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On-Line Technique for the Determination of the δ^{37}Cl of Inorganic and Total Organic Cl in Environmental Samples

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Here we describe an on-line method for measuring δ^{37}Cl values of chloride bearing salts, waters, and organic materials using multicollector continuous-flow isotope ratio mass spectrometry (CF-IRMS). Pure AgCl quantitatively derived from total Cl in water, inorganic Cl salts, and biological samples was reacted with iodomethane in evacuated 10-mL stopper sealed glass vials to produce methyl chloride gas. A GV Instruments Multicollector CF-IRMS with CH_3Cl optimized collector geometry was modified to accommodate a headspace single-sample gas injection port prior to a GC column. The GC column was a 2-m Porapak-Q packed column held at 160 °C. The resolved sample CH_3Cl was introduced to the IRMS source in a helium stream via an open split. δ^{37}Cl values were calculated by measurement of CH_3Cl at m/z 52/50 and by comparison to a reference pulse of CH_3Cl calibrated to standard mean ocean chloride. Sample CH_3Cl analysis time was ∼6 min. Injections of 40 μL of pure CH_3Cl gas yielded a repeatability (±SD) of ±0.06‰ for δ^{37}Cl (n = 10). Combined GC and IRMS source linearity for CH_3Cl was <0.2‰/nA (V) peak height. External repeatability, based on processing of seawater and NaCl reference solutions, was better than ±0.08‰. The smallest sample for δ^{37}Cl analysis by this method was ∼0.2 μmol of Cl. Selected results from a river basin and biological samples study illustrate the potential of on-line chlorine isotope assays in environmental pollution studies.

Chloride is a ubiquitous anionic species dissolved in seawater, lakes, rivers, and groundwater and is considered to be a conservative tracer of both natural and anthropogenic chloride sources.[1−5] Moreover, some chlorinated organic and inorganic compounds are serious environmental pollutants. Some of these Cl bearing organic contaminants (e.g., PCBs, TCE, PCE) are known to bioconcentrate in tissues and fat of higher trophic level organisms.[6] While the stable chlorine isotope composition (^{37}Cl/^{35}Cl) of seawater Cl^- is isotopically homogeneous and used as the primary δ^{37}Cl reference (standard mean ocean chloride, SMOC), the δ^{37}Cl of natural water Cl^- varies only over a range of ∼4 parts per thousand (‰) and from −2 to +2‰ relative to SMOC.[1−3,7,8] Chlorinated organic compounds, on the other hand, have a wider range of δ^{37}Cl values, presumably due to chlorine isotope fractionation during high-temperature manufacturing processes, resulting in a larger range of δ^{37}Cl values from −7 to +3‰ SMOC.[4,5,9,10] Some chlorine bearing contaminants, such as perchlorate and TCE, experience kinetic isotope effects during biodegradation, and therefore, chlorine isotope compositions may be a good indicator of bioremediation.[11,12] For PCBs, on the other hand, no fractionation is observed during biodegradation.[13] Thus, there is an interest in improving the analysis procedures and application of high-precision δ^{37}Cl assays in hydrologic tracer studies and for fingerprinting sources and bioremediation of Cl bearing contaminants in the environment.[4,7,11,13,14]

Overall, the stable isotopic composition of Cl has not been very extensively used as a tracer in environmental studies. We are unaware of any Cl isotope research on total organic chlorine in the tissues, fats, or contaminants of higher level organisms. Part of the reason is that conventional off-line preparation techniques for extracting Cl^- from environmental matrixes and the conversion to pure AgCl salt is both time-consuming and costly, but also requires stable isotope laboratory facilities capable of conducting natural abundance Cl isotope measurements. The chemical reaction of sample AgCl with iodomethane to produce CH_3Cl and the subsequent purification and isolation of CH_3Cl as the analysis gas requires precise and time-consuming cryogenic separations

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or GC purification or cryogenic transfer into glass ampules prior to conventional dual-inlet analysis.[3−5,7] Considerable amounts of Cl are typically required for off-line dual-inlet isotope ratio mass spectrometry (IRMS)-based analyses (40−100 μmol of Cl[3,7,11]), requiring either relatively high Cl content samples or concentration of Cl^- via preparative processing of large samples.[15] Another impediment is that conventional dual-inlet IRMS triple collector assemblies used for CH_3Cl analyses are not optimized for high-mass CH_3Cl gas (mass 50), resulting in dispersion of the mass 50 (^{35}Cl) signal into the mass 52 (^{37}Cl) collector and potentially adversely affecting results.[3,7] The stable isotope abundance of ^{37}Cl relative to ^{35}Cl is ∼0.25, very different from that of the typical light isotopes (e.g., ^{13}C,^{2}H,^{15}N,^{18}O,^{34}S), and therefore, custom IRMS head amplifiers are required.

Continuous-flow (CF)-IRMS has reduced the sample size requirement of conventional dual-inlet analyses by several orders of magnitude. Generally, this small sample capability comes at the cost of lower precision; however, new CF-IRMS technologies are currently able to produce data that are equivalent or superior to off-line dual-inlet assays.[16] A benefit of CF-IRMS is that it is suited to chromatographic purification of the sample analyte gas and subsequent rapid on-line stable isotopic analysis. The goal of this paper is to (1) provide a simplified laboratory protocol for preparation of CH_3Cl from environmental Cl bearing samples suitable for headspace sampling and (2) to demonstrate a new on-line multicollector CF-IRMS analytical procedure to measure the $\delta^{37}Cl$ of environmental samples. Sample data from rivers in a large basin and total Cl in polar bear (*Ursa maritimus*) abdominal fat are presented to demonstrate the utility of this Cl isotope analysis technique as a hydrologic tracer and potential forensic tracer of chlorinated contaminants in food webs.

METHODOLOGY AND RESULTS

Aqueous Cl^- Sample Preparation. The standard argentometric isolation of dissolved Cl^- in seawater, groundwater, and river water and from dissolved Cl salt samples and its conversion to pure AgCl was described previously.[3,7] Briefly, a preparative target mass of Cl was isolated (we targeted 100 μmol of Cl^-) from water samples by volume adjustment to 100-mL aqueous solutions, either by dilution of samples with high Cl^- concentrations using Milli-Q deionized water or by boiling down samples with low Cl^- concentrations. Alternately, Cl^- from dilute natural water samples was preconcentrated by strong base anion exchange liquid chromatography as described in ref 15. Silver chloride salt was precipitated from this preparative solution (or from the anion exchange column eluent) by increasing the ionic strength (adding 4 mL of 1 M KNO_3) and acidifying the sample with 2 mL of 5% (v/v) HNO_3. The sample solution was placed on a hot plate and heated to 85 °C, upon which 1 mL of 0.5 M $AgNO_3$ was added and a pure AgCl precipitate formed. The beaker was wrapped with aluminum foil to avoid light exposure and cooled for ∼20 min to allow full precipitation of the AgCl salt. Samples were then filtered through a 0.7-μm Whatman glass fiber filter (GF/F), retaining the AgCl precipitate on the filter. The sample beaker and filter paper were rinsed with dilute nitric acid (0.2 vol % HNO_3). Following filtration, the GF filters containing sample AgCl precipitate were dried at 60 °C in the dark and afterward wrapped in aluminum foil and stored in dark vials. The GF filter paper for each sample was weighed prior to AgCl precipitation and after drying so that the mass and recovery of Cl was quantified gravimetrically. Gravimetric yields were typically 95−100%.[3]

Total Organic Cl Samples. The quantitative isolation of total Cl from biological tissue samples (e.g., bear fat, fish muscle) was conducted using a modification of the Parr Bomb ASTM (American Society for Testing and Materials) procedure D808-00,[17] as previously described[4] for organic solvents. Combustion of samples was done using a Parr Bomb (model 1108 oxygen combustion bomb), which energetically and quantitatively releases all Cl from the organic matrix into a sodium carbonate solution as NaCl.

Freeze-dried salmon muscle and bear fat tissue samples were preground or homogenized and precisely weighed (∼1.0 g) into a cleaned type 1108 Parr oxygen bomb combustion capsule, with the bomb fuse wire just touching the top of the sample. A 5-mL solution of reagent grade 5% sodium carbonate was pipetted into bottom half of Parr Bomb assembly, and the bomb was sealed and filled with O_2 to a pressure of ∼34 atm. Following sample ignition and combustion, the bomb was slowly depressurized and opened, and the combustion product solution (containing the recovered Cl as NaCl) was decanted into a clean beaker. The bomb was further rinsed with 50 mL of Milli-Q deionized water to the beaker. The NaCl in solution was converted to pure AgCl using the argentometric procedure described above. The total mass of Cl in organic samples was determined gravimetrically by comparing the mass of AgCl recovered to the mass of organic sample material combusted. Care was taken in all procedural steps to avoid Cl^- contamination by careful use of gloves, cleaning of all utensils, glassware, and bomb devices, and triple rinsing with Milli-Q deionized water.

Conversion of AgCl to CH_3Cl for On-Line Isotopic Analysis. All AgCl samples on GF filter papers were stored in aluminum foil and kept in dark vials to avoid photodecomposition.[7] For the chemical conversion to CH_3Cl, the GF filter papers were carefully folded into 10-mL serum bottles (Wheaton Catalog No. 223739). The serum bottles were aluminum crimp sealed with thick 13 × 20 mm butyl blue stoppers (Catalog No. 2048-11800, Bellco Biological Glassware, Vineland, NJ) and wrapped with aluminum foil. With the stoppers inserted, the effective volume of the vials was reduced to ∼9 mL. The use of thin stoppers or plastic septa vial caps was unsuccessful due to pressure of the conversion reaction causing failure or severe leakage. The crimp-sealed bottles with the GF/F containing AgCl were evacuated using a rotary vacuum pump to <0.001 atm using a 21-gauge needle coupled to a high-vacuum manifold. Following evacuation, 150 μL (or ∼8 times the stoichiometric amount required for conversion[7]) of liquid iodomethane was injected through the stopper into the evacuated vial using a 21-gauge needle and syringe. The vials were then placed in a convection oven at 80 °C to thermally convert AgCl to CH_3Cl. Although a 48-h reaction time was recommended previously,[7] a reaction time test showed that a 30-h reaction time was sufficient, and no changes in CH_3Cl yield or $\delta^{37}Cl$ values were observed after 30 h (Table 1). Furthermore, if immediate analysis was not possible due to IRMS scheduling, unreacted sample vials

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Table 1. Effect of Reaction Time between AgCl and Iodomethane on CH_3Cl Yield and $\delta^{37}Cl$[a]

reaction time (h)	fractional yield (%)	$\delta^{37}Cl$ (‰)
3	10	−5.1
5	15	−7.4
24	45	−6.3
30	98, 99	−4.5, −4.4
48	100	−4.5

[a] Fractional yield is based on 150-μL CH_3Cl injections and is reported relative to the 48-h reaction time. $\delta^{37}Cl$ is relative to tank reference gas.

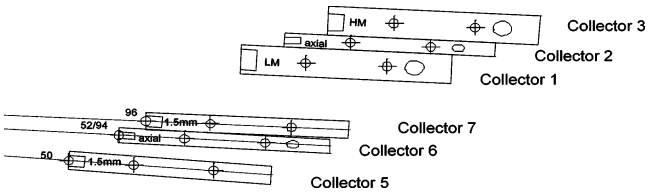

Figure 1. Schematic of the GV Instruments Isoprime CF-IRMS multicollector array. Collectors 1−3 show the standard triple collector array. Mass 52 and mass 50 (CH_3Cl) are monitored on additional Faraday collectors 5 and 6. Hydrogen collector 4 not shown.

containing the iodomethane could be stored frozen prior to the heating reaction step. We found no difference in $\delta^{37}Cl$ results between samples reacted immediately and those stored frozen with iodomethane and reacted 1 week later. Vials containing blanks or only iodomethane injection and no filters yielded no CH_3Cl. Similarly, minute amounts of atmospheric gases introduced with the iodomethane injection had no measurable effect.

For the $\delta^{37}Cl$ isotope analysis, 150−700 μL of headspace gas in the reaction vial (amount depending on the mass of Cl in the GF/F), containing both CH_3Cl and residual iodomethane, was drawn into a 1000-μL gastight syringe and injected into the CF-IRMS system, described below. Because the gastight syringe exhibited residual sample memory effects, all syringes were vacuum purged between samples using a Hamilton vacuum syringe cleaner. No syringe memory effects were observed when this procedure was used.

IRMS Analytical Procedures. The $\delta^{37}Cl$ values of Cl in water and organic samples were measured by a multicollector CF-IRMS using a GV Instruments Isoprime. The standard triple collector array was complemented by the addition of two additional Faraday collectors for monitoring masses 50 and 52, as shown in Figure 1. A third additional collector installed could be used for bromine isotopes (mass 96/94). The head amplifier design was modified so that the resistors on both mass 50 and mass 52 amplifiers were 1×10^9 Ω.

The Isoprime MassLynx instrument control software was modified to integrate mass 50 and 52 beam currents. Data acquisition maps and instrument source tuning files were developed for CH_3Cl as well as a direct data interface to log results into a laboratory information management system for light stable isotopes[18] for subsequent data normalization, reporting, and archiving.

Pure CH_3Cl was used as the analysis gas and the reference gas. A Eurovector 3000 elemental analyzer was modified to

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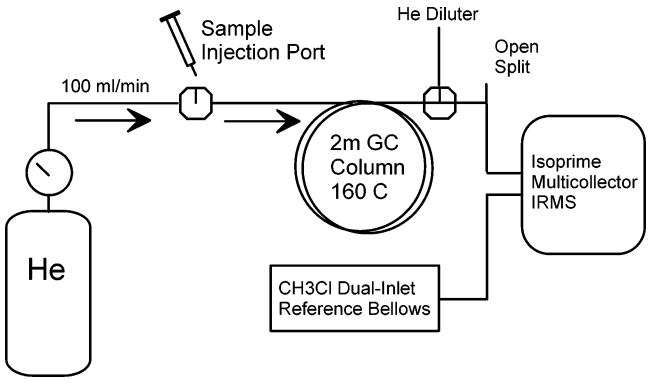

Figure 2. Schematic flow diagram of the CF-IRMS system used for $\delta^{37}Cl$ stable isotopic measurements. See text for detailed description.

accommodate an on-line headspace gas sample injection port prior to the GC column. The injection port was a standard Swagelock $^1/_4$-in. UltraTorr union tee, connected to the existing $^1/_{16}$-in. stainless steel sample flow line with Swagelock adapters. The third fitting on the $^1/_4$-in. UltraTorr tee comprised the sample injection port. The steel nut and ferrule were removed from the third fitting, a Supelco Thermogreen 12.5-mm LB-2 septa was inserted, and the nut and ferrule were replaced and tightened enough to form a gastight seal. This sample injection port was attached in-line prior to the GC column as shown schematically in Figure 2. The injection septa were replaced after ∼20−25 sample injections.

The GC column used to resolve CH_3Cl from residual iodomethane was a standard 2-m Porapak-Q $^1/_4$-in. SS packed column (Elemental Microanalysis) that could remain in place for routine C/N isotope analyses. The GC column temperature was held at 160 °C. The sample helium flow rate was 100 mL/min using a column head pressure of ∼150 kPa.

At the beginning of the analysis run, a 30-s reference CH_3Cl pulse was automatically introduced using the dual-inlet bellows of the GV Instruments Isoprime starting at 15 s. A sample of headspace CH_3Cl taken from the sample vial using a gastight syringe was manually injected ∼20 s after the initiation of the analysis run. This 20-s injection delay was required to allow the small amount of air (N_2, O_2) in the syringe needle to be resolved after CH_3Cl reference gas injection pulse (Figure 3). The GC-resolved sample CH_3Cl peak appeared at ∼3.8 min on the TCD and was introduced to the mass spectrometer via an open split capillary. About 0.3 mL/min (∼0.3% of the sample CH_3Cl) from the sample capillary flow line of 100 mL/min was diverted to the IRMS source; the remaining 99.7% was diverted to waste through the open split. At approximately 13−14 min, the residual iodomethane peak appeared on the TCD. To prevent iodomethane from entering the IRMS source, a GV Instruments Diluter was used to direct this peak to a waste line. This diluter waste line was connected to the laboratory fume hood to avoid iodomethane venting into the laboratory air. A schematic diagram of the entire gas introduction and handling system is shown in Figure 2. While the actual analysis time for CH_3Cl is only ∼6 min, a delay to separate iodomethane from CH_3Cl resulted in an overall run time of 14.5 min. We note this overall analysis time could probably be improved by 3−4 min by using a 1-m or shorter packed GC column. We also note that for testing purposes in this paper we targeted a very large mass of Cl (∼100 μmol of Cl), which allowed

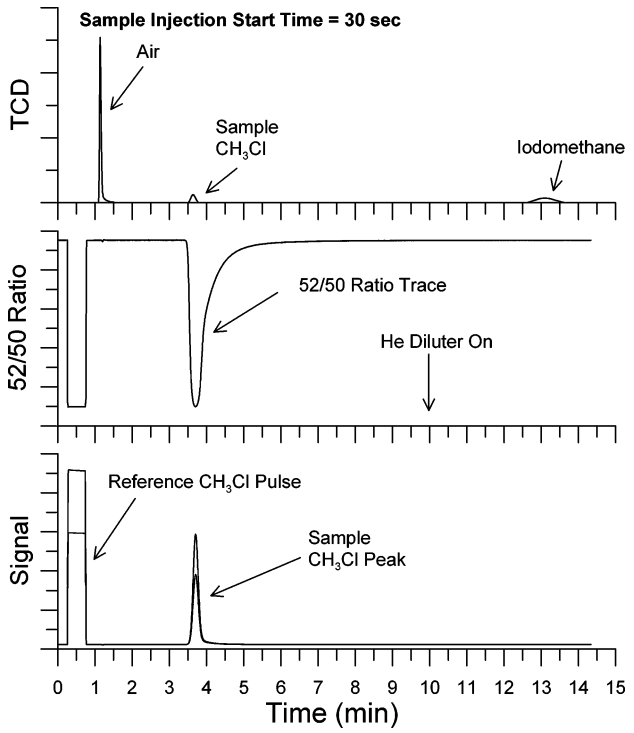

Figure 3. Typical CF-IRMS sample and reference signal chromatogram and ion current ratios (mass 50/52) and TCD trace for CH_3Cl and iodomethane. The reference gas used here was research grade CH_3Cl introduced via the dual-inlet bellows.

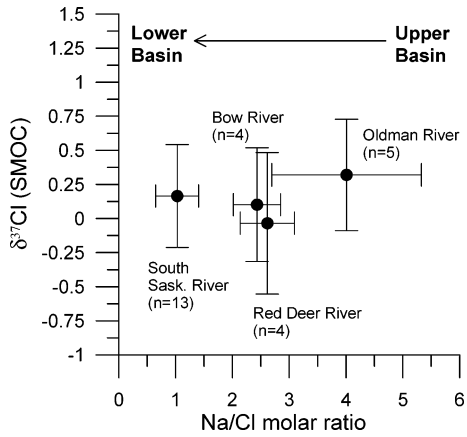

Figure 4. Relationship between δ^{37}Cl values and Na/Cl molar ratios in sub-basinal watersheds of the South Saskatchewan Basin, Western Canada.

for numerous injections (6−10) of sample gas from the same reaction vial. Practically, for studies where Cl concentrations are routinely low, the target mass of Cl^- can be scaled down by a factor of 10 or more to ~10 μmol of Cl^-. All spent sample reaction vials and stoppers were sent to accredited chemical disposal facilities and were not reused.

The δ^{37}Cl value of sample Cl was calculated by measurement of CH_3Cl at m/z 52/50 and comparison to a reference pulse of research grade (99.99%) CH_3Cl. A typical TCD, 52/50 ratio trace, and mass 50 and 52 ion trace from a 150-μL sample injection are shown in Figure 3. The IRMS source was empirically tuned for maximum linearity for CH_3Cl using a source trap current of 400 μA. Combined GC and instrument source linearity, while instrument specific, was derived by injecting CH_3Cl samples over a size range from 0.4 to 3.2 μmol of CH_3Cl. This was experimentally determined to be <0.2‰/nA (V) and highly linear ($r^2 = 0.97$, $n = 20$). This sample size linearity may be improved by using a lower source trap current (200 μA) and larger sample injections; however, we did not test this option. The smallest sample for δ^{37}Cl determinations using the current source-tuning parameters was ~0.2 μmol of Cl (or 7 μg of Cl^-), suitable for most environmental applications.

Repeated 30-μL injections of 100% CH_3Cl sampled from a Tedlar gas sampling bag yielded a repeatability (±SD) of ±0.06‰ for δ^{37}Cl ($n = 10$). To correct our δ^{37}Cl relative to the SMOC reference we used (1) 100 mL of seawater obtained from H. Eggenkamp (personal communication) and (2) a carboy (5L) of seawater obtained from the Atlantic Ocean near Halifax, Canada. There was no significant difference observed between the δ^{37}Cl of both of the seawater samples, as was previously observed by others.[4,19] Thereafter, our seawater carboy reference sample was assumed to have a δ^{37}Cl value of 0‰ with respect to SMOC water and was subsequently included in all sample batch preparations to correct sample and reference gas values relative to SMOC.

Selected Field Results. Here we present some δ^{37}Cl isotopic data prepared using the above technique to illustrate results using the on-line method. Cl^- and δ^{37}Cl data collected in February 2004 (base flow conditions) for the entire South Saskatchewan River Basin (1400 km of river length) including the South Saskatchewan River, Bow River, Oldman River, and Red Deer River sub-basins are shown in Figure 4. The range of δ^{37}Cl values, as expected, was low but they were variable within the basin. The riverine chloride concentrations progressively increased from the upper reaches of the basin at 0.5−10 mg/L Cl^- at the outflow primarily due to cumulative effects of urban sewage effluent inputs. Not surprisingly, a cross-plot of Na/Cl ratios versus δ^{37}Cl shows this riverine progression of Cl^- chemistry toward Na/Cl ratios of 1 and δ^{37}Cl toward zero (SMOC).

Total fat Cl and δ^{37}Cl data for 30 individual polar bear fat samples collected from across the Canadian arctic region are shown in Figure 5. Each sample was prepared and analyzed in triplicate or duplicate and shown are the mean Cl and δ^{37}Cl (±SD) for 30 individual bears. The abdominal fats reveal a wide range of total Cl contents from 670 to 12 000 ppm. Most fat samples contained less than 4000 ppm Cl. The δ^{37}Cl values had a very wide range of values from −3.1 to +1.2‰ (SMOC). The ecological and contaminant significance of these results is the subject of an ongoing study.

Pacific salmon dorsal muscle was prepared from two separate fillets (2 individuals). The two fillets were freeze-dried and blended to powder form in an attempt to homogenize the material as a working natural organic Cl isotope reference material. The salmon yielded a δ^{37}Cl of −3.4 ± 0.5 ($n = 10$). The high variance in this powder compared to seawater samples, however, suggests that the sample was not sufficiently homogenized and that the two individuals comprising this composite sample may have had slightly different δ^{37}Cl values.

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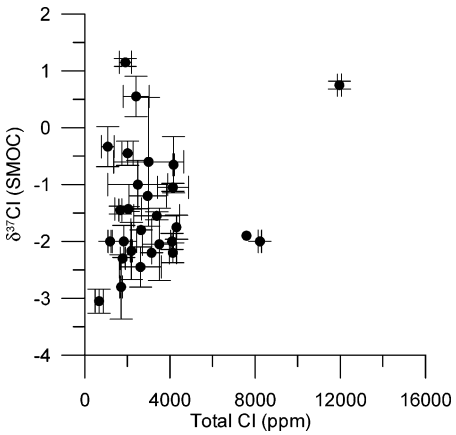

Figure 5. Relationship between mean δ^{37}Cl values and total Cl content (in ppm) of abdominal fat samples taken from polar bears in the Canadian arctic.

CONCLUSIONS

There are significant advantages of the multicollector CF-IRMS technique over conventional dual-inlet methods for δ^{37}Cl isotopic measurements. The on-line technique does not require off-line preparative cryogenic separation or GC isolation of CH_3Cl from iodomethane, thereby significantly reducing technical costs and potential for isotopic fractionation during gas handling and cleanup. The construction and use of costly vacuum and cryogenic manifolds for sample processing are not required, resulting in significant hardware cost savings. Samples are processed directly and rapidly by headspace analysis, in contrast to off-line gas separations and dual-inlet methods. Finally, the use of new multicollector CF-IRMS technology optimized for CH_3Cl analyses improves analytical precision by addressing the dispersion and isotope abundance problems arising with measuring CH_3Cl on conventional triple collector assemblies.

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