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$\delta^{13}\text{C}$ and $\delta^2\text{H}$ measurement of methane from ecological and geological sources by gas chromatography/combustion/pyrolysis isotope-ratio mass spectrometry

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RATIONALE: The carbon and hydrogen isotopes of methane are useful in differentiating biological (e.g. wetlands, ruminants, biomass burning) and geological methane sources (e.g. fossil fuels, gas hydrates), as well as quantifying pathways of methanotrophism. Continuous-flow isotopic measurements of methane present a set of analytical challenges, including sample size restrictions and separation of CH_4 from atmosphere, hydrocarbons, and CO_2 .

METHODS: Small-scale modifications were made to a commercial trace-gas preconcentration and sampling unit (Thermo Scientific PreCon-GasBench) for improved isotopic analysis of methane ($\delta^{13}\text{C}/\delta^2\text{H}$) across a range of gas concentrations.

RESULTS: The long-term reproducibility of $\delta^{13}\text{C}\text{-CH}_4$ values is less than $\pm 0.2\%$ (1σ). The limit-of-quantitation of $\delta^{13}\text{C}\text{-CH}_4$ values is less than 0.8 nmol, conveniently measurable within standard gas sampling vials. A reproducibility of better than $\pm 4\%$ (1σ) is regularly achieved for $\delta^2\text{H}$ values from sample sizes greater than 2 nmol. The range of measurement, for both $\delta^{13}\text{C}$ and $\delta^2\text{H}$ values, is easily extended from ambient concentration (~ 1.7 ppm-v) for preconcentrated samples to percent methane concentrations under subsampling.

CONCLUSIONS: The automated measurement of $\delta^{13}\text{C}\text{-CH}_4$ and $\delta^2\text{H}\text{-CH}_4$ values, from ambient to percentage concentrations, is possible with minimal modifications to a commercial preconcentration/gas chromatography inlet. Sample matrix interferences (CO_2 , C_nH_y , air) are eliminated and simultaneous isotopic measurements of methane and CO_2 and/or $\text{C}_1\text{-C}_4$ light hydrocarbons are possible, while still retaining functionality for isotopic measurements of other gas species (e.g. CO_2 , N_2 , O_2). Copyright © 2013 John Wiley & Sons, Ltd.

Atmospheric methane, the third most abundant greenhouse gas behind carbon dioxide and water vapor, has risen in concentration by more than 150% over the past 2000 years.^[1] Methane also reduces atmospheric oxidation capacity through its interaction with hydroxyl radicals (OH^\bullet) in the troposphere, further enhancing atmospheric methane concentrations through positive feedback.^[2] Methane production is a significant contributor to anthropogenic climate impacts.^[3] However, because of methane's short residence time in the atmosphere (~ 9 years) and relative strength as a greenhouse gas, reductions in methane emissions may yield rapid results in slowing climate change.^[4] The key to addressing concerns over increased methane emissions is improving our understanding of the relative contribution of and links between natural sources of methane, such as wetlands, lakes, terrestrial arthropods, and anthropogenic sources, including agriculture, energy production, and waste management.^[5] The impact of recent increases in natural gas extraction to the global methane budget will also require evaluation.^[6,7]

Expanding isotopic measurements of methane is critical to quantifying key components of the global methane budget.^[8] While atmospheric concentrations of methane are regularly monitored,^[9] much additional information concerning the sources and sinks of methane can be gained through the measurement of carbon ($^{13}\text{C}/^{12}\text{C}$) and hydrogen ($^2\text{H}/^1\text{H}$) isotopes.^[10–14] Carbon and hydrogen isotopes of methane are valuable in differentiating specific biological (e.g. wetlands, ruminants, biomass burning) and geological methane sources (e.g. fossil fuels, gas hydrates), as well as elucidating methanogenic and methanotrophic pathways.^[15,16]

Methane emissions tend to be patchy in both time and space,^[14,17] and most ecological studies of methane will produce samples with concentrations ranging from that of ambient air (~ 1.7 ppm-v) to hundreds of parts per million. The same is true for geochemical studies,^[18] albeit at different concentration scales, ranging from parts per thousand to percentage concentrations. These broad ranges of analyte concentration are unique to methane, as other major greenhouse gases (e.g. CO_2 and N_2O) are generally measurable under a uniform set of analytical conditions. Therefore, instrumentation with the capacity to make quality measurements across a broad range of methane concentrations is essential.

Historically, there have been two general analytical strategies for the stable isotopic measurement of methane. The approach of the instrument manufacturers has been the

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chemical and cryogenic trapping of sample CO_2 and H_2O followed by the combustion of CH_4 to CO_2 and H_2O and subsequent cryofocusing of the CH_4 -derived CO_2 for $\delta^{13}\text{C}$ analysis. For atmospheric and ecological samples, the minimum sample size is typically restricted to ≥ 60 mL, and sample inlets are not generally automated. In addition, the technique is sensitive to the presence of background CO_2 , additional organics (C_nH_y), and CO . These instruments, consisting of a preconcentration unit and associated gas chromatograph, are designed to analyze a range of gases, not just methane, and the adoption of this strategy retains full instrument functionality for the multidisciplinary laboratory. A second, customized, approach involves the preconcentration and separation of methane from other sample components prior to combustion/pyrolysis. For the unequivocal separation of CH_4 from a variety of potential interferences (e.g. N_2 , O_2 , CO , CO_2 , C_nH_y), this approach is clearly superior. Moreover, sample size requirements can often be reduced through preconcentration.^[19,20] However, adopting this strategy typically involves extensive modification of the instrumentation, including extension of automated valve controls, chemical traps, and/or inlets. The downside to this approach is that overall measurement capabilities are severely reduced and are often limited to the measurement of the $\delta^{13}\text{C}$ and/or $\delta^2\text{H}$ values of methane or hydrocarbons.

This study describes a suite of small-scale modifications to a commercially available gas chromatograph and preconcentration unit for the fully automated analysis of $\delta^{13}\text{C}$ and $\delta^2\text{H}$ values in methane from ecological samples across a range of methane concentrations by gas chromatography/combustion/pyrolysis isotope-ratio mass spectrometry (GC/C/Pyr/IRMS) with or without preconcentration. The design presented here achieves precise measurements of methane samples ($\delta^{13}\text{C}$: 12 mL; $\delta^2\text{H}$: 20 mL) from a range of sources following the preconcentration and separation of CH_4 in an expedient analytical time (16 minutes), yet without the requirement of additional automation, traps, or inlets. Furthermore, through the addition of a set of manual switching valves, the instrument retains all the analytical functionality of the original instrument design. The employment of this fully automated design may facilitate isotopic measurements of methane in a greater number of laboratories and research facilities, thereby supporting additional research applications of isotopic measurements of methane.

EXPERIMENTAL

The instrument described here is a ThermoFinnigan PreCon-GasBench preconcentration unit-gas chromatograph interfaced with a Delta V Plus isotope-ratio mass spectrometer (Thermo Scientific, Bremen, Germany). The IRMS instrument has an eight-cup collector configuration, including ^1H and ^2H collectors. The instrument is also fitted with a GC PAL autosampler (CTC Analytics, Zwingen, Switzerland). The manual sampling ports in the PreCon-GasBench are adapted to accept the flush and transfer lines of a two-hole sampling needle (Thermo Scientific).

Modifications of the PreCon-GasBench

Modifications to the PreCon-GasBench include changes to the cryogenic traps, flow paths, the open-split design, and the addition of a second column oven (Fig. 1). However, no additional automation is required. In order to provide a substrate for the trapping of CH_4 at liquid nitrogen (LN_2) temperatures (-196°C), the first automated cryogenic trap (T1) on the PreCon is replaced by a coiled 90 cm length of divinylbenzene capillary column (0.32 mm ID, GS-Q, J & W Scientific, Folsom, CA, USA); the trap slits on the Dewar cover of the PreCon were removed to facilitate the movement of the coiled trap. The second cold trap (T2) is also replaced with capillary of the same composition and of the same length as the original deactivated fused silica capillary. The original Trap 1, a 0.5 mm ID stainless steel (SS) tube containing nickel wires, can be easily reinstalled for the analysis of other trace gases (i.e. N_2O , CO_2). It is not necessary to reinstall the original Trap 2, a shielded 0.3 mm ID defused silica capillary, for the analysis of other trace gases.

A second GC column is added for the analysis of methane. Originally, this column was a Rt-Q-BOND (30 m \times 0.32 mm \times 10 mm; Restek, Bellefonte, PA, USA). This column provided reasonable separation of $\text{N}_2/\text{O}_2/\text{CO}$ and CO_2 from CH_4 , but was occasionally prone to baseline disturbances ($\text{N}_2/\text{O}_2/\text{CO}$) prior to the elution of CH_4 , depending on the sample composition. The present column of choice is an Agilent J & W GS-CarbonPlot column (30 m \times 0.32 mm \times 3 mm; Agilent Technologies, Folsom, CA, USA). This column provides superior separation of CH_4 and air components (65 s versus 45 s at 30°C) and no appreciable change in CO_2 background is observed before and after the actuation of the open split. Temperature ramping is not required, as elsewhere.^[20] The original CP-PoraPLOT-Q column (25 m \times 0.32 mm \times 10 mm; Varian, Lake Forest, CA, USA) remains within the GasBench oven for use in other analyses, while the GS-CarbonPlot is housed in a second GC oven fitted with an isothermal temperature controller (Digi-Sense R/S; Cole-Parmer, Vernon Hills, IL, USA).

In order to incorporate these components and preserve functionality of the GasBench for non-methane measurements, the flow path must be altered from the original PreCon-GasBench (Fig. 1). Four manual switching valves (VICI Valco, Houston, TX, USA) must be added to allow for the proper changes in instrumental configuration needed for the analysis of different analytes (e.g. CH_4 , atmospheric CO_2). The instrumental configuration appropriate for the desired analysis is determined by selection of different positions of these valves prior to analysis. The first valve (four-port), A, selects the sampling inlet from either the GasBench or the PreCon. This allows the user to select the PreCon for trace gas measurements (i.e. CH_4) or the GasBench for the analysis of air components ($\text{N}_2/\text{CO}_2/\text{O}_2$). A second valve (four-port), B, functions as a column selection valve, diverting gas to the column (GS-CarbonPlot) and furnaces for methane analysis using the PreCon or, in the case where the measurement of air components (via GasBench) or preconcentrated trace gases (PreCon: $\text{N}_2\text{O}/\text{CO}_2$) is desired, the gas is passed to the CP PoraPLOT Q. For methane analysis, a third valve (six-port), M1, controls the flow of gases from the GS CarbonPLOT column

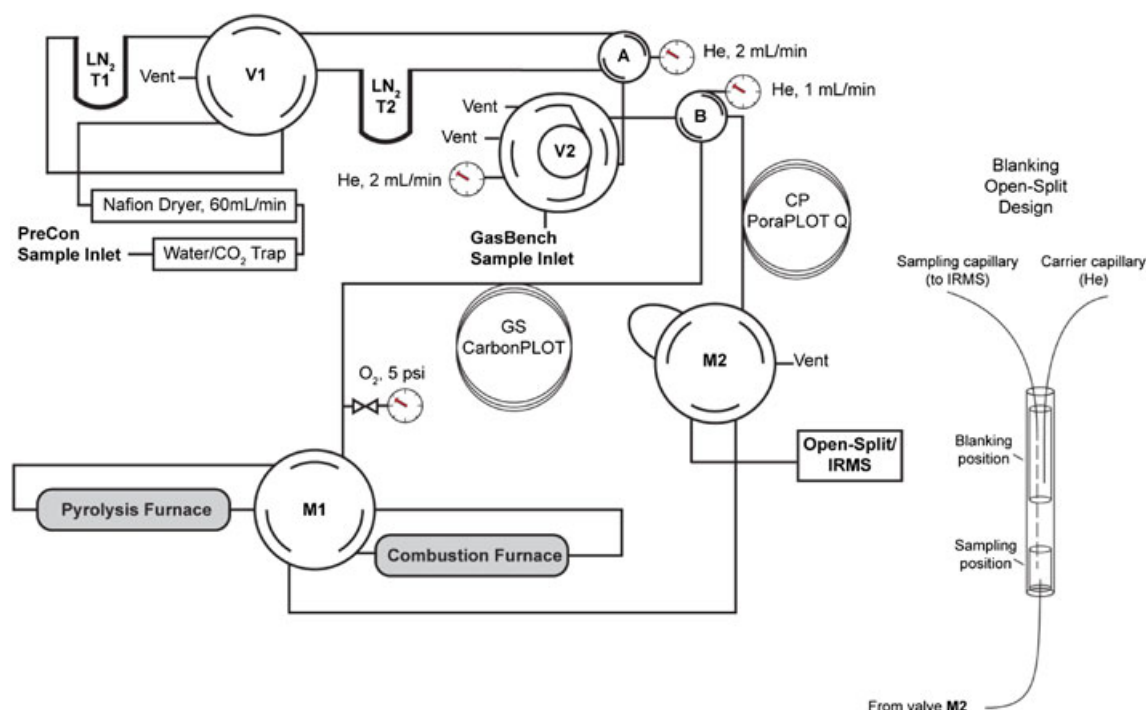


Figure 1. Schematic diagram of modified Finnigan PreCon-GasBench PC/GC/C/Pyr/IRMS system. As displayed, the flow diagram illustrates the unloading of Trap 1 (T1) to Trap 2 (T2) prior to pyrolysis of preconcentrated methane. The open-split design used in this instrument is also displayed: A, four-port manual inlet selection valve (GasBench or PreCon); B, four-port manual column selection valve; M1, six-port manual reactor selection valve; M2, six-port manual eluent selection valve; V1, six-port pneumatic valve (native to PreCon); V2, eight-port pneumatic valve (native to GasBench).

to the (i) combustion furnace or (ii) the pyrolysis furnace, acting as a reactor selection valve for switching between combustion and pyrolysis measurements. A fourth valve (six-port), M2, determines which column eluent (GS CarbonPLOT or CP PoraPLOT Q) passes to the isotope ratio mass spectrometer through the open-split, while the other column's eluent is vented to atmosphere. Please note that for safety reasons, all oxidizers (i.e. O_2 , N_2O) must be isolated from the multi-gas reference open-split during hydrogen measurements.

In order to minimize the disturbance of air components ($N_2/O_2/CO$) to the baseline prior to the elution of CH_4 , the current open-split design of the Finnigan GasBench II required modification. The current GasBench split design, essentially a narrow-bore GC injection liner, does not provide sufficient dilution of the air components. Conveniently, the original open-split design of the Finnigan GasBench,^[21] which allows for "blanking" through the use of an interior glass capillary (Fig. 1), is still presently available from Thermo Scientific, and is utilized here.

For increased throughput, this design includes two furnaces: (i) a combustion furnace (maximum temperature: 1000 °C), which is a standard component of the PreCon, and (ii) a pyrolysis furnace ("GC-TC"; maximum temperature: 1550 °C) that was purchased separately from Thermo Scientific. Oxygen is supplied via a pressure regulator to allow for reoxidation of the combustion reactor (Fig. 1). For cost considerations, the pyrolysis furnace could be used alone, but this would require the exchange of reactors between $\delta^{13}C$ and δ^2H analysis.

Analysis timings for $\delta^{13}C$ and δ^2H of low concentration methane

For low-concentration methane samples (<170 nmol), a two-hole sampling needle is back-flushed with the helium carrier gas (UHP: 99.999%; Airgas, Inc., Sacramento, CA, USA) until vial penetration, at which time T1 is inserted into a Dewar of LN_2 . The sample is purged at 20 mL/min, passing through a chemical trap composed of granulated NaOH and $Mg(ClO_4)_2$ to reduce the abundance of CO_2 and H_2O , respectively, before being further dried by a Nafion[®] dryer (30 cm, 60 mL/min) and collected in T1. At 400 s, T2 begins cooling in the LN_2 bath. At 460 seconds, Valve 1 (V1; PreCon) is switched to "INJECT" mode and T1 is subsequently removed from the LN_2 and the sample transferred to T2 for 160 s. Longer trapping times do not increase signal strength or reproducibility, as determined by incremental extensions to the sampling time of 30 s. The sample transfer line and needle are back-flushed once again after V1 is set to "INJECT". At 575 s, V1 is switched back to "LOAD", where the carrier flow rates through T2 and the GS-CarbonPlot analytical column are set to 1.2 mL/min. T2 is removed from the LN_2 55 s later. The sample stream is then separated on the analytical column and passes through either the combustion or the pyrolysis reactor. The combustion reactor contains NiO wires heated to 1000 °C; the pyrolysis reactor is an empty alumina tube heated to 1350 °C after conditioning with several samples containing ~150 nmol of CH_4 . At a column temperature of 25 °C, residual air components elute at approximately

875 s, CH_4 at 940 s, and CO_2 at 1040 s. The open-split is activated at 925 s to capture the methane peak, and deactivated once again at 1000 s. The total analysis time is 1100 s.

Analysis timings for $\delta^{13}\text{C}$ and $\delta^2\text{H}$ of high concentration methane

For high-concentration methane (>170 nmol), the analysis begins with the GasBench two-hole sampling needle, flushed with the helium carrier gas (UHP: 99.999%; Airgas, Inc.) inserted into the sample via at time 0 s. The sample is purged at 1 mL/min, passing through a sampling loop mounted on an eight-port pneumatic sampling valve (V2; Valco VICI), supplied with the GasBench. The volume of the sampling loop is selected based upon the expected concentration of methane. At 90 s, V2 is switched to "INJECT" mode, where a second source of carrier gas unloads the sample loop to the GS-CarbonPlot analytical column. At 120 s, V2 is switched back to the "LOAD position". Following separation, the sample gases then pass through the combustion or pyrolysis reactor, as described earlier. The open-split is activated at 300–370 s to capture the methane peak ($t_R = 330$ s) and exclude the CO_2 peak ($t_R = 390$ s). If CO_2 is also of interest, the open-split activation time can be extended. The total analysis time is 400 s.

Methane standards and samples

Three certified $\delta^{13}\text{C}$ isotopic working standards: B-iso1 ($\delta^{13}\text{C}$: -54.5 ± 0.2), H-iso1 ($\delta^{13}\text{C}$: -23.9 ± 0.2 ‰), and L-iso1 ($\delta^{13}\text{C}$: -66.5 ± 0.2 ‰), were purchased from Isometric Instruments (Victoria, British Columbia, Canada) and prepared at 25 nmol CH_4 . Two calibrated $\delta^{13}\text{C} + \delta^2\text{H}$ reference materials (NIST: National Institute of Standards and Technology, Gaithersburg, MD, USA): NGS-1 ($\delta^{13}\text{C}$: -29 ± 0.2 ; $\delta^2\text{H}$: -138 ± 6 ‰), NGS-2 ($\delta^{13}\text{C}$: -44.7 ± 0.4 ; $\delta^2\text{H}$: -173 ± 4 ‰) were used to calibrate all $\delta^2\text{H}$ measurements and verify the $\delta^{13}\text{C}$ values of the Isometric Instruments standards. Because of the small volume of NGS-1 and NGS-2 available, these gases were strictly reserved for use in $\delta^2\text{H}$ measurements, for which commercially available working standards are presently unavailable. Another gas, Mamm Creek ($\delta^2\text{H} \approx -190.4$ ‰), was used to evaluate

$\delta^2\text{H}-\text{CH}_4$ corrections (courtesy of Robert Dias, US Geological Survey, Denver, CO, USA). Three methane mixtures were purchased from Scott Specialty Gases (Plumsteadville, PA, USA), an equimolar mix of $\text{CH}_4 + \text{CO}_2$, and Airgas Inc.: (i) UCDM1: 10 ppm-v methane in air; and (ii) UCDM2: 500 ppm-v methane in helium. To evaluate the isotopic linearity of this instrument, a range of methane concentrations was made from UCDM2 and helium (99.999% purity) using a custom-made two-port gas-mixing unit. Provisional values were calculated by comparison with a pure reference gas (CO_2 or H_2). Final calibration of the methane $\delta^{13}\text{C}$ and $\delta^2\text{H}$ values was performed using NGS-1 and NGS-2 (International Atomic Energy Agency (IAEA); courtesy of Robert Dias, US Geological Survey). The isotopic abundances are reported as δ -values ("per mil", ‰) on the V-PDB scale (Vienna Pee Dee Belemnite) for $\delta^{13}\text{C}$ values, and the V-SMOW scale (Vienna Standard Mean Ocean Water) for $\delta^2\text{H}$ values, as determined by the IAEA (Vienna, Austria).

Air samples were collected at 1 m above dry soil using a 25 mL gas-tight syringe during April 2012 on the campus of the University of California in Davis, California, USA. Natural dissolved methane and air samples were also collected from a regulated stretch of Putah Creek in Yolo Co., in north-central California, USA, during October 2012. Due to strict regulation of flows for agricultural water conservation, the section of Putah Creek below Monticello Dam, the primary water storage facility in the drainage, is characterized by high sedimentation rates and an expanding aquatic plant community. Samples were collected approximately 1.5 km downstream of Monticello Dam below a small plunge pool. For dissolved methane, samples were collected in evacuated 12 mL Exetainers (Labco Ltd., Lampeter, UK) containing 1 mL 85% phosphoric acid. The sampling procedures followed Bastkiven *et al.*^[22] For air samples, 15 mL of air were injected into a previously evacuated (<10 mTorr) 12 mL Exetainer using a gas-tight syringe (SGE Analytical, Melbourne, Australia). Three sample types were collected for the analysis of methane: (i) water, 20 cm below still surface water, over a sediment-rich 5 m² patch of *Elodea nutallii* (waterweed) and *Myriophyllum spicatum* (Eurasian watermilfoil); (ii) air above the water containing *E. nutallii* and *M. spicatum*; and (iii) ambient air collected ~20 m from the stream. For dissolved methane samples, 4 mL of water was replaced with helium and the headspace equilibrated for 24 h prior to analysis.

Table 1. $\delta^{13}\text{C}$ and $\delta^2\text{H}$ measurements of methane from working standards and ambient air at the Stable Isotope Facility at the University of California, Davis. All δ -values (‰) are reported at $\pm 1\sigma$

Sample	<i>n</i>	$\delta^{13}\text{C}$	$\delta^2\text{H}$
NGS-1 (IAEA)	3	-29.02 ± 0.38	-138.09 ± 2.03
NGS-2 (IAEA)	3	-44.31 ± 0.51	-177.12 ± 0.39
Mamm Creek	3	-41.29 ± 0.40	-223.69 ± 2.67
B-iso1 (Isometric Instruments)	3	-54.29 ± 0.26	Not measured
H-iso1 (Isometric Instruments)	3	-23.88 ± 0.27	Not measured
L-iso1 (Isometric Instruments)	3	-66.44 ± 0.42	Not measured
UCDM1 (Airgas)	4	-36.59 ± 0.13	-131.86 ± 2.19
UCDM2 (Airgas)	12	-36.78 ± 0.27	-146.96 ± 1.09
Scott (Scott Specialty Gases)	3	-41.63 ± 0.47	-176.65 ± 1.23
Air (Davis, CA, USA)	4	-45.21 ± 0.45	-84.09 ± 2.96

RESULTS

The measurement of methane $\delta^{13}\text{C}$ values was both accurate and reproducible (0.13‰ – 0.51‰ ; $\pm 1\sigma$) for a wide range of methane sources (Table 1) and concentrations (Fig. 2). The range of measurement possible for $\delta^{13}\text{C}$ values following preconcentration was 0.8 – 130 nmol, corresponding to peak areas of 3 – 210 volt-seconds (Vs). The upper range of measurement was extended either through sample dilution (to 170 nmol) or valve-switching (to 100 nmol) following introduction through the GasBench (Fig. 3). The preconcentration of methane on the GS-Q column yielded a response factor for $\delta^{13}\text{C}$ values of 2.7 Vs/nmol (± 0.07 ; 1σ , $n=12$). This was better than the response factor observed for $\delta^{13}\text{C}$ values in the original configuration (1.3 ± 0.17 Vs/nmol; 1σ , $n=10$). Significant system carryover between samples was not observed for $\delta^{13}\text{C}$ values (Fig. 4). Importantly, the instrument was able to measure the $\delta^{13}\text{C}$ values of methane from atmosphere (~ 0.8 nmol) within a standard 12 mL Exetainer, a nearly universal sampling vial for ecological studies of gases.

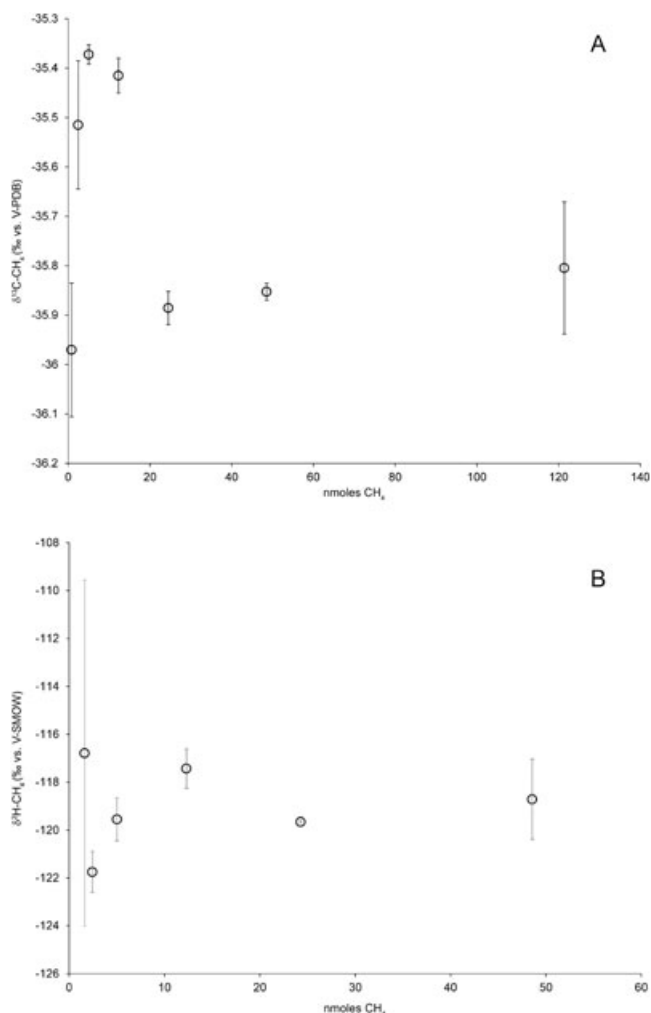


Figure 2. Duplicate (A) $\delta^{13}\text{C}$ and (B) $\delta^2\text{H}$ measurements of methane ($n=2$, $\pm\text{SD}$) from ~ 1.6 – 48 or 120 nmol CH_4 , respectively. Measurements are corrected for instrument linearity ($\text{D}\delta^{13}\text{C}/\text{DCH}_4$).

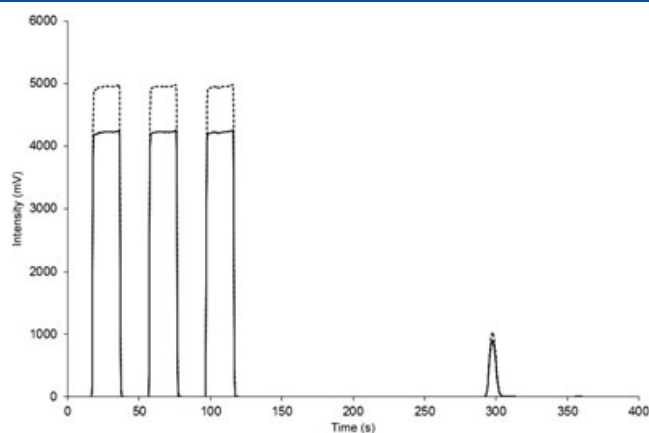


Figure 3. Chromatography of CH_4 (242 nmol) on the J & W GS-CarbonPLOT column under valve sampling on the GasBench II.

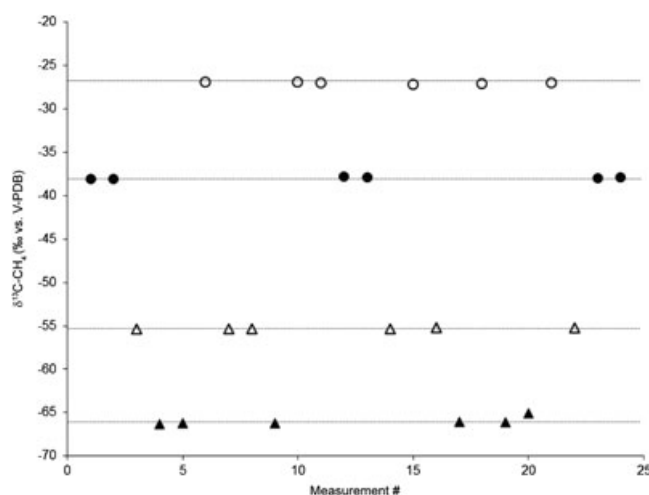


Figure 4. Sequential measurements of $\delta^{13}\text{C}$ values in methane by PC/GC/C/IRMS. Samples were prepared at 2.5 nmol in 12 mL of helium and ordered randomly (5 ppm-v: UCDM1, solid circle; H-iso1, open circle; L-iso1, solid triangle; B-iso1, open triangle).

The precision of $\delta^2\text{H}$ measurements ranged from 1.09‰ to 2.96‰ (1σ ; Table 1). The accuracy of $\delta^2\text{H}$ measurements is more difficult to evaluate given the current lack of commercially available isotopic standards of methane. However, the $\delta^2\text{H}$ measurements of methane from air in Davis, California, USA, were found to be quite close to published values for atmospheric methane.^[3,23] The reproducibility of $\delta^2\text{H}$ measurements of CH_4 in atmospheric samples was $\sim 3\text{‰}$ (Table 1) and this is consistent with $\delta^2\text{H}$ - CH_4 measurements reported elsewhere.^[24,25] Significant system carryover between samples was not observed for $\delta^2\text{H}$ measurements (Fig. 5). Despite a low H_3 -factor (2.91 ppm/nA), linearity was significant for $\delta^2\text{H}$ values ($+1.4\text{‰}/\text{Vs}$), probably a limitation of background correction routines available in the ISODAT software (Thermo Scientific);^[19] the $\delta^2\text{H}$ data was evaluated using the *Individual* background correction with a 2 s interval. The linearity of the $\delta^2\text{H}$

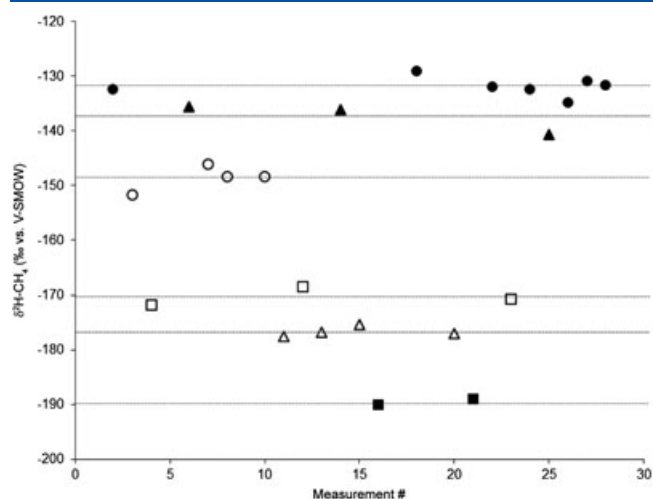


Figure 5. Sequential measurements of $\delta^2\text{H}$ values in methane by PC/GC/Pyr/IRMS. Samples were prepared at 2.4 nmol in 12 mL of helium and ordered randomly (5 ppm-v: UCDM1, solid circle; UCDM2, open circle; NGS 1, solid triangle; NGS 2, open triangle; Mamm Creek, solid square; Scott gas, open square).

measurements was found to be quite stable over time and was used in post-analysis data reduction. The response factor for $\delta^2\text{H}$ values was 3.0 Vs/nmole (± 0.03 ; 1σ , $n=12$); the $\delta^2\text{H}$ values were not measured using the original Finnigan PreCon configuration. The $\delta^2\text{H}$ measurements were more sensitive to low sample concentrations (Fig. 2). The drop in precision was precipitous, from $\pm 1\%$ for duplicate samples containing 2.4 nmol, to greater than 7% for duplicate samples of 1.6 nmol. Samples should contain, at minimum, >2 nmol of CH_4 for reasonable $\delta^2\text{H}$ analysis by this instrument. The range of measurement possible for $\delta^2\text{H}$ values without dilution was 2–260 nmol, and this was extendable to 180 mmol by using the GasBench loop-sampling inlet. For $\delta^2\text{H}$ measurements, samples in Exetainers must contain a minimum of 5 ppm-v CH_4 in order to meet the 2 nmol requirement, thus precluding ambient $\delta^2\text{H}$ measurements in these vials. Measurements of $\delta^2\text{H}$ values at ambient concentrations are facilitated by sample collection with a 20 mL headspace vial, in place of the 12 mL Exetainer. However, headspace vials are not nearly as leak-tight as Exetainers and samples must be measured quickly following sample collection.

Concentration measurements from IRMS data, using $\delta^{13}\text{C}$ data only, were evaluated through a comparison with GC flame ionization detector (FID) data for samples from several laboratory and field projects. The sample methane concentrations were generally well estimated by IRMS compared with GC-FID measurements (Fig. 6), especially when the potential variation in sample vial integrity and effects of prior measurement by GC-FID are considered. While the precision of GC-FID data ($\% \text{RSD} \leq 2$) is generally better than measurements by IRMS ($\% \text{RSD} = 6.2$; $\delta^{13}\text{C}$ measurements at 5 nmol; $n=5$), in studies where the concentration is less important, IRMS data may be substituted.

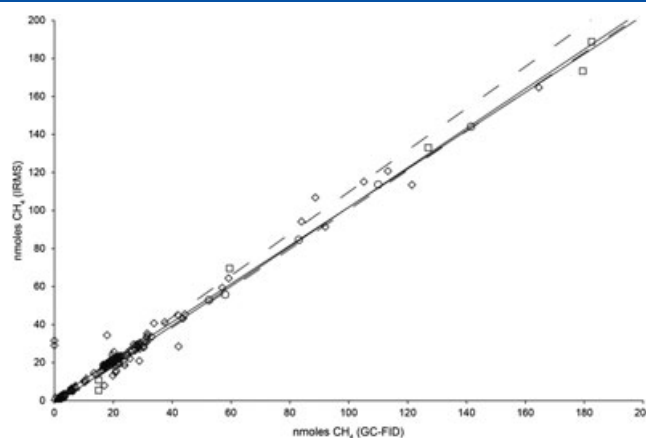


Figure 6. Methane concentration measurements by PC/GC/C/IRMS compared with concentration measurements by GC-FID. Symbols represent data from four separate studies.

By trapping methane prior to chromatography and subsequent combustion to CO_2 , this instrumental design greatly reduces the influence of C_nH_y and CO_2/CO contamination in CH_4 measurements requiring preconcentration. The signal-to-blank ratio for an air sample in the conventional Finnigan PreCon design was measured as (9.5:1.5 Vs, 100 mL; 15.7%; Fig. 7(A)), while the same ratio for the modified Finnigan PreCon was (4.8:0.3 Vs, 20 mL; 6.3%). The total CO_2 present in the original system, as determined by a measurement of helium-flushed container, constitutes ~35% of the total signal produced by a measurement of ambient air (9.5:3.5 Vs, 100 mL; Fig. 7(B)). Based on the reduction in background CO_2 measured when placing a manual cryotrap prior to combustion, as is required of the original instrument design, about 20% of the system CO_2 comes from the sample introduction train. In addition, if the combustion train is then bypassed, the total background CO_2 of a helium-flushed container drops to near 2%. It appears that much of the CO_2 blank in the original design was attributable to the alumina tube used in the combustion reactor; thicker diameter alumina tubes significantly reduced the amount of CO_2 present in the blank. Clearly, designs where combustion occurs before cryotrapping are ultimately system leak concentrators; slight changes to system integrity can cause a measureable change in the CO_2 blank associated with the CH_4 measurement. At high concentrations, this may not contribute significantly to measurement error, but near ambient concentration this issue becomes difficult to ignore. Designs, such as that described here, that utilize chromatography to separate CH_4 from any residual air components or interferences from non- CH_4 hydrocarbons are critical for samples that contain CO_2 and/or $\text{C}_2\text{--C}_4$; this would include almost all ecological and geochemical samples.

The use of a “blanking” open-split greatly diminished any $\text{N}_2/\text{O}_2/\text{CO}$ -based baseline disturbances prior to the elution of CH_4 ; the baseline disturbance prior to methane is negligible for $\delta^{13}\text{C}$ measurements and well separated for $\delta^2\text{H}$ measurements (Fig. 8). Previous measurements using the standard open-split resulted in a rise in the m/z 46 background, probably due to the formation of NO_2 in the

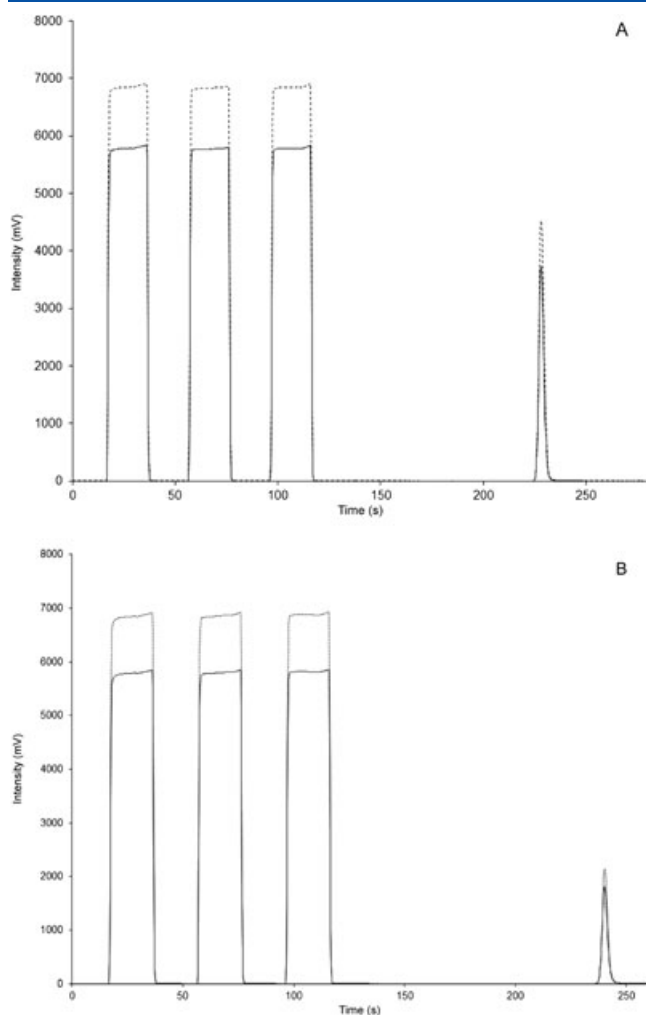


Figure 7. $\delta^{13}\text{C}$ values of CH_4 in (A) air and system blank (helium flushed vial, no manual LN_2 trap); (B) using the original PreCon-GasBench configuration (sample size = 100 mL sample flask).

source following the co-elution of N_2 and O_2 . Furthermore, there was no measureable effect of the presence of air components on the overall precision of measurement; the precision of a mix of methane in air, UCDM1 (CH_4 in air), was $\pm 0.13\%$ (1σ), while the precision of a mix of methane in helium, UCDM2 (CH_4 in He), was measured to be $\pm 0.27\%$ (1σ). If present, excessive concentrations of $\text{C}_2\text{-C}_4$ were simply blanked by the open-split at the beginning of the subsequent sample. For those researchers interested in isotopic measurements of parts-per-million concentrations of $\text{C}_2\text{-C}_4$, it is possible to extend the analysis time and activation of the open-split to capture these data. This open-split design also allows the analyst to control the co-measurement of CO_2 to a much greater degree than a single-bore design.

Methane $\delta^{13}\text{C}$ measurements from stream water, emissions, and adjacent environments on Putah Creek (California, USA) provide just one example of the potential applications facilitated by this instrument design. The methane concentrations differed significantly between air, methane-enriched air, and stream water, and ranged from ambient (~ 1.7 ppm-v) to 10 times ambient concentrations.

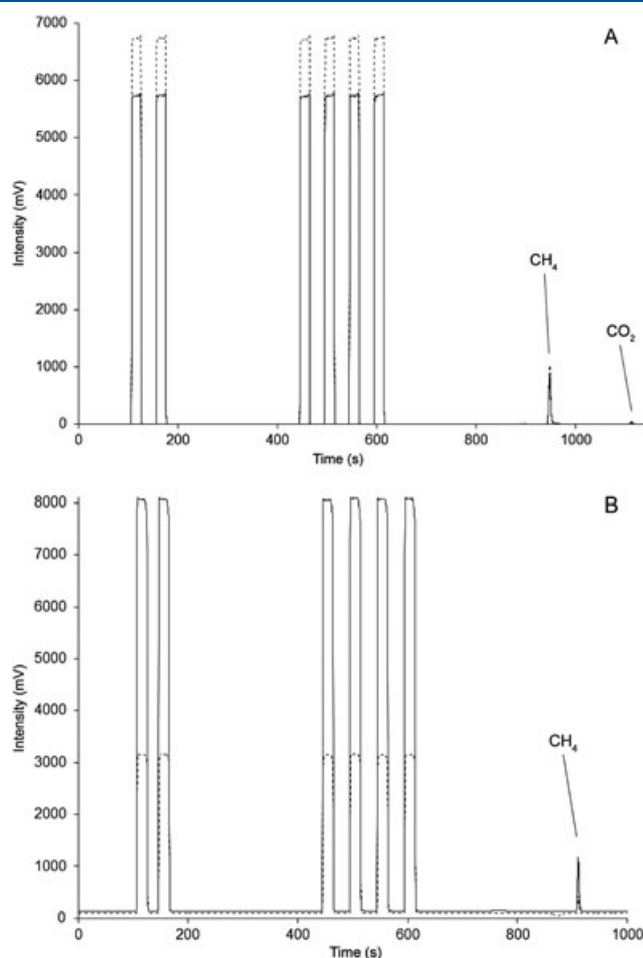


Figure 8. Chromatographic separation of CH_4 from CO_2 and residual air in an ambient air sample on the J & W GS-CarbonPLOT column for (A) $\delta^{13}\text{C}$ values (12 mL) and (B) $\delta^2\text{H}$ values (20 mL).

The highest concentrations were measured in the stream water sampled above organic-rich sediments occupied by aquatic vegetation. The carbon isotopic signature of the streamside air ($\delta^{13}\text{C} = -42.24 \pm 0.12\%$) was significantly different from that of the emitted methane ($-46.24 \pm 2.1\%$), while the $\delta^{13}\text{C}$ value of dissolved methane was lower ($-51.87 \pm 1.2\%$), consistent with isotopic signatures of freshwater sediments.^[15] Furthermore, a Keeling plot of the emitted methane identified the underlying dissolved methane as a probable source of excess methane in air (Fig. 9).

CONCLUSIONS

Critical to the improvement of preconcentration/gas chromatography systems for isotopic measurements of methane was the cryofocusing of methane prior to separation and combustion. This eliminated the contribution of background CO_2/CO and/or non-methane hydrocarbons and dramatically lowered the system CO_2 blank. In addition, with the inclusion of a high-temperature furnace, $\delta^2\text{H}$ measurements were made possible. The chromatography was specifically targeted to provide clear separation of

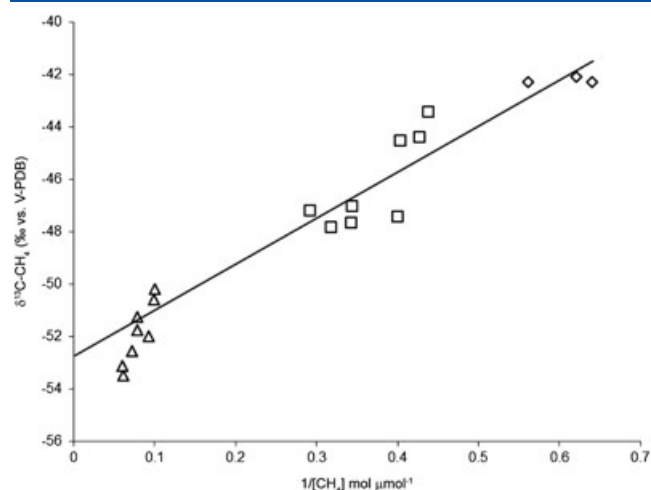


Figure 9. Plot of $\delta^{13}\text{C}\text{-CH}_4$ values versus the inverse of CH_4 mole fractions from air (open diamonds) and CH_4 emissions (open squares) collected on Putah Creek, California, USA. The intercept of the plot (-52.76‰) indicates the $\delta^{13}\text{C}$ value of the source for the CH_4 emissions. The mean $\delta^{13}\text{C}$ values of the dissolved methane collected over the creek sediment (open triangles, overlain) was found to be $-51.87 \pm 1.2\text{‰}$.

residual air components, CH_4 and CO_2 , thereby decreasing measurement error of CH_4 near or at ambient concentration in comparison with the original design. In addition, the integration of the PreCon and the GasBench greatly extended the measurement range of CH_4 , while also maintaining functionality for CO_2 and N_2O measurements, and allowing for isolation of various system components, thereby facilitating troubleshooting.

The difficulty of making quality, automated measurements of $\delta^{13}\text{C}$ and $\delta^2\text{H}$ values in methane, especially in ecological samples, has hindered progress in the application of stable isotope data to methane studies. The instrument described here is able to provide isotopic measurements of $\delta^{13}\text{C}$ and $\delta^2\text{H}$ values in methane, from ambient to percentage concentrations, with minimal modifications to a commercial preconcentration/gas chromatography inlet. Potential interferences are avoided; indeed, simultaneous measurements of methane and CO_2 and/or $\text{C}_1\text{-C}_4$ light hydrocarbons may be performed with this design. While the specific modifications described here provide extensibility for the measurement of $\delta^{13}\text{C}$ and $\delta^2\text{H}$ values in methane to the ThermoFinnigan PreCon-GasBench, similar modifications could be performed on the other major commercial systems that share the same basic design.

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