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Working

Lipid Biomarker Extraction and Elution into different Fractions from sediment

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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.

PROTOCOL STATUS

Working

We use this protocol in our group and it is working

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 N_2

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 Elude acids and diols with 2 DV of Ethyl acetate

18 Elude other compounds with 2 DV of Methanol

19 Evaporate all fractions using N_2 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 N_2

Preparation of fatty acids

23 Derivatize fatty acids by adding **5 ml** of MeOH, **400 µl** of H_2SO_4 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 5α-androstane (8mg/L) and **150 µl** of DCM

29 Reconstitute aromatics with 5α-androstane (8mg/L) and **50 µl** of DCM

30 Reconstitute ketones with 5α-androstan-3-ol (8mg/L) and **50 µl** of DCM

31 Reconstitute alcohols with 5α-androstan-3-ol (8mg/L) and **40 µl** of DCM and **10 µl** of Ethyl acetate

32 Reconstitute acids and diols with Methyl C19:0 (8mg/L) and **40 µl** of DCM and **10 µl** of Ethyl acetate

33 Reconstitute other compounds with Methyl C19:0 (8mg/L) and **40 µl** of DCM and **10 µl** of Ethyl acetate