FAME Derivatization and Analysis of Fatty Acids

MATERIALS

- 3 M Methanolic-HCl (Supelco, 33355)
- Methanol (Sigma, 322415)
- Chloroform (Sigma, 288306)
- Sodium chloride solid (Sigma, S9888)
- Anhydrous Sodium Sulphate (Sigma, 239313)
- Hexane (Sigma, 296090)
- Methyl pentadecanoate (C15:0) (Restek, 35047)
- Methyl heptadecanoate (C17:0) (Restek, 35050)
- Fatty acid methyl ester standard (Restek, Food Industry FAME Mix, 35077)
- Glass Tube, 13 x 100 mm, with Teflon lined Cap (Pyrex, 9826-13)
- Sodium Chloride (Sigma, S9888)

1 M Methanolic-HCl:

- Slowly dissolve 1 mL of 3 M Methanolic-HCl with 2 mL of cold Methanol in a glass screw-top tube on ice-bath. Slowly mix the solution by tapping (do not vortex)

Half-Saturated NaCl:

- 13 g of NaCl

Prepare a Saturated NaCl solution by adding the 13 g of NaCl to 15 mL of Milli-Q water in a 50 mL centrifuge tube. Add more Milli-Q water to bring the volume to 30 mL and then vortex the solution until no further dissolution of NaCl occurs. Transfer 15 mL of the Saturated NaCl to a clean 50 mL centrifuge tube and bring the volume to 30 mL with Milli-Q water.

PROCEDURE

1. Transfer a known amount of sample to a glass screw-top tube. To this add a known amount of methyl pentadecanoate or methyl heptadecanoate, as an internal standard and dry the mixture with dry nitrogen flush.

2. Add 100-200 µL of 1 M Methanolic-HCl to the sample, mix slowly and methanolyze the sample on a heating block at 80°C for 18-20 hr.

3. Following methanolysis, cool the sample to room temperature and evaporate Methanolic-HCl by dry nitrogen flush till approximately half the sample is evaporated. Place the remaining sample in an ice bath for 2-3 min.

4. Add 1 mL of half-saturated NaCl solution and vortex to mix the solution and then place it on ice bath of 2-3 min.

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5. Extract the methyl esters of the fatty acids by adding 1 mL of Chloroform. Vortex thoroughly and place the solution on ice bath for 2-3 min.

6. Next, centrifuge the sample at 2000 rpm for 2 min at 10 °C and then transfer the bottom chloroform layer to a clean glass screw-top tube and store on ice.

7. Repeat the chloroform extracted a further two times using 500 µL of chloroform, collecting the chloroform layer from each extraction into the same glass screw-top tube.

8. Remove trace amounts of salts from the pooled chloroform extract with the addition of 1 mL of Milli-Q water; vortex to mix.

9. Discard the top aqueous layer and then remove residual water by adding anhydrous sodium sulphate and vortex to mix.

10. Centrifuge the sample at 2000 rpm for 2min to precipitate the sodium sulfate crystals. Carefully remove the chloroform layer and filter it over a glass Pasteur pipette packed at the tip with glass wool. Prepare the glass wool by passing a small amount of hexane prior to filtering the sample.

11. Evaporate the chloroform using dry nitrogen flush carefully till the sample is just dried. Do not over-dry the sample.

12. Dissolve the dried sample in 100 µL of hexane and transfer to a GC-MS vial for analysis.

GC-MS SETTINGS:

Carrier Gas: Helium
Inlet Conditions:

- Injection mode: Splitless
- Temperature: 200 ^{o}C
- Pressure: 10.39 psi

GC-MS Transfer Line Temp: 280 ^{o}C
Column: Restek 5MS or Equivalent, 30 m x 0.25 mm x 0.25 µm
Column Flow: 1.1971 mL/min
Injection Volume: 1 µL
Run Time: 55 min

Conditions	^{o}C/min	^{o}C	Hold Time (min)	Run Time (min)
Initial		60	0	0
Ramp1	15	120	1	5
Ramp2	3	240	10	55

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