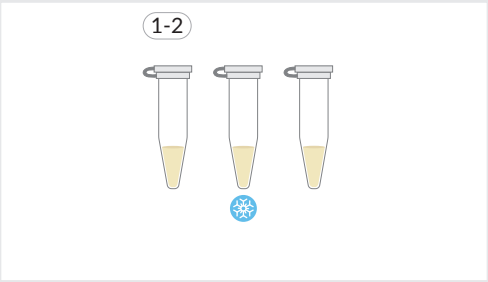
URINE

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

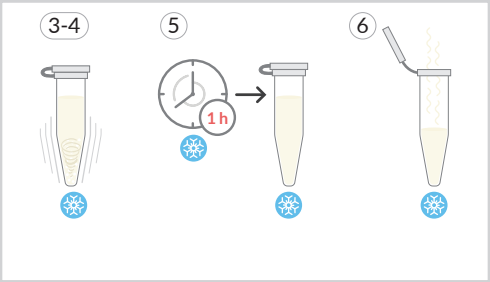
STEPS 1-2



SAMPLE COLLECTION

1. Obtain 24 h or time point urine samples; aliquot cold samples as soon as possible before storing at -80°C
2. Reserve 5 µl for creatinine quantification

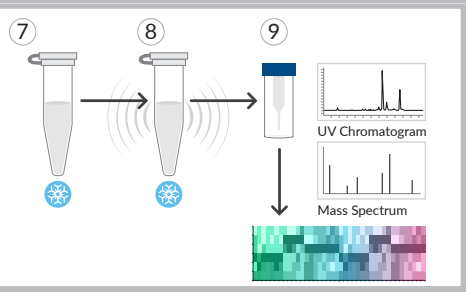
STEPS 3-6



EXTRACTION

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

STEPS 7-9



ANALYSIS

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

SAMPLE COLLECTION

① Collect fasting urine sample from one time point or 24 h urine (collection performed over 24 h to eliminate interindividual variations). Keep the sample at 4°C and aliquot it as soon as possible prior to storing at −80°C

METABOLITE EXTRACTION

② Save 5 µl urine for measuring creatinine concentrations using a creatine (urinary) colorimetric assay kit (*Specific gravity should be used in the case of kidney disease or similar diseases that impact creatinine levels*).

③ Add 800 µl ice cold MeOH to 200 µl of urine. The final methanol/urine ratio should be 4:1, by volume. For example, an alternative would be to add 80 µl ice cold MeOH to 20 µl of urine

④ Vortex for 30s

⑤ Incubate samples for 1 hour at -20°C, then centrifuge at 16,000 g at 4°C for 15 minutes

⑥ 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

⑦ Reconstitute the dry extracts in 200 µl of MeOH/water (1:1, v/v)

⑧ Sonicate for 1 minute in an ice-cold bath and centrifuge for 15 minutes at 16,000 g and 4°C to remove debris

⑨ Transfer supernatants to UPLC vials with inserts and inject them for LC/MS. (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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