Standard Operating Procedure - Extraction of Liver Tissue Samples for GC-MS

1. Purpose

To extract metabolites from liver tissue for general metabolomics analysis by GC-MS. This procedure covers processing the actual samples, preparation of process blanks (PB) samples to determine sources of possible contamination, and quality control (QC) samples.

2. Scope

This SOP applies to all liver tissue samples submitted for GC-MS analysis. Samples may come from academic laboratories or outside companies.

3. Prerequisites

Agreement between the client and our lab.

4. Responsibilities

Dr. James Cox is the primary researcher responsible for this SOP and the assays involved herein. Dr. Alan Maschek and Steven Bell is also covered in this SOP.

5. Procedure

Liver tissue sample preparation-Researcher

a) Sample Preparation. Liver tissue should be collected so the volume of the sample is estimated to be around 25 mg, snap frozen and kept at -80˚C.
b) Submit samples to Core with a hard copy of sample submission form. This will be placed into sample box for identification. The Researcher will be responsible for sending this Excel sheet as an email.

Liver tissue sample extraction-Core

c) Determine the quantity of 90% methanol (MeOH) solution needed for extraction. 18 parts of 90% HPLC grade methanol is added to each liver tissue sample. **Example:** If one has 12 samples of liver tissue then each sample will need 25X18 = 450 µL 90% MeOH; totally 12 X 450 µL 90% MeOH= 5,400 µL is needed. Include 2 extra samples for process blanks. For accurate measurement assume that you will need enough for 15 samples, 15 X 450 µL= 6,750 µL or 6.75 mL. Make this up in a 15 mL or 50 mL conical tube. ***Use 10 mL pipette.***

d) Determine quantity of d4-succinate internal standard to add from stock solution. Total amount of d4-succinate per sample is always 1 µg. The stock solution is made of 2 mg/mL of d4-succinate made up in nanopure water. Add to 90% methanol solution and chill to -20°C in freezer. ***Use positive displacement tip for this.*** **Example:** For methanol prepared in c) add 7.5 µL of stock solution of d4-succinate.

e) Label a set of bead tube (bioExpress, Cat. #: G-3290-1, Ceramic Bead Tube Kit, 1.4mm) according to the sample names. Chill the tubes in -80°C freezer. Label appropriate number of bead tubes as PB (Process Blank). For 12 samples make up 2, for 18 make up 3, for 24 make up 4, etc. These PB tubes will not receive tissue samples, but will be otherwise processed in the same way and at the same time as real samples.

f) While the liver tissue samples are still in frozen state, quickly transfer the samples to the corresponding labeled bead tube. Put the bead tube containing the tissue back to -80°C freezer.

g) Using **piston pipette** add the cold 90% methanol solution containing the internal standard into each tube including the PB. ***It is important to use the piston pipette for accurate addition. Make certain no bubbles are in the tip!***

h) Make sure the cap of the bead tube is securely screwed on. Put bead tubes onto OMNI Bead Ruptor 24. Use the setting #3 for homogenizing. This setting will homogenize samples at S=6.45 (MHz) for C=01 (1 cycle) for T=0:30 (30 seconds) with D=0:00 (0 waiting time between cycles). After homogenization place the tubes into -20°C freezer and incubate for 1 hour. ***Use timer!***

i) Chill Beckman centrifuge and rotor to 4°C during this time. Label a fresh set of 1.5 mL microcentrifuge tubes with the exact label as found on the sample tubes.

j) After incubation in step i) centrifuge at 20,000 x g for 5 minutes at 4°C.

k) Transfer supernatant from each sample tube into the labeled fresh microcentrifuge tubes.

l) Make up QC samples by removing 20% volume of each sample and adding to a single microcentrifuge tube. From this aliquot an amount equal to the final volume of the actual samples. **Example**: For the 12 liver tissue samples used in step c) remove 90 µL from the samples in j). This gives a final volume of each sample of 1080 µL. Mix the pooled QC by vortex then aliquot 360 µL into 3 tubes labeled QC. *THIS STEP IS CRITICAL-DO NOT SKIP!*

m) Dry samples in speed-vac using *–OH* setting overnight. It is critical that extracted samples are completely dry.

n) Store at room temperature in box containing several desiccant bags.

GC-MS sample preparation

o) Prepare GC-MS using operating SOP

p) Make up a 40 mg/mL solution of N-methoxy amine (MOX) in dry pyridine. The MOX is stored in room temperature desiccators. The dry pyridine is stored in the hood. Dry the needle and syringe prior to use.

Waste disposal

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

6. Definitions

GC: gas chromatography
MS: mass spectrometry
PB: process blank
QC: quality control