Protocol MSU_MSMC_011

Extraction of lipids and conversion to fatty acid methyl esters (FAMEs)
Purpose: Simultaneous extraction and transesterification of total fatty acids and detection by GC/MS.
Last modified: July 7th, 2019

Reagents:
Methanol - Sigma, Cat# 646377
Chloroform – VWR, Cat# MK4443 (06 for 1 L, 10 for 4 L bottle)
Butylated hydroxytoluene (BHT) – Sigma, Cat# W218405 (Also available in the MSMC)
Formic acid, 98% – Sigma, Cat#FX0440
Potassium chloride, KCl
Sodium chloride, NaCl
Pentadecanoic acid (C15:0), analytical standard – Sigma, Cat# 91446; an alternative (for milk, animal, or microbial samples) is [13C16]palmitic acid (MSMC standard IS_0015)
3M Methanolic hydrogen chloride - Sigma, Cat# 90964
n-Hexane, Spartan marketplace, Cat#15541750 (hexane can also be replaced with isooctane)

Materials:
2.0 mL Eppendorf Safe-Lock tubes
3 mm Chrome Stainless steel ball bearings (#Kit12064, VXB.com) or suitable substitute (may be available from Mass Spec Core
Borosilicate glass (pyrex) tubes: 13x100 mm or another tube of sufficient volume - Fisher Scientific, Cat# 14-933A
Phenolic caps with PTFE liners for glass tubes – Sigma, Cat# CLS999813
Glass autosampler vials, caps and limited volume inserts (available in the BMB store)

Equipment:
Pipettor (1000 μL and 200 μL) and pipet tips
Lab shaker or vortexer
Tabletop centrifuge
Speed vac or nitrogen gas evaporator
Water bath
Centrifuge with a rotor that will fit the pyrex glass tubes

Notes:
Recommendations for tissue amount to be extracted:

- Plant tissue (leaf): 10-50 mg of fresh tissue.
- Animal tissue: 10-50 mg
- Bacterial cells: 10-50 mg of fresh cells

Folch, Lee and Stanley extraction protocol (chloroform/methanol):

1. Prepare the extraction solvent of 2:1 chloroform/methanol containing 0.01% (w/v) BHT, 1% (v/v) formic acid and pentadecanoic acid (10 μg/mL) as an internal standard. Alternatively, [13C16] palmitic acid (also 10 μg/mL) may be added in place of pentadecanoic acid for samples where pentadecanoic acid may be expected as a constituent of the lipids.

2. Prepare a 0.88% (w/v) solution of potassium chloride in water (8.8 g/L).
3. Add one 3-mm ball bearing per Eppendorf tube and homogenize frozen sample using a lab shaker or vortexer. Spin down briefly to collect the sample at the bottom of the tube. Make sure tissue stays frozen during the homogenization process.
4. Add 1000 μL of extraction solvent (from Step 1) and cap the tube. This will add 10 μg of internal standard to the tube. Vortex thoroughly for 3 mins.
5. Add 333 μl of 0.88% potassium chloride in water and cap the tube. Vortex carefully and thoroughly.
6. Centrifuge for 10 min at 13500 x *g* in a tabletop centrifuge at ambient temperature. There should be two phases present after centrifugation. The upper layer is the water/methanol fraction while the lower will be the chloroform/methanol fraction. The lipids should be present in the lower layer.
7. Carefully remove a fixed volume (e.g. 500 μL) of the lower chloroform/methanol layer to a pyrex glass tube. Be careful in not removing any of the upper aqueous layer.
8. Dry the chloroform/methanol to dryness using either a speed-vac or nitrogen gas evaporator (both are available in the MSMC facility)
9. The dried lipid extracts can be stored sealed at -80 °C until the trans-esterification reaction is ready to be performed.

Trans-esterification protocol:

1. Prepare 1M methylation reagent. For example, mix 20 mL methanol with 10 mL of 3 M methanolic hydrogen chloride. You will need 1 mL for each sample to be transesterified.
2. Prepare 0.9% (w/v) sodium chloride in water (9 g/L).
3. Add 1000 μL of the methylation reagent (1 M methanolic HCl) to each dried lipid extract (from above). Ensure that the tube is capped and sealed.
4. Heat tubes in an 80°C water bath for 1 hour (note, this is above the boiling point of methanol). Check the tubes after 5 min to ensure that the caps are sealing well and that solvent is not evaporating during the trans-esterification reaction. It is often helpful to open the tube briefly to allow air to escape before re-sealing the cap.
5. Remove the tubes from the water bath. Let them cool to room temperature before opening.
6. Add 150 μL hexane (or isooctane) and 1000 μL 0.9% (w/v) sodium chloride in water. Vortex thoroughly for 1 min.
7. Spin 10 min at 1,500 x g in a centrifuge with an appropriate rotor for pyrex glass tubes.
8. Transfer ~100 μL hexane layer (top) to an autosampler vial with insert for GC/MS analysis.

Reference:

1. Christie, W.W. and Han, X. *Lipid Analysis - Isolation, Separation, Identification and Lipidomic Analysis* (4th edition), 446 pages (Oily Press, Bridgwater, U.K.) (2010).