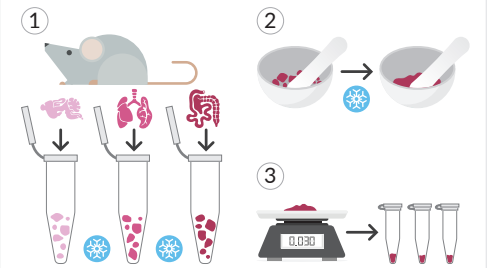
TISSUE

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

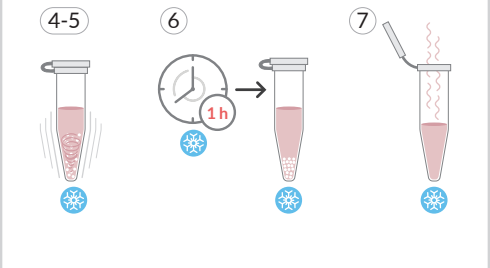
STEPS 1-3



SAMPLE COLLECTION

1. Harvest tissues and store at -80°C
2. Crush tissues with mortar and pestle under liquid N_2
3. Weigh and divide ~30 mg/tube

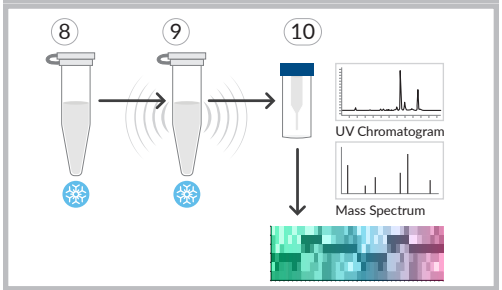
STEPS 4-7



EXTRACTION

4. Add cold MeOH:H_2O (4:1) and beads
5. Homogenize
6. Incubate for 1 h at 20°C; centrifuge for 15 min at 4°C
7. Transfer supernatant to a new tube and evaporate solvent

STEPS 8-10



ANALYSIS

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

SAMPLE COLLECTION

① Harvest and dissect the tissue as soon as possible following the sacrifice and store it at –80°C prior to further processing.

② Crush the tissue into a homogeneous powder with a mortar and pestle, under liquid nitrogen.

③ Weigh 30 +/- 5 mg of frozen tissue "aliquot(s)" in a 2 ml lysis tube (that fits tissue homogenizer). Note tissue weight.

METABOLITE EXTRACTION

④ Add 450 µl (150 µl/10 mg of tissue) of cold MeOH:H_2O (4:1, v/v) and glass or ceramic beads. Solvent volume needs to be adjusted accordingly to tissue weight.

⑤ Homogenize the solution in tissue homogenizer (Precellys®) 3 x 20s at 6,000 rpm (with 5s between each homogenization). A cold trap (Cryolys) of the homogenizer should be filled with dry ice to keep the sample cold.

⑥ Incubate samples for 1 hour at -20°C, then centrifuge at 16,000 g at 4°C for 15 minutes

⑦ Transfer the supernatants to another 1.5 ml Eppendorf 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*)

⑧ Reconstitute the dry extracts of MeOH/water (1:1, v/v) normalized to mass of tissue

⑨ Sonicate for 1 minute in an ice-cold bath and centrifuge for 15 minutes at 16,000 g and 4°C to remove debris

⑩ Transfer supernatants to UPLC vials with inserts and inject them for LC/MS analysis. (*The storage at –80°C should be avoided at this stage because it might cause the 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/)

Scripps Research METLIN XCMS Online Cayman CHEMICAL