Technical Bulletin

^{13}C-Short Chain Fatty Acids Stool Mixture

Catalog Number SBR00035

Product Description

Short chain fatty acids (SCFAs) are saturated fatty acids with less than 6 carbons. SCFAs are produced in the gut by microbial fermentation of dietary fibers.[1] The most important and researched SCFAs are Acetate (Ac), Propionate (Pr) and Butyrate (Bu).[2] SCFAs are predominantly found in the colon and cross the intestinal epithelium into the blood stream. SCFAs have been found to be beneficial and effective in prevention of obesity, non-alcoholic fatty liver disease, and insulin resistance.[3,4]

The concentrations of SCFAs in the stool are varied from subject to subject in relation to the experiment parameters. The average concentration for Acetate, Propionate and Butyrate are 30, 10, and 10 µM, respectively.

The ^{13}C-SCFA Mixture can serve in numerous applications:

- Used as a spiking mixture to track sample preparation errors.
- Used as a QC system control, to exclude run to run variations and to normalize the runs.
- Generation of a calibration curve to quantify SCFAs in stool samples.

Components

The ^{13}C-Short Chain Fatty Acids Stool Mixture contains the following components (Table 1):

Table 1. ^{13}C-SCFAs components concentrations.

Name	Linear formula	Conc. [mM]
^{13}C-Sodium Acetate	$^{13}CH_3{}^{13}COONa$	3
^{13}C-Sodium Propionate	$^{13}CH_3{}^{13}CH_2{}^{13}COONa$	1
^{13}C-Sodium Butyrate	$^{13}CH_3(^{13}CH_2)_2{}^{13}COONa$	1

Reagents and Equipment Required but Not Provided

- Titan™ C18 UHPLC Column, 1.9 micron HPLC column (Catalog Number 577124-U)
- N-(3-Dimethylaminopropyl)-N′-ethylcarbodiimide HCl (EDC) (Catalog Number 03450 or equivalent)
- 3-Nitrophenylhydrazine HCl (3-NPH) (Catalog Number N21804 or equivalent)
- Pyridine (Catalog Number 270407 or equivalent)
- Acetonitrile (ACN) (Catalog Number 1.00029 or equivalent)
- Methanol (Catalog Number 1.06035 or equivalent)
- Sodium sulfate (Catalog Number 239313 or equivalent)
- 7-mL screw top graduated vials (Catalog Number 27507)
- Threaded vial contactor (Catalog Number Z502855)
- Connecting Adapter for sample vials (Catalog Number Z510807)

Precautions and Disclaimer

For Research Use Only. Not for use in diagnostic procedures. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The reagent is shipped at an ambient temperature. For long-term storage, store at -20 °C.

Procedure

Experimental procedures provided here are an example of how to use the products. The results may vary under different experimental parameters.

Instruments and methods

Detection by LC-MS

When analyzing by LC-MS, derivatization should take place. 3-NPH and EDC can be used to derivatize SCFAs in an aqueous acetonitrile mixture.

LC conditions:

Column: Titan™ C18 UHPLC 1.9 µm
Column oven temperature: 45 ºC
Flow rate: 0.5 ml/min
Detection at 280 nm
Injection volume 2 µL

Table 2. LC gradient table.

Time [min]	Acetonitrile [%]	Water + 0.1% Formic acid [%]
0	30	70
5	40	60
6	80	20
7	80	20
8	30	70
10	30	70

Mass Spectrometry Conditions:

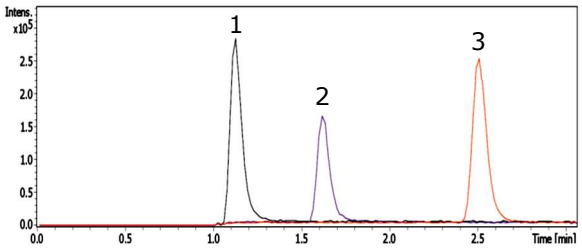
Instrument: Bruker™ Q-Tof Impact II
Source Type: ESI
Ion Polarity: Positive
Capillary: 4500 V
Nebulizer: 2 Bar
Dry gas temperature: 185 ºC
Dry gas: 6 L/min

The mobile phase is introduced to the MS source from 1-4.2 min, before and after this range the Mobile phase is discarded to the waste.

Example Procedure 1 for ^{13}C-SCFA Stool Mixture Derivatization

1. Prepare the following solutions:
   a. 200 mM solution of 3-NPH·HCl in ACN:water (1:1) (57 mg in 1.5 ml).
   b. 120 mM solution of EDC·HCl in ACN:water (1:1) (34.5 mg in 1.5 ml). Add 90 µL of Pyridine (6% final concentration) to this solution.
2. Mix 100 µL ^{13}C-SCFA Stool Mixture with 900 µL ACN:water (1:1) in a small screwcap glass vial.
3. Add 72 µL of 200 mM 3-NPH solution from Step 1a to the ^{13}C-SCFA Mixture from Step 2.
4. Add 72 µL of 120 mM EDC solution from Step 1b to the ^{13}C-SCFA Mixture from Step 3.
5. Heat mixture to 40 ºC for 40-50 minutes. Analyze by LC-MS following procedure as detailed above.

Figure 1. XIC chromatogram of derivatized stool MIX: 1. ^{13}C-Acetic-3-NPH 30 µM; 2. ^{13}C-Propionic-3-NPH 10 µM; 3. ^{13}C-Butyric-3-NPH 10 µM. Analysis was performed using Example Procedure 1.



Example Procedure 2 for ^{13}C-SCFA Stool Mixture: Spike in mouse stool

1. In a centrifuge tube, hydrate 10-20 mg of the stool sample in 0.3 mL water for 10 minutes.
2. Add 3 µL ^{13}C-SCFA Stool Mixture as a spike to the sample.
3. Add 2.7 mL acetonitrile to the stool-SCFA mixture.
4. Add 72 µL of 3-NPH solution to the stool-SCFA mixture.
5. Add 72 µL of EDC solution to the stool-SCFA mixture.
6. Heat to 40 °C for 40-50 minutes.
7. Centrifuge for 5 minutes at 4000 RPM.
8. Transfer the supernatant to a glass vial (such as Catalog Number 27507) and evaporate the solvents using a vial contactor (such as Catalog Numbers Z510807 and Z502855).
9. Dissolve the residue in 2 mL methyl tert-butyl ether (MTBE) and dry with sodium sulfate.
10. Transfer the solution to a clean glass vial and use another 1 mL of MTBE to wash and add to the transferred solution.
11. Evaporate the MTBE as in Step 8. Dissolve the residue in 0.3 mL ACN:water (1:1) for LC-MS analysis.

Figure 2. XIC Chromatogram of spiked mouse stool: 1. Acetic-3-NPH; 2. ^{13}C-Acetic-3-NPH 30 µM; 3. Propionic-3-NPH; 4. ^{13}C-Propionic-3-NPH 10 µM; 5. Butyric-3-NPH; 6. ^{13}C-Butyric-3-NPH 10 µM.

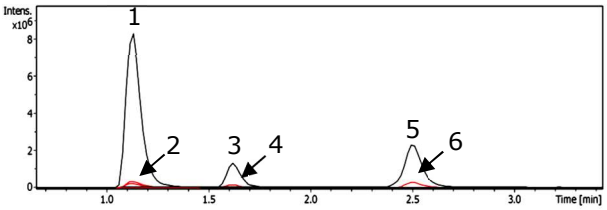
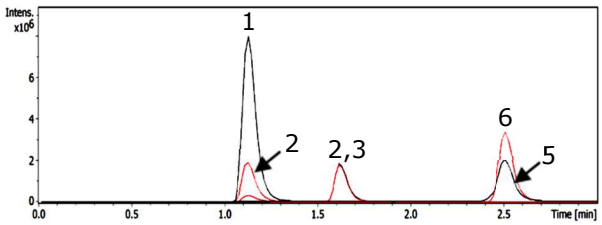


Figure 3. XIC Chromatogram of spiked mouse stool: 1. Acetic-3-NPH; 2. ^{13}C-Acetic-3-NPH 300 µM; 3. Propionic-3-NPH; 4. ^{13}C-Propionic-3-NPH 100 µM; 5. Butyric-3-NPH; 6. ^{13}C-Butyric-3-NPH 100 µM.



Example Procedure 2 for 13C-SCFA Stool Mixture: SCFAs in Plasma and MRM transitions

See the technical bulletin for related product ^{13}C-Short Chain Fatty Acids Plasma Mixture (Catalog Number SBR00034) for further information regarding spiking SCFA mixtures to plasma samples and MRM transitions.

Example Procedure 3 for ^{13}C-SCFA Stool Mixture: Detection by GC-MS

For GC-MS analysis, it is recommended to use Nukol™ Capillary GC Column (Catalog Number 24107).

Size × I.D.: 30 m × 0.25 mm
df 0.25 µm

GC conditions:

Oven: 185 ºC
Carrier gas: helium 20 cm/sec
Injection: 1 µL

See the technical bulletin for related product Short Chain Fatty Acid Kit (Catalog Number SBR00030) for further information.

References

1. Koh, A., et al., From dietary fiber to host physiology: short-chain fatty acids as key bacterial metabolites. *Cell*, **165(6)**, 1332-1345 (2016).
2. Cummings, J., et al., (1987). Short chain fatty acids in human large intestine, portal, hepatic and venous blood. *Gut*, **28(10)**, 1221-1227 (1987).
3. Canfora, E.E., et al., Gut microbial metabolites in obesity, NAFLD and T2DM. *Nature Reviews Endocrinology*, 1 (2019).
4. Henagan, T.M., Sodium butyrate epigenetically modulates high-fat diet-induced skeletal muscle mitochondrial adaptation, obesity and insulin resistance through nucleosome positioning. *British Journal of Pharmacology*, **172(11)**, 2782-2798 (2015).

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