Short Chain Fatty Acid Profile
Service Code: SCFA

Summary: Cold extraction of short chain fatty acids, measured by EI- GCMS without derivatization. SCFA species are reported as uM, with CV's generally ~10%.

Container: Eppendorf Tube or equivalent
Normal Volume: Plasma (100 ul); Tissue (50-100 mgs); Cells (2E7), Feces (50 mg)
Minimal Volume: Plasma (50 uL); Tissue (30 mg); Cells (~5E6); Feces (40 mg)
Special Handling: If human or primate, note any known presence of infectious agents.
Sample Collection: Snap freeze by liquid nitrogen. For tissues, resect and snap-freeze as soon as practical in tared centrifuge tube. Provide both sample weight and tared vial weight on sample submission

Reference:

Table I: Analytes reported. Others on special request:

Analyte	Abbr.	PubCHEM	LOQ(uM)
Acetic acid	(C2:0)	176	
Propionic acid	(C3:0)	1032	
Butyric acid	(C4:0)	264	
Isobutyric acid	(C4:0i)	6590	
Valeric acid	(C5:0)	7991	
Isovaleric acid	(C5:0i)	10430	
Caproic acid (Hexanoic)	(C6:0)	8892	
Heptanoic acid	(C7:0)	8094	
Caprylic acid (Octanoic)	(C8:0)	379	
Nonanoic acid	(C9:0)	8158	

Table II: Internal standards and corresponding analytes

Internal Standards	Source	Cat#	Analytes quantified	mM
Butyric-d_7 acid	Sigma	488399	Butyric, Isobutyric, Valeric, Isovaleric Acids	4
Acetic-d_3 acid	Sigma	487856	Acetic, propionic Acids	4
Hexanoic-d_{11} acid	Sigma	448168	Caproic, Heptanoic, Caprylic, Nonanoic Acids	4

Materials

1. Agilent 6890 with 5973 MSD, autosampler

2. Vortexer
3. Refrigerated centrifuge, capable of 13,000g with eppendorf tube compatible rotor
4. ice bucket, ice
5. Balance
6. Prepared stock solutions of short chain fatty acid standards and isotope-labeled short chain fatty acid internal standards.
7. LCMS grade water, diethyl ether, hydrochloric acid

PROCEDURES:

Preparation of Standards

IS stock solution preparation: (a 4 mM solution of isotopically labeled hexanoic, butyric, acetic acid)

1. Add 1455 uL of water to a vial
2. Add 15 uL of D11 hexanoic acid (50 mM)
3. Add 15 uL of D7 butyric acid (400 mM)
4. Add 15 uL of D2 acetic acid (400 mM stock)
5. Vortex to mix. Final volume should be 1500 uL.

Standard stock preparation: a 15 mM solution (for most compounds)

1. Add 737.5 uL of water to a vial
2. Add 37.5 uL of 400mM acetic
3. Add 37.5 uL of 400mM propionic
4. Add 37.5 uL of 400mM n-butyric
5. Add 37.5 uL of 200mM n-valeric
6. Add 37.5 uL of 200 mM i-valeric
7. Add 37.5 uL of 50mM n-caproic
8. Add 37.5 uL of 15mM n-heptanoic

Vortex to mix (final volume should be 1000 uL)

Preparation of Extraction Solvent

Extraction solvent: a solution of 30 mM hydrochloric acid plus isotopically-labeled acetate (0.25mM), butyrate (0.25mM),and hexanoate (0.03mM) in water. Prepare a volume sufficient for 300 uL per sample to be extracted (plus some extra for safety margin)

For 50mM D11 hexanoic acid stock: 0.05*1x10-3L*127.22=6.36mg/0.93 (density) = 6.84ul

1. Transfer 9680 uL of water into a glass vial
2. Add 666.7 uL of 4mM IS stock
3. Add 320 uL of 1M HCl
4. Vortex to mix

Fecal and Chyme Sample Preparation

1. Remove all samples from -80 °C freezer. Thaw and store on ice throughout extraction procedure.
2. If not pre-weighed, weigh ~50 mg sample and add 600 uL of cold extraction solution (with internal standards) to labeled iced 1.5 mL eppendorf tube.

3. Sonicate each tube using a probe sonicator (power level 3, 20% duty cycle) for 15-20 seconds. Keep tube on ice while sonicating. Make sure the sample is completely homogenized.
4. Vortex all samples, centrifuge 15,000g for 10min. 4°C
5. Transfer 300 uL of supernatant to a 1.5ml iced CLEAR eppendorf tube.
6. Add 300 uL of cold diethyl ether.
7. Vortex vial for 10 seconds to emulsify, incubate 5 min.
8. Centrifuge 15000g, 1 min at 4°C. After layers have separated, transfer upper layer to autosampler vial with insert and immediately cap
9. For reference standard mixtures prepared above: prepare in eppendorf tube, perform steps 5-10 exactly as with samples in order to prepare calibration samples for injection on GC-MS.
10. Promptly analyze all samples by GC-MS

Plasma/Serum Sample Preparation

1) Prepare two set of stds if >20 samples
2) Remove all samples from -80 C freezer. Thaw all samples and store on ice throughout extraction procedure.
3) Transfer 100ul of plasma to glass tube (15x75mm).
4) Make 2 pooled sample from samples
5) Add 200ul of extraction solvent to each tube.
6) Vortex all samples for 10s.
7) Add 300uL of diethyl ether. Process standard samples from this step.
8) Vortex vial for 10 seconds to emulsify, wait 5min at 4C, vortex again for 10s
9) Centrifuge all tubes 1 minutes to help separate layers.
10) After layers have separated, transfer upper layer to autosampler vial with insert, cap vials immediately (glued caps).
11) Promptly analyze all samples by GC-MS at **SIM mode**
12) Put all samples and Stds back to -80 freezer

Cell Sample Preparation

1. Put samples in a box with dry ice. Put extraction solvent on dry ice.
2. Working one plate at a time, remove plate from the cooler and place on a surface of regular ice.
3. Clean cell scraper with MeOH and kimwipe.
4. Add 540ul of extraction solvent to the plate.
5. Scrape cells with cell scraper, then scrape solvent to one corner of the plate.
6. Transfer debris to a labeled 2mL eppendorf vial. Put vial on dry ice, incubate 5 minutes.
7. Repeat procedure with all additional eppendorf vials.
8. Centrifuge all vials at 15,000g for 10 minutes
9. Transfer 100ul of supernatant to an autosampler vial (with insert)

Preparation of GCMS Standard Curve

Authentic SCFA standards to be quantitated (non-isotopically labeled) at the following concentrations: 0uM, 100uM, 300uM, 1000uM, 3000uM (for acetate, propionate, butyrate); 0uM, 50uM, 150uM, 500uM, 1500uM (for n-valeric+i-valeric); 0uM, 25uM, 75uM, 250uM, 750uM (for i-butyric); 0uM, 12.5uM, 37.5uM, 125uM, 375uM (for n-caproic); 0uM, 3.75uM, 11.25uM, 37.5uM, 112.5uM.

Standard	**Volume of STD mix (15mM)**	**Volume of IS mix (4mM)**	**Volume of water**	**Volume of HCl (1M)**
0	0 uL	18.75 uL	272 uL	9 uL
0.1	2	18.75	270	9
0.3	6	18.75	266	9
1	20	18.75	252	9
3	60	18.75	212	9
10	200	18.75	72	9

GC-MS procedure

1. GC column: ZB-WAX*plus*, 30m x0.25mmx0.25um (Phenomenex Cat#7HG-G013-11)
2. Carrier gas: He, flow rate: 1.1ml/min constant
3. Autosampler: room temperature
4. Injector: 250°C, 1 uL injection with 1:10 split ratio
5. Agilent 6890 with 5973 MSD: EI, 240 °C, Auxiliary: 310°C