Protocol MSU_MSMC_010a

Quantification of pentafluorobenzyl (PFB) derivatives of short-chain fatty acids (SCFA) [C1 to C7] using gas chromatography-mass spectrometry (GC/MS protocol)

Equipment

Gas chromatography-mass spectrometry instrument (e.g. Agilent 7890 GC coupled to 7010) mass spectrometer with autosampler or Thermo Scientific Trace GC Ultra coupled to Thermo DSQII mass spectrometer)

Experimental design

For quantification, a calibration curve with concentrations covering the expected range of sample concentration should be analyzed.

Each batch to be analyzed should include the following:

Solvent blank (hexane or isooctane)
Internal standard blank or procedural blank
Samples containing derivatized SCFAs

Experimental parameters for GC/MS analysis

Column	Agilent VF5ms GC column, 30 m x 0.25 mm x 0.25 μm with 10 m guard column (Part number: CP9013)
Carrier gas	Helium
Carrier gas flow rate	1.0 mL/min (Constant flow)
Injector type	Split/splitless injector
Inlet parameters	Split injection, recommended starting at 1:10 or 1:20 split at 250°C
Column temperature gradient	40°C (1 min hold), increase at 20°C/min to 130°C, increase 40°C/min to 320°C, hold at 320°C for 4.75 minutes (total 15 minutes)
Ionization method	Negative chemical ionization (electron capture mode)
Ionization source temperature	150°C for both Agilent and Thermo instrument
Transfer line temperature	300°C
Data acquisition type	SIM
SIM ions	Formate *m/z* 45, [$^{13}C_1$]formate *m/z* 46, Acetate *m/z* 59, [$^{13}C_2$]acetate *m/z* 61, Propionate *m/z* 73, [$^{13}C_3$]propionate *m/z* 76, Butanoate *m/z* 87, [$^{13}C_4$]butanoate *m/z* 91, Pentanoate *m/z* 101, Hexanoate *m/z* 115, Heptanoate *m/z* 129 With 20 ms dwell time for each ***m/z*** value
Solvent delay (min)	4
Detector gain	1
Mass calibration procedure	Tune instrument with positive chemical ionization first, followed by negative chemical ionization before running a sequence