Plasma: From Sample Collection to LC-MS Analysis of Moderately Polar Metabolites

Sample Collection

1. Draw blood into a tube coated with EDTA or lithium heparin *(BE CONSISTENT in using the same type of tubes for the entire batch of samples you wish to analyze, since the background from differnet tubes is different and will interfere with metabolite measurement)*
2. Mix the tube in the vacutainer (or invert the tube ten times by hand to ensure blood mixing) and place the tube immediately on wet ice for transport
3. Centrifuge at ~ 3000 rpm for 10 minutes at 4°C within 30 minutes (if possible)
4. Aliquot the plasma into storage tubes (*usually 200-500uL*) to avoid freeze-thaw cycles in case you wish to perform different types of analyses on same blood sample
5. Store the aliquots at −80°C before further analysis

Remark: *It is mandatory to keep track of sample ID numbers, physiological parameters, day & time of blood sampling*

Metabolite Extraction

6. Add 800µL ice cold MeOH to 200µL of plasma.The final methanol/plasma ratio should be 4:1, by volume. For example, an alternative would be to add 80µL ice cold MeOH to 20µL of plasma.
7. Vortex for 30s
8. Incubate samples for 1 hour at -20°C, then centrifuge at 16,000g at 4°C for 15 minutes
9. Transfer the supernatants to another 1.5mL microcentrifuge tube and evaporate the supernatant to dryness in a vacuum concentrator (LABCONCO CentriVap Benchtop) at 10°C *(If the time is limited, the dry extracts can be stored at –80°C at this stage, prior to further processing*)
10. Reconstitute the dry extracts in 200µL of MeOH/water (1:1, v/v)
11. Sonicate for 1 minute in an ice-cold bath and centrifuge for 15 minutes at 16,000g and 4°C to remove debris
12. Transfer supernatants to UPLC vials with inserts and inject them for LC/MS analysis. (*Storage at –80°C should be avoided at this stage because it might cause metabolite precipitation and therefore compromise the coverage)*

Liquid Chromatography Conditions

- LC System: UPLC
- Column & Guard: WATER ACQUITY UPLC BEH C18 Column, 130Å, 1.7µm, 2.1mm X 100mm BEH C18, 2.1x 100mm, 1.7µm & WATER ACQUITY UPLC BEH C18 VanGuard Pre-column, 130Å, 1.7µm, 2.1mm X 5mm
- Flow rate: 400uL/min
- Mobile phases:

Time (minutes)	A (H_20 + 0.1% FA)	B (ACN + 0.1% FA)
0	99	1
1	99	1
10	1	99
13	1	99
14	99	1
17	99	1

Mass Spectrometry Conditions

- Ionization modes: ESI (+) and ESI (-)
- Acquisition mode: MS and Auto MS/MS
- *m/z* range: 50-1000 Da
- Spectra rate: 500 milliseconds per MS spectra, 130 milliseconds per MS/MS spectra (10-50 eV)

Materials

- All solvents are HPLC grade
- UPLC Column Waters ACQUITY PREMIER BEH C18, 130Å, 1.7µm VanGuard FIT 2.1 x 100mm MVK
- Glass vials Thermo Fisher, part# 4000-S1W
- Vial caps Agilent, Part#5182-0717
- Glass inserts Agilent, Part# 5181-8872
- 1.5 mL tubes (natural) Eppendorf, part# 022363204

- Standards mixture Cayman quality control standards mixture