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Standard Operating Procedure-Sample Preparation for GC-MS

1. Purpose

To prepare and analyze samples for short chain fatty acid GC-MS analysis.

2. Scope

This SOP applies to all GC-MS samples submitted for short chain fatty acid (SCFA) analysis. Samples may come from academic laboratories or outside companies.

3. Prerequisites

Agreement between the client and our lab.

4. Responsibilities


Dr. Alan Maschek is the primary researcher responsible for this SOP and the procedures involved herein. Dr. James Cox, Tyler Van Ry and Leon Catrow in this SOP.

5. Procedures

Sample extraction from biological sources

- a. Samples are extracted from their source by the addition of 400 μL dd- H_2O , 10 μL of 5-sulfosalicylic acid (1 mg/ μL) and 2 μL of internal standard (1 M pivalic acid). The samples are vortexed and/or sonicated for 1 min, rested on ice for 1 hour, and briefly vortexed every 15 min. The samples are then centrifuged at 2,000 g for 10 min. The supernatant is transferred into glass vials with PTFE caps containing 10 μL of conc. HCl following by the addition of 3 mL of ether. After vortexing (30 sec), the samples are centrifuged at 1,200 g for 10 min. The upper (organic) phases are carefully transferred to new labeled glass vials with PTFE caps and then 50 μL of TBDMS is added to derivatize the samples. The samples are then vortexed and placed in a 60 $^\circ\text{C}$ sand bath for 30 min. The samples are then cooled to room temperature and reduced under a gentle stream of nitrogen down to approximately 200–300 μL (20-40 min) and transferred to GC-MS vial with insert for analysis.
- b. Concurrently a process blank sample is brought forward as well as a quality control sample (~5-50 μL per sample) was prepared by taking equal volumes from each sample.

Sample extraction from tissue

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- a. Same as above except for the use of bead mill tubes and homogenization in one 30-sec cycle.

Maintenance of GC-MS instrument

- a. Approximately once a week the instrument is re-tuned. Approximately once every two weeks the liner and seals are replaced.

GC-MS lipid analysis

- a. SCFAs are separated on a DB-1 column.

Data analysis

- a. Results from GC-MS experiments are collected using Chemstation and analyzed using the software packages Mass Hunter Qual B.05.00 and Mass Hunter Quant (Agilent Technologies, Inc.).

Waste disposal

- a. Chemical waste generated from sample extraction and LC-MS analysis are collected and pooled for collection from University of Utah Environmental Health and Safety.

6. Definitions

GC: Gas Chromatography

MS: Mass Spectrometry