21GRD04 - isoMET

D5: Good practice guide for the analysis of CH_4 source gases for $\delta^{13}C(CH_4)$ and $\delta^{2}H(CH_4)$ (part1) or $\Delta^{13}CH_3D$ and $\Delta^{12}CH_2D_2$ (part 2) by OIRS and IRMS, including sample handling, purification, optimised analytical procedures and traceability to the international standards and target uncertainties $\delta^{13}C(CH_4)$: 0.2 ‰, $\delta^{2}H(CH_4)$: 2 ‰, $\Delta^{13}CH_3D$: 0.5 ‰, $\Delta^{12}CH_2D_2$: 2 ‰.

Organisation name of the lead participant for the deliverable: Empa

Due date of the deliverable: 31.05.2025

Actual submission date of the deliverable: 13.08.2025

Confidentiality Status: PU - Public, fully open (remember to deposit public deliverables in a trusted repository)

Deliverable Cover Sheet

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The project has received funding from the European Partnership on Metrology, co-financed from the European Union's Horizon Europe Research and Innovation Programme and by the Participating States.

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1 Summary

The aim of task 2.3 is to provide CH_4 isotope source data traceable to the VPDB and VSMOW/SLAP scales for emission categories relevant at European scales but currently understudied and underrepresented. To support this task, protocols towards traceable and representative CH_4 isotope source signatures were developed. Work builds on OIRS and IRMS techniques and CH_4 isotope reference materials developed in the EMPIR JRP 19ENV05 STELLAR. In addition, analytical methods to analyse $\Delta^{13}CH_3D$ and $\Delta^{12}CH_2D_2$ were developed and evaluated to provide additional dimensions to databases and to evaluate their potential for source appointment

This good practice guide for accurate analysis of $\delta^{13}C(CH_4)$ and $\delta^{2}H(CH_4)$ (part 1, UHEI) and $\Delta^{13}CH_3D$ and $\Delta^{12}CH_2D_2$ (part 2, VTT) by OIRS and IRMS provides a number of key findings in order to achieve accurate analysis within target uncertainties $\delta^{13}C(CH_4)$: 0.2 ‰, $\delta^{2}H(CH_4)$: 2 ‰, $\Delta^{13}CH_3D$: 0.5 ‰, $\Delta^{12}CH_2D_2$: 2 ‰.

Part 1: $\delta^{13}C(CH_4)$ and $\delta^{2}H(CH_4)$:

- *Sampling techniques:* Sufficient enhancement of CH_4 concentration above background levels is required to extract accurate delta values of source signatures. Materials applied for gas handling / storage have to be tested to assure minimal artifacts; as an example, glass or stainless steel flasks generally offer preferable performance over multi-layer sampling bags.
- *Instrument characterisation:* To assure accurate analysis the following performance characteristics have to be determined; Allan precision, amount fraction dependence, matrix gas effects, water vapour and other interferant effects.
- *Calibration:* High-purity CH_4 source characterised for $\delta^{13}C(CH_4)$ and $\delta^{2}H(CH_4)$ by IRMS or ORIS are required to prepare reference materials diluted in an appropriate gas matrix for analyser calibration. This is particularly challenging for biogenic sources. Agreement of analytical techniques within uncertainty targets is assured by inter-laboratory comparison (see D4).
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Part 2: $\Delta^{13}CH_3D$ and $\Delta^{12}CH_2D_2$:

- *Sampling techniques:* Different manual and automated sample extraction and purification techniques have been developed for use on different sample qualities (ambient air to fossil CH_4) and analytical requirements (1 to 20 mL of pure CH_4). Details are provided in section 3.2.
- *Instrument development, characterisation and data correction:* A major challenge of $^{13}CH_3D$ and $^{12}CH_2D_2$ analysis is the low abundance of the clumped isotope analyte molecules, which requires careful selection of instrumental parameters (see section 3.3). In addition, optimized automated setups for gas handling / injection into the HR-IRMS / OIRS devices have been established to facilitate reproducible sample / reference switching. Artifacts on apparent clumped isotope abundances based on differences in bulk isotopic composition have to be corrected.
- *Calibration:* Referencing of $\Delta^{13}CH_3D$ and $\Delta^{12}CH_2D_2$ is provided by thermal equilibration of CH_4 gases over a catalyst. Successful equilibration can be assured by dissipating of an isotope spike over time. Agreement of analytical techniques within uncertainty targets is assured by inter-laboratory comparison (see D4).

2 Good practice guide for the analysis of CH_4 source gases for $\delta^{13}C(CH_4)$ and $\delta^{2}H(CH_4)$ by OIRS and IRMS (A2.3.1; Martina Schmidt (UHEI))

2.1 Objective

UHEI, RHUL, Empa, PTB and UU developed a protocol to determine CH4 isotopic source signatures traceable to VPDB ($\delta^{13}C(CH_4)$) and VSMOW/SLAP ($\delta^{2}H(CH_4)$). The protocol encloses sampling techniques, preconcentration in particular for $\delta^{2}H(CH_4)$, removal of interferant gases, calibration by diluted CH_4 RMs prepared in A1.1.2 and data correction algorithms as well as procedures to extract source signatures. It builds on the good practice guide for accurate methane isotope ratio measurements using laser spectroscopy: analyser characterisation and statement of uncertainty with a target precision of 0.2 ‰ for $\delta^{13}C(CH_4)$ and 1 ‰ for $\delta^{2}H(CH_4)$ written within EMPIR JRP 19ENV05 STELLAR.

Within the EMPIR JRP 19ENV05 STELLAR project a “Good practice guide for accurate methane isotope ratio measurements using laser spectroscopy” was already developed, which is taken as the basis for this report (Rennick 2023). Additional experience from more recent publications as well as from the European ICOS measurement network and an IAEA Guideline for the accurate measurement of CH_4 isotopes are also included (Sperlich, Camin et al. 2024).

2.2 Sampling techniques

In order to determine $\delta^{13}C(CH_4)$ and $\delta^{2}H(CH_4)$ from specific CH_4 emitter using flask or bag samples, air must be collected both downwind of the CH_4 source—across the emission plume—and upwind, to establish background concentrations of CH_4. For accurate determination of the CH_4 source signature, the Keeling plot approach requires a sufficient enhancement of CH_4 concentration above the background level to provide a precise sources signature determination. The minimum required CH_4 enhancement depends on the precision of the measurement instrumentation as shown in (Hoheisel, Yeman et al. 2019).

It is essential that the collected air samples remain unaffected by sampling artefacts. Therefore, all materials used in the air inlet system, as well as the sampling flasks and bags, must not alter the composition of the sample and must store the air without modification until analysis to avoid isotope fractionation effects. For isotopic studies air samples are typically collected in glass flasks or multi-layer foil sampling bags (Menoud, Van Der Veen et al. 2022). Glass flasks offer high sample stability for CH_4 measurements but are fragile and therefore require careful handling and transport. In contrast, multi-layer foil bags are lightweight and easy to transport, making them suitable for fieldwork under challenging conditions. However, they are more susceptible to storage-related effects compared to metal or glass containers. Careful handling is essential, and storage tests are necessary to determine how long samples can be stored without changing their chemical or isotopic composition prior to analysis.

Flask and bag samples can be analysed in the laboratory using isotope ratio mass spectrometry (IRMS) or optical isotope ratio spectrometers (OIRS). The measurement sequence includes the use of reference gases calibrated against international standards—VPDB for $\delta^{13}C(CH_4)$ and VSMOW/SLAP for $\delta^{2}H(CH_4)$. When analysing air samples collected near CH_4 emission sources and measured subsequently in the laboratory, the following aspects should be considered:

- Gas samples taken directly from CH_4 emitters or in close proximity (e.g., biogas plants, landfill gas collection systems, or wastewater treatment facilities) often contain CH_4 mole fractions that exceed the calibrated measurement range of the analyser. In such cases, samples must be diluted to fall within the instrument's operational range. Hoheisel, Yeman et al. (2019)described a practical dilution method, in which 30–100 µL of a natural gas sample is transferred using a gas-tight syringe into a 3 L sample bag filled with synthetic air.
- Samples collected near emission sources may also contain elevated levels of interfering trace gases, such as ethane, which can affect isotopic analyses. These compounds must either be removed prior to analysis or their influence must be corrected (see Section 2.2.3). Additionally, samples with high water vapour content must be dehumidified during collection to prevent condensation inside the flasks or bags.

- Samples with low CH_4 mole fractions, such as soil-air samples, often yield poor data quality and require preconcentration to enable accurate isotopic measurements. This is particularly critical for $\delta^2H(CH_4)$ analyses. Preconcentration techniques using laser spectroscopy, as described by (Eyer, Tuzson et al. 2016), are recommended in such cases (see Section 2.2.4).

2.3 Instrument Characterisation for Optical Isotope Ratio Spectrometers (OIRS)

Each OIRS instrument—even within the same model series—exhibits individual characteristics that can significantly influence measurement accuracy and precision. Therefore, every device must be characterised and calibrated individually. The 19ENV05 STELLAR Guideline provides a comprehensive overview of recommended testing procedures and known influencing factors (Rennick 2023). Below, key aspects of instrument characterisation are summarised, with reference to more detailed descriptions in the guideline and other publications.

Instrument stability (Allan-Werle Variance and temperature stability)

To assess long-term precision and drift, each OIRS should undergo stability testing over a period of at least 36 hours, using a stable reference gas from a high-pressure cylinder. The data should be evaluated using the Allan-Werle variance analysis (Werle 2011), which helps determine the optimal averaging time for minimising noise without introducing drift effects. This analysis provides input for defining the measurement averaging time and calibration frequency. Instruments may show sensitivity to room temperature fluctuations, and temperature control or compensation may be necessary to ensure short-term stability.

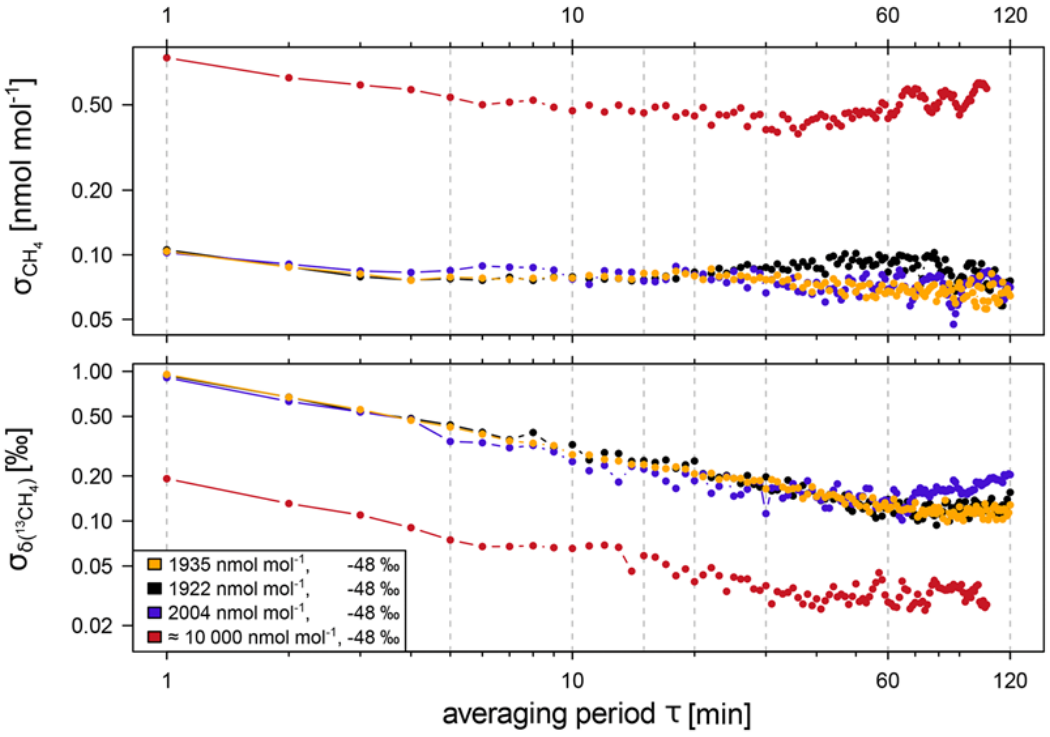

Figure 1: Allan standard deviations for CH_4 mole fraction and $\delta^{13}C(CH_4)$ determined for the CRDS G2201-i analyser and different CH_4 mole fractions and isotope ratios. The Allan standard deviations are based on ambient air mole fractions (1922-2004 ppb CH_4) measurements (orange, black and blue) and on a 10 000 ppb CH_4 (red). This figure is taken from the study of (Hoheisel and Schmidt 2024).

Figure 1 shows an example of the Allan-Werle variance analysis for CH_4 mole fraction and $\delta^{13}C(CH_4)$ measurements, determined using a CRDS G2201-i analyser (Hoheisel and Schmidt 2024). The Allan standard deviations are presented for different CH_4 mole fractions and isotopic compositions. The data include measurements of high-pressure cylinders with CH_4 mole fractions ranging from 1922 to 2004 ppb (shown in orange, black, and blue), as well as measurements from a high-pressure cylinder containing 10,000 ppb CH_4 (shown in red). This analysis illustrates how the instrument's noise characteristics depend on both averaging time and CH_4 mole fraction. Notably, measurements at elevated CH_4 concentrations (e.g., 10,000 ppb CH_4, shown in red) result in a significantly improved precision for $\delta^{13}C(CH_4)$ compared to measurements at ambient CH_4 levels, highlighting the positive effect of higher signal strength on isotope ratio accuracy.

Amount fraction dependency of isotope ratio (linearity)

Changes in the CH_4 amount fraction may produce feedback on apparent isotope delta values due to a non-linear response of the analyser on changes in isotopologue amount fraction, this is often referred to as 'linearity calibration'.

OIRS instruments can show a dependency of measured isotopic ratios on the CH_4 mole fraction, commonly referred to as a linearity effect. This occurs when changes in CH_4 concentration cause nonlinear responses in the detector's signal, often due to nonlinear absorption of individual isotopologues, cell conditions (pressure, temperature) and instrument-specific factors such as laser line width, fitting algorithms, and background correction. While many instruments include a factory-applied linearity correction, these corrections are often based on standard test conditions and may not hold true under all field conditions (Rella, Hoffnagle et al. 2015).

Therefore, it is recommended to perform an independent linearity calibration by stepwise diluting a high-pressure cylinder containing CH_4 at a concentration of 10 ppm or higher to produce a series of samples with varying CH_4 mole fractions. These samples should then be analysed to assess how the measured isotopic ratios ($\delta^{13}C(CH_4)$ and $\delta^{2}H(CH_4)$) respond to changes in CH_4 concentration. This procedure helps identify non-linearities in the analyser's response and ensures that appropriate correction factors can be applied.

Matrix gas effects

Changes in the gas matrix composition—particularly in the proportions of N_2, O_2, Ar, and CO_2—can influence absorption spectra due to changes in pressure broadening and line shape parameters. This can shift the apparent isotope ratio, especially in high-resolution laser-based systems, when the reference material and sample have different gas matrix. To minimise matrix effects reference gases should closely match the ambient air matrix composition. If this is not (e.g., use of synthetic air), the impact should be quantified, and matrix-specific correction factors applied.

Water vapor effect

Water vapour is a major interfering species in CH_4 isotope measurements. It affects both the spectral baseline and isotopologue-specific absorption features, especially for $\delta^{2}H(CH_4)$. Although most OIRS instruments include a built-in water vapour correction, studies have shown that this is often insufficient to reach the required precision for isotopic analysis (Eyer, Tuzson et al. 2016, Hoheisel, Yeman et al. 2019).

The best method to avoid cross-interferences from H_2O is physical drying of the sample air using a nafion dryer or cryogenic trap prior to analysis. Alternatively, conducting humidity tests to quantify the water vapour influence and applying empirical correction factors.

2.4 Conclusion

High-precision measurements of CH_4 source signatures—achieving uncertainties of approximately ±0.2‰ for $\delta^{13}C(CH_4)$ and ±2‰ for $\delta^{2}H(CH_4)$—are feasible with Optical Isotope Ratio Spectrometers (OIRS), provided a dedicated measurement protocol is followed.

For $\delta^{2}H(CH_4)$ analysis, preconcentration is essential, as current laser systems require CH_4 mole fractions around 550 ppm to achieve sufficient sensitivity. In addition to increasing CH_4 levels, preconcentration also removes major air components (N_2, O_2, Ar) and potential interferants such as CO_2 and H_2O.

$\delta^{13}C(CH_4)$ measurements benefit from the elevated CH_4 concentrations typically found in emission plumes, often allowing direct analysis without preconcentration. However, source-proximal samples may contain interfering trace gases, such as ethane. which can bias isotope readings and must be accounted for.

Each OIRS device shows individual response characteristics, including sensitivity to matrix composition, water vapour, and drift. Therefore, every analyser must undergo instrument-specific characterisation, including tests for stability (e.g., Allan variance), linearity with CH_4 concentration, and interference effects.

Because source samples can exhibit large variations in CH_4 mole fractions, linearity testing is especially important. OIRS systems often display a concentration-dependent bias in isotopic readings that must be quantified and corrected.

Calibration of these instruments relies on primary reference materials (PRMs)—gravimetrically prepared CH_4-in-N_2 mixtures—derived from a high-purity CH_4 source characterised for $\delta^{13}C(CH_4)$ and $\delta^{2}H(CH_4)$ by IRMS or ORIS.

3 Good practice guide for the analysis of CH4 source gases for $\Delta^{13}CH_3D$ and $\Delta^{12}CH_2D_2$ by OIRS and IRMS (A2.3.2; Mehr Fatima (VTT))

3.1 Objective

VTT, Empa, UU and UofG developed a protocol to determine CH4 isotopic source signatures, $\Delta^{13}CH_3D$ and $\Delta^{12}CH_2D_2$, traceable to stochastic distribution. The protocol encloses sampling techniques, extraction of large amounts of CH_4 for analysis of rare isotopologues, removal of interferant gases, correction for any remaining interferences, calibration using pure CH4 to be value assigned in A2.2.5 and data correction algorithms.

3.2 CH_4 extraction, purification and removal of interferants

Automated preconcentration (Empa)

At Empa, an automated preconcentration device, named CleanEx, was developed, which is based on cryogenic adsorption and temperature-controlled desorption and isolates methane from other atmospheric gases [1]. The maximum adsorption capacity of the device is around 18 L, mainly dependent on trap temperatures and sample flow rates. At high CH_4 concentrations in the sample gas, sufficient CH4 amounts for $\Delta^{13}CH_3D$ (0.12 mmol) or even $\Delta^{12}CH_2D_2$, analysis (0.41 mmol) can be separated (Prokhorov and Mohn 2022). The schematic for the device is shown in Figure 2. The following steps describe the device operation:

Sample preparations: Before introduction into the CleanEx device the sample gas is dehumidified using permeation drying and pressurized to 4 to 5 bars overpressure to allow sufficient flows over the primary trap.

Adsorption on primary trap (T1): The primary trap consists of an electro-polished stainless-steel tube (4 mm o.d., total length 1 m) packed with HayeSep D and coiled around an aluminum standoff. During CH_4 adsorption the trap is cooled down to approx. –170°C by pressing the aluminum standoff against a copper baseplate, maintained at −215 °C using a Stirling cryocooler. At the provided temperature CH_4 is adsorbed together with other trace gases with higher boiling points, e.g., CO_2 and N_2O, while permanent gases with similar boiling point to CH_4, such as O_2 and Kr are enriched, i.e. partly retained.

Temperature-controlled desorption: For this, T1 is detached from the baseplate and heated by a polyamide heating pad. The temperature of T1 is gradually increased and purged with N_2, to first desorb O_2, second CH_4 and finally high-boiling trace gases (CO_2, N_2O, H_2O).

Secondary cryo-focusing trap (T2): Following desorption from T1 the gas fraction containing CH_4 gas is directed towards a secondary cryo-focusing trap (1/16” o.d., 5.5 mg HayeSep D). This trap is mounted on an aluminum stand-off permanently mounted on the copper baseplate. The main function of T2 is to separate CH_4 from residual atmospheric contaminants (O_2).

Sample transfer to QCLAS: After desorption from T2 the CH_4 sample gas is introduced into the QCLAS multipass cell for analysis applying a flow of high-purity N_2 gas.

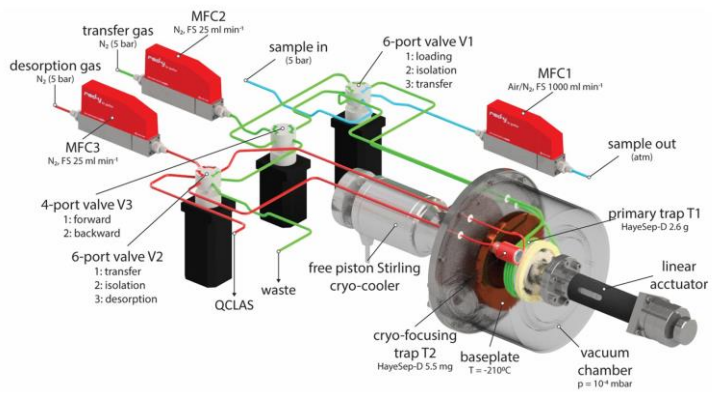

Fig. 2: Simplified depiction of the CleanEx flow layout for methane preconcentration and introduction into a QCLAS device (Prokhorov and Mohn 2022). Multiport valve V1 controls the sample flow through the primary trap (T1) or isolates the trap. V2 changes the flow direction (forward vs backward) through T1. V3 controls the sample transfer onto the cryo-focusing trap (T2) by connecting the traps in series or isolates T2. Mass flow controllers MFC1−3 regulate the flows of the sample, transfer, and desorption gas, respectively. The stainless-steel vacuum chamber, shown transparent for clarity, is evacuated with a turbo-molecular pump to avoid condensation on the cooled parts.

CH_4 extraction and purification at Utrecht University

The extraction and purification methodology developed at Utrecht University for methane works for a wide concentration spectrum, from high (>1%) and medium (0.1-1%) to low concentrations (<0.1%), including atmospheric air with CH_4 concentrations as low as ~2 ppm (0.0002%). This approach is valid for clumped isotope analysis, specifically $\Delta^{13}CDH_3$ and $\Delta^{12}CD_2H_2$ [3]. The schematic for the extraction and purification of methane used by Utrecht University is presented in Figure 3 (Sivan, Röckmann et al. 2024).

LCES

The extraction of methane from large volume of air is achieved by cryogenic trapping through a series of steps using charcoal traps. This ensures methane concentration and is employed in low-concentration extraction system (LCES). The system comprises a glass tube fitted with high-vacuum valves, an empty glass trap (GT), two Russian Doll Traps (RDT1 and RDT2), and two charcoal traps (CT1 and CT2). This system was designed for carbon isotope analysis but works perfectly for methane samples also. It removes water and carbon dioxide from air samples, followed by a pre-concentration phase that selectively collects CH_4, thereby increasing its concentration for further analysis.

The air samples from the environment or the cylinder, undergo initial drying step. It is done in the glass trap at -70°C with a dry ice-ethanol slurry and further drying is carried out with a $Mg(ClO_4)_2$ tube. RDT1 and RDT2 are cooled to -196°C with liquid nitrogen to scrub out condensable gases like CO_2, N_2O, H_2O from the sample. The CO_2 removal using this step and the sample is sent to the CT1 where, CH_4 is trapped at -196°C. The pressure and temperature are monitored throughout to prevent condensation and freezing within the system, using a Mass Flow Controller (MFC) to maintain optimal flow and pressure.

Once a substantial volume of air has been processed, the collected air in CT1 is warmed to 70°C to separate N_2 and O_2 from CH_4. The system then transfers the CH_4 to CT2, which is cooled again to -196°C for further concentration. These controlled conditions ensure efficient collection and concentration of CH_4 for high-concentration extraction.

For medium CH_4 concentration samples (0.1-1%), the initial stages are bypassed, and the sample is directly trapped in CT2, following the same subsequent procedure. To prevent contamination from previous samples, RDTs and CTs are thoroughly cleaned with pure N_2 while being heated. This comprehensive extraction method allows for the efficient collection of CH_4 from large volumes of air, facilitating further high-concentration extraction and analysis.

HCES

The High-Concentration Extraction System (HCES) is designed for extracting methane from samples with methane concentration above 1%. 200 mL of sample gas can be handled efficiently by the system. It is based on two silica gel-filled traps (Trap A,B), and two empty traps (Trap C,D) with a gas chromatograph (GC). It also includes a passive thermal cavity detector (TCD). All these components are connected using stainless steel tubing and Swagelok valves, inspired by the design from (Young, Kohl et al. 2017). The extraction process is started by the GC separation of methane from the sample gas. It is then cryogenically collected on the silica gel in trap A, which is cooled down to -196 °C with liquid nitrogen. The precise control of helium flow and temperature adjustments facilitates the transfer of CH_4 from Trap A to the GC for further purification.

IN HCES, the purification stage employs two specially designed GC columns: a 5-meter column for removing gases like H_2, Ar, O_2, and N_2, and a 2-meter column for isolating CH_4 from heavier hydrocarbons, ensuring the extraction of pure CH_4 within 55 minutes.

For samples with CH_4 concentrations exceeding 5% in air, CH_4 is collected post the elution of gases like O_2 and N_2 in Trap B, where it is cryogenically isolated. In cases where the initial GC run does not resolve CH_4 from O_2 and N_2 peaks due to the large sample size, a secondary purification run is required. Additionally, for atmospheric samples containing krypton (Kr), the GC columns are operated at a reduced temperature of 40 °C, as opposed to the usual 50 °C, to achieve the necessary separation of CH_4 from Kr, showcasing the system's adaptability to various sample compositions.

Post-extraction, the GC columns are subjected to a baking process to eliminate any residual impurities, preparing them for subsequent extraction cycles. Similarly, Traps A and B are thoroughly cleaned and prepared for future use, ensuring the system's readiness and reliability for precise CH_4 extraction.

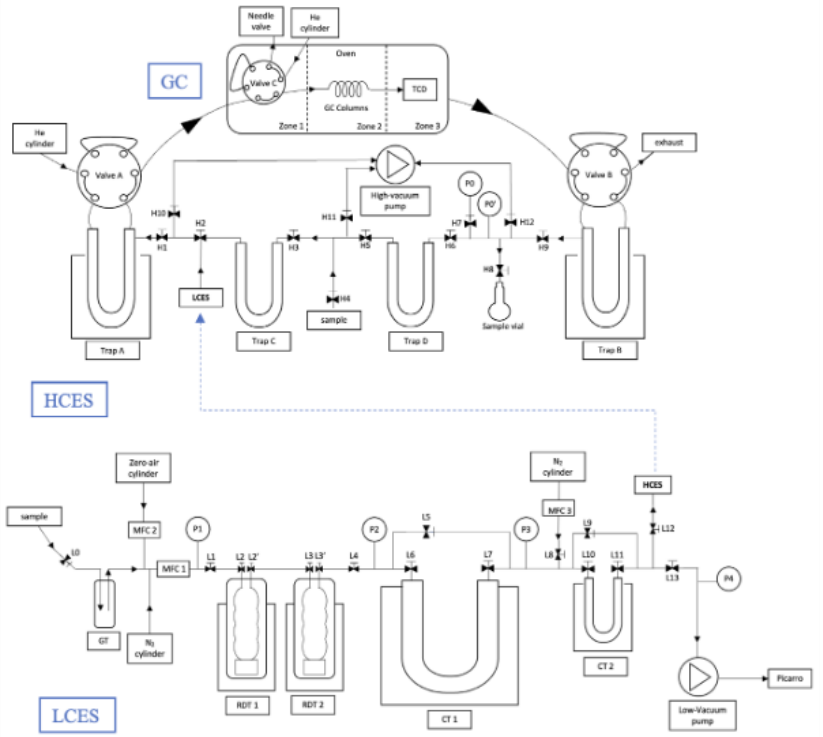

Fig. 3: Schematic of high-concentration extraction system (HCES) and low-concentration extraction system (LCES) and the GC setup at IMAU. Samples are introduced to the HCES via H4 and to the LCES via L0. The pre-concentrated sample in CT2 is transferred to Trap A via a connection between L12 and H2 (Sivan, Röckmann et al. 2024).

CH_4 extraction and purification at the University of Glasgow

At the University of Glasgow, methane samples are purified using a Janis helium-cooled cryostat with precise temperature controls to remove selectively gases with different boiling temperatures, similar to the method described in (Stolper, Martini et al. 2015), shown in Figure 3. The method described below is suitable for samples with methane concentrations of >1%.

System Description

The purification system is a glass vacuum line with an oil-less pumping system (turbo pump backed by a scroll pump) and Teflon valves. The line is equipped with a Janis cryostat, cooled with liquid He and able to reach temperatures of 10K. The trap itself does not use charcoal or adsorbents to trap the gases and the volume that can reach T<20K is sufficient to trap more than 400 mL of gas at ambient temperature and 1 atmosphere. This means that for a gas sample with a 1% methane content, it is possible to trap an amount of methane sufficient for a measurement with the HR-IRMS (~2 mL). The line has two spiral U-traps (shown as U shapes in the schematics) to allow for trapping other gases (e.g., water vapour), and two traps filled with silica gel.

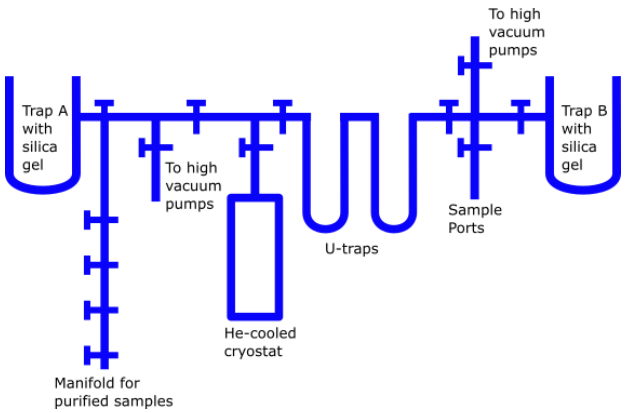

Fig. 3: Schematics of the methane purification glass line at the University of Glasgow

Purification Process

Sample vessels are connected to the sample port and the cryostat is set to 20K. The first U-trap is immersed in dry ice – propanol slush to trap water vapor and the second in liquid nitrogen to trap N_2O, CO_2 and higher alkanes that could be present. Methane and other gases such as N_2 and O_2 will be trapped in the cryostat, with only potentially H_2 and He left in the gas phase. The pressure is monitored with a wide-range gauge until a steady value is obtained, and He and H2 can then be evacuated. Methane is then separated from other gaseous species by cycling the temperature of the cryostat between 45 and 75K. At 45K, the partial pressure of N_2, Ar and O_2 allows for evacuation of these gases without affecting methane. We observed however that one purification step was not enough to quantitatively remove N_2 and O_2 from methane, possibly because a fraction of these gases stayed trapped in the condensed methane at 45K. We therefore heat the cryostat to 75K, wait for 5 minutes and cool back to 45K repeatedly until the pressure observed at 45K above the cryostat is below 5.10-3 mbar.

Methane is then transferred to sample tubes containing molecular sieves or silica gel and immersed in immersed in liquid nitrogen by setting the temperature of the cryostat to 75K. The sample tube can then be disconnected from the line and connected to the HR-IRMS for analysis of the gas.

Methane purification using a Gas Chromatography system that is also connected to the vacuum line is currently being tested and validated and will be detailed in the coming months. The main limitations of the cryogenic method described above is the separation of Kr from methane for atmospheric samples, and repeated cycles and long time necessary for the separation of methane and O_2 when the latter is more than ten times more abundant than methane in the samples.

3.3 Sample analysis and calibration using pure CH_4

High-resolution IRMS (UU, UofG)

Instrument Overview

Methane (CH_4) isotopic compositions are measured using the Thermo Scientific Ultra HR-IRMS at Utrecht University, operated with Qtegra™ software. The instrument allows for high-resolution measurements with selectable slit widths of 250, 16, and 5 µm, as well as an additional HR+ “aperture” option for enhanced resolution. The Ultra employs a mass analyser with a variable detection system comprising nine Faraday detectors and compact discrete dynode (CDD) ion counting detectors, with resistances ranging from 3 × 108 Ω to 1013 Ω, enabling precise isotope detection. Clumped isotope measurements are conducted in high-resolution mode (HR+) across three specific configurations that focus on different isotopologues. A full measurement cycle takes approximately 20 hours. Maintenance includes replacing narrow source slits every six months or sooner, as carbon deposits reduce ion transmission and sensitivity, especially if ion counts fall below 50% of initial levels.

Instrumental Stability

According to the Thermo manual, the instrumental stability for $\delta^{13}C$ measurements involves typical intensities of 13CH4 at ~ 1 × 107cps, resulting in a shot noise of σ = 3×103cps for 1-second integrations, combined with Johnson-Nyquist noise of 6×103cps, achieving an expected standard error of 0.01% [7]. For δD, the long method yields shot noise of σ = 2 × 102cps for $^{12}CH_3D$ intensities of ~ 6 × 104cps, with a standard error of 0.12‰. In the short method, δD intensities of ~ 5 × 105cps produce a shot noise of σ = 7 × 102cps, with a standard error of 0.15‰. Clumped isotope measurements include $\Delta^{13}CH_3D$ at 5 × 103cps (σ = 7 × 101cps, 0.28‰ standard error) and $\Delta^{12}CH_2D_2$ at ~ 1 × 102cps, achieving a standard error of 1‰ for 18-hour integrations or 2‰ for shorter durations of 7 hours.

Measurements

Clumped isotope measurements are performed across three configurations over 20 hours. Peak centers remain stable, with mass shifts corrected hourly using the software. For D(CH_4), collectors include L1 (10 Ω) for $^{12}CH_4$ and H4-CDD for $^{12}CH_3D$, focusing on their respective abundances. For C(CH_4) and CH_3D, the collectors include L3 (10 Ω) for $^{12}CH_4$, L1 (10 Ω) for $^{13}CH_4$, and H4-CDD for $^{13}CH_3D$, targeting their abundances. For CH_2D_2, L3, L1, and H4-CDD measure $^{12}CH_4$,$^{13}CH_4$, and $^{12}CH_2D_2$, respectively. Achieved precisions

approach the shot noise limit for $\delta^{13}C$, $\delta^{13}CDH_3$, and $\delta^{12}CD_2H_2$, while δD precision is approximately twice as noisy due to high count rates near the CDD detector limit.

Temperature Equilibration Experiments

These experiments evaluate isotopologue equilibrium using the AP613 reference gas. Measured values for $\Delta^{13}CDH_3$ and $\Delta^{12}CD_2H_2$ are 2.23 ± 0.12‰ and 3.1 ± 0.9‰, respectively. Measurements deviate from thermodynamic equilibrium curve; formation temperature for AP613 cannot be assigned.

Internal Precision and Reproducibility

For the AP613 zero-enrichment gas, mean values over three years are $\Delta^{13}CDH_3$ = 2.3 ± 0.1‰ and $\Delta^{12}CD_2H_2$ = 3.2 ± 0.3‰. Precision values for $\Delta^{13}CDH_3$ are 0.3 ± 0.1‰, and for $\Delta^{12}CD_2H_2$, they are 2.4 ± 0.8‰. A modified procedure for small samples (< 2 mL CH_4) involves longer measurements of $^{12}CD_2H_2$ and shorter durations for $^{12}CDH_3$ to optimise precision, with a ”Factor worse” metric comparing measurements to the noise limit.

Inter-Laboratory Calibration

Isotopic measurements of AP613, CAL1549, and IMAU-3 gases were compared between Utrecht University (Thermo Ultra) and the University of Maryland (Nu Panorama). The values derived from heating experiments at Utrecht align well with measurements of non-heated AP613 on the Panorama, confirming inter-laboratory consistency.

Extraction Test with Known Gas

Extraction tests evaluated isotopic fractionation and contamination, showing most measurements within the expected 1 uncertainty range. Outliers beyond 2 were resolved by extending silica gel conditioning at 150°C. The presence of krypton (Kr) was identified as a source of significant bias and must be removed prior to CH_4 measurements.

Chromatographic Separation

Chromatographic separation requires 3 ± 1 mL CH_4 for precise $\Delta^{13}CDH_3$ and $\Delta^{12}CD_2H_2$ measurements. Samples undergo purification via gas chromatography (GC). For high-volume samples (> 100 mL), an additional GC round separates O_2 and N_2. For atmospheric CH_4, GC columns are maintained at 40°C to facilitate Kr removal.

Quantum cascade laser absorption spectroscopy (Empa, VTT)

Laser spectroscopy:

At Empa a customized dual-laser trace gas monitor (QCLAS, Aerodyne Research Inc., USA) was employed (Zhang, Prokhorov et al. 2025). Using the high resolution FTIR spectra of $^{13}CH_3D$ and $^{12}CH_2D_2$ recorded by PTB (A2.2.1) improved spectral windows for clumped isotope analysis were identified and implemented. Continuous-wave (cw) quantum cascade lasers (QCL, Alpes Lasers, Switzerland) were installed (L1: 1076.83 – 1077.06 cm^{-1}, L2: 1163.45 – 1163.65 cm^{-1}). The analyzer uses an astigmatic Herriott multipass cell with 413 m optical path length (2.5 L of volume). A 20 Torr heated capacitance manometer monitors the pressure in the optical cell. Temperature stability of the spectrometer is achieved by a two-stage procedure: the interior temperature of the spectrometer is maintained with a recirculating water chiller, while the entire instrument is enclosed in a custom-made plexiglass thermal shield, cooled with a high-power Peltier-element assembly. Temperature stabilization of about 0.1 K is achieved in the enclosure, despite temperature fluctuations in the laboratory environment of 2.5 K, while the temperature stability of the multipass cell and optical module is at the level of 2 mK over timescales of days.

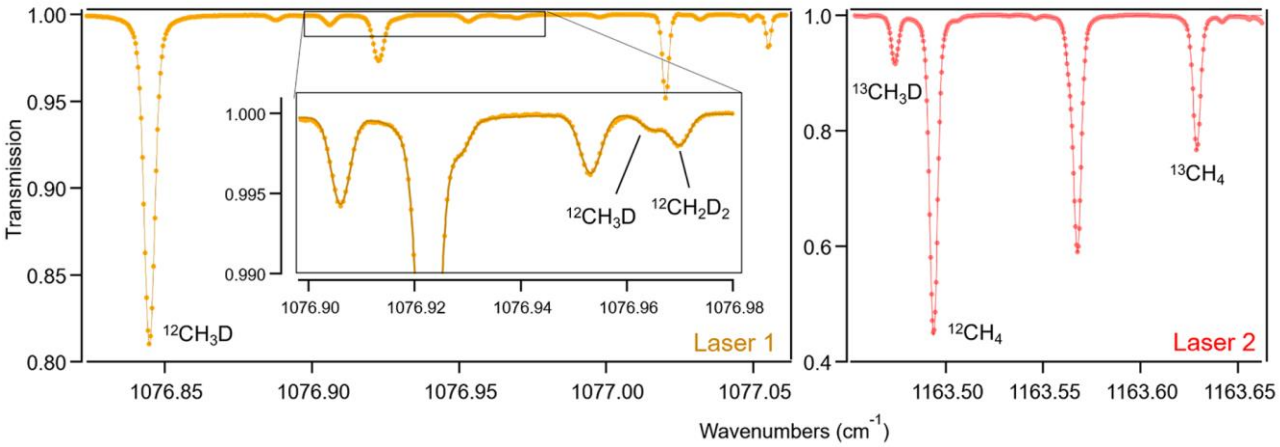

Fig. 4: Measured (point) and fitted (line) transmission spectrum in the spectral range covered by laser 1 and laser 2 at 7.5 Torr cell pressure of pure methane (Zhang, Prokhorov et al. 2025).

Gas inlet system of spectrometer:

Gases are introduced to the spectrometer with a custom-built fully-automated gas inlet system shown in Figure 5. It consists of four normally closed below-sealed pneumatically actuated valves (SS-48K-1C, Swagelok) with 0.25 inch fittings to deliver high-purity (99.9999%) N_2, sample, or reference gas to the intermediate expansion volume (~50 mL), and eight valves of the same type with 0.5 inch VCR-gasket-sealed fitting (SS-8BK-VCR-1C, Swagelok) for gas handling, including injection to or extraction from the spectrometer cell. A screw vacuum pump (PDV 500, Ebara Corporation, Japan) is used for evacuating larger amounts of gas from the cell and inlet system, down to 0.1 Torr, and a turbo-molecular pump backed up by a diaphragm pump (HiCube 80 Eco, Pfeiffer Vacuum, Germany) for evacuation down to 0.1 mTorr. Reference gases are introduced into the system through a 16-port dead-end selector valve (Valco Vici AG, USA). Every second input port of the valve is blinded and used as a parking position, while reference gases are plumbed to intermediate positions.

Calibration procedure:

Under standard operation each reference / sample pair is preceded by a background spectrum measurement of N_2 collected at 1.5 times the target reference / sample pressure to compensate for the different refractive index between gases. Next, the laboratory working reference gas (EP6) is analyzed at the target pressure (200 s), followed by the measurement of the sample gas at the same pressure (200 s). Optimal spectral averaging times and maximum time gaps between sample and reference gas measurements were chosen based on the results of Allan-Werle deviation measurements. During background, reference or sample preparation, gas is expanded into the intermediate volume until a target pressure is reached (15.5 Torr per mL CH_4), controlled using a 0-1000 Torr manometer (Baratron AA02A, MKS, USA) and a critical orifice.

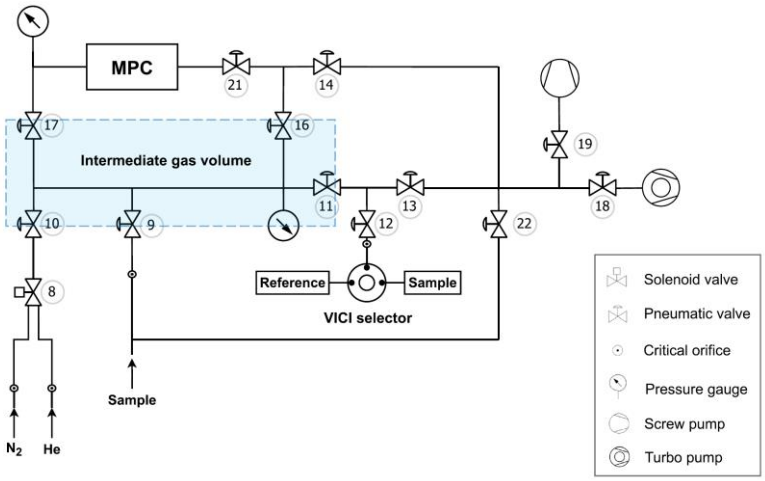

Fig. 5: Schematic drawing of the custom-built inlet system for reference and sample injection / withdrawal into the spectrometer multipass cell (MPC). First, the gas fills the intermediate gas volume (light blue shaded area) via either valve 9 or the VICI selector valve. The gas flow into the intermediate volume is restricted by critical orifices and the filling process is terminated once the set pressure is reached. Valves No. 17 and 21 control the respective injection and extraction of the analyte gas into the MPC, respectively. Evacuation is accomplished with a screw pump and a turbomolecular pump operated sequentially (Zhang, Prokhorov et al. 2025).

3.4 Data correction algorithms for remaining interferant gases

High-resolution IRMS (UU, UofG)

An intuitive check is applied where the mass 17 – mass 16 and mass 18 – mass 16 counts from the Thermo Ultra are plotted against the bellow pressure along the duration of the experiment to identify pressure dependence. If there is a minor dependence (defined threshold) the counts are corrected through means of a simple regression.

Quantum cascade laser absorption spectroscopy (Empa, VTT)

In principle, clumped isotope values should be independent of bulk isotope values. However, a significant nonlinearity effect, primarily influenced by $\delta D\text{-}CH_4$ values, has been reported in previous spectroscopic analyses. To address this potential nonlinearity effects, we prepared a suite of in-house standard gases, including three commercially available pure methane gases (EP1, EP6, and EP7), and a $^{12}CH_3D$-spiked sample gas (EP4). CH_4 gases covering $\delta D\text{-}CH_4$ values ranging from -204‰ to -40‰ facilitate to characterize dependencies of $\Delta^{12}CH_2D_2$ and $\Delta^{13}CH_3D$ on $\delta D\text{-}CH_4$.

Figure 6, illustrates the correlations between apparent clumped isotope values ($\Delta^{13}CH_3D$ and $\Delta^{12}CH_2D_2$) and $\delta D\text{-}CH_4$ for four CH_4 samples equilibrated at 300 °C. At a cell pressure of 7.5 Torr, we observed a bias in $\Delta^{13}CH_3D$ of 0.034‰ and in $\Delta^{12}CH_2D_2$ of -0.108‰ for each 1‰ difference in $\delta D\text{-}CH_4$ of the sample compared to the reference gas. This effect might be related to imperfect spectral fitting, for the interfering $^{12}CH_3D$ line on $^{12}CH_2D_2$ and/or inaccuracies in baseline corrections. Relationships remain constant over time, unless major adjustments to the spectroscopic setup or spectral fitting are undertaken, underlines the robustness of our analytical platform.

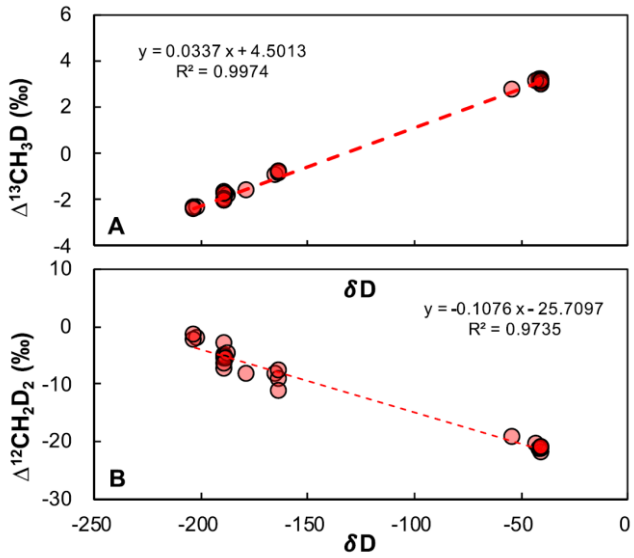

Fig. 6: Apparent (A) $\Delta^{13}CH_3D$ and (B) $\Delta^{12}CH_2D_2$ values for CH_4 gas with varying δD-CH_4, equilibrated at 300 °C. EP6 was used as the reference for all measurements, while sample gases with different δD-CH_4 were applied.

3.5 Equilibration of CH_4 gas

The calibration with pure methane is performed through a series of experiments conducted at various temperatures to create a calibration scale. All measurements are made relative to a reference gas, which is a stainless-steel canister filled from a high purity (>99.999%) CH_4 reference gas cylinder (AP613). The sample and the reference are measured alternately, and then the bulk and clumped isotopic composition of the samples are calculated from the isotopologue ratios as follows:

$$\delta^{13C}_{\text{sam}-\text{VPDB}} = \delta^{13C}_{\text{sam}-\text{ref}} + \delta^{13C}_{\text{ref}-\text{VPDB}} + \left(\delta^{13C}_{\text{sam}-\text{ref}} * \delta^{13C}_{\text{ref}-\text{VPDB}}\right)$$

$$\delta^{D}_{\text{sam}-\text{VSMOW}} = \delta^{D}_{\text{sam}-\text{ref}} + \delta^{D}_{\text{ref}-\text{VPDB}} + \left(\delta^{D}_{\text{sam}-\text{ref}} * \delta^{D}_{\text{ref}-\text{VSMOW}}\right)$$

$$\Delta^{13_{CDH_3}}_{\text{sam}} = \frac{\left(1 + \delta^{13_{CDH_3}}_{\text{sam}-\text{ref}}\right) * \left(1 + \Delta^{13_{CDH_3}}_{ref}\right)}{\left(1 + \delta^{13C}_{\text{sam}-\text{ref}}\right) * \left(1 + \Delta^{D}_{\text{sam}-\text{ref}}\right)} - 1$$

$$\Delta^{12_{CD_2H_2}}_{\text{sam}} = \frac{\left(1 + \delta^{12_{CD_2H_2}}_{\text{sam}-\text{ref}}\right) * \left(1 + \Delta^{12_{CD_2H_2}}_{ref}\right)}{\left(1 + \delta^{D}_{\text{sam}-\text{ref}}\right)^2} - 1$$

$\delta^{13C}_{\text{sam}-\text{ref}}$, $\delta^{D}_{\text{sam}-\text{ref}}$, $\delta^{13_{CDH_3}}_{\text{sam}-\text{ref}}$ and $\delta^{12_{CD_2H_2}}_{\text{sam}-\text{ref}}$ are the values of the sample measured against the reference calculated from the measured ion intensities on the Ultra. These values are converted to the standard scales: $\delta^{13C}_{\text{sam}-\text{VPDB}}$, $\delta^{D}_{\text{sam}-\text{VSMOW}}$, $\Delta^{13_{CDH_3}}_{\text{sam}}$ and $\Delta^{12_{CD_2H_2}}_{\text{sam}}$ using the 300 formulae above. The clumping anomalies of

the reference gas used for the measurements, AP613, denoted as $\Delta_{ref}^{13_{CDH_3}}$ and $\Delta_{ref}^{12_{CD_2H_2}}$, were assigned using temperature-equilibration experiments which are explained in detail in the next section. The bulk isotopic composition of AP613 denoted as $\delta_{\mathrm{ref-VPDB}}^{13_C}$ and $\delta_{\mathrm{ref-VSMOW}}^{D}$, was obtained by measurements using a conventional continuous flow IRMS system (Menoud, Van Der Veen et al. 2021).

Temperature calibration scale:

Temperature-variation experiments:

Methane from the AP613 cylinder is equilibrated at temperatures ranging from 50 to 450 °C using two different catalysts: γ-Al_2O_3 for temperatures below 200 °C and Pt on Al_2O_3 for temperatures between 200-450 °C.

Catalyst Activation:

The catalysts are activated by heating them with pure O_2 at 550 °C for about 5 hours to remove adsorbed air and moisture. After heating, the catalysts are left overnight at 550 °C and then cooled to room temperature. This avoids exposure to outside air when activated.

Equilibration Process:

For each temperature experiment, about 10 pellets of the activated catalyst are placed in a glass tube with a Teflon valve and evacuated. The tube is then filled with methane from AP613 and heated to the desired temperature for the specified duration to allow isotope exchange.

Measurement of Equilibrated Gases:

The equilibrated gases are measured on the Thermo Ultra high-resolution isotope ratio mass spectrometer (HR-IRMS) against unmodified methane from the AP613 cylinder. This step involves calculating the raw $\Delta^{13}CDH_3$ and $\Delta^{12}CD_2H_2$ values relative to the reference gas, assuming the clumped values of the reference are zero.

Assignment of Clumped Isotope Values to AP613:

The measured values from the equilibrated gas experiments are compared to the theoretical equilibrium curve, and the $\Delta^{13}CDH_3$ and $\Delta^{12}CD_2H_2$ values of AP613 are estimated using Monte Carlo simulations. This involves applying random errors based on the uncertainty of each measurement, fitting the polynomial functions for $\Delta^{13}CDH_3$ and $\Delta^{12}CD_2H_2$ with an added parameter for the offset, and averaging the parameters obtained from all runs to assign the absolute clumped isotope values to AP613.

3.6 Conclusion

To conclude, this chapter lists down good practices for the analysis of CH_4 source gases $\Delta 13CH_3D$ and $\Delta^{12}CH_2D_2$ (part 2) by OIRS and IRMS, including sample handling, purification, optimised analytical procedures and traceability to the international standards and target uncertainties $\delta^{13}C(CH_4)$: 0.2 ‰, $\delta^{2}H(CH_4)$: 2 ‰, $\Delta^{13}CH_3D$: 0.5 ‰, $\Delta^{12}CH_2D_2$: 2 ‰.

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