Implementation of traceability to measurements of air pollution

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To my parents, my sister, and Mathi.
Thank you for all the love and support.

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ABSTRACT

Air pollution is a critical global issue, with carbon monoxide (CO) and carbon dioxide (CO_2) playing significant roles in both environmental degradation and public health risks. Accurate measurement of these gases is essential for air quality monitoring, regulatory compliance, and climate change mitigation. However, achieving reliable and internationally comparable measurements requires robust metrological traceability, well-characterized reference materials, and standardized calibration procedures.

This thesis focuses on the implementation of metrological traceability in air pollution measurements through the development and certification of reference gas mixtures. A Primary Reference Material (PRM) for CO_2 and Certified Reference Materials (CRMs) for both CO and CO_2 were prepared using gravimetric methods, ensuring direct traceability to the International System of Units (SI). The study includes a comprehensive evaluation of homogeneity, short- and long-term stability, and measurement uncertainty, as well as an in-depth comparison of calibration methods, including calibration curves, two-point bracketing, and single-point exact-match calibration (SPEM).

The findings confirm that high-accuracy reference gas mixtures can be reliably produced and maintained, significantly improving the quality and consistency of air pollution measurements. The results also highlight key factors influencing gas mixture stability, particularly the effects of adsorption and desorption on cylinder walls and temperature variations during storage and transport. By strengthening metrological capabilities in gas analysis, this research supports more precise environmental assessments, enhances the reliability of pollution data, and contributes to the development of evidence-based air quality policies.

RESUMEN

La contaminación del aire es un problema global crítico, con el monóxido de carbono (CO) y el dióxido de carbono (CO_2) desempeñando roles significativos tanto en la degradación ambiental como en los riesgos para la salud pública. La medición precisa de estos gases es esencial para el monitoreo de la calidad del aire, el cumplimiento de normativas y la mitigación del cambio climático. Sin embargo, lograr mediciones confiables y comparables a nivel internacional requiere una trazabilidad metrológica sólida, materiales de referencia bien caracterizados y procedimientos de calibración estandarizados.

Esta tesis se centra en la implementación de la trazabilidad metrológica en las mediciones de contaminación del aire mediante el desarrollo y certificación de mezclas de gases de referencia. Se prepararon un Material de Referencia Primario (PRM) para CO_2 y Materiales de Referencia Certificados (CRMs) para CO y CO_2 utilizando métodos gravimétricos, lo que garantiza la trazabilidad directa al Sistema Internacional de Unidades (SI). El estudio incluye una evaluación exhaustiva de la homogeneidad, la estabilidad a corto y largo plazo, y la incertidumbre de medición, así como una comparación detallada de los métodos de calibración, incluyendo curvas de calibración, *bracketing* de dos puntos y calibración exacta en un solo punto (SPEM).

Los resultados confirman que las mezclas de gases de referencia de alta precisión pueden producirse y mantenerse de manera confiable, mejorando significativamente la calidad y la consistencia de las mediciones de contaminación del aire. Además, los hallazgos destacan los factores clave que influyen en la estabilidad de las mezclas de gases, en particular los efectos de adsorción y desorción en las paredes de los cilindros y las variaciones de temperatura durante el almacenamiento y transporte. Al fortalecer las capacidades metrológicas en el análisis de gases, esta investigación respalda evaluaciones ambientales más precisas, mejora la confiabilidad de los datos sobre la contaminación y contribuye al desarrollo de políticas de calidad del aire basadas en evidencia.

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ABBREVIATIONS

BIPM – Bureau International des Poids et Mesures
CCC – Calibration Curves Computing
CCQM – Consultative Committee for Amount of Substance: Metrology in Chemistry and Biology
CIPM – International Committee for Weights and Measures
CO – Carbon monoxide
CO_2 – Carbon dioxide
CRM – Certified Reference Material
FID – Flame Ionization Detector
GC – Gas Chromatography/Chromatograph
GDR – Generalized Distance Regression
GGMR – Generalized Gauss-Markov Regression
GHG – Greenhouse Gas
GLS – Generalized Least Squares
H_2O – Water
INMETRO - National Institute of Metrology Standardization and Industrial Quality (Brazil NMI)
INRiM – Istituto Nazionale di Ricerca Metrologica (Italy NMI)
IUPAC – International Union of Pure and Applied Chemistry
LATU – Technological Laboratory of Uruguay (Uruguay NMI)
NMI – National Metrology Institute
NO_x – Nitrogen oxides
NPL – National Physical Laboratory (UK NMI)
OLS – Ordinary least squares
PRM – Primary Reference Materials
RM – Reference Material
SI – International System of Units
SO_2 – Sulfur dioxide
SPEM – Single-Point Exact-Match
TCD – Thermal Conductivity Detector
VSL – Van Swinden Laboratory (The Netherlands NMI)
WTLS – Weighted Total Least Squares

CHAPTER 1. INTRODUCTION

1.1. Climate Change and the Role of Carbon Dioxide and Carbon Monoxide

1.1.1. Climate change

Climate change is one of the most urgent global issues of the 21st century, driven primarily by the increase in greenhouse gas (GHG) concentrations in the atmosphere. Among these gases, carbon dioxide (CO_2) and carbon monoxide (CO) play essential roles in influencing the Earth's climate and atmospheric chemistry. Their presence and accumulation are directly linked to anthropogenic activities, particularly the combustion of fossil fuels, industrial emissions, and biomass burning ((World Meteorological Organization [WMO], 2024)).

1.1.2. The Impact of CO_2 on Climate Change

Carbon dioxide is the most significant anthropogenic GHG contributing to climate change. Its concentration in the atmosphere has been rising at an alarming rate, with 2023 marking a record high of 420.0 ppm, an increase of approximately 151% compared to pre-industrial levels, before the year 1750 (WMO, 2024). The primary driver of this increase is the continued reliance on fossil fuels for energy production, alongside land-use changes such as deforestation. CO_2 is responsible for nearly 66% of radiative forcing by long-lived GHGs, significantly impacting global temperatures (WMO, 2024).

The consequences of rising CO_2 levels are profound. The warming of the atmosphere has led to more frequent and intense heatwaves, disruptions in precipitation patterns, and increased occurrences of extreme weather events. Additionally, the ocean absorbs a substantial fraction of emitted CO_2, leading to acidification, which adversely affects marine ecosystems and biodiversity (Intergovernmental Panel on Climate Change [IPCC], 2023; WMO, 2024).

1.1.3. The Role and Consequences of CO

While carbon monoxide is not a GHG itself, it plays an indirect role in climate change by influencing the atmospheric concentrations of methane and tropospheric ozone, both potent GHGs. CO is primarily emitted from incomplete combustion of fossil fuels, biomass burning, and wildfires. In 2023, the world witnessed a significant rise in CO levels, particularly due to extreme wildfire events in Canada and Australia, which emitted substantial amounts of CO and CO_2 into the atmosphere (WMO, 2024).

Elevated CO levels also contribute to poor air quality, posing serious health risks such as respiratory issues and cardiovascular diseases. Given its relatively short atmospheric lifetime, CO serves as a valuable indicator of pollution from burning fuels and biomass, making its monitoring crucial for both climate and air quality management (WMO, 2024) .

1.1.4. The Need for Continuous Monitoring and Measurement

Accurate and continuous monitoring of CO_2 and CO is essential for understanding their long-term trends, evaluating the effectiveness of mitigation strategies, and predicting future climate scenarios. Organizations such as the World Meteorological Organization (WMO) and the Intergovernmental Panel on Climate Change (IPCC) emphasize the need for robust observational networks to monitor the concentration levels of these gases across different regions (IPCC, 2023; WMO, 2024).

Recent advancements in satellite measurements, monitoring stations, and atmospheric modelling have improved our ability to quantify emissions and assess their impact. However, significant challenges remain, particularly in identifying sources and understanding the interactions between these gases and the Earth's climate (IPCC, 2023).

In response to these challenges, there has been increasing emphasis on the development and deployment of novel sensors and low-cost measurement techniques aimed at addressing observational gaps in GHG monitoring (Bureau International des Poids et Mesures [BIPM], 2023). Closing this gap is essential for advancing climate science and requires an integrated GHG monitoring system. Such a system should include not only traditional high-precision techniques, but also widely distributed low-cost sensor networks and satellite observations. The scientific community is encouraged to rapidly implement these systems in collaboration with key organizations such as WMO. The inclusion of low-cost sensors can expand spatial coverage and enable more accessible, decentralized environmental monitoring, which complements and enhances traceable and high-accuracy measurements.

Addressing climate change requires a complex approach, including reducing emissions, enhancing carbon sinks, and improving technological capabilities for precise measurement and analysis. Accurate quantification of CO_2 and CO emissions is crucial for ensuring that the results of measurements are reliable and can guide effective action. Trust in these measurements is essential for making informed decisions and developing policies that can mitigate the impact of these gases on global climate systems (Rolle et al., 2017; Brewer et al., 2019).

1.2. Metrological traceability and the International System of Units (SI)

Metrological traceability is the property of a measurement result whereby the result can be related to a reference through a documented unbroken chain of calibrations, each contributing to the measurement uncertainty (JCGM, 2012). This chain is vital for maintaining consistency, comparability, and accuracy across different measurement systems globally. Traceability supports the harmonization of measurements worldwide, enabling consistent, trustworthy results and promoting fair practices in international trade and regulatory compliance.

Metrological traceability can be visualized as a pyramid. At the top lies the International System of Units (SI), the global reference framework for measurement standards. The SI provides a set of consistent definitions for seven fundamental quantities: the meter (length), kilogram (mass), second (time), ampere (electric current), kelvin (temperature), mole (amount of substance), and candela (luminous intensity) (BIPM, 2019). These units are the building blocks of all measurement activities, ensuring that measurements across the world are based on universally accepted definitions. The development and maintenance of the SI are the responsibility of National Metrology

Institutes (NMIs), which ensure that measurement standards within their respective countries are traceable to the SI (BIPM, 2019).

The Bureau International des Poids et Mesures (BIPM) plays a central role in overseeing the SI and ensuring that every NMI in the world work together to maintain and promote consistent measurement standards. The BIPM is an intergovernmental organization founded by the Meter Convention in 1875 and is tasked with ensuring the global harmonization of measurement systems. Through the BIPM, the International Committee for Weights and Measures (CIPM) and various technical committees such as the Consultative Committee for Amount of Substance (CCQM), countries cooperate to develop and enhance measurement standards, ensuring that measurement results are equivalent and consistent across borders.

Below the SI, are the NMIs, which are responsible for the development, maintenance, and dissemination of national measurement standards. Each NMI contributes to ensuring that measurements performed in its country are traceable to the SI through various calibration steps (BIPM, 2019). This responsibility includes the creation of Primary Reference Materials (PRMs) and Certified Reference Materials (CRMs), which are critical components in chemical metrology. PRMs are developed using primary measurement methods, which are the highest-level measurement techniques with direct traceability to SI units (BIPM, 2019).

The traceability chain operates by linking each level of measurement to the previous one. For instance, a laboratory's measurement results are traceable to the SI through secondary laboratories, which perform calibration using CRMs and PRMs. However, as the number of steps in this chain increases, the measurement uncertainty also increases (De Bièvre & Taylor, 2003). This means that the more calibration steps are involved, the more uncertainty is associated with the final measurement result. To minimize uncertainty, it is crucial to develop PRMs and CRMs with the lowest possible uncertainty. In this sense, NMIs must maintain the highest level of measurement accuracy and precision (De Bièvre & Taylor, 2003).

As the uncertainty increases with each calibration step, it becomes imperative for NMIs and laboratories to carefully manage and document the measurement uncertainty at each stage of the calibration process. It is crucial that by the end of the process, both analytical laboratories and the end user have a reasonable level of uncertainty that ensures confidence in the reliability of their results (De Bièvre & Taylor, 2003).

To verify the accuracy and competence of the NMIs it is important their participation in key comparisons, which are international interlaboratory measurement comparisons. Key comparisons, organized by international bodies such as the CCQM, serve to establish the degree of equivalence between NMIs, ensuring that their measurement capabilities are globally recognized and comparable.

Through these activities, NMIs play a critical role in maintaining global measurement standards. The Mutual Recognition Arrangement (CIPM MRA), which is an agreement between NMIs of participating countries, recognizes each other's calibration certificates and measurement standards, facilitating the acceptance of measurement results across borders (CIPM, 2003). This agreement ensures that a calibration certificate issued by an NMI in one country is recognized and trusted internationally, promoting consistency in global trade, scientific research, and regulatory frameworks.

1.3. Reference gas mixtures

1.3.1. Definition of a reference gas mixture

A reference gas mixture is a precisely prepared blend of gases, with known and certified amount fractions, which is prepared for particular use and should have characteristics such as homogeneity, stability, unit, associated uncertainty and metrological traceability. These mixtures are usually used for the calibration and validation of gas measurement instruments and essential in ensuring their accuracy, reproducibility, and traceability (Słomińska et al., 2010; JCGM, 2012).

Usually these mixtures are classified depending on the preparation process and their value assignment. In this study we will be using the terms Primary Reference Materials (PRMs) or Certified Reference Materials (CRMs).

1.3.2. Gas mixture primary reference material

A PRM is a calibration gas mixture prepared gravimetrically in accordance with the International Standard ISO 6142-1 and validated against independent PRMs according to the International Standard ISO 6143 (International Organization for Standardization [ISO], 2015; International Organization for Standardization [ISO], 2001). These mixtures are at the top of the traceability chain, providing the smallest achievable uncertainties. The gravimetric preparation ensures accurate determination of amount fractions by precisely weighing pure gases, liquids, or pre-mixed components, converting their masses to amount of substances based on chemical purity and atomic/molecular masses, and verifying the final mixture against independent reference standards (International Organization for Standardization [ISO], 2015).

PRMs serve as the foundation for traceability in metrology, providing certified values with the lowest possible uncertainties, as documented in their certificates. These certificates include the gravimetric amount fraction value, associated uncertainty, and an expiration date determined through stability studies.

1.3.3. Gas mixture certified reference material

A CRM is a reference material characterized by a metrologically valid procedure for one or more specified properties, accompanied by a reference material certificate that provides the value of the specified property, its associated uncertainty, and a statement of metrological traceability. Uncertainties for such attributes may be expressed as probabilities or confidence levels (International Organization for Standardization [ISO], 2016). A reference material (RM) is a substance that is sufficiently homogeneous and stable concerning one or more specified properties and has been established as fit for its intended use in a measurement process. The certified value is the assigned value of the reference material, documented with an uncertainty statement and metrological traceability, as specified in the reference material certificate (International Organization for Standardization [ISO], 2016).

In the case of a gas mixture CRM, preparation is performed by introducing different gases into a cylinder, typically by controlling their pressures, weighing them on an analytical balance, and then analysing the resulting mixture using an appropriate instrument, such as a gas chromatograph. The assigned value is determined by comparison against independent PRMs injected alongside the mixture. The value can be assigned using methods such as a calibration curve of PRMs, two-point calibration with bracketing, or single-point exact-match calibration (SPEM) (International Organization for Standardization [ISO], 2017, ISO 12963).

1.4. Gravimetry

Gravimetry is a precise technique used for preparing primary reference gas mixtures, crucial for ensuring traceability in gas metrology. NMIs commonly use gravimetric methods to prepare high-accuracy gas mixtures by accurately weighing gas components based on their mass, molar mass, and purity. The mass of each gas component is determined by weighing cylinders before and after filling them with gas. This method ensures that gas mixtures are traceable to the SI, providing reliable standards for various applications such as emissions control and air quality monitoring (Matsumoto, 2004; International Organization for Standardization [ISO], 2015).

Gravimetry is essential for producing reference gas mixtures with known compositions, but its accuracy is limited by the smallest mass that can be measured. To overcome this limitation, gravimetric techniques often involve multiple dilution steps to improve precision. These dilution procedures ensure that the final gas mixture meets the required uncertainty specifications, even for highly diluted mixtures (Milton et al., 2011).

One challenge in gravimetric measurements is the buoyancy effect, which can cause errors in mass determination. Changes in environmental factors such as temperature, pressure, and humidity affect the buoyancy of gas cylinders, leading to inaccuracies. To minimize this, gravimetric methods employ comparative weighing, where the sample cylinder is weighed against a reference cylinder. This technique helps reduce the impact of buoyancy variations and enhances the accuracy of mass measurements (Milton et al., 2011; Amico di Meane et al., 2009; Alink & van der Veen, 2000).

For gas metrology to maintain reliable standards, traceability to the SI unit of the mole is crucial. Gravimetry provides a direct way to measure the amount of substance, ensuring that results are traceable. This is achieved through precise weighing, careful consideration of component purity, and verification against independent reference mixtures. The accuracy of gravimetric methods makes them ideal for creating certified reference materials used in calibration and metrological applications (De Bièvre & Taylor, 2003).

1.5. Methods for determining or checking the composition of reference gas mixture

1.5.1. Calibration Curve

The calibration curve method, as described in ISO 6143:2001, is a multipoint calibration technique used to establish the relationship between instrumental response and the analyte content in calibration gas mixtures. This method requires selecting a series of reference gas mixtures with known analyte amount fractions, ensuring that the amount fraction range covers the expected values of the unknown sample in which the analyte content has to be determined. The instrument's response to each reference gas mixture is measured, generating a calibration function. The relationship between instrument response and analyte amount fraction is modelled using regression analysis, which can be linear, polynomial, or another non-linear approach, depending on the system's response characteristics (International Organization for Standardization [ISO], 2001). Advanced regression techniques are applied to account for uncertainties in both the instrument response and the reference gas composition.

Once the calibration function is established, the uncertainty of the measurements is calculated using statistical propagation methods, and the model undergoes validation to ensure reliability before being used for sample analysis (International Organization for Standardization [ISO], 2001).

1.5.2. Two-point calibration with bracketing – bracketing

The bracketing method, also referred to as two-point calibration with bracketing in ISO 12963:2017, is a simplified approach that requires only two calibration gas mixtures. One mixture has a lower amount fraction of the analyte, while the other has a higher amount fraction, effectively "bracketing" the unknown sample. The instrument's response to both calibration gases is recorded, followed by the response of the unknown sample. The analyte amount fraction in the unknown sample is then determined through linear interpolation between the two reference points. This method assumes a linear relationship between response and analyte content, and its accuracy depends on the reliability of this assumption.

Uncertainty is evaluated by considering potential deviations from linearity and variations between the two reference gases. Compared to the calibration curve method, bracketing is faster and uses less PRMs. However, in gas analysis its accuracy is lower than that of multipoint calibration, particularly when the system shows non-linearity (International Organization for Standardization [ISO], 2017, ISO 12963).

1.5.3. Single-point exact-match calibration (SPEM)

The single-point exact-match calibration (SPEM) method, as described in ISO 12963:2017, is the simplest calibration technique and is used when the calibration gas composition closely matches that of the unknown sample. The process begins with selecting a calibration gas mixture whose analyte amount fraction is nearly identical to the sample one. The instrument's response to the calibration gas is measured multiple times to ensure stability. If the responses of the calibration gas and the unknown sample are statistically indistinguishable, the sample's composition is assumed to be the same as that of the calibration gas. This method avoids the need for regression or interpolation, as the uncertainty of the calibration gas mixture is directly transferred to the sample measurement.

Although SPEM is the fastest and most straightforward calibration method, its applicability is limited to cases where a closely matching calibration gas is available. When such a match is possible, SPEM provides highly accurate results with minimal calibration effort, making it particularly useful for high-precision applications requiring rapid analysis (International Organization for Standardization [ISO], 2017, ISO 12963).

1.6. Gas chromatography

Gas chromatography (GC) was the method selected for the analysis of the prepared gas mixtures in the present work. This analytical technique is used to separate and analyse volatile compounds in a mixture. The sample is vaporized and transported through a chromatographic column by an inert carrier gas, such as helium, nitrogen, or hydrogen. The separation of the components occurs based on their differential interactions with the stationary phase inside the column, leading to distinct retention times. This technique is widely used in environmental analysis, petrochemical testing, and food quality control.

Direct gas injection in GC is employed when working with gaseous samples, allowing for the introduction of the sample without requiring a liquid phase. The injection is typically performed using a gas sampling valve with a pressure regulator or a gas-tight syringe, ensuring precise volume control. This method is particularly useful in analysing permanent gases, volatile hydrocarbons, and atmospheric pollutants. It eliminates the need for solvent extraction or derivatization, making it an efficient and straightforward approach for analysing gas-phase samples (Skoog et al., 2021; Flores et al., 2019b).

GC relies on various detectors to identify and quantify the separated components.

1.6.1. Thermal conductivity detector (TCD)

The TCD is a universal detector used in gas chromatography to measure changes in the thermal conductivity of the carrier gas due to the presence of analytes. As different compounds elute from the column, they alter the thermal conductivity of the carrier gas, which is detected as a signal. This detector consists of an electrically heated source, typically a fine wire made of platinum, gold, or tungsten, or a small thermistor. The temperature of the heated element depends on the thermal conductivity of the surrounding gas, and its electrical resistance changes accordingly. The TCD is non-destructive, allowing for sample recovery, making it ideal for detecting both organic and inorganic gases, such as hydrogen, nitrogen, oxygen, and carbon dioxide (Skoog et al., 2021).

Although the TCD has a wide dynamic range, typically spanning about five orders of magnitude, and responds to a broad range of organic and inorganic species, it has a relatively low sensitivity compared to other detectors. This lower sensitivity makes the TCD more suitable for samples with higher analyte amount fractions, and it often cannot be used effectively with capillary columns when sample quantities are very low. The TCD's main advantage lies in its simplicity and ability to detect changes in thermal conductivity, but its limitations arise when the thermal conductivities of the carrier gas and sample components are similar, leading to less reliable detection (Skoog et al., 2021).

Helium and hydrogen, with thermal conductivities approximately six to ten times greater than most organic compounds, cause significant decreases in the thermal conductivity of the effluent when organic species are present. This results in an increase in the temperature of the detector. However, thermal conductivity detection is less effective when the thermal conductivities of the carrier gas and the sample components are similar. Despite its limitations, the TCD's non-destructive nature and broad applicability to various types of gases make it a useful tool in many chromatographic analyses (Skoog et al., 2021).

1.6.2. Flame ionization detector (FID)

The FID is a highly sensitive and widely used detector in gas chromatography, particularly for analysing organic compounds. The FID works by combusting the sample in a hydrogen-air flame, which produces ionized carbon species. These ions generate an electrical current that is proportional to the number of carbon atoms in the sample, enabling accurate quantification (Skoog et al., 2021).

The FID is especially effective for detecting hydrocarbons due to its high sensitivity, wide linear dynamic range, and excellent stability. Functional groups such as carbonyl, alcohol, halogen, and amine produce fewer ions (or none) in the flame, and the detector is insensitive to non-combustible gases like H_2O, CO_2, SO_2, and NO_x. Despite this limitation, the FID remains a very useful detector for organic samples, including those contaminated with water and nitrogen and sulphur oxides.

To enhance the FID's ability to detect gases like CO and CO_2, a methanizer can be incorporated into the system. The methanizer is a catalytic reactor that converts CO and CO_2 into methane using a nickel catalyst at elevated temperatures, around 350°C. Once converted to methane, these gases can be detected by the FID, improving its sensitivity for these compounds. The methanizer is particularly useful in environmental monitoring and industrial applications, where detecting trace levels of CO and CO_2 is critical (Skoog et al., 2021).

1.7. Method validation

To make sure that the methodology is adequate to quantify the analytes in the gas mixture, it is necessary to perform a method validation. Method validation is a fundamental step in the development and application of calibration gas mixtures, ensuring the reliability, accuracy, and reproducibility of measurement results. In gas metrology, where traceability to international standards is required, validation provides confidence in the measurement process by assessing key performance parameters.

1.7.1. Precision

Precision is the closeness of agreement between indications or measured quantity values obtained by replicate measurements on the same or similar objects under specified conditions. It is usually expressed numerically by measures of imprecision, such as standard deviation, variance, or coefficient of variation under the specified conditions of measurement (JCGM, 2012).

1.7.2. Accuracy

Accuracy is the closeness of agreement between a measured quantity value and a true quantity value of a measurand (JCGM, 2012). In metrology, it is usually evaluated with a normalised error (Formula 54) between the value being measured and the certified value of the PRM. (Becerra, 2003).

1.7.3. Selectivity

Selectivity ensures the method can accurately distinguish the target analyte from other potential interferences. This is particularly important in gas mixtures where multiple components may be present.

1.7.4. Range

Range is the measuring interval, a set of values of quantities of the same kind that can be measured by a given measuring instrument or measuring system with specified instrumental measurement uncertainty, under defined conditions (JCGM, 2012).

1.7.5. Linearity (Regression)

Linearity assesses whether the measurement response is proportional to the analyte amount fraction across the calibration range. This is typically evaluated using regression analysis, such as goodness-of-fit tests, where a calibration curve is fitted to experimental data points. A key statistical metric for evaluating linearity is the residual sum of weighted squared deviations, which quantifies the total deviation of observed values from the fitted regression model, weighted by their uncertainty. A residual sum of weighted squared deviation below 2 indicates a good fit of the data to the regression model, ensuring accurate quantification across the calibration range (International Organization for Standardization [ISO], 2015).

1.7.6. Robustness

Robustness determines the method’s ability to maintain its performance when small variations in experimental conditions occur, such as temperature fluctuations, carrier gas flow rate changes, or minor instrument adjustments.

1.7.7. Uncertainty

Uncertainty quantifies potential sources of error affecting the measurement, using uncertainty propagation techniques. This includes contributions from calibration gas preparation, instrumental response, and environmental factors.

CHAPTER 2. JUSTIFICATION AND OBJECTIVES

2.1. Justification

The accurate measurement of CO and CO_2 is crucial due to their significant impact on both environmental and public health. CO is a toxic gas that poses serious health risks even at low concentrations, while CO_2 is a major contributor to climate change. At a national level, ensuring the traceability of measurements for these gases is essential to guarantee the reliability and comparability of data obtained from different monitoring systems. Without proper traceability, measurement discrepancies can lead to incorrect assessments of pollution levels, potentially hindering effective decision-making and regulatory compliance (Almirón et al., 2024).

Implementing traceability in CO and CO_2 measurements allows for the establishment of standardized methods that improve accuracy, reproducibility, and long-term monitoring reliability. This is particularly important for air quality control, industrial emissions monitoring, and climate studies, where precise quantification is necessary for developing mitigation strategies and policies.

To achieve this, the use of certified reference gas mixtures is essential. These mixtures serve as reliable controls for environmental measurements, method validation, and instrument calibration, all of which are fundamental to ensuring the accuracy, safety and consistency of gas analysis. By ensuring that measurements are traceable to recognized standards, authorities, researchers, and industry professionals can make informed decisions that contribute to environmental protection, process safety, and human health.

In the present work, CRM and a PRM with an amount fraction of 0.35 mol/mol of CO_2 in N_2 were prepared, alongside a CRM of CO with an amount fraction of 1500 µmol/mol of CO in N_2. These certified gas mixtures are designed to support environmental monitoring and air quality assessment efforts in Uruguay. The 0.35 mol/mol CO_2 reference mixture serves as a reference standard for emissions control from different sources, such as industrial chimneys and exhaust systems. Meanwhile, the 1500 µmol/mol CO mixture is intended for air quality applications, simulating realistic levels of carbon monoxide pollution that, while not immediately lethal, pose health risks with prolonged exposure. Both mixtures were specifically requested by environmental laboratories in Uruguay, which require accurate and traceable reference materials for calibrating gas analysers, sensors, and atmospheric monitoring equipment. For these mixtures, a relative expanded uncertainty of 2% was established for the CRMs and 1% for the PRM. These uncertainty levels were set more as targets to evaluate feasibility and assess whether such precision could be achieved in practice. Feedback from local buyers, particularly environmental laboratories in Uruguay, indicated that these uncertainty levels were acceptable and considered fit for purpose within the context of their routine monitoring activities.

2.2. General objective

- To develop a CRM with an amount fraction of 1500 µmol/mol of CO in N_2 and a CRM with an amount fraction of 0.35 mol/mol of CO_2 in N_2 in a 5-liter aluminium cylinder.

- To develop a PRM with an amount fraction of 0.35 mol/mol of CO_2 in N_2 in a 5-liter aluminium cylinder.

2.3. Specific objectives

- To develop a system for vacuuming cylinders and introducing gases into an aluminium cylinder, while controlling both the mass and pressure within the system.

- To develop reference gas mixtures (CRM and PRM) with established metrological traceability to ensure the accuracy and international comparability of the measurements in our country.

- To develop and validate quantification methods using GC-TCD/FID for value assignment and verification of the mixtures.

- To evaluate the uncertainty associated with the preparation of the gas mixtures.

CHAPTER 3. METHODOLOGY

3.1. Materials

- 5-liter Luxfer® aluminium cylinders
- Pressure regulators with a maximum pressure of 200 bar and DIN 1 connection
- Direct injection to GC via 1/16” stainless steel tubing
- Gas chromatograph Thermo Scientific TRACE 1300 with TCD and FID detectors with methanizer
- PC with Chromeleon 7 software, Thermo Fisher Scientific
- Rt-Q-Bond PLOT chromatographic column (30 m, 0.53 mm ID, 20 μm)
- ShinCarbon ST 100/120 chromatographic column (2 m, 1 mm ID, 1/16” OD)
- 250 μL and 500 μL loop
- Filling and emptying station
- Pfeiffer mechanical and turbomolecular pump
- Mass comparator Mettler Toledo XPR10003SC
- Balance Ohaus model RANGER™ 3000
- Gas extraction hood
- Leak detectors
- PRMs from other NMIs
- N_2 (99.9999% mol/mol of purity), batch number: P0212:20200201105. Linde®
- CO_2 (99.9% mol/mol of purity), batch number: 407540-3. Linde®
- Premixture CO 4% in N_2, batch number: 304113031704, Praxair®
- Nitrogen (99.99% mol/mol purity) for blanks, batch number: 305170-1. Linde®
- Snoop® soapy solution for leak detection

3.2. Planning stage of a reference gas mixture

During the planning phase, calculations are performed to ensure the target composition of the final gas mixture is achieved, based on the required masses of each component to be added.

The first step involves assessing the feasibility of preparing the gas mixture. This includes evaluating potential safety risks, such as hazardous reactions between the components, interactions with the cylinder materials, and ensuring the final pressure does not exceed the cylinder's maximum working pressure. A risk analysis, based on existing literature, must be conducted to assess the stability of the gas mixture and identify any potential chemical reactions among its components (International Organization for Standardization [ISO], 2015). For example, oxidizing gases could corrode the cylinder's internal walls. Addressing these concerns helps minimize hazards during the filling process and ensures the final mixture composition remains stable over time.

The parent gases, which are the starting materials, should be pure gases of high purity or pre-mixtures from high-purity gases to minimize the presence of impurities in the final gas mixture.

The next step is calculating the target masses for each component in the mixture. This calculation is performed using the ideal gas law, considering the target values of the analytes in the final gas mixture, the molar masses of the parent gases, the cylinder volume, and the working temperature (International Organization for Standardization [ISO], 2015). The method for calculating the target masses is the same, regardless of whether the reference mixture is a Certified Reference Material (CRM) or a Primary Reference Material (PRM). The formula used is as follows (Equation 1):

$$m_j = \frac{y_k \times M_k}{\sum_{i=1}^{q} y_i \times M_i} \times m_\Omega \tag{1}$$

Where m_Ω is calculated as:

$$m_\Omega = \frac{p_{F,\Omega} \times V_{cyl}}{Z_\Omega \times R \times T_F} \sum_{i=1}^{q} y_i \times M_i \tag{2}$$

Being,

m_j: mass added of parent gas *j* (mass to be introduced in the cylinder)

y_k: amount-of-substance fraction of component k in the prepared mixture
$\bar{M}_k$: molar mass of component k
y_i: amount-of-substance fraction of component *i* in the prepared mixture

$P_{f,\Omega}$: filling pressure of the final mixture
V_{cil}: cylinder volume
Z_Ω: compressibility of the final mixture
R: Ideal gas constant
T_f: Filling temperature

The balance should have a maximum capacity suitable for weighing the mass of the cylinders and a resolution sufficient to accurately measure even the smallest gas mass. It is crucial to ensure that the calculated mass aligns with the balance's tolerances. The balance used for weighing the cylinders (Ohaus model RANGER™ 3000) can measure masses with an appropriate uncertainty for values above 25 g. Therefore, if the calculated mass is below this threshold, the mixture planning must be adjusted, either by modifying the composition or using a premixture (International Organization for Standardization [ISO], 2015). Premixtures are intermediate-amount fraction gas mixtures prepared from pure gas cylinders, using the same balance gas intended for the final mixture.

3.3. Preparation of a gas reference mixture

3.3.1. Cylinder conditioning and emptying

For the preparation of all gas mixtures, aluminium cylinders manufactured by Luxfer® (UK) were used, fitted with Rotarex® valves sourced from Rotarex® Brazil. These cylinders underwent leak and safety testing to ensure proper performance, as they will be subjected to high pressures during its use. Aluminium was selected due to its high quality and proven compatibility with the gases used in this study (CO, CO_2, and N_2), ensuring no chemical reactions or material interactions occur, in accordance with ISO 16664 (International Organization for Standardization [ISO], 2017, ISO 16664).

The aluminium cylinder which will contain the mixture is initially connected to the outlet of the filling/emptying station to remove any residual gas (Figure 2). To do this, the valves directing gas flow to the extraction hood are opened, and the purge valve is used to completely empty the cylinder. The gas filling station setup is illustrated in the valve diagram shown in Figure 1.

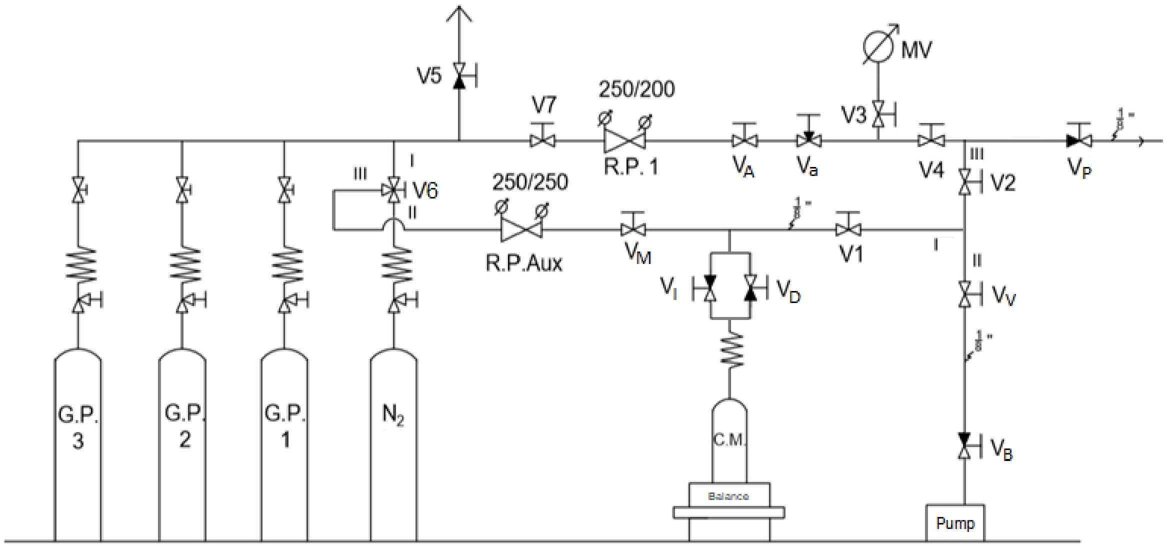


Figure 1: Gas filling station valve diagram

Next, a nitrogen 6.0 cylinder (99.9999% purity) is connected to the inlet of the filling/emptying station to fill the sample cylinder and clean it. First, the system lines are purged by allowing low-pressure N_2 to flow through all the system's pipes multiple times. The system is further purged by opening the purge valve while keeping the extraction hood running. Once the system is clean, the sample cylinder is connected to the station's outlet, and the nitrogen flow is directed into it by opening the appropriate valves. The cylinder is filled with approximately 30 g of gas, and to monitor this, it is placed on a balance that is tared before the filling process begins.

Figure 2: Aluminium cylinder connected to the gas emptying/filling station

After filling, the cylinder is disconnected from the station and placed in a homogenizer (Figure 3), a rotating cylinder device, to ensure the nitrogen comes into contact with the internal walls, removing any remaining impurities. After 30 minutes in the homogenizer, the cylinder is reconnected to the station.

Figure 3: Cylinder homogenizer

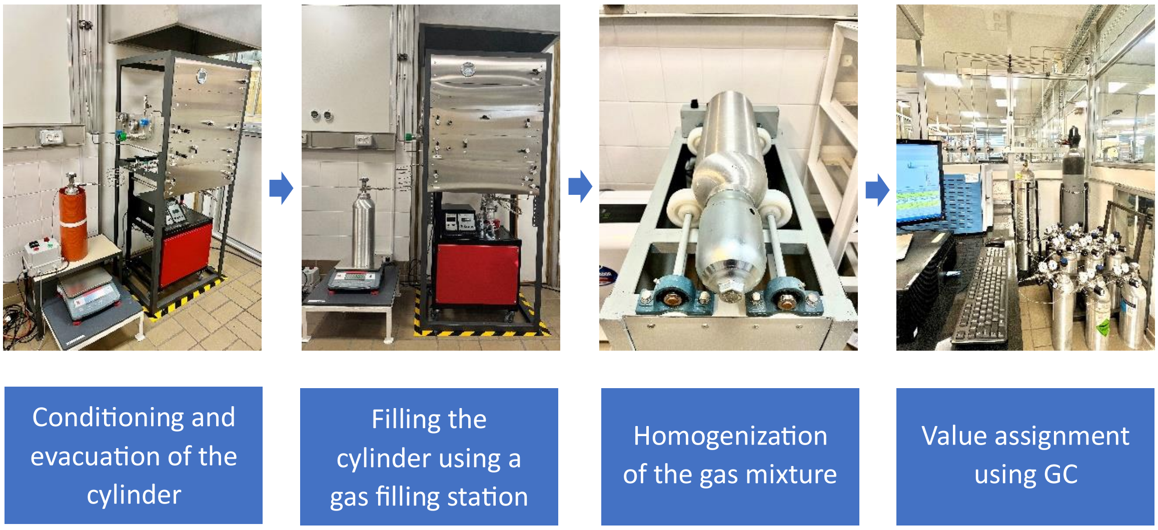
The cylinder is then emptied again by connecting it to the station’s outlet. To facilitate this, a thermal blanket set to 40°C is wrapped around the cylinder (Figure 4), helping achieve a higher vacuum level. The emptying process begins by turning on the mechanical pump and slowly opening the cylinder valve, ensuring the pump’s pressure does not exceed 30 mbar to prevent damage. Once the mechanical pump reaches a pressure below 1 mbar, the turbomolecular pump is activated to achieve a deeper vacuum. When the pressure drops below 1×10^{-5} mbar, or after at least 12 hours under vacuum, the cylinder valve is closed, and the cylinder is disconnected from the system, ready to be filled with the gas mixture.

Finally, the entire system is purged with N_2 6.0, leaving a small amount of gas in the system to prevent air impurities from entering the station.

Figure 4: Cylinder surrounded by thermal blanket connected to the filling/emptying station to be emptied

3.3.2. Preparation of a gas mixture CRM

The whole preparation process of a CRM follows the scheme showed in Figure 5.



Conditioning and evacuation of the cylinder

Filling the cylinder using a gas filling station

Homogenization of the gas mixture

Value assignment using GC

Figure 5: Preparation process of a CRM

Gases are introduced into the cylinder in order of increasing pressure, meaning the gas with the lowest pressure, i.e. the less component at the lowest amount fraction in the final mixture, is added first, followed by gases with gradually higher pressures; the balance gas or matrix gas will be the last to be introduced. Since gases naturally flow from places of higher pressure to lower pressure, this method ensures a complete and homogeneous mixture. Additionally, this approach facilitates corrections when the added gas mass deviates from the target value, as controlling the exact mass entering the cylinder is not always straightforward.

The process begins by connecting the parent gas, the pure gas used to start filling the cylinder, to the inlet of the filling/emptying system. This gas is selected because it has the lowest amount fraction in the final mixture. Any residual gas in the station is purged, and the system is flushed with the pure gas to remove any traces of other gases that could affect the composition of the CRM candidate.

Next, the sample cylinder is placed on a balance and connected to the system's outlet. The system is pressurized with the parent gas, ensuring it is slightly above the intended filling pressure. To accurately monitor the mass of gas added, the balance must be tared with all gas in the line up to the point just before the cylinder valve. The cylinder is then filled while carefully monitoring the added mass on the balance to ensure it does not exceed the target value.

Once the cylinder is filled, it is left to rest for at least one hour, as the gas introduction process generates heat. In the preparation of a Primary Reference Material (PRM), which will be discussed in section 3.3.3, at this stage, the cylinder would be weighed using a mass comparator to precisely determine the amount of gas added, so that its value can then be assigned. However, since this is a CRM preparation, the cylinder is simply left to rest before proceeding with the next gas addition.

Before introducing the next gas, the station must be purged multiple times with the incoming gas to prevent contamination. Additionally, since the cylinder now contains pressurized gas, the pressure of the newly added gas must always be higher than the current pressure inside the cylinder. Also, to ensure accurate mass measurement, the balance must again be tared just before opening the cylinder valve to introduce the next gas.

A CO CRM with an amount fraction of 1500 µmol/mol in N_2 and a CO_2 CRM with an amount fraction of 0.35 mol/mol in N_2 were prepared using this methodology.

3.3.3. Preparation of a gas mixture PRM

The whole preparation process of a PRM follows the scheme showed in Figure 6.

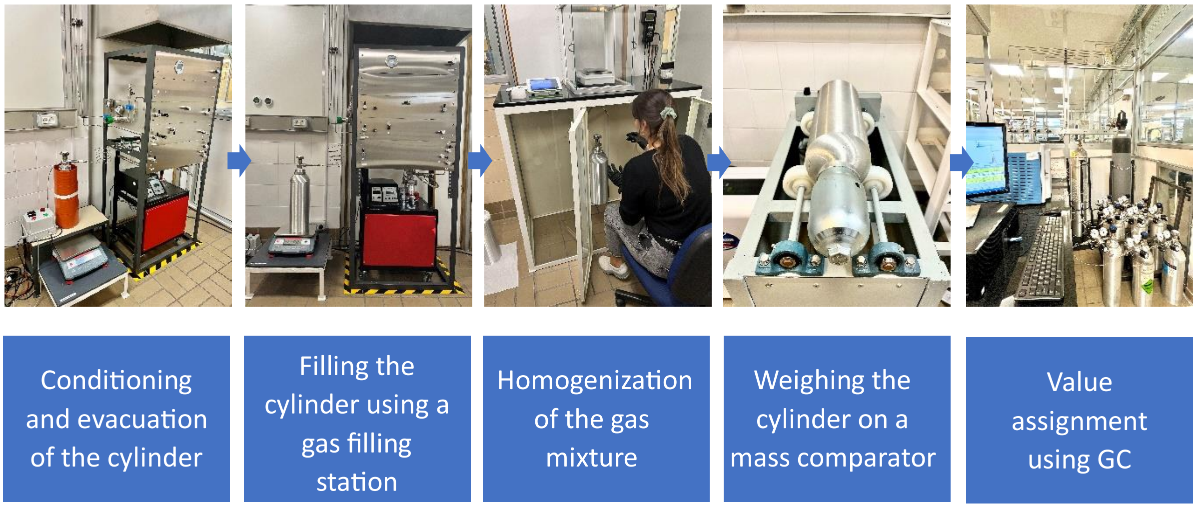


Figure 6: Preparation process of a PRM

This procedure is similar to that of a CRM. The gases are introduced into the cylinder sequentially using the gas filling station, following an ascending order based on the amount fraction that will be present of each gas in the final mixture. The main difference is that at the beginning of the preparation and after each filling stage, the cylinder undergoes several weighing cycles (Flores et al., 2019a).

Before starting the process, the empty cylinder is placed in the mass comparator room for at least an hour to reach thermal equilibrium and prevent weight fluctuations due to temperature differences (Figure 7). This room is maintained under controlled conditions of temperature, humidity, and pressure and is protected from vibrations and air currents to ensure measurement stability.

Figure 7: Mass comparator room, where the cylinder is waiting to be weighed

Since gas cylinders are large objects that displace a significant volume of air, their mass varies proportionally with temperature and humidity. To correct the buoyancy effect caused by variations in atmospheric pressure, a comparative weighing is performed against a reference cylinder of the same material and external volume (International Organization for Standardization [ISO], 2015).

The reference cylinder is prepared similarly to a CRM but contains only nitrogen 5.0 and is not characterized. Its primary function is to reduce the corrections needed in the weighing result of the sample cylinder. During each weighing cycle, the mass difference between the two cylinders is determined, allowing for an accurate calculation of the added mass.

The weighing procedure follows the substitution or Borda method, in which the sample and reference cylinders are weighed alternately on a comparator balance (Alink & van der Veen, 2000). The purpose of this method is to minimize any potential systematic errors by repeatedly weighing the cylinder and its contents, alternating between a reference cylinder and the sample cylinder (Becerra, 2003).

The mass comparator used is a single-pan balance where the cylinders must be placed in a below-balance weighing position (Figure 8). This type of manual mass comparator requires careful centring of the loads, waiting for thermal and vibration effects to subside, and monitoring the beam's oscillations. As a result, achieving the highest accuracy, with uncertainties below 1 mg, requires weighing cycles as long as 10 hours. However, measurements with uncertainties of 20 mg to 30 mg can be obtained with shorter weighing cycles of approximately 1 hour (Milton et al., 2011).

Figure 8: Cylinder being place in a below-balance weighing position of the single-pan balance

To ensure precise measurements within a narrow mass range, the mass difference between the cylinders is compensated by adding or removing calibrated mass pieces (Alink & van der Veen, 2000). These weights are placed on top of a cap positioned above the cylinder. The goal is to minimize the mass difference between the sample and reference cylinders. Depending on the stage of the preparation process, the weights may need to be placed on either the reference or the sample cylinder (Figure 9). The balance is tared at the beginning of every weighing cycle, with the cap positioned on top of the below-balance weighing position to subtract its weight.

Figure 9: Weights placed on top of a cap positioned above the cylinder

Before and after each weighing cycle, a 1 g mass piece is used as a reference. This mass is placed on the balance to verify and calibrate the system, ensuring that the measurements taken throughout the process are accurate and consistent. The use of a 1 g mass at both the beginning and end of each cycle serves to stabilize and verify the accuracy of the balance, correcting for any drift that may occur during the weighing process (Alink & van der Veen, 2000).

It is important to emphasize that traceability to the mole derives from the weighing procedure itself, the analysis of the purity of the source gases, and knowledge of the relative molecular masses (RMMs) of the components (International Organization for Standardization [ISO], 2015). Therefore, the mass pieces used during the weighing process or in the calibration of the balance must be traceable. Similarly, the instruments used to measure ambient temperature and pressure, which are necessary for buoyancy correction, must also be traceable to national or international standards. All of these physical quantities are calibrated by the Physical Metrology Department of LATU.

A CO_2 PRM with an amount fraction of 0.35 mol/mol in N_2 was prepared using this methodology (Figure 10).

Figure 10: PRM prepared in aluminium cylinder

3.4. Homogeneity evaluation

Ensuring the homogeneity of the gas mixture is a crucial step in the preparation process, as any inhomogeneity can result in inaccurate measurements and compromise the traceability of the CRM/PRM. After the final parent gas has been added, and weighed in the case of a PRM, the cylinder must undergo homogenization to achieve a uniform composition throughout (Dias & Costa, 2019; Almirón et al., 2024).

This homogenization is carried out by rolling the cylinder in a horizontal orientation using a homogenizer instrument (Figure 3). The minimum homogenization duration should be determined based on prior experimental knowledge. Density differences between the gas components are particularly important to consider; when a component has a significantly higher relative density than the balance gas, short rolling times may not ensure complete (International Organization for Standardization [ISO], 2015). Moreover, re-homogenization may be necessary after prolonged storage to maintain mixture integrity, as factors such as density differences, temperature variations, adsorption, partial condensation, and chemical reactions can affect homogeneity over time. Failure to do so may lead to compositional drift and variations in the assigned value of the gas mixture (International Organization for Standardization [ISO], 2017, ISO 16664).

Since the gas mixture undergoes proper homogenization through rolling and is composed of gases with similar densities that do not condense, inhomogeneity is not expected to contribute to measurement uncertainty. Therefore, no additional component related to inhomogeneity needs to be included in the uncertainty budget.

3.5. Long term stability evaluation

The long-term stability of a gas mixture is important to make sure its composition stays the same over time. Several factors can impact stability, including adsorption onto the cylinder walls, chemical reactions between components, leaks through seals, and changes in pressure or temperature. Over time, these effects can slightly change the amount fraction of some components, leading to variations from the original composition (International Organization for Standardization [ISO], 2015).

To account for these possible changes, the uncertainty related to stability is included in the overall uncertainty calculation. This helps measure any potential drift in composition over time and ensures the mixture remains reliable. Stability studies usually involve checking the mixture at different time intervals to track any changes, often modelled as a slow drift in the amount fraction (Durbiano et al., 2024). If significant changes are detected, adjustments may be needed, such as applying corrections or reducing the validity period or enlarging the uncertainty associated with the mixture composition.

The gas mixtures were tested at various points throughout their lifespan and continue to be monitored.

3.6. Evaluation of short-term stability

Short-term stability, also known as, transportation stability is a property of the material referring to stability under expected transport conditions. For this, the behaviour of the material and its property values are studied under (as a minimum) the intended conditions for packaging and transport. The more restrictive the intended conditions for transport are, the smaller is the range of conditions that should be included in the transportation stability study (International Organization for Standardization [ISO], 2024). Factors such as temperature fluctuations, pressure changes, and exposure to moisture can affect the stability of the reference gas mixtures (International Organization for Standardization [ISO], 2017, ISO 16664).

If there is no prior experience with a specific material, a short-term stability study should be conducted to determine suitable transport conditions. The study should simulate the expected transport duration and conditions, including possible extreme temperatures. Generally, the study period should match or slightly exceed the actual transport time. For example, if transport is limited to 3 weeks, a 3 to 4 week short-term stability study would be sufficient (International Organization for Standardization [ISO], 2024).

It is assumed in the ISO 33405 standard that transport conditions will usually be sufficiently closely controlled that the effect of transport does not significantly affect the property values and does not require an increase in uncertainty for certified values. Transportation stability studies are therefore assumed to be designed primarily to check for significant change rather than to provide an accurate quantitative estimate of change (International Organization for Standardization [ISO], 2024).

If a reference gas mixture is found to be susceptible to instability under transport conditions, adjustments such as selecting an appropriate cylinder treatment or maintaining the temperature within a controlled range may be necessary to preserve its composition. The mixtures prepared in this work, 1500 µmol/mol of CO in N_2 and 0.35 mol/mol of CO_2 in N_2 are both susceptible to temperature related effects that could compromise their integrity during storage and transportation.

In the case of mixtures of CO_2, adsorption onto cylinder walls is a well-documented phenomenon, particularly if the cylinder has not undergone proper surface treatment or has been exposed to humidity before filling. Variations in temperature can increase desorption, which may lead to slight changes in the gas mixture composition over time (Miller et al., 2015). Additionally, at high pressures, CO_2 can partially condense if the temperature drops significantly, leading to phase separation. Since obtaining pre-treated cylinders is challenging, and the cylinders used in this work are aluminium cylinders with no special treatment, maintaining a controlled temperature range during transport is crucial to preserving the integrity of the mixture. Another important precaution taken during gas mixture preparation is avoiding the use of Snoop® for leak testing. Snoop® is only applied to the connections between the pressure regulators and the direct injection system of the gas chromatograph, ensuring that it does not interfere with the composition of the prepared mixture.

On the other hand, CO is a reactive gas and can slowly oxidize to CO_2 in the presence of residual oxygen, a reaction that is accelerated by elevated temperatures. If trace amounts oxygen are present in the cylinder or valve, prolonged exposure to high temperatures during transport may accelerate this oxidation, leading to a reduction in CO amount fraction over time. Furthermore, CO is known to weakly adsorb onto cylinder walls, particularly when aluminium or untreated steel cylinders are used. As with CO_2, temperature increases can promote desorption, causing slight fluctuations in the measured CO amount fraction immediately after transport.

For this reason, a short-term stability study was performed by placing the PRM of CO_2 in N_2 in a refrigerator at 4ºC for two weeks. Afterward, it was analysed and then placed in a stove at 35ºC for two additional weeks, after which it was analysed again. In this study, the thermal stability of CO_2 was evaluated experimentally, as it is more susceptible to temperature effects such as adsorption, desorption, and condensation.

However, extreme thermal stability tests were not conducted for CO, since only a single reference standard was available, and it was not possible to prepare a second cylinder due to the lack of pure CO. Exposing the standard to conditions that could permanently change its composition would have made it unusable for future stability checks. Since CO_2 successfully passed the thermal stability test and is more predisposed to temperature effects than CO, it is reasonable to assume that CO would also remain stable within the same temperature range. However, due to the lack of direct experimental tests on the thermal stability of CO, this conclusion is based on theoretical considerations rather than experimental verification.

3.7. Value assignment of a reference gas mixture

3.7.1. CRM certification

3.7.1.1. CRM characterization

Characterization refers to the determination of the values of the relevant properties of a reference material, as part of the production process (International Organization for Standardization [ISO], 2024).

The ISO 33405 is intended mainly for the measurements performed to assign the certified values for a material (y). It is important to note that a certified value shall be a good estimate of the true value and not just the average of a population. The certified value can be the same for many individual reference material units, if it is a batch, or an individual value can be assigned to each unit (International Organization for Standardization [ISO], 2024), such as the case of preparing a reference gas mixture in a filling station with just one outlet for connecting the aluminium cylinder.

For certified values, the associated uncertainty of characterization (u_{char}) shall be determined. The ISO 17034 standard requires a reference material producer to provide evidence of the metrological traceability of the certified value to a stated reference (International Organization for Standardization [ISO], 2016). This means that, whatever the approach chosen, the metrological traceability of the certified values shall be clearly defined. Traceability can only be achieved if the values that are combined have been shown to provide valid estimates of the value of the measurand (as defined) within the claimed uncertainty and the results are traceable to the same metrological reference. Ideally, the International System of Units (SI) is the preferred metrological reference, but other internationally agreed references can be used. Metrological traceability also applies to operationally defined measurands; it remains essential to ensure traceability to defined metrological references by proper calibration (International Organization for Standardization [ISO], 2016).

Characterization can be carried out using one or multiple methods across one or more laboratories. One approach is characterization through a single reference measurement procedure (JCGM, 2012) conducted in a single laboratory. Within this category, one option is value transfer characterization, where a “secondary CRM” is value assigned by directly comparing its results with those of an already characterized and closely matched CRM (International Organization for Standardization [ISO], 2024).

When calibrating a candidate CRM, each measurement inherently compares it to another CRM (the calibrator). In this study, the primary approach will be the calibration curve method, using a set of PRMs with amount fractions both below and above the candidate CRM (International Organization for Standardization [ISO], 2001). Additionally, differences in the assigned value of the candidate CRM will be explored using the bracketing technique and the SPEM method (International Organization for Standardization [ISO], 2017, ISO 12963). In all three cases, it is essential that the primary and secondary CRMs share the same gas balance to prevent matrix effects from influencing the measurement instrument’s response.

3.7.1.2. CRM uncertainty evaluation

The uncertainty associated with the characterization value consists of a combination of the uncertainty of the certified value of the PRM(s) used, the uncertainty of calibration according to the chosen calibration model (which includes contribution due to the selectivity of the technique) and the effect of repeatability on the results of the candidate CRM. The calculated uncertainty should take account of the statistical treatment used to obtain the assigned value (International Organization for Standardization [ISO], 2024).

3.7.1.3. Value assignment

Value assignment is the process of combining the results from the homogeneity and stability assessment with the results from the characterization studies to determine the assigned values and their uncertainties (International Organization for Standardization [ISO], 2024).

As previously stated, for gas reference mixtures prepared individually, there is no need to include uncertainty components related to inhomogeneity or short-term stability. These values are primarily informative and provide insights into the behaviour of the prepared mixtures. The uncertainty budget for a gas mixture CRM will include the characterization uncertainty component and the long-term stability component.

These values are then documented in a certificate, following the requirements of the ISO 6141 standard, which specifies the minimum requirements for the contents of certificates for homogeneous gas mixtures in gas cylinders intended for use as reference gas mixtures (International Organization for Standardization [ISO], 2015, ISO 6141).

3.7.2. PRM certification

3.7.2.1. PRM characterization

PRMs are prepared by transferring pure gases, pure liquids, or gravimetrically prepared mixtures of known composition quantitatively into a cylinder in which the calibration gas will be contained. The traceability to the SI of amount fractions of these mixtures arises from the correct execution of the determination of the added gas masses, the conversion of the added masses to amounts of substance by knowledge of their chemical purity and appropriate relative molecular masses, and the verification of the final mixture against independent reference gas mixtures (International Organization for Standardization [ISO], 2015).

The amount fractions of the components in the final mixture, y_k, can be calculated using Equation 3, according to the ISO 6142-1:2015 standard. In the formula $x_{k,j}$ is the mole fraction of component k in parent gas or liquid j, $x_{i,j}$ is the mole fraction of component *i* in parent gas or liquid j, m_j is the mass added of parent gas or liquid *j*, and M_i is the molar mass of component *i* (International Organization for Standardization [ISO], 2015).

$$y_k = \frac{\sum_{j=1}^{r}\left(\frac{x_{k,j} \times m_j}{\sum_{i=1}^{q} x_{i,j} \times M_i}\right)}{\sum_{j=1}^{r}\left(\frac{m_j}{\sum_{i=1}^{q} x_{i,j} \times M_i}\right)} \tag{3}$$

Equation 3 provides a general approach for calculating the amount fractions of the components in the mixture. However, considering that PRM prepared in this work is a binary mixture, consisting only of CO_2 and N_2, the formula was simplified accordingly. As a result, the amount fraction of CO_2 was determined using Equation 4, while the amount fraction of N_2 was calculated using Equation 5.

$$y_{CO2} = \frac{\left[\left(\frac{x_{CO2,CO2} \times m_{CO2}}{M_{CO2}}\right) + \left(\frac{x_{CO2,N2} \times m_{N2}}{M_{N2}}\right)\right]}{\left[\left(\frac{x_{CO2,CO2} \times m_{CO2}}{M_{CO2}}\right) + \left(\frac{x_{CO2,N2} \times m_{N2}}{M_{N2}}\right) + \left(\frac{x_{N2,N2} \times m_{N2}}{M_{N2}}\right)\right]} \tag{4}$$

$$y_{N2} = \frac{\left(\frac{x_{N2,N2} \times m_{N2}}{M_{N2}}\right)}{\left[\left(\frac{x_{CO2,CO2} \times m_{CO2}}{M_{CO2}}\right) + \left(\frac{x_{CO2,N2} \times m_{N2}}{M_{N2}}\right) + \left(\frac{x_{N2,N2} \times m_{N2}}{M_{N2}}\right)\right]} \tag{5}$$

In both Equations, y_{CO2} and y_{N2} represent the amount fractions of CO_2 and N_2, respectively, in the final mixture. $x_{CO2,CO2}$ is the mole fraction of carbon dioxide in the pure CO_2 cylinder, $x_{CO2,N2}$ is the mole fraction of an impurity of carbon dioxide in the pure N_2 cylinder and $x_{N2,N2}$ is the mole fraction of nitrogen in the pure N_2 cylinder. Additionally, m_{CO2} and m_{N2} are the masses of CO_2 and N_2 introduced into the preparation cylinder, while M_{CO2} and M_{N2} are the molar masses of CO_2 and N_2, respectively.

To calculate each parameter from the Equations above, it is necessary to consider the gravimetric calculations, the impurities in the gases, and the molar masses.

3.7.2.1.1. *Gravimetry*

The chosen weighing procedure used is known as the substitution or Borda method. In this method, the reference cylinder and the sample cylinder are weighed sequentially on a comparator balance. Since both cylinders are made of the same material and have similar masses, the influence of laboratory air buoyancy on the results is significantly reduced. To achieve high accuracy, the mass difference between the reference and sample cylinders is compensated by adding or removing small mass pieces. This approach allows precise measurements within a narrow mass range (Alink & van der Veen, 2000; Sega et al., 2008).

The added masses of gases, m_j, were calculated by determining the difference between the results of consecutive weighing cycles, as expressed in Equation 6 (Alink & van der Veen, 2000). In the first weighing cycle, w_0, the mass difference between the reference cylinder and the empty sample cylinder was measured. In the second weighing cycle, w_1, the mass difference between the reference and sample cylinder containing the first added gas, CO_2, was determined. In the final weighing cycle, w_2, the difference between the cylinders was computed, with the sample cylinder containing both CO_2 and the balance gas, N_2.

$$m_j = w_j - w_{j-1} \tag{6}$$

The result of each weighing cycle, w_j, was calculated using Equation 7. In this expression, e_j is the calibration factor, q_j is the reading of the sample cylinder, p_j is the reading of the reference cylinder, W_j is the total mass of mass pieces added to the reference cylinder and M_j is the total mass of mass pieces added to the sample cylinder. $\rho_{air,j}$ is the density of the air, ρ_S is the density of the mass pieces, $V_{S,j}$ is the volume of the sample cylinder and V_R is the volume of the reference cylinder. The calibration factor is used to convert the reading values into units of mass. This calibration is performed at the beginning and end of each weighing cycle by adding a 1 g mass piece to the reference cylinder (Alink & van der Veen, 2000).

$$w_j = e_j(q_j - p_j) + (W_j - M_j)\left(1 - \frac{\rho_{air,j}}{\rho_s}\right) + \rho_{air,j}(V_{S,j} - V_R) \tag{7}$$

The calculation of $(q_j - p_j)$ and e_j considered the sequence of the weighing process, as detailed in Table 1. In this table, R is the reference cylinder, S the sample cylinder and Q is the 1g calibration mass piece.

Table 1: Weighing process for the reference and sample cylinder.

Weighing number	System	Reading
0	$R + W + Q$	$m_{C,0}$
1	$R + W$	$m_{C,1}$
2	$S + M$	$m_{C,2}$
3	$R + W$	$m_{C,3}$
4	$S + M$	$m_{C,4}$
5	$R + W$	$m_{C,5}$
6	$S + M$	$m_{C,6}$
7	$R + W$	$m_{C,7}$
8	$R + W + Q$	$m_{C,8}$

The term $(q_j - p_j)$ was calculated using Equation 8 and e_j using Equation 9 (Alink & van der Veen, 2000).

$$(q_j - p_j) = \frac{1}{6}(2m_{C,2} + 2m_{C,4} + 2m_{C,6} - m_{C,1} - 2m_{C,3} - 2m_{C,5} - m_{C,7}) \tag{8}$$

$$e_j = \frac{2m_Q}{(m_{C,0} - m_{C,1} + m_{C,8} - m_{C,7})} \tag{9}$$

The volume difference was calculated according to *Alink et al.* Both cylinders used are the same at the start of the preparation process, but while preparing the gas mixture the pressure in the sample cylinder increases from vacuum to over 100 bar. For an aluminium-alloy cylinder with an internal volume of 5 L and a pressure difference of 120 bar, the volume increases by (12 ± 1) cm³ (Alink & van der Veen, 2000).

The density of the weights used was determined through their calibration certificate, following the guidelines of the International Recommendation OIML R 111-1 for E2 stainless steel weights. Its assigned value was 7950 ± 0.02 kg/m³ (International Organization of Legal Metrology [OIML], 2004).

The air density was calculated using Equation 10 based on *Jones et al*:

$$\rho_{air} = \frac{PM_a}{ZRT}\left[1 - x_v\left(1 - \frac{M_v}{M_a}\right)\right] \tag{10}$$

In this Equation, P is the atmospheric pressure, T the thermodynamic temperature, x_v the mole fraction of water vapour, M_a the molar mass of dry air, M_v the molar mass of water, R the molar gas constant, and Z the compressibility factor (Jones, 1978).

As a result, according to formula 6, the mass of CO_2 added to the cylinder was calculated as the difference between the masses obtained in the second weighing cycle and in the first weighing cycle. In the same way, the mass of N_2 added to the cylinder was determined as the difference in mass between the third and second weighing cycle.

3.7.2.1.2. Purity of parent gases

The determination of the impurities present in the different gases is crucial, as they have a direct impact in the composition of the gas mixture.

The level of purity assessment required depends on the number of impurities that could be critical and/or significant to the final composition of the mixture. A critical impurity is an impurity in the parent gas that is also present as a minor component in the same mixture at low amount fractions. A significant impurity is an impurity that is predicted to contribute more than 10 % to the target uncertainty (International Organization for Standardization [ISO], 2019).

For the most accurate results, traceable purity analysis results are preferred. Regardless of the specific impurities present in the parent gases, a traceable purity analysis ensures that all impurities are accurately quantified and properly considered in the final mixture calculation. Performing a traceable purity analysis requires calibrating the analyser using reference gas mixtures with defined uncertainties and quantifying the impurities by direct comparison with these calibration mixtures, following the methods described in ISO 6143 and ISO 12963 (International Organization for Standardization [ISO], 2019).

However, quantifying every possible impurity in a pure gas requires multiple instruments and different reference gas mixtures at amount fraction levels suitable for each impurity, making the process complex and costly.

In the field of gas analysis, it is common to perform an indicative purity analysis when full metrological traceability is not established or when the necessary resources for a traceable purity analysis are not available. One typical example of indicative purity analysis is relying on data from the “certificate of analysis” provided by the gas manufacturer, even when metrological traceability is not explicitly stated. Any data without established traceability must be considered indicative (International Organization for Standardization [ISO], 2019).

When reporting the results of an indicative purity analysis, any potential bias in the data must be accounted for by either including an appropriate uncertainty component to reflect the possible bias or applying a correction for the bias. Gas manufacturers often quantify the purity of their gases based on the analytical capabilities used for purity assessment or by monitoring the production process. If an impurity is likely to be present in the “pure” parent gas but is below the detection limit of the analytical method, the detection limit is typically stated in the manufacturer’s specifications (International Organization for Standardization [ISO], 2019; Mulyana et al., 2020).

In this case, the amount-of-substance fraction of the expected impurity, $x_{ij},$ shall be set equal to half of the value of the detection limit of the analytical method, *Lij*, as shown in Equation 11. The uncertainty associated with x_{ij} is evaluated assuming a rectangular distribution with L_{ij} being the upper limit of the rectangle, and zero the lower limit (Equation 12).

$$x_{ij} = \frac{L_{ij}}{2} \tag{11}$$

$$u(x_{ij}) = \frac{L_{ij}}{2 \times \sqrt{3}} \tag{12}$$

The amount fraction of the most abundant component was calculated by subtracting the sum of all impurities in the parent gas from 1, as expressed in Equation 13. Its uncertainty was determined using the law of propagation of uncertainty described in *GUM-6* (Joint Committee for Guides in Metrology [JCGM], 2020) (Equation 14).

$$x_{ij} = 1 - \sum_{i=1, i \neq k}^{n} x_{ij} \tag{13}$$

$$u^2(w_{kj}) = \sum_{i=1, j \neq k}^{n} u^2(x_{ij}) \tag{14}$$

It is important that if an impurity is considered critical and/or significant, a purity analysis with traceable results must be performed. Since nitrogen typically contains carbon dioxide impurities, and the main component of the mixture is carbon dioxide, a purity analysis was conducted using the table provided by the gas manufacturer to confirm that the CO_2 impurity is neither critical nor significant.

3.7.2.1.3. <u>Molar masses</u>

Molar masses are essential for converting mass fractions to amount fractions in gas mixtures. The values of atomic weights used to calculate molar masses must be taken from the latest IUPAC publications, as atomic weights and molecular masses depend on isotopic composition and nuclide masses (International Organization for Standardization [ISO], 2015).

When determining atomic weights and molecular masses, two key factors must be considered: whether the isotopic composition of an element in a specific material can be measured and whether literature data are available for that element. If either condition is met, these data should be used alongside the latest IUPAC values. Otherwise, standard atomic weights from IUPAC should be used (International Organization for Standardization [ISO], 2015; Aoki et al., 2019).

In this work, the data for calculating the molar masses and the associated uncertainties for CO_2 and N_2 were calculated using the most recent atomic masses and isotopic abundances reported by the IUPAC (Prohaska et al., 2022).

3.7.2.2. PRM verification

The composition of a calibration gas mixture shall be verified experimentally to demonstrate that the composition of the calibration gas sampled from the cylinder is consistent with the composition calculated from the gravimetric preparation process. The verification is relevant to detect possible errors in the preparation process of the individual gas mixture, the presence of any chemical reaction between the components of the mixture or between any component and the cylinder. The composition of a calibration gas mixture can only be considered traceable to the SI when it has been verified (International Organization for Standardization [ISO], 2015).

The verification of the composition of a calibration gas mixture may be achieved by demonstrating consistency between the prepared mixture and appropriate reference gas mixtures. This verification must be performed by analysing the prepared mixture against independent reference mixtures. These reference standards must be prepared separately, ensuring they are not derived from the same parent gases or produced within the same batch, particularly in cases of simultaneous cylinder preparation, thus assuring different traceability chains.

The verification process follows the same methodology outlined for gas mixture calibration, in accordance with ISO 6142 when using a calibration curve, or ISO 12963 when assigning values through the two other validated procedures in this study: bracketing and/or SPEM (International Organization for Standardization [ISO], 2001;

International Organization for Standardization [ISO], 2017, ISO 12963). Additionally, it is crucial to use reference gas mixtures with the same balance gas to minimize matrix effects in the analysis. If mixtures with different matrices are used, the procedure must undergo thorough validation.

The uncertainty calculation for verification considers the number and uncertainty of standards used, process repeatability, and the number of repetitions of the verification. It may also include method performance in previous analyses, proficiency testing participation, and quality system monitoring to ensure reliability and corrective actions when necessary (International Organization for Standardization [ISO], 2015).

The calibration mixture passes the verification if it passes the criterion:

$$\left|y_{k,prep} - y_{k,ver}\right| \leq 2\sqrt{u^2\left(y_{k,prep}\right) + u^2\left(y_{k,ver}\right)} \tag{15}$$

In Equation 15, $y_{k,prep}$ represents the amount fraction obtained from the preparation process, while $y_{k,ver}$ denotes the amount fraction determined through verification by measuring the mixture. The terms $u^2\left(y_{k,prep}\right)$ and $u^2\left(y_{k,ver}\right)$ correspond to their associated uncertainties, respectively.

3.7.2.3. PRM uncertainty evaluation

The combined standard uncertainty of the amount fraction of component *k* in the final mixture shall be calculated using Equation 16 (International Organization for Standardization [ISO], 2015).

$$u_c(y_k) = \frac{1}{2}\sqrt{u^2\left(y_{k,prep}\right) + u^2\left(y_{k,ver}\right) + \left(y_{k,prep} - y_{k,ver}\right)^2} \tag{16}$$

Where $u^2\left(y_{k,ver}\right)$ is calculated according to the methodology used, and $u^2\left(y_{k,prep}\right)$ is calculated according to Equation 17.

$$u(y_{k,prep}) = \sqrt{u^2(y_{k,grav}) + u^2(y_{k,stab})} \tag{17}$$

The uncertainty computed from gravimetry $u^2(y_{k,grav})$ is calculated by the application of the law of propagation of uncertainty to Equation 7 and is calculated according to Equation 18.

$$u^2(y_{k,grav}) = \sum_{i=1}^{q}\left(\frac{\partial y_k}{\partial M_i}\right)^2 \times u^2(M_i) + \sum_{j=1}^{r}\left(\frac{\partial y_k}{\partial m_j}\right)^2 \times u^2(m_j) + \sum_{j=1}^{r}\sum_{i=1}^{q}\left(\frac{\partial y_k}{\partial x_{i,j}}\right) \times u^2(x_{i,j}) \tag{18}$$

Finally, the expanded uncertainty is calculated multiplying $u_c(y_k)$ by the coverage factor, *k* = 2 (Equation 19). This uncertainty corresponds to the reported uncertainty in the PRM certificate.

$$U(y_k) = k \times u_c(y_k) \tag{19}$$

3.8. Comparison of methods for determining the composition of reference gas mixtures

3.8.1. Calibration curve method

The calibration of a measurement system based on input and output variable measurements, along with their associated uncertainties, involves estimating parameters that best describe the relationship between them. In this case, the calibration of the gas chromatograph with FID/TCD detectors is done using PRMs, and in this way, the parameters defining the calibration curve associated with this system are estimated.

While there are mathematically straightforward algorithms when the input variable, in this case, the amount-of-substance fraction of the PRMs, is considered “exact” compared to the output variable, this assumption is not valid in several applications, such as gas metrology (International Organization for Standardization [ISO], 2001). In such scenarios, the mathematical algorithms become more complex, and specific strategies exist based on the classification of the regression problem. Furthermore, the mathematical function describing the relationship between the variables is not always linear; in fact, it is often a second-order polynomial.

As a consequence of adopting non-linear response models, advanced regression techniques (errors in both variables) and uncertainty propagation, the main calculation procedures can only be performed on a computer, using a specific program.

3.8.2. Bracketing method

The bracketing method, also known as two-point calibration, consists in reducing as much as possible the interval over which the linearity of the calibration function is assumed. This leads to use two reference materials having property values surrounding as tightly as possible (or bracketing) the nominal value of the unknown quantity. Because of the tight surrounding of each unknown quantity by two materials, and because of the short period of time needed for this procedure (time to measure the unknown quantity and the two standards), the bracketing technique usually yields greater accuracy in determining the transformed value of an unknown quantity. The unknown quantity and the two reference materials are measured together. The value of the unknown quantity is estimated directly, based on a linear interpolation between the values of the two standards (International Organization for Standardization [ISO], 1996).

Whereas the comparison methods described in ISO 6143 based on multipoint calibration are in principle suitable for all applications in gas analysis for determining the composition of calibration gas mixtures, in many cases simpler calibration methods can be used. These methods typically require a smaller number of calibration gas mixtures with a traceable composition (International Organization for Standardization [ISO], 2017, ISO 12963).

One- and two-point calibration of instruments is widely used in the gas industry and in national metrology institutes. They often constitute fair compromise between costs and efforts on one hand, and accuracy on the other (International Organization for Standardization [ISO], 2017, ISO 12963). Although the bracketing technique can significantly reduce uncertainty in many applications, gas analysis often involves nonlinear response models. Therefore, proper validation is essential to ensure its applicability and to account for the necessary uncertainty components arising from nonlinearity.

The procedure for performing the bracketing technique in gas analysis is described in ISO 12963 – Gas Analysis – Comparison Methods for the Determination of the Composition of Gas Mixtures Based on One- and Two-Point Calibration. (International Organization for Standardization [ISO], 2017, ISO 12963).

The first step is to select two reference gas mixtures, with certified values x_{r1}, and x_{r2}, within the range of the sample mixture for calibration, one above and one below the sample mixture. Then, at least three replicate measurements are taken for each calibration gas mixture. The mean response values of these replicates, y_{r1} and y_{r2}, are calculated along with their respective standard uncertainties, $u(y_{\mathrm{r}1})$ and $u(y_{\mathrm{r}2})$. Similarly, at least three replicate measurements of the sample are performed, and the mean response, y_{s}, is computed along with its standard uncertainty, $u(y_{\mathrm{s}})$.

It is assumed in general that the responses to the two bracketing gases are uncorrelated. It is also assumed that the two bracketing gases are independent, i.e. not derived from a common source (International Organization for Standardization [ISO], 2017, ISO 12963).

The value of the unknown, x_s, is estimated using Equation 20, by assuming a straight-line analysis function with a nonzero intercept that approximates the true calibration function within the specified range.

$$x_s = \frac{y_{r2} \times x_{r1} - y_{r1} \times x_{r2}}{y_{r2} - y_{r1}} + \frac{x_{r2} - x_{r1}}{y_{r2} - y_{r1}} \times y_s \qquad (20)$$

To verify system stability, the measurements should be performed following the sequence illustrated in Figure 11: first measuring the PRM with the lower value, followed by the PRM with the higher value, then the sample, then repeating the PRM with the higher value, and finally the PRM with the lower value. The same number of replicates should be used for each gas mixture; although Figure 11 shows only three replicates per gas, this number can be adjusted.

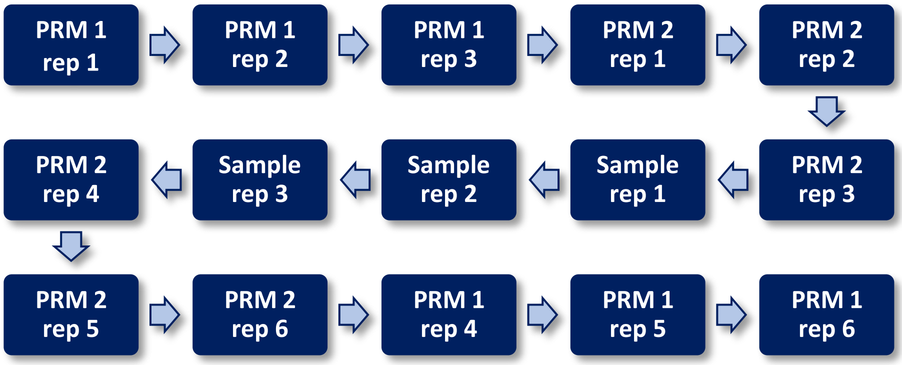


Figure 11: Sample and reference mixtures measurement sequence

The uncertainty associated with the amount fraction value of the sample mixture, considered as unknown, is derived from the uncertainty of the calibration gas mixtures, the uncertainty of the measurement responses and the contributions of the prediction errors (Equation 21).

$$\begin{aligned} u^2(x_s) = \left(\frac{\partial x_s}{\partial y_s}\right)^2 \times u^2(y_s) + \left(\frac{\partial x_s}{\partial y_{r2}}\right)^2 \times u^2(y_{r2}) + \left(\frac{\partial x_s}{\partial y_{r1}}\right)^2 \times u^2(y_{r1}) \\ + \left(\frac{\partial x_s}{\partial x_{r2}}\right)^2 \times u^2(x_{r2}) + \left(\frac{\partial x_s}{\partial x_{r1}}\right)^2 \times u^2(x_{r1}) + u^2(\Delta) \end{aligned} \tag{21}$$

The analytical expressions for the sensitivity coefficients are calculated as follows:

$$\left(\frac{\partial x_s}{\partial y_s}\right) = \frac{x_{r2} - x_{r1}}{y_{r2} - y_{r1}}$$

$$\left(\frac{\partial x_s}{\partial y_{r2}}\right) = \frac{x_{r2} - x_{r1}}{(y_{r2} - y_{r1})^2} \times (y_{r1} - y_s)$$

$$\left(\frac{\partial x_s}{\partial y_{r1}}\right) = \frac{x_{r2} - x_{r1}}{(y_{r2} - y_{r1})^2} \times (y_s - y_{r2})$$

$$\left(\frac{\partial x_s}{\partial x_{r2}}\right) = \frac{y_s - y_{r1}}{y_{r2} - y_{r1}}$$

$$\left(\frac{\partial x_s}{\partial x_{r1}}\right) = \frac{y_{r2} - y_s}{y_{r2} - y_{r1}}$$

The estimation of the term $u(\Delta)$ can be calculated following two different approaches: One of them is the performance evaluation of the measuring system and the other one is an alternative approach to assessing the nonlinearity contribution based on *GUM-6* (Joint Committee for Guides in Metrology [JCGM], 2020).

It is important to check whether the criterion in Equation 22 is fulfilled, where the subscripts *b* and *e* denote the result for the unknown sample calculated using the reference measurements taken at the beginning and at the end respectively (International Organization for Standardization [ISO], 2017, ISO 12963).

$$\frac{\left|x_{s,b} - x_{s,e}\right|}{2 \times \sqrt{u^2\left(x_{s,b}\right) + u^2\left(x_{s,e}\right)}} \leq 1 \tag{22}$$

If the criterion is fulfilled, $x_{s,b}$ is stated as the value and $u\left(x_{s,b}\right)$ as the uncertainty of the unknown composition of the mixture.

3.8.1.1. Performance evaluation of the measuring system

The performance evaluation aims at quantifying the effects of nonlinearity of the analyser system on its performance when using bracketing. This evaluation shall be performed at the time of implementing and validating the method and each time after the system have been altered, maintained or replaced (International Organization for Standardization [ISO], 2017, ISO 12963).

Nonlinearity of the measuring system would require a content-dependent correction throughout the analytical range. To avoid such a correction, there are several alternatives. In the ISO 12963 standard, a possible nonlinearity is treated according to ISO 15796:2005, 5.3.4 (International Organization for Standardization [ISO], 2005), by including the maximum possible deviation in the uncertainty budget of the reported value.

First, select at least seven calibration gas mixtures covering the intended analytical range and measure their response, *y*. If prior information on the analyser's response function is available, this number may be reduced to five for quadratic and three for linear systems. In this work, 5 PRM were selected since the analyser's response was already validated for the range of amount fractions under consideration, and its response behaves like a second-order polynomial.

Using GLS regression, the best-available analysis function is tested. If the residual sum of weighted squared deviations (SSD) is less than twice the number of calibration points and Γ<2, the system is linear, and $u(\Delta)$ is set to zero. The Goodness of Fit, Γ, of a statistical model describes how well it fits into a set of observations (Maydeu-Olivares & García-Forero, 2010). The term $u(\Delta)$ represents the uncertainty contribution due to zero-deviations and analyser nonlinearity (International Organization for Standardization [ISO], 2017, ISO 12963).

If linearity is not confirmed, a second-order polynomial should be used for the fitting and a reassessment carried out. If criteria are met, the system is slightly nonlinear, and the uncertainty of $u(\Delta)$ should be assessed.

To calculate $u(\Delta)$, Equation 23 should be used:

$$\Delta = (\beta_0 + \beta_1 \times y + \beta_2 \times y^2) - b_0 - b_1 \times y \tag{23}$$

Being β_0, $\beta_1$and β_2 the parameters of the second-order polynomial, and b_0 and b_1 the parameters of the linear regression model used to approximate it.

The parameter $u(\Delta)$ would be the maximum absolute value calculated when using the formula with y_{r1} and when using y_{r2}.

3.8.1.2. Nonlinearity contribution based on ISO/IEC Guide 98-3:2008

In this approach, it is necessary to calculate the true calibration function for the analyser according to ISO 6143. The analytical range of interest will be specified by a lower amount-of-substance fraction, x_1, and an upper fraction, x_2. Then, the lower and upper responses will be computed, and the coefficients of the true analysis function will be calculated using a second-order polynomial. Afterward, the coefficients of the simplified analysis function will be calculated using linear regression (International Organization for Standardization [ISO], 2017, ISO 12963).

Then the bias between both regressions is calculated using the Equation 24:

$$\bar{\delta} = \frac{F(y_2) - F(y_1)}{y_2 - y_1} \tag{24}$$

Where,

$$F(y) = (\beta_0 - b_0)y + \frac{1}{2}(\beta_1 - b_1)y^2 + \frac{1}{3}(\beta_2)y^3 \tag{25}$$

The squared standard uncertainty of the average bias is calculated using Equation 26.

$$u^2(\bar{\delta}) = \frac{G(y_2) - G(y_1)}{y_2 - y_1} + \frac{H(y_2) - H(y_1)}{y_2 - y_1} \tag{26}$$

Where,

$$\begin{aligned} G(y) = \frac{1}{5}{\beta_2}^2 y^5 &+ \frac{1}{4} \times 2(\beta_1 - b_1)\beta_2 y^4 \\ &+ \frac{1}{3} \times 2\left((\beta_0 - b_0 - \bar{\delta})\beta_2 + (\beta_1 - b_1)^2\right)y^3 \\ &+ (\beta_0 - b_0 - \bar{\delta})(\beta_1 - b_1)y^2 + (\beta_0 - b_0 - \bar{\delta})^2 y \end{aligned} \tag{27}$$

and

$$\begin{aligned} H(y) = yu^2(b_0) + \frac{1}{3}y^3u^2(b_1) + y^2u(b_0, b_1) + yu^2(\beta_0) + \frac{1}{3}y^3u^2(\beta_1) \\ + \frac{1}{5}y^5u^2(\beta_2) + y^2u(\beta_0, \beta_1) + \frac{2}{3}y^3u(\beta_0, \beta_2) + \frac{1}{2}y^4u(\beta_1, \beta_2) \end{aligned} \tag{28}$$

Equations 25 and 27 are adapted to second-order polynomial regression.
Finally, Equation 29 is used to calculate $u(\Delta)$.

$$u(\Delta) = \sqrt{\bar{\delta}^2 + u^2(\bar{\delta})} \tag{29}$$

3.8.3. SPEM method

Single-Point Exact-Match Calibration (SPEM) is a method used in analytical chemistry and in general in calibration techniques where the calibration is based on a single reference point, and the goal is for the measured value to match exactly with a known standard or reference value.

According to the ISO 12963 standard, the steps to check the composition of a gas mixture are the following. First, a calibration gas mixture is selected such that the measuring system produces, on this mixture, responses which are statistically indistinguishable from those obtained on the unknown sample (International Organization for Standardization [ISO], 2017, ISO 12963).

Then, at least three replicate measurements of the calibration gas mixture are carried out and the mean response, y_r, is calculated along with its standard uncertainty, $u(y_r)$. Similarly, at least three replicate measurements of the sample are taken and the mean response value, y_s, and its standard uncertainty, $u(y_s)$ are calculated. Both uncertainties are determined from the standard deviation of the measurements.

The sample mixture and the reference gas mixture are indistinguishable if they meet the requirements of Equation 30.

$$\frac{|y_r - y_s|}{2 \times \sqrt{u^2(y_r) + u^2(y_s)}} \leq 1 \tag{30}$$

If the condition is fulfilled, then the amount fraction of the sample gas mixture, x_s, is calculated according to Equation 31, and its standard uncertainty, $u(x_s)$, as the square root of the value obtained by applying Equation 32.

$$x_s = x_r \times \frac{y_s}{y_r} \tag{31}$$

$$u^2(x_s) = u^2(x_r) + \frac{x_r^2}{y_r^2}[u^2(y_s) + u^2(y_r)] \tag{32}$$

In an exact-match situation, the signal ratio for the measurements of the unknown and the reference should ideally be unity, yielding to the “exact” match, x_s = x_r. If the criterion is not met, the ISO 12963 standard recommends using an alternative calibration method, such as bracketing or a calibration curve (International Organization for Standardization [ISO], 2017, ISO 12963).

To check system stability, the measurements of the reference gas mixture should be repeated after measuring the sample mixture (International Organization for Standardization [ISO], 2017, ISO 12963).

3.9. Software

3.9.1. XLGENLINE

The XLGENLINE 1.1 software, developed by the National Physical Laboratory (NPL), the National Metrology Institute of the United Kingdom, allows us to perform regression calculations using ordinary least squares (OLS) and generalized least squares (GLS) methods with polynomial calibration curves. The ordinary least-squares regression is a commonly used method for determining a calibration curve. This method has the advantage that there are many software implementations available. However, implicit in its use is the assumption that the values realized by the standards in the calibration have negligible associated uncertainties relative to those of the measured responses of the system. (Milton, Harris et al., 2006). In many applications, such as gas metrology, this assumption is inappropriate. Ignoring the uncertainties associated with the values realized by the standards can yield biased results and unreliable uncertainties associated with estimates of quantities derived from the calibration curve. Unlike ordinary least squares regression, the generalized least squares method permits a valid treatment of calibration problems for which there are uncertainties associated with measured values both of the stimulus and the corresponding response of the measuring system, as well as correlations associated with pairs of such values (Milton, Harris et al., 2006). Essentially, to determine the behaviour of a response variable v as a function of a stimulus t, a model must be constructed involving establishing the functional relationship h between v and t, which includes a set of model parameters. The parameters a parameterize the range of possible response behaviours, and the actual behaviour is specified by determining values for these parameters based on measurement data (Milton, Guenther et al., 2006).

The measurement data (x) includes the measured values of v and their corresponding values of t (International Organization for Standardization [ISO], 2001). The uncertainty matrices U_x, U_v, U_t, and U_{vt} contain information about the uncertainties and covariances associated with the measurements. The parameters of the analysis function a can be estimated through generalized least squares analysis, which, for measurement data with a Gaussian distribution, provides maximum likelihood estimators.

The calculation of the parameter estimators depends on the classification of the regression problem based on the behaviour of the variables. In gas metrology, two common options are often used (Milton, Guenther et al., 2006).

a) Generalized Distance Regression (GDR) with negligible covariances, estimated using the Deming method.

b) Generalized Gauss-Markov Regression (GGMR) with complete covariance information, estimated through the minimization of the same expression.

Since the primary reference materials used for value assignment are obtained independently, it can be assumed that there are no associated covariances. Therefore, to obtain the parameter estimations, the XLGENLINE 1.1 software, which is based on GDR can be used (Smith, 2010). Once the estimators of the model's parameters a are obtained, it is necessary to verify that the proposed model is adequate through validation using a goodness-of-fit test. The selected model should have a goodness-of-fit estimator (Γ, gamma) less than or equal to 2 to ensure adequacy.

After establishing and validating the model that relates the variables "v" and "t", the model is then utilized to estimate a sample value "t_{sample}" from a problem value "v_{sample}," a process known as inverse evaluation or prediction. The amount-of-substance fraction of the problem gas mixtures is determined by performing an inverse evaluation of a regression model obtained through generalized least squares, constructed with PRMs.

As an acceptance criterion, it must be verified that the normalised error, En, calculated by using Equation 33, is less than or equal to 1, and that the percentage difference, $\Delta\%$, between the value obtained with the inverse evaluation and the certified value calculated with Equation 34, is less than or equal to the relative expanded uncertainty, $U_{\Delta}\%$.

Criterion 1:

$$En = \frac{|F_{PRM} - x_{value.\ IE}|}{\left(U_F^2 + (2u_{x\ value,IE})^2\right)} \leq 1 \tag{33}$$

Criterion 2:

$$|\Delta\%| \leq |U_{\Delta}\%| \tag{34}$$

Bearing in mind that:

$$\Delta\% = \frac{x_{value.\ IE} - F_{PRM}}{F_{PRM}} \times 100 \tag{35}$$

$$U_{\Delta}\% = \frac{U_{\Delta}}{F_{PRM}} \times 100 \tag{36}$$

$$U_{\Delta} = 2\left(u_F^2 + u_{x\ value,IE}^2\right) \tag{37}$$

Being:

$x_{value.\ IE}$: the inverse evaluation of the value of x

$u_{\text{x value, IE}}$: the uncertainty associated with the inverse evaluation of the value of x

F_{PRM}: amount fraction value in the certificate of the PRM

U_{F}: expanded uncertainty of the certified value

The use of XLGENLINE v1.1 ensures reliable and standardized regression analysis in gas metrology, providing robust uncertainty estimations and adherence to international calibration guidelines.

3.9.2. CCC Software

Calibration Curves Computing – CCC Software (Release 1.3), is a software, developed in MATLAB environment, for the evaluation of instrument calibration curves, and developed at the Istituto Nazionale di Ricerca Metrologica (INRiM) (Lecuna et al., 2020).

The software determines estimates of the calibration curve parameters and their associated covariance matrix, as well as estimates of values on the calibration curve and their associated covariance matrix. The software can perform the following kinds of regression:

- Ordinary least-squares regression (OLS)
- Weighted least-squares regression (WLS)
- Weighted total least-squares regression (WTLS)

3.9.3. GravCalc2

GravCalc2 is a software program designed by NPL to calculate the amount fraction and uncertainty of all components in a gravimetrically prepared gas mixture, using the method outlined in the International Standard ISO 6142 (Brown, 2009).

The ISO 6142 method determines the amount fraction and uncertainty of each component given knowledge of:

- The mass of each component added to the mixture, and its uncertainty
- The purity of each component, and the uncertainty of the purity analysis
- The relative molar mass of each species, and its uncertainty

CHAPTER 4. RESULTS AND DISCUSSION

4.1. Preparation and characterization of CO in N_2 CRM

4.1.1. Preparation of CO in N_2 CRM

A mixture of CO in N_2 was prepared with a target amount fraction of 1500 µmol/mol.

The CO mixture was prepared from a pre-mixture of CO at 0.04017 mol/mol in N_2, since using pure CO would have required a dilution. Additionally, this was the only CO in N_2 gas cylinder available at that moment, so the tests for preparing a CRM and optimizing the filling station began with this gas. The N_2 used for preparation was of 6.0 purity.

Initially, it was difficult to control the amount of gas entering the cylinder, resulting in mixtures that deviated from the calculated molar fraction, either exceeding or below the target value. To address this, the filling station was optimized by adding a pressure regulator inside the system, connected to the station, to better control the balance gas. Additionally, valves were installed near the cylinder valve for more precise gas flow control, and the flexible hose connecting the system directly to the cylinder valve was replaced with a 1/8” threaded pipeline. These modifications allowed for better regulation of the gas entering the cylinder.

Figure 12 shows how the cylinder was initially connected using a flexible hose. At that time, Luxfer® cylinders were not available, so a Catalina Cylinders® cylinder was used for testing. Since the cylinder was not new, it had to be cleaned multiple times.

Figure 13 illustrates the upgrades made to the filling station, including the pressure regulator for the balance gas, the new inlet and outlet valves near the cylinder for improved gas flow control, and the new pipeline with greater sealing integrity, ensuring more precise gas regulation and preventing leaks.

Figure 12: Cylinder connected to the filling station directly with a flexible hose

Figure 13: Pressure regulator for the balance gas, the new inlet and outlet valves near the cylinder, and new pipeline of 1/8”

The CO in N_2 gas mixture was prepared in a Catalina cylinder before the system was fully optimized. However, the resulting molar fraction was suitable for its intended use.

The mixture was intended to serve as a reference gas mixture in a proficiency testing scheme for the measurement of CO, with the aim of ensuring that national laboratories accurately quantify this gas at amount fractions relevant to air quality monitoring. Equation 1 was applied to calculate the required number of gases to introduce into the cylinder to obtain the final mixture at the desired CO amount fraction. The conditions were a cylinder pressure of 120 bar, a volume of 4.6 liters, and a working temperature of 293 K.

At this stage in the CRM preparation, purity calculations were not necessary, as the amount fraction would be determined through measurements using a GC-TCD/FID (Figure 14) along with five PRMs.

The calculated amount of CO, using Equation 1, to be introduced into the cylinder was 22.1 g (4.18 bar), and 612.6 g (115.5 bar) of N_2.

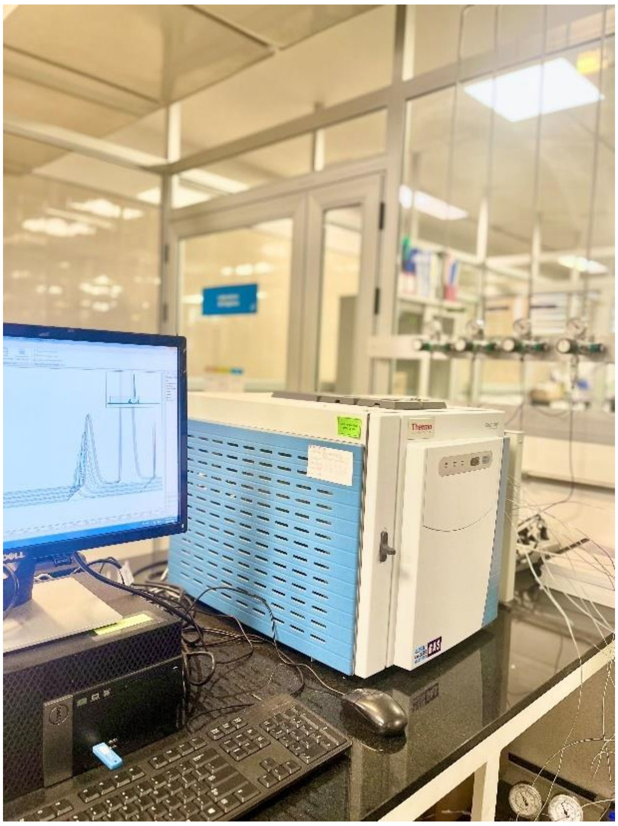


Figure 14: Gas chromatograph with TCD and FID detectors

4.1.2. Characterization of CO in N_2 CRM

Due to the lack of pure CO, which is critical for the preparation of a PRM since its purity must be determined for the amount fraction calculation, it was not possible to produce a PRM of CO in N_2. As a result, only a CRM of CO in N_2 was prepared.

The method of characterization used was a calibration curve with 5 PRMs.
Four PRMs from the National Metrology Institute of the Netherlands (VSL) and one PRM from the National Metrology Institute of Brazil (INMETRO) were used for the mixture characterization (Botha et al., 2010). Their certificated values are shown in table 2.

Table 2: PRMs from VSL and INMETRO used for characterization of the CO in N_2 mixture

PRM code	Amount fraction (mol/mol)	Expanded uncertainty, $k = 2$ (mol/mol)
D887513 (VSL)	5.0×10^{-4}	1.0×10^{-6}
D887492 (VSL)	1.0×10^{-3}	2.0×10^{-6}
D887607 (VSL)	1.5×10^{-3}	3.0×10^{-6}
D887616 (VSL)	2.0×10^{-3}	4.0×10^{-6}
M692238 (INMETRO)	2.5×10^{-3}	9.0×10^{-6}

For the characterization of this gas mixture, a gas chromatograph with a FID detector and a methanizer was used under the following conditions:

- Chromatographic column: Rt-Q-BOND PLOT 30m, 0.53mm ID, 20µm
- Carrier gas: Helium 5.0 (99.999%)
- Loop: 500 µL
- Constant column temperature: 40 ºC
- Retention time: (1.98 ± 0.01) min, GC run: 5 min.
- Software: Chromeleon 7. Thermo Fischer Scientific.
- Type of fit: GLS, second-order polynomial regression

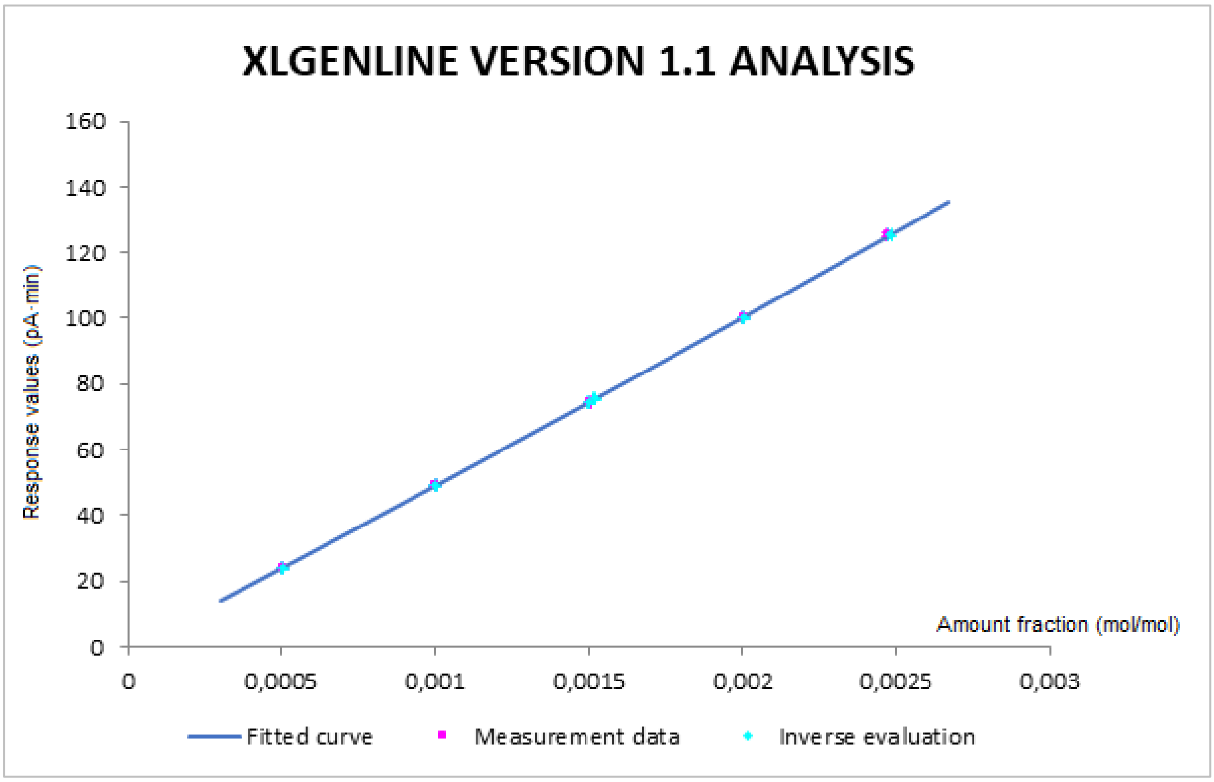


Figure 15: Fitted curved of the characterization of the CO in N_2 mixture, with the measured data and their inverse evaluation

The characterization values obtained from the XLGENLINE v1.1 regression are the following:

Amount fraction of CO = 1517.4 mol/mol

Characterization standard uncertainty = 2.4 mol/mol

The Figure 15 shows the calibration curve obtained.

The data for the method validation for the determination of CO in a CO in N_2 gas mixture in the measurement range of interest which contains the sample mixture amount fraction, are shown in table 3.

The validation of the chromatographic method was conducted over two different days of measurement. The entire range of PRMs, from 501x10^{-6} mol/mol to 2471x10^{-6} mol/mol, was analysed. Six replicates were performed at each amount fraction level to ensure statistical reliability across the calibration curve.

Repeatability and intermediate precision were assessed by analysing the data collected over the two measurement days, evaluating the consistency of the results under the same operational conditions but at different times.

The accuracy of the method was confirmed by ensuring that the normalised error between the amount fraction and its associated uncertainty, calculated via inverse evaluation using the XLGENLINE v1.1 software, and the certified value of each PRM remained below 1. Additionally, that the percentage difference, $\Delta\%$, between the value obtained with the inverse evaluation and the certified value is less than or equal to the relative expanded uncertainty of the difference, $U_\Delta\%$.

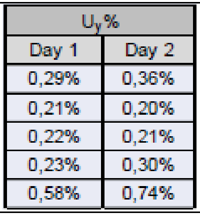
Selectivity was evaluated by analysing the chromatographic resolution between adjacent peaks and calculating the selectivity coefficient. This ensured the method's ability to distinguish the target analyte from potential interferences or neighbouring compounds in the matrix.

Linearity was confirmed by calculating the goodness-of-fit for the calibration curve, with a linear regression model demonstrating the best correlation across the measured amount fraction range.

Robustness was evaluated by introducing small variations in critical operational parameters, specifically the carrier gas flow rate and the split ratio. These variations did not significantly affect the retention times or peak shapes, and acceptable peak asymmetry factors were consistently obtained, indicating the method’s resilience to minor changes.

Lastly, the relative uncertainty of the calibration was demonstrably kept at or below 1.5%, as a predefined acceptance criterion to confirm the method's suitability for accurate quantification.

Table 3: Validation parameters of the CO in N_2 measurement

<table>
<tr><th>Parameter</th><th>Criteria</th><th>Result</th></tr>
<tr><td>Precision</td><td>Repeatability: (<i>n</i>=6)
<i>RSD</i> ≤ 0.5 %
Intermediate precision:
$RSD_{p.int}$ ≤ 1%</td><td>RSD_{max} day 1 = 0.25%
RSD_{max} day 2 = 0.33%
$RSD_{p.int\ max}$ = 0.72 %</td></tr>
<tr><td>Accuracy</td><td>$E_n \leq 1$
$|\Delta\%| \leq |Uy\ \%|$</td><td>Max E_n day 1 = 0.675
Max E_n day 2 = 0.458
$|\Delta\%| < |Uy\ \%|$
in every calibration point, during both days.</td></tr>
<tr><td>Selectivity</td><td>Resolution ≥ 1,5
Selectivity ≥ 1</td><td>Minimum resolution obtained, <i>R</i> = 2.4
Minimum selectivity obtained, <i>S</i> = 1.2</td></tr>
<tr><td>Range</td><td>The calibration curve must be valid for the working range.</td><td>$(501 – 2471)\times10^{-6}$ mol/mol</td></tr>
<tr><td>Linearity (Regression)</td><td>Goodness-of-fit test, $\Gamma \leq 2$</td><td>Γ day 1 = 1.2
Γ day 2 = 0.9</td></tr>
<tr><td>Robustness</td><td>Introduced modifications that do not affect the accuracy or precision of the method (carrier, split).
<i>As</i> ≤ 2</td><td>The modifications did not affect the accuracy or precision of the method.
Max <i>As</i> obtained = 2.0</td></tr>
<tr><td>Uncertainty</td><td>$U_y\% \leq 1.5\%$</td><td>
<table>
<tr><th colspan="2">U_y%</th></tr>
<tr><th>Day 1</th><th>Day 2</th></tr>
<tr><td>0,29%</td><td>0,36%</td></tr>
<tr><td>0,21%</td><td>0,20%</td></tr>
<tr><td>0,22%</td><td>0,21%</td></tr>
<tr><td>0,23%</td><td>0,30%</td></tr>
<tr><td>0,58%</td><td>0,74%</td></tr>
</table></td></tr>
</table>

4.2. Preparation and characterization of CO_2 in N_2 CRM

4.2.1. Preparation of CO_2 in N_2 CRM

A CO_2 in N_2 mixture was prepared with a target amount fraction of 0.35 mol/mol.

The CO_2 in N_2 mixture was prepared immediately after the system was optimized, making the process more efficient. The mixture was prepared in an aluminium Luxfer® cylinder. The CO_2 mixture was prepared using pure industrial grade CO_2 (99.9%) and the N_2 used for preparation was of 6.0 purity. This CO_2, produced locally, is the highest purity commercially available in the country, which is why it was chosen for the preparation of the mixture.

The conditions were a cylinder pressure of 120 bar, a volume of 5 L, and a working temperature of 295 K.

The calculated amount of CO_2, using Equation 1, to be introduced into the cylinder was 378.7 g (42.2 bar), and 444.2 g (77.79 bar) of N_2.

4.2.2. Characterization of CO_2 in N_2 CRM

The method of characterization used was a calibration curve with 5 PRMs and one extra PRM as a quality control.

The PRMs of CO_2 in N_2 used for the characterization of the mixture were purchased from the National Institute of Metrology of The Netherlands (VSL). Their certified values are shown in table 4.

Table 4: PRMs from VSL used for characterization of the CO_2 in N_2 mixture

PRM code	Amount fraction (mol/mol)	Expanded uncertainty, k = 2 (mol/mol)
D562955	0.05003	0.00005
D562939	0.09996	0.00010
D563094	0.20006	0.00020
D563020	0.30042	0.00030
5603670	0.40000	0.00040

For the characterization of this gas mixture, a gas chromatograph with a TCD detector was used under the following conditions:

- Chromatographic column: ShinCarbonST 100/120 2m, 1mm ID, 1/16”OD
- Carrier gas: Helium

- Loop: 250 µL
- Temperature program: 40 ºC (3 min), ramp 8.3 ºC/min to 140 ºC (12 min)
- Retention time: 9.5 ± 0.5 min, GC run: 15 min.
- Software: Chromeleon 7. Thermo Fischer Scientific.
- Type of fit: GLS, second-order polynomial regression

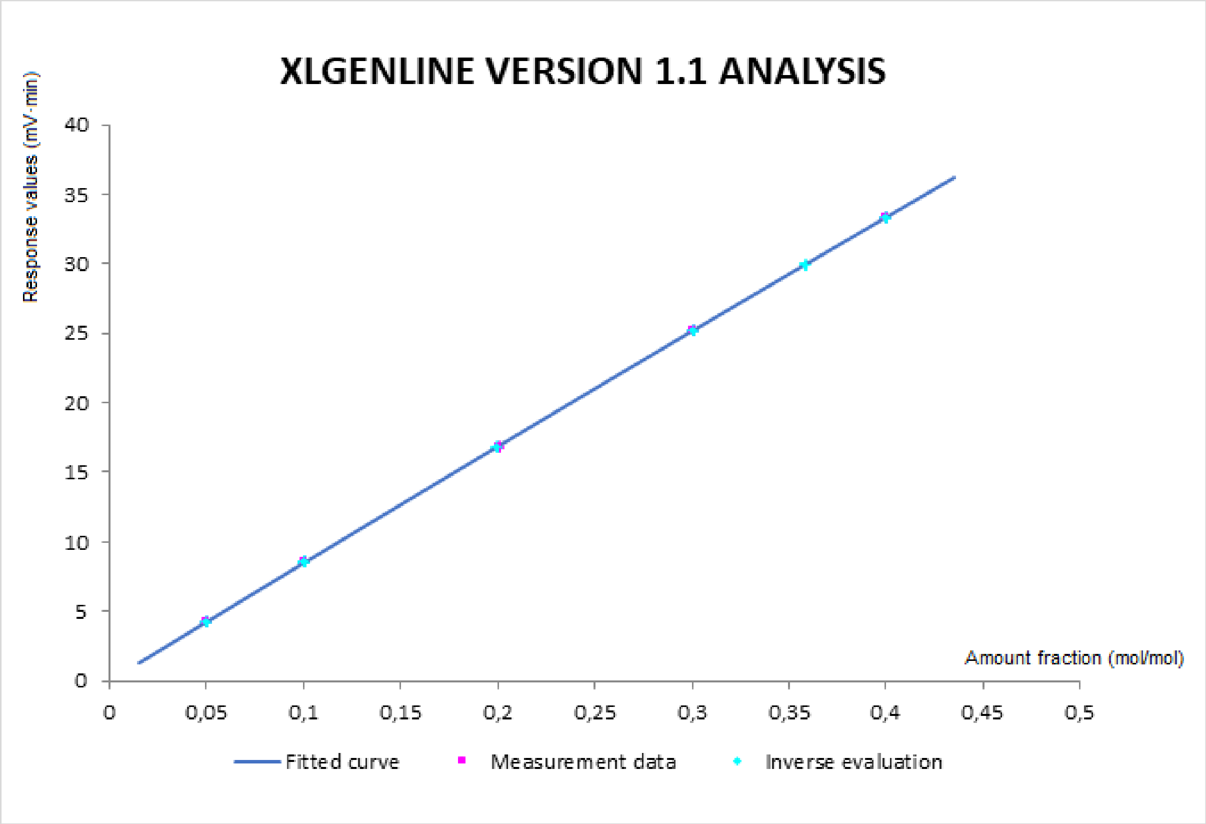


Figure 16: Fitted curved of the characterization of the CO_2 in N_2 mixture, with the measured data and their inverse evaluation

The characterization values obtained from the XLGENLINE v1.1 regression are the following:

Amount fraction of CO_2 = 0.35714 mol/mol

Characterization standard uncertainty = 0.00033 mol/mol

The Figure 16 shows the calibration curve obtained.

The data for the method validation for the determination of a CO_2 in N_2 gas mixture in the range containing the sample mixture are shown in table 5.

The validation of the chromatographic method for CO_2 was conducted in a manner similar to that used for CO validation, spanning two separate days of measurement. The entire range of PRMs, from 2.0×10^{-3} mol/mol to 4.0×10^{-1} mol/mol, was analysed. Six replicates were performed at each amount fraction level to ensure statistical reliability across the calibration curve.

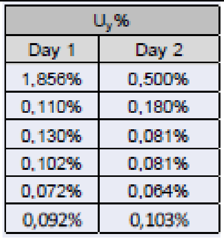
The evaluation of repeatability, intermediate precision, accuracy and selectivity was carried out using the same procedures employed in the CO validation.

Linearity was confirmed by calculating the goodness-of-fit for the calibration curve, with a second-order polynomial regression model providing the best correlation across the evaluated amount fraction range.

Robustness was tested by comparing two loop volumes (2 mL vs. 250 µL), with no significant effect on accuracy or precision. A t-student test comparison between 5 and 20 replicates showed equivalent results, confirming that 5 replicates are sufficient for routine analysis. Initially, carryover was observed in the first replicate; therefore, it was decided to discard that replicate and use only the subsequent five. However, after method optimization, including the insertion of blank runs between samples, carryover was eliminated, and all replicates could be used.

Uncertainty was also handled differently for CO_2. Since the PRM at 2.0×10^{-3} mol/mol was significantly lower than the other PRMs, the relative uncertainty criterion was adapted accordingly.

Table 5: Validation parameters of the CO_2 in N_2 measurement

<table>
<tr><th>Parameter</th><th>Criteria</th><th>Result</th></tr>
<tr><td>Precision</td><td><u>Repeatability: (<i>n</i>=6)</u>
Amount fraction $< 5x10^{-2}$ mol/mol, $RSD \leq 1$ %
Amount fraction ≥ $5x10^{-2}$ mol/mol, $RSD \leq 0.3$ %
<u>Intermediate precision:</u>
Amount fraction $< 5x10^{-2}$ mol/mol, $RSD_{p.int} \leq 1$ %
Amount fraction ≥ $5x10^{-2}$ mol/mol, $RSD_{p.int} \leq 0.3$ %</td><td>RSD_{max} day 1 = 0.68%
RSD_{max} day 2 = 0.17%
$RSD_{p.int} = 1$ % for 0.2x10-2 mol/mol
$RSD_{p.int} < 0.2$ % for the rest of the calibration points</td></tr>
<tr><td>Accuracy</td><td>$E_n \leq 1$
$|\Delta\%| \leq |Uy\%|$</td><td>Max E_n day 1 = 0.711
Max E_n day 2 = 0.335
$|\Delta\%| < |Uy\%|$ in every calibration point, during both days.</td></tr>
<tr><td>Selectivity</td><td>Resolution ≥ 1,5
Selectivity ≥ 1</td><td>Minimum resolution obtained, $R = 26.3$
Minimum selectivity obtained, $S = 5.8$</td></tr>
<tr><td>Range</td><td>The calibration curve must be valid for the working range.</td><td>($2.0x10^{-3}$ – $4.0x10^{-1}$) mol/mol</td></tr>
<tr><td>Linearity (Regression)</td><td>Goodness-of-fit test, $\Gamma \leq 2$</td><td>Γ day 1 = 1.5
Γ day 2 = 0.63</td></tr>
<tr><td>Robustness</td><td>Introduced modifications that do not affect the accuracy or precision of the method (loop, replicants).
$As \leq 2$</td><td>The modifications did not affect the accuracy or precision of the method.
Max As obtained = 2.0</td></tr>
<tr><td>Uncertainty</td><td>Amount fraction $< 5x10^{-2}$ mol/mol, $Uy\% \leq 3\%$
Amount fraction ≥ $5x10^{-2}$ mol/mol, $Uy\% \leq 1\%$</td><td><table><tr><th colspan="2">U_y%</th></tr><tr><th>Day 1</th><th>Day 2</th></tr><tr><td>1,856%</td><td>0,500%</td></tr><tr><td>0,110%</td><td>0,180%</td></tr><tr><td>0,130%</td><td>0,081%</td></tr><tr><td>0,102%</td><td>0,081%</td></tr><tr><td>0,072%</td><td>0,064%</td></tr><tr><td>0,092%</td><td>0,103%</td></tr></table></td></tr>
</table>

4.3. Preparation and gravimetric uncertainty of CO_2 in N_2 PRM

4.3.1. Gravimetric preparation of CO_2 in N_2

A PRM of CO_2 at 0.35 mol/mol in N_2 was prepared, allowing for comparison with the CRM of the same amount fraction.

The values obtained from the first weighing cycle, the one including the empty cylinder and the reference cylinder, are shown in table 6. In the table, R is the reference cylinder, S the sample cylinder, Q is the 1g calibration mass piece, W is the total mass of mass pieces added to the reference cylinder and M is the total mass of mass pieces added to the sample cylinder.

Table 6: First weighing cycle

Measurement	System	Reading (g)
$m_{C,0}$	$R + W + Q$	8057.418
$m_{C,1}$	$R + W$	8056.427
$m_{C,2}$	$S + M$	8056.860
$m_{C,3}$	$R + W$	8056.425
$m_{C,4}$	$S + M$	8056.862
$m_{C,5}$	$R + W$	8056.429
$m_{C,6}$	$S + M$	8056.864
$m_{C,7}$	$R + W$	8056.428
$m_{C,8}$	$R + W + Q$	8057.432

The environmental conditions during the first weighing cycle were as follows: the initial temperature was 20.6°C, relative humidity 54.1 %, and pressure 1014.0 hPa. Final conditions were the same for temperature (20.6°C), with slight changes in humidity (53.7%) and pressure (1014.0 hPa). Therefore, the average conditions throughout the cycle were 20.6 °C for temperature, 53.9 % for humidity, and 1014.0 hPa for pressure.

The values obtained from the second weighing cycle, the one including the cylinder filled only with CO_2 and the reference cylinder, are shown in table 7. In the table, R is the reference cylinder, S the sample cylinder, Q is the 1g calibration mass piece, W is the total mass of mass pieces added to the reference cylinder and *M* is the total mass of mass pieces added to the sample cylinder.

Table 7: Second weighing cycle

Measurement	System	Reading (g)
$m_{C,0}$	$R + W + Q$	8307.488
$m_{C,1}$	$R + W$	8306.484
$m_{C,2}$	$S + M$	8306.683
$m_{C,3}$	$R + W$	8306.487
$m_{C,4}$	$S + M$	8306.684
$m_{C,5}$	$R + W$	8306.489
$m_{C,6}$	$S + M$	8306.682
$m_{C,7}$	$R + W$	8306.485
$m_{C,8}$	$R + W + Q$	8307.489

The environmental conditions during the second weighing cycle were as follows: an initial temperature of 20.4°C, relative humidity of 52.5 %, and pressure of 1005.0 hPa. At the end of the cycle, the temperature was 20.6°C, humidity 52.9 %, and pressure remained at 1005.0 hPa. Therefore, the average conditions throughout the cycle were 20.5°C for temperature, 52.7 % for humidity, and 1005.0 hPa for pressure.

The values obtained from the third weighing cycle, the one including the cylinder filled with CO_2 and N_2, and the reference cylinder, are shown in table 8. In the table, *R* is the reference cylinder, *S* the sample cylinder, *Q* is the 1g calibration mass piece, *W* is the total mass of mass pieces added to the reference cylinder and *M* is the total mass of mass pieces added to the sample cylinder.

Table 8: Third weighing cycle

Measurement	System	Reading (g)
$m_{C,0}$	$R + W + Q$	8714.419
$m_{C,1}$	$R + W$	8713.422
$m_{C,2}$	$S + M$	8713.754
$m_{C,3}$	$R + W$	8713.421
$m_{C,4}$	$S + M$	8713.753
$m_{C,5}$	$R + W$	8713.421
$m_{C,6}$	$S + M$	8713.754
$m_{C,7}$	$R + W$	8713.423
$m_{C,8}$	$R + W + Q$	8714.425

The environmental conditions during the third weighing cycle were as follows: an initial temperature of 20.7°C, relative humidity of 53.4 %, and pressure of 1014.0 hPa. At the end of the cycle, the temperature was 20.6°C, humidity 50.9 %, and pressure remained at 1014.0 hPa. Therefore, the average conditions throughout the cycle were 20.7 °C for temperature, 52.2 % for humidity, and 1014.0 hPa for pressure.

The uncertainties associated with each parameter are calculated through uncertainty propagation, in accordance with the Guide to the Expression of Uncertainty in Measurement (GUM, ISO/IEC Guide 98-3), by deriving Equation 7 with respect to each term and combining the results as appropriate. The uncertainty budget for each weighing cycle is presented in tables 9, 10 and 11.

$$w_j = e_j(q_j - p_j) + (W_j - M_j)\left(1 - \frac{\rho_{air,j}}{\rho_S}\right) + \rho_{air,j}(V_{S,j} - V_R) \tag{7}$$

Table 9: Uncertainty budget for the first weighing cycle

Quantity	Estimate	Sensitivity coefficient (c_s)	c_s value	Probability distribution	Standard uncertainty	Uncertainty contribution	Unit
e_1	1.0025	$(q_1 - p_1)$	0.4348	Normal	2.9x10^{-4}	1.3x10^{-4}	-
q_1	8056.862	-	-	-	-	-	g
p_1	8056.427	-	-	-	-	-	g
$(q_1 - p_1)$	0.4348	e_j	1.0025	Normal	1.5x10^{-3}	1.5x10^{-3}	g
W_1	0	-	-	-	-	-	g
M_1	104.0	-	-	-	-	-	g
$(W_1 - M_1)$	-104.0	$\left(1 - \frac{\rho_{air,1}}{\rho_S}\right)$	0.99985	Normal	2.6x10^{-5}	2.6x10^{-5}	g
$\rho_{air,1}$	1.1936	$\left(\frac{(W_1 - M_1)}{\rho_S}\right) + (V_{S,1} - V_R)$	-1.082	Normal	8.9x10^{-7}	-9.6x10^{-7}	kg/m^3
$V_{S,1}$	5.012	-	-	-	-	-	L
V_R	5.0	-	-	-	-	-	L
$(V_{S,1} - V_R)$	12x10^{-3}	$\rho_{air,j}$	1.1936	Rectangular	5.0x10^{-4}	6.0x10^{-4}	L
ρ_S	7950	$\left(\frac{1}{\rho_S}\right)^2$ $(W_1 - M_1)$ $(\rho_{air,1})$	-1.96x10^{-3}	Normal	0.02	-3.9x10^{-5}	kg/m^3
w_1	-	-	-	-	-	**0.0016**	**g**

Table 10: Uncertainty budget for the second weighing cycle

Quantity	Estimate	Sensitivity coefficient (c_s)	c_s value	Probability distribution	Standard uncertainty	Uncertainty contribution	Unit
e_2	0.9960	$(q_2 - p_2)$	0.1962	Normal	$2.9\text{x}10^{-4}$	$5.6\text{x}10^{-5}$	-
q_2	8306.683	-	-	-	-	-	g
p_2	8306.487	-	-	-	-	-	g
$(q_2 - p_2)$	0.1962	e_j	0.9960	Normal	$1.3\text{x}10^{-3}$	$1.3\text{x}10^{-3}$	g
W_2	250.0	-	-	-	-	-	g
M_2	0	-	-	-	-	-	g
$(W_2 - M_2)$	250.0	$\left(1 - \frac{\rho_{air,2}}{\rho_S}\right)$	0.99985	Normal	$1.2\text{x}10^{-4}$	$1.2\text{x}10^{-4}$	g
$\rho_{air,2}$	1.1836	$\left(\frac{(W_2 - M_2)}{\rho_S}\right)$ $+ \left(V_{S,2} - V_R\right)$	43.45	Normal	$9.1\text{x}10^{-7}$	$3.9\text{x}10^{-5}$	kg/m^3
$V_{S,2}$	5.012	-	-	-	-	-	L
V_R	5.0	-	-	-	-	-	L
$\left(V_{S,2} - V_R\right)$	$12\text{x}10^{-3}$	$\rho_{air,j}$	1.1836	Rectangular	$5.0\text{x}10^{-4}$	$5.9\text{x}10^{-4}$	L
ρ_S	7950	$\left(\frac{1}{\rho_S}\right)^2$ $(W_2 - M_2)$ $\left(\rho_{air,2}\right)$	$4.7\text{x}10^{-3}$	Normal	0.02	$9.4\text{x}10^{-5}$	kg/m^3
w_2	-	-	-	-	-	**0.0014**	**g**

Table 11: Uncertainty budget for the third weighing cycle

Quantity	Estimate	Sensitivity coefficient (c_s)	c_s value	Probability distribution	Standard uncertainty	Uncertainty contribution	Unit
e_3	1.0005	$(q_3 - p_3)$	0.3322	Normal	2.89x10^{-4}	9.6x10^{-5}	-
q_3	8713.754	-	-	-	-	-	g
p_3	8716.422	-	-	-	-	-	g
$(q_3 - p_3)$	0.3322	e_j	1.0005	Normal	6.05x10^{-5}	6.1x10^{-4}	g
W_3	0	-	-	-	-	-	g
M_3	657.0	-	-	-	-	-	g
$(W_3 - M_3)$	657.0	$\left(1 - \frac{\rho_{air,3}}{\rho_S}\right)$	0.99985	Normal	1.19x10^{-4}	1.2x10^{-4}	g
$\rho_{air,3}$	1.1935	$\left(\frac{(W_3 - M_3)}{\rho_S}\right) + \left(V_{S,3} - V_R\right)$	94.64	Normal	8.88x10^{-7}	8.4x10^{-5}	kg/m^3
$V_{S,3}$	5.012	-	-	-	-	-	L
V_R	5.0	-	-	-	-	-	L
$\left(V_{S,3} - V_R\right)$	12x10^{-3}	$\rho_{air,j}$	1.1935	Rectangular	5.00x10^{-4}	6.0x10^{-4}	L
ρ_S	7950	$\left(\frac{1}{\rho_S}\right)^2 (W_3 - M_3) \left(\rho_{air,3}\right)$	1.24x10^{-2}	Normal	0.02	2.5x10^{-4}	kg/m^3
w_3	-	-	-	-	-	**0.00090**	**g**

The weighted mass of CO_2 was determined using Equation 6 with the weighing cycles 2 and 1, while the weighted mass of N_2 was determined using the same equation but with the weighing cycles 3 and 2.

The combined uncertainty for the weighing process of CO_2 was determined as the square root of the sum of the squared standard uncertainties for cycles 1 and 2. In the same way, the uncertainty associated with the weighing process of N_2 was calculated as the sum of the squared standard uncertainties for cycles 2 and 3 (Alink & van der Veen, 2000; Flores et al., 2019a).

The determined masses and their associated standard uncertainties are shown in table 12.

Table 12: Determined masses and their associated standard uncertainties

m_{CO2} (g)	$u_{weigh}(m_{CO2})$ (g)	m_{N2} (g)	$u_{weigh}(m_{N2})$ (g)
353.7067	0.0021	407.0757	0.0017

According to Alink et al., calculated uncertainties on numerous weighing cycles typically vary between 0.7 mg and 1.5 mg. Additionally, tests under reproducibility conditions for a comparator balance have shown that the results of weighing cycles performed by six individuals on different days, using two pairs of sample and reference cylinders, agree within 2 mg. Since we are using a manual mass comparator, which is more difficult to handle and stabilize during measurements, a standard deviation of ≤ 2 mg was chosen as a parameter to ensure reliability and consistency in the weighing process, in accordance with the comparator's control requirements.

The standard deviation for each weighing cycle was calculated from the differences between consecutive readings: ($m_2 - m_1$), ($m_2 - m_3$), ($m_4 - m_3$), ($m_4 - m_5$), ($m_6 - m_5$), and ($m_6 - m_7$). The resulting standard deviations were 1.6 mg for the first cycle, 2.0 mg for the second cycle and 0.8 mg for the third cycle.

4.3.2. Purity assessment of CO_2 and N_2

The data from the certificate of analysis issued by the gas manufacturer is presented in Table 13 for N_2 and Table 14 for CO_2.

Table 13: Impurities of the N_2 6.0 according to the gas manufacturer

Component	**Specification (µmol/mol)**
Nitrogen	>999999
Carbon dioxide	≤ 0.1
Hydrocarbons	≤ 0.1
Water	≤ 0.2
Carbon monoxide	≤ 0.1
Oxygen	≤ 0.5

Table 14: Impurities of the pure industrial CO_2 according to the gas manufacturer

Component	**Specification (µmol/mol)**
Carbon dioxide	>999000
Water	≤ 150

Based on the specification tables, the CO_2 impurity in the nitrogen pure cylinder appears to be small enough not to be considered critical. It is also unlikely to be considered significant unless the target uncertainty for CO_2 is extremely low.

To quantify the effect of these impurities on the uncertainty of the prepared gas mixtures, the impurity levels reported in the manufacturer's specifications were used as the basis for estimation. In accordance with ISO 19229, when an impurity is below the limit of detection of the analytical method, its molar fraction (International Organization for Standardization [ISO], 2019), x_{ij}, is conventionally calculated as the half of the value of the limit of detection, L_{ij} (Equation 11). The associated standard uncertainty is then evaluated assuming a rectangular distribution with L_{ij} being the upper limit of the rectangle, and zero the lower limit (Formula 12) (Aoki et al., 2019; Shehata et al., 2021).

The molar fractions and associated uncertainties of N_2 and CO_2 are shown in tables 15 and 16, respectively.

Table 15: Molar fractions and associated uncertainties of the N_2 impurities

Component	**x_{ij} (µmol/mol)**	**$u(x_{ij})$ (µmol/mol)**
Nitrogen	0.99999950	0.00000016
Carbon dioxide	0.000000050	0.000000029
Hydrocarbons	0.000000050	0.000000029
Water	0.000000100	0.000000058
Carbon monoxide	0.000000050	0.000000029
Oxygen	0.00000025	0.00000014

Table 16: Molar fractions and associated uncertainties of the CO_2 impurities

Component	**x_{ij} (µmol/mol)**	**$u(x_{ij})$ (µmol/mol)**
Carbon dioxide	0.999925	0.000043
Water	0.000075	0.000043

From this estimation, it can be concluded that the impurity of CO_2 present in the pure nitrogen cylinder is not considered a critical impurity. The mole fraction of $5x10^{-8}$ mol/mol is extremely small relative to the major component (0.35 mol/mol) in the mixture and, therefore, does not contribute significantly to the overall composition. Furthermore, it is concluded that the CO_2 impurity is not significant, as its uncertainty is much smaller than the target uncertainty of the mixture (1 % mol/mol).

4.3.3. Calculation of molar masses

In addition to the purity of the components, appropriate relative atomic and/or molecular masses must be used to convert the added masses into amounts of substance.

The atomic weights of the elements C, O, and N were determined as the averages of the atomic weight intervals, where *a* represents the lower limit and *b* the upper limit, as provided by (Prohaska et al., 2022). The associated uncertainties were calculated using a rectangular distribution, with *a* and *b* being the end points of the distribution. Results are shown in Table 17.

Table 17: IUPAC atomic weights of elements and their uncertainties (numerically equivalent to g/mol)

Element	*a*	*b*	Atomic weight	Standard uncertainty
N	14.00643	14.00728	14.00686	0.00025
O	15.99903	15.99977	15.99940	0.00021
C	12.0096	12.0116	12.01060	0.00058

The molar masses of CO_2 and N_2 were calculated using Equation 38 and their associated uncertainties were determined using Equation 39. In both equations ν_{zi} is the stoichiometric coefficient for the element z, Z is the number of atoms present in all components in the composition of the mixture, and A_z the atomic weight of element z.

$$M_i = \sum_{z=1}^{Z} \nu_{zi} A_z \tag{38}$$

$$u^2(M_i) = \sum_{z=1}^{Z} \nu_{zi}^2 u^2(A_z) \tag{39}$$

The molar masses calculated for both gases are shown in table 18.

Table 18: Molar masses and associated uncertainties

M_{CO2} (g/mol)	44.00940	$u(M_{CO2})$ (g/mol)	0.00072
M_{N2} (g/mol)	28.01371	$u(M_{N2})$ (g/mol)	0.00049

4.3.4. Calculation of gravimetric uncertainty

The calculation of the sensitivity coefficients from Equation 21, i.e., the partial derivates: $\left(\frac{\partial y_k}{\partial M_i}\right), \left(\frac{\partial y_k}{\partial m_j}\right), \left(\frac{\partial y_k}{\partial x_{i,j}}\right)$, for each parameter are presented in table 19. The calculations of these partial derivatives are provided below, in Equations from 40 to 53.

Table 19: Sensitivity coefficients from formula 21

Partial derivates y_{CO2}	Values of derivates y_{CO2}	Partial derivates y_{N2}	Values of derivates y_{N2}
$\left(\frac{\partial y_{CO2}}{\partial x_{CO2,CO2}}\right)$	1.14656E-08	$\left(\frac{\partial y_{CO2}}{\partial x_{CO2,CO2}}\right)$	-0.229311112
$\left(\frac{\partial y_{CO2}}{\partial x_{CO2,N2}}\right)$	2.07301E-08	$\left(\frac{\partial y_{CO2}}{\partial x_{CO2,N2}}\right)$	-0.414602296
$\left(\frac{\partial y_{CO2}}{\partial m_{CO2}}\right)$	-0.643896324	$\left(\frac{\partial y_{CO2}}{\partial m_{CO2}}\right)$	0.229294049
$\left(\frac{\partial y_{CO2}}{\partial m_{N2}}\right)$	3.2413E-11	$\left(\frac{\partial y_{CO2}}{\partial m_{N2}}\right)$	-0.00064826
$\left(\frac{\partial y_{CO2}}{\partial M_{CO2}}\right)$	-0.00158176	$\left(\frac{\partial y_{CO2}}{\partial M_{CO2}}\right)$	0.000563271
$\left(\frac{\partial y_{CO2}}{\partial M_{N2}}\right)$	-2.60506E-10	$\left(\frac{\partial y_{CO2}}{\partial M_{N2}}\right)$	0.005210112

The calculation of the amount fraction of CO_2 and N_2 was carried out using Equations 4 and 5, respectively, and their gravimetric uncertainty were determined using Equations 21, with the sensitivity coefficients from above. The results are presented in table 20.

Table 20: Amount fractions and gravimetric uncertainties of CO_2 and N_2

y_{CO2} (mol/mol)	$u_{grav}(y_{CO2})$ (mol/mol)	y_{N2} (mol/mol)	$u_{grav}(y_{N2})$ (mol/mol)
0.356104	0.000012	0.643896	0.000011

$$\left(\frac{\partial y_{CO2}}{\partial x_{CO2,CO2}}\right)=\frac{\left[\left(\frac{x_{CO2,CO2}\times m_{CO2}}{M_{CO2}}\right)+\left(\frac{x_{CO2,N2}\times m_{N2}}{M_{N2}}\right)+\left(\frac{x_{N2,N2}\times m_{N2}}{M_{N2}}\right)\right]\times\left(\frac{m_{CO2}}{M_{CO2}}\right)-\left[\left(\frac{x_{CO2,CO2}\times m_{CO2}}{M_{CO2}}\right)+\left(\frac{x_{CO2,N2}\times m_{N2}}{M_{N2}}\right)\right]\times\left(\frac{m_{CO2}}{M_{CO2}}\right)}{\left[\left(\frac{x_{CO2,CO2}\times m_{CO2}}{M_{CO2}}\right)+\left(\frac{x_{CO2,N2}\times m_{N2}}{M_{N2}}\right)+\left(\frac{x_{N2,N2}\times m_{N2}}{M_{N2}}\right)\right]^{2}} \tag{40}$$

$$\left(\frac{\partial y_{CO2}}{\partial x_{CO2,N2}}\right)=\frac{\left[\left(\frac{x_{CO2,CO2}\times m_{CO2}}{M_{CO2}}\right)+\left(\frac{x_{CO2,N2}\times m_{N2}}{M_{N2}}\right)+\left(\frac{x_{N2,N2}\times m_{N2}}{M_{N2}}\right)\right]\times\left(\frac{m_{N2}}{M_{N2}}\right)-\left[\left(\frac{x_{CO2,CO2}\times m_{CO2}}{M_{CO2}}\right)+\left(\frac{x_{CO2,N2}\times m_{N2}}{M_{N2}}\right)\right]\times\left(\frac{m_{N2}}{M_{N2}}\right)}{\left[\left(\frac{x_{CO2,CO2}\times m_{CO2}}{M_{CO2}}\right)+\left(\frac{x_{CO2,N2}\times m_{N2}}{M_{N2}}\right)+\left(\frac{x_{N2,N2}\times m_{N2}}{M_{N2}}\right)\right]^{2}} \tag{41}$$

$$\left(\frac{\partial y_{CO2}}{\partial x_{N2,N2}}\right)=\frac{-\left[\left(\frac{x_{CO2,CO2}\times m_{CO2}}{M_{CO2}}\right)+\left(\frac{x_{CO2,N2}\times m_{N2}}{M_{N2}}\right)\right]\times\left(\frac{m_{N2}}{M_{N2}}\right)}{\left[\left(\frac{x_{CO2,CO2}\times m_{CO2}}{M_{CO2}}\right)+\left(\frac{x_{CO2,N2}\times m_{N2}}{M_{N2}}\right)+\left(\frac{x_{N2,N2}\times m_{N2}}{M_{N2}}\right)\right]^{2}} \tag{42}$$

$$\left(\frac{\partial y_{CO2}}{\partial m_{CO2}}\right)=\frac{\left[\left(\frac{x_{CO2,CO2}\times m_{CO2}}{M_{CO2}}\right)+\left(\frac{x_{CO2,N2}\times m_{N2}}{M_{N2}}\right)+\left(\frac{x_{N2,N2}\times m_{N2}}{M_{N2}}\right)\right]\times\left(\frac{x_{CO2,CO2}}{M_{CO2}}\right)-\left[\left(\frac{x_{CO2,CO2}\times m_{CO2}}{M_{CO2}}\right)+\left(\frac{x_{CO2,N2}\times m_{N2}}{M_{N2}}\right)\right]\times\left(\frac{x_{CO2,CO2}}{M_{CO2}}\right)}{\left[\left(\frac{x_{CO2,CO2}\times m_{CO2}}{M_{CO2}}\right)+\left(\frac{x_{CO2,N2}\times m_{N2}}{M_{N2}}\right)+\left(\frac{x_{N2,N2}\times m_{N2}}{M_{N2}}\right)\right]^{2}} \tag{43}$$

$$\left(\frac{\partial y_{CO2}}{\partial m_{N2}}\right)=\frac{\left[\left(\frac{x_{CO2,CO2}\times m_{CO2}}{M_{CO2}}\right)+\left(\frac{x_{CO2,N2}\times m_{N2}}{M_{N2}}\right)+\left(\frac{x_{N2,N2}\times m_{N2}}{M_{N2}}\right)\right]\times\left(\frac{x_{CO2,N2}}{M_{N2}}\right)-\left[\left(\frac{x_{CO2,CO2}\times m_{CO2}}{M_{CO2}}\right)+\left(\frac{x_{CO2,N2}\times m_{N2}}{M_{N2}}\right)\right]\times\left(\frac{x_{CO2,N2}+x_{N2,N2}}{M_{N2}}\right)}{\left[\left(\frac{x_{CO2,CO2}\times m_{CO2}}{M_{CO2}}\right)+\left(\frac{x_{CO2,N2}\times m_{N2}}{M_{N2}}\right)+\left(\frac{x_{N2,N2}\times m_{N2}}{M_{N2}}\right)\right]^{2}} \tag{44}$$

$$\left(\frac{\partial y_{CO2}}{\partial M_{CO2}}\right) = \frac{\left[\left(\frac{x_{CO2,CO2}\times m_{CO2}}{M_{CO2}}\right)+\left(\frac{x_{CO2,N2}\times m_{N2}}{M_{N2}}\right)+\left(\frac{x_{N2,N2}\times m_{N2}}{M_{N2}}\right)\right]\times\left(-\frac{x_{CO2,CO2}\times m_{CO2}}{M_{CO2}^{2}}\right)-\Bigg[\left(\frac{x_{CO2,CO2}\times m_{CO2}}{M_{CO2}}\right)+\left(\frac{x_{CO2,N2}\times m_{N2}}{M_{N2}}\right)\Bigg]\times\left(-\frac{x_{CO2,CO2}\times m_{CO2}}{M_{CO2}^{2}}\right)}{\left[\left(\frac{x_{CO2,CO2}\times m_{CO2}}{M_{CO2}}\right)+\left(\frac{x_{CO2,N2}\times m_{N2}}{M_{N2}}\right)+\left(\frac{x_{N2,N2}\times m_{N2}}{M_{N2}}\right)\right]^{2}} \tag{45}$$

$$\left(\frac{\partial y_{CO2}}{\partial M_{N2}}\right) = \frac{\left[\left(\frac{x_{CO2,CO2}\times m_{CO2}}{M_{CO2}}\right)+\left(\frac{x_{CO2,N2}\times m_{N2}}{M_{N2}}\right)+\left(\frac{x_{N2,N2}\times m_{N2}}{M_{N2}}\right)\right]\times\left(-\frac{x_{CO2,N2}\times m_{N2}}{M_{N2}^{2}}\right)-\Bigg[\left(\frac{x_{CO2,CO2}\times m_{CO2}}{M_{CO2}}\right)+\left(\frac{x_{CO2,N2}\times m_{N2}}{M_{N2}}\right)\Bigg]\times\left(-\frac{x_{CO2,N2}\times m_{N2}}{M_{N2}^{2}}-\frac{x_{N2,N2}\times m_{N2}}{M_{N2}^{2}}\right)}{\left[\left(\frac{x_{CO2,CO2}\times m_{CO2}}{M_{CO2}}\right)+\left(\frac{x_{CO2,N2}\times m_{N2}}{M_{N2}}\right)+\left(\frac{x_{N2,N2}\times m_{N2}}{M_{N2}}\right)\right]^{2}} \tag{46}$$

$$\left(\frac{\partial y_{N2}}{\partial x_{N2,N2}}\right) = \frac{\left[\left(\frac{x_{CO2,CO2}\times m_{CO2}}{M_{CO2}}\right)+\left(\frac{x_{CO2,N2}\times m_{N2}}{M_{N2}}\right)+\left(\frac{x_{N2,N2}\times m_{N2}}{M_{N2}}\right)\right]\times\left(\frac{m_{N2}}{M_{N2}}\right)-\left[\left(\frac{x_{N2,N2}\times m_{N2}}{M_{N2}}\right)\times\left(\frac{m_{N2}}{M_{N2}}\right)\right]}{\left[\left(\frac{x_{CO2,CO2}\times m_{CO2}}{M_{CO2}}\right)+\left(\frac{x_{CO2,N2}\times m_{N2}}{M_{N2}}\right)+\left(\frac{x_{N2,N2}\times m_{N2}}{M_{N2}}\right)\right]^{2}} \tag{47}$$

$$\left(\frac{\partial y_{N2}}{\partial m_{N2}}\right) = \frac{\left[\left(\frac{x_{CO2,CO2}\times m_{CO2}}{M_{CO2}}\right)+\left(\frac{x_{CO2,N2}\times m_{N2}}{M_{N2}}\right)+\left(\frac{x_{N2,N2}\times m_{N2}}{M_{N2}}\right)\right]\times\left(\frac{x_{N2,N2}}{M_{N2}}\right)-\left[\left(\frac{x_{N2,N2}\times m_{N2}}{M_{N2}}\right)\times\left(\frac{x_{CO2,N2}+x_{N2,N2}}{M_{N2}}\right)\right]}{\left[\left(\frac{x_{CO2,CO2}\times m_{CO2}}{M_{CO2}}\right)+\left(\frac{x_{CO2,N2}\times m_{N2}}{M_{N2}}\right)+\left(\frac{x_{N2,N2}\times m_{N2}}{M_{N2}}\right)\right]^{2}} \tag{48}$$

$$\left(\frac{\partial y_{N2}}{\partial M_{N2}}\right) = \frac{\left[\left(\frac{x_{CO2,CO2}\times m_{CO2}}{M_{CO2}}\right)+\left(\frac{x_{CO2,N2}\times m_{N2}}{M_{N2}}\right)+\left(\frac{x_{N2,N2}\times m_{N2}}{M_{N2}}\right)\right]\times\left(-\frac{x_{N2,N2}\times m_{N2}}{M_{N2}^{2}}\right)-\left[\left(\frac{x_{N2,N2}\times m_{N2}}{M_{N2}}\right)\times\left(\frac{-x_{CO2,N2}\times m_{N2}-x_{N2,N2}\times m_{N2}}{M_{N2}^{2}}\right)\right]}{\left[\left(\frac{x_{CO2,CO2}\times m_{CO2}}{M_{CO2}}\right)+\left(\frac{x_{CO2,N2}\times m_{N2}}{M_{N2}}\right)+\left(\frac{x_{N2,N2}\times m_{N2}}{M_{N2}}\right)\right]^{2}} \tag{49}$$

$$\left(\frac{\partial y_{N2}}{\partial x_{CO2,CO2}}\right) = \frac{-\left(\frac{x_{N2,N2} \times m_{N2}}{M_{N2}}\right) \times \left(\frac{m_{CO2}}{M_{CO2}}\right)}{\left[\left(\frac{x_{CO2,CO2} \times m_{CO2}}{M_{CO2}}\right) + \left(\frac{x_{CO2,N2} \times m_{N2}}{M_{N2}}\right) + \left(\frac{x_{N2,N2} \times m_{N2}}{M_{N2}}\right)\right]^2} \tag{50}$$

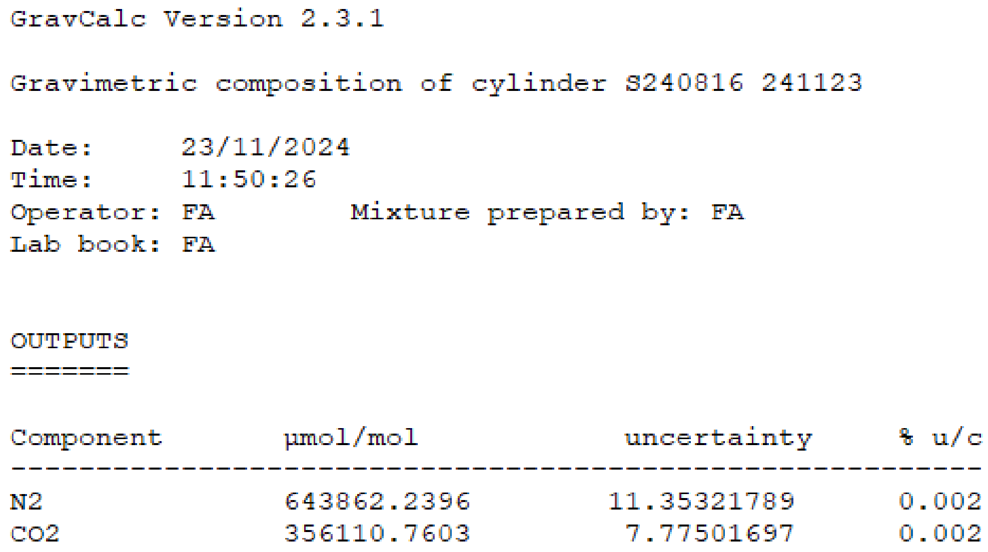
$$\left(\frac{\partial y_{N2}}{\partial m_{CO2}}\right) = \frac{-\left(\frac{x_{N2,N2} \times m_{N2}}{M_{N2}}\right) \times \left(\frac{x_{CO2,CO2}}{M_{CO2}}\right)}{\left[\left(\frac{x_{CO2,CO2} \times m_{CO2}}{M_{CO2}}\right) + \left(\frac{x_{CO2,N2} \times m_{N2}}{M_{N2}}\right) + \left(\frac{x_{N2,N2} \times m_{N2}}{M_{N2}}\right)\right]^2} \tag{51}$$

$$\left(\frac{\partial y_{N2}}{\partial M_{CO2}}\right) = \frac{-\left(\frac{x_{N2,N2} \times m_{N2}}{M_{N2}}\right) \times \left(-\frac{x_{CO2,CO2} \times m_{CO2}}{M_{CO2}^2}\right)}{\left[\left(\frac{x_{CO2,CO2} \times m_{CO2}}{M_{CO2}}\right) + \left(\frac{x_{CO2,N2} \times m_{N2}}{M_{N2}}\right) + \left(\frac{x_{N2,N2} \times m_{N2}}{M_{N2}}\right)\right]^2} \tag{52}$$

$$\left(\frac{\partial y_{N2}}{\partial x_{CO2,N2}}\right) = \frac{-\left(\frac{x_{N2,N2} \times m_{N2}}{M_{N2}}\right) \times \left(\frac{m_{N2}}{M_{N2}}\right)}{\left[\left(\frac{x_{CO2,CO2} \times m_{CO2}}{M_{CO2}}\right) + \left(\frac{x_{CO2,N2} \times m_{N2}}{M_{N2}}\right) + \left(\frac{x_{N2,N2} \times m_{N2}}{M_{N2}}\right)\right]^2} \tag{53}$$

4.3.5. Comparison of results obtained with software GravCalc2

Given that calculating the amount fraction of CO_2 and N_2, along with their associated gravimetric uncertainties, according to ISO 6142 is a complex process involving many calculations and steps, a software developed by the National Physical Laboratory (NPL), the National Metrology Institute of the United Kingdom, was employed to validate the results. The software, GravCalc2, is a Windows-based application designed to calculate the amount fraction and uncertainty of all components in gravimetrically prepared gas mixtures, following the method described in ISO 6142. This program can be applied to any gas mixture (Brown, 2009). The results obtained for the amount fraction and standard uncertainty of CO_2 and N_2, using GravCalc2 v2.3.1, are presented in Figure 17.



```
GravCalc Version 2.3.1

Gravimetric composition of cylinder S240816 241123

Date:      23/11/2024
Time:      11:50:26
Operator: FA         Mixture prepared by: FA
Lab book: FA


OUTPUTS
=======

Component        µmol/mol            uncertainty     % u/c
----------------------------------------------------------
N2               643862.2396        11.35321789      0.002
CO2              356110.7603         7.77501697      0.002
```

Figure 17: Calculated data using software GravCalc2.

To determine whether the results show statistically insignificant differences, a normalised error (Equation 54) was calculated between the values obtained using GravCalc2 and those calculated manually. The values from both approaches are presented in Table 21.

Table 21: Comparison between GravCalc2 and manually calculated results

	CO_2		**N_2**	
Method	Amount fraction (mol/mol)	Standard uncertainty (mol/mol)	Amount fraction (mol/mol)	Standard uncertainty (mol/mol)
Manual calculation	0.356104	0.000012	0.643896	0.000011
GravCalc2	0.3561108	0.0000078	0.643862	0.000011

The normalised error calculated by applying Equation 54 was 0.24 for CO_2 and 1.0 for N_2, thus proving that the results obtained from both approaches do not show a statistically significant difference.

4.4. Verification of CO_2 in N_2 PRM

To perform the verification of the PRM candidate, the calibration curve method was used with five independent PRMs at proper CO_2 amount fraction purchased from VSL. The amount fractions and expanded uncertainties of the PRMs used are shown in table 22.

Table 22: PRMs of CO_2 in N_2 from VSL used for the calibration curve

PRM code	Amount fraction (mol/mol)	Expanded uncertainty, k = 2 (mol/mol)
D562955	0.05003	0.00005
D562939	0.09996	0.00010
D563094	0.20006	0.00020
D563020	0.30042	0.00030
5603670	0.40000	0.00040

The type of fit and polynomial used for this regression was the same as the one validated for CO_2 in N_2 within this amount fraction range: GLS and a second-degree polynomial. The Γ obtained was 0.65 and the calibration curve obtained from the XLGENLINE v1.1 is shown in Figure 18.

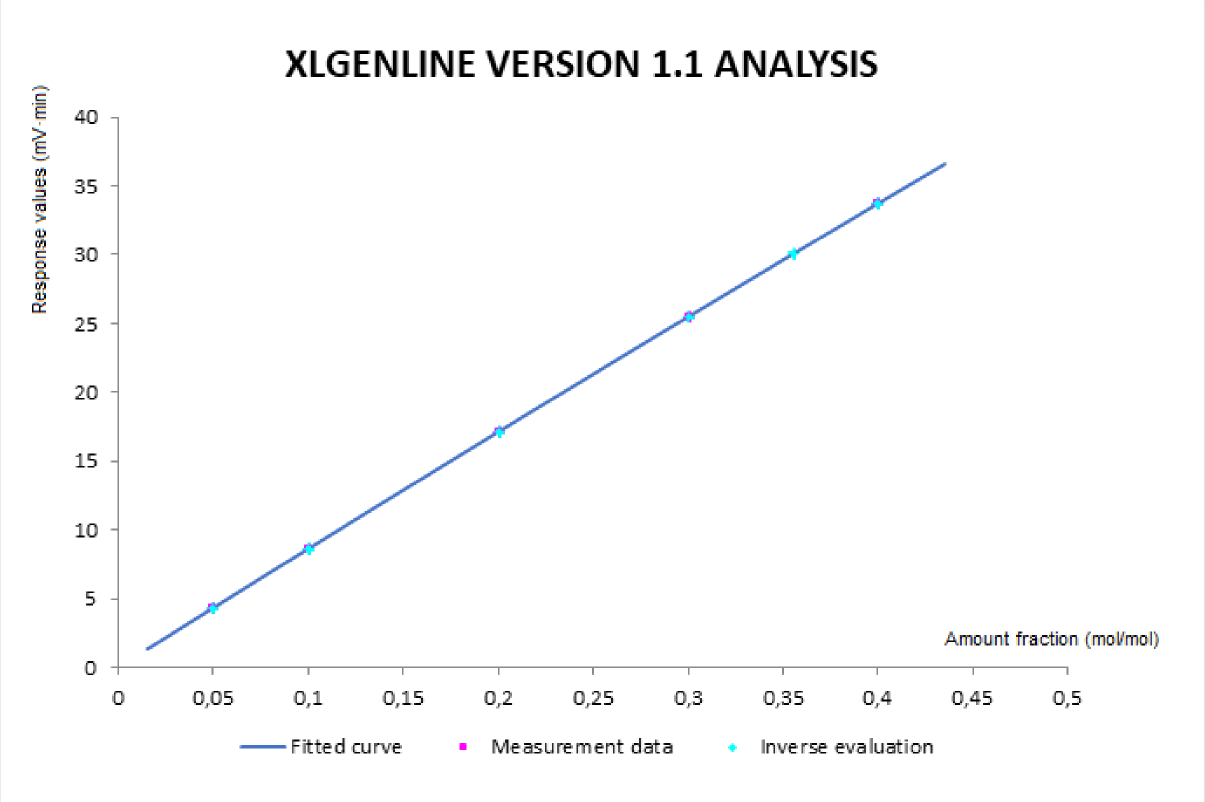


Figure 18: Calibration curve obtained for the verification of the prepared PRM candidate

The amount fraction and its standard uncertainty determined by the software was 0.35603 ± 0.00044 mol/mol (k = 1).

The gravimetric value of 0.356104 ± 0.000012 mol/mol (k = 1) has a significantly lower uncertainty than the verification result of 0.35603 ± 0.00044 mol/mol (k = 1), confirming the higher precision and reliability of the gravimetric method. Also, both amount fractions are very similar, so gravimetry results and verification are in good agreement within their respective uncertainties. This consistency is further supported by the low normalized error of 0.084, confirming the comparability between both results.

4.5. Homogeneity

The CO in N_2 gas mixture, prepared when the cylinder homogenizer was first set up, was rolled for approximately 8 hours to ensure uniform distribution of the CO gas within the mixture. However, for the CO_2 in N_2 mixture, a homogeneity study was conducted

(Dias, 2019). Given the viscosity of carbon dioxide at a molar fraction of 0.35 mol/mol, homogeneity is a critical parameter to characterize.

The CO_2 in N_2 mixture was analysed using gas chromatography coupled with a thermal conductivity detector after different homogenization times. The results obtained are shown in Table 23. From these results, it can be concluded that after half an hour of rolling, the mixture did not completely homogenize, and the obtained value resulted from stratification of the gas mixture in the cylinder.

Table 23: Different homogenization times for a gas mixture of carbon dioxide in nitrogen, of amount fraction equal to 0.35 mol/mol

Homogenization time (h)	**Amount fraction (mol/mol)**	**Standard uncertainty (mol/mol)**
0.5	0.08554	0.00065
2	0.35398	0.00052
3	0.35714	0.00033
4	0.35644	0.00017
5	0.35710	0.00019
8	0.35803	0.00040

When a gas mixture contains components with different densities, stratification can occur if the mixture is not properly homogenized. In such cases, the denser gas (in this case, CO_2) tends to accumulate at the bottom of the cylinder, while the lighter component, N_2, remains toward the top. If the gas sample is taken before complete homogenization, particularly from the upper part of the cylinder, the sample may contain a disproportionately high amount of N_2 and an underrepresentation of CO_2. This could explain the abnormally low amount fraction observed after only 0.5 h of rolling (0.08554 mol/mol), despite the target value being approximately 0.35 mol/mol (Figure 19).

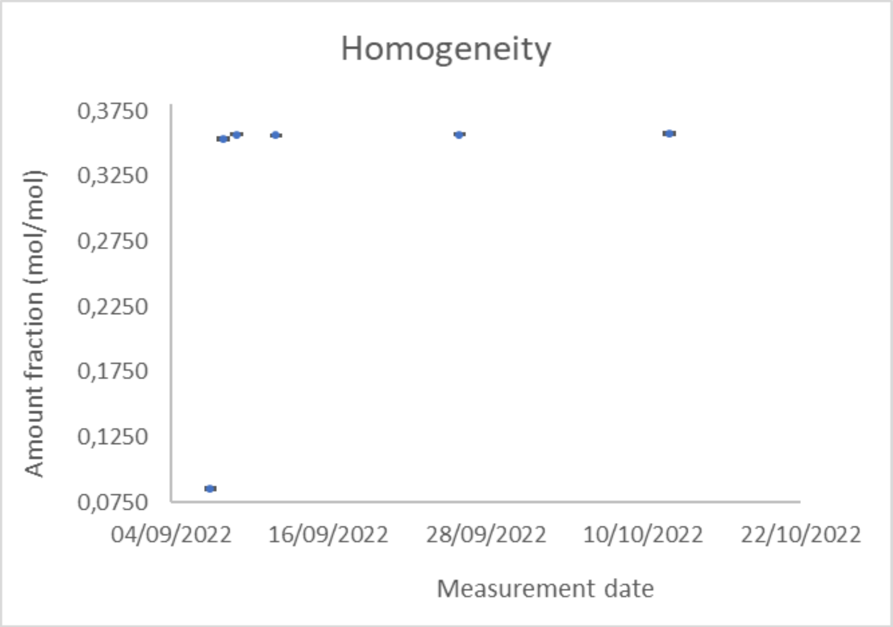
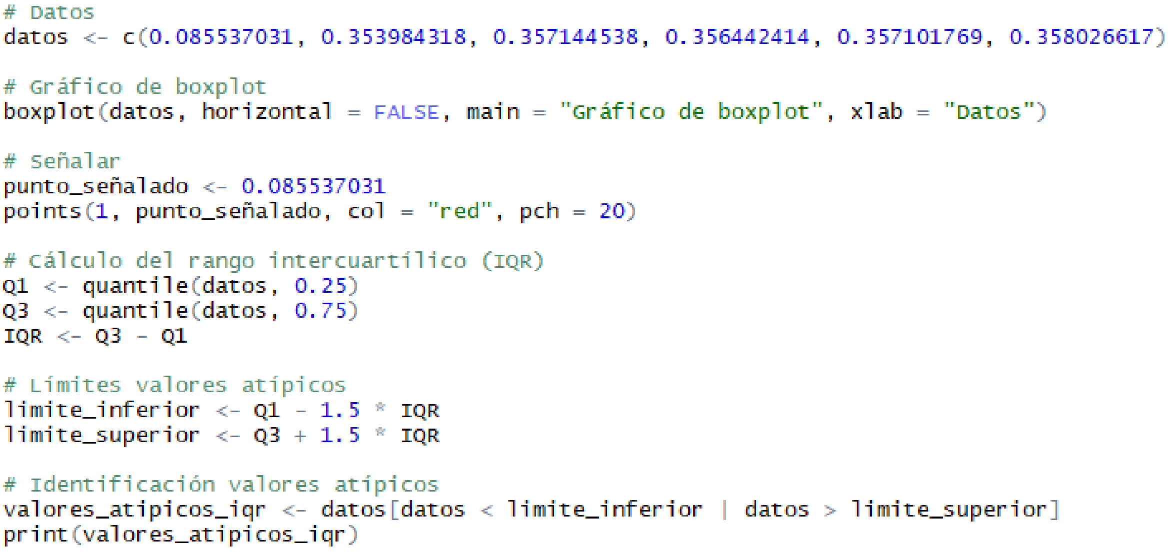


Figure 19: Homogeneity graph including all values

As the homogenization time increased, the molar fraction measurements stabilized, indicating that stratification had been resolved and the mixture had become uniform. To ensure selecting an optimal rolling time that guarantees the homogeneity of the mixture, a boxplot graph was created, and the interquartile range (IQR) was calculated. A boxplot graph is a graphical representation of the distribution of data. It helps to visualize the spread of data points, the central tendency, and any potential outliers. The boxplot displays the median, the first quartile, the third quartile, and the minimum and maximum values, excluding outliers. An outlier is a data point that is significantly different from the rest of the values in a dataset (Miller & Miller, 2000). The IQR which is the difference between the third quartile and the first quartile. This range captures the middle 50 % of the data, providing in this case, an indication of how much variation exists in the rolling times that result in a homogeneous mixture.

The R Studio software and the script in Figure 20 were used for these calculations. The script identified the first molar fraction value, 0.08554 mol/mol, as an outlier, and the boxplot graph (Figure 21) also indicated it as an outlier. After discarding this data point, the interquartile range was recalculated, and a new boxplot graph was generated, showing that the molar fraction corresponding to 0.35398 mol/mol is also an outlier (Figure 22).

The molar fraction value corresponding to 2 h of homogenization was discarded, and the outliers were checked again. By calculating the interquartile range, it was found that the last molar fraction value is an outlier; however, when examining the boxplot graph, it was observed that all molar fraction values are within the graph's range (Figure 23). The values obtained for 3, 4, 5 and 8 hours of rolling are showed in Figure 24.



```
# Datos
datos <- c(0.085537031, 0.353984318, 0.357144538, 0.356442414, 0.357101769, 0.358026617)

# Gráfico de boxplot
boxplot(datos, horizontal = FALSE, main = "Gráfico de boxplot", xlab = "Datos")

# Señalar
punto_señalado <- 0.085537031
points(1, punto_señalado, col = "red", pch = 20)

# Cálculo del rango intercuartílico (IQR)
Q1 <- quantile(datos, 0.25)
Q3 <- quantile(datos, 0.75)
IQR <- Q3 - Q1

# Límites valores atípicos
limite_inferior <- Q1 - 1.5 * IQR
limite_superior <- Q3 + 1.5 * IQR

# Identificación valores atípicos
valores_atipicos_iqr <- datos[datos < limite_inferior | datos > limite_superior]
print(valores_atipicos_iqr)
```

Figure 20: R Studio script for calculating the interquartile range

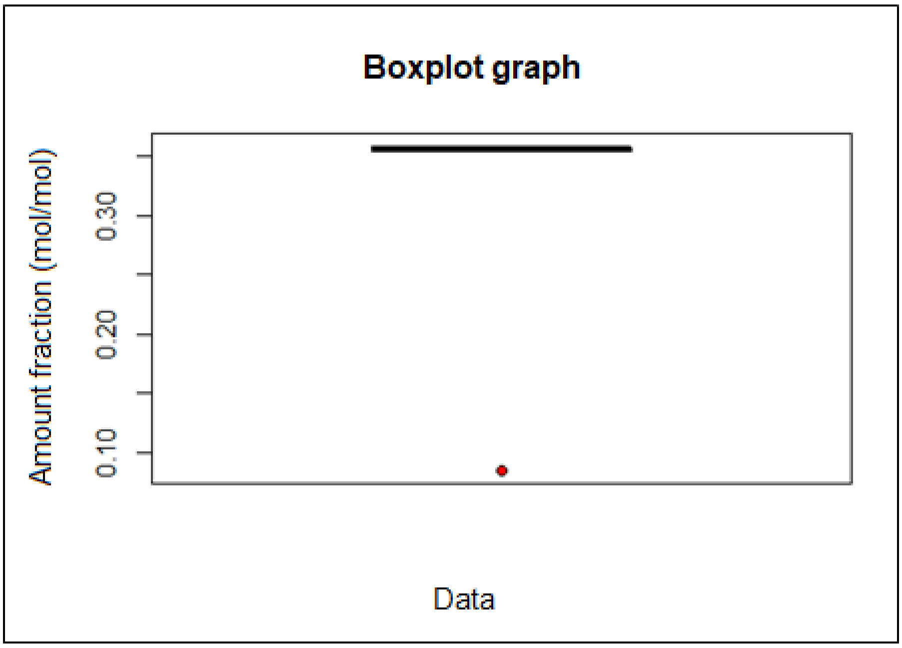
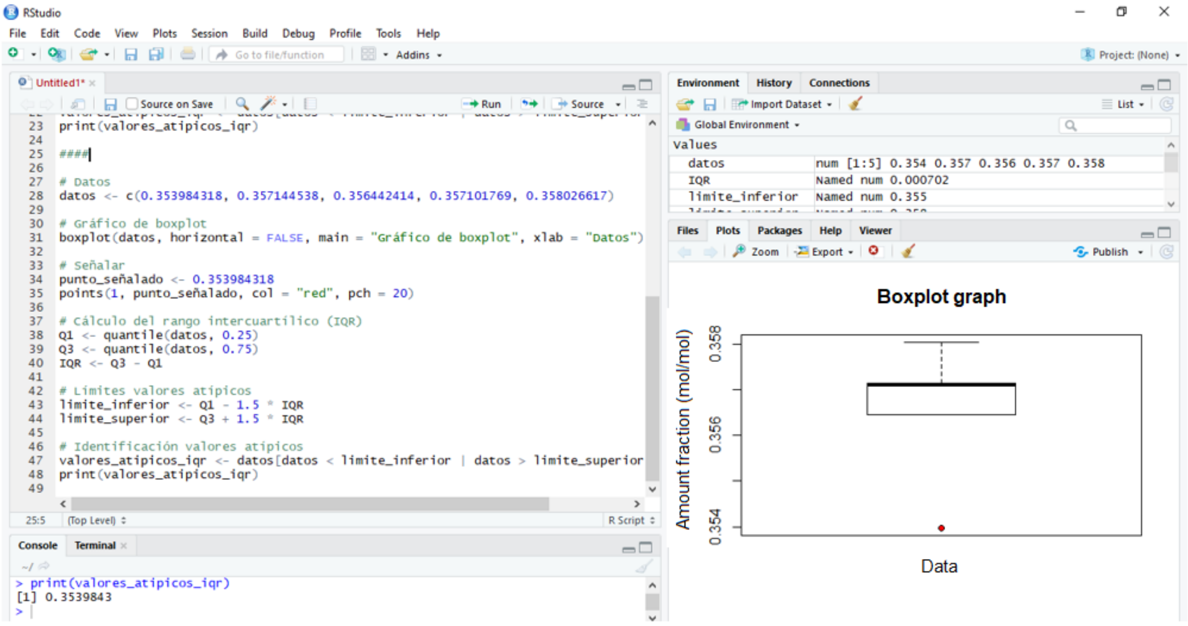


Figure 21: Boxplot graph for molar fraction values



```r
print(valores_atipicos_iqr)

####

# Datos
datos <- c(0.353984318, 0.357144538, 0.356442414, 0.357101769, 0.358026617)

# Gráfico de boxplot
boxplot(datos, horizontal = FALSE, main = "Gráfico de boxplot", xlab = "Datos")

# Señalar
punto_señalado <- 0.353984318
points(1, punto_señalado, col = "red", pch = 20)

# Cálculo del rango intercuartílico (IQR)
Q1 <- quantile(datos, 0.25)
Q3 <- quantile(datos, 0.75)
IQR <- Q3 - Q1

# Límites valores atípicos
limite_inferior <- Q1 - 1.5 * IQR
limite_superior <- Q3 + 1.5 * IQR

# Identificación valores atípicos
valores_atipicos_iqr <- datos[datos < limite_inferior | datos > limite_superior
print(valores_atipicos_iqr)
```

```
Values
  datos              num [1:5] 0.354 0.357 0.356 0.357 0.358
  IQR                Named num 0.000702
  limite_inferior    Named num 0.355
```

```
> print(valores_atipicos_iqr)
[1] 0.3539843
>
```

Figure 22: R studio script for outlier calculation, discarding the molar fraction value corresponding to the half hour of homogenization

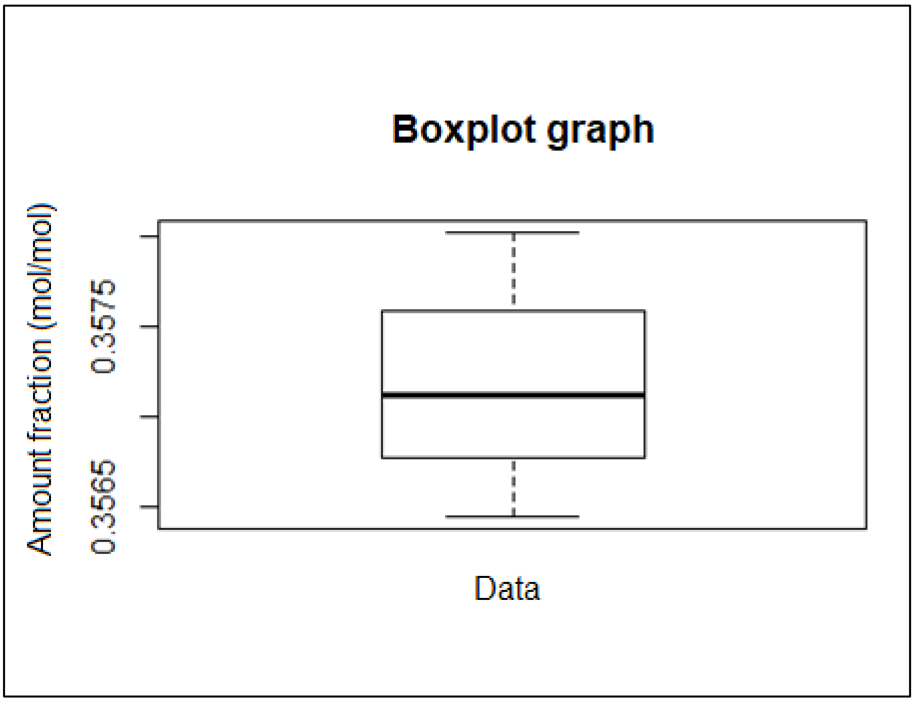


Figure 23: Boxplot graph eliminating the amount fraction values corresponding to the half hour and two hours of homogenization.

The difference in criteria may have been due to the limited amount of data available for analysis. The interquartile range is a measure of dispersion based on quartiles in a dataset and does not take into account the distribution of data beyond those quartiles.

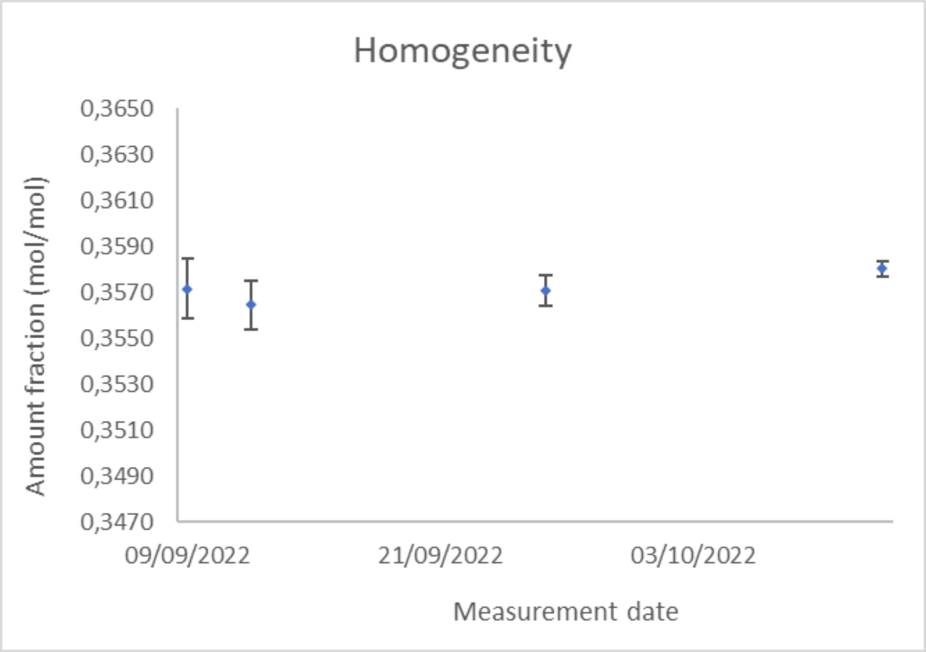


Figure 24: Values from 3, 4, 5 and 8 hours of rolling time.

To confirm that there is no statistically significant difference in the homogeneity of the mixture when rolling the gas cylinder for at least 3 hours, the normalised error was calculated between the molar fraction obtained at 3 hours and the different fractions obtained at other rolling times: 4, 5, and 8 hours. Equation 54 was used for this calculation, yielding the following normalised errors: $E_{n\,(3h-4h)} = 0{,}942$, $E_{n\,(3h-5h)} = 0{,}056$ and $E_{n\,(3h-8h)} = 0{,}847$. Since all the normalised errors were less than 1, it can be concluded that it is enough to roll the gas cylinder for 3 hours to obtain a homogeneous mixture.

$$E_{n\,(t1-t2)} = \frac{|x_{t1} - x_{t2}|}{\sqrt{U_{x,t1}^2 + U_{x,t2}^2}} \tag{54}$$

As stated before, both gas mixtures underwent proper homogenization through rolling and consist of gases with similar densities that do not condense. Consequently, inhomogeneity is not expected to impact measurement uncertainty, and no additional uncertainty component needs to be included for this factor.

4.6. Long term stability evaluation

To calculate the uncertainty associated with the instability of the CO in N_2 mixture, the data from table 24 was used, as shown in Figure 25. The evaluation of this uncertainty component (International Organization for Standardization [ISO Guide 35], 2017) should be based on the uncertainty associated with predicting the change in value between the first and last measurement. For a simple linear model applied to a classical stability study with multiple points in time, the uncertainty associated with the predicted change is given by Equation 55.

Table 24: Stability data for mixture of carbon monoxide in nitrogen, 1517 µmol/mol

Time (weeks)	Molar fraction (mol/mol)	Expanded uncertainty (mol/mol)
0	0.0015174	0.0000024
4	0.0015195	0.0000043
6	0.0015193	0.0000042
8	0.0015097	0.0000062
32	0.0015247	0.0000041
74	0.0015026	0.0000065

$$u_{est} = s(b_1)(t) \tag{55}$$

In Equation 55, $s(b_1)$ is the standard error of the slope, calculated as in Equation 56, and t is the time interval between the value assignment and the last monitoring.

$$s(b_1) = \frac{s}{\sqrt{\sum_{i=1}^{n}(x_i - \bar{x})^2}} \tag{56}$$

In the equation above, s represents the residual standard error, calculated as the sum square of the residuals, divided by the degrees of freedom, *n*-2 (Equation 57), where *n* is the number of data points.

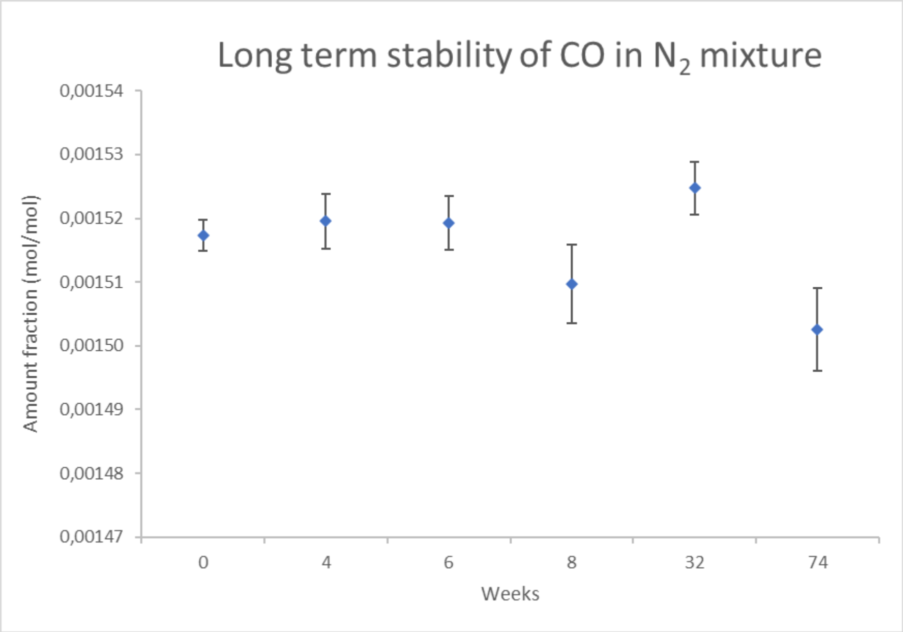


Figure 25: Values obtained from the long term stability evaluation of the CO in N_2 mixture

$$s^2 = \frac{\sum_{i=1}^{n}(y_i - b_0 - b_1 x_i)^2}{n-2} \tag{57}$$

Using these data, a standard uncertainty value of 8.4×10^{-6} mol/mol was obtained for the instability.

Since, at the time of preparing the CO_2 mixture, the only available data were the ones associated to the above mentioned characterized mixture of CO in N_2 and considering that a CO in N_2 mixture behaves similarly to a CO_2 in N_2 mixture in terms of reactivity under controlled temperature and humidity conditions, the calculated instability uncertainty for obtained for the mixture of CO in N_2 was applied to the CO_2 mixture. Therefore, a stability uncertainty of 0.0020 mol/mol was preliminary associated to the CO_2 in N_2 mixture.

The CO_2 gas mixture was then measured at 46, 64, and 111 weeks (Figure 26). The previously estimated stability uncertainty was re-evaluated using the data obtained from these measurements (Table 25), by applying Equation 58.

$$u(y_{k,stab}) = b_s \times t_s \tag{58}$$

This equation is less conservative than the one used before, since, instead of considering the standard error of the slope, it directly uses the value of the slope (International Organization for Standardization [ISO], 2015). In this way, the instability of the mixture is directly related to the drift rate of component in the mixture, in this case, CO_2.

Table 25: Stability data for mixture of carbon dioxide in nitrogen, 0.35 mol/mol

Time (weeks)	**Amount fraction (mol/mol)**	**Expanded uncertainty (mol/mol)**
0	0.35714	0.00066
46	0.35828	0.00072
64	0.35611	0.00070
111	0.35643	0.00072

Based on these data, the calculated uncertainty contribution for instability was 0.0010 mol/mol. This value is lower than the previously evaluated instability uncertainty of 0.0020 mol/mol. This reduction in instability uncertainty can be justified by considering that the initial estimate of 0.0020 mol/mol was based on data from a CO in N_2 mixture at a much lower amount fraction, 1500 µmol/mol. Applying this uncertainty value to a significantly higher amount fraction of CO_2 in N_2 mixture, 0.35 mol/mol, may have been an overly conservative approach.

In summary, the long term instability uncertainty, u_{stab}, is $8.4\text{x}10^{-6}$ mol/mol for the CO mixture and 0.0010 mol/mol for the CO_2 mixture.

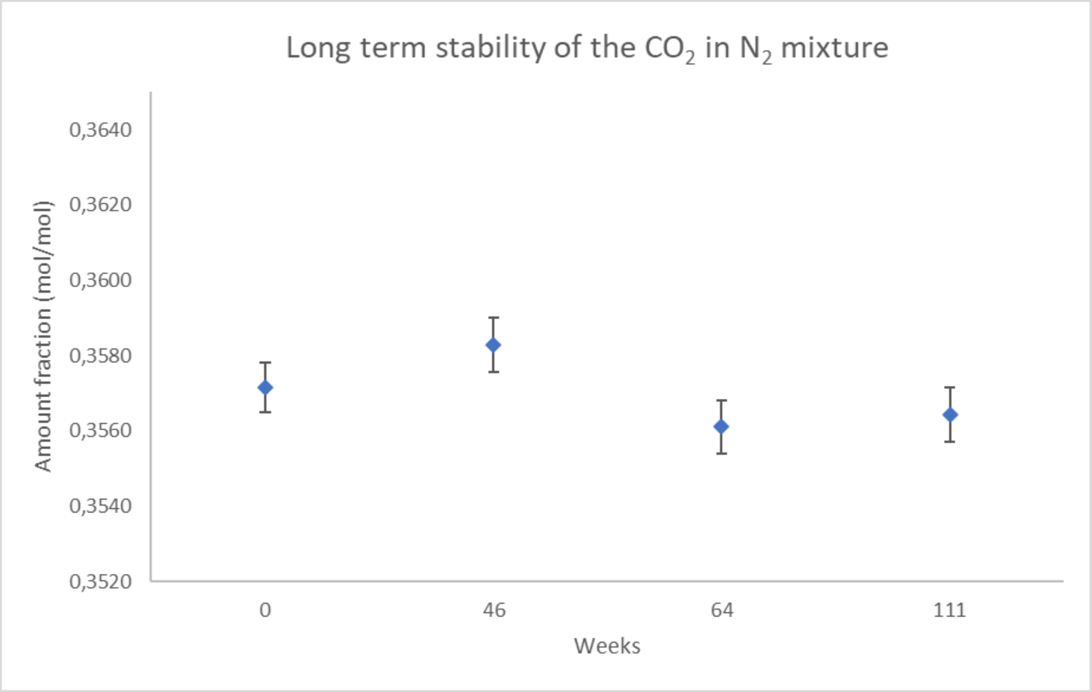


Figure 26: Values obtained from the long term stability evaluation of the CO$_2$ in N$_2$ mixture

4.7. Short-term stability evaluation

In order to carry out the short term stability evaluation, the cylinder containing a 0.35 mol/mol CO$_2$ in N$_2$ gas mixture was placed in a refrigerator at 4ºC for two weeks. After this period, it was returned to room temperature before analysis. The same procedure was followed after storing the cylinder in a stove at 35ºC for an additional two weeks.

In both cases, the measured values were compared to the initial value obtained before temperature exposure. The cylinder was analysed by GC-TCD calibrated with the PRMs purchased from VSL mentioned before (Table 22). The obtained data is presented in Table 26 and Figure 27.

Table 26: Short-term stability measurements

Measurement date	Temperature	Amount fraction (mol/mol)	Standard uncertainty (mol/mol)
02/09/2024	20 ºC	0.35603	0.00044
30/10/2024	4 ºC	0.35512	0.00045
03/12/2024	35 ºC	0.35508	0.00037

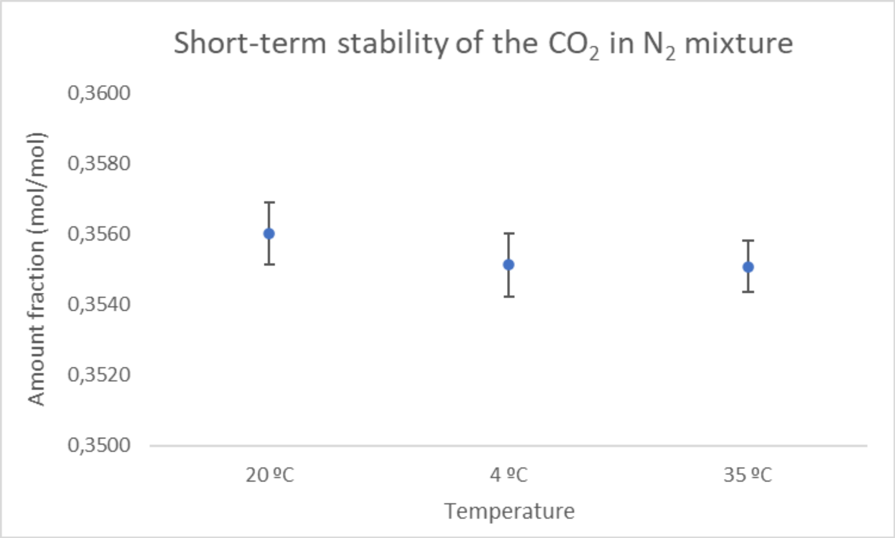


Figure 27: Short-term stability of the CO_2 in N_2 mixture

The results were analysed calculating the normalised error (Equation 54) between the analytical amount fraction value of the gravimetrically prepared PRM measured at 20ºC and the value obtained after the exposure to 4ºC for two weeks, a second normalised error between the measurements at 20ºC and those taken after exposure to 35ºC, and a third normalised error comparing the values from the post 4ºC and post 35ºC analyses. Results are shown in table 27.

Table 27: Comparison of the amount fractions after being exposed the sample at different temperatures

Amount fraction (x)	E_n
$x_{20ºC-4ºC}$	0.719
$x_{20ºC-35ºC}$	0.829
$x_{35ºC-4ºC}$	0.039

Results show that there are no significant differences between the values measured at the different temperatures. Therefore, it is concluded that the PRM of CO_2 in N_2 prepared can be exposed to a temperature range of 4°C to 35°C without altering its assigned value. As a result, there is no need to include a component of short-term instability in the certified value.

4.8. Certification of CRMs and PRM

4.8.1. Certification of CRMs

The expanded uncertainty for both mixtures was calculated using Equation 59, according to the ISO 33405 standard (International Organization for Standardization [ISO], 2024). The certification uncertainty was calculated using Equation 60 (95% confidence level, *k* = 2).

$$u_{CRM} = \sqrt{u_{char}^2 + u_{stab}^2} \tag{59}$$

$$U_{CRM} = 2 \times u_{CRM} \tag{60}$$

The relevant values for the mixture of CO are reported below:

- $y_{char,CO}$ = 1517.4 µmol/mol
- $u_{char,CO}$ = 2.4 µmol/mol
- $u_{stab,CO}$ = 8.4x10^{-6} mol/mol
- $U_{CRM,CO}$ = 17x10^{-6} mol/mol

Therefore, the certified value for the **CRM of CO in N_2 is 1517 ± 17 µmol/mol**.

The relevant values for the mixture of CO_2 are reported below:

$y_{char,CO2}$ = 0.35714 mol/mol

$u_{char,CO2}$ = 0.00033 mol/mol

$u_{stab,CO2}$ = 0.0010 mol/mol

$U_{CRM,CO2}$ = 0.0022 mol/mol

Therefore, the certified value for the **CRM of CO_2 in N_2 is 0.3571 ± 0.0022 mol/mol.**

The CRM of CO in N_2 has a validity period of 18 months from the time of certification and the CRM of CO_2 in N_2 has a validity period of 2 years and 2 months from the time of its certification.

Considering that the uncertainty of certified reference materials should be as low as possible, the objective was to ensure that both materials do not exceed 2% relative expanded uncertainty, a requirement that is met for both mixtures.

The relative expanded uncertainty for the CO in N_2 CRM is 1.2%, while for the CRM of CO_2 in N_2 is 0.61%.

4.8.2. Certification of PRM of CO_2 in N_2

The uncertainty component associated with the preparation was calculated using Equation 17. Hence, u_{prep} was determined as 0,0010 mol/mol. Uncertainty components are shown in table 28.

Table 28: Uncertainty components of CO_2 in N_2 PRM

Uncertainty component	**Value (mol/mol)**
$u_{grav}(y_{CO2})$	0.000012
$u_{stab}\ (y_{CO2})$	0.0010
$u_{prep}(y_{CO2})$	0,0010
$u_{verif}(y_{CO2})$	0.00044
$u_c(y_{CO2})$	0.00056
$U_c(y_{CO2})$, *k*=2	0.0011

Therefore, the certified value for the **PRM of CO_2 in N_2 is 0.3561 ± 0.0011 mol/mol.**

To confirm that the PRM is accurate and reliable, it must pass the verification from Equation 15, mentioned above.

$$\left|y_{k,prep} - y_{k,ver}\right| \leq 2\sqrt{u^2\left(y_{k,prep}\right) + u^2\left(y_{k,ver}\right)} \qquad (15)$$

The values obtained from the Equation are shown below:

$\left|y_{k,prep} - y_{k,ver}\right|$ = 0.000079 mol/mol

$2\sqrt{u^2\left(y_{k,prep}\right) + u^2\left(y_{k,ver}\right)}$ = 0.0022 mol/mol

According to the values from above, the verification criterion is passed and the gas mixture prepared is appropriate to be used as a PRM.

It is important to highlight that the certification uncertainty determined for the PRM of CO_2 in N_2 is half of the uncertainty obtained for the CRM of the same amount fraction gas mixture, 0.35 mol/mol. This is because PRMs have a higher level of traceability to the SI units, utilizing standards with the best uncertainty and the shortest traceability chain, whereas the value of a CRM is assigned by comparison against PRMs.

4.9. Bracketing method analysis

In addition to the analysis of the prepared CO and CO_2 reference gas mixtures using a calibration curve with VSL PRMs, other quantification techniques were also explored for comparison purposes. Among them, the bracketing method was applied to a different gas mixture to assess its accuracy and suitability for the verification of reference mixtures. The following section presents the results obtained using this technique, which offers an alternative to the calibration curve approach previously described.

Two PRMs of CO_2 in N_2 were selected to assign the value by analysis to a gas mixture using the bracketing method. To validate the process, the unknown gas mixture was also a PRM, allowing us to assess the accuracy of this estimation approach.

The selected PRMs are listed in Table 29 and were purchased from the National Metrology Institute of Brazil (INMETRO).

Table 29: PRMs used for bracketing

Reference mixture	**PRM code**	**Amount fraction, *x* (mol/mol)**	**Expanded uncertainty, *u(x)* (mol/mol)**
1	M692264	0.06000	0.00027
2	M692252	0.11916	0.00039
Sample	M692265	0.10010	0.00078

The two reference gas mixtures were measured five times, followed by five measurements of the sample mixture, and then five additional measurements of the reference gas mixtures, according to the sequence represented in Figure 11. The measurement data is presented in Table 30, where the averages and their standard uncertainties were calculated.

Table 30: Measurements obtained from the PRMs

Replicate	y_{PRM1} initial	y_{PRM2} initial	y_{sample}	Replicate	y_{PRM1} final	y_{PRM2} final
1	5.07	9.97	8.40	6	5.08	10.00
2	5.09	9.99	8.42	7	5.08	10.02
3	5.08	9.98	8.42	8	5.07	10.01
4	5.08	9.98	8.43	9	5.08	10.01
5	5.08	9.99	8.43	10	5.09	10.01
$\bar{y}$	5.08	9.98	8.42	$\bar{y}$	5.08	10.01
$u(\bar{y})$	0.0033	0.0035	0.0051	$u(\bar{y})$	0.0020	0.0036

Using Equation 20, and data from tables 29 and 30, the amount fraction, x_s, obtained from the sample gas mixture was 0.1003 mol/mol. To calculate its associated uncertainty is necessary to address the estimation of *u*(*Δ*).

1- *u*(*Δ*) using the performance evaluation of the measuring system:

Using the two PRMs for bracketing, the parameters of the best-fitting linear function were determined using the XLGENLINE v1.1 software. The results are presented in Table 31.

Table 31: Parameters of the linear regression

Parameter	Value
b_0	-0.00139045
b_1	0.01207736
$u(b_0)$	0.00035358
$u(b_1)$	4.98424E-05
$u(b_0, b_1)$	-1.66657E-08

For the performance evaluation of the measuring system, six PRMs of CO_2 in N_2 were measured within the analysis range, with six replicates for each, to assess the results. As demonstrated in the validation of the method of analysis of CO_2 in N_2 using GC-TCD/FID, the relationship within the measurement range of (0.002 to 0.40) mol/mol follows a second-order polynomial behaviour. The regression was performed using the XLGENLINE v1.1 software, confirming that the regression behaves as a second-order polynomial with a *Γ* value of 1.5 (Smith, 2010).

Since the XLGENLINE v1.1 software does not display the regression parameters for a second-order polynomial, only indicating whether it is a good fit, another software, Calibration Curves Computing (CCC Software), developed by the National Metrology Institute of Italy (INRIM), was used. This allowed for obtaining the results shown in Table 32 (Lecuna et al., 2020).

Table 32: Parameters from second-order polynomial

Parameter	Value	Parameter	Value	Parameter	Value
$ß_0$	3.79864E-05	$u(ß_0)$	1.61E-05	$u(ß_0,ß_1)$	-2.88E-11
$ß_1$	1.19897E-02	$u(ß_1)$	8.29E-06	$u(ß_0,ß_2)$	9.51E-13
$ß_2$	1.02502E-05	$u(ß_2)$	3.48E-07	$u(ß_1,ß_2)$	-2.09E-12

From the data in Tables 31 and 32, and using Equation 23, the values obtained for Δ are 0.0012 for the lower PRM, and 0.0016 for the upper PRM. Therefore, the value of $u(\Delta)$ is 0.0016.

To ensure that the CCC software was being used correctly and to perform a validation check, the values from *clause D.4 Performance evaluation* of the ISO 12963 standard were entered, and the software's output was analysed. The results are presented in Table 33. It was confirmed that the values are similar and within the same order of magnitude. The minor differences between the results in the standard and those generated by the CCC software are likely due to the different assumptions and mathematical approaches that each method uses to get a result.

Additionally, since XLGENLINE for second-order polynomial regressions only indicates whether the fit is acceptable or not, a comparison was made between the Γ value provided by XLGENLINE using the data from the standard, the Γ value obtained from the CCC software, and the Γ value listed in the standard. The Γ values were as follows: 1.49 in the standard, 1.56 from CCC, and 1.46 from XLGENLINE. These results suggest that the fit is comparable across the different software.

Table 33: Comparison between data from the ISO 12963 standard and CCC software

	ISO 12963:2017 standard	**CCC Software**
a	-3.7660E-03	-3.7933E-03
b	2.7387E-04	2.7389E-04
c	2.4027E-10	2.3910E-10
u(a)	1.7900E-03	1.7691E-03
u(b)	1.4270E-06	1.4278E-06
u(c)	6.2980E-11	6.3188E-11
c (a,b)	-2.0070E-09	-1.9961E-10
c (a,c)	7.6110E-14	7.5923E-14
c (b,c)	-8.0640E-17	-8.0908E-17

Below in this report, the values obtained from XLGENLINE and CCC Software are compared.

2- $u(\Delta)$ using contribution based on ISO/IEC Guide 98-3:2008

Using the same values from tables 31 and 32, $F(y_1)$, $F(y_2)$, $G(y_1)$, $G(y_2)$, $H(y_1)$ and $H(y_2)$ were calculated, using the initial and final values of y. The results are shown in Table 34.

Table 34: Values of the different functions for the calculation of u(Δ)

Initial values					
$F(y_1)$	$F(y_2)$	$G(y_1)$	$G(y_2)$	$H(y_1)$	$H(y_2)$
0.0066	0.0133	4.53E-08	9.06E-08	3.17E-07	4.26E-07
Final values					
$F(y_1)$	$F(y_2)$	$G(y_1)$	$G(y_2)$	$H(y_1)$	$H(y_2)$
0.0066	0.0133	4.62E-08	9.27E-08	3.17E-07	4.27E-07

From the table 34 it is possible to calculate $\bar{\delta}$=0.0014, $u(\bar{\delta})$=0.00018 and $u(\Delta)$=0.0014. The results from the initial and final values are the same. Therefore, $u(\Delta)$ using this method is 0.0014.

Both approaches yielded similar results; however, the latter is considered more accurate, so this value is chosen for the uncertainty evaluation.

The expressions for the sensitivity coefficients were calculated and expressed in table 35.

Table 35: Expression of the sensitivity coefficients for the uncertainty evaluation

Expression	**Value**
$\left(\frac{\partial x_s}{\partial y_s}\right)$	0.01207736
$\left(\frac{\partial x_s}{\partial y_{r2}}\right)$	-0.00822389
$\left(\frac{\partial x_s}{\partial y_{r1}}\right)$	-0.00385348
$\left(\frac{\partial x_s}{\partial x_{r2}}\right)$	0.68093385
$\left(\frac{\partial x_s}{\partial x_{r1}}\right)$	0.31906615

The uncertainty of the mixture, calculated using Equation 29, was then assessed as 0.0014 mol/mol.

To confirm whether the values chosen for value assignment by bracketing, i.e., using only the initial value, are appropriate, it is necessary to verify that there is no instrumental drift in the measurement. For this purpose, the calculated data for $x_{s,b}$, $x_{s,e}$, $u(x_{s,b})$ and $u(x_{s,e})$, as shown in Table 36, are considered.

Table 36: Amount fractions and standard uncertainties calculated from the beginning of the analysis and at the end

$x_{s,b}$ (mol/mol)	0.1003	$u(x_{s,b})$ (mol/mol)	0.0014
$x_{s,e}$(mol/mol)	0.1001	$u(x_{s,e})$ (mol/mol)	0.0014

The value obtained from Equation 22 was 0.058, which is less than 1, confirming that there is no drift. Therefore, the values used to assess the amount fraction and its uncertainties should be those calculated using the data from the beginning of the analysis, $x_{s,b}$ and $u(x_{s,b})$. So, the determined value using bracketing was: 0.1003 ± 0.0028 mol/mol (k = 2).

Finally, since the sample mixture used was a PRM with a certified value of 0.10010 ± 0.00078 mol/mol, a normalised error (Equation 54) analysis was performed using the certified value and the value calculated from the bracketing method, 0.1003 ± 0.0028 mol/mol (with k = 2). The obtained E_n value was 0.064, confirming that the bracketing method is accurate.

4.10. SPEM method analysis

As with the bracketing method, the SPEM technique was also applied to a different gas mixture to evaluate its potential for verifying reference gas amount fractions. The following section presents the results obtained using this method.

For the SPEM analysis, five replicates of a PRM of CO_2 in N_2 were measured, and their mean response and standard uncertainty were calculated. After that, five replicates of a gas mixture of CO_2 in N_2 were measured, and the same values were determined. The chosen gas mixture was also a PRM, allowing for accuracy verification of the method. Additionally, five more replicates of the PRM used as the reference gas mixture were measured after the sample mixture to check system stability.
The certified value of the PRM used as reference was 0.09996 ± 0.00010.

Results from the analysis are shown in table 37.

Table 37: Amount fractions for five replicates of the initial and final PRM measurements, and of the sample mixture measurement, obtained using the bracketing method

Replicant	$y_{PRM, I}$ **(mol/mol)**	$y_{PRM, f}$ **(mol/mol)**	y_{sample} **(mol/mol)**
1	8.52	8.41	8.39
2	8.42	8.43	8.41
3	8.40	8.44	8.41
4	8.39	8.43	8.42
5	8.39	8.43	8.42

Once the measurements are done, it is necessary to check if the reference gas mixture and the sample mixture are indistinguishable. For that, Equation 30 was used.

Since the ISO 12963 standard states that the criterion should be fulfilled for the measurements done before and after the sample measurement, the relevant results are shown in table 38.

Table 38: Results obtained from PRMs and sample

	PRM $_{\text{initial}}$	**PRM** $_{\text{final}}$	**Sample**
y_{prom} (mol/mol)	8,42	8,43	8,41
$u(y_{prom})$ (mol/mol)	0.023	0.0046	0.0068
Criterion value	0.31	1.0	-

Since criterion was fulfilled for measurements done before and after the sample measurement, the determination of the amount fraction of the sample mixture was performed using formula 31 and its uncertainty using Equation 32.

The determined value using SPEM for the gas mixture was 0.09978 ± 0.00058 ($k = 2$).

Since the sample mixture used was a PRM with a certified value of 0.10010 ± 0.00078 mol/mol, a normalised error (Equation 54) analysis was performed using the certified value and the value calculated from SPEM. The obtained E_n value was 0.33, confirming that the SPEM method is accurate.

4.11. Calibration curve analysis

To compare the uncertainty associated with different verification methods, a PRM of CO_2 in N_2 purchased from INMETRO was analysed using the calibration curve technique. This PRM is the same that was analysed using SPEM and bracketing methods, mentioned above. It is important to clarify that this PRM is not the one prepared in this work, but an independent standard used here as a sample to assess the performance of the different verification methods.

The PRMs used for the calibration curve were all purchased from VSL, while the PRM used as a sample was purchased from INMETRO, thus having two independent traceability sources for the standards and the sample. The amount fractions and expanded uncertainties of the PRMs from VSL are shown in Table 39.

Table 39: PRMs of CO_2 in N_2 from VSL used for the calibration curve

PRM code	Amount fraction (mol/mol)	Expanded uncertainty, k = 2 (mol/mol)
D562955	0.05003	0.00005
D562939	0.09996	0.00010
D563094	0.20006	0.00020
D563020	0.30042	0.00030
5603670	0.40000	0.00040

The type of fit and polynomial used for this regression was the same as the one validated for CO_2 in N_2 within this amount fraction range: GLS and a second-degree polynomial. The Γ obtained was 1.88 and the calibration curve is shown in Figure 28.

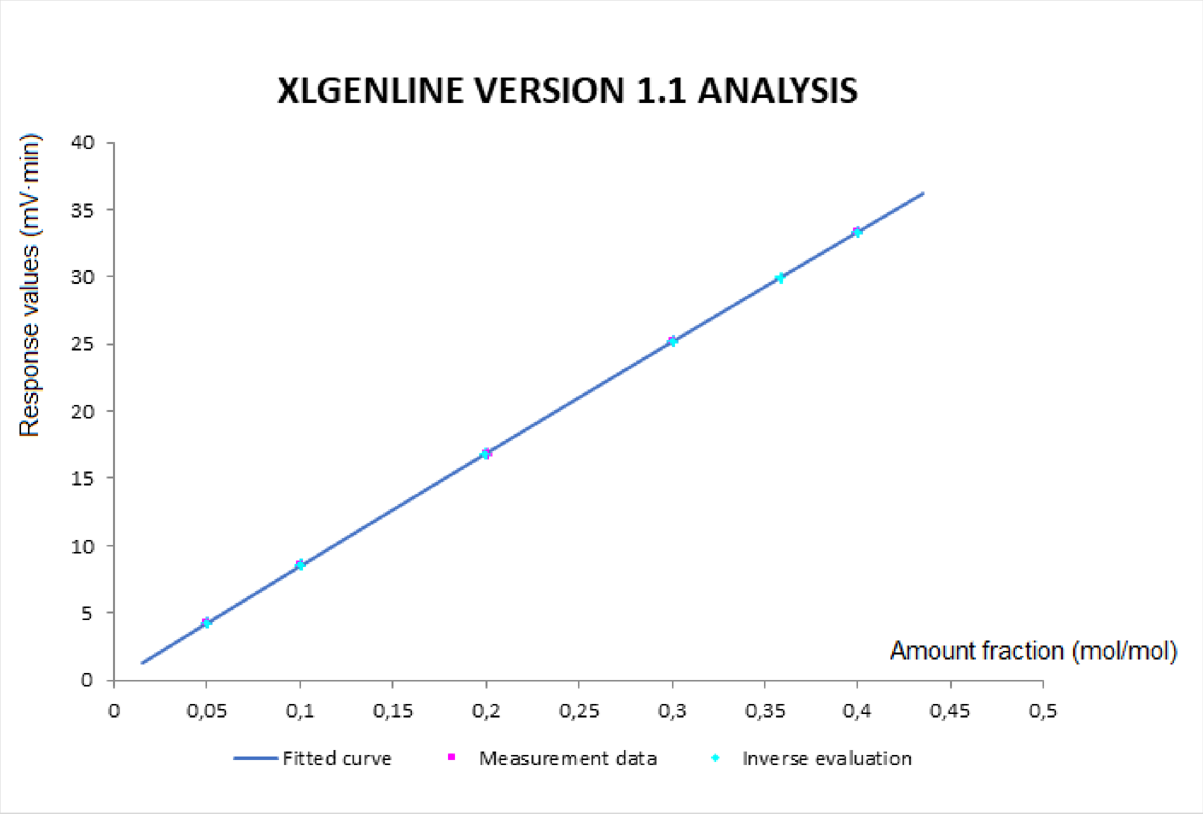


Figure 28: Calibration curve obtained for the PRMs of VSL and the sample cylinder

The amount fraction with its standard uncertainty determined by the calibration curve method was 0.10021 ± 0.00018 mol/mol (k = 2).

Given the fact that the sample mixture used was a PRM with a certified value of 0.10010 ± 0.00078 mol/mol, a normalised error analysis was performed using the certified value and the value calculated from the calibration curve method. The resulting E_n value of 0.137 confirms the accuracy of the measurement.

Table 40 shows the different values obtained from the characterization of the same PRM using three types of calibration methods.

Table 40: Results from different methods of characterization

Method	**Amount fraction (mol/mol)**	**Expanded uncertainty, *k* = 2 (mol/mol)**
SPEM	0.09978	0.00058
Bracketing	0.1003	0.0028
Calibration curve	0.10021	0.00018

When comparing the expanded uncertainties determined for each of the methods used, it is observed that the calibration curve method yields the lowest uncertainty (Figure 29). This is advantageous, as additional uncertainty components, such as those related to instability and preparation will be subsequently added. These additional components will further increase the final uncertainty of the reference mixture. It is important to remember that in metrology, minimizing uncertainty is essential to ensure that appropriate values are maintained throughout the traceability chain. For this reason, the calibration curve method is an effective approach for achieving lower uncertainties in gas analysis.

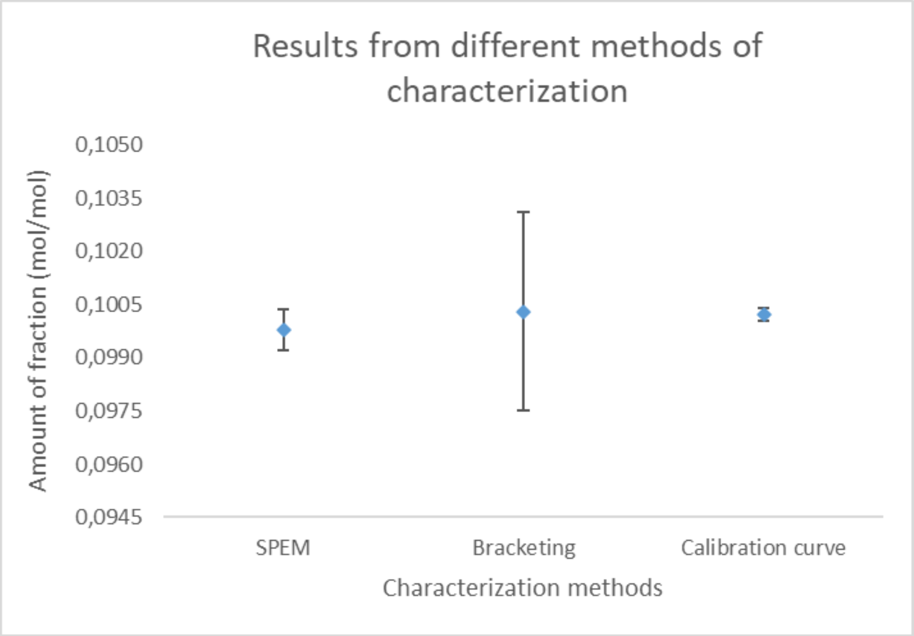


Figure 29: Graph of the values obtained by the different methods of characterization

However, since gases typically exhibit linear regressions, this method requires the use of at least five reference gas mixtures. This involves obtaining five independent standards and longer measurement time. In this regard, the SPEM method would be the best option, as it allows for low uncertainties. However, its application requires a reference mixture that is nearly identical to the sample being calibrated, strictly meeting the criterion reported in Equation 22. If a suitable standard is available, this method is ideal, as it significantly reduces measurement time by measuring just two gas cylinders and it only requires one single standard. Otherwise, an additional characterization method would be necessary, resulting in extra time and the use of additional resources.

The bracketing method is effective when reference standards are very close in amount fraction to the sample being characterized. However, if the analyte being calibrated is already well studied and known to deviate from a linear relationship, this method results in higher uncertainty compared to other approaches. Therefore, it is advisable only when a well-established linear relationship exists within a measurement range that covers the sample amount fraction.

In conclusion, selecting the most suitable calibration method for gas mixtures depends on the available reference standards, the time available for analysis, and the intended purpose of the calibration. It is essential to assess whether achieving the lowest possible uncertainty is necessary or if a slightly higher uncertainty remains acceptable for the client or end user.

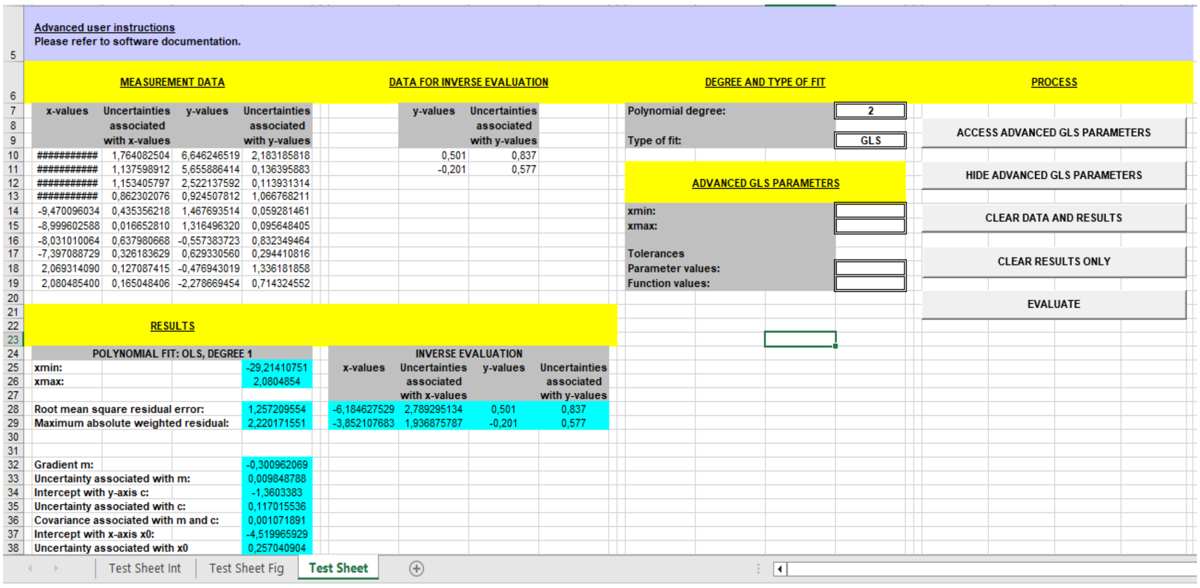
4.12. Comparison of results between XLGENLINE and CCC software

For the calculation of amount fractions using calibration curves, the software most commonly used was XLGENLINE v1.1. However, another software was used to validate the calculations made by the first one.

XLGENLINE (Figure 30) is very user friendly as it works using an Excel spreadsheet. It also allows for the inverse evaluation of results, uses polynomial regressions of order 2 or 3, and employs GLS. One of the most convenient aspects of the software is that it provides the result of the sample to be interpolated directly within the function, without the need to solve for the regression that best fits. However, when dealing with nonlinear regressions, it does not give you the parameters of the function and it only provides the Root Mean Square Residual Error and the Maximum Absolute Weighted Residual, which means that you can only determine whether the fit is good or not. On the other hand, it provides the regression plot including the measured values, the fitted data and the inverse function.

In contrast, CCC Software (Figure 31), which uses Weighted Total Least Squares (WTLS), optimizes the estimates of both x and y, providing more accurate results in cases where uncertainties are present in both variables. WTLS is more advanced as it iterates through optimization processes to minimize errors in both variables, unlike GLS, which focuses primarily on adjusting weights based on uncertainty in y. Therefore, CCC is better suited for situations where the uncertainty in x is significant, as it allows for more precise estimations by adjusting both x and y values to minimize the total error (Lecuna et al., 2020).

One of the advantages of CCC Software is that it always provides the parameters of the fitted function, including the values of variances and covariances. However, while CCC allows for performing the regression, it does not directly allow you to input the value to be interpolated into the regression. This means that the interpolation step must be done manually, which adds extra effort compared to XLGENLINE, where the interpolation can be directly calculated, and the result is obtained in the same software.



Advanced user instructions
Please refer to software documentation.

MEASUREMENT DATA

x-values	Uncertainties associated with x-values	y-values	Uncertainties associated with y-values
###########	1,764082504	6,646246519	2,183185818
###########	1,137598912	5,655886414	0,136395883
###########	1,153405797	2,522137592	0,113931314
###########	0,862302076	0,924507812	1,066768211
-9,470096034	0,435356218	1,467693514	0,059281461
-8,999602588	0,016652810	1,316496320	0,095648405
-8,031010064	0,637980668	-0,557383723	0,832349464
-7,397088729	0,326183629	0,629330560	0,294410816
2,069314090	0,127087415	-0,476943019	1,336181858
2,080485400	0,165048406	-2,278669454	0,714324552

DATA FOR INVERSE EVALUATION

y-values	Uncertainties associated with y-values
0,501	0,837
-0,201	0,577

DEGREE AND TYPE OF FIT

Polynomial degree: 2
Type of fit: GLS

ADVANCED GLS PARAMETERS

xmin:
xmax:
Tolerances
Parameter values:
Function values:

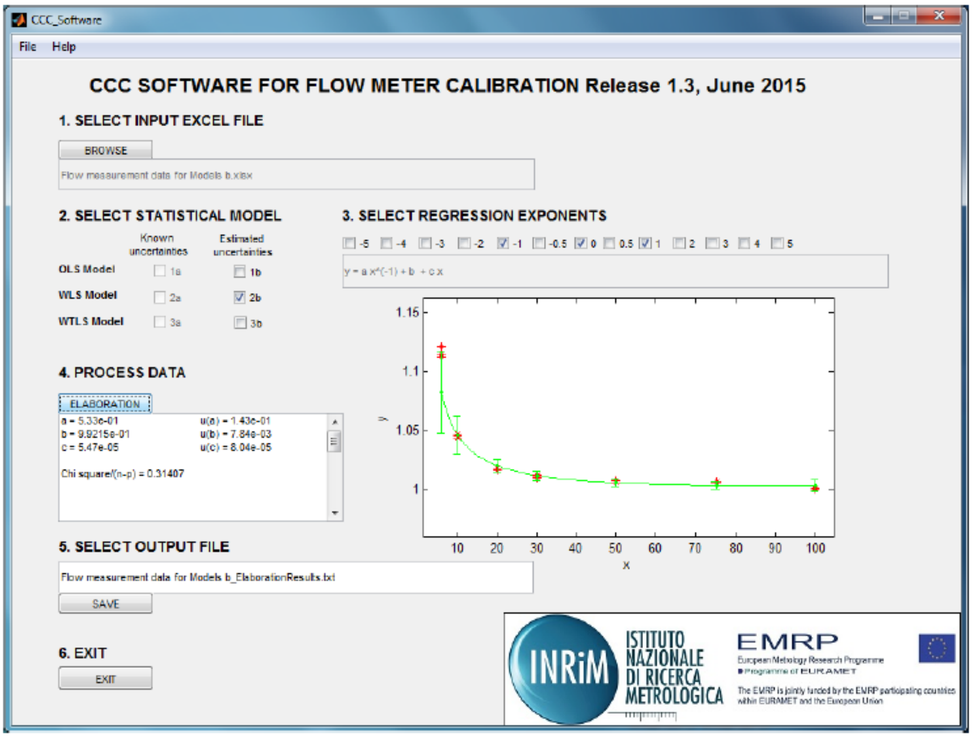
RESULTS

POLYNOMIAL FIT: OLS, DEGREE 1	
xmin:	-29,21410751
xmax:	2,0804854
Root mean square residual error:	1,257209554
Maximum absolute weighted residual:	2,220171551
Gradient m:	-0,300962069
Uncertainty associated with m:	0,009848788
Intercept with y-axis c:	-1,3603383
Uncertainty associated with c:	0,117015536
Covariance associated with m and c:	0,001071891
Intercept with x-axis x0:	-4,519965929
Uncertainty associated with x0	0,257040904

INVERSE EVALUATION

x-values	Uncertainties associated with x-values	y-values	Uncertainties associated with y-values
-6,184627529	2,789295134	0,501	0,837
-3,852107683	1,936875787	-0,201	0,577

Figure 30: XLGENLINE v1.1 software



CCC SOFTWARE FOR FLOW METER CALIBRATION Release 1.3, June 2015

1. SELECT INPUT EXCEL FILE

Flow measurement data for Models b.xlsx

2. SELECT STATISTICAL MODEL

	Known uncertainties	Estimated uncertainties
OLS Model	☐ 1a	☐ 1b
WLS Model	☐ 2a	☑ 2b
WTLS Model	☐ 3a	☐ 3b

3. SELECT REGRESSION EXPONENTS

☐ -5 ☐ -4 ☐ -3 ☐ -2 ☑ -1 ☐ -0.5 ☑ 0 ☐ 0.5 ☑ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5

y = a x^(-1) + b + c x

4. PROCESS DATA

```
a = 5.33e-01                u(a) = 1.43e-01
b = 9.9215e-01              u(b) = 7.84e-03
c = 5.47e-05                u(c) = 8.04e-05

Chi square/(n-p) = 0.31407
```

5. SELECT OUTPUT FILE

Flow measurement data for Models b_ElaborationResults.txt

6. EXIT

Figure 31: CCC Software v.1.3

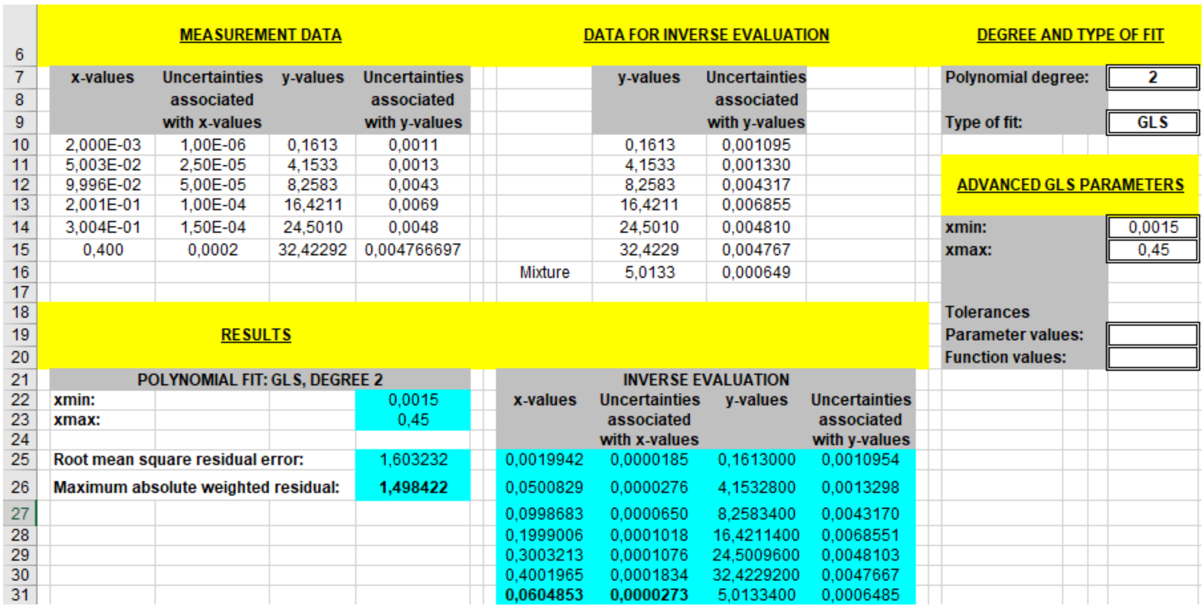
To compare the results obtained from both software programs, the data from Table 41 was used. This data was derived from measurements of different PRMs of CO_2 in N_2 and a sample mixture of the same gases with an unknown amount fraction and associated uncertainty.

Table 41: Data from a measurement of a sample mixture of CO_2 in N_2 along with 6 PRMs

Amount fraction (mol/mol)	Standard uncertainty of the amount fraction (mol/mol)	Measured response values expressed as integrated area (pA*min)	Standard uncertainty of the response (pA*min)
2.000E-03	1.00E-06	0.1613	0.0011
5.003E-02	2.50E-05	4.1533	0.0013
9.996E-02	5.00E-05	8.2583	0.0043
2.001E-01	1.00E-04	16.4211	0.0069
3.004E-01	1.50E-04	24.5010	0.0048
0.400	0.0002	32.4229	0.0047

The unknown sample was measured along whit the PRMs in a GC-TCD and the obtained area was 5,0133 mV*min with a standard uncertainty 0,000648537 mV*min.

Using XLGENLINE, the results were 0.060485 ± 0.000055 mol/mol (*k*=2), as highlighted in bold at the bottom of the inverse evaluation output (Figure 32), below the x-values and their associated uncertainty. The uncertainty shown in the table represents the standard uncertainty. As shown in the figure, the residual sum of weighted squared deviations, which reflects the overall discrepancy between the observed data and the fitted regression line, accounting for measurement uncertainty, remains below 2. This value (1.498), also highlighted in bold, indicates a strong agreement between the model and the data, supporting reliable quantification throughout the calibration range. A second-degree polynomial was selected for the fit, based on prior validation results for CO_2 indicating it as the most appropriate model.



MEASUREMENT DATA

x-values	Uncertainties associated with x-values	y-values	Uncertainties associated with y-values
2,000E-03	1,00E-06	0,1613	0,0011
5,003E-02	2,50E-05	4,1533	0,0013
9,996E-02	5,00E-05	8,2583	0,0043
2,001E-01	1,00E-04	16,4211	0,0069
3,004E-01	1,50E-04	24,5010	0,0048
0,400	0,0002	32,42292	0,004766697

DATA FOR INVERSE EVALUATION

	y-values	Uncertainties associated with y-values
	0,1613	0,001095
	4,1533	0,001330
	8,2583	0,004317
	16,4211	0,006855
	24,5010	0,004810
	32,4229	0,004767
Mixture	5,0133	0,000649

DEGREE AND TYPE OF FIT

Polynomial degree:	2
Type of fit:	GLS

ADVANCED GLS PARAMETERS

xmin:	0,0015
xmax:	0,45
Tolerances	
Parameter values:	
Function values:	

RESULTS

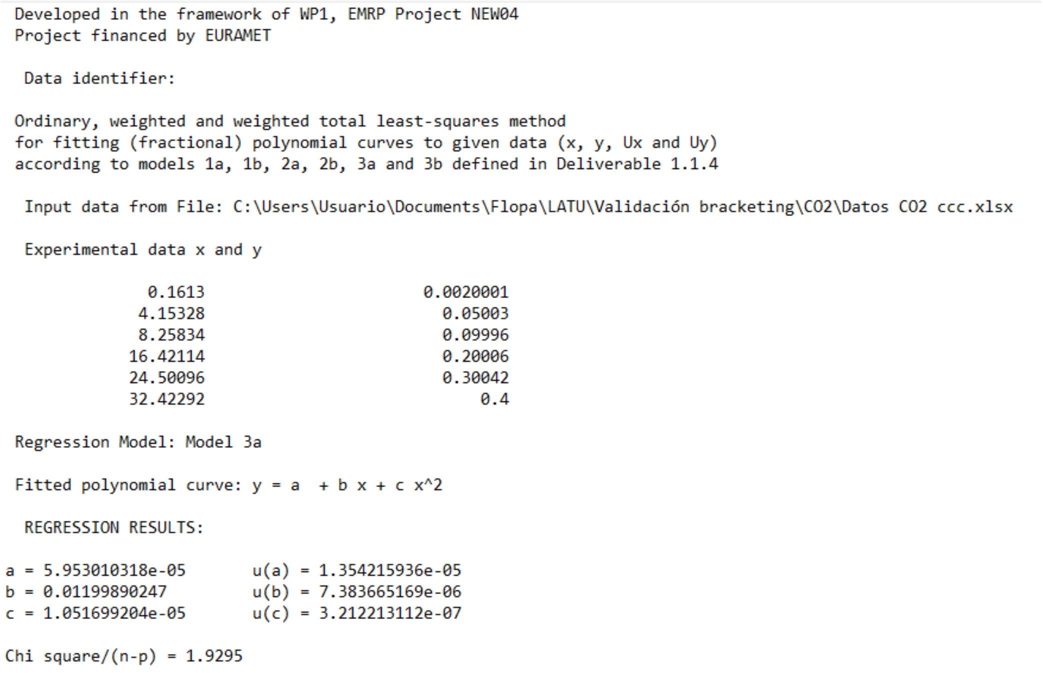
POLYNOMIAL FIT: GLS, DEGREE 2	
xmin:	0,0015
xmax:	0,45
Root mean square residual error:	1,603232
Maximum absolute weighted residual:	**1,498422**

INVERSE EVALUATION

x-values	Uncertainties associated with x-values	y-values	Uncertainties associated with y-values
0,0019942	0,0000185	0,1613000	0,0010954
0,0500829	0,0000276	4,1532800	0,0013298
0,0998683	0,0000650	8,2583400	0,0043170
0,1999006	0,0001018	16,4211400	0,0068551
0,3003213	0,0001076	24,5009600	0,0048103
0,4001965	0,0001834	32,4229200	0,0047667
0,0604853	**0,0000273**	5,0133400	0,0006485

Figure 32: Results obtained from interpolating a mixture in XLGENLINE

Using the CCC software, the regression that best fits the data was determined, as shown in Figure 33 and its graph in Figure 34. With these values and an R script, the amount fraction and the uncertainty of the unknown mixture was calculated. The script from the Figure 35 was used.



```
 Developed in the framework of WP1, EMRP Project NEW04
 Project financed by EURAMET

  Data identifier:

 Ordinary, weighted and weighted total least-squares method
 for fitting (fractional) polynomial curves to given data (x, y, Ux and Uy)
 according to models 1a, 1b, 2a, 2b, 3a and 3b defined in Deliverable 1.1.4

  Input data from File: C:\Users\Usuario\Documents\Flopa\LATU\Validación bracketing\CO2\Datos CO2 ccc.xlsx

  Experimental data x and y

              0.1613                    0.0020001
             4.15328                      0.05003
             8.25834                      0.09996
            16.42114                      0.20006
            24.50096                      0.30042
            32.42292                          0.4

 Regression Model: Model 3a

 Fitted polynomial curve: y = a  + b x + c x^2

  REGRESSION RESULTS:

a = 5.953010318e-05        u(a) = 1.354215936e-05
b = 0.01199890247          u(b) = 7.383665169e-06
c = 1.051699204e-05        u(c) = 3.212213112e-07

Chi square/(n-p) = 1.9295
```

Figure 33: Regression results using CCC software

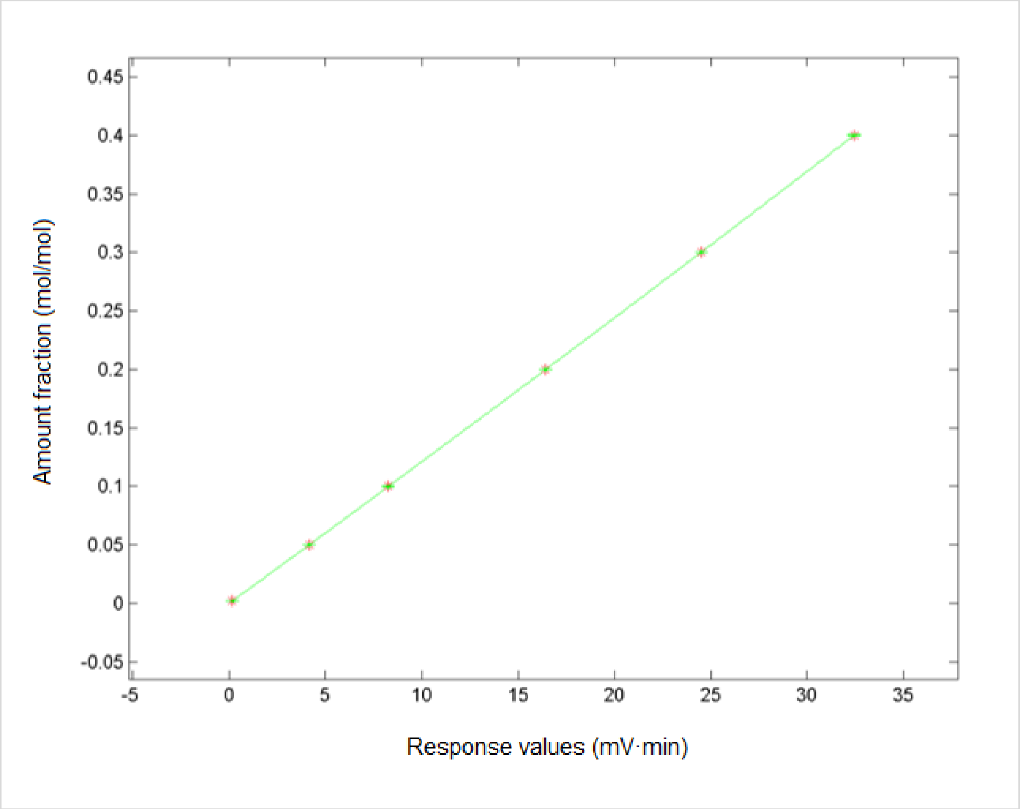
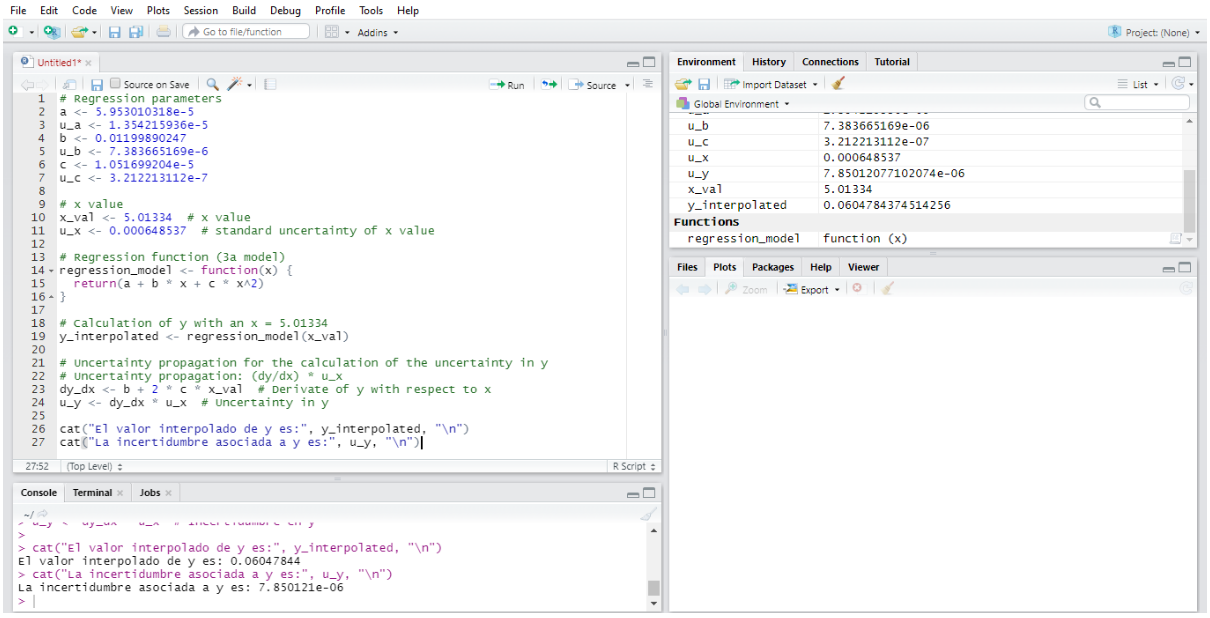


Figure 34: Calibration curve using CCC software



```r
# Regression parameters
a <- 5.953010318e-5
u_a <- 1.354215936e-5
b <- 0.01199890247
u_b <- 7.383665169e-6
c <- 1.051699204e-5
u_c <- 3.212213112e-7

# x value
x_val <- 5.01334  # x value
u_x <- 0.000648537  # standard uncertainty of x value

# Regression function (3a model)
regression_model <- function(x) {
  return(a + b * x + c * x^2)
}

# Calculation of y with an x = 5.01334
y_interpolated <- regression_model(x_val)

# Uncertainty propagation for the calculation of the uncertainty in y
# Uncertainty propagation: (dy/dx) * u_x
dy_dx <- b + 2 * c * x_val  # Derivate of y with respect to x
u_y <- dy_dx * u_x  # Uncertainty in y

cat("El valor interpolado de y es:", y_interpolated, "\n")
cat("La incertidumbre asociada a y es:", u_y, "\n")
```

```
>
> cat("El valor interpolado de y es:", y_interpolated, "\n")
El valor interpolado de y es: 0.06047844
> cat("La incertidumbre asociada a y es:", u_y, "\n")
La incertidumbre asociada a y es: 7.850121e-06
>
```

```
u_b              7.383665169e-06
u_c              3.212213112e-07
u_x              0.000648537
u_y              7.85012077102074e-06
x_val            5.01334
y_interpolated   0.0604784374514256
Functions
regression_model function (x)
```

Figure 35: R script for the determination of the amount fraction and uncertainty of the unknown sample

The calculated value using CCC software and the R studio script was 0.06047844 mol/mol with a standard uncertainty of 7.850121×10^{-6}.

Table 42 shows values obtained from both software.

Table 42: Software comparison

Software	Amount fraction (mol/mol)	Standard uncertainty (mol/mol)	Expanded uncertainty, *k*=2 (mol/mol)
XLGENLINE	0.060485	2.7289×10^{-5}	0,000055
CCC Software + R studio	0.060478	7.85×10^{-6}	0,000016

To determine whether the results show statistically insignificant differences, a normalised error was calculated between the values obtained using both software. The calculated En value was 0.12, indicating that the results obtained with both software are equivalent within their uncertainties.

CHAPTER 5. **CONCLUSIONS**

The system for vacuuming cylinders and introducing gases into aluminium cylinders was successfully developed, achieving precise control over both mass and pressure, which is crucial for ensuring precise preparation and accurate reference gas mixtures,

The reference gas mixtures, CRMs and PRM, were successfully developed with metrological traceability, ensuring their accuracy and enabling international comparability of measurements within the country. It is important to highlight that the PRMs developed exhibit significantly lower uncertainty than CRM of the same amount fraction and analyte, confirming that they are superior in the metrological chain for achieving more precise and reliable measurements.

The quantification methods using GC-TCD/FID were successfully developed and validated for value assignment and verification of the gas mixtures, ensuring reliable and consistent results in gas mixture analysis.

The calibration methods used for the gas mixtures (calibration curve, bracketing, and SPEM) were found to be effective, each with its advantages depending on the context. The choice of the most suitable calibration method depends on the available reference gas mixtures, the time available for determination, and the intended purpose of the calibration.

The software used for uncertainty determination were validated against each other, demonstrating that both XLGENLINE and CCC Software provide reliable and accurate results.

CHAPTER 6. PERSPECTIVES

The development of PRMs for gas mixtures holds significant promise for advancing measurement capabilities across various sectors, particularly in environmental monitoring, energy production, and industrial processes. A key aspect of these developments is achieving greater precision and direct traceability in measurements, especially for gases like CO. While the production of CO PRMs could not be achieved in this particular work due to the unavailability of pure CO within the required timeframe, developing this PRM remains essential to ensure greater accuracy in applications such as air quality monitoring and emissions control.

Similarly, PRMs for CO_2 are essential for both high-level and trace-level applications. At elevated amount fractions, they are needed for the support of climate change and greenhouse gas monitoring programs, enabling accurate and comparable data for global emissions inventories. On the other hand, at lower amount fraction ranges, the preparation of CO_2 PRMs becomes more challenging due to the limitations in the purity of commercially available $CO_{2.}$ Developing reliable PRMs at these levels is crucial for ambient air monitoring and for calibrating instruments used in indoor air quality assessments and sensitive analytical systems.

In addition, the use of PRMs in PT schemes is crucial to assess the performance of laboratories and to ensure the comparability of measurement results across institutions. This is particularly important in the context of air pollutant monitoring, where reliable and traceable measurements are essential for regulatory compliance and public health protection. The availability of well-characterized PRMs supports the validation of analytical methods and reinforces national capabilities in environmental measurement and control.

Another important area where the development of PRMs can contribute to global progress is the hydrogen industry. As the world shifts toward renewable energy, hydrogen plays a key role as a clean energy carrier. To safely and effectively use hydrogen, it is important to have a solid metrological infrastructure in place. Developing PRMs for hydrogen impurities is essential to ensure that hydrogen used in applications like fuel cells and industrial processes meets the necessary purity standards. Accurate measurements of impurities will be crucial for ensuring the reliability of hydrogen-based technologies and for safely calibrating sensors to detect any potential leaks. This will also help standardize hydrogen production and use across industries, making sure hydrogen can be used safely and efficiently.

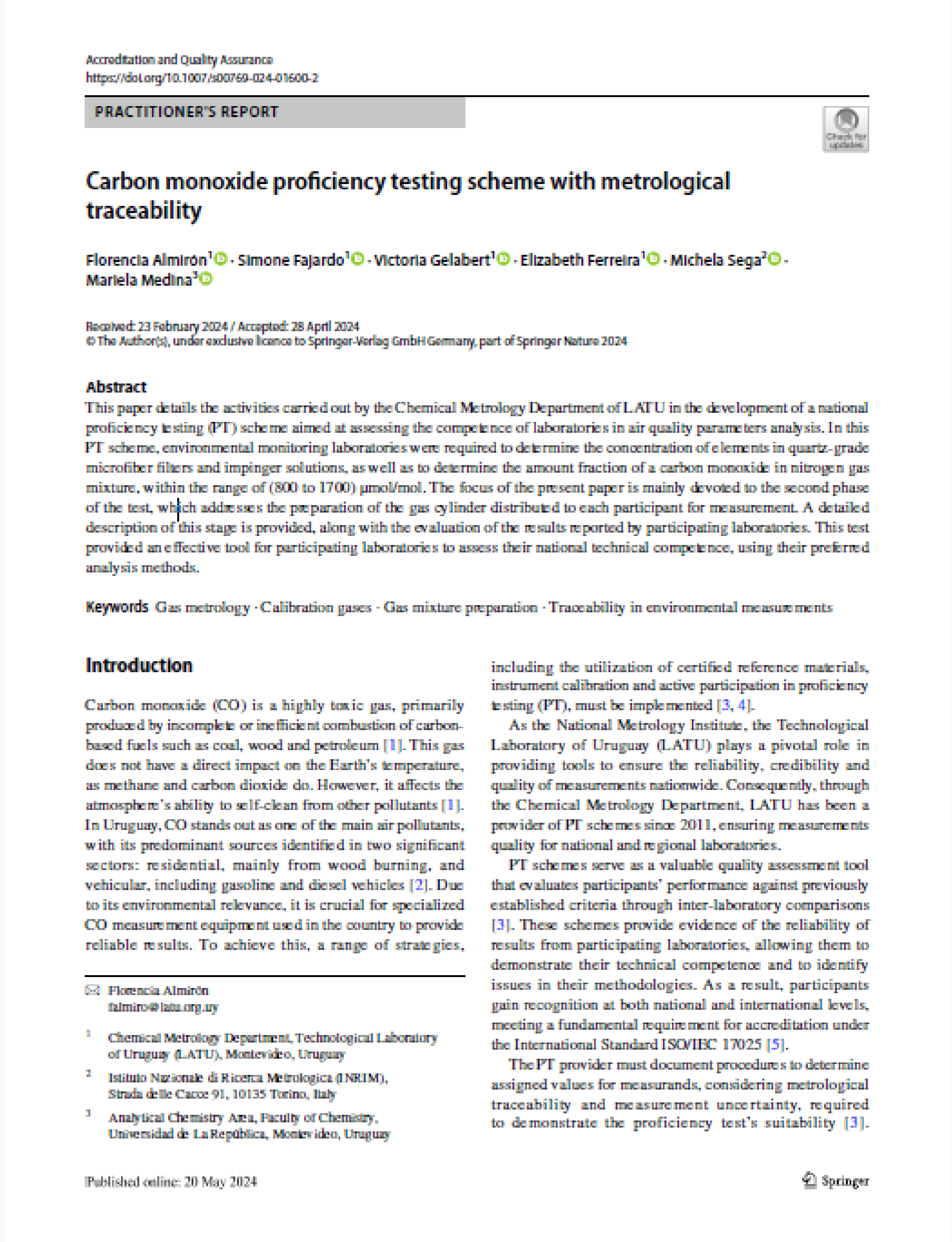
CHAPTER 7. **APPENDIX**

APPENDIX I: Published papers in international scientific journals.

APPENDIX II: Oral presentations at conferences or CCQM working group meetings.

APPENDIX III: Posters presented at conferences.

APPENDIX I



Accreditation and Quality Assurance
https://doi.org/10.1007/s00769-024-01600-2

PRACTITIONER'S REPORT

Carbon monoxide proficiency testing scheme with metrological traceability

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Received: 23 February 2024 / Accepted: 28 April 2024

Abstract

This paper details the activities carried out by the Chemical Metrology Department of LATU in the development of a national proficiency testing (PT) scheme aimed at assessing the competence of laboratories in air quality parameters analysis. In this PT scheme, environmental monitoring laboratories were required to determine the concentration of elements in quartz-grade microfiber filters and impinger solutions, as well as to determine the amount fraction of a carbon monoxide in nitrogen gas mixture, within the range of (800 to 1700) µmol/mol. The focus of the present paper is mainly devoted to the second phase of the test, which addresses the preparation of the gas cylinder distributed to each participant for measurement. A detailed description of this stage is provided, along with the evaluation of the results reported by participating laboratories. This test provided an effective tool for participating laboratories to assess their national technical competence, using their preferred analysis methods.

Keywords Gas metrology · Calibration gases · Gas mixture preparation · Traceability in environmental measurements

Introduction

Carbon monoxide (CO) is a highly toxic gas, primarily produced by incomplete or inefficient combustion of carbon-based fuels such as coal, wood and petroleum [1]. This gas does not have a direct impact on the Earth's temperature, as methane and carbon dioxide do. However, it affects the atmosphere's ability to self-clean from other pollutants [1]. In Uruguay, CO stands out as one of the main air pollutants, with its predominant sources identified in two significant sectors: residential, mainly from wood burning, and vehicular, including gasoline and diesel vehicles [2]. Due to its environmental relevance, it is crucial for specialized CO measurement equipment used in the country to provide reliable results. To achieve this, a range of strategies, including the utilization of certified reference materials, instrument calibration and active participation in proficiency testing (PT), must be implemented [3, 4].

As the National Metrology Institute, the Technological Laboratory of Uruguay (LATU) plays a pivotal role in providing tools to ensure the reliability, credibility and quality of measurements nationwide. Consequently, through the Chemical Metrology Department, LATU has been a provider of PT schemes since 2011, ensuring measurements quality for national and regional laboratories.

PT schemes serve as a valuable quality assessment tool that evaluates participants' performance against previously established criteria through inter-laboratory comparisons [3]. These schemes provide evidence of the reliability of results from participating laboratories, allowing them to demonstrate their technical competence and to identify issues in their methodologies. As a result, participants gain recognition at both national and international levels, meeting a fundamental requirement for accreditation under the International Standard ISO/IEC 17025 [5].

The PT provider must document procedures to determine assigned values for measurands, considering metrological traceability and measurement uncertainty, required to demonstrate the proficiency test's suitability [3].

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The International Vocabulary of Metrology (VIM) defines metrological traceability as the property of a measurement result by which the result can be related to a reference through an unbroken and documented chain of calibrations, each contributing to the measurement uncertainty [6]. Direct metrological traceability to the International System of Units (SI) is obtained through primary methods, which have the highest metrological hierarchy. In chemical metrology, the applicable unit is the mole, and traceability is achieved through primary methods such as gravimetry [7], which can be applied for the realization of reference gas mixtures in accordance with the International Standard ISO 6142-1 [8]. This method is highly accurate and can be employed for certifying reference materials as well as assigning reference values for proficiency testing. Within the framework of this PT scheme, a gas mixture of CO in nitrogen (N_2) with a nominal amount fraction value of 1500 µmol/mol was prepared. The metrological traceability of the CO amount fraction value of this mixture was established by using primary reference materials produced by other National Metrology Institutes.

Materials and methods

Gas mixture development

The gas mixture preparation plan aimed at achieving an amount fraction of CO at a nominal value of 1500 µmol/mol, starting from a pre-mixture of CO in N_2 with a nominal value of 4.0 % mol/mol and a high purity N_2 (6.0) as balance gas. Preparation was carried out in an aluminum cylinder with a 4.6 L internal volume and a working pressure of 139 bar. It is noteworthy that this cylinder material is compatible with both CO and N_2, ensuring non-reactivity with the contained gases [8, 9].

To determine the amount of mass to be added for each component in the cylinder, the ideal gas law equation, along with the molar mass of the corresponding compounds, was employed. The working temperature was maintained at (21 ± 1) °C.

The cylinder intended for containing the mixture was conditioned and evacuated prior to usage. To achieve this, a specialized filling and vacuum station was employed. This station features a set of valves facilitating gas passage for cylinder filling or purging. Additionally, it is equipped with a mechanical pump and a turbomolecular pump for effective cylinder evacuation. To enhance impurity removal, a thermal blanket set at 40 °C is applied around the cylinder, ensuring an improved vacuum (Fig. 1).

Fig. 1 Cylinder connected to the station for empting

Fig. 2 Cylinder homogenizer equipment

In accordance with the prescriptions of ISO 6142-1 [8], the analyte gas CO was introduced into the cylinder, followed by the balance gas N_2. The resulting mixture was then placed in a specialized equipment designed for cylinder rotation, ensuring effective homogenization (Fig. 2) [10].

Gas mixture value assignment

The molar fraction of a gas mixture, prepared through partial pressures, must be determined by analysis performed on specific equipment, such as a gas chromatograph. This equipment should be properly calibrated using primary reference materials (PRMs) of the same gases present in the gas mixture, ensuring that the assigned value is metrologically traceable to the SI. These PRMs hold the highest metrological hierarchy, as their preparation and value assignment are conducted using the gravimetric method [8, 11, 12] and verified in accordance with ISO 6143 standards [13].

The prepared mixture was introduced into a gas chromatograph with a flame ionization detector equipped with a methanizer, and its value was assigned using a calibration curve determined by using PRMs produced by the National Metrology Institute of the Netherlands (VSL) and the National Metrology Institute of Brazil (INMETRO). Table 1 shows the reference standards used for calibration.

Selection of proficiency testing scheme and reference value

This proficiency testing, focused on determining parameters for air quality assessment, was developed in collaboration with the Environmental Laboratory Division of DINACEA—Ministry of the Environment of Uruguay. The aim was to assess the existing analytical capabilities in the country, concerning the composition of the air. Participants were tasked with determining the concentration of elements in quartz microfiber filters and impinger solutions. Additionally, they were required to measure the molar fraction of CO in a N_2 gas mixture within the range of (800 to 1700) μmol/mol. In the latter part of the test, three laboratories that are members of the Uruguayan Environmental Laboratories Network (RLAU) participated. Measurements were performed by the participants by using their routine techniques for assessing CO levels for air quality.

The implemented strategy for this PT follows a sequential scheme and meets the requirements of the ISO/IEC 17043 [3, 14]. After measuring the gas mixture for the first time, the cylinder is sent to the first laboratory, where the corresponding measurement is conducted. After this initial determination, the cylinder is returned to the organizing laboratory, LATU, for a new analysis. Once LATU completes its measurement, the cylinder is transferred to the second laboratory for further determination. This sequential pattern continues, involving all participating laboratories, with the objective of obtaining a comprehensive assessment of the gas mixture through multiple consecutive measurements. This model facilitates monitoring the stability of the mixture over time and assessing for any significant changes in the gas composition.

The gas mixture used for this scheme was the first mixture developed by the Chemical Metrology Department, so the stability study was based on bibliography and in the historical data from other carbon monoxide gas mixtures in nitrogen. The strategy for selecting the reference value involved averaging every measurement performed by LATU throughout the duration of the proficiency testing [14].

Results and discussion

Reference value

Table 2 shows the amount fraction and uncertainty values obtained at LATU during the PT scheme; the measurement dates are also reported. Figure 3 illustrates these data in a graph. The reference value and associated expanded uncertainty at 95 % confidence level for the CO gas mixture in N_2 were 1516.5 ± 9.2 μmol/mol. The reference value was calculated as the average of the four measurements, and its uncertainty was determined by combining the standard uncertainties obtained from each measurement, u_{meas}, along with a rectangular component, u_{rec} (Eq. 1) [14]. The latter was considered to account for potential variations that may have occurred on the different measurement days. The combined uncertainty of the reference value was multiplied

Table 1 Calibration primary reference materials

PRM cylinder	CO amount fraction (μmol/mol)	Expanded uncertainty, $k = 2$ (μmol/mol)
D887513	500.6	1.0
D887492	1000.5	2.0
D887607	1501.5	3.0
D887616	2001.8	4.0
M692238	2471.0	9.0

Table 2 Results of the measurements carried out at LATU during the PT scheme

Date	Amount fraction (μmol/mol)	Standard uncertainty (μmol/mol)	Cylinder pressure (psi)
07/09/2021	1517.4	2.4	1600
06/10/2021	1519.5	4.3	1400
21/10/2021	1519.3	4.2	1100
01/11/2021	1509.7	6.2	500

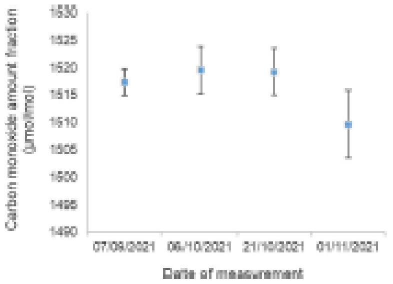

Fig. 3 Amount fractions obtained at LATU, in 4 days of analysis

by a coverage factor of $k = 2$ to determine the expanded uncertainty at approximately 95 % level of confidence.

$$u_{\text{ref value}} = \sqrt{u_{\text{meas}}^2 + u_{\text{rec}}^2} \quad (1)$$

Laboratory performance evaluation

The performance evaluation of participants was conducted using the z'-score (Eq. 2) [14].

$$z' - \text{score} = \frac{E}{\sqrt{\sigma_{PT}^2 + u_{\text{ref value}}^2}} \quad (2)$$

where E represents the error (i.e., the difference between the value reported by the laboratory and the reference value), σ_{PT} the standard deviation of the PT (set at 10 % of the reference value) and $u_{\text{ref value}}$ the standard uncertainty associated with the reference value.

A z'-score equal or less than 2 implies that the laboratory value is statistically comparable to the reference value, considering the assumptions made in the evaluation. A value between 2 and 3 signals an alert regarding the obtained value and warrants a study of the results in case this situation repeats. A value equal or greater than 3 indicates a significant error in the measurement, requiring an examination of potential error sources for correction [14].

Table 3 Reported values by participants in the PT

Laboratory	Reported value (µmol/mol)	Error (µmol/mol)	|z'- score|
1A	1685	168.5	1.1
1B	1699	182.5	1.2
3C	1390	−126.5	0.8
5C	1450	−66.5	0.4

Table 3 presents the values reported by the three participating laboratories identified by a numerical code, ensuring the confidentiality of the results. Laboratory 1 participated using two different methods, while laboratories 3 and 5 participated using only one technique. The letter accompanying each laboratory number indicates the type of methodology used. Letter A represents Fourier transform infrared spectroscopy (FTIR), letter B non-dispersive infrared spectroscopy (NDIR) and letter C the electrochemical method.

All laboratories obtained absolute z'-scores below 2, indicating good agreement between the conducted measurements and the reference value. Figure 4 displays a graph constructed from the reported results by the laboratories. This representation visually illustrates how all laboratories remained within the warning limits of the graph, confirming their good performance. For the graph construction, the warning limits were calculated as the reference value ± two times the denominator of the z'-score, which is the square root of the sum of the squares of σ_{PT} and $u_{\text{ref value}}$. The action limits were determined as the reference value ± three times the denominator of the z'-score [14].

Conclusion

The results obtained reveal a strong performance by the participating laboratories in the proficiency testing, showcasing their ability to carry out precise measurements of CO emissions. This reaffirms the significant contribution of PT schemes in preserving and enhancing the quality of environmental measurements.

It is important to highlight that this PT ensures the reliability of results published by laboratories but also guarantees international comparability in the measurement of this

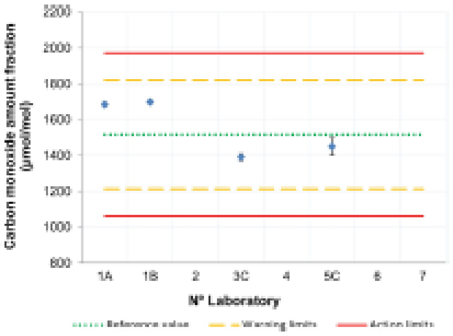

Fig. 4 Participants' reported values

gas component since the reference value selected is traceable to the SI.

This PT has provided valuable insights into the current capabilities of environmental laboratories in determining carbon monoxide levels. With ongoing participation in this initiative, we anticipate a collective improvement among participants, thereby promoting a more accurate assessment over time.

Acknowledgements We would like to express our gratitude to the dedicated team at the Environmental Laboratory Division of DINACEA—Ministry of the Environment and the personnel from the Chemical Metrology Department of LATU.

Author contributions F.A. wrote the manuscript and prepared figures and tables.F.A., S.F., V.G. and EF contributed to the study design, revised and edited the manuscript.M.S. provided expertise in gas metrology, reviewed and edited the manuscript.All authors revised and approved the manuscript.

Declarations

Conflict of interest Michela Sega, co-author of this manuscript, is a member of the Intercontinental Advisory Board.

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APPENDIX II

Collaboration Project INTI-PTB (2025)

Seminar Presentation: The Role of LATU Supporting the Hydrogen Industry

Type of Participation: Invited Speaker

Organising Institution: INTI. Argentina

Geographic Scope: International

SURAMET SIM Projects on Hydrogen and Biogas (2024)

Seminar Presentation: The Role of LATU Supporting the Hydrogen Industry

Type of Participation: Invited Speaker

Organising Institution: INMETRO. Brazil.

Geographic Scope: Regional - Online

Gas Analysis Working Group - 48th Meeting (2023)

Type: Presentation about new developments at LATU

Organising Institution: LATU. Montevideo, Uruguay.

Geographic Scope: International

Gas Analysis Working Group - 46th Meeting of the CCQM (2022)

Type: Presentation about development at LATU

Organising Institution: National Physical Laboratory (NPL). Teddington, United Kingdom.

Geographic Scope: International

Gas Analysis Working Group - 39th Meeting of the CCQM (2018)

Type: Introducing LATU Gas Laboratory

Organising Institution: Centro Nacional de Metrología (CENAM). Queretaro, Mexico.

Geographic Scope: International

APPENDIX III

Poster presentation at Congreso Uruguayo de Química Analítica - CUQA (2024)

Preparación gravimétrica de mezcla gaseosa de dióxido de carbono

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INTRODUCCIÓN

La contaminación atmosférica es la principal causa del cambio climático. En particular, en nuestro país, los vehículos automotores son uno de los más importantes emisores de gases contaminantes como el monóxido de carbono y el dióxido de carbono. Para controlar que los niveles emitidos de estos gases a la atmósfera estén dentro de los límites permitidos, se deben tener equipos especializados en su medición, que emitan resultados precisos y confiables. Esto se puede lograr mediante el desarrollo de mezclas gaseosas de referencia, para la calibración de equipos de análisis y para el desarrollo de métodos de medición adecuados. Estas mezclas contienen fracciones de masa exactamente conocidas de distintos gases, lo cual permite brindar trazabilidad metrológica a los contaminantes atmosféricos. Las mezclas gaseosas de referencia pueden ser materiales de referencia primarios (PRM) o materiales de referencia certificados (MRC). Los PRM se preparan utilizando métodos de medición primarios, que son los métodos de más alto nivel utilizados para la realización de unidades y tienen trazabilidad directa a una unidad del Sistema Internacional de Unidades (SI). En este caso, los PRM de mezclas gaseosas se preparan mediante gravimetría, que es una técnica trazable directamente al mol. Los MRC de mezclas gaseosas, por su parte, obtienen su trazabilidad al ser calibrados en comparación con los PRM.

OBJETIVO

Desarrollar un material de referencia primario de mezcla gaseosa de dióxido de carbono (CO_2) en nitrógeno, con el fin de proporcionar medidas precisas y confiables de CO_2 en el país.

METODOLOGÍA

Para la preparación de un PRM, se utilizan dos cilindros del mismo material y volumen. Uno de ellos actúa como cilindro de referencia, mientras que el otro, a preparar, se llena con los gases componentes (gases padres). La masa agregada de cada gas se determina como la diferencia de masa entre los cilindros de muestra y de referencia de cada ciclo de pesaje.

La trazabilidad de estos PRM al SI de las fracciones de cantidad de sustancia se logra a través de la correcta ejecución de tres pasos: la determinación precisa de las masas agregadas, la conversión de estas masas a cantidades de sustancia, considerando su pureza química y las masas relativas atómicas y/o molares, y la verificación de la mezcla final utilizando PRM independientes.

RESULTADOS

Se preparó un material de referencia primario de 0,35 mol/mol de CO_2 en balance N_2, en un cilindro de aluminio. Para su preparación, el cilindro se conectó a una estación de vaciado y llenado de cilindros donde primero se acondicionó y luego fue llenado con ambos gases. El cilindro fue pesado en una comparadora de masas en sus tres etapas: vacío, luego del agregado de CO_2 y luego del agregado de N_2. Se calculó la fracción de cantidad de sustancia (y_k) de cada gas utilizando la ecuación 1, que incluye la fracción molar de los componentes de la mezcla (*x*), sus masas molares (*M*) y las masas agregadas de cada gas (*m*).

$$y_k = \frac{\sum_{j=1}^{r}\left(\frac{x_{k,j} \times m_j}{\sum_{i=1}^{q} x_{i,j} \times M_i}\right)}{\sum_{j=1}^{r}\left(\frac{m_j}{\sum_{i=1}^{q} x_{i,j} \times M_i}\right)}$$

Ecuación 1

El valor determinado por la ecuación es el valor certificado de la mezcla gaseosa primaria. Su incertidumbre asociada (u_c) es una combinación de la incertidumbre de preparación (u_{prep}), junto a la de verificación (u_{ver}). La incertidumbre de preparación es raíz de la suma de los cuadrados de la incertidumbre gravimétrica y la incertidumbre por inestabilidad.

$$u_c(y_k) = \frac{1}{2}\sqrt{u^2(y_{k,prep}) + u^2(y_{k,ver}) + (y_{k,prep} - y_{k,ver})^2}$$

Ecuación 2

CONCLUSIÓN

Los resultados obtenidos confirman la alta eficacia del método gravimétrico en la preparación de mezclas gaseosas de referencia, fundamentales para la calibración de equipos, la certificación de estándares gaseosos y la validación de métodos analíticos. Esta aplicación garantiza una trazabilidad directa al Sistema Internacional de Unidades (SI), asegurando la comparabilidad internacional.

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AGRADECIMIENTOS

Agradecemos al Istituto Nazionale di Ricerca Metrologica de Italia (INRIM) y al Instituto Nacional de Metrologia, Qualidade e Tecnologia de Brasil (INMETRO) por las capacitaciones recibidas, las cuales fueron esenciales para el desarrollo de la preparación de materiales de referencia primarios de mezclas gaseosas.

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Fortalecimiento de la infraestructura metrológica para impulsar la industria del hidrógeno

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Jornada Académica

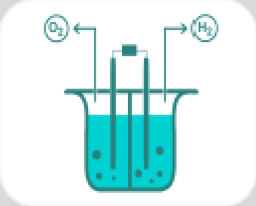
INTRODUCCIÓN

En un mundo que busca soluciones energéticas sostenibles, el hidrógeno ha surgido como un candidato prometedor para la transición hacia un futuro más limpio y eficiente. A medida que su demanda crece en diversos sectores, garantizar su calidad, seguridad y confiabilidad se vuelve esencial. La calidad del hidrógeno como combustible es crítica, ya que las impurezas pueden reducir la eficiencia y vida útil de las celdas de combustible, aumentando los costos operativos y de mantenimiento. Por ello, asegurar la pureza del hidrógeno optimiza el rendimiento de los sistemas de combustión, reduce las emisiones contaminantes y promueve un uso más sostenible de este recurso.

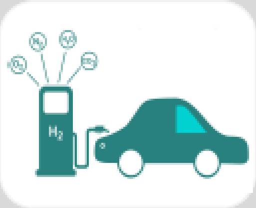
El desarrollo de una cadena de valor sólida para el hidrógeno depende no solo de los avances tecnológicos, sino también de una infraestructura metrológica robusta. En este póster, presentamos dos estrategias innovadoras que el Departamento de Metrología Química del LATU implementará, con el objetivo de fortalecer el marco metrológico que respalda la industria del hidrógeno y asegurar su sostenibilidad a largo plazo.

OPTIMIZACIÓN CELDA ELECTROLÍTICA

Algunos de los principales componentes de los electrolizadores modernos son los electrocatalizadores catódico y anódico, junto con la membrana de intercambio. Estos elementos forman en conjunto lo que se denomina conjunto membrana-electrodo (MEA), una estructura crucial que determina el rendimiento y la eficiencia general del electrolizador. La MEA desempeña un papel central tanto en la cinética de las reacciones electroquímicas como en el transporte de iones a través de la membrana, lo cual impacta directamente en la eficiencia del proceso de separación de agua y en la pureza del hidrógeno generado. Para evaluar su rendimiento en la electrólisis del agua, se caracterizarán distintos materiales comerciales y experimentales para electrodos mediante análisis fisicoquímico (difracción de rayos X, microscopía electrónica de barrido, microscopía electrónica de transmisión, análisis químico, entre otros) y electroquímico. La curva de polarización característica de la MEA se determinará en una configuración de celda completa, utilizando una celda de prueba con control de temperatura. Además, se estudiará la eficiencia farádica a diferentes densidades de corriente, midiendo el flujo y la pureza del hidrógeno producido.



CUANTIFICACIÓN DE IMPUREZAS



Dado el gran potencial de Uruguay para la producción de hidrógeno verde mediante electrólisis del agua, en esta primera etapa se identificarán y cuantificarán las impurezas que pueden estar presentes en el hidrógeno producido a través de este proceso. La pureza del hidrógeno es crucial, ya que incluso pequeñas cantidades de ciertos contaminantes pueden afectar negativamente el rendimiento de celdas de combustible y otros sistemas de aplicación. Las impurezas clave que se analizarán incluyen nitrógeno, oxígeno, agua y dióxido de carbono. La estrategia de cuantificación propuesta utiliza un cromatógrafo de gases acoplado a un detector de conductividad térmica y un detector de ionización de llama con metanizador. Además, se utilizará un segundo equipo para determinar con alta precisión el contenido de agua, un parámetro especialmente relevante para asegurar la estabilidad del hidrógeno. Con el cromatógrafo de gases, también se cuantificarán otras impurezas de interés, como metano, monóxido de carbono, hidrocarburos C2-C18, argón y helio. Estos instrumentos serán calibrados utilizando mezclas gaseosas de referencia cubriendo un rango alineado con los valores límite establecidos en la norma ISO 14687, garantizando así la confiabilidad de la cuantificación.

CONCLUSIONES

El establecimiento de un marco metrológico robusto dentro de la industria del hidrógeno es esencial para su desarrollo sostenible. Aunque existe un costo inicial significativo asociado con esta infraestructura, la inversión resulta altamente beneficiosa a largo plazo. Al optimizar el proceso de producción de hidrógeno, podemos lograr un gas de alta pureza, lo que mejora directamente la eficiencia del sistema y reduce los desafíos operativos. Además, el cumplimiento estricto de las regulaciones y el mantenimiento de los niveles de impurezas dentro de límites aceptables extiende la vida útil de las celdas de combustible, minimizando la necesidad de reemplazos frecuentes y reduciendo los costos de mantenimiento. A medida que avanzamos hacia la próxima fase, nuestro objetivo se centrará en cuantificar las impurezas restantes enumeradas en la norma ISO 14687, reforzando aún más nuestro compromiso de asegurar la más alta calidad y fiabilidad en la producción de hidrógeno.

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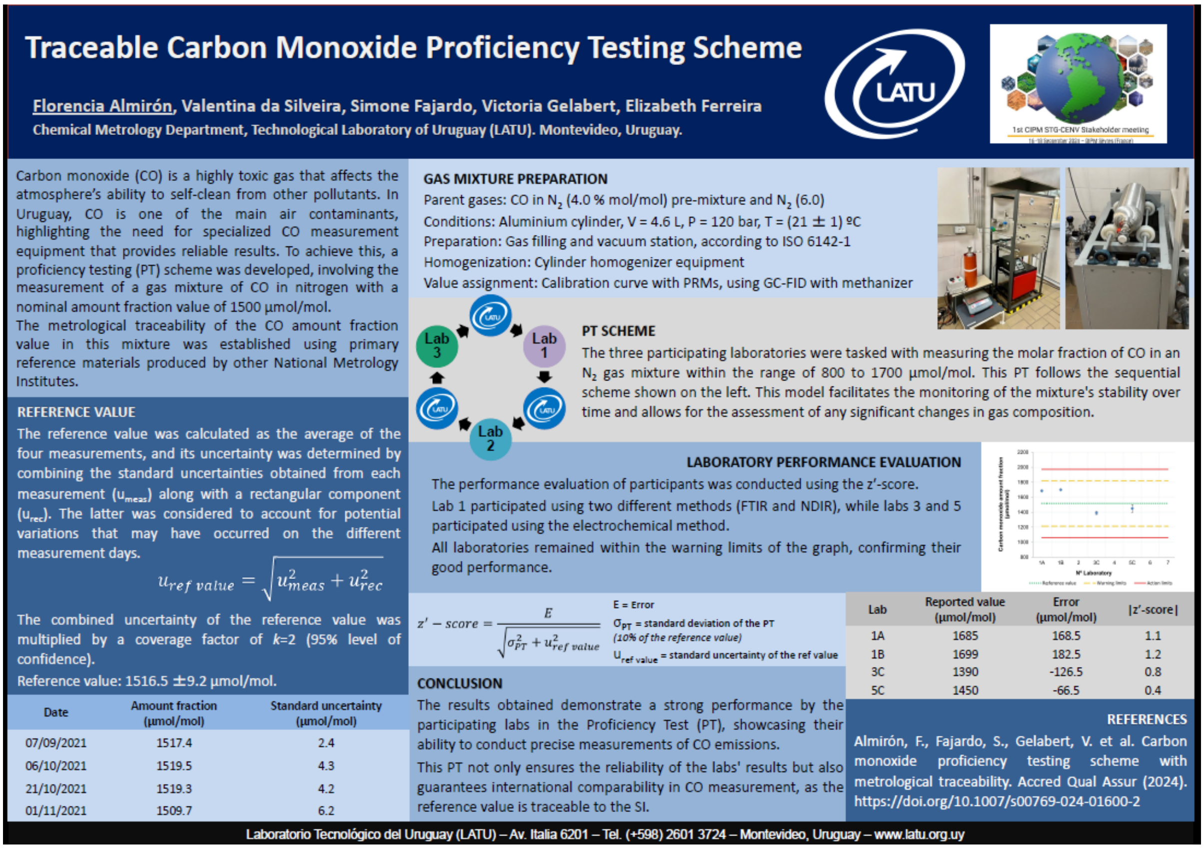
Poster presentation at 1st CIPM STG-CENV Stakeholder Meeting (2024)
Organizing Institution: BIPM (online), France.



Traceable Carbon Monoxide Proficiency Testing Scheme

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Carbon monoxide (CO) is a highly toxic gas that affects the atmosphere's ability to self-clean from other pollutants. In Uruguay, CO is one of the main air contaminants, highlighting the need for specialized CO measurement equipment that provides reliable results. To achieve this, a proficiency testing (PT) scheme was developed, involving the measurement of a gas mixture of CO in nitrogen with a nominal amount fraction value of 1500 µmol/mol.
The metrological traceability of the CO amount fraction value in this mixture was established using primary reference materials produced by other National Metrology Institutes.

REFERENCE VALUE

The reference value was calculated as the average of the four measurements, and its uncertainty was determined by combining the standard uncertainties obtained from each measurement (u_{meas}) along with a rectangular component (u_{rec}). The latter was considered to account for potential variations that may have occurred on the different measurement days.

$$u_{ref\ value} = \sqrt{u_{meas}^2 + u_{rec}^2}$$

The combined uncertainty of the reference value was multiplied by a coverage factor of *k*=2 (95% level of confidence).
Reference value: 1516.5 ±9.2 µmol/mol.

Date	Amount fraction (µmol/mol)	Standard uncertainty (µmol/mol)
07/09/2021	1517.4	2.4
06/10/2021	1519.5	4.3
21/10/2021	1519.3	4.2
01/11/2021	1509.7	6.2

GAS MIXTURE PREPARATION

Parent gases: CO in N_2 (4.0 % mol/mol) pre-mixture and N_2 (6.0)
Conditions: Aluminium cylinder, V = 4.6 L, P = 120 bar, T = (21 ± 1) ºC
Preparation: Gas filling and vacuum station, according to ISO 6142-1
Homogenization: Cylinder homogenizer equipment
Value assignment: Calibration curve with PRMs, using GC-FID with methanizer

PT SCHEME

The three participating laboratories were tasked with measuring the molar fraction of CO in an N_2 gas mixture within the range of 800 to 1700 µmol/mol. This PT follows the sequential scheme shown on the left. This model facilitates the monitoring of the mixture's stability over time and allows for the assessment of any significant changes in gas composition.

LABORATORY PERFORMANCE EVALUATION

The performance evaluation of participants was conducted using the z′-score.
Lab 1 participated using two different methods (FTIR and NDIR), while labs 3 and 5 participated using the electrochemical method.
All laboratories remained within the warning limits of the graph, confirming their good performance.

$$z' - score = \frac{E}{\sqrt{\sigma_{PT}^2 + u_{ref\ value}^2}}$$

E = Error
σ_{PT} = standard deviation of the PT
(10% of the reference value)
$U_{ref\ value}$ = standard uncertainty of the ref value

Lab	Reported value (µmol/mol)	Error (µmol/mol)	|z′-score|
1A	1685	168.5	1.1
1B	1699	182.5	1.2
3C	1390	-126.5	0.8
5C	1450	-66.5	0.4

CONCLUSION

The results obtained demonstrate a strong performance by the participating labs in the Proficiency Test (PT), showcasing their ability to conduct precise measurements of CO emissions.

This PT not only ensures the reliability of the labs' results but also guarantees international comparability in CO measurement, as the reference value is traceable to the SI.

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Poster presentation at Workshop on Global and National GHG Monitoring Initiatives (2024)

Organizing Institution: Korea Research Institute of Standards and Science - KRISS (online)

The Role of LATU in Supporting the Hydrogen Industry

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Uruguay has embarked on its second energy transition, a major step towards reducing fossil fuel consumption and minimizing greenhouse gas emissions that drive climate change. This effort is an important part of Uruguay's goal to reach carbon neutrality by 2050, as described in the country's 2021 Long-Term Climate Strategy. To meet this ambitious objective, significant and accelerated changes are needed in energy sources, particularly in industrial processes.

Having already decarbonized nearly 94% of its electricity matrix, placing Uruguay among global leaders in renewable energy, there remains a local challenge: decarbonizing the remaining energy matrix, particularly in the transport and industrial sectors. Achieving this will require the adoption of low-emission energy sources. In this regard, the production of green hydrogen, generated from water and renewable energies, has emerged as a highly relevant solution on the global agenda.

Ensuring environmental sustainability in the development and use of clean energy technologies is essential. Within this framework, hydrogen fuel quality is critical. Impurities in hydrogen can degrade the efficiency and lifespan of fuel cells, leading to system failures and increased operational and maintenance costs.

Images extracted from Uruguay's Roadmap for Green Hydrogen and Derivatives – H2U – October 2023

Guaranteeing hydrogen purity enhances combustion system efficiency, thereby reducing pollutant emissions and promoting a cleaner and more efficient use of this energy resource. Moreover, using high-purity hydrogen supports a circular economy, where resources are used more efficiently and sustainably.

This work highlights two strategies being developed by LATU's Chemical Metrology Department to strengthen the metrological infrastructure supporting the hydrogen industry. The first strategy is to optimize the key components of PEM and alkali electrolyzers to achieve a high degree of purity in the produced hydrogen, as well as high efficiency and long-term cyclability of the electrolyzer materials. The second approach focuses on quantifying the impurities in locally produced hydrogen to ensure they are below the specified limits outlined in the ISO 14687 standard, thus preventing any adverse effects on the fuel cells in hydrogen-powered vehicles and other applications.

Some of the main components of modern electrolyzers are the cathodic and anodic electrocatalysts, along with the exchange membrane. Together, these elements form the so-called membrane-electrode assembly (MEA), a crucial structure that determines the overall performance and efficiency of the electrolyzer. The MEA plays a central role in both the kinetics of the electrochemical reactions and the transport of ions through the membrane, which directly impacts the efficiency of the water-splitting process and the purity of the generated hydrogen. In order to evaluate their performance for water electrolysis, different commercial and experimental electrode materials will be characterized through physicochemical (X-ray diffraction, scanning electron microscopy, transmission electron microscopy, chemical, among others) and electrochemical analysis. The characteristic polarization curve of the MEA will be determined in a complete cell configuration, using a temperature-controlled test cell. Additionally, the Faradaic efficiency at different current densities will be studied by measuring the flow and purity of the produced hydrogen.

In our initial phase, we will evaluate the potential impurities in hydrogen production through Proton Exchange Membrane (PEM) water electrolysis and chlor-alkali membrane electrolysis. These two methods are the predominant approaches to hydrogen generation in our country, making them the primary focus of our research. The major impurities that may be present include nitrogen, oxygen, water, and carbon dioxide.

Our quantification strategy will involve a gas chromatograph coupled with a thermal conductivity detector and a flame ionization detector, along with a methanizer. Additionally, we will use a second instrument for accurate water content determination. Using the gas chromatograph, we will also quantify other impurities of interest, such as methane, carbon monoxide, C2-C18 hydrocarbons, argon, and helium.

These instruments will be calibrated using reference gas mixtures covering a range that aligns with the threshold values outlined in ISO 14687, ensuring the reliability of the quantification.

CONCLUSION

The establishment of a robust metrological framework within the hydrogen industry is essential for its sustainable development. While there is a significant upfront cost associated with this infrastructure, the investment proves highly advantageous in the long term. By optimizing the hydrogen production process, we can achieve high-purity gas, which directly enhances system efficiency and reduces operational challenges. Additionally, strict compliance with regulations and maintaining impurity levels within acceptable limits extends the lifespan of fuel cells, minimizing the need for frequent replacements and lowering maintenance costs. As we move forward into the next phase, our objective will focus on quantifying the remaining impurities listed by the ISO 14687 standard, further reinforcing our commitment to ensuring the highest quality and reliability in hydrogen production.

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Bacquart, T. et al. (2018) Probability of occurrence of ISO 14687-2 contaminants in hydrogen: Principles and examples from steam methane reforming and electrolysis (water and chlor-alkali) production processes model. International Journal of Hydrogen Energy, 43(26), 11872–11883.

Laboratorio Tecnológico del Uruguay (LATU) – Av. Italia 6201 – Tel. (+598) 2601 3724 – Montevideo, Uruguay – www.latu.org.uy

Poster presentation at Green Hydrogen Workshop (2023)

Organizing Institution: Laboratorio Tecnológico del Uruguay, Uruguay

Enhancing the Hydrogen Value Chain through Metrology Infrastructure: LATU's perspectives

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INTRODUCTION

In a world that seeks sustainable energy solutions, hydrogen has emerged as a promising candidate for the transition towards a cleaner and more efficient energy future. As the demand for hydrogen continues to grow across various sectors, ensuring its quality, safety, and reliability becomes essential.

The development and optimization of the hydrogen value chain depend not only on technological advancements but also on the robust underpinning of metrological infrastructure. In this poster, we introduce two forward-thinking strategies that the Chemical Metrology Department of LATU will put into practice, both with the aim of strengthening the metrological framework within the hydrogen industry.

The first strategy is to optimize the key components of PEM and alkali electrolyzers, to achieve a high degree of purity in the produced green hydrogen as well as a high efficiency and long term cyclability of the electrolyzer materials.

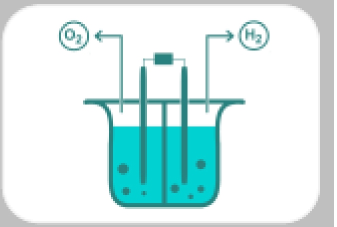
The second approach is to quantify the impurities in locally produced hydrogen to ensure that they are below the specified limits outlined in the ISO 14687 standard, thus preventing any adverse effects on the fuel cell in hydrogen-powered vehicles and other fuel applications.

ELECTROLYSIS CELL OPTIMIZATION

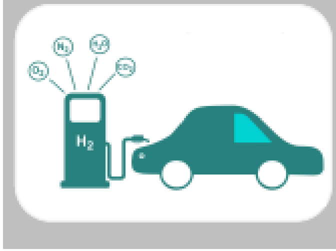
Some of the main components of the modern electrolyzers are the cathodic and anodic electrocatalysts, together with the exchange membrane. These constitute the so-called membrane-electrode assembly (MEA), which determine the electrolyzer behavior.

In order to evaluate their performance for water electrolysis, different commercial and experimental electrode materials will be characterized through physicochemical (XRD, SEM, TEM, chemical, among others) and electrochemical analysis.

The characteristic polarization curve of the MEA will be determined in a complete cell configuration, using a temperature-controlled test cell. Additionally, the Faradaic efficiency at different current densities will be studied by measuring the flow and purity of the produced H_2.



H_2 IMPURITIES QUANTIFICATION



In our initial phase, we will assess the potential impurities in hydrogen production via Proton Exchange Membrane (PEM) water electrolysis and Chlor-alkali membrane electrolysis. These two methods are the predominant approaches to hydrogen generation in our country, making them the primary focus of our investigation. The impurities that could be present include Nitrogen, Oxygen, Water, and Carbon dioxide.

Our quantification strategy will include a Gas Chromatograph coupled with a thermal conductivity detector and a flame ionization detector. This setup comprises three gas columns for the analysis of Nitrogen, Oxygen, and Carbon dioxide. Additionally, we will use an FTIR instrument for accurate water content determination. These instruments will be calibrated using a set of five reference gas mixtures of these gases, covering a range that aligns with the ISO 14687 standard threshold value, ensuring the reliability of the quantification.

CONCLUSIONS

The establishment of a robust metrological framework within the hydrogen industry is essential. While there is a significant upfront cost, this investment proves advantageous in the long term. Optimizing the hydrogen production process results in high-purity gas, enhancing efficiency. Moreover, compliance with regulations and maintaining impurity levels within limits extends fuel cell lifespan, reducing the need for frequent replacements.

As a next phase, our objective will focus in quantifying the remaining impurities listed by the ISO 14687 standard, reinforcing our commitment to hydrogen production quality and reliability.

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Poster presentation at SETAC Latin America 15th Biennial Meeting (2023)

Gas Analyser Calibration for Reliable Air Pollutants Measurement and Minimization of Environmental Risk

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INTRODUCTION

Carbon dioxide and carbon monoxide are substantially polluting gases that are released into the atmosphere mainly by automotive vehicles and by industry-related activities. The presence of these contaminants at high concentration levels contributes negatively to air quality, affecting human health and the future of our planet.

Reliable measurement of air pollutants is crucial for accurately assessing the environmental impact, as obtaining an inaccurate result can lead to misinterpretations. One way to achieve this, is by using gas analysers that were calibrated against certified reference materials of gas mixtures to ensure metrological traceability.

In this study, a flue gas analyser was calibrated in carbon dioxide and carbon monoxide in the instrument measurement range, quantifying the error with its uncertainty at each calibration level.

Calibration is the process of comparing the measurement values of an instrument against known reference values to characterise the error in different readings.
When calibrating a gas analyser, the reference values could be obtained using primary reference materials of gas mixtures (PRMs). These mixtures are highly accurate well-characterized standards, which allow establishing direct traceability to the International System of Units (SI).

One calibration strategy is to use several calibration points. These points are typically chosen to cover the intended operating range of the instrument or to target specific areas of interest where accuracy is critical.

OBTAINED DATA

Carbon dioxide

Readings (% mol/mol)	Level 1	Level 2	Level 3	Level 4
1	0.41	5.2	9.8	19.7
2	0.42	5.3	9.8	19.1
3	0.41	5.2	9.8	20.0
4	0.42	5.3	9.8	19.7
5	0.42	5.3	9.9	19.6
6	0.42	5.3	10	19.7
7	0.42	5.4	9.8	19.7
8	0.42	5.3	9.8	19.6
9	0.41	5.2	9.9	19.7
10	0.42	5.2	9.7	19.6
Reference value (% mol/mol)	**0.20**	**5.0**	**10.0**	**20.0**
Average (% mol/mol)	0.42	5.3	9.8	19.6
Error (% mol/mol)	0.2210	0.267	-0.166	-0.37
Standard deviation (% mol/mol)	0.0015	0.0313	0.0260	0.0703
Expanded uncertainty (% mol/mol)	0.0065	0.073	0.078	0.15

Carbon monoxide

Readings (ppm)	Level 1	Level 2	Level 3	Level 4
1	486	978	1466	1948
2	488	978	1467	1948
3	489	978	1467	1948
4	489	979	1468	1949
5	489	979	1468	1949
6	489	980	1468	1949
7	490	980	1468	1949
8	490	980	1468	1950
9	490	981	1468	1950
10	490	981	1468	1950
Reference value (ppm)	**501**	**1001**	**1502**	**2002**
Average (ppm)	489	979	1468	1949
Error (ppm)	-11.6	-21.1	-33.9	-52.8
Standard deviation (ppm)	0.38	0.17	0.22	0.26
Expanded uncertainty (ppm)	1.8	2.2	3.1	4.1

METHODOLOGY

Gas analyser calibration was performed by using:

- 4 PRMs of Carbon Monoxide in Nitrogen – (500 to 2000) ppm.
- 4 PRMs of Carbon Dioxide in Nitrogen – (0.2 to 20) % mol/mol.

These concentrations cover the full span of the instrument's measurement capability and encompass a range commonly encountered in environmental monitoring scenarios. The gas analyser was kept in the laboratory along with the PRMs for at least one hour to achieve thermal equilibrium. The calibration procedure began with the PRM containing the lowest amount fraction and after stabilizing its flow, ten measurements were taken. This process was repeated for each PRM, increasing the amount of fraction. Following PRMs measurement, a zero gas (nitrogen 5.0) was measured ten times.

The error was determined as: $Error = \overline{Measurement} - PRM_{Ref\ value}$

The uncertainty associated to the $Error$ was determined as:

$$u_{Error,\,reading} = \sqrt{u^2_{Repeatibility} + u^2_{Division} + u^2_{Ref\ value}}$$

RESULTS AND CONCLUSIONS

The gas analyzer was calibrated using PRMs, establishing traceability to the SI and ensuring reliable and accurate measurements. This calibration process ensures the data quality in environmental monitoring, supports the development of effective mitigation strategies and promotes the adoption of sustainable practices for environmental protection.

Accurate quantification of CO and CO_2 concentrations enables thorough environmental impact assessments, facilitating informed decision-making and policy formulation to protect ecosystems, public health and overall environmental well-being.

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Poster presentation at Congreso Uruguayo de Química Analítica - CUQA (2022)

Desarrollo de un material de referencia certificado de dióxido de carbono en nitrógeno.

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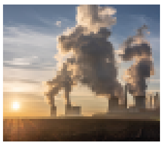
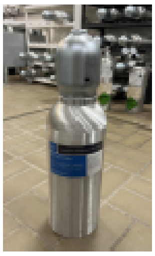
Introducción



La concentración de dióxido de carbono en la atmósfera está incrementando año a año, viéndose reflejada en un aumento de la temperatura de la tierra, lo que se conoce como "efecto invernadero". Por este motivo, es de suma importancia que este gas pueda medirse de manera precisa y confiable. Una forma de lograr esto, es mediante el desarrollo de materiales de referencia certificados (MRC) de mezclas gaseosas, para la calibración de equipos de análisis de calidad del aire. Estos contienen una fracción de cantidad de masa exactamente conocida de distintos gases, y permiten brindar trazabilidad metrológica a las mediciones de contaminantes atmosféricos. En este trabajo se desarrolló un MRC de dióxido de carbono de fracción de cantidad de masa igual a 35 %mol/mol, en balance nitrógeno.



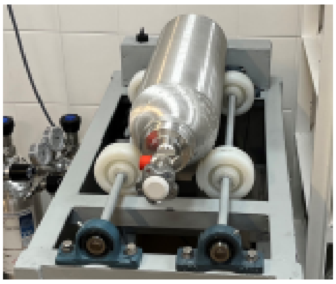
Desarrollo de MRC de mezclas gaseosas

La primer etapa en el desarrollo de un MRC de mezcla gaseosa es la planificación. Se debe evaluar:

- ✓Presión final de la mezcla < Presión de trabajo cilindro.
- ✓Presión de llenado < Presión de vapor del punto de rocío.
- ✓Reacciones entre los componentes de la mezcla.
- ✓Reacciones de los gases con el material del cilindro (ISO 16664 e ISO 11114).
- ✓Masa mínima a agregar > 20 g (ISO 6142).

Estos puntos minimizan peligros asociados al proceso de llenado y previenen efectos significativos en la composición de la mezcla.

El cálculo de la cantidad de gas a agregar en el cilindro se realiza utilizando la ley de los gases ideales.



La segunda etapa es la de preparación. La mezcla gaseosa fue preparada en un cilindro de aluminio.

- Debe limpiarse mediante el agregado de nitrógeno 6.0 y homogeneización por al menos 30 minutos.
- Se conecta a la estación de llenado/vaciado para hacerle un vacío de al menos 12 horas, de manera de remover las impurezas retenidas.
- Se llena primero con el gas analito, dióxido de carbono, y luego con el gas balance, nitrógeno. La cantidad agregada se controla en el display de la balanza.
- La mezcla se homogeneiza por 6 horas.

Por último se realiza la asignación de valor, donde se inyectan a un cromatógrafo de gases Thermo Scientific modelo Trace 1300, con detector de conductividad térmica:

- Mezcla gaseosa preparada
- 6 PRM de CO_2 en N_2 del Instituto Nacional de Metrología de los Países Bajos, VSL.

Los PRM (materiales de referencia primarios) tienen la mayor jerarquía metrológica, dado que son preparados mediante gravimetría según la norma ISO 6142 y verificados según la norma ISO 6143, y son trazables directamente al Sistema Internacional de Unidades.

<u>Metrología de Gases:</u>

- Incertidumbre en ambas variables (estímulo y respuesta)
- Función matemática que describe la relación entre variables no lineal

➔Uso de software XLGENLINE versión 1.1 para cálculo de la fracción de cantidad de masa e incertidumbre.

Fracción de cantidad de sustancia obtenida: (0,3449 ± 0,0027) mol/mol

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