PROTOCOL

A new automated method for high-throughput carbon and hydrogen isotope analysis of gaseous and dissolved methane at atmospheric concentrations

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Rationale: The dual isotope ratio analysis, carbon ($\delta^{13}C$ value) and hydrogen (δ^2H value), of methane (CH_4) is a valuable tracer tool within a range of areas of scientific investigation, not least wetland ecology, microbiology, CH_4 source identification and the tracing of geological leakages of thermogenic CH_4 in groundwater. Traditional methods of collecting, purification, separating and analysing CH_4 for $\delta^{13}C$ and δ^2H determination are, however, very time consuming, involving offline manual extractions.

Methods: Here we describe a new gas chromatography, pyrolysis/combustion, isotope ratio mass spectrometry (IRMS) system for the automated analysis of either dissolved or gaseous CH_4 down to ambient atmospheric concentrations (2.0 ppm). Sample introduction is via a traditional XYZ autosampler, allowing either helium (He) purging of gas or sparging of water from a range of suitable, airtight bottles.

Results: The system routinely achieves precision of <0.3‰ for $\delta^{13}C$ values and <3.0‰ for δ^2H values, based on long-term replicate analysis of an in-house CH_4/He mix standard (BGS-1), corrected to two externally calibrated reference gases at near atmospheric concentrations of methane. Depending upon CH_4 concentration and therefore bottle size, the system runs between 21 (140-mL bottle) and 200 samples (12-mL exetainer) in an unattended run overnight.

Conclusions: This represents the first commercially available IRMS system for dual $\delta^{13}C$ and δ^2H analysis of methane at atmospheric concentrations and a step forward for the routine (and high-volume) analysis of CH_4 in environmental studies.

1 | INTRODUCTION

Atmospheric methane (CH_4) is the second most abundant and potent greenhouse gas after carbon dioxide (CO_2).[1] Methane concentrations in the atmosphere have more than doubled (to 1858 ppb in 2018) since pre-industrial times,[2–5] with a current growth rate in the atmosphere of 10 ppm/year.[1] Therefore, understanding CH_4

formation pathways and sources of emission is critical to informing effective mitigation strategies and limiting the role of CH_4 in future climate change.[1] Important natural emission sources include shallow wetlands and water-saturated soils,[4,6,7] the digestive systems of ruminants and termites, and natural geological sources.[4] However, changes in the flux associated with anthropogenic sources represent the major factor responsible for the significant post-industrial CH_4

increase, with approximately 60% of CH_4 emissions attributed to human activities, both direct and indirect.[4] These include energy production (gas and coal), biomass burning, agriculture[8] (including rice farming), waste management, and leakages associated with subsurface natural gas extraction,[9] gas storage and piped delivery.[10] Evaluation of their relative contributions to the global CH_4 budget is, however, complex to determine and requires a robust set of geochemical tools to accurately identify methane sources and sinks.[5,11]

Alongside aiding global warming within the atmosphere, high concentrations of dissolved CH_4 in groundwater degas rapidly and can build up to cause risk of explosion or asphyxiation when confined.[12] Recent investments in the shale gas and coal bed CH_4 industries have meant that fingerprinting CH_4 sources within groundwater is becoming a critical concern for operators and regulators alike.[9] Under natural or "baseline" conditions the majority of UK groundwater CH_4 is bacterially derived,[12] but leakages of thermogenic gas associated with hydraulic fracturing of shale deposits have been identified as a potential polluter of groundwater supplies within the USA,[13] as have leakages associated with gas wells[9] and buried pipework.[14] With future development of the shale gas industry in some regions, this issue requires careful monitoring pre-, during and post-hydraulic extraction.[9,12,15,16] The carbon ($\delta^{13}C$-CH_4) and hydrogen (δ^2H-CH_4) isotope compositions of CH_4 can help identify mechanisms and sites of CH_4 formation and destruction both in atmospheric and in dissolved gas samples.[5] Stable isotope composition is a powerful tracer tool because of the unique isotope fractionations imparted during the different CH_4 production pathways.[5,8,17,18]

CH_4 production is derived from three main sources, biogenic, thermogenic and pyrogenic (mainly biomass burning), with different average isotope ratios for each process, allowing for source attribution.[5,11] Biogenic production occurs either through the reduction of CO_2 or by the fermentation of reduced carbon substrates such as acetate and methanol.[18,19] The reduction of CO_2 to CH_4 discriminates against the heavier ^{13}C and ^2H isotopes, producing CH_4 with distinct, isotopically low $\delta^{13}C$-CH_4 (< $-110‰$) and δ^2H-CH_4 signatures (-150 to $-250‰$). The fermentation of acetate and methylated substrates also causes kinetic fractionation, this time more pronounced in the δ^2H-CH_4 composition: $\delta^{13}C$-CH_4 (< -50 to $-60‰$) and δ^2H-CH_4 (-300 to $-400‰$).[18] It should be noted that recent work has demonstrated that some reduction processes, such as that during nitrogen fixation by nitrogenase, can lead to small amounts of CH_4 produced with much lower δ^2H-CH_4 values ($-560‰$).[20] Differences in the δ^2H-CH_4 values of microbially derived CH_4 can be used to distinguish CH_4 source, where terrestrial sources (marsh and glacial tills) have lower values than those for CH_4 derived from marine environments; much of this is associated with the δ^2H composition of the surrounding water.[17] Post CH_4 formation, secondary processes of CH_4 consumption (both aerobic and anaerobic) can cause isotopic fractionations, resulting in the ^{13}C and ^2H enrichment of the residual CH_4 pool.[18] These processes can lead to the misinterpretation of residual CH_4 isotope ratios as this residual pool can reflect isotopic composition more characteristic of thermogenic CH_4.

Thermogenic CH_4 is derived from diagenesis which produces gas with relatively high $\delta^{13}C$-CH_4 values (-45 to $-55‰$).[11] This $\delta^{13}C$-CH_4 range observed in thermogenic methane[11] is controlled by a combination of factors including the precursor kerogen $\delta^{13}C$ composition, the thermal maturity, the contribution of coal bed microbial methanogenisis[11] and the source rock type. The CH_4 becomes progressively enriched in ^{13}C with increasing thermal maturity, eventually approaching the original $\delta^{13}C$ composition of the source organic material.[17,18,21] Further scrutiny of the δ^2H-CH_4 composition of thermogenic CH_4 has been used on a site-specific basis to help to differentiate between the original organic matter sources of thermogenic CH_4[17] and to show heterogeneities in this source material.[21] Where complications may arise between thermogenic or oxidised CH_4 pools it is of importance to consider other geochemical tracers including C_{2+} hydrocarbon abundance and noble gases.

Whilst the dual isotope approach offers one of the key geochemical tracers of CH_4 sources, accurate and precise measurements have been difficult to make.[22] Originally, CH_4 isotope measurements required a number of "offline" manual gas clean-up stages, the addition of an oxygen source and the analysis of resulting CO_2 and H_2O via well-established isotope ratio mass spectrometry (IRMS) methods. These processes are time consuming (hours) and often limit the number of samples which can reasonably be collected and analysed (max 10–20 per day). The time and cost involved are therefore problematic for long-term baseline or high-resolution studies. Recently, developments in cavity ring-down spectroscopy and a number of bespoke "lab built" instruments utilising GC/continuous flow IRMS methods for the automated clean up and analysis of methane for either $\delta^{13}C$-CH_4 or δ^2H-CH_4 values or both have improved this situation.[2,22–24] However, many of the recently developed GC/IRMS systems require lab-based expertise to develop bespoke instrumentation. To overcome this problem, Sercon (Crewe, UK) have produced a CryoGas preparation module coupled to a 20-22 isotope ratio mass spectrometer (CG-2022), which is the first commercially available automated system that combines gas chromatography, cryogenic trapping, pyrolysis/combustion, and continuous flow IRMS for the high-precision, high-throughput measurement of the $\delta^{13}C$ and δ^2H values of CH_4 down to atmospheric concentrations.

2 | SAMPLE PREPARATION

Through extensive testing of the CG-2022 we have seen that accurate isotope measurement of methane relies upon the use of appropriate vessels, which are known to be totally gas tight (for >1 month) from gas collection but also during sample introduction, reducing contamination issues to negligible levels. To ensure this and to enable the automated introduction of sample into the CG-2022 system we use 12-mL exetainers (Labco, Lampeter, UK) for high-concentration CH_4 samples, or custom-made 140-mL sample bottles (Cambridge Glassblowing, Cambridge, UK) which have Labco

exetainer caps blown directly onto the larger bottle size (PN 25365/NAP) for near-atmospheric samples. This requirement for custom-made bottles is a result of clear leakage from off-the-shelf "gas-tight" bottles. Labco (chlorobutyl septa) bottles were chosen due to the robustness of the septa under variable sampling conditions and multiple needle injections.

For atmospheric sample collection (approximately 2 ppm CH_4) we recommend collecting 140 mL of air for measurement of $\delta^{13}C$-CH_4 values and a further 140-mL sample for δ^2H-CH_4 values. This can be undertaken by simply leaving the bottles to exchange with ambient air or by injecting a collected sample into a pre-evacuated bottle. For water samples we suggest collection of 140-mL samples (collected with no headspace) to ensure a high enough concentration of CH_4 after headspace equilibration. Water samples should be refrigerated, both to avoid microbial activity within the bottle and to reduce the likelihood of thermal expansion causing breakages to some full water samples.

In the lab the samples are refrigerated until analysis. The sample equilibration process for dissolved samples is as follows. (1) Sample bottles (140-mL) are removed from the fridge and weighed. (2) Approximately 20 mL of sample water is removed and helium (He) gas introduced via a gas-tight syringe system, creating 20 mL of inert gas headspace for dissolved CH_4 to degas into, the bottles are reweighed, and the exact volume of sample remaining is calculated. (3) Samples are shaken vigorously for 30 s then left at room temperature (21°C) overnight for complete equilibration to be achieved. (4) A subsample of the gas (2 mL) is removed via a gas-tight syringe and introduced into a model 6890 gas chromatograph (Agilent Technologies, Santa Clara, CA, USA) for hydrocarbon separation and CH_4 concentration analysis using detection by flame ionisation detection (FID). (5) If storage is required the remaining headspace is removed from the sample bottle via a gas-tight syringe into a pre-evacuated 12-mL Labco exetainer, removing any issues with bacterial processing of CH_4 in situ during storage of a water sample. (6) If isotope analysis is to be conducted immediately, an appropriate volume of headspace gas (based on the measured CH_4 concentration)

is transferred into a He-flushed 12-mL Labco exetainer and loaded into the XYZ autosampler. (7) Where concentrations are high enough samples are run in triplicate. If, however, the concentration is low, the entire contents of the 140-mL bottle can be introduced into the autosampler and the fluid sparged with He, completely extracting the headspace gas.

3 | INSTRUMENT DESIGN

The CryoGas is a versatile gas sample preparation module coupled to a 20-22 isotope ratio mass spectrometer (CG-2022), configured in this case for the high-precision isotope analyses of CO_2 or H_2 (Figure 1). The following sections describe sample introduction, the automated separation of CH_4 from other gas components, conversion into CO_2 or H_2, and isotope measurement, developed from the designs of "lab built" instruments.[22,23] We include a detailed methodology for the two configurations of the CG-2022 required for the measurement of $\delta^{13}C$-CH_4 and δ^2H-CH_4 values. From here on these two modes of operation are described as "carbon mode" or "hydrogen mode" for simplicity. Under both modes of operation, sample introduction is consistent. Gas is introduced via an ASX-7400 autosampler (Cetac, Omaha, NE, USA) with sample racks to accommodate bottle sizes of 12 mL and 140 mL. Also consistent to both modes are a series of cryogenic traps which are automatically lowered into liquid nitrogen (−196°C) to remove the condensable gases (i.e. N_2O and H_2O) from a He carrier flow. Liquid nitrogen delivery uses a micro dosing system (Norhof LN2; Microdosing Systems, Ede, The Netherlands), delivering liquid nitrogen slowly to maintain a set level within an open-topped Dewar, which houses all the cryotraps. After trapping, the GC columns separate the sample gases from any potential contaminants and ensure complete sample delivery to the isotope ratio mass spectrometer. Switching between carbon and hydrogen modes requires manual changes to the system configuration, as described in section 3.2. An average run time in both modes is 12–22 min, but this depends upon flow rates and sample

FIGURE 1 The CryoGas 2022 IRMS system. The unit on the left-hand side "CryoFlex" is the basic CryoGas system which has been adapted for CH_4 analysis. This unit contains the GC columns, water CO and CO_2 traps as well as the open Dewar liquid nitrogen system. The unit on the right is the benchtop 20-22 mass spectrometer

flush time. All the timings described in the following sections are for a 140-mL bottle flush, with a 12-mL sample run being quicker.

3.1 | Carbon (δ^{13}C) mode

The configuration for carbon mode (δ^{13}C-CH$_4$) is shown in Figure 2. The sample bottle is flushed with lab grade 99.9% pure He (approx. 50 mL/min, 13 psi), ensuring recovery of all sample CH$_4$ and precise and accurate isotope ratios (140 mL = 600–800 s, 12 mL = 80–100 s) based on recovery tests (Figure 3). The flow of He and sample passes through chemical CO (Schuetze Reagent, Sercon PN: SC0364) and CO$_2$ (EMASorb, Sercon PN: SC0236) scrubbers. The gas then enters T1 (1.6 mm OD, 0.75 mm ID stainless-steel capillary, 110 cm length), which is lowered into liquid nitrogen at the start of the analysis. T1 traps any remaining condensable gases (e.g. N$_2$O, H$_2$O) as well as other hydrocarbons (ethane, propane, see supporting information) while allowing CH$_4$ and any non-condensable gases (mainly N$_2$ and O$_2$) to pass through to the variable temperature furnace (combustion) set at 860°C.

The 1.6 mm OD, 0.8 mm ID alumina combustion furnace tube (Sercon PN: SC0091) is packed with one 200 mm platinum wire (Sercon PN: SC0121) and ten 200 mm copper wires (Sercon PN:SC0088), twisted together and trimmed to 160 mm. On the installation of a new tube, the copper wire is oxidised at 600°C for at least 2 h using a pure O$_2$ source via V1. Once conditioned the furnace is set to temperature (860°C) and left overnight before commencing analysis. We have seen that the addition of a 30–50 s O$_2$ flush from V1 can be helpful in retaining the CuO$_2$; this is added at the end of the sample run period (1000 s) and O$_2$ is vented through V2. As the carrier gas passes through the furnace, methane is combusted in the presence of CuO$_2$ (as an O$_2$ source) to CO$_2$ + H$_2$O; the combustion is at or very near to 100% efficient, see supporting information for more details. The water is immediately removed using a Nafion™ membrane with a counter-flow of He to sweep the water away (Figure 2), allowing the dry CO$_2$ to pass onto the pre-concentration loop (T2), which is cooled in liquid nitrogen from the start of the analysis. Any non-condensables are not retained in T1 or T2 and are vented via valve V2 (Figure 2). At the end of the bottle flush time the needle is removed from the bottle; the He pressure is retained in the system with a He flow rate of approx. 20 mL/min.

T2 consists of approximately 90 cm of a 1.6 mm OD, 0.75 mm ID stainless-steel capillary tube formed into three turns and has a constant flow of 20 mL/min. The CO$_2$ from CH$_4$ is condensed and retained in the T2 loop. After 860 s, T2 is raised out of the liquid nitrogen allowing the condensed CO$_2$ to evaporate and transfer to the cryofocus loop (T3), which is submerged in liquid nitrogen from the start of the run. The transfer time from T2 to T3 is 140 s with a carrier flow of 20 mL/min. T3 consists of 60 cm of the same capillary as T2; this smaller volume focuses the CO$_2$ gas. Once the cryofocused sample has been transferred to T3 the Divert valve (D2) is switched (VICI Valco, Houston, TX, USA), reducing the flow from T3 to GC2 to 1–2 mL/min and isolating the first part of the system (autosampler – D2). This isolation means that T1 and T2 can be warmed to room temperature for the remainder of the sample run time by removal from the liquid nitrogen, allowing any condensables to be vented via V2 to atmosphere, ready for the next sample acquisition.

Before T3 is lifted, V3 switches to align GC2 with the isotope ratio mass spectrometer and a constant flow rate of 1–2 mL/min is established on GC2. At this point the cryofocus loop T3 is lifted. As the CO$_2$ from T3 evaporates it is introduced into this much slower flow (−10× less than the initial carrier flow), causing a concentrated pulse of CO$_2$ to transfer from T3 to GC2. GC2 is a 30 m × 0.53 mm ID Rt-Q-Bond column, for the separation of trace gases, housed in a heating box maintained at 30°C to remove any issues of variable flow resulting from ambient temperature fluctuations. The GC2 flow is set at approximately 1.5 mL/min, slowing the CO$_2$ and enabling any final residual air components to pass through the mass spectrometer prior to the CO$_2$ peak. The lack of any baseline fluctuation prior to the CO$_2$ peak does, however, indicate that any contaminants cause little or no interference with the m/z 44, 45, 46 measurement. The δ^{13}C values of the CO$_2$ produced from combustion of CH$_4$ is then measured on the 20-22 isotope ratio mass spectrometer.

3.2 | Switching between carbon and hydrogen mode

Switching from carbon to hydrogen mode involves manually replacing the cryotraps T2 and T3 with short packed GC columns, replacing GC1 and GC2, reconfiguring them in tandem and replacing the combustion tube with a packed pyrolysis tube
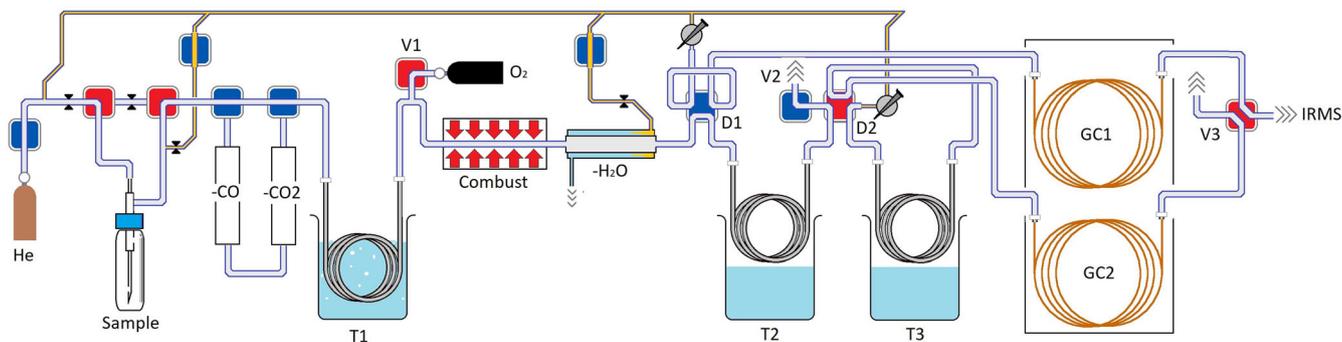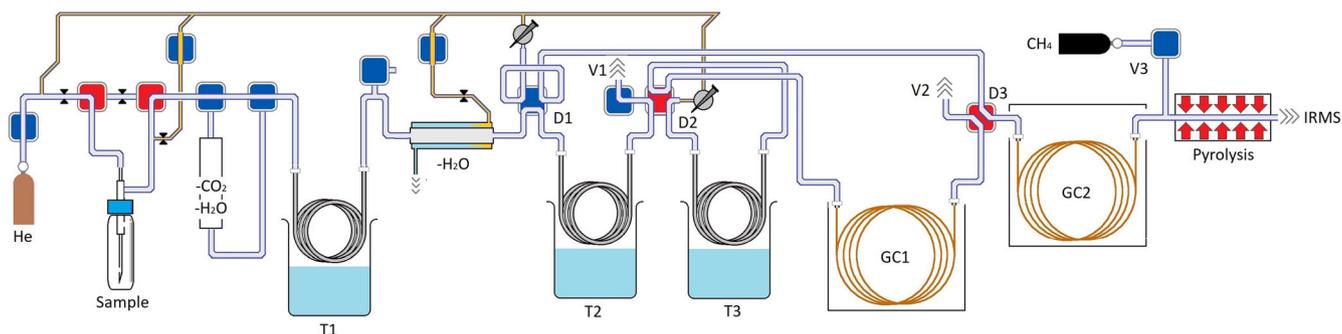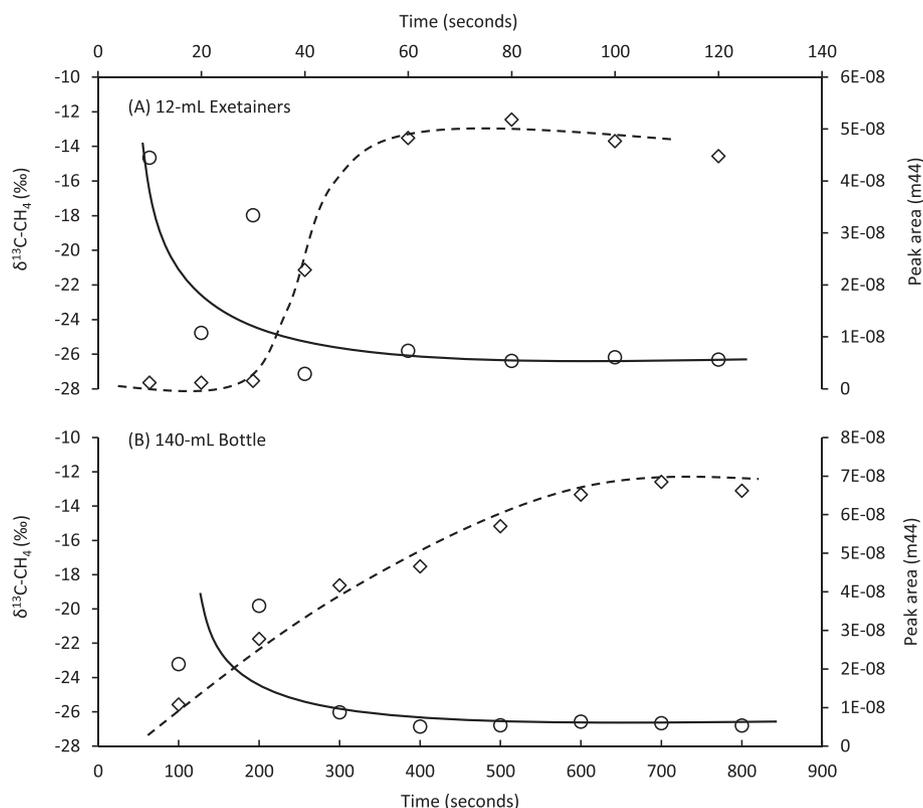

FIGURE 2 Schematic of the Cryogas system in carbon mode, relevant valves, traps and GC columns are labelled

FIGURE 3 Required bottle flush
times needed to reach full recovery of
CH_4 (diamonds), and acceptable precision
(circles) from A, the 12-mL exetainers
and B, the 150-mL bottles. Tests used
40 μL of 1% CH_4 balance air, approx.
18 nmol of CH_4

FIGURE 4 Schematic of the Cryogas system in hydrogen mode, relevant valves, traps and GC columns are labelled

(details of all configurations are given below). This takes between 2 and 4 h. It is recommended that the system is left to stabilise, and the oven to reach operational temperature overnight, before running samples for δ^2H measurements from CH_4. The operational configuration for δ^2H-CH_4 measurements is shown in Figure 4. The sample introduction and bottle flush times are consistent with carbon mode operation. However, the backpressure caused by the cryotrap and focus packed columns means that the pressure of the He supply on the unit has to be increased from 13 psi to approximately 21 psi to obtain the appropriate working flow rates (approximately 15–20 mL/min carrier and approx. 50 mL/min flush flow rate).

3.3 | Hydrogen (δ^2H) mode

As the sample is purged from the bottle, it passes through a CO_2 (EMASorb, Sercon PN: SC0236) and chemical water scrubber (perchloride, Sercon PN: SC0023). The sample bypasses T1 which is not utilised in hydrogen mode and has any residual H_2O removed by the Nafion™ membrane (Figure 4). The sample gas is then pre-concentrated at T2, which is submerged into liquid nitrogen at the start of the run. T2 is a 1/8" packed Hayesep D column, connected in position with short pieces of 1.6 mm × 0.75 mm OD tubing. T2 is retained in liquid nitrogen with D2 venting from V1 for the full time of the bottle flush (Figure 4), removing any non-condensables. After

400 s, the Cryofocus loop (T3) drops into liquid nitrogen and then 2 min later D2 switches, placing T2 and T3 in tandem. T2 is warmed by removing it from liquid nitrogen, transferring the CH_4 to T3, which is a 100-cm piece of a GS-Q column looped twice; the transfer time from T2 to T3 is 150 s. After the transfer is complete, D2 switches again, moving CH_4 and any remaining air components on to GC1.

GC1 is a HayeSep D 2 m × 1/16" OD column used to slow CH_4 flow, while venting air components to waste via V2 (D3 is in the off position).[24] This venting stage is critical to ensure that any remaining air components (especially O_2) are removed from the system before the sample is sent on to the pyrolysis tube. If the timing is incorrect and large volumes of O_2 enter the pyrolysis tube the carbon deposit is stripped away, affecting the reductive condition needed for pyrolysis. The exact time for switching of D3 is sensitive to the flow rates on GC1; adequate separation of air components and CH_4 requires a flow rate of approx. 1.5 mL/min and a D3 switch time of 90–100 s, but this may vary from instrument to instrument and requires optimisation. Once venting is complete, D3 switches, aligning GC1 in tandem with GC2, and transferring the CH_4 to GC2, which is a Carbon Plot 30 m × 0.32 μm column, used to separate CH_4 from any trace contaminants that may have got through the system. Both GC columns are housed in a heating box maintained at 30°C to remove any issues of variable flow resulting from ambient temperature fluctuations. From GC2 the cleaned CH_4 transfers to the pyrolysis tube.

The pyrolysis tube is a 1.6 mm OD, 0.8 mm ID alumina tube (Sercon PN: SC0091) with a packing of nickel carbon yarn (Sercon PN: SC0327). After the tube has been fitted, the furnace temperature is slowly increased in 300°C steps to a working temperature of 1350°C. On initial instillation the pyrolysis tube requires "conditioning", where a supply of tank CH_4 (5% CH_4 balance He; Air Liquide UK, Stoke-on-Trent, UK) is used to create a carbon coating on the inside of the ceramic tube. The 5% CH_4 cylinder is connected to the CryoGas SGE-MOVPT valve, V3 (Figure 4). The CH_4 cylinder output must be at a low pressure <15 psi and further crimped or connected via a needle valve to have a flow of approximately 3–5 mL/min going into the back of the MOVPT valve. One side arm of this SGE valve is connected to the mass spectrometer line out of the CryoGas, and the other side arm to the input of the pyrolysis tube, allowing the 5% CH_4 to be used at any time to automatically condition/re-condition the pyrolysis tube.

Once conditioned the tube is stable without the requirement for regular re-conditioning. If, however, the pyrolysis tube has been exposed to a large amount of O_2 (i.e. the D3 switch time is incorrect), it may need to be re-conditioned via the above procedure. Under normal working conditions the CH_4 sample transfers from GC2 to the pyrolysis tube where CH_4 is broken down into C and H. Carbon is retained in the tube and H_2 passes through to the 20-22 isotope ratio mass spectrometer. The 20-22 is tuned for $^1H^2H$ using a low electromagnet field setting. The source conditions are optimised for the best $^1H^2H$ 2/1 ratio trace, stability and sensitivity.

4 | METHANE STANDARDS

There are currently no commercially available international standards for methane isotopes, although there are standards currently in development.[25] The original set of international standards produced by the National Institute of Standards and Technology (NIST, Gaithersburg, MD, USA) are 8559 (NGS-1), 8560 (NGS-2) and 8561 (NGS-3). These gases are now exhausted and out of date, leaving the community lacking a fully calibrated commercially available alternative.

To calibrate our system, we purchased two isotopically distinct, calibrated CH_4 standards (Air Liquide) and named these AL-high and AL-low. AL-high is a 1% CH_4 in synthetic air mix with supplied values of $\delta^{13}C = -24.2 \pm 0.3$‰ and $\delta^2H = -118 \pm 5.0$‰; AL-low is a 1% CH_4 in synthetic air mix with supplied values of $\delta^{13}C = -60.9 \pm 0.3$‰ and $\delta^2H = -260 \pm 5.0$‰. We also purchased a 4.5% CH_4 balance He tank gas (Air Liquide UK), with no attributed values (BGS-1). Aliquots of these sample gases were sent to UC Davis (UCD) Stable Isotope Facility (Davis, CA, USA) for cross checking of the purchased standards and external calibration of BGS-1 following the method described in the literature.[23] Repeat analysis of BGS-1 at UCD gave values of -42.3 ± 0.4‰ for $\delta^{13}C$-CH_4 (n = 6) and -188.5 ± 0.5‰ for δ^2H-CH_4 (n = 6). BGS-1 is now used as our in-house check standard after a two-point calibration to the externally verified values of AL-high and AL-low. Long-term analysis of BGS-1 vs AL-high and AL-low using the CryoGas at the British Geological Survey (BGS, Keyworth, Nottingham, UK) gives an average $\delta^{13}C$-CH_4 value of -41.9 ± 0.5‰ and -187.0 ± 3.1‰ for the δ^2H-CH_4 value, confirming the accuracy of the system in comparison with the well-established UCD system.[23]

5 | SYSTEM OPERATION

5.1 | Isotope measurements

Isotope measurements are carried out on a 20-22 high-precision isotope ratio mass spectrometer tuned either to CO_2 or to H_2. For each sample the isotope composition of the sample peak is compared with that of a working reference gas (either CO_2 or H_2) delivered directly to the mass spectrometer from a series of reference gas ports; reference gas injection occurs concurrently to the CryoGas purifying the sample. Whilst this working reference gas is delivered directly to the mass spectrometer (i.e. does not pass through the CryoGas), all the external reference gases (AL-high, AL-low and BGS-1) are treated in exactly the same way as the samples, following the identical treatment principle. These reference gases are included within the run, where an appropriate volume of reference gas is added to He-flushed bottles (or exetainers) at atmospheric pressure, via a gas-tight syringe. At near-atmospheric concentrations, we inject 40 μL of 1% CH_4 (AL-high and AL-low) and 10 μL of BGS-1 (~4% CH_4) to ensure a near matched peak area to that expected from 140 mL of atmosphere (1.8 ppm CH_4, beam area approximately 3.0E-8). The reference gases are extracted by the autosampler, cleaned, trapped, converted into CO_2 or H_2 and then

analysed in the same way as any sample. The precision of the GC-2022 instrument is defined by the replication of these gas measurements, not the precision of the mass spectrometer defined by the directly injected working gas (n = 10: typically, <0.02‰ for $\delta^{13}C$-CO_2 values; and 0.05‰ for δ^2H-H_2 values). The majority of the associated error with both isotope measurements is, as would be expected, related to the gas purification within the CryoGas system.

Background detection is undertaken by specifying an integration interval (Figure 5, yellow bars) pre- and post-peak, while the peak area is measured within the set integration period (Figure 5, purple and grey boxes). Manual positioning of the integration period is preferred but the position remains stable once the run method and gas flows are set (Figure 5).

5.2 | Isotope ratio, ^{17}O and H_3^+ corrections

Callisto software (Sercon) automatically applies the necessary ion corrections for ^{17}O in CO_2[26] and calculates the peak area and the isotope ratio of the peaks. The software allows two modes of isotope
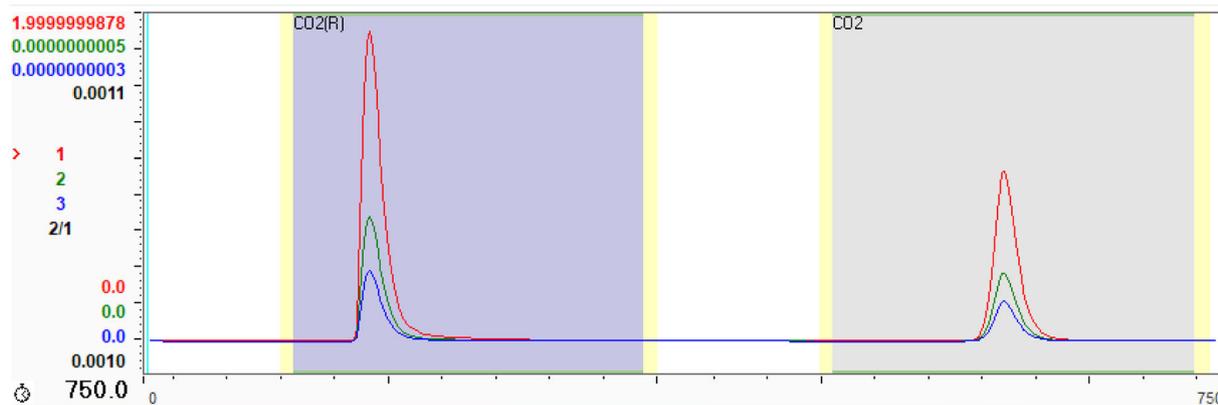
FIGURE 5 Standard MS peak showing CO_2 reference gas injection peak (purple box) 5 s baseline integration intervals for both reference and sample (yellow bars) and sample peak (grey box) for a $\delta^{13}C$-CH_4 measurement. The stable baseline highlights efficient contaminant trapping, showing no baseline disturbance pre- or post-peak and the sharp sample peak indicates effective cryofocusing post-combustion
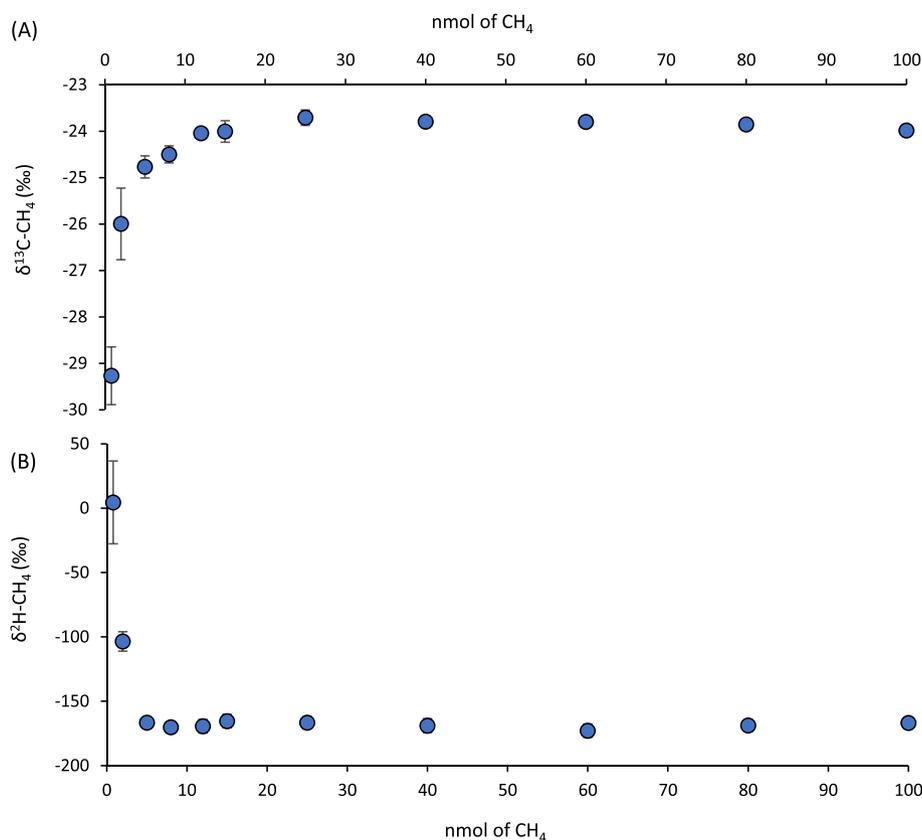
FIGURE 6 A, Stable $\delta^{13}C$-CH_4 and B, δ^2H-CH_4 data (raw vs reference gas) for samples between 100 and 0.8 nmol CH_4 (atmospheric values in 12 mL of air[23]) highlighting the limit of detection of the CG-2022 system

ratio characterisation. First, a single-point calibration can be applied automatically against one of the reference gases within the run. This is achieved by characterising this gas as a "Standard" within the run sheet and attributing the known isotope ratio to this standard gas. The Callisto system then corrects all "unknowns" including other reference gases and samples to this single calibration gas. The second, and preferred method, is to run all the reference and sample gases as "unknowns", with the Callisto system correcting these to the working reference gas delta value (tank CO_2 or H_2). This allows for an initial calculation of the isotope ratio vs working gas within the software. Assignment of the isotope ratio vs calibrated standard materials is then undertaken offline, following blank and drift correction via a two-point calibration to AL-high and AL-low with BGS-1 acting as a check standard, for both within-run and long-term accuracy, and precision characterisation. Especially for natural CH_4 samples this two-point correction is highly recommended due to the wide range of possible $\delta^{13}C$-CH_4 and δ^2H-CH_4 values found in natural gases and the need to bracket this range as well as possible with references.

For H_2, the H_3^+ factor is determined via one of two methods. First, this can be done by running a series of different sized reference gas peaks spanning the range of the samples and plotting the beam area incidence in nano amps against the uncorrected 2/1 ratio. The slope will give the H_3^+ value which is then fixed in the software for sample H_3^+ factor calculations. This value is valid for that set of source parameters. This method is sometimes called peak-wise H_3^+ correction and is our preferred method when major changes in source settings occur. However, a second method is to use the automatic non-linear regression Marquardt which calculates the H_3^+ factor based on point-to-point measurements of beams 1 and 2 increasing and then decreasing as the peak elutes. An average of these values can be taken from several peaks and then fixed in the software. A combination of methods can be utilised to check and fix the H_3^+ factor when major changes in the source settings occur.

6 | RESULTS

6.1 | Limit of quantification

The system was designed specifically to offer high-precision, high-throughput analysis of CH_4 at atmospheric concentrations, from a reasonable size sample container for most field collection scenarios.
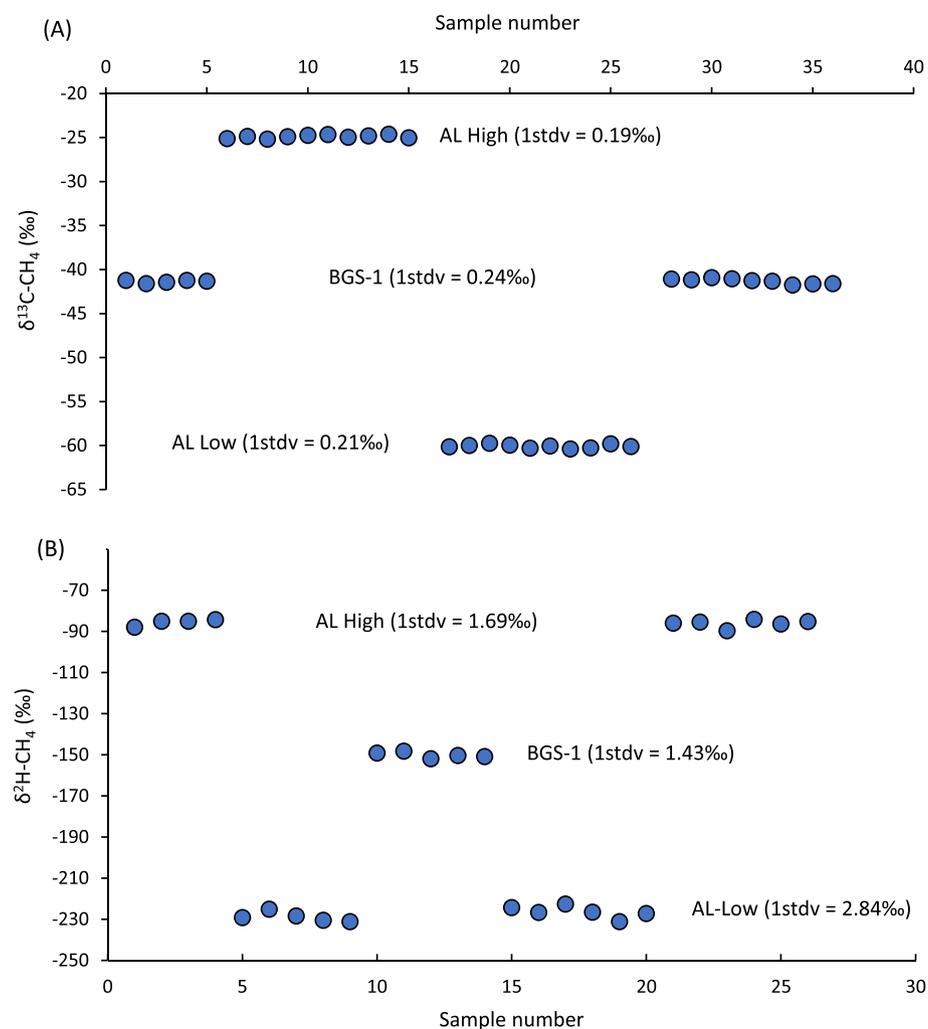
FIGURE 7 7Isotope carryover test for A, $\delta^{13}C$-CH_4 and B, δ^2H-CH_4 standards. The range of isotope values was 35.9‰ for $\delta^{13}C$-CH_4 and 141‰ for δ^2H-CH_4; the data show no carry-over effects between samples

Following other recent developments in high-precision CH_4 analysis,[22-24] we aimed to analyse background concentrations of CH_4 in <150 mL of air as a sensible trade-off between sample collection/ storage in the field and analytical complexity. As suggested in section 2, for this system (based on significant prototype testing) we recommend collection of 140 mL of air for both $\delta^{13}C$-CH_4 and δ^2H-CH_4 samples, equating to approximately 11 nmol of CH_4 and resulting in beam areas of approximately 4.0E-08 for $\delta^{13}C$-CH_4 values and 2.5.0E-08 for δ^2H-CH_4 values.

Figures 6A ($\delta^{13}C$-CH_4) and 6B (δ^2H-CH_4) show tests on the limits of quantification on the CryoGas system with accurate and precise measurements of $\delta^{13}C$-CH_4 values down to 8 nmol and δ^2H-CH_4 values down to 5 nmol. There is a significant reduction in precision at 5 nmol for $\delta^{13}C$-CH_4 values and at 2 nmol for δ^2H-CH_4 values (Figure 6), to which we attribute a reasonable cut-off of 1stdv 0.3‰ for $\delta^{13}C$-CH_4 and 3% for δ^2H-CH_4, comparable with other existing instruments. These tests indicate the system is capable of measuring $\delta^{13}C$-CH_4 and δ^2H-CH_4 values in approximately 100 mL of air (2 ppm), to a useful precision for source identification (0.3‰ and 3.0‰, respectively),[27] without significant changes to the system or improvements in sensitivity within the mass spectrometer. Where lower concentration analyses are required the system offers lower precision data for $\delta^{13}C$-CH_4 values down to around 2 nmol of CH_4 (1stdv = ±0.7‰); once beam areas drop below 1E-08, significant reductions in precision are observed, although slightly smaller beam sizes still yield acceptable precision on the δ^2H-CH_4 measurement within the 3‰ tolerance.

6.2 | Carryover

Large variations in methane isotope ratios are seen within natural samples, >10s of ‰ in $\delta^{13}C$-CH_4 values and >100s of ‰ in δ^2H-CH_4 values.[1,27] It is therefore fundamental that instruments regularly analysing CH_4 to attribute the source, either of gas or of dissolved gas, are able to cope with a large dynamic isotope range with minimal carryover effects between samples. To test this for the CG-2022 system we analysed our external standards and internal reference gas to ascertain the extent of carryover from one sample to the next across a large isotope ratio range. Figures 7A and 7B show the results for $\delta^{13}C$-CH_4 values and δ^2H-CH_4 values, respectively, all run against the instrument working gas. Ordering of gases in this manner (AL-high, AL-low, BGS-1) enabled the largest possible isotope jumps to be made based on the available gases; no obvious indication of sample carryover is observed, with each gas falling within the stated error range of the instrument (section 6.1).

7 | CONCLUSIONS

The CG-2022 IRMS system offers a versatile "off the shelf" option for the dual measurement of $\delta^{13}C$-CH_4 and δ^2H-CH_4 values. The system operates unattended using an autosampler with up to 200 sample slots, drastically reducing staff time and costs, improving efficiency and enabling the high sample throughput required for many modern environmental monitoring programs, including both groundwater pollution and greenhouse gas emission tracing. The system operates using 140-mL or 12-mL Labco capped bottles, offering good precision for environmental monitoring, similar to that of bespoke lab-built systems: <0.3‰ for $\delta^{13}C$-CH_4 values and <3.0 for δ^2H-CH_4 values at atmospheric concentrations of CH_4. The system has been designed to enable the sparging of water samples as well as the measurement of gas only samples, facilitating the routine analysis of ground and fresh waters. Minimal carryover makes this an ideal system for applications in pollution tracing, source attribution and greenhouse gas monitoring, where a wide range of CH_4 isotope ratios could be expected.

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