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# GC2 PFT Analysis: User Guide

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## Introduction

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The major concern in shipboard microbiological study is whether microbes from the drilling fluid are introduced into the recovered core material during coring. Therefore, it is critical to verify whether recovered cores are contaminated. Perfluorocarbon tracer (PFT) can be used to quantify the amount of contamination due to drilling fluid. It is strongly recommended that this test be routinely conducted when coring for microbiological studies.

PFT is chemically inert and can be detected with high sensitivity. Perfluoro(methylcyclohexane) is used as the chemical tracer to monitor potential seawater contamination of sediment and rock samples on the *JOIDES Resolution*. This compound has a molecular weight of 350.05 g, a boiling point of 76°C, and a density of 1.76 g/mL. Its solubility is ~2 mg/L in water and 104 mg/L in methanol. The low solubility in water facilitates gas-phase partitioning and quantitative headspace analysis.

PFT is continuously fed into the stream of drilling fluid using a high-performance liquid chromatography pump. The tracer is delivered into the drilling fluid stream through a valve on the low-pressure side of the mud charge pump. The rate of the tracer injection is adjusted to maintain a final concentration of ~1 mg/L in the drilling fluid through the entire drill string.

After core retrieval, samples for PFT measurement are immediately taken from selected sections. Headspace vials containing the collected sediment are heated 5–10 min in an oven to evaporate and release the tracer, and then an aliquot of the vial headspace is injected onto a gas chromatograph equipped with a micro electron capture detector, which is extremely sensitive to halogenated compounds.

## Apparatus, Reagents, & Materials

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### Laboratory Apparatus

- 20 mL headspace vials (HP 5182-0837) and metal caps with Teflon seals
- Manual vial crimper
- 10 mL, 1 mL, and 200 µL syringes
- 0.1–1.0 mL gas-tight syringes
- Oven gloves and metal tray
- GC septa: 11 mm diameter, usable up to 250°C or 400°C
- GC column: Agilent column (15 m x 0.250 mm x 5 µm)

### Reagents

- Perfluorocarbon tracer
- Methanol: HPLC purity
- Nitrogen gas: ultra high purity, 50 psi max

## Calibration Standards

Dilute concentrated perfluorocarbon tracer in methanol in the following dilution series:

| Solute (mL)          | Solvent (mL) | Concentration |
|----------------------|--------------|---------------|
| 0.1 of conc. PFT     | 9.9 methanol | $10^{-2}$     |
| 0.1 of $10^{-2}$ PFT | 9.9 methanol | $10^{-4}$     |
| 0.1 of $10^{-4}$ PFT | 9.9 methanol | $10^{-6}$     |
| 0.1 of $10^{-6}$ PFT | 9.9 methanol | $10^{-8}$     |
| 0.1 of $10^{-8}$ PFT | 9.9 methanol | $10^{-10}$    |

## Hardware

The GC2 system comprises an HP 6890 gas chromatograph (GC) with a micro-electron capture detector ( $\mu$ ECD).

The GC inlet is operated in splitless mode. PFT gas samples obtained using the headspace extraction method are injected manually using Hamilton CR-700-200 auto syringes directly into injection port B. The injection port liner assembly is connected to an alumina-coated capillary column (15 m x 0.250 mm x 5  $\mu$ m), and then to a  $\mu$ ECD detector, which requires both carrier and makeup gases (nitrogen).

### Nitrogen Supply

Nitrogen gas is used in all three flow lines (column carrier, detector carrier, and makeup gases). Nitrogen suffices as the detector makeup gas for this procedure because chromatographic efficiency is not an issue and it is readily available aboard ship because of the nitrogen generator.

The  $\mu$ ECD is designed to operate best with a flow rate of at least 20 mL/min. Carrier flow of capillary columns, typically 10 mL/min, requires make-up gas to ensure the optimum total flow rate for the detector.

Nitrogen supply settings are:

- Tank pressure: 50 psi.
- Supply tubing: copper equipped with 1/8 inch Swagelock fitting.
- Flow rate is crucial to prevent damage to the  $^{63}\text{Ni}$  foil in the ECD.
- Both the  $\mu$ ECD and column are sensitive to oxygen; therefore, an oxygen/moisture trap and oxygen indication trap are highly recommended for the nitrogen supply lines.

### Electron Capture Detector

The  $\mu$ ECD cell contains  $^{63}\text{Ni}$ , a radioactive isotope emitting high-energy electrons ( $\beta$ -particles). These undergo repeated collisions with carrier gas molecules, producing  $\sim 100$  secondary electrons for each initial  $\beta$ -particle.

Further collisions reduce the energy of these electrons into thermal range. These low-energy electrons are then captured by suitable sample molecules, which reduces the total electron population within the cell. Therefore, with higher sample concentration the conductivity of an existing gas will drop noticeably, which is recorded by the ECD outcoming signal detector.

## Sample Preparation & Analysis

PFT is pumped into the drilling fluid during coring. When core is delivered to the deck, small core samples are placed in headspace vials, sealed, and heated before headspace analysis on the GC2. The presence of a PFT peak from a sample from the interior of a core indicates core contamination from drill fluid, which may contain contaminating microbes.

### Sample Collection

Sediment samples are collected from the edge and center of the core on the catwalk immediately after cores are retrieved. The sample from the outer edge is used to confirm successful delivery of the tracer to the core, whereas the interior sample is used to estimate the quantity of intrusion of drill water into the core. Because the exterior of

the core liner is coated with drilling fluid, contact with the liner should be avoided while collecting core samples for PFT analysis.

### **Unconsolidated Sediments**

1. After cutting the core liner, break up sediment core by pulling sections apart rather than cutting with a knife to ensure that the tracer is not dragged through the core with the knife.
2. Cut the luer end off of 5 mL plastic syringes (one for each sample to be collected).
3. Collect one plug sample ( $\sim 3 \text{ cm}^3$ ) using a cut-off syringe from the outer edge of the sample along the core liner.
4. Collect another plug sample ( $\sim 3 \text{ cm}^3$ ) using another cut-off syringe from near the center of the core.
5. Immediately extrude each sample into a 20 mL headspace vial and seal with gas-tight cap and septa.

### **Consolidated Sediments**

1. After cutting the core liner, place the core on a fresh sheet of aluminum foil.
2. Pare the exterior of the core using hammer, chisel, and tongs. Before using these tools, pass them through a flame torch to remove any PFT contaminant.
3. Collect one sample ( $\sim 3 \text{ cm}^3$ ) from the outer edge of the sample along the core liner.
4. Collect another sample ( $\sim 3 \text{ cm}^3$ ) from near the center of the core.
5. Immediately place each sample into a 20 mL headspace vial and seal with gas-tight cap and septa.

### **Igneous Rock**

1. Immediately after the core liner is split in the core lab, choose pieces of core for PFT analysis.
2. Place several small pieces of rock from the exterior of the core into a 20 mL headspace vial and seal with gas-tight cap and septa.
3. Alternatively, wipe the interior of the core liner with a cotton swab and place the swab into the 20 mL headspace vial and seal with gas-tight cap and septa.
4. Remove PFT from the surface of the rock before sampling the interior:
  - Rinse the exterior with water or methanol
  - Hold the piece with tongs under the flame of a handheld propane torch until it appears dry.
5. After cleaning, hold the rock on a fresh sheet of aluminum foil and pare away the exterior using a cleaned hammer and chisel or the hydraulic rock splitter. Use cleaned tongs to handle the rock pieces.
6. After each paring, pass tools through the flame of the torch and place rock pieces on fresh aluminum foil.
7. When the entire exterior of the rock is removed, crush the interior of the rock in a mortar.
8. Immediately place an aliquot of the crushed rock into a 20 mL headspace vial and seal with gas-tight cap and septa.

## Sample Analysis

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Sample analysis includes the following steps:

- Prepare GC and syringes
- Prepare standards and run calibration curve
- Approve calibration
- Run samples
- Analyze results

### Preparing GC and Syringes

1. Check nitrogen gas availability, tank pressure > 500 psi, and delivery pressure = 50 psi.
2. Change septum on GC.
3. Clean and bake all syringes at 70°C for 10 min.
4. Prepare foil plate to heat syringes in oven at 70°C.

### Preparing and Running Calibration Standards

1. Rinse syringes with methanol.
2. Prepare dilutions of PFT as per instructions in Reagent Solutions.
3. Transfer 10 µL of the  $10^{-4}$ ,  $10^{-6}$ ,  $10^{-8}$ , and  $10^{-10}$  dilutions into duplicate 20 mL headspace vials and seal with gas-tight cap and septa.
4. In open GC2 control window, choose **View > Method & Run Control**.
5. In the method box choose **PFT.M** method file and wait until **Ready** message is lit.
6. Heat the lowest dilution calibration headspace vial for 1 min at 70°C.
7. Using the heated Hamilton auto-syringe, withdraw 0.5 mL of headspace from the vial.
8. Immediately inject into injection port B and press **Start** on the GC keypad or software dialog window. Use a consistent injection technique to maximize consistency in the PFT peaks.
9. Wait for temperature program to cycle and GC to return to ready (~10 min).
10. Repeat heating and standard injections until all calibration standards have been run.

### Approving Calibration

1. Navigate to **Calibration > Data Analysis** and open the calibration files.
2. Enter required parameters into the **Calibration** table and view calibration curve and correlation coefficient.
3. If calibration is acceptable, continue with sample analysis.

## Running Samples

1. Heat each headspace vial containing sample in an oven at 70°C for 5–10 min.
2. Using the gas-tight syringe, withdraw 5 mL headspace from the heated vial, inject directly into GC injection port B, and press **Start** on the GC keypad or software dialog box.
3. Wait for GC temperature program to cycle and GC to return to ready (~10 min).
4. Repeat steps 1–3 for each sample to be analyzed.

## Analyzing Samples

- The area of the PFT peak is integrated and converted to the amount of PFT using values from the standard curve.
- The amount of sample is determined by weighing each vial and subtracting the weight of an empty vial.
- Total headspace volume is calculated by subtracting the volume of sample from the total volume of the vial.
- Total tracer concentration in the sample is corrected to account for the fraction of the headspace that is injected.
- The amount of drilling fluid in the sample is calculated assuming that the tracer was present at 1 mg/L.

## Calculations

Use the following equations to determine the amount of drill-water intrusion in a sample:

$$(\text{Drill water, L}) / \text{Core material, g} / [(P_s - P_b) / (C_{DW} \times a \times W \times F_i)]$$

where

$P_s$  = integrated peak area of PFT in sample (in arbitrary units),

$P_b$  = integrated peak area of PFT in blank (in arbitrary units),

$a$  = slope derived from the calibration curve (in arbitrary units per gram),

$C_{DW}$  = concentration of PFT in drilling fluid (in grams per liter),

$W$  = weight of sample (in grams), and

$F_i$  = fraction of the total headspace gas injected:

$$V_{inj} / [V_{vial} - (W / \rho_{bulk})]$$

where

$V_{inj}$  = volume of sample injected (in liters),

$V_{vial}$  = volume of vial (in liters),

$\rho_{bulk}$  = sample density (in grams per liter), and

$W$  = weight of sample (in grams).

## Quality Assurance/Quality Control

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### Analytical Batch

The analytical batch is a group of samples run together with a single set of QC parameters, such as calibration/calibration verification, blank, and other QA/QC samples.

### Blanks

Blanks are analyzed to determine the instrumental and procedural backgrounds. These blanks consist of 5 mL injections of air collected in the gas-tight syringe from outside the laboratory or headspace gas from empty vials prepared at the same time and location the samples are taken.

## Calibration

Calibrating the instrument produces instrument response factors to absolute component concentrations. To prepare a calibration for quantitation of unknown samples, the retention time(s) for the peak(s) of interest and the amount of component injected must be known.

### Calibration Cuve

The graphical representation of the amount and response (peak area) for PFT from the calibration samples defines the calibration curve. Because the ECD is not linear across its range of detection, multiple calibration standards are run to calibrate for PFT. Various curve-fit calculations are available to determine optimum regression coefficient including linear, log, power, exponential, quadratic, and cubic.

### Correlation Coefficient

The correlation coefficient is the square root of the regression coefficient and gives a measure of the fit of the calibration curve to the data points. The value of the correlation coefficient ranges from 0.000 (no fit) to 1.000 (perfect fit). The calibration coefficient for PFT must be >0.995 to be considered an acceptable calibration.

### Calibration Range

A multilevel calibration is valid over the range of concentrations used in the calibration samples. Extrapolation of a calibration curve, especially if it is not linear, gives at best an approximation result.

## LIMS Integration

Results are manually entered into the LIMS database associated with an analysis code and an analysis component. Analysis codes and their components, definitions, and units are listed below.

| Analysis | Component       | Definition                               | Unit |
|----------|-----------------|--|------|
| GC2      | PFT             | Concentration of PFT                     | PPMV |
|          | dat_filename    |  |      |
|          | run_test        | Pointer to related calibration           |      |
| GC2_QAQC | PFT             | Concentration of PFT                     | PPMV |
|          | dat_filename    |  |      |
|          | run_test        | Pointer to related calibration           |      |
|          | PFT_corr2       | Calibration correltaion coefficient (R2) |      |
|          | PFT_intercept   | Intercept of the calibration curve       |      |
|          | PFT_slope       | Slope of the calibration curve           |      |
|          | method_filename |  |      |

## Health, Safety, & Environment

### Safety

Primary safety issues include the following:

–Electron capture detector

- PFT chemical compound handling
- Oven operation

### Electron Capture Detector

The electron capture detector measures the current flow caused by  $\beta$ -particle emission from the  $^{63}\text{Ni}$  foil source. This source is completely contained and is considered to be safe for humans if the system is properly maintained.

If the ECD detector is heated above  $\sim 150^\circ\text{C}$  without supply gas flow or is overheated above  $\sim 400^\circ\text{C}$ , the  $^{63}\text{Ni}$  foil internal to the detector may oxidize and be damaged. Such damage may result in a release of  $^{63}\text{Ni}$  into the laboratory atmosphere.

- Never disassemble the ECD detector to ensure containment of the  $^{63}\text{Ni}$  material.
- Do not modify the ECD in any manner including removing its sheet metal cover.
- The **Caution – Radioactive Materials** label must be attached to the detector at all times. The label must identify the following: Type of radioactive material the ECD contains, Activity of the radioactive material, Reference date.
- Never use a damaged detector – notify the HSE director and the Supervisor of Technical support on the shore or Third Mate on the ship immediately upon discovery.
- When the ECD is not in use, cap the inlet and outlet fittings and keep the detector temperature at room temperature.
- Solvents (including water) or corrosive chemicals in contact with the ECD may compromise its integrity.
- Interfering with the overheat circuitry could result in the release of radioactive material from the ECD.
- Vent ECD effluent to a filter or a fume hood – not ambient room air. Wear disposable gloves when removing or attaching vent lines.
- The ECD must be available for leak testing every six months.

If the ECD must be shipped or relocated, contact associate director for HSE for instructions.

### PFT Chemical Compound Handling

Perfluoromethylcyclohexane is used as the perfluorocarbon tracer compound. This volatile compound is chemically inert and of reasonably low toxicity. Although it is relatively harmless, it can permeate widely if not used under properly ventilated conditions and cross-contamination of environment-to-sample can occur.

Safe handling guidelines for the PFT chemical compound consist of the following:

- Perform PFT chemical dilution and standards preparation procedures under a ventilated hood.
- Avoid direct contact with PFT chemicals by wearing gloves.
- Do not leave open PFT solutions in unventilated areas.
- Make sure no vials with PFT are left in the oven during and after PFT analysis procedure.
- Wear personal protective equipment including gloves and labcoat when working with PFT.

#### Health Hazards:

- Contact: irritant
- Inhalation: irritant
- Ingestion: irritant

#### Chemical Hazards:

- Incompatible substances: oxidizing agents, strong acids, strong bases
- Emits toxic fumes under fire conditions

## General Chemistry Laboratory Safety

- Methanol is flammable, take precautions around heat sources.
- Always label interim and stock solutions with chemical name, concentration, operator name, and date.
- Ensure gas tanks are secure. Do NOT drop the gas tanks, the head could break off!
- Ensure the tank head and tank types match.
- Check cable settings.
- Ensure cable settings are tight and correctly loaded.
- Turn power off before repairing or checking instrument electronics inside the machine to avoid electric shock.

## Oven Safety

- Make sure the oven is switched off after PFT analysis routing is finished to avoid fire hazards.
- Do not exceed the flame or explosion value of any chemicals used in the oven.
- Be careful not to touch heated oven parts during operation.
- Wear oven gloves and/or use forceps when working inside the oven and adding or removing vials. Provide instant access to oven gloves and oven forceps during PFT analysis.

## Pollution Prevention

- Provide adequate ventilation of the ECD exhaust line to ensure proper evacuation of possible hazardous and radioactive residues.
- Perform a radiation leak wipe test every 6 months of ECD operation. Contact the senior chemistry technician or the Lab Officer.

## Maintenance & Troubleshooting

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For procedure or GC operation problems, call a chemistry technician for help.

- Before each operational session, check the GC cable and tubing connections.
- The Total Flow knob should be open and stay in that position for all analyses; otherwise, the EPPC and EPPB pressure and, thus, the column flow may not reach the desirable values.
- The 11 mm injection port septa needs to be changed every day. This is a common cause of leaks in the flow line. If the GC safety alarm starts beeping, check the injection septa.
- If the column flow is low, the GC safety alarm will beep. If the column flow is adequate, check the nitrogen supply. The valve may be closed or the line empty. Check the N<sub>2</sub> supply pressure and the close valve on a regular basis.

## Capillary Column Maintenance

### *Inadequate Carrier Gas Flow*

To prevent permanent damage, never heat the column without adequate carrier gas flow through the column. In most flow failure cases, the system will give a warning beep followed by emergency shutdown procedures. Lack of carrier gas flow may be caused by:

- Leak above the column connection with injection port
- Broken column
- Empty or closed gas supply line

### *Oxygen Contamination*

A critical issue is column degradation due to oxygen penetration, especially at high temperatures

- If a column needs to be removed and stored, tightly cover each end of the column with capillary column caps to prevent oxygen penetration.



- After column installation, condition the column (i.e., leave under carrier gas flow at room temperature, followed by gradual temperature increase).
- Eliminate the possibility of flow line leaks before increasing the oven temperature. Proper connection of tubing and column parts should decrease the chance of a leak.
- Do not bend the column at a sharp angle or apply significant pressure to the column tubing during installation to prevent breakage.

### Column End Cuts

Before installing the column, inspect the quality of the cut end with a magnifying glass. If the cut is not square and smooth, recut the column with a column cutter until quality is satisfactory. Ensure the correct length of the inner column end part for each connection.

### Conditioning Column

For ECD, the column should be conditioned with the detector end disconnected. To condition the column, follow these steps:

- Connect the column to the injection port
- Open the carrier gas line and start gas flow
- Check if gas is flowing out of the detector end of the column
- After 5–10 min of “cold” conditioning, increase oven temperature to 250°–300°C
- Allow column gas to flow at 250°–300°C for 3 hr
- Lower oven temperature to 50°C, cut off 10-20 cm of column at detector end, install column to ECD, and let carrier gas flow again through the system at low temperature to evacuate any oxygen residues from system flow line.

### Column Contamination

If column contamination is suspected, rinse the column with a small injection of pure methanol. If the baseline does not improve significantly try the following:

- Replace the column
- Clean the column with methanol injection assemblies
- Cut off 0.5–1.0 m of column at the injector end

**Warning:** Never rinse or inject the capillary column with inorganic acids or bases!

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