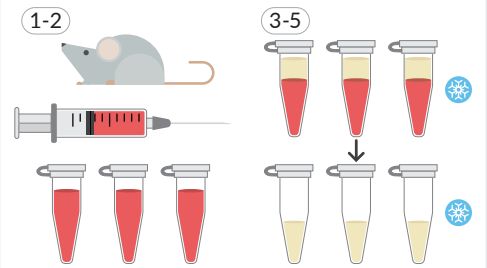
PLASMA

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

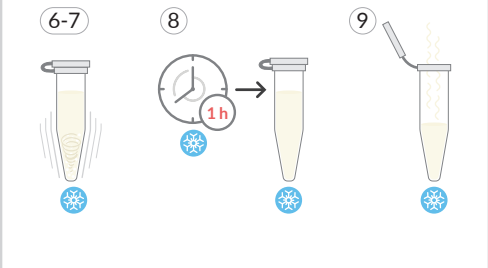
STEPS 1-5



SAMPLE COLLECTION

1. Draw blood
2. Thoroughly mix the samples
3. Centrifuge at ~3,000 rpm for 10 min at 4°C as soon as possible
4. Aliquot plasma to avoid freeze-thaw cycles
5. Store at -80°C

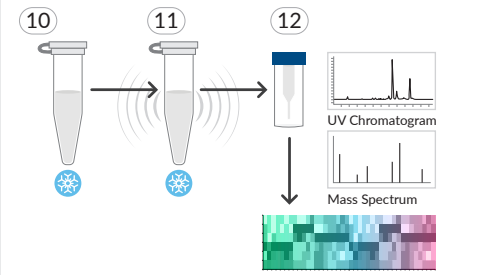
STEPS 6-9



EXTRACTION

6. Add cold MeOH to plasma (4:1)
7. Vortex for 30 s
8. Incubate for 1 h at 20°C; centrifuge for 15 min at 4°C
9. Transfer to a new tube and evaporate solvent

STEPS 10-12



ANALYSIS

10. Reconstitute extracts in MeOH:H_2O (1:1)
11. Sonicate for 1 min in ice-cold bath; centrifuge for 15 min at 4°C
12. Transfer supernatant to UPLC vials and inject for LC-MS analysis

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 different 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 ~ 3,000 rpm for 10 minutes at 4°C within 30 minutes (if possible)

④ Aliquot the plasma into storage tubes (usually 200-500 ul) 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 800 µl ice cold MeOH to 200 µl of plasma.The final methanol/plasma ratio should be 4:1, by volume. For example, an alter native would be to add 80 µl ice cold MeOH to 20 µl of plasma

⑦ Vortex for 30s

⑧ Incubate samples for 1 hour at -20°C, then centrifuge at 16,000 g at 4°C for 15 minutes

⑨ Transfer the supernatants to another 1.5 ml 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 200 µl of MeOH/water (1:1, v/v)

⑪ Sonicate for 1 minute in an ice-cold bath and centrifuge for 15 minutes at 16,000 g 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, 1.7 µm, 2.1 mm X 100 mm BEH C18, 2.1x 100 mm, 1.7 µm & WATER ACQUITY UPLC BEH C18 VanGuard Pre-column, 130Å, 1.7 µm, 2.1 mm X 5 mm

· Flow rate: 400 ul/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: 50-1,000 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, 1.7 µm VanGuard FIT 2.1 x 100 mm MVK

· Glass vials Thermo Fisher, Part No. 4000-S1W

· Vial caps Agilent, Part No. 5182-0717

· Glass inserts Agilent, Part No. 5181-8872

· 1.5 ml tubes (natural) Eppendorf, Part No. 022363204

· Standards mixture Cayman, Vascular Eicosanoid Urinary Metabolite LC-MS Mixture, Part No. 19668

REFERENCE

https://www.nature.com/articles/s41592-021-01197-1
https://www.nature.com/articles/s41592-020-0942-5

RESOURCES

METLIN Gen2 (https://massconsortium.com/)
XCMS Online (https://xcmsonline.scripps.edu/)
Cayman Chemical Lipidomic Standards (https://www.caymanchem.com/)

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