Protocol MSU_MSMC_004, version 1.0
Two-phase extraction of metabolites from animal tissues

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Purpose

This SOP describes procedures for extraction of polar- and lipid-based metabolites from animal (including human) tissue for subsequent metabolomic analysis via GC/MS and LC/MS respectively. Before tissues are collected, all appropriate ethical oversight approvals and safety training (e.g. for handling of biohazardous materials) must be in place.

Supplies needed

Nitrile gloves
Eye protection (e.g. safety glasses/goggles)
3 mm Chrome Stainless steel ball bearings (#Kit12064, VXB.com) or suitable substitute
Pipetters (1000 µL) and pipet tips (positive displacement pipetters preferred)
2-mL polypropylene microcentrifuge tubes
1.6-mL polypropylene microcentrifuge tubes
Labels for microcentrifuge tubes and vials
Vial storage boxes
Methanol, HPLC grade
Chloroform, HPLC grade
Formic acid, 98%, Millipore-Sigma (Fluka) #94318
Butylated hydroxytoluene (BHT), solid

GENERAL REQUIREMENTS

- Gloves must be worn at all times when handling tissue specimens, removing lids from microcentrifuge tubes, centrifugation, pipetting, disposal of contaminated tubes, and cleanup of spills. All plasticware must be properly disposed of in biohazard containers in accordance with institutional requirements.
- All other institutional requirements and local health and safety procedures for working with tissue samples should be followed, including gloves, eye protection or working in a biosafety cabinet for subsequent processing.
- All equipment and samples (during storage and shipping) must be labeled as biohazard.
- Steps should be taken to prevent thawing of samples during transportation (i.e. keeping on dry-ice during transportation).

Equipment

1. Lab shaker or vortexer
2. Ultrasound water bath
3. Centrifuge with rotor for 2-mL polypropylene tubes
4. Vacuum centrifuge with rotor for 2-mL tubes (e.g. SpeedVac) and cold trap
5. Explosion-proof or flammable storage freezer (-20˚C)

Preparation of Reagents and Supplies

1. 3-mm stainless steel ball bearings should be cleaned by sonication in methanol followed by drying on clean paper towels.
2. Prepare a mixture of 2:1 methanol/chloroform containing 0.01% BHT and 1% formic acid (by volume). Store in explosion-proof or flammable-storage freezer (-20˚C).

Metabolite Extraction

1. If specimen is frozen, initially defrost in refrigerator (4˚C) or on ice prior to exposure to room temperature.
2. Transfer 100 mg of tissue to a labeled 2-mL polypropylene microcentrifuge tube (Safe-Lock)
3. Add internal standard cocktail to each tube (composition of internal standard to be defined as appropriate for metabolites of interest).
4. Add 600 µL of methanol/chloroform (2:1 v/v) + 1% formic acid and 0.01% BHT and one 3-mm ball bearing per tube; seal the tube
5. Homogenize using a lab shaker or vortexer for 3 minutes.
6. Sonicate in water bath for 15 minutes
7. Add 500 µL of Milli-Q water (> 18 MΩ)
8. Centrifuge at 13500 x *g* for 15 minutes at ambient temperature
9. Transfer the upper (aqueous layer) to an additional 1.5 mL polypropylene centrifuge tube
10. Repeat steps 7-10, pooling the two aqueous volumes into the same receiving tube (~ 1000 µL).
11. Remove 200 µL of the combined aqueous extracts and transfer to a labeled microcentrifuge tube for storage (at -80 ˚C). This aliquot may be used for analysis of volatile metabolites that would otherwise be lost during solvent evaporation.
12. Evaporate the remaining aqueous fraction (~800 µL) to dryness in the SpeedVac without application of heat.
13. The lower (chloroform) layer may be evaporated to dryness under a stream of nitrogen.
14. After solvent evaporation, seal the microcentrifuge tubes from both the aqueous and chloroform phases, and store them at -20˚C for up to 30 days (store at -80˚C) if storage for longer is required.