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UC Davis - Metabolomics: Sample preparation for Lipidomics

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Protocol status: Working
We use this protocol and it's working

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Abstract

Summary:

This SOP describes sample extraction and sample preparation for lipid profiling by liquid chromatography / quadrupole time of flight mass spectrometry (LC-QTOF) or nanoelectrospray ion trap-FTICR MS.

Materials

MATERIALS

- Centrifuge **Eppendorf Catalog #**5415 D
- Calibrated pipettes 1-200 ul and 100-1000ul **Catalog #**1-200 ul and 100-1000ul
- Eppendorf tubes 1.5 mL uncolored **Eppendorf Catalog #**022363204
- ThermoElectron Neslab RTE 740 cooling bath at – 20°C **Catalog #**RTE 740 cooling bath
- MiniV ortexer **VWR International (Avantor) Catalog #**58816-121
- Orbital Mixing Chilling/Heating Plate **Torrey Pines Scientific Instruments**
- Speed vacuum concentration system **Labconco Centrivap cold trap**
- Eppendorf tips for organic solvents such as acetonitrile methanol and MTBE
- Glass Amber Vials **National Scientific Catalog #**C4000-2W
- Glass Inserts **Supelco Catalog #**27400-U
- Blue Tops for Vials **Agilent Technologies Catalog #**5182-0717
- Crushed ice
- Nitrogen line with pipette tip
- Pure water
- MTBE: Sigma Chromasolv 99.8% for HPLC 100mL (smallest available) (34875-100mL) **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**34875-100ML
- Methanol: J.T. Baker LC/MS Grade (9830-03) **JT Baker Catalog #**9830-03
- CUDA (12-[[(cyclohexylamino)carbonyl]amino]- dodecanoic acid) **Cayman Chemical Company Catalog #**10007923

Note:

Sigma-Aldrich RRID:SCR_008988
Cayman Chemical RRID:SCR_008945

1 **Starting material:**
Plasma/serum: 30 µl sample volume or aliquot

2 **Sample Preparation:**
Switch on bath to pre-cool at –20°C (±2°C validity temperature range)

Extraction solvents

♦ Purge both MeOH and MTBE for 5 min with nitrogen.
♦ Store solvents in the –20°C freezer to pre-chill

Homogenization and extraction

♦ Thaw plasma on ice, and gently rotate or invert the blood samples for about 10s to obtain a homogenized sample.
♦ Take out 60 µL and add 220 µL cold MeOH. Add 5 µL of QC mix as internal standard (see SOP "QC mix for LC-MS lipid analysis").
♦ Vortex each sample for 10s, keeping the rest on ice
♦ Add 750 µL MTBE
♦ Vortex for 10s
♦ Shake for 6min at 4°C
♦ Add 187.5 µL distilled water
♦ Vortex for 20s
♦ Centrifuge for 2 min @ 14000 rcf
♦ Remove supernatant, splitting into two aliquots of 300 µL, keeping one at –20°C for backup
♦ Dry samples to complete dryness in the speed vacuum concentration system

Preparation for analysis

♦ Re-suspend dry samples in 70 µL MeOH containing CUDA (10 µM), degassed using the above method.
♦Transfer 30 µL to two separate amber glass vial with micro-insert. Cap vials with Agilent blue top.
♦ Use independent vials for positive and negative mode acquisitions.

3 **Quality assurance**

♦ For each sequence of sample extractions, perform one blank negative control extraction by applying the total procedure (i.e. all materials and plastic ware) without biological sample.

♦ Use one commercial plasma/serum pool sample per 10 authentic subject samples as control. If no suitable commercial blood sample is available, prepare a large pool sample during the thawing/mixing step by aliquoting 100 ul per 1 ml plasma sample, and aliquot such pool sample for 1 pool extract per 10 authentic subject samples.

♦ Prepare at least six NIST plasma extracts in the same manner as positive controls

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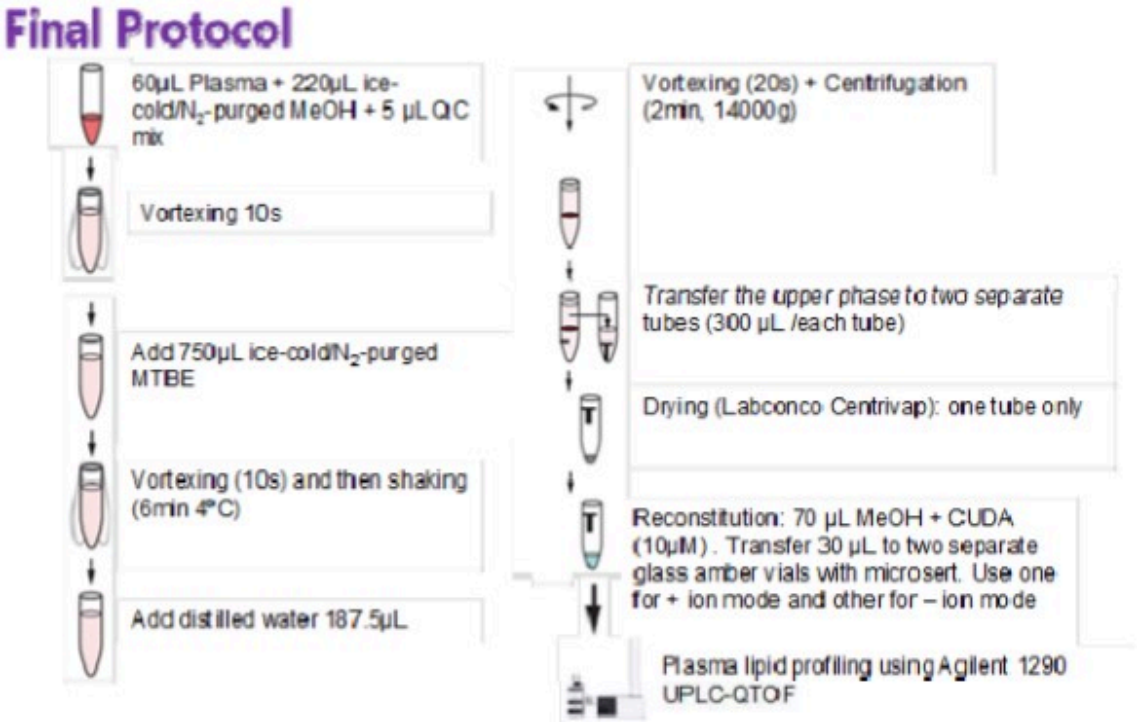

IMPORTANT: To prevent contamination disposable material is used. To prevent inhalation of toxic ether vapor, use fume hood during lipid extraction.

DISPOSAL OF WASTE: Collect all chemicals in appropriate bottles and follow the disposal rules. Collect residual plasma / serum samples in specifically designed red 'biohazard' waste bags.