Dr. Lampe’s Adipose Tissue

Platform: GCTOF

Adipose Tissue: ~15mg

Tissue was cut on Dry Ice to approximately 15mg (15.35mg). Remaining Tissue was placed back into the dummy tube and stored at -80C in the original box

***Extraction Protocol:***

1. Degased Pre-Chilled Extraction Solvent (3:3:2 Acetonitrile/Isopropanol/ddH2O)
2. Kept Extraction Solvent on Ice
3. Homogenized Cell Pellets using Cryo GenoGrinder to a fine dust
   1. 3-4 balls used
   2. It should be noted that the tissue sample was not entirely homogenized. This is likely a consequence of its small size. Alternative extraction methods should be conceived if initial trial shows low abundance of metabolites
4. Added 1mL of ice-cold extraction solvent to homogenized cell pellet
5. Vortexed briefly for 5 seconds
6. Shook for 5 minutes at 4C using the Orbital Mixing Chilling/Heating Plate
7. Transfered Supernatant (all) to a new 1.5mL eppendorf tube and placed on ice
8. Vortexed for 10seconds
9. Shook for 5 minutes at 4C using the Orbital Mixing Chilling/Heating Plate
10. Centrifuge samples for 2 minutes at 14,000 rcf
11. Aliquoted two 450µL portions of the supernatant into new 1.5mL eppendorf tubes. One for analysis one for a backup sample. Store Backup in -20C.
12. Evaporate one 450µL aliquot of the sample in the Labconco Centrivap cold trap concentrator to complete dryness
13. Resuspend the dried aliquot with 450µl of 50/50 Acetonitrile/ddH2O (degassed) as a clean-up step to remove lipids
14. Centrifuge for 2 minutes at 14,000 rcf
15. Remove supernatant to a new 1.5mL eppendorf tube
16. Evaporate one 450µL aliquot of the sample in the Labconco Centrivap cold trap concentrator to complete dryness
17. Submit for derivatization according to SOP (see attached)
18. GCTOF parameter alterations:
    1. \*\*\*injection volume increased to 1µL rather than 0.5µL. This is due to low abundance of compounds. See Figure Below

