Technical Bulletin

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

Catalog Number SBR00034

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 plasma are varied from subject to subject in relation to the experiment parameters. The average concentration for Acetate, Propionate and Butyrate are 125, 35, and 17 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 plasma samples.

Components

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

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

Name	Linear formula	Conc. [mM]
^{13}C-Sodium Acetate	$^{13}CH_3{}^{13}COONa$	12.5
^{13}C-Sodium Propionate	$^{13}CH_3{}^{13}CH_2{}^{13}COONa$	3.5
^{13}C-Sodium Butyrate	$^{13}CH_3(^{13}CH_2)_2{}^{13}COONa$	1.7

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	15	85
5	40	60
6	80	20
7	80	20
8	15	85
10	15	85

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 minutes, before and after the time range the mobile phase is discarded to the waste.

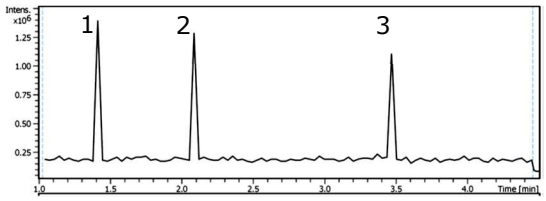
Example Procedure 1 for ^{13}C-SCFA Plasma Mixture Derivatization

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 Plasma 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.
6. Analyze by LC-MS following procedure as detailed above using the MRM transitions in Table 3.

Table 3. MRM transition of 3-NPH derivatized SCFAs

Compound	RT [min]	Exact mass + H^+	Qualifier 1	Qualifier 2
Acetate	1.38	196.072	154.061	137.035
^{13}C-Acetate	1.35	198.079	154.061	137.035
Propionate	2.07	210.087	154.061	137.035
^{13}C-Propionate	2.10	213.098	154.061	137.035
Butyrate	3.34	224.103	154.061	137.035
^{13}C-Butyrate	3.51	228.117	154.061	137.035

Figure 1. TIC chromatogram of the ^{13}C-SCFA Plasma Mixture after 3-NPH derivatization. Peaks: 1. Acetate; 2. Propionate; 3. Butyrate. Analysis was performed using Example Procedure 1.



Example Procedure 2 for ^{13}C-SCFA Plasma Mixture: Generation of a Calibration Curve

The ^{13}C-SCFA Plasma Mixture can be used to form a calibration curve.

1. Derivatize SCFA as described in Example Procedure 1 above.
2. Prepare several dilutions for the calibration curve. In this example the derivatized mixture is diluted by factors of 10, 20, 100, 200, and 1000 (Table 4).
3. Analyze the samples according the LC-MS method. (Figures 2-4).

Table 4. Concentrations table for calibration curve.

Dilution Factor	13C-Acetate [µM]	13C-Propionate [µM]	13C-Butyrate [µM]
1000	1.09	0.31	0.15
200	5.46	1.53	0.74
100	10.92	3.06	1.49
20	54.63	15.30	7.43
10	109.26	30.59	14.86

Figure 2. Calibration curve for Acetic-3-NPH.

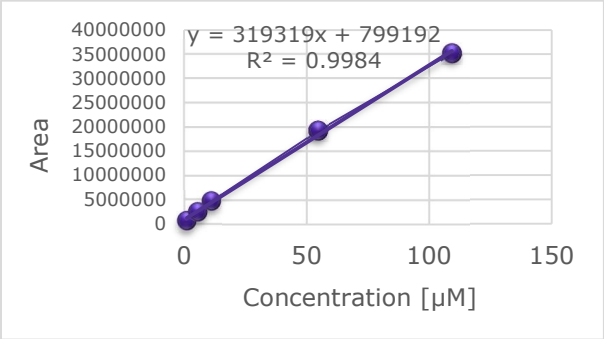


Figure 3. Calibration curve for Propionic-3-NPH.

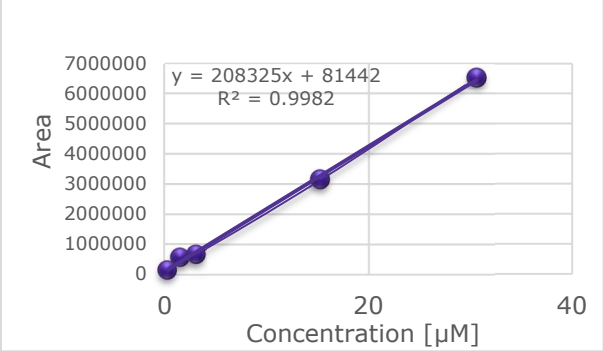
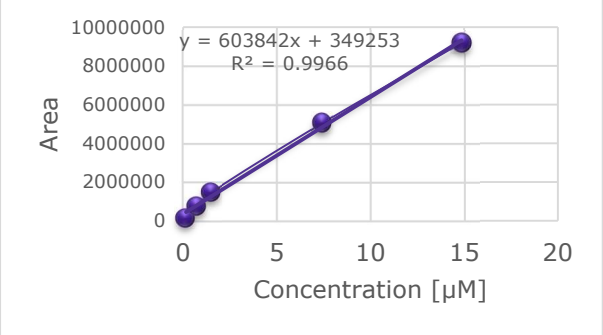


Figure 4. Calibration curve for Butyric-3-NPH.



Example Procedure 3 for ^{13}C-SCFA Plasma Mixture: Spike in plasma

1. To 0.3 mL Plasma sample in a centrifuge tube, add 3 µL ^{13}C-SCFA Plasma Mixture.
2. Add 2.7 mL acetonitrile. Sonicate the mixture for 5 minutes at 40 °C to precipitate the protein.
3. Add 72 µL of 3-NPH solution to the plasma-SCFA mixture.
4. Add 72 µL of EDC solution to the plasma-SCFA mixture.
5. Heat to 40 °C for 40-50 minutes.
6. Centrifuge for 5 minutes at 4000 RPM.
7. 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).
8. Dissolve the residue in 2 mL methyl tert-butyl ether (MTBE) and dry with sodium sulfate.
9. Transfer the solution to a clean glass vial and use another 1 mL of MTBE to wash and add to the transferred solution.
10. Evaporate the MTBE as in Step 7. Dissolve the residue in 0.3 mL methanol for LC-MS analysis.

Figure 5. TIC of the spiked plasma with the integration to the Acetate 196.072.

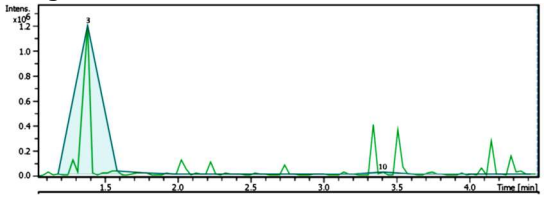
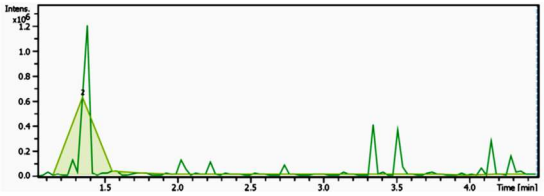


Figure 6. TIC of the spiked plasma with the integration to the ^{13}C Acetate 198.079.



Example Procedure 3 for ^{13}C-SCFA Plasma 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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