation and measurement uncertainty (MU) estimation for monitoring of greenhouse gases (CO_2 and CH_4) using in-house prepared gas standard mixture. The CGMs of CO_2 and CH_4 were prepared by following ISO 6142-1, and verification was done using standard protocol of ISO 6143. Each result in validation parameters provides sufficient evidence that this method is fit for usage for greenhouse gases measurement. The linearity parameter is confirmed by many statistical inputs such as graphical plots, statistical significance and from numerical parameter, which prevails that there is no any deviation from linearity in the prepared range of calibration gas mixtures. In this analytical procedure, all the uncertainty factors are incorporated which include repeatability, reproducibility, calibration gas mixtures, calibration curve and limit of detection. Relative uncertainty in this analytical procedure for CH_4 (124.8 μmol mol^{-1}) and CO_2 (1099.8 μmol mol^{-1}) was observed 0.019 and 0.01 at 95 % confidence interval with coverage factor (k) 2.16 and 1.96, respectively. The experimental evidence provided in this case study establishes a degree of confidence for accuracy of results in analytical methods.

Author contributions Ms. Komal did all the experimental analysis, data curation and writing manuscript. Dr Daya Soni supervised in all experimental part , constructing the manuscript and data processing. Dr. Shankar G. Aggarwal reviewed the manuscript.

Data availability The data supporting the findings of this study are available from the corresponding author on request.

Declarations

Conflict of interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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