Issued: 06/24/2013 Valid from: 06/24/2013	Validity area: UC Davis Genome Center, Metabolomics Core and Research Laboratories
Responsible: Bill Wikoff	Secondary: Carlos Leon, Brian DeFelice
This SOP supersedes: Sample preparation of blood plasma or serum samples for lipidomics analysis 12/17/2012	approved: Oliver Fiehn

Sample preparation of blood plasma/serum samples for lipidomic analysis

1. Purpose

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.

2. References

Matyash V, Liebisch G, Kurzchalia TV, Shevchenko A and Schwudke D (2008) Lipid extraction by methyl-*tert*-butyl ether for high-throughput lipidomics. J Lip Res 2008, 49: 1137-1146

3. Starting material

Plasma/serum: 20 µl sample volume or aliquot (see Aliquoting TEDDY Plasma SOP)

Control: Pooled Sterile Human Plasma in Sodium Citrate from Rockland Institute (aka "Citrate Plasma"). Citrate Plasma 1 mL aliquots are stored in the Revco -80ºC freezer at 2-6.

4. Equipment

- Centrifuge (Eppendorf 5415 D)
- Calibrated pipettes 20–200 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)

5. Chemicals:

- Crushed ice
- Nitrogen line with pipette tip
- Pure LC/MS Grade Water (Fisher Optima W6-4)
- 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
- Ethanol absolute (Sigma-Aldrich)

6. Sample Preparation

<u>Extraction solvents</u>

- Degas both "MeOH with QCmix" and MTBE with Cholesterol Ester 22:1 (see SOP "QC mix for LC-MS lipid analysis") by sonication
- Store solvents in the –20°C freezer to pre-chill

Homogenization and extraction

- Thaw each 20 µL plasma aliquot at room temperature (see Aliquoting TEDDY samples SOP). Once thawed (~10min) place liquid plasma samples on ice.

- Add 225 µL cold "MeOH with QC mix" (see SOP "QC mix for LC-MS lipid analysis"). Keep MeOH on ice during extraction
- Vortex each sample for 10s, keeping the rest on ice during all the extraction.
- Add 750 µL of cold MTBE with 22:1 CE, keep MTBE on ice during extraction
- Vortex for 10s
- Shake for 6min at 4°C in the orbital mixer.
- Add 188 µL room temperature LC/MS grade water.
- Vortex for 20 s
- Centrifuge for 2 min @ 14,000 rcf (12300 rpm)
- Remove supernatant, splitting into two aliquots of 350 µL, keeping one at –20°C for backup
- Dry samples to complete dryness in the speed vacuum concentration system

Preparation of resuspension solvent

- Weight 2mg of CUDA and dissolve in 2mL of ethanol. This is the CUDA stock solution (1mg/mL).
- Prepare 120 mL of MeOH:Toluene 90:10 (108 mL MeOH+12 mL Toluene). Degas by sonication for 5min
- Sonicate CUDA stock solution (1mg/mL) for 5 min, then dilute by adding 50uL (1mg/mL CUDA) in 950 µL MeOH:Toluene 90:10. This is CUDA 0.05mg/mL.
- Dilute 100 µL CUDA 0.05mg/mL in 9.9 mL MeOH:Toluene 90:10. This is CUDA 0.5 µg/mL.
- Dilute 10mL of CUDA 0.5 µg/mL in 90 mL MeOH;Toluene 90;10. This is the final resuspension solvent, MeOH:Toluene 90:10 with CUDA 50ng/mL.
- Store the solutions in the freezer at -20ºC until use.

Preparation for analysis

- Re-suspend dry samples in 108.6 µL MeOH:Toluene 90:10 with CUDA (50ng/mL), 24 samples at a time, degassed using the above method.
- After adding MeOH:Toluene to all 24 samples, vortex the rack on low speed as to not "whip" air into the liquid for 20 seconds.
- Then Sonicate at RT for 5 min, and centrifuge samples for 2 min at max speed.
- Transfer 50 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. Use the QTOF 6530 for positive ion analysis and the QTOF 6550 for negative analysis.

7. Problems

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

8. 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 TEDDY citrate plasma standard aliquot per 10 authentic subject samples as control.
- Prepare at least six NIST plasma extracts in the same manner as positive controls

9. 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.