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Caltech-SCFA-methods fecal

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This protocol details the Caltech-SCFA-methods for measurement of short-chain fatty acids in mouse fecal samples.

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Sample preparation

1 Extract mouse fecal samples and derivatize as described previously.

(J.C. Chan, DH.Y. Kioh, G.C. Yap, B.W. Lee, E.C. Chan A Novel LCMSMS Method for Quantitative Measurement of Short-Chain Fatty Acids in Human Stool Derivatized With ^{12}C- And ^{13}C-labelled Aniline.J. Pharm. Biomed Anal., 138 (2017), pp. 43-53).

2  18m

Briefly add, ice-cold extraction solvent (1:1 v/v ACN/water) to fecal sample at a ratio of **2 µL** : **1 mg** sample and internal standard mix to a final concentration of **100 micromolar (µM)** and subject to vortex mixing for **00:03:00** at **Room temperature** and sonicate for **00:15:00** .

3  15m

Centrifuge the suspension at **18000 x g** for **00:15:00** at **4 °C** .

4  2h

Derivatize an aliquot of **100 µL** subsequently using a final concentration of **10 millimolar (mM)** aniline and **5 millimolar (mM)** EDC for **02:00:00** at **4 °C** .

5 Quench derivatization reaction using a final concentration of **18 millimolar (mM)** succinic acid and **4.6 micromolar (µM)** 2-mercaptoethanol for **02:00:00** at **4 °C** . 2h

6 Store all samples at **4 °C** until analysis on the same day.

7 Mix calibrators of acetic acid, propanoic acid, butyric acid and isobutyric acid (10 nM - 10×10^3 nM) together with single- and double- blanks, spiked with internal standard mix (Acetic acid-d3, propanoic acid-d2, butyric acid-d2) to a final concentration of **100 micromolar (µM)** , prepare and subject to the same sample preparation procedure as fecal samples.

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Liquid Chromatography Mass Spectrometry (LC-MS): 16m

8 Analyze derivatized samples using an ultra-high performance liquid chromatography (UHPLC) system 1290 connect to a quadrupole time of flight (Q-TOF 6545) mass spectrometer from Agilent Technologies (Santa Clara, CA, USA) equipped with an orthogonal DUAL AJS-ESI interface.

9 Subject the samples to reverse phase C18 separation (Phenomenex Scherzo SS-C18) 100 x 2 mm and collect data in positive ion mode.

10 Acquire the data from 50 to 750 m z^{-1} at 2 spectra s^{-1}. Set Electrospray ionization (ESI) source conditions as follows:

A	B
Gas temperature	325 °C
Drying gas	9 L min-1
Nebulizer	35 psi
Fragmentor	125 V
Sheath gas temperature	350 °C
Sheath gas flow	8 L min-1
Nozzle voltage	1000V

11 

Use, a two-solvent gradient running at **0.3 mL** min^{-1} (Mobile Phase: A: 100:0.1 Water: Formic Acid, B: 100:0.1 Isopropanol: Formic Acid) for reverse phase C18 chromatographic separation.

12 Equilibrate the column at 15% B for **00:01:00** and introduce a sample. 1m

13  15m

Increase the solvent ratio from 15% B to 100% B over **00:13:00** and reduce back to 15% B over **00:02:00** . Injection volume is **5 µL** and column temperature of **45 °C** .

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14 Acquire the LC-MS/MS data using Agilent Mass Hunter Workstation (.d files) and process in quantitative analysis software (Agilent Technologies) for quantitative analysis of samples.

15 Construct the linear calibration plots for acetic acid, propanoic acid, butyric acid and isobutyric acid using peak area ratios of each analyte to the IS versus the concentrations of calibrators (x) with 1/x weighting, and obtain the least squares linear regression equations as the calibration equations for individual analytes.

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