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Determination of $\delta^{13}C$-CO_2 in ambient air by gas preconcentration-isotope ratio mass spectrometry: methodological development and optimization at sub-milliliter injection volumes

Tianyu Bian[1,2], Xingliang He[2,*], Xiaoyong Duan[2,*], Feng Li[2], Huajie Wu[2], Linlin Cui[3]

1. School of Chemistry and Chemical Engineering, Ocean University of China, Qingdao 266100, China
2. Qingdao Institute of Marine Geology, China Geological Survey, Qingdao 266237, China
3.Institute of Geology and Geophysics, Chinese Academy of Sciences, Beijing 100029, China

* Corresponding author: Xingliang He

Postal address: Qingdao Institute of Marine Geology, China Geological Survey, Qingdao 266237, Shandong, China

E-mail: 76791772@qq.com

* Corresponding author: Xiaoyong Duan

Postal address: Qingdao Institute of Marine Geology, China Geological Survey, Qingdao 266237, Shandong, China

E-mail: dxiaoyong@mail.cgs.gov.cn

Abstract. The stable carbon isotope composition of atmospheric CO_2 ($\delta^{13}C$-CO_2) is a critical tracer for distinguishing the contributions of different carbon sources and sinks. However, the atmospheric background CO_2 concentration is only approximately 420 ppm, which renders direct injection and measurement unachievable using conventional continuous-flow isotope ratio mass spectrometry and limits the widespread application of this technique in high-frequency monitoring scenarios. In this study, a gas preconcentration unit (PreCon) was coupled to an isotope ratio mass spectrometer (IRMS). Key parameters, including the trapping duration of the two-stage liquid nitrogen cold traps (T2/T3), sample injection volume, and sample vial pretreatment protocol, were systematically optimized, and methodological validation was conducted using multiple reference materials via multiple pretreatment pathways. The results demonstrated that the optimal trapping time for both T2 and T3 cold traps was 200 s, under which quantitative CO_2 trapping was achieved without detectable isotopic fractionation. The system background signal accounted for approximately 0.4% of the signal intensity of a typical sample, exerting no significant interference on the measurement results. Over the injection volume range of 0.5-5.0 mL, a strong linear

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correlation was observed between injection volume and signal response (R^2 = 0.9998). The minimum effective injection volume was 0.5 mL (corresponding to approximately 8.9 nmol CO_2), with a replicate measurement precision of 0.02‰ for $\delta^{13}C$. Helium flush was identified as the optimal pretreatment approach for air samples stored in Labco vials. Measurements via manual and automatic injection showed excellent consistency, and the results agreed well with the certified values of gas matrix reference materials. The maximum deviation among the three pretreatment pathways (PreCon, GasBench, and dual inlet (DI)) was 0.11‰, and the method enabled accurate determination of both solid carbonate and gas matrix reference materials. This proposed method achieves online high-precision $\delta^{13}C$ measurement of atmospheric background CO_2 at the milliliter scale, and provides reliable technical support for atmospheric carbon cycle tracing and isotopic monitoring of urban carbon emissions.

Keywords. gas preconcentration; isotope ratio mass spectrometry; $\delta^{13}C$-CO_2; carbon isotope

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1 Introduction

The continuous rise in atmospheric CO_2 concentration is the core driver of global climate change (Le Quéré et al., 2016), and the Sixth Assessment Report (AR6) of the Intergovernmental Panel on Climate Change (IPCC) clearly points out its irreversible impact on the global climate system (Intergovernmental Panel On Climate Change (Ipcc), 2023). Against the practical demand of the "Dual Carbon" goal, accurately partitioning the contributions of various source and sink processes-including fossil fuel combustion, terrestrial ecosystem respiration, and ocean-atmosphere exchange-is a critical prerequisite for formulating emission reduction policies (Wang, 2025). Stable carbon isotope ($\delta^{13}C$) is widely used as an independent tracer for atmospheric CO_2 source-sink analysis, as different emission sources bear characteristic isotopic "fingerprints": approximately -28‰ for fossil fuels, -26‰ for C3 plants, and +1‰ to +2‰ for marine sources (Keeling et al., 2017; Sauze et al., 2026; Zhou et al., 2006). However, the ambient air matrix is highly complex, comprising abundant N_2, O_2, Ar, and water vapor. The $H_2{}^{17}O^+$ and $H_2{}^{18}O^+$ fragments generated from water vapor in the IRMS ion source cause mass interference with the target ions $^{13}CO_2{}^+$ and $C^{18}O^{16}O^+$, leading to systematic measurement biases (Leckrone and Hayes, 1998). Meanwhile, CO_2 is present in the atmosphere at a trace level of approximately 420 ppm, far below the detection requirement of conventional continuous-flow isotope ratio mass spectrometry (Leitner et al., 2023; Manaj and Kim, 2025). Accordingly, the central challenge for high-precision $\delta^{13}C$-CO_2 determination is that samples must undergo thorough water removal, purification, and enrichment before being introduced into the isotope ratio mass spectrometer (IRMS) (Kantnerová et al., 2022).

To tackle this technical challenge, researchers have developed multiple pretreatment methods. The offline cryogenic vacuum method can produce high-purity CO2, but it features cumbersome operations, low throughput (1-2 hours per sample), and high susceptibility to fractionation from numerous manual procedures (Sakai and Matsuda, 2017). The dual-inlet IRMS (DI-IRMS) method achieves a precision of 0.005‰-0.010‰, yet it imposes strict requirements on sample quantity (milligram-scale carbon equivalent), making it difficult to meet the needs of large-scale monitoring (Srivastava and Michael Verkouteren, 2018). The GasBench continuous-flow IRMS (CF-IRMS) approach enables automated sample injection, but it requires CO2 mole fractions at the percentage level and thus cannot be directly applied to samples at atmospheric background concentrations (Yu and Lee, 2020). The coupling of a gas preconcentration system (PreCon) with IRMS (Brand, 1995) performs online two-stage (T2/T3) cryogenic enrichment of CO2 using liquid nitrogen cold traps (-196 °C). This technique reduces sample volume requirements to the milliliter scale and shortens the analytical cycle to approximately 15 minutes per sample, while effectively removing non-condensable matrix gases (Brand, 1995; Liu et al., 2012). Nevertheless, existing studies have mainly focused on the application of PreCon systems. Key methodological issues still lack comprehensive empirical support, including the systematic optimization of cold trap enrichment efficiency during preconcentration, quantitative assessment of isotopic fractionation during freeze-release cycles, boundary conditions for the linear response of signal intensity to injection volume, and comparative validation of measurement consistency across different pretreatment pathways. In particular, whether measurement

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results obtained via the PreCon method can be seamlessly traced to the international VPDB scale has not been systematically documented.

To address these methodological gaps, this study carried out systematic methodological validation using IRMS as the core instrument and PreCon as the pretreatment platform. Key aspects investigated included cold trap enrichment efficiency, system background, linear response range, calibration curve establishment, and comparison across different pretreatment pathways. This study aims to provide a complete and traceable methodological foundation for high-precision monitoring of $\delta^{13}C\text{-}CO_2$ in ambient air, and reliable analytical technical support for atmospheric carbon source-sink research and isotopic verification of carbon emission inventories.

2 Experimental Section

2.1 Experimental Apparatus and Methods

2.1.1 Instrument System and Analytical Principle

In this study, a MAT 253 gas stable isotope ratio mass spectrometer (Thermo Fisher Scientific, USA) was employed. The instrument is equipped with three mass detectors for simultaneous measurement of ion beams at m/z 44, 45 and 46, achieving an internal measurement precision better than 0.06‰. It was coupled to a PreCon trace gas preconcentrator fitted with liquid nitrogen cold traps (T2 and T3, -196 ℃) and a magnesium perchlorate-packed chemical trap, as well as a GasBench II multi-purpose online gas preparation and introduction system configured with an HP-PLOT Q capillary column (30 m × 0.32 mm × 20.0 μm). A schematic diagram of the instrumental setup is presented in Fig.1.

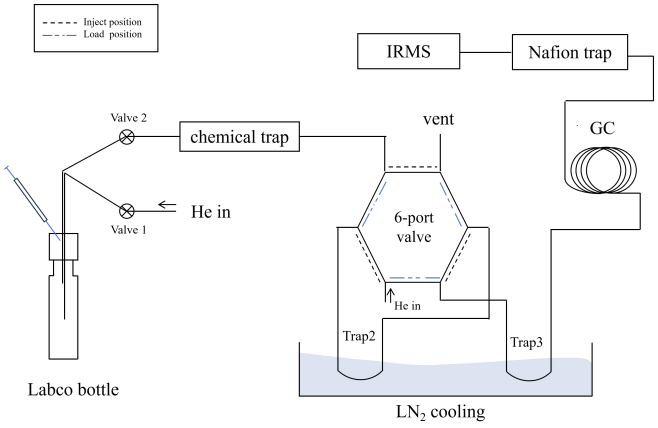

Fig. 1. Schematic diagram of the PreCon-GC-IRMS instrumental system

After water vapor was removed via a chemical trap packed with magnesium perchlorate, air samples entered the T2 liquid nitrogen cold trap (-196 ℃) for the first stage of cryogenic preconcentration. Upon

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desorption, the CO_2 was delivered to the T3 liquid nitrogen cold trap (-196 ℃) for a second stage of cryogenic trapping to achieve further purification and enrichment. The purified CO_2 was transported by helium carrier gas, separated on an HP-PLOT Q capillary column maintained at an isothermal temperature of 40 ℃, and introduced into the ion source of the isotope ratio mass spectrometer, where it was ionized into ions with mass-to-charge ratios (m/z) of 44, 45 and 46. The stable carbon isotope composition ($\delta^{13}C$) of sample CO_2 was calculated relative to the VPDB scale based on the corresponding ion beam intensities.

Typical operating parameters of the PreCon-GC-IRMS system were set as follows: the GC column oven temperature of the GasBench was 40 ℃, the carrier gas pressure was approximately 15 psi, the CO_2 reference gas pressure was approximately 23 psi, the PreCon carrier gas pressure was approximately 16 psi, and both T2 and T3 liquid nitrogen cold traps were held at -196 ℃.

The instrumental stability of the IRMS was tested in on-off mode. High-purity CO_2 (purity ≥ 99.999%) was used as the reference gas, which was alternately introduced with a blank through the IRMS reference gas inlet in an on-off cycle. Ten consecutive measurements were acquired for each group; the ion beam intensities at m/z 44, 45 and 46 were recorded, and the corresponding $\delta^{13}C$ values on the VPDB scale were calculated. The short-term instrumental stability was evaluated using the standard deviation of the measurement results.

Vacuum line sample preparation method (McCrea, 1950): Approximately 6 mg of carbonate reference material was weighed into a reaction vial connected to the vacuum line system. After the system was evacuated to a pressure below 0.1 Pa, excess 100% orthophosphoric acid was added, and the reaction was carried out in a constant-temperature water bath at 25 ℃ ± 0.1 ℃ for 24 h. The generated CO_2 was purified via cold trapping and flame-sealed in quartz glass tubes (100 mm in length, 6 mm in outer diameter) for subsequent DI-IRMS and PreCon-GC-IRMS determinations.

Dual-inlet (DI) measurement: Purified CO_2 prepared via the vacuum line method was introduced into the IRMS through a dual-inlet system and measured alternately against the reference gas to determine the $\delta^{13}C$-CO_2 values.

2.1.2 Gas Sample Pretreatment and Injection Procedures

Two injection modes, manual and automatic, were employed for sample analysis.

For the manual injection mode, a locking gas-tight syringe was used to deliver precise sample volumes. Gaseous reference samples of 0.35 mL and 0.70 mL were injected directly into the system through the PreCon inlet for preconcentration and measurement. For CO_2 produced from the phosphoric acid digestion of solid carbonate reference materials, a 50 μL aliquot was manually introduced into the GasBench inlet for isotopic analysis.

For the automatic injection mode, 12 mL Labco borosilicate glass vials served as sample vessels. Prior to sample loading, two blank pretreatment protocols were applied to empty vials: 1. vials were evacuated under vacuum and then filled with reference or sample gas; 2. air in the vials was repeatedly flushed with high-purity helium to eliminate background CO_2 interference. Following pretreatment, 0.35 mL and 0.50

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mL of gaseous reference materials were accurately injected into the vials. The sealed vials were loaded onto the sample tray of a CTC autosampler, and automatic injection, preconcentration and measurement were performed according to the predefined program.

2.1.3 Determination of Solid Carbonate Reference Materials

To systematically validate the precision and trueness of the proposed method, comparative experiments were performed via four distinct pretreatment-injection pathways:

1. PreCon + Labco vial method: Approximately 0.5 mg of each reference material was weighed into the bottom of 12 mL Labco vials, with 3-5 replicates prepared per sample, and the vials were sealed with stoppers. The vial headspace was flushed with high-purity helium at 150 mL/min for ≥ 10 min to fully displace ambient air. More than 10 drops of 100% H_3PO_4 were added using a disposable syringe; the vials were vortexed and then incubated at a constant temperature of 25 °C ± 0.1 °C for ≥ 24 h. A 100 μL locking gas-tight syringe was used to withdraw 50 μL of the CO_2 generated by the reaction, which was injected into the PreCon-GC-IRMS system for measurement. The instrumental operating parameters were consistent with those specified in Sect 2.1.1.

2. GasBench + Labco vial method: Approximately 0.5 mg of each reference material was weighed into the bottom of 12 mL Labco vials, with 3 replicates prepared per sample, and the vials were sealed. Helium flushing, acid addition, and the 24 h constant-temperature reaction were conducted following the same protocol as Method 1. The sample vials were loaded onto the GasBench autosampler tray, and $\delta^{13}C$ determination was completed via automatic sampling with a dual-needle assembly. The chromatographic operating parameters were consistent with those specified in Sect 2.1.1.

3. Dual-inlet (DI) + vacuum line method: Approximately 10 mg of each reference material was weighed into the bottom of the main arm of glass reaction manifolds, with 5 replicates prepared per sample. Approximately 2 mL of 100% H_3PO_4 was added to the bottom of the side arm of each manifold. The manifolds were connected to a vacuum line, evacuated for 2 h to a pressure below 0.001 Pa, and then flame-sealed. Each manifold was tilted to allow the phosphoric acid in the side arm to flow into the main arm and submerge the sample, and the reaction proceeded in a constant-temperature water bath at 25 °C ± 0.1 °C for ≥ 24 h. The manifolds were reconnected to the vacuum line, and the generated CO_2 was collected via liquid nitrogen cryotrapping; the purified CO_2 was encapsulated in quartz tubes by flame sealing. The quartz tubes were fitted into stainless steel bellows, connected to the dual-inlet system, and $\delta^{13}C$ was measured using the DI-IRMS method.

4. PreCon + vacuum line method: Approximately 1 mg of each reference material was weighed, and acid digestion, purification and quartz tube encapsulation were performed following the vacuum line procedure described in Method 3, with 3-4 replicates prepared per sample. The CO_2-sealed quartz tubes were scored and placed inside 12 mL Labco vials. After sealing, the vials were flushed with high-purity helium for ≥ 10 min to displace ambient air. The vials were tapped to fracture the quartz tubes and release CO_2 into the vial headspace. A 100 μL gas-tight syringe was used to withdraw 30 μL of the gas, which was injected into the

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PreCon-GC-IRMS system for measurement. The instrumental parameters were consistent with those specified in Sect 2.1.1.

2.2 Main Materials and Reagents

Helium (purity ≥ 99.999%), carbon dioxide (purity ≥ 99.999%), phosphoric acid (100%, prepared from 98% H_3PO_4 and P_2O_5), liquid nitrogen (cryogen for T2/T3 cold traps), magnesium perchlorate (analytical grade, packing material for chemical traps), and gas-tight syringes (100 μL and 1000 μL, with locking function) were employed in the experiments. Carbonate isotope reference materials included solid carbonate standards from the International Atomic Energy Agency (IAEA), the National Institute of Standards and Technology (NIST) and the United States Geological Survey (USGS): IAEA-603, IAEA-610, IAEA-611, IAEA-612, NBS 18, NBS 19, USGS 44 and IAEA-CO-8. Chinese national primary solid carbonate reference materials comprised GBW04405, GBW04406, GBW04416 and GBW04417. Chinese national secondary reference materials were CO_2 gas stable carbon isotope standards GBW(E)040027 and GBW(E)040028.

2.3 Calibration Method

The stable carbon isotope composition of CO_2 in samples is expressed as $\delta^{13}C$ values in per mil (‰) relative to the international VPDB reference scale. A multi-point calibration approach was adopted, and the raw measurements were corrected following Eq. (R1):

$$\delta^{13}C_{sam,VPDB\text{-}CO2}=m\cdot\delta^{13}C_{sam,WRG}+c \quad (R1)$$

where $\delta^{13}C_{sam,VPDB\text{-}CO2}$ is the calibrated $\delta^{13}C$ value of sample CO_2 (‰), $\delta^{13}C_{sam,WRG}$ is the raw measured $\delta^{13}C$ value of sample CO_2 (‰), m represents the slope of the calibration curve, and c represents the intercept of the calibration curve. All $\delta^{13}C$ results are reported to two decimal places.

3 Results and Discussion

3.1 IRMS Instrumental Stability

Test Ten consecutive on-off stability tests were performed on the isotope ratio mass spectrometer (IRMS) using high-purity CO_2 reference gas, and the results are presented in Table 1. The mean precision (expressed as standard deviation s) of $\delta^{13}C_{VPDB}$ measurements was approximately 0.008‰. According to the Chinese national standard GB/T 42490-2023 Isotope Ratio Mass Spectrometry, the internal measurement precision of an IRMS analytical system is required to be ≤ 0.06‰ (National Technical Committee for Soil Quality Standardization, 2023). The WMO/GAW Greenhouse Gas Measurement Guide (GAW Report No. 229) sets the inter-laboratory compatibility target for $\delta^{13}C$-CO_2 at better than 0.01‰ (Sperlich et al., 2022; Tans, P and Zellweger, C, 2016). The $\delta^{13}C$ precision of 0.008‰ achieved in the on-off stability test of the developed system is approximately one order of magnitude superior to the above

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criteria, demonstrating that the baseline performance of the MAT 253 IRMS provides a solid hardware foundation for subsequent methodological optimization of the PreCon system.

Table 1. On-off test results for IRMS instrumental stability

Number of measurements	On-off-1	On-off-2	On-off-3
Number of measurements	$\delta^{13}C_{VPDB}$	$\delta^{13}C_{VPDB}$	$\delta^{13}C_{VPDB}$
Unit	‰	‰	‰
1	-0.012	-0.005	-0.008
2	-0.018	-0.015	-0.005
3	0.000	0.000	0.000
4	-0.030	-0.009	0.024
5	-0.021	-0.006	0.003
6	-0.026	0.001	-0.002
7	-0.012	-0.014	0.009
8	-0.013	0.002	-0.005
9	-0.025	0.001	0.013
10	-0.021	0.007	0.004
Standard deviation, *s*	0.009	0.007	0.010

3.2 Optimization of Preconcentration Conditions for the PreCon Two-Stage Liquid Nitrogen Cold Traps

A self-developed national secondary reference material GBW(E)040028 - a stable carbon isotope standard for CO_2 in synthetic air, with a matrix composition of 1000 ppm CO_2, 1% Ar, 21% O_2 and approximately 78% N_2 -was employed as the test sample. Prior to $\delta^{13}C$-CO_2 determination, Ar, O_2 and N_2 were removed from the sample matrix, and CO_2 was cryogenically enriched via cold trapping. To assess the preconcentration efficiency and confirm whether isotopic fractionation occurred during the enrichment process, key operating parameters, including the trapping duration of the T2 and T3 cold traps and the sample injection volume, were systematically optimized for the PreCon system.

3.2.1 Evaluation of T2 Cold Trap Enrichment Efficiency

The GasBench GC oven temperature was set at 40 °C, the carrier gas pressure at 0.10 MPa, the reference gas pressure at 0.16 MPa, and the PreCon carrier gas pressure at 0.12 MPa. The 1000 ppm CO_2 synthetic air reference material GBW(E)040028 was used as the test sample. With the T3 trap maintained at -196 °C and a fixed trapping duration of 320 s, the CO_2 enrichment efficiency and variation trend of $\delta^{13}C$-CO_2 were investigated for the T2 trap (-196 °C) at trapping durations of 60 s, 120 s, 200 s and 320 s, respectively.

As presented in Fig. 2. at an injection volume of 0.5 mL, the peak area increased gradually with prolonged trapping time and plateaued at full enrichment after 200 s. When the injection volume was increased to 2.0 mL, complete enrichment was also achieved at 200 s. These results indicate that no further extension of trapping duration is required for larger injection volumes. However, signal saturation occurred at higher injection volumes (manifested as a nonlinear response of m/z 46), which precluded reliable assessment of

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δ-value variations. By comprehensively considering enrichment efficiency and peak shape, the optimal trapping duration for the T2 cold trap was determined to be 200 s.

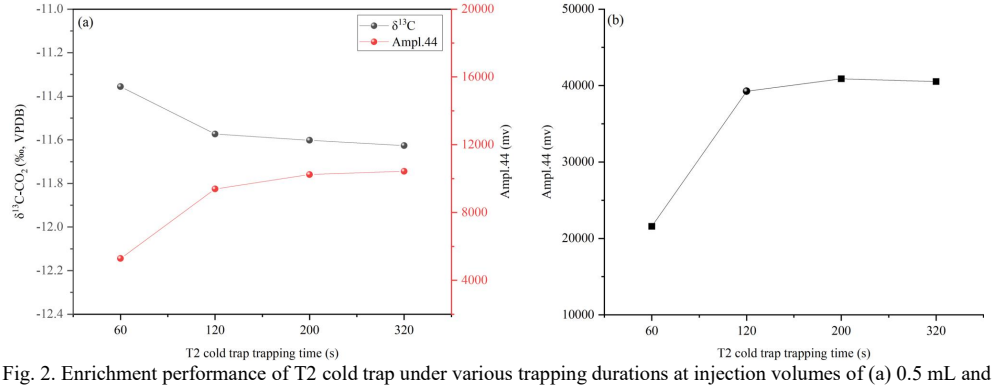

Fig. 2. Enrichment performance of T2 cold trap under various trapping durations at injection volumes of (a) 0.5 mL and (b) 2.0 mL

3.2.2 Evaluation of T3 Cold Trap Enrichment Efficiency

Under the aforementioned experimental conditions, the 1000 ppm CO_2 synthetic air reference material GBW(E)040028 was used as the test sample. With the T2 trap maintained at -196 °C and a fixed trapping duration of 200 s, the enrichment efficiency of the T3 trap (-196 °C) was further examined at trapping durations of 200 s and 320 s, respectively (Fig. 3). The results indicated that at an injection volume of 0.5 mL, the m/z 44 signal intensities and $\delta^{13}C$ values of CO_2 obtained with T3 trapping durations of 200 s and 320 s were largely consistent. Prolonging the trapping duration provided no notable improvement in enrichment performance. Accordingly, a trapping duration of 200 s was selected for the T3 cold trap, which adequately satisfies the demands of subsequent analyses. In summary, the optimal trapping duration for both T2 and T3 cold traps in the two-stage preconcentration system was 200 s. Under this condition, quantitative trapping of CO_2 was achieved, and no detectable carbon isotopic fractionation was introduced during the process.

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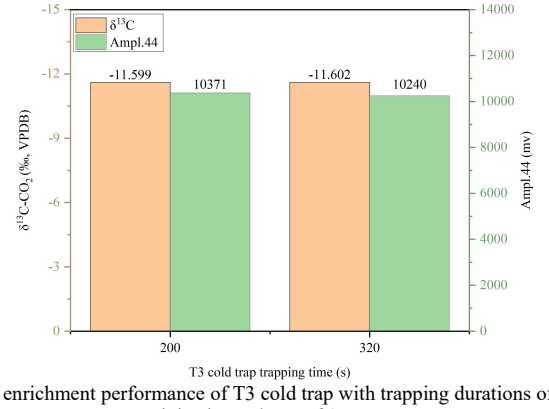

Fig. 3. Comparison of enrichment performance of T3 cold trap with trapping durations of 200 s and 320 s at an injection volume of 0.5 mL

3.2.3 Background Assessment of the PreCon System

Under the optimized conditions (200 s of trapping for both T2 and T3), no sample gas was injected into the PreCon system (injection volume = 0 mL), and the background CO_2 signal intensity of the helium carrier gas after preconcentration and purification via the T2 and T3 cold traps was investigated. The results (Table 2) showed that the m/z 44 signal intensity of the blank background (mean Ampl. 44 ≈ 48 mV) accounted for only approximately 0.4% of the sample signal intensity, indicating that the system background exerts a negligible effect on the measurement results.

Table 2. Measured signals of PreCon blank background and standard samples

Test sample	Ampl. 44mv	Ampl. 45mv	Ampl. 46mv	BGD 44mv	BGD 45mv	BGD 46mv	Area all Vs	$\delta^{13}C$‰
Blk-0.0 mL	45	53	63	5.60	6.20	8.50	0.107	-18.45
	47	56	65	5.50	6.50	8.80	0.108	-18.92
	52	61	72	3.50	3.80	5.40	0.122	-18.24
Blk-Mean	48	57	67	4.87	5.50	7.57	0.11	-18.54
1000 ppm-0.35 mL	11265	13517	15918	4.20	4.60	6.60	34.14	-11.65
	12544	15107	17647	4.20	4.60	6.60	34.24	-11.67
	11479	13830	16204	4.30	4.60	6.60	32.32	-11.67
500 ppm-0.70 mL	12348	14736	17484	4.20	4.60	6.60	33.85	-23.37
	12325	14708	17496	4.30	4.70	6.70	34.14	-23.39
	11445	13603	16277	4.30	4.70	6.70	32.78	-23.39

3.3 Evaluation of Minimum Effective Injection Volume

Injection volume is a key metric for assessing the applicability limit of online $\delta^{13}C$-CO_2 analytical methods. Observation networks such as WMO/GAW and ICOS require that monitoring methods minimize the sample volume per analysis while preserving measurement precision. A minimum injection volume of 1 mL has been reported for the GasBench II single cold trap protocol by Sauze et al.(2026) (Sauze et al., 2026) and Leitner et al. (2023)(Leitner et al., 2023). In this study, the 500 ppm CO_2 synthetic air reference

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material GBW(E)040027 was used as the test sample, and variations in $\delta^{13}C$ across a range of injection volumes (0, 0.25, 0.4, 0.5, 1.0, 2.0, 4.0 and 5.0 mL) were investigated (Table 3).

Table 3. Variations in $\delta^{13}C$ values under different injection volumes

Sample	V_{inject}（mL）	C mass（nmol）	Ampl.44 （mV）	$\delta^{13}C_{VPDB}$（‰）
500 ppm CO_2	0.0	0.0	46	/
	0.25	4.5	1979	-22.95
	0.4	7.1	3063	-23.07
	0.5	8.9	3753	-23.20
	1.0	17.9	7575	-23.21
	2.0	35.7	13925	-23.19
	4.0	71.4	28515	-23.16
	5.0	89.3	35715	-23.21
0.5-5.0mL Precision (‰)				0.02

The results (Fig. 4) demonstrated that within the 0.25-5.0 mL injection range, the ion beam intensity at m/z 44 (Ampl. 44) showed a strong linear correlation with injection volume (R^2 = 0.9998), with signal intensity increasing proportionally with sample volume. This finding confirms that CO_2 volumes up to 5.0 mL can be completely trapped by the two-stage liquid nitrogen cold traps, yielding stable and consistent enrichment efficiency. With respect to $\delta^{13}C$ measurements, at injection volumes below 0.5 mL, the measured values gradually deviated from the certified value and data stability declined as injection volume decreased, attributable to insufficient total carbon content and low signal intensity. At injection volumes ≥ 0.5 mL, $\delta^{13}C$ measurements remained stable, with minor fluctuations between -23.16‰ and -23.21‰. The replicate measurement precision (standard deviation) across the 0.5-5.0 mL range was 0.02‰, which satisfies the requirements for high-precision isotopic analysis.

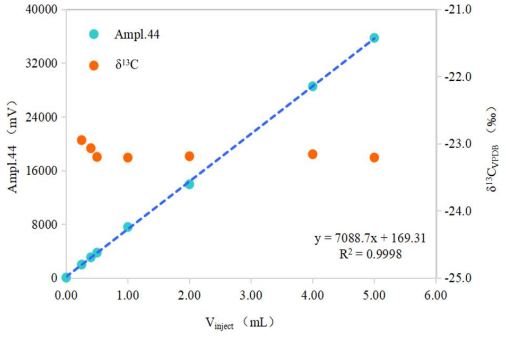

Fig. 4. Changes in $\delta^{13}C$ and Ampl.44 signals as a function of injection volume

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By integrating signal linearity and isotopic stability, the minimum effective injection volume of the proposed method was determined to be 0.5 mL, corresponding to a 50% reduction relative to the optimal protocol reported by Sauze et al. (2026)(Sauze et al., 2026). This result highlights the superiority of the two-stage cold trap series configuration in CO_2 enrichment efficiency.

3.4 Effects of Injection Mode and Headspace Treatment on $\delta^{13}C$ Determination

The synthetic air CO_2 stable carbon isotope reference material GBW(E)040027, with a certified $\delta^{13}C$-CO_2 value of -23.12‰ and an expanded uncertainty of 0.25‰ (k = 2), was used as the test sample. The effects of two injection modes (manual injection and automatic injection) and two background air removal treatments for Labco vials (vacuum evacuation and helium flush) on the measurement results were investigated, and the results are presented in Table 4.

Table 4. Comparison of $\delta^{13}C$-CO_2 results obtained with different injection modes and vial pretreatment protocols

Test No.	Manual injection + vacuum evacuation	Manual injection + He flush	Automatic injection + vacuum evacuation	Automatic injection + He flush
1	-22.96	-23.08	-22.54	-23.20
2	-22.86	-23.11	-22.68	-23.20
3	-22.72	-23.12	-22.50	-23.27
4	-22.46	-23.08	-22.90	-23.19
5	-22.97	-23.06	-23.00	-23.18
Mean value	−22.794	−23.090	−22.724	−23.208
Standard deviation	0.212	0.025	0.220	0.036

Deviations from the certified values for manual and automatic injection in the vacuum evacuation group reached 0.212‰ and 0.22‰, respectively, representing substantial biases. In contrast, measurements from the helium flush group agreed closely with the certified values, with deviations of only 0.024‰ and 0.036‰, yielding higher analytical accuracy.

Overall, helium flush was identified as the optimal pretreatment method for removing background air from Labco vials. Under this condition, measurements obtained via both manual and automatic injection showed good consistency with the certified values of GBW(E)040027 reference material and exhibited high analytical precision, demonstrating that automatic injection can replace manual injection to improve the analytical throughput of the system.

3.5 Method Precision and Trueness

Five reference materials with a gradient of $\delta^{13}C$ values spanning the isotopic composition range of the target samples were selected, comprising four international standards (IAEA-603, IAEA-610, IAEA-612,

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NBS 19) and one Chinese national primary standard (GBW04416). The carbonate reference materials were converted to CO_2 by reaction with phosphoric acid at 25 ℃ ± 0.1 ℃ for 24 h, using two preparation protocols: 0.5 mg of sample in 12 mL Labco vials, and 6 mg of sample processed via vacuum line. The reaction equation is as follows (R2):

$$CaCO_3 + 2H_3PO_4 \rightarrow Ca^{2+} + 2H_2PO_4^- + CO_2\uparrow + H_2O \qquad (R2)$$

No carbon isotopic fractionation occurs during this acid digestion process; therefore, the $\delta^{13}C$ value of the produced CO_2 is equivalent to the certified $\delta^{13}C$ value of the corresponding carbonate reference material. Accordingly:

$$\delta^{13}C_{VPDB\text{-}CO2} = \delta^{13}C_{VPDB}$$

A recent study by Chartrand et al. (2026)(Chartrand et al., 2026) demonstrated that calibration with a panel of carbonate reference materials including IAEA-603, IAEA-610 and IAEA-612 can significantly reduce the measurement uncertainty of δ13C compared with calibration using VPDB-LSVEC alone. The reference material suite selected in this study aligns with this optimized calibration strategy. Three pretreatment systems (PreCon, GasBench and DI) were each coupled to the IRMS for δ13C measurement of CO2 released from each reference material, to evaluate the precision and trueness of the established method.

As presented in Table 5, the standard deviation of $\delta^{13}C$-CO_2 measured by the PreCon-GC-IRMS method was approximately 0.05‰, satisfying the methodological requirements for high-precision isotopic analysis. A calibration curve was plotted with the average measured $\delta^{13}C$ values of IAEA-603, IAEA-610, IAEA-612, NBS 19 and GBW04416 on the x-axis and their certified values on the y-axis. The linear correlation coefficient R^2 was 1 (Fig. 5), indicating favorable trueness of the method.

Table 5. Evaluation of method precision and trueness

CRMs	$\delta^{13}C_{VPDB}$ （‰）				Certified
	Labco bottle（0.5 mg）		Vacuum line（6 mg）		
	PreCon	GasBench	DI	PreCon	
IAEA603	2.22	2.23	2.26	1.91	2.46
	2.15	2.27	2.25	1.98	
	2.13	2.25	2.26		
	2.10		2.25		
			2.26		
stdev	0.05	0.02	0.004	0.05	
IAEA610	-9.13	-9.11	-9.21		-9.109
	-9.19	-9.14	-9.21		
	-9.17	-9.09	-9.21		
	-9.18		-9.20		
	-9.10		-9.19		
stdev	0.04	0.02	0.008		
IAEA612	-36.71	-36.84	-36.85	-36.88	-36.722

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	-36.80	-36.84	-36.84	-36.77	
	-36.63	-36.85	-36.85		
	-36.75				
	-36.68		-36.85		
stdev	0.06	0.01	0.005	0.08	
NBS19	1.67				1.95
	1.69				
	1.74				
stdev	0.04				
GBW04416				1.46	1.61
				1.46	
				1.40	
				1.43	
stdev				0.03	
Reference gas calibration result	-21.04	-21.01	-20.98	-20.93	-21.11

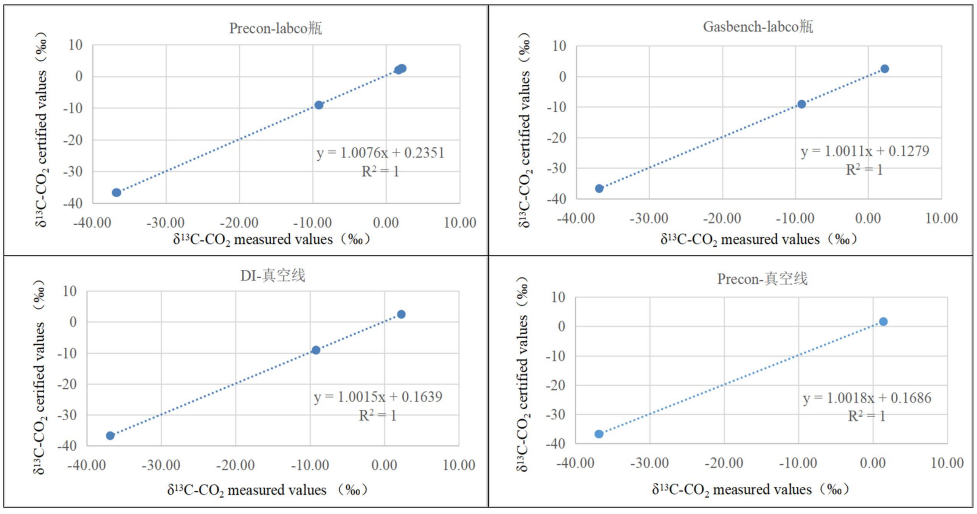

Fig. 5. Calibration curves acquired by coupling IRMS with different peripheral pretreatment devices

The calibration results for the same IRMS reference gas obtained via the three pretreatment pathways were -21.04‰, -21.01‰, -20.98‰ and -20.93‰, respectively, with a maximum range of 0.11‰ (Fig. 5), reflecting excellent measurement consistency among the pathways. The WMO/GAW specifies an inter-laboratory compatibility target of better than 0.01‰ for $\delta^{13}C\text{-}CO_2$ measurements (Sperlich et al., 2022; Tans, P and Zellweger, C, 2016), while ICOS requires a station-level precision of better than 0.1‰ (Levin et al., 2020). The replicate injection precision of the proposed method was 0.02‰, and the inter-pathway precision ranged from 0.04‰ to 0.06‰. This performance exceeds the ICOS station-level precision requirement and is notably superior to the 0.09‰-0.10‰ reported for the single cold trap protocol by (Leitner et al., 2023). The range of reference gas calibration values across the three pathways was only 0.11‰, and all pathways are traceable to the VPDB scale via multi-reference-material calibration. Such

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systematic validation has not been documented in previous methodological studies on PreCon systems. Compared with optical techniques such as OA-CEAS (Yang Yubing et al., 2025), IRMS still retains unique advantages in direct traceability to VPDB and long-term measurement consistency. Dual validation using both gas matrix and solid carbonate reference materials confirms that the proposed method enables high-precision and high-trueness determination for both categories of reference materials.

4 Conclusions

In this study, a systematic analytical method for $\delta^{13}C\text{-}CO_2$ in ambient air was established and optimized based on the coupled PreCon-GC-IRMS technique. The optimal trapping duration for the two-stage liquid nitrogen cold traps was determined (T2 = T3 = 200 s). It was verified that quantitative CO_2 trapping can be achieved without parameter adjustment when the injection volume increases from 0.5 mL to 5.0 mL, with a minimum effective injection volume of 0.5 mL, and no detectable carbon isotopic fractionation was observed throughout the entire analytical process. Helium flush was identified as the optimal pretreatment protocol for air samples stored in Labco vials. The measurement results showed good agreement with the certified values of gas matrix reference materials with minimal measurement uncertainty, and no significant difference was found between manual and automatic injection modes. Dual validation using both solid carbonate and gas matrix reference materials confirmed that the proposed method enables high-precision and high-trueness determination for both types of reference materials. The maximum deviation among the three pretreatment pathways (PreCon, GasBench and DI) was only 0.11‰, and all measurement results are traceable to the international VPDB scale. These findings demonstrate that the proposed method has comprehensive advantages in sample volume requirement, analytical throughput and traceability reliability, and can provide reliable methodological support for high-precision monitoring of atmospheric $\delta^{13}C\text{-}CO_2$.

Author contributions

Tianyu Bian: Conceptualization, Methodology, Formal analysis, Writing-original draft, Visualization. Xingliang He, Xiaoyong Duan: Conceptualization, Supervision, Project administration, Funding acquisition, Writing - review & editing. Feng Li, Huajie Wu, Linlin Cui: Validation, Resources.

Competing interests

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

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