Protocol MSU_MSMC_010

Extraction of short chain fatty acids (SCFAs) and conversion to pentafluorobenzyl derivatives.

Purpose: Simultaneous extraction and derivatization of SCFAs (C_1 to C_7) and detection by negative chemical ionization GC/MS.

Last modified: Nov 12th, 2019

Reagents:
Methanol - Sigma, Cat# 646377
n-Hexane, Spartan marketplace, Cat#15541750 (hexane can also be replaced with isooctane)
2,3,4,5,6-pentafluorobenzyl bromide (PFBBr), Sigma, Cat# 101052
N,N-diisopropylethylamine – Sigma, Cat# D125806
Sodium chloride – Sigma, Cat# S9888

Internal standards:
Dispense 7.6 μL of each 100 mM sodium formate ($^{13}C_1$), sodium acetate ($^{13}C_2$), sodium propionate ($^{13}C_3$) and sodium butyrate ($^{13}C_4$) stock into a final volume of 10 mL 1:1 methanol:water. 100 μL will dispense 0.35 μg of IS, which will be equivalent to 50 μM in final hexane extract.

External standards:
10 mM volatile free acid mix in water – Supelco, cat# CRM46975

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)
Glass autosampler vials, caps and limited volume inserts (available at the BMB store)

Equipment:
Pipettor (1000 μL and 200 μL) and pipet tips
Lab shaker or vortexer
Tabletop centrifuge
Oven

Notes:

- Recommendations for tissue amount to be extracted:
- Plant tissue (leaf): **100** mg of fresh tissue.
- Fecal sample: **10** mg
- Bacterial cells: **100** mg of fresh (pelleted) cells

- Acetone and acetonitrile often contain trace amounts of acetic acid that contributes to the background. We recommend avoiding use of these solvents when measuring short-chain fatty acids.
- The derivatization reagent, PFBBr also has been known to contain trace amount of formate, propionate and butyrate and high levels of acetate. In order to remove these background

contaminants, make a solution of 172 mM pentafluorobenzyl bromide (PFBBr) dissolved in hexane (26.2 μL in 1 mL hexane) and wash 3 times with equal volume of milliQ H_2O. Only retain the upper hexane layer. Note the volume of hexane transferred. Evaporate the hexane layer with N_2 without heating. There should be a small volume of PFBBr after hexane has been evaporated. Resuspend into an equal volume of methanol.

Protocol:

1. Prepare a 0.9% (w/v) solution of sodium chloride in water (9 g/L).
2. Add one 3-mm ball bearing per Eppendorf tube and homogenize a weighed amount (10-100 mg) of frozen sample using a lab shaker or vortexer. Centrifuge briefly (5000 x g; 1 minute) to collect the sample at the bottom of the tube. Make sure tissue stays frozen during the homogenization process.
3. Add 400 μL methanol, 100 μL internal standard solution, 100 μL 172 mM PFBBr in methanol, 10 μL diisopropylethylamine. This changes the pH to approximately pH 8. Vortex for 3 mins to mix and spin down briefly.
4. Incubate at 60 °C for 30 minutes. Let tubes cool down on ice. Spin down briefly before opening the tubes.
5. Add 150 μL hexane and 150 μL 0.9% (w/v) sodium chloride in water. Vortex again and spin down briefly.
6. Pipet the upper hexane layer into autosampler vials, cap the vials, and store in the freezer until the extracts are analyzed.