

**APPENDIX G**  
**UNIVERSITY OF WATERLOO LABORTORY PROTOCOLS**  
**FOR STABLE ISOTOPE ANALYSIS**

This page intentionally left blank.

ENVIRONMENTAL ISOTOPE LABORATORY  
DEPARTMENT OF EARTH SCIENCES  
UNIVERSITY OF WATERLOO

Technical Procedure 38.0

DRAFT DRAFT DRAFT DRAFT DRAFT DRAFT DRAFT DRAFT DRAFT  
**CARBON-13 BY GC-IRMS**  
DRAFT DRAFT DRAFT DRAFT DRAFT DRAFT DRAFT DRAFT DRAFT

**AUTHOR (S):**

\_\_\_\_\_  
Daniel Hunkeler

\_\_\_\_\_  
Heide A. Flatt

\_\_\_\_\_  
Robert J. Drimmie

**APPROVAL:**

\_\_\_\_\_  
Laboratory Manager

**January, 2001**

## 1.0 Introduction

The gas chromatography combustion isotope ratio mass spectrometry (GC-C-IRMS) system consists of the gas chromatograph (GC) connected to an isotope ratio mass spectrometer (IRMS) via a combustion interface. In the combustion interface, the separated compound pulses are combusted to CO<sub>2</sub> and carried to the mass spectrometer by a continuous helium stream. In the mass spectrometer, CO<sub>2</sub> is ionized, the ions are accelerated, and CO<sub>2</sub> with mass 44, 45 and 46 is separated in a magnetic field that bends the ion beams depending on the mass of the ions. The separated ion beams hit Faraday cups and produce currents that are registered and displayed on the computer screen. In addition to the ion beam intensities, the FID signals in the 2/1 ratio (mass 45/mass 44) and 3/1 ratio (mass 46/mass 44) are displayed. The 2/1 ratios during peak elution are typically S-shaped due to the slightly faster elution of compound containing <sup>13</sup>C compared to compounds containing <sup>12</sup>C. The peaks are automatically integrated and the isotope ratio is calculated for each peak. It is important that the compounds are completely resolved (baseline separation) and the whole peak is integrated. If only part of the peak is integrated (e.g. due to overlapping) the result will be biased towards <sup>13</sup>C or <sup>12</sup>C. The dynamic range of the system is at the maximal one order of magnitude, i.e. it is much smaller than that of a GC-FID. Therefore, concentrations of compound in the samples have to be known in advance to be able to inject an appropriate amount of compound or to be able to adjust the concentration by dilution of the samples. Typically, 2 to 12 nmol carbon is required for a reproducible carbon isotope ratio analysis.

### 1.1 Precautions

The precautions listed are not comprehensive. Use in conjunction with the relevant Material Safety Data Sheets (MSDS) from the manufacturer. Generic MSDS's from Aldrich Chemicals Company are available through the science Watstar network on any computer attached to ethernet. Follow acceptable safety procedures including the wearing of safety glasses at all times and gloves if necessary.

Whenever feasible, keep waste separate and retain for processing in the Hazardous Waste Handling Facility (HWHF) in ESC 150. Acids and bases not containing toxic substances may be poured down the drain provided they are between pH 5.5 and 9 or can be brought to pH 5.5-9 by dilution with water in a maximum ratio of 5:1 (water to solution). **WHEN IN DOUBT, RETAIN ALL WASTE IN SEPARATE LABELLED CONTAINERS AND TAKE TO THE HWHF.**

1. Exercise caution when handling concentrated acids. Open bottles in fumehood, wearing protective gloves and safety glasses. Always add acid to water.
2. Ammonia (NH<sub>3</sub>) is a colourless, corrosive and alkaline gas with a very pungent odour (characteristic of drying urine). Mixtures of ammonia and air will explode when ignited under favourable conditions but NH<sub>3</sub> is generally considered non-flammable. It is exothermic in solution with water. Inhalation of concentrated vapour causes edema of the respiratory tract, spasm of the glottis and asphyxia. Prompt treatment is required to prevent death.
3. Ammonium Hydroxide (NH<sub>4</sub>OH) is a colourless liquid with an intense, pungent, suffocating odour. It has an acrid taste and produces a strong alkaline reaction. Reaction with sulphuric or other strong acid is exothermic and will cause the mixture to boil. It is irritating to the eyes,

- respiratory tract and skin. It causes burns, is harmful if inhaled and **MAY BE FATAL IF SWALLOWED**. Use only in fumehood and wear gloves, lab coat and glasses. Dispose as corrosive, reactive waste.
4. Helium (He) is a colourless and odourless gas at room temperature. It is a simple asphyxiant. Use only in well ventilated areas.
  5. Methyl Chloride (CH<sub>3</sub>Cl), commonly called chloromethane, is a colourless gas that compresses to a colourless liquid of ethereal odour and sweet taste that burns with a smoky flame. It is **POISONOUS** and may cause injury to the liver, kidneys and central nervous system. Vent all waste to the fumehood.
  6. Methyl Iodide (CH<sub>3</sub>I), commonly called iodomethane, is a colourless, transparent liquid that turns brown on exposure to air. It is **EXTREMELY POISONOUS** and may produce severe narcosis and acute lung irritation. Wear nitrile (green) gloves when handling. Make sure when the column is stripped of this gas that it is being vented into the fume hood.
  7. Nitric Acid (HNO<sub>3</sub>) is a **POISONOUS**, colourless liquid that fumes in moist air with a characteristic choking odour. **IT REACTS VIOLENTLY WITH ORGANICS**. Liquid and vapour may cause severe burns. It may be fatal if swallowed or inhaled. Wear neoprene gloves, glasses and lab coat with apron and use only in the fumehood. Dispose as ignitable, corrosive waste.
  8. Nitrogen (N<sub>2</sub>) is a colourless, odourless gas at room temperature. In liquid form (condensation temperature -196°C), it can produce severe frostbite. Wear safety glasses and thermal, impervious gloves to prevent frostbite. It is a simple asphyxiant; use under well-ventilated conditions.
  9. Silver Carbonate (Ag<sub>2</sub>CO<sub>3</sub>) is a light yellow powder when freshly precipitated, but becomes darker on drying and exposure to light. It decomposes at 220°C to Ag<sub>2</sub>O and CO<sub>2</sub> and to metallic Ag at higher temperature. It has low health and safety risks.
  10. Silver Chloride (AgCl) is a white powder that darkens on exposure to light. It may be harmful by inhalation, ingestion or skin absorption. Symptoms are eye and skin irritation and argyria (a slate-grey or bluish discolouration of the skin).
  11. Silver Iodide (AgI) is a light yellow odourless powder that slowly darkens under light. It has low health and safety risks.
  12. Silver Nitrate (AgNO<sub>3</sub>) is a **POISONOUS**, corrosive and odourless, white crystalline powder. It is irritating to the respiratory tract and may cause death if ingested. Wear safety glasses and rubber gloves and use fumehood when handling. Dispose as toxic waste. Store solutions in amber containers to avoid light exposure.
  13. Sulphuric Acid (H<sub>2</sub>SO<sub>4</sub>) is a clear, odourless, colourless, dense, hygroscopic and oily liquid with a marked acid taste when pure. It produces a violent exothermic reaction with water and may ignite other materials such as paper, oil etc. **SULPHURIC ACID IS CORROSIVE AND HIGHLY TOXIC, WITH SEVERE EYE, SKIN AND MUCOUS MEMBRANE IRRITATION**. It is immediately dangerous to life or health at 80 mg/m<sup>3</sup>.

## 2.0 General start-up and shutdown procedure

### 2.1 Getting the interface ready

1. Verify that cold trap is connected  
If cold trap has been backflushed overnight, reconnect it:  
Set FID helium flow close to zero and close needle valve at back of interface  
Disconnect steel capillary from outlet of cold trap  
Reconnect inlet and outlet capillaries. Verify with magnifying glass that capillaries are not blocked by particles before reconnecting them.
2. Set cold trap temperature control to minus 100 C.
3. Add 1/2 Dewar of liquid nitrogen to cold trap.
4. When cold trap has reached minus 100 C, set temperature control of furnace to 850 C.

### 2.2 Conditioning GC oven

1. Verify that the appropriate liner is installed in the inlet
2. Bake the GC oven for at least 30 minutes. Set temperature at least 20C above the highest temperature used in the method, but below the temperature limit of the column (Note: different columns have different upper temperature limits).

### 2.3 Getting the mass spectrometer ready

1. After having baked the GC oven for 30 minutes, set GC oven to 50C.
2. Verify that cold trap is connected; the cold trap temperature has reached -100C. and the furnace temperature is 850C.
3. Switch from standby to mass spec(**Menu: Inlet>Mass Spec**).
4. Verify that the Penning pressure is about 5 to 6\*10<sup>-6</sup> mbar. If the pressure is higher return immediately to standby and check for leaks. After the pressure has stabilized write it down in the instrument logbook.
5. 15 minutes after having switched to mass spec mode, center the peak (ion-beam) of the mass spec: Open reference gas valve: Double-click **RG** on diagram on-screen and wait until reading stabilizes. Perform a peak scan. Choose from menu: **Scan>Peak Center**. A peak scan is performed and the center of the peak is automatically detected if the peak is within the scan window. Save the new peak location: Choose from menu: **Mass Spec>Tune Source**. Overwrite CO2 file. Close peak center window. Close reference gas valve (double-click **RG**)
6. Verify the intensity of the mass 44 current. It should be between 2 and 5\*10<sup>-11</sup> A at an oven temperature of 50 C.. Write down the background intensity in the instrument logbook.
7. Perform a stability run: Choose from menu: **Analysis>Single Run**  
Choose method within single run window: **CO2-STAB**  
Define name for folder and filename (STAB)  
Press **Run**. Ten reference gas pulses are automatically injected. The height of the reference gas pulses (mass-44=major) should be between 3 and 5\*10<sup>-9</sup>.  
Check printout. The calculated standard deviation of fit should be smaller than 1\*10<sup>-6</sup>. The fourth digit of the 2/1 ratios should not vary by more than five units. Write down the

standard deviation and the deviation between the smallest and highest ratio in the instrument logbook.

## **2.4 Setting the heart split balance point**

### ***2.4.1 background information***

#### ***Heart split valve***

The heart split the valve controls the flow to the FID. If the heart split valve is closed, the flow is blocked, if it is open, flow is possible according to pressure gradients.

#### ***Sample line helium pressure***

The purpose of the sample line (SLN) Helium is i) to push the sample pulses across the furnace tube if the heart split is closed and ii) to keep the column flow way from the furnace tube and direct it to the FID if the heart split is open. The sample line pressure should be sufficiently high to keep the column flow away when the heart split is open but not too high to avoid excessive dilution when the sample pulses flow towards the mass spec. The required sample line helium pressure to keep the flow away from the furnace depends on the oven temperature (which influences the viscosity of the gases and thus the flow characteristics). At higher temperature, a higher pressure is required. It is particularly important to keep the flow away at lower temperatures when the solvent peak elutes.

#### ***FID helium pressure***

The FID helium flow pushes the sample pulses across the small dead volume of the heart split towards the FID and avoids excessive broadening of the FID peaks due to this dead volume. However, if the FID helium pressure is increased, higher sample line helium pressure is required to direct the column flow towards the FID. Since the peak shape in FID is usually not important, the FID helium can be set close to zero in order to minimize the sample line helium flow.

### ***2.4.2 Setting the sample line helium and FID helium pressures***

1. set to FID helium pressure close to zero.
2. verify that GC is at 50 C. and HC is open (float to GC)
3. measure the SLN helium flow using the electronic flow meter. The split flow should be between 0.7 and 0.8 ml/min (corresponds approximately to a pressure of 2 to 3 psi). If the split flow is outside this range adjust it using the SLN helium knob on the interface box.
4. Close the heart split and measure the split flow again. It should remain between 0.7 and 0.8 ml/min.
5. A careful setting of the SLN and FID helium pressures is particular important if solvent injections are made, to prevent that the solvents peak enters the furnace tube which significantly reduces the lifetime of the furnace tube. Verify that no compound reaches the furnace tube/isotope ratio mass spectrometer when the HC is open before injecting solvent extracts. To check this, inject methane using the same GC temperature program as for samples and keep the HC open during the entire run. A methane peak should appear in the FID trace but nothing should be detected by the IRMS.

Note: the required pressure may change after replacement of the furnace tube and may change during the lifetime of the furnace tube. Usually at SLN setting that yields of split flow of 0.7 ml/min is sufficient.

## 2.5 Running external standards

1. Run external standards that have a similar matrix as the samples that are about to be analyzed. For more details, refer to the method-specific sections.
2. Compare the obtained isotope ratio with the known isotope ratio. The average of two measurements should not deviate by more than 0.5 per mil from calibrated value. Report the name of the compound, calibrated and measured value in the instrument logbook.
3. Check the peak area. Under optimal flow conditions the peak area should be about 5 to 8 \*10<sup>-9</sup> the As per nmol carbon. Lower peak areas indicate the presence of a leak.

## Shut down procedure

1. Set the mass spec in standby mode **menu: Inlet > Standby**
2. Set cold trap temperature to 25 C.
3. Set furnace temperature to 300 C.
4. Switch FID detector off, switch GC oven off.
5. Every second or third day, back flush the cold trap.
  - Set cold trap temperature to 50 C.
  - Disconnect in and out capillary (make very sure mass spec is on standby)
  - Connect metal capillary to outlet of cold trap
  - Open needle valve at back of interface
  - Set FID helium to 2psi
  - Verify that helium flows out at inlet of cold trap

## 3.0 SPME method

### 3.1 Introduction

The SPME method is more sensitive than the headspace method (see below) with detection limits as low as 5 ppb. While the method is more sensitive, it has the disadvantage that the injection volume is given by the volume of the fiber coating. Therefore, samples have to be diluted to appropriate concentration levels. This sensitivity of the method can be improved by adding salt or in case of headspace SPME, heating the samples. The samples can be extracted by immersing the fiber directly into the aqueous phase of the sample or by creating a headspace and exposing the fiber to it. The headspace method should be used for all compounds with Henry coefficients above 0.02 since I) with the headspace method volatile compounds are extracted preferentially, making the baseline quieter, II) it prolongs the lifetime of the fiber since the fiber does not come into contact with the matrix, and III) the equilibration time it is shorter than for direct SPME.

### 3.2 Choice of column

All columns except for the GS–GasPro are suited for the SPME method. The column has to be chosen depending on the composition of the samples (see column selection guide).

### 3.3 Inlet

An SPME-liner (0.85-mm inner diameter) has to be installed. The inlet temperature depends on which type of fiber is used (see manufacturers guide for appropriate temperatures).

### 3.4. Temperature programming

Generally the temperature program should be started at 40 to 50C. A low initial temperature leads to increased stationary phase focusing of volatile compounds and thus an increase in peak height, resolution and sensitivity.

### 3.5 Preparation of standards

Prepared standard stock solutions by dissolving pure organic phase compounds or mixtures of pure organic phases in NANOpure water

1. Rinse bottles and magnetic stirrer with methanol and place in oven at 100C. for at least two hours.
2. Fill bottle with NANOpure water.
3. Add organic compounds with a microliter glass syringe. For DNAPL, open bottle and inject organic compounds while immersing the needle of the syringe underwater. For LNAPL, inject organic compounds through septum.
4. Place bottle on magnetic stirrer at 600 to 1,000 rpm for at least 12 hours (e.g. over night).
5. Prepare standards. Dilute standard stock solution with NANOpure water in vials containing a magnetic stirrer that are similar to those used for samples. Stir standards for at least 15 min.
6. Open vial and introduce a small headspace if direct SPME is used or a larger headspace (10% of vial volume ) if headspace SPME is used. Add a magnetic stirrer. In case of head space SPME, stir for 15 min at 1100 rpm before starting the SPME extraction.

### 3.6 Preparation of samples

1. Open vial and introduce a small headspace if direct SPME is used or a larger headspace (10% of vial volume ) if headspace SPME is used. Add a magnetic stirrer. In case of head space SPME, stir for 15 min at 1100 rpm before starting the SPME extraction.
2. Prepare a replicate sample for one out of five samples.

### 3.7 Analysis of standards and samples

1. Start instrument according to general start-up section (above).
2. Place vials on magnetic stirrer at 1100 rpm
  1. Retract fiber in needle, retract needle completely in fiber holder
  2. Place fiber holder on top of vial. Move needle out of fiber holder by turning inner part of fiber holder (metal) while holding outer part (black), until septa is penetrated.
  3. Move fiber out of needle. Adjust needle such that the fiber coating is completely immersed in the aqueous phase (direct SPME) or that fiber does not contact the aqueous phase (headspace SPME).
  4. Two minutes before finishing the extraction, make injector ready for injection by pressing prep run.

5. After the extraction period is over, retract fiber into needle and remove fiber holder from vial.
6. Adjust needle position to 4 by turning inner part of fiber holder.
7. Insert needle into injector and immediately move fiber out of needle.
8. Start GC run.
9. Leave fiber in injector for 15 min.
10. Verify that all peaks of interest are completely separated by examining the 2/1 trace, which should show a typical S-shape caused by earlier elution of compounds with 13C compared to compounds with 12C. The S-shape curve should be bordered by a horizontal line on both sides.

### **3.6 Sequence of analysis of samples and standards**

1. Analyze two separate standard vials. The measured 13C. of compounds should agree with the calibrated values within 0.5 per mille.
2. Analyze samples.
3. analyze a duplicate for one out of five samples. The duplicate should be injected at least five injections before or after the sample.
  - a. Flush the bottles with helium for two min.
  - b. Add compounds as pure phase or as mixtures of pure phases. Choose the concentration in such a way as to obtain an average peak size on the mass spec. Example: Add each compound at a concentration of 120 microM C which yields a compound pulse of about 6 nmol C if 0.5ml it is injected at a split of 10:1.
  - c. Wait for at least ½ hour to allow for evaporation of liquid phases. A piece of aluminum foil can initially be added to mix the gas by shaking the bottle.

### **3.7 Preparation of samples**

- a. Introduce a headspace of helium with a volume of at least 5ml. Use 2 syringes, an empty one and one with helium. Insert both syringes at the same time and push in helium and pull out water at the same time. Note that the concentration in the headspace is the smaller the larger the headspace is and thus the sensitivity of the method decreases with increasing headspace size.
- b. Place the bottles or vials on the shaker for at least an hour and no more than 24 hours if septum sealed vials are used and the septum has been punctured. Place the bottles horizontally to avoid contact of the headspace with the punctured septum. It is usually best to prepare the samples in the afternoon of the previous day and shake the samples overnight.

### **3.8 Selection of injection volume**

The amount of carbon entering the column should be between 2 and 12 nmol per compound. The required amount may very dependent on the flow settings. For a first try, select the injection size in a way to obtain compound pulses of 6 nmol C. The injection size can be calculated based on

the concentration of the compound in the headspace which is given by equation 1 and the split ratio:

$$C_g = C_o / \left( \frac{1}{K} + \frac{V_g}{V_w} \right)$$

Where  $C_g$  is the concentration in the headspace,  $C_o$  the initial concentration of the compound in the aqueous phase (before and production of a headspace),  $K$  the dimensionless Henry coefficient,  $V_g$  the volume of the headspace and  $V_w$  the volume of the aqueous phase.

If the sample contains several compounds for which isotope ratios should be obtained, select an injection size that yields a compound pulse of 10 nmols C for the compound with the highest concentration. If the peak sizes for the other compounds are too small, make a second injection using a larger volume and direct the larger peaks (for which isotope ratios have been obtained in the first run) to the FID using the heart split.

### 3.9. Typical Procedure

#### Two days before running samples.

- Prepare a standard stock solution by dissolving pure organic phase compounds or mixtures of pure organic phases in NANOpure water.
- Rinse bottles and magnetic stirrer with methanol and place into in oven that at 100C for at least two hours
- Fill bottle with NANOpure water
- Add organic compounds with a microliter glass syringe. For DNAPL, open bottle and inject organic compounds while immersing the needle of the syringe underwater. For LNAPL, inject organic compounds through septum.
- Place bottle on magnetic stirrer at 600 to 1,000 rpm for at least 12 hours (e.g. over night).

#### One day before running samples

1. Prepare standards. Dilute standard stock solution with NANOpure water in vials that are similar to those used for samples. T
2. Prepare samples in the afternoon, introducing a helium headspace and place samples on shaker overnight. Make sure you only prepare samples that contain compounds in sufficient quantities for headspace analysis

#### Day of analysis

1. Get GC oven, interface and mass spec ready according to general start-up procedure.
2. Analyze standards. Inject gas phase standard twice and check if isotope ratio corresponds to know that you and is reproducible (deviation < 0.5 per mille). Verify that peak area is about  $5 \text{ to } 8 \times 10^{-9} \text{ As per nmol C}$ .
3. Inject first sample twice and check reproducibility of isotope ratio (deviation < 0.5 per mille). Verify that all peaks of interest are completely separated by examining the 2/1 trace, which should show the typical S-shaped curve. The S-shape curve should be bordered by a horizontal line on both sides.
4. Inject each sample once, repeat each fifth sample and check reproducibility.

5. Repeat standard injection after having performed nine sample injections.
6. Keep an eye on cold trap temperature. Note: the temperature display on the screen is not functioning. After about six hours use, add another ½ Dewar of liquid nitrogen to cold trap.

## 4.0 Quality-control

### 4.1 BI weekly quality-control activities

#### 4.1.1. Linearity test

Perform a linearity test as follows:

1. Set the GC oven temperature to 50C and open that each sea.
2. Stability test to verify that the system to stable (see below). If the criteria of the stability test has been met proceed.
3. Select: **Analysis>Single run**
4. Choose the CO<sub>2</sub>-LIN method and press **run**.
5. Vary the height of reference gas pulse by manually changing the CO<sub>2</sub> gas pressure at the reference gas box. The height of the mass-44 ion trace should be between 1.5\*10<sup>-9</sup> to 1.5\*10<sup>-8</sup> and vary randomly.

**Acceptance criteria:** the standard deviation of the 2/1 ratio of 10 consecutive reference gas pulses passed to be smaller than 1.0\*10<sup>-7</sup>. The deviation between the smallest and highest value should be less then 5.0\*10<sup>-6</sup>.

**Reporting:** The standard deviation and deviation between the smallest and highest value are reported in the instrument logbook.

Corrective actions:

- a) Verify that background intensity remains constant throughout the run. If background intensity changes the system may not be at equilibrium yet. Wait for one-hour and repeat the linearity test.
- b) Contact Bill Mark for retuning of IRMS parameters.

#### 4.1.2 Checking the efficiency of the furnace tube

Determine the efficiency of the furnace by injecting propane. Measuring carbon isotope ratios of propane provides insensitivity means to verify that combustion is complete since non-combusted propane contributes to mass 44 and thus strongly biases the measured eyes to ratio.

1. Prepare a propane gas standard with a concentration of 100 micro-M C.
2. Set the GC oven at 100C.
3. Inject 0.5 ml of standard gas and start the run. Inject standard gas three more times with a spacing of one min.

**Acceptance criteria:** The 13C values should not deviate by more than 0.5 per mille from the calibrated value.

**Reporting:** Report the 13C values of propane in instruments log book.

**Corrective actions:**

Verify mass-44 background intensity levels at a column temperature of 50C(<math>5 \times 10^{-11}</math> A), mass 44 background levels during the run (<math>1 \times 10^{-10}</math> A) and the shape of the peaks (2/1-ratio trace). If all criteria are met and deviations persist, re-oxidize the furnace tube.

**4.1.3 Checking the oxygen level of furnace tube**

Monitor the state of the furnace to by measuring the oxygen level using the isotope ratio mass spec.

1. Tune the IRMS to oxygen by selecting the **Mass Spec>Tune Source**. Load the O2 tuning file.
2. Open the HC.
3. Perform the scan. Select **Scan>Peak Scan**. Load to O2 scan file. Press **GO**.

**Reporting:** Report the mass-32 and mass-34 intensity levels at the center of the peaks in the instrument logbook.

**4.1.4 Checking cleanness of vacuum**

A magnet scan is performed to determine the cleanness of the vacuum and particularly to evaluate the presence of H<sub>2</sub>O.

**4.2. Daily quality-control activities****4.2.1 Determination of background intensity**

After starting up the instrument (section 1), the background intensity of the mass-44 ion beam is determined at a GC oven temperature of 50C.

**Frequency:** Daily before starting to analyze samples.

**Acceptance criteria:** The intensity should be smaller than  $5 \times 10^{-9}$ .

**Reporting:** The date of determination, type of GC column, oven temperature and background intensity level are reported in the instrument logbook.

**Corrective actions:**

- a) Heat the GC column to 20C below the upper temperature limit for 1-hour. Afterwards, set the GC temperature to 50C and determine the background activity again. The oven temperature during backing, duration of backing and the new background intensity are reported in the instrument logbook.
- b) Replace the inlet liner and set the oven temperature to 20C below the upper temperature limit for 30 min. Afterwards set the GC temperature to 50C and determine the mass-44 background intensity. Report the duration of backing and the background intensity enhancement logbook.

**4.2.2 Stability test**

A stability test is performed as described in section 1.3.

**Frequency:** Daily before starting to analyze samples.

**Acceptance criteria:** the standard deviation of the 2/1 ratio of 10 consecutive reference gas pulses has to be smaller than  $1.0 \times 10^{-7}$ . The deviation between the smallest and highest value should be less than  $5.0 \times 10^{-6}$ .

**Reporting:** The date of determination, the standard deviation and the deviation between the smallest and highest value are reported in the instrument logbook

**Corrective actions:**

- a) Wait for 15 minutes and repeat the stability test, as the system may not have reached equilibrium. The time of measurement, standard deviation and the deviation between the smallest and highest value are reported in the instrument logbook.
- b) Verify the reference gas flow, which should be between 8 and 12 ml/min. Adjust the flow if necessary and repeat the stability test. The corrected reference gas flow, the standard deviation and the deviation between the smallest and highest value are reported in an instrument logbook.

#### ***4.2.3 Analysis of blank***

A blank containing organic free NANOpure water is analyzed identically as the samples are analyzed. The method for analyzing samples is described in section 2 (SPME method) and section 3 (headspace method).

**Frequency:** Daily before starting to analyze samples.

**Acceptance criteria:** The 44-ion trace should remain below  $1 \times 10^{-10}$  A during the entire time of the run, except during injection of reference gas pulses.

**Reporting:** If the acceptance criteria are met, “blank test passed” is noted in the instrument logbook, if the criteria are not met, “blank test failed” is noted in the instrument logbook.

**Corrective actions:**

- a) Set oven to 20C below upper temperature limit for one-hour. Afterwards repeat blank test. Report result in instrument logbook.
- b) Clean syringe (headspace method) or clean SPME fiber according to manufacturers instruction and repeat blank test. Report actions and results in instrument logbook.

#### ***4.2.4 Analysis of external standard***

Standard solutions containing reference compounds with known carbon isotope ratio are analyzed twice before starting to analyze samples and once after each 6 injections of samples. The standard solutions are prepared and analyzed as described in section 2 (SPME method) and in section 3 (headspace method).

**Frequency:** Daily.

**Acceptance criteria:** The  $^{13}\text{C}$  values should not deviate more than 0.5 per mil from the calibrated value.

**Reporting:** The measured  $^{13}\text{C}$  values of standard solution and calibrated value are reported in reference compound logbook.

**Corrective actions:**

Verify that 2/1 ratio trace has typical S-shaped curve bordered by straight baseline on both sides. Verify that mass-44 background intensity is below  $1 \times 10^{-10}$ A before and after the peak. Verify that 44-mass background intensity is below  $5 \times 10^{-11}$  A at a GC oven temperature of 50C.

#### ***4.2.5 Analysis of duplicates***

For one out of every five samples, a duplicate is analyzed. The duplicate is analyzed at least five injections before or after analyzing the sample.

**Frequency:** Daily.

**Acceptance criteria:** The deviation in  $^{13}\text{C}$  values between sample and duplicate should be  $<0.5$  per mille.

**Reporting:** The  $^{13}\text{C}$  values of samples and duplicates are reported on the data sheet.

**Corrective actions:**

Verify that 2/1 ratio trace has typical S-shaped bordered by straight baseline on both sides.

Verify that mass-44 background intensity is below  $1 \times 10^{-10}$ A before and after the peak.

Verify that 44-mass background intensity is below  $5 \times 10^{-11}$  A at a GC oven temperature of 50C.

ENVIRONMENTAL ISOTOPE LABORATORY  
DEPARTMENT OF EARTH SCIENCES  
UNIVERSITY OF WATERLOO

Technical Procedure 41.0

DRAFT DRAFT DRAFT DRAFT DRAFT DRAFT DRAFT DRAFT DRAFT  
**CHLORINE ISOTOPE ANALYSIS BY GC-IRMS**  
DRAFT DRAFT DRAFT DRAFT DRAFT DRAFT DRAFT DRAFT DRAFT

**AUTHOR(S):**

_____	_____
Robert J. Drimmie	
_____	_____
_____	_____

**APPROVAL:**

---

	<u>March, 2001</u>
_____	Date
Laboratory Manager	

---

## 1.0 Introduction

The gas chromatography isotope ratio mass spectrometry (GC-IRMS) system consists of the gas chromatograph (GC) connected to an isotope ratio mass spectrometer (IRMS) via glass capillary interface. In the mass spectrometer, sample gas is ionized, the ions are accelerated, and sample gas with mass  $XX$ , and  $XX+2$  is separated in a magnetic field that bends the ion beams depending on the mass of the ions. The separated ion beams hit Faraday cups and produce currents that are registered and displayed on the computer screen. In addition to the ion beam intensities, the FID signals in the 2/1 ratio (mass  $XX+2$ /mass  $XX$ ) are displayed. The 2/1 ratios during peak elution are typically S-shaped due to the slightly faster elution of compound containing  $^{37}\text{Cl}$  compared to compounds containing  $^{35}\text{Cl}$ . The peaks are automatically integrated and the isotope ratio is calculated. It is important that the whole peak is integrated. If only part of the peak is integrated (e.g. due to overlapping) the result will be biased towards  $^{37}\text{Cl}$  or  $^{35}\text{Cl}$ . The dynamic range of the system is at the maximal one order of magnitude, i.e. it is much smaller than that of a GC-FID. The actual peaks measured will depend on the compound and the ion fractions that form in the Mass Spectrometer. As for example TCE will split off one chlorine and one hydrogen to form a peak at 96 and 94. Therefore, concentrations of compound in the samples have to be known in advance to be able to inject an appropriate amount of compound or to be able to adjust the concentration by dilution of the samples. Typically, 2 to 12 nmol chlorine is required for a reproducible chlorine isotope ratio analysis.

### 1.1 Precautions

The precautions listed are not comprehensive. Use in conjunction with the relevant Material Safety Data Sheets (MSDS) from the manufacturer. Generic MSDSs from Aldrich Chemicals Company are available through the science Watstar network on any computer attached to ethernet. Follow acceptable safety procedures including the wearing of safety glasses at all times and gloves if necessary.

Whenever feasible, keep waste separate and retain for processing in the Hazardous Waste Handling Facility (HWHF) in ESC 150. Acids and bases not containing toxic substances may be poured down the drain provided they are between pH 5.5 and 9 or can be brought to pH 5.5-9 by dilution with water in a maximum ratio of 5:1 (water to solution). **WHEN IN DOUBT, RETAIN ALL WASTE IN SEPARATE LABELLED CONTAINERS AND TAKE TO THE HWHF.**

1. Exercise caution when handling concentrated acids. Open bottles in fumehood, wearing protective gloves and safety glasses. Always add acid to water.
2. Ammonia ( $\text{NH}_3$ ) is a colourless, corrosive and alkaline gas with a very pungent odour (characteristic of drying urine). Mixtures of ammonia and air will explode when ignited under favourable conditions but  $\text{NH}_3$  is generally considered nonflammable. It is exothermic in solution with water. Inhalation of concentrated vapour causes edema of the respiratory tract, spasm of the glottis and asphyxia. Prompt treatment is required to prevent death.

3. Ammonium Hydroxide ( $\text{NH}_4\text{OH}$ ) is a colourless liquid with an intense, pungent, suffocating odour. It has an acrid taste and produces a strong alkaline reaction. Reaction with sulphuric or other strong acid is exothermic and will cause the mixture to boil. It is irritating to the eyes, respiratory tract and skin. It causes burns, is harmful if inhaled and **MAY BE FATAL IF SWALLOWED**. Use only in a fumehood and wear gloves, lab coat and glasses. Dispose as corrosive, reactive waste.
4. Helium ( $\text{He}$ ) is a colourless and odourless gas at room temperature. It is a simple asphyxiant. Use only in well ventilated areas.
5. Methyl Chloride ( $\text{CH}_3\text{Cl}$ ), commonly called chloromethane, is a colourless gas which compresses to a colourless liquid of ethereal odour and sweet taste that burns with a smoky flame. It is **POISONOUS** and may cause injury to the liver, kidneys and central nervous system. Vent all waste to the fumehood.
6. Methyl Iodide ( $\text{CH}_3\text{I}$ ), commonly called iodomethane, is a colourless, transparent liquid that turns brown on exposure to air. It is **EXTREMELY POISONOUS** and may produce severe narcosis and acute lung irritation. Wear nitrile (green) gloves when handling. Make sure when the column is stripped of this gas that it is being vented into the fume hood.
7. Nitric Acid ( $\text{HNO}_3$ ) is a **POISONOUS**, colourless liquid that fumes in moist air with a characteristic choking odour. **IT REACTS VIOLENTLY WITH ORGANICS**. Liquid and vapour may cause severe burns. It may be fatal if swallowed or inhaled. Wear neoprene gloves, glasses and lab coat with apron and use only in the fumehood. Dispose as ignitable, corrosive waste.
8. Nitrogen ( $\text{N}_2$ ) is a colourless, odourless gas at room temperature. In liquid form (condensation temperature  $-196^\circ\text{C}$ ), it can produce severe frostbite. Wear safety glasses and thermal, impervious gloves to prevent frostbite. It is a simple asphyxiant; use under well-ventilated conditions.
9. Silver Carbonate ( $\text{Ag}_2\text{CO}_3$ ) is a light yellow powder when freshly precipitated, but becomes darker on drying and exposure to light. It decomposes at  $220^\circ\text{C}$  to  $\text{Ag}_2\text{O}$  and  $\text{CO}_2$  and to metallic Ag at higher temperature. It has low health and safety risks.
10. Silver Chloride ( $\text{AgCl}$ ) is a white powder that darkens on exposure to light. It may be harmful by inhalation, ingestion or skin absorption. Symptoms are eye and skin irritation and argyria (a slate-gray or bluish discolouration of the skin).
11. Silver Iodide ( $\text{AgI}$ ) is a light yellow odourless powder that slowly darkens under light. It has low health and safety risks.
12. Silver Nitrate ( $\text{AgNO}_3$ ) is a **POISONOUS**, corrosive and odourless, white crystalline powder. It is irritating to the respiratory tract and may cause death if ingested. Wear safety glasses and rubber gloves and use fumehood when handling. Dispose as toxic waste. Store solutions in amber containers to avoid light exposure .
13. Sulphuric Acid ( $\text{H}_2\text{SO}_4$ ) is a clear, odourless, colourless, dense, hygroscopic and oily liquid with a marked acid taste when pure. It produces a violent exothermic reaction with water and may ignite other materials such as paper, oil etc.. **SULPHURIC ACID IS CORROSIVE AND HIGHLY TOXIC, WITH SEVERE EYE, SKIN AND**

**MUCOUS MEMBRANE IRRITATION.** It is immediately dangerous to life or health at 80 mg/m<sup>3</sup>.

## **2.0 General start-up and shutdown procedure**

### **2.1 Conditioning GC oven**

1. Verify that the appropriate liner is installed in the inlet
2. Bake the GC oven for at least 30 minutes. Set temperature at least 20C above the highest temperature used in the method, but below the temperature limit of the column (Note: different columns have different upper temperature limits).

### **2.2 Getting the mass spectrometer ready**

1. After having baked the GC oven for 30 minutes, set GC oven to 50C.
2. Switch from standby to mass spec(**Menu: Inlet>Mass Spec**).
3. Verify that the Penning pressure is about 5 to 6\*10<sup>-6</sup> mbar. If the pressure is higher return immediately to standby and check for leaks. After the pressure has stabilized write it down in the instrument logbook.
4. 15 minutes after having switched to mass spec mode, center the peak (ion-beam) of the mass spec: Open reference gas valve: Double-click **RG** on diagram on-screen and wait until reading stabilizes. Perform a peak scan. Choose from menu: **Scan>Peak Center.** A peak scan is performed and the center of the peak is automatically detected if the peak is within the scan window. Save the new peak location: Choose from menu: **Mass Spec>Tune Source**. Overwrite CH3CL file. Close peak center window. Close reference gas valve (double-click **RG**)
5. Verify the intensity of the mass 52 current. It should be between 2 and 5\*10<sup>-11</sup> A at an oven temperature of 50 C.. Write down the background intensity in the instrument logbook.
6. Perform a stability run: Choose from menu: **Analysis>Single Run**  
Choose method within single run window: **CH3CL-STAB**  
Define name for folder and filename (STAB)  
Press **Run**. Ten reference gas pulses are automatically injected. The height of the reference gas pulses (mass-52=major) should be between 3 and 5\*10<sup>-9</sup>.  
Check printout. The calculated standard deviation of fit should be smaller than 1\*10<sup>-6</sup>. The fourth digit of the 2/1 ratios should not vary by more than five units. Write down the standard deviation and the deviation between the smallest and highest ratio in the instrument logbook.

### **2.4 Setting the heart split balance point**

#### **2.4.1 Background Information**

##### ***Heart split valve***

The heart split the valve controls the flow to the FID. If the heart split valve is closed, the flow is blocked, if it is open, flow is possible according to pressure gradients.

### ***Sample line helium pressure***

The purpose of the sample line (SLN) helium is to push the sample pulses into the IRMS if the heart split is closed and to direct the column flow to the FID if the heart split is open. The sample line pressure should be sufficiently high to keep the column flow away when the heart split is open but not too high to avoid excessive dilution when the sample pulses flow towards the mass spec. It is particularly important to keep the flow away from the IRMS when the solvent peak elutes.

### ***FID helium pressure***

The FID helium flow pushes the sample pulses across the small dead volume of the heart split towards the FID and avoids excessive broadening of the FID peaks due to this dead volume. However, if the FID helium pressure is increased, higher sample line helium pressure is required to direct the column flow towards the FID. Since the peak shape in FID is usually not important, the FID helium can be set close to zero in order to minimize the sample line helium flow.

#### ***2.4.2 Setting the sample line helium and FID helium pressures***

1. Set to FID helium pressure close to zero.
2. Verify that GC is at 50 C. and HC is open (float to GC)
3. Measure the SLN helium flow using the electronic flow meter. The split flow should be between 0.7 and 0.8 ml/min (corresponds approximately to a pressure of 2 to 3 psi). If the split flow is outside this range adjust it using the SLN helium knob on the interface box.
4. Close the heart split and measure the split flow again. It should remain between 0.7 and 0.8 ml/min.
5. A careful setting of the SLN and FID helium pressures is particular important if solvent injections are made, to prevent that the solvents peak enters the IRMS, which significantly reduces, cleanliness of the IRMS. Verify that no compound reaches the isotope ratio mass spectrometer when the HC is open before injecting solvent extracts. To check this, inject methane using the same GC temperature program as for samples and keep the HC open during the entire run. A methane peak should appear in the FID trace but nothing should be detected by the IRMS.

## **2.5 Running external standards**

1. Run external standards that have a similar matrix as the samples that are about to be analyzed. For more details, refer to the method-specific sections.
2. Compare the obtained isotope ratio with the known isotope ratio. The average of two measurements should not deviate by more than 0.3 per mil from calibrated value. Report the name of the compound, calibrated and measured value in the instrument logbook.
3. Check the peak area. Under optimal flow conditions the peak area should be about 5 to 8 \*10<sup>-9</sup> As per nmol chlorine. Lower peak areas indicate the presence of a leak.

## **2.6 shut down procedure**

1. Set the mass spec in standby mode **menu: Inlet > Standby**
2. Set cold trap temperature to 25 C.
3. Switch FID detector off, switch GC oven off.

## **3.0 SPME method**

### **3.1 Introduction**

The SPME method is more sensitive than the headspace method (see below) with detection limits as low as 5 ppb. While the method is more sensitive, it has the disadvantage that the injection volume is given by the volume of the fiber coating. Therefore, samples have to be diluted to appropriate concentration levels. This sensitivity of the method can be improved by adding salt or in case of headspace SPME, heating the samples. The samples can be extracted by immersing the fiber directly into the aqueous phase (dSPME) of the sample or by creating a headspace (hSPME) and exposing the fiber to it. The headspace method should be used for all compounds with Henry coefficients above 0.02 since I) with the headspace method volatile compounds are extracted preferentially, making the baseline quieter, II) it prolongs the lifetime of the fiber since the fiber does not come into contact with the matrix, and III) the equilibration time it is shorter than for dSPME.

### **3.2 Choice of column**

All columns except for the GS–GasPro are suited for the SPME method. The column has to be chosen depending on the composition of the samples (see column selection guide).

### **3.3. Inlet**

An SPME-liner (0.85 mm inner diameter) has to be installed. The inlet temperature depends on which type of fiber is used (see manufacturers guide for appropriate temperatures).

### **3.4. Temperature programming**

Generally the temperature program should be started at 40 to 50C. A low initial temperature leads to increased stationary phase focusing of volatile compounds and thus an increase in peak height, resolution and sensitivity.

### **3.5 Preparation of standards**

Prepared standard stock solutions by dissolving pure organic phase compounds or mixtures of pure organic phases in NANOpure water.

1. Rinse bottles and magnetic stirrer with methanol and place in oven at 100C. for at least two hours.
2. Fill bottle with NANOpure water.
3. Add organic compounds with a microliter glass syringe. For DNAPL, open bottle and inject organic compounds while immersing the needle of the syringe underwater. For LNAPL, inject organic compounds through septum.
4. Place bottle on magnetic stirrer at 600 to 1,000 rpm for at least 12 hours (e.g. over night).
5. Prepare standards. Dilute standard stock solution with NANOpure water in vials containing a magnetic stirrer that are similar to those used for samples. Stir standards for at least 15 min.
6. Open vial and introduce a small headspace if direct SPME is used or a larger headspace (10% of vial volume ) if headspace SPME is used. Add a magnetic stirrer. In case of head space SPME, stir for 15 min at 1100 rpm before starting the SPME extraction.

### 3.6 Preparation of samples

1. Open vial and introduce a small headspace if dSPME is used or a larger headspace (10% of vial volume) if hSPME is used. Add a magnetic stirrer. In case of hSPME, stir for 15 min at 1100 rpm before starting the SPME extraction.
2. Prepare a replicate sample for one out of five samples.

### 3.7 Analysis of standards and samples

1. Start instrument according to general start-up section (above).
2. Place vials on magnetic stirrer at 1100 rpm
  1. Retract fiber in needle, retract needle completely in fiber holder
  2. Place fiber holder on top of vial. Move needle out of fiber holder by turning inner part of fiber holder (metal) while holding outer part (black), until septa is penetrated.
  3. Move fiber out of needle. Adjust needle such that the fiber coating is completely immersed in the aqueous phase (direct SPME) or that fiber does not contact the aqueous phase (headspace SPME).
  4. Two minutes before finishing the extraction, make injector ready for injection by pressing prep run.
  5. After the extraction period is over, retract fiber into needle and remove fiber holder from vial.
  6. Adjust needle position to 4 by turning inner part of fiber holder.
  7. Insert needle into injector and immediately move fiber out of needle.
  8. Start GC run.
  9. Leave fiber in injector for 15 min.
  10. Verify that all peaks of interest are completely separated by examining the 2/1 trace, which should show a typical S-shape caused by earlier elution of compounds with  $^{37}\text{Cl}$  compared to compounds with  $^{35}\text{Cl}$ . The S-shape curve should be bordered by a horizontal line on both sides.

### 3.8 Sequence of analysis of samples and standards

1. Analyze two separate standard vials. The measured  $^{37}\text{Cl}$  of compounds should agree with the calibrated values within 0.3 per mil.
2. Analyze samples.
3. Analyze a duplicate for one out of five samples. The duplicate should be injected at least five injections before or after the sample.

- a. Flush the bottles with helium for two min.
- b. Add compounds as pure phase or as mixtures of pure phases. Choose the concentration in such a way as to obtain an average peak size on the mass spec. Example: Add each compound at a concentration of 120 microM C which yields a compound pulse of about 6 nmol C if 0.5ml it is injected at a split of 10:1.
- c. Wait for at least ½ hour to allow for evaporation of liquid phases. A piece of aluminum foil can initially be added to mix the gas by shaking the bottle.

### 3.9 Preparation of samples

- a. Introduce a headspace of helium with a volume of at least 5ml. Use 2 syringes, an empty one and one with helium. Insert both syringes at the same time and push in helium and pull out water at the same time. Note that the concentration in the headspace is the smaller the larger the headspace is and thus the sensitivity of the method decreases with increasing headspace size.
- b. Place the bottles or vials on the shaker for at least an hour and no more than 24 hours if septum sealed vials are used and the septum has been punctured. Place the bottles horizontally to avoid contact of the headspace with the punctured septum. It is usually best to prepare the samples in the afternoon of the previous day and shake the samples overnight.

### 3.10 Selection of injection volume

The amount of chlorine entering the column should be between 2 and 12 nmol per compound. The required amount may very dependent on the flow settings. For a first try, select the injection size in a way to obtain compound pulses of 6 nmol C. The injection size can be calculated based on the concentration of the compound in the headspace which is a given by equation 1 and the split ratio:

$$C_g = C_o / \left( \frac{1}{K} + \frac{V_g}{V_w} \right)$$

Where  $C_g$  is the concentration in the headspace,  $C_o$  the initial concentration of the compound in the aqueous phase (before and production of a headspace),  $K$  the dimensionless Henry coefficient,  $V_g$  the volume of the headspace and  $V_w$  the volume of the aqueous phase.

If the sample contains several compounds for which isotope ratios should be obtained, select an injection size that yields a compound pulse of 10 nmols Cl for the compound with the highest concentration. If the peak sizes for the other compounds are too small, make a second injection using a larger volume and direct the larger peaks (for which isotope ratios have been obtained in the first run) to the FID using the heart split.

### 3.11. Typical Procedure

Two days before running samples.

- Prepare a standard stock solution by dissolving pure organic phase compounds or mixtures of pure organic phases in NANOpure water.
- Rinse bottles and magnetic stirrer with methanol and place into in oven that at 100C for at least two hours
- Fill bottle with NANOpure water
- Add organic compounds with a microliter glass syringe. For DNAPL, open bottle and inject organic compounds while immersing the needle of the syringe underwater. For LNAPL, inject organic compounds through septum.
- Place bottle on magnetic stirrer at 600 to 1,000 rpm for at least 12 hours (e.g. over night).

#### One day before running samples

1. Prepare standards. Dilute standard stock solution with NANOpure water in vials that are similar to those used for samples.
2. Prepare samples in the afternoon, introducing a helium headspace and place samples on shaker overnight. Make sure you only prepare samples that contain compounds in sufficient quantities for headspace analysis

#### Day of analysis

1. Get GC oven, interface and mass spec ready according to general start-up procedure.
2. Analyze standards. Inject gas phase standard twice and check if isotope ratio corresponds to know that you and is reproducible (deviation < 0.5 per mil). Verify that peak area is about  $5 \text{ to } 8 \times 10^{-9} \text{ As per nmol Cl}$ .
3. Inject first sample twice and check reproducibility of isotope ratio (deviation < 0.3 per mil). Verify that all peaks of interest are completely separated by examining the 2/1 trace, which should show the typical S-shaped curve. The S-shape curve should be bordered by a horizontal line on both sides.
4. Inject each sample once, repeat each fifth sample and check reproducibility.
5. Repeat standard injection after having performed nine sample injections.
6. Keep an eye on cold trap temperature. Note: the temperature display on the screen is not functioning. After about six hours use, add another  $\frac{1}{2}$  Dewar of liquid nitrogen to cold trap.

## **4.0 Quality-control**

### **4.1 BI weekly quality-control activities**

#### **4.1.1. Linearity test**

Perform a linearity test as follows:

1. Set the GC oven temperature to 50C and open that each sea.
2. Stability test to verify that the system to stable (see below). If the criteria of the stability test has been met proceed.
3. Select: **Analysis>Single run**
4. Choose the CH<sub>3</sub>Cl-LIN method and press **run**.
5. Vary the height of reference gas pulse by manually changing the CH<sub>3</sub>Cl gas pressure at the reference gas box. The height of the mass-52 ion trace should be between  $1.5 \times 10^{-9}$  to  $1.5 \times 10^{-8}$  and vary randomly.

**Acceptance criteria:** the standard deviation of the 2/1 ratio of 10 consecutive reference gas pulses passed to be smaller than  $1.0 \times 10^{-7}$ . The deviation between the smallest and highest value should be less than  $5.0 \times 10^{-6}$ .

**Reporting:** The standard deviation and deviation between the smallest and highest value are reported in the instrument logbook.

Corrective actions:

- a) Verify that background intensity remains constant throughout the run. If background intensity changes the system may not be at equilibrium yet. Wait for one-hour and repeat the linearity test.
- b) Contact Orfan Shouakar-Stash for retuning of IRMS parameters.

#### ***4.1.2 Checking cleanness of vacuum***

A magnet scan is performed to determine the cleanness of the vacuum and particularly to evaluate the presence of H<sub>2</sub>O.

### **4.2. Daily Quality-Control Activities**

After starting up the instrument (section 1), the background intensity of the mass-XX ion beam is determined at a GC oven temperature of 50C.

**Frequency:** Daily before starting to analyze samples.

**Acceptance criteria:** The intensity should be smaller than  $5 \times 10^{-9}$ .

**Reporting:** The date of determination, type of GC column, oven temperature and background intensity level are reported in the instrument logbook.

**Corrective actions:**

- a) Heat the GC column to 20C below the upper temperature limit for 1-hour. Afterwards, set the GC temperature to 50C and determine the background activity again. The oven temperature during backing, duration of backing and the new background intensity are reported in the instrument logbook.
- b) Replace the inlet liner and set the oven temperature to 20C below the upper temperature limit for 30 min. Afterwards set the GC temperature to 50C and determine the mass-52 background intensity. Report the duration of backing and the background intensity enhancement logbook.

#### ***4.2.2 Stability test***

A stability test is performed as described in section 1.3.

**Frequency:** Daily before starting to analyze samples.

**Acceptance criteria:** the standard deviation of the 2/1 ratio of 10 consecutive reference gas pulses has with the to be smaller than  $1.0 \times 10^{-7}$ . The deviation between the smallest and highest value should be less than  $5.0 \times 10^{-6}$ .

**Reporting:** The date of determination, the standard deviation and the deviation between the smallest and highest value are reported in the instrument logbook

**Corrective actions:**

- a) Wait for 15 minutes and repeat the stability test, as the system may not have reached equilibrium. The time of measurement, standard deviation and the deviation between the smallest and highest value are reported in the instrument logbook.
- b) Verify the reference gas flow, which should be between 8 and 12 ml/min. Adjust the flow if necessary and repeat the stability test. The corrected reference gas flow, the standard deviation and the deviation between the smallest and highest value are reported an instrument logbook.

#### ***4.2.3 Analysis of blank***

A blank containing organic free NANOpure water is analyzed identically as the samples are analyzed. The method for analyzing samples is described in section 2 (SPME method) and section 3 (headspace method).

**Frequency:** Daily before starting to analyze samples.

**Acceptance criteria:** The XX-ion trace should remain below  $1 \cdot 10^{-10}$  A during the entire time of the run, except during injection of reference gas pulses.

**Reporting:** If the acceptance criteria are met, “blank test passed” is noted in the instrument logbook, if the criteria are not met, “blank test failed” is noted in the instrument logbook.

**Corrective actions:**

a) Set oven to 20C below upper temperature limit for one-hour. Afterwards repeat blank test. Report result in instrument logbook.

b) Clean syringe (headspace method) or clean SPME fiber according to manufacturers instruction and repeat blank test. Report actions and results in instrument logbook.

#### ***4.2.4 Analysis of external standard***

Standard solutions containing reference compounds with known chlorine isotope ratio are analyzed twice before starting to analyze samples and once after each 6 injections of samples. The standard solutions are prepared and analyzed as described in section 2 (SPME method) and in section 3 (headspace method).

**Frequency:** Daily.

**Acceptance criteria:** The  $^{37}\text{Cl}$  values should not deviate more then 0.3 per mil from the calibrated value.

**Reporting:** The measured  $^{37}\text{Cl}$  values of standard solution and calibrated value are reported in reference compound logbook.

**Corrective actions:**

Verify that 2/1-ratio trace has typical S-shaped curve bordered by straight baseline on both sides. Verify that mass-XX background intensity is below  $1 \cdot 10^{-10}$ A before and after the peak. Verify that XX-mass background intensity is below  $5 \cdot 10^{-11}$  A at a GC oven temperature of 50C.

#### ***4.2.5 Analysis of duplicates***

For one out of every five samples, a duplicate is analyzed. The duplicate is analyzed at least five injections before or after analyzing the sample.

**Frequency:** Daily.

**Acceptance criteria:** The deviation in  $^{37}\text{Cl}$  values between sample and duplicate should be  $<0.3$  per mil.

**Reporting:** The  $^{37}\text{Cl}$  values of samples and duplicates are reported on the data sheet.

**Corrective actions:**

Verify that 2/1-ratio trace has typical S-shaped bordered by straight baseline on both sides.

Verify that mass-XX background intensity is below  $1 \cdot 10^{-10}\text{A}$  before and after the peak.

Verify that XX-mass background intensity is below  $5 \cdot 10^{-11}\text{A}$  at a GC oven temperature of 50C.

#### ***5.0 Quality Assurance Requirements***

Samples are logged into a computer database where each sample is assigned a unique laboratory number and the file, a number YYxxxx, where YY is the year and xxxx is a sequential number starting at 1 for the first file of that year. This information is also entered into a sample log book as a back-up. All information pertaining to that file is stored in a file cabinet that is locked when the laboratory office is not staffed. The samples are placed on shelves within the lab and their location entered on the Laboratory Traveller Form generated for the file. Any information that the technician might require for analysis is hand written on the traveller or it is indicated that special instructions are in the file.

Additional and detailed description of the sample handling can be found in the EIL QA/QC Document 1.0.