Sample preparation of blood plasma or serum samples for lipidomic analysis

Version: 1
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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.

Reagents and Materials:

Reagent/Material	Vendor	Stock Number
Centrifuge	Eppendorf	5415 D
Calibrated pipettes 1-200 µl and 100-1000µl		
Eppendorf tubes 1.5 mL, uncolored		Cat. No. 022363204
ThermoElectron Neslab RTE 740 cooling bath at –20°C		
MiniVortexer	VWR	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	C4000-2W
Glass Inserts	Supelco	27400-U
Blue Tops for Vials	Agilent	5182-0717
Crushed ice		
Nitrogen line with pipette tip		
Pure water		
MTBE: Sigma, Chromasolv 99.8% for HPLC 100mL (smallest available) (34875-100mL)		
Methanol: J.T. Baker LC/MS Grade (9830-03)		
CUDA (12-[[(cyclohexylamino)carbonyl]amino]-dodecanoic acid) from Cayman Chemical Item Number 10007923		

Protocol:

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

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

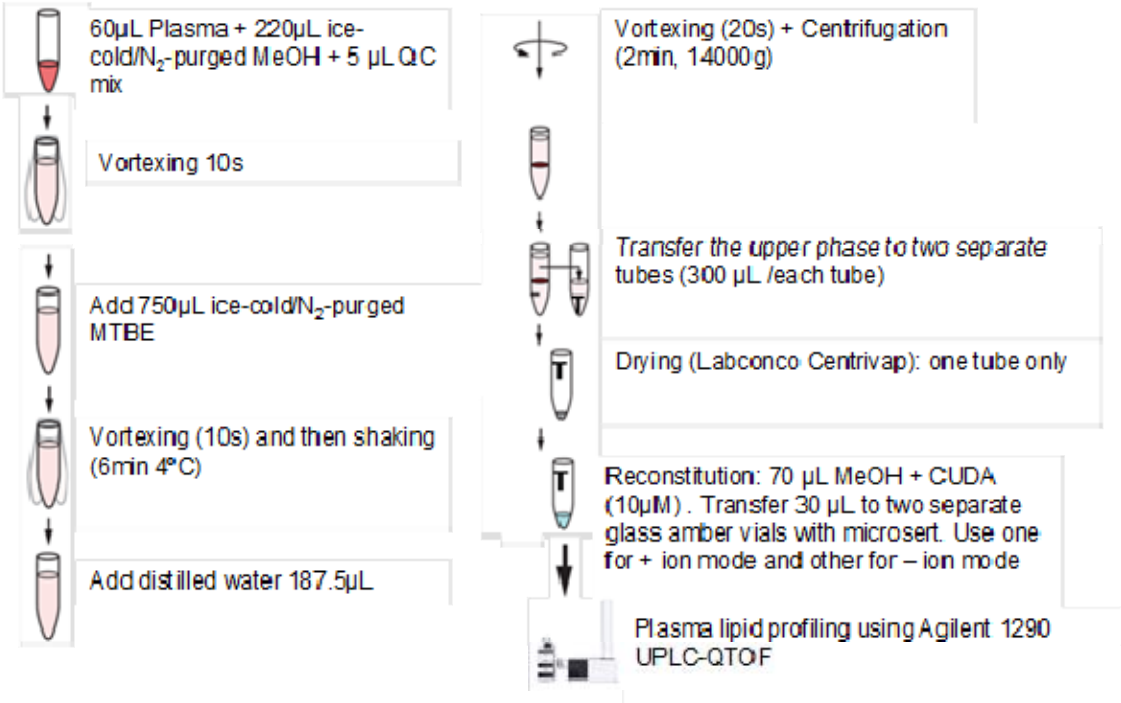
Preparation for analysis

- 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.

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

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.