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Working

UC Davis - Metabolomics: Sample preparation for Lipidomics

Oliver Fiehn[1]

[1]University of California, Davis

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Mouse Metabolic Phenotyping Centers
Tech. support email: info@mmpc.org

Lili Liang

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.

EXTERNAL LINK

https://mmpc.org/shared/document.aspx?id=122&docType=Protocol

MATERIALS

NAME	CATALOG #	VENDOR
Centrifuge	5415 D	Eppendorf Centrifuge
Calibrated pipettes 1-200 ul and 100-1000ul	1-200 ul and 100-1000ul	
Eppendorf tubes 1.5 mL uncolored	022363204	Eppendorf Centrifuge
ThermoElectron Neslab RTE 740 cooling bath at – 20°C	RTE 740 cooling bath	
MiniV ortexer	58816-121	VWR Scientific
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	C4000-2W	National Scientific
Glass Inserts	27400-U	Supelco
Blue Tops for Vials	5182-0717	Agilent Technologies
Crushed ice		
Nitrogen line with pipette tip		
Pure water		
MTBE: Sigma Chromasolv 99.8% for HPLC 100mL (smallest available) (34875-100mL)	34875-100ML	Sigma Aldrich
Methanol: J.T. Baker LC/MS Grade (9830-03)	9830-03	JT Baker
CUDA (12-[[(cyclohexylamino)carbonyl]amino]- dodecanoic acid)	10007923	Cayman Chemical Company

MATERIALS TEXT

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.

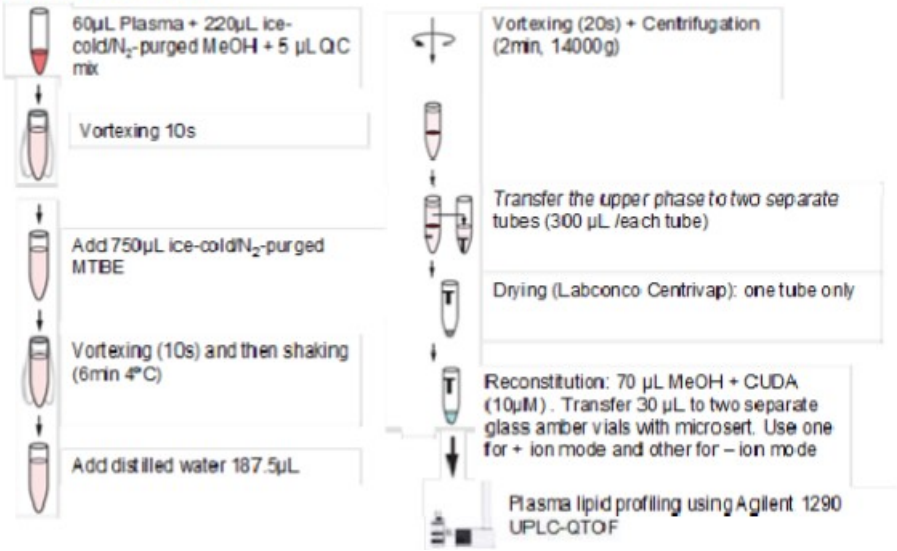
3 **Quality assurance**

- 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

4 Final Protocol



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.