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Lipid Biomarker Extraction and Elution into different Fractions from sediment

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We use this protocol and it's working

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ABSTRACT

This is a protocol of a method used to obtain the total lipid extract (TLE) from archaeological sediment samples and the further elution of the TLE into different fraction and their preparation for GC-MS analysis.

Keywords: Total lipid extract,
lipid biomarker in sediment,
archaeological sediment
samples

Preparation

- 1 Oven drying of sediment samples at 60 °C during 48:00:00 and subsampling to 5 g of homogenized sediment

Lipid Extraction

- 2 Add 40 mL dichloromethane/methanol (DCM/MeOH) 9:1 v/v to the sediment
- 3 Mix for 00:30:00 in a sonicator (below 30 °C)
- 4 Transfer the solvent into a centrifuge tube and centrifugate for 00:10:00 at 4700 rpm
- 5 Repeat step 2-5 two more times
- 6 Filter centrifuged solvent through annealed glass wool and evaporate with N₂

Prepare silica gel column for SPE extraction

- 7 Add small ball of anniled glass wool to column

8 Add 0.1 g of sand (50-70 mesh, previously fired at 450 °C during 10:00:00) to column

9 Add 1 g of silica (70-230 mesh, previously fired at 450 °C during 10:00:00) to column

Adding lipids to column

10 Add lipids reconstituted in DCM with about 9 drops every 10 minutes

11 Leave the column covered in aluminium foil for at least 12 hours

Elution

12 Calculate dead volume (DV) using n-hexane

13 Elute n-alkanes with 3/8 DV using n-Hexane

14 Elute aromatics with 2 DV 8/2 v:v n-hexane/DCM

- 15 Elute ketones with 2 DV DCM
- 16 Elute alcohols with 2DV of 1/1 v:v DCM/Ethyl acetate
- 17 Elute acids and diols with 2 DV of Ethyl acetate
- 18 Elute other compounds with 2 DV of Methanol
- 19 Evaporate all fractions using N₂ at 30 °C

Preparation of alcohols

- 20 Add 100 µL of N,O-Bis(trimethylsilyl)trifluoroacetamide (BSTFA) + trimethylchlorosilane (TCMS) 99:1 v/v and 100 µL of pyridine to the extract
- 21 Derivatize at 80 °C for 01:00:00
- 22 Dry the sample under N₂

Preparation of fatty acids

- 23 Derivatize fatty acids by adding 5 mL of MeOH, 400 μ L of H₂SO₄ to the extract
- 24 Heat at 70 °C for 04:00:00
- 25 Neutralize with saturated sodium bicarbonate solution
- 26 Extract three times with 3 mL n-hexane and dry under nitrogen

Adding standard and reconstituting

- 27 Store at -20 °C until measurement
- 28 Reconstitute n-alkanes with 5 α -androstane (8mg/L) and 150 μ L of DCM
- 29 Reconstitute aromatics with 5 α -androstane (8mg/L) and 50 μ L of DCM

- 30** Reconstitute ketones with 5 α -androstan-3-ol (8mg/L) and 50 μ L of DCM
- 31** Reconstitute alcohols with 5 α -androstan-3-ol (8mg/L) and 40 μ L of DCM and 10 μ L of Ethyl acetate
- 32** Reconstitute acids and diols with Methyl C19:0 (8mg/L) and 40 μ L of DCM and 10 μ L of Ethyl acetate
- 33** Reconstitute other compounds with Methyl C19:0 (8mg/L) and 40 μ L of DCM and 10 μ L of Ethyl acetate