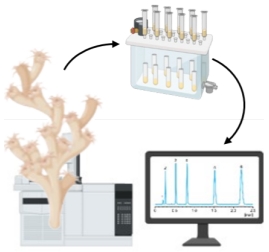
Aug 18, 2025

# Selective Isolation of Lipid Classes from cold-water corals via SPE for Quantitative Fatty Acid Profiling



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**Protocol status:** Working
**We use this protocol and it's working**

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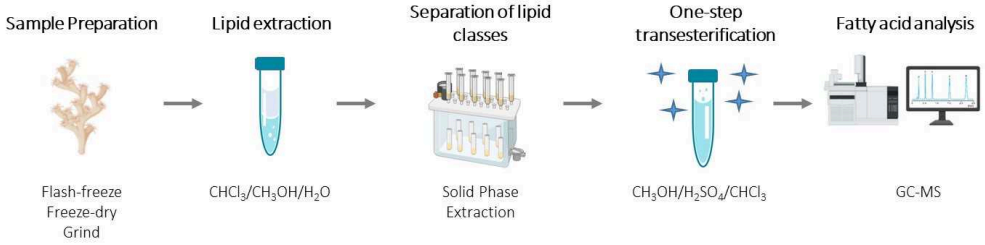
**Keywords:** Lipids, Fatty acids, Solid Phase Extraction, Corals, extraction of total lipid, water corals via spe, coral polyp sample, selective isolation of lipid class, separation of lipid class, quantitative fatty acid profiling, quantitative fatty acid profiling this protocol, fatty acid methyl ester, water coral, total lipid, lipid class, extraction, solid phase extraction, step acidic transesterification

## Abstract

This protocol describes the extraction of total lipids from coral polyp samples using the method of Bligh and Dyer (1959), followed by the separation of lipid classes via solid phase extraction (SPE) on aminopropyl ($NH_2$) silica cartridges as described by Kaluzny et al. (1985). A one-step acidic transesterification, adapted from Indarti et al. (2005) and Christie (2003), is then applied to produce fatty acid methyl esters (FAMEs) for GC-MS analysis.




Protocol flowchart

## Image Attribution

Pruski Audrey M.

## Guidelines

**Sample Handling:** Handle sample quickly after collection to prevent lipolysis

**Equipment:** Ensure all glassware, tubes, and Pasteur pipettes are pre-burnt ( 450 °C ) to avoid contamination

**Chemicals and solvents:** Use GC-MS or HPLC reagent grade products

**Internal Standard:** Nonadecanoic acid (C19:0) is used to correct for extraction losses during transesterification

**Temperature Control:** Maintain precise heating and cooling conditions to ensure consistent results

**Storage:** Keep eluates and FAME extracts at -20 °C if not analyzed immediately

## Materials

**Equipments:**

- Freeze dryer (Christ Alpha 1-4)
- TissueLyser II (QIAGEN)
- Solid phase extraction manifold (Phenomenex®)
- Hand-operated vacuum pump
- Rotary shaker (Bioblock)
- Ultracentrifuge (Sigma 6K-15)
- Speed-Vac concentrator (Thermo Scientific™ Savant™)
- GC-MS system (Agilent)

**Materials:**

- Strata $NH_2$ cartridges (Phenomenex®)
- 20 and 30 ml Borosilicate glass tubes round bottom with PBT red screw cap & PTFE liner
- 20 ml Borosilicate glass tubes conical bottom with PBT red screw cap & PTFE liner
- 2ml GC vials with 200µl insert
- Polar capillary column (e.g. VF-Wax-MS or ZB-WAX-MS)

**Chemicals and reagents (HPLC or GC grade):**

- Acetic acid ($CH_3CO_2H$)
- Butylated hydroxytoluene (BHT)
- Chloroform ($CH_3Cl$)
- Dichloromethane ($CH_2Cl_2$)
- Diethyl ether ($(C_2H_5)_2O$)
- Ethyl acetate ($C_4H_8O_2$)
- Hexane ($C_6H_{14}$)
- Hydrochloric acid (HCl)
- Methanol ($CH_3OH$)
- Nonadecanoic acid (C19:0= internal standard) ($C_{19}H_{38}O_2$)
- Phosphoric acid ($H_3PO_4$)
- Potassium bicarbonate ($KHCO_3$)
- 2-Propanol ($C_3H_8O$)
- Sodium chloride (NaCl)
- Sodium hydroxide (NaOH)
- Sulphuric acid ($H_2SO_4$)
- Water (ultrapure)

- Commercial FAME standards (Qualmix Fish Synthetic, Ladoran Fine Chemicals, INTERCHIM, France; Supelco 37, PUFA No. 1 and No. 3, SUPELCO France) for GC-MS calibration

**Note: use GC/HPLC grade reagents**

**Biological samples:**

- Coral nubbins

## Safety warnings

Safety information

- Always wear safety goggles and gloves
- Work in a fume hood when handling solvents
- Dispose of waste in appropriate containers

# Lipid Extraction from the Biological Matrix (Cold-water coral nubbins)

1d 9h 6m

1 **Sample Preparation**

1.1 Flash-freeze coral nubbins on board to prevent degradation 10m

1.2 Freeze-dry samples and store at -80 °C until analysis 1d

1.3 Grind freeze-dried coral nubbins into a fine powder using a bead mill homogenizer while keeping the chamber filled with liquid nitrogen to prevent heating 10m

2 **Preparation of Working Solutions** 4h

2.1 Chloroform/methanol/water (1:2:0.8) reagent: 5.265 mL $CHCl_3$, 10.525 mL $CH_3OH$ and 4.21 mL $H_2O$. Keep at 4 °C in a flammable storage refrigerator

2.2 1M NaCl solution in ultrapure water (58g.$L^{-1}$). Keep at 4 °C in a refrigerator

2.3 0.3N NaOH solution (12 g.L-1). Keep at 4 °C in a refrigerator

2.4 0.2M $H_3PO_4$ solution (19.6g.L-1). Keep at 4 °C in a refrigerator

2.5 BHT (butylated hydroxytoluene) at 50 mg.mL-1 in Chloroform. Keep at 4 °C in a flammable storage refrigerator

3 **Total lipid extraction** 4h

Note

Total lipids are extracted using a modified version of the Bligh and Dyer method (1959), optimized to improve extraction efficiency.

3.1 Place the freeze-dried coral nubbins (~ 3 g ) in a 30 mL glass tube (round bottom, screw tap with PTFE liner)

3.2 Add 20 mL of $CHCl_3/CH_3OH/H_2O$ (1:2:0.8) with 1M NaCl + 0.2M $H_3PO_4$

Note

The solution of NaCl 1M-$H_3PO_4$ 0.2M increases protein unwinding and lipid recovery

3.3 Sonicate 00:05:00 ( On ice if needed ), then rotate 00:20:00 at Room temperature 25m

3.4 Add 2.5 mL $CHCl_3$, then 2.5 mL $H_2O$. Shake 00:05:00 5m

3.5 2500 rpm, 4°C, 00:05:00 . Recover the organic phase (bottom) using a Pasteur pipette 5m

3.6 Repeat extraction 3 times with 5 mL $CHCl_3$

3.7 Pool all organic phases (final volume ≈ 22.765 mL) and vortex. ⚠ Keep organic phase cold On ice

3.8 Wash organic phase with 2.5 mL cold 0.3N NaOH. Shake 00:01:00 1m

3.9 2500 rpm, 4°C, 00:05:00 and recover the organic phase 5m

3.10 Take 20 mL or more into a new glass tube (conical bottom, screw tap with PTFE liner) and evaporate at 40 °C under vacuum (rotary evaporator)

Note

⚠ Lipid extracts should never be allowed to dry out!

3.11 Cool at -20 °C and redissolve in 400 µL $CHCl_3$ + BHT (50 $mg.L^{-1}$)

Note

BHT is added optionally to chloroform to prevent lipid oxidation during extraction!

3.12 Centrifuge 2000 rpm, 4°C, 00:05:00 5m

Store at -20 °C

## Lipid Class Separation by Solid Phase Extraction (SPE) 1d

4 **Solvent Preparation**

4.1 Solvent A: Chloroform:2-propanol (2:1 v/v)
Solvent B: 2% Acetic acid in diethyl ether
Solvent C: Methanol
Solvent D: Hexane
Solvent E: 1% Diethyl ether + 10% Dichloromethane in hexane
Solvent F: 5% Ethyl acetate in hexane
Solvent G: 15% Ethyl acetate in hexane
Solvent H: Chloroform:Methanol (2:1 v/v)

Note

Keep all solvents at 4 °C in a flammable storage refrigerator!

5 **Separation of neutral lipids, fatty acids and phospholipids** 1d

Note

3 Strata $NH_2$ (aminopropyl silica) cartridges (Phenomenex) are needed per sample.
7 lipid fractions are efficiently recovered from the initial chloroform extract with minimal impurities.

5.1 Condition the first cartridge (C1) with 2 × 2 mL hexane, vacuum dry 2–5 min

5.2 Load 390 µL lipid extract. Vacuum to dryness

5.3 Elute neutral lipids (E1): 4 × 2 mL solvent A

5.4 Elute free fatty acids (E2): 4 × 2 mL solvent B

5.5 Elute phospholipids (E3): 4 × 2 mL solvent C

5.6 Store eluates at -20 °C .

6 **Neutral Lipid Fractionation**

Note

In the original method by Kaluzny et al. (1985), the elution of steryl esters was demonstrated using commercially available lipid standards, which did not include wax esters. However, based on our experimental results, wax esters appear to co-elute with steryl esters under the same solvent conditions used for fraction E4.

6.1 Condition a second cartridge (C2) as above with hexane

6.2 Load 390 µL from E1. No need to dry

6.3 Elute wax esters and sterol esters (E4): 4 × 2 mL solvent D

Note

Note: Mono-, di-, and triglycerides and sterols are retained on C2.

6.4 Condition a third cartridge (C3) as above

6.5 Stack C2 onto C3 using an adapter

6.6 Elute triglycerides (E5): 6 × 2 mL solvent E

6.7 Elute sterols (E6): 9 × 2 mL solvent F

6.8 Remove and discard C3, place C2 back on manifold

6.9 Elute diglycerides (E7): 4 × 2 mL solvent G

6.10 Elute monoglycerides (E8): 4 × 2 mL solvent H

## 7 Evaporation of lipid fractions

7.1 Evaporate under vacuum all eluates ( 45 °C )

7.2 Redissolve in 100 µL $CHCl_3$ + BHT. Store at -20 °C

Note

Eluates for GC-MS analysis of fatty acids are E2 (Free Fatty Acids), E3 (Phospholipids), E4 (Wax esters and Sterol esters), E5 (Triglycerides), E7 (Diglycerides), and E8 (Monoglycerides). E6 may be used for sterol analysis!

## One-Step Acidic Transesterification

1d

8 **Lipid extraction and Transesterification**

8.1 **Preparation of Working Solutions**

- Transmethylation reagent: 3.4 mL Methanol + 0.6 mL $H_2SO_4$ (98%), and 4.0 mL $CHCl_3$ with BHT (50 mg/L). Keep at 4 °C in a flammable storage refrigerator

Note

Reagent should be cold and freshly prepared

- C19:0 (internal standard) stock solution ~1 $mg.mL^{-1}$ in hexane. The solution is diluted before use (1/9). Keep at -20 °C

Note

Adjust the concentration of the internal standard so that its peak on the chromatogram falls within the range of peak intensities, ideally between the most abundant and the least abundant fatty acids. This ensures accurate quantification across the entire dynamic range

8.2 To the eluates: add 20 µL internal standard C19:0 + 8 mL of transmethylation reagent. Cap and shake

8.3 Incubate 01:30:00 at 90 °C. Shake occasionally (3-4 times)

1h 30m

9 **Phase Separation**

9.1 Cool ( On ice or -20 °C ). Open carefully

9.2 Add 2 mL cold $H_2O$. Vortex. 300 rpm, 4°C, 00:05:00 Centrifuge 5 min, 3000 rpm, 4°C

9.3 Recover lower organic phase in a new glass tube

10 **Organic Phase Wash**

10.1 Re-extract aqueous phase 2 times with 2 mL cold hexane:$CHCl_3$ (4:1)

10.2 Combine all organic phases (~8–9 mL)

11 **Final Purification**

11.1 Wash with 4 mL cold 2% $K_2CO_3$. 3000 rpm, 4°C, 00:05:00

11.2 Discard the upper polar layer and transfer 6-9 mL of the lower organic phase to a clean screw-cap tube

12 **Drying and Dissolving FAMEs**

12.1 Evaporate solvent in a SpeedVac concentrator at Room temperature . Cool at -20 °C

12.2 Redissolve in 30-50 µL pure hexane. Vortex, 3000 rpm, 4°C, 00:05:00 10m

12.3 Transfer to GC vials with a 200 µl insert. Store at -80 °C

12.4 Fatty acid methyl extracts are ready for GC-MS analysis

Note

In this study, the analyses were carried out using the instrumental conditions described in a companion protocol developed by our group (Pruski & Vétion, 2024).

## Protocol references

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