

Jun 26, 2025

Sampling and Extraction of Lyophilized Plant Tissue for LC-MS Analysis

DOI

<https://dx.doi.org/10.17504/protocols.io.kxygxqr44v8j/v1>

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Protocol Citation: Kateřina Kučerová, Tito Damiani, Fede B 2025. Sampling and Extraction of Lyophilized Plant Tissue for LC-MS Analysis. protocols.io <https://dx.doi.org/10.17504/protocols.io.kxygxqr44v8j/v1>

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Protocol status: Working

We use this protocol and it's working

Created: June 25, 2025

Last Modified: June 26, 2025

Protocol Integer ID: 221008

Keywords: Plant extraction, LC-MS, lyophilization, metabolomics, sample preparation, plant tissue, extraction of lyophilized plant tissue, extraction of plant tissue, ms metabolite analysis, sterile sampling of fresh plant material, lyophilized plant tissue, reproducibility across multiple plant tissue type, fresh plant material, ms vials for analysis, step solvent extraction, extraction, extract, multiple plant tissue type, polar metabolite, ms vial, ms analysis, including leaf

Abstract

This protocol outlines a standardized procedure for the collection, processing, and extraction of plant tissues for downstream LC-MS metabolite analysis. The method includes sterile sampling of fresh plant material in the field, immediate cryopreservation on dry ice, lyophilization, grinding to a fine powder using an IKA mill, and a two-step solvent extraction optimized for polar metabolites. The extracts are then clarified and transferred into LC-MS vials for analysis. This protocol ensures sample integrity and reproducibility across multiple plant tissue types, including leaf, stem, root, flower, and fruit.

Guidelines

- Ensure that all equipment (e.g., TissueLyser, SpeedVac, centrifuge, ultrasonic bath) is clean, calibrated, and functioning properly before starting the protocol.
- Use pre-labeled tubes and vials throughout the procedure to maintain sample traceability.
- Handle all samples consistently to minimize biological and technical variability, especially during sampling and extraction steps.

Materials

Reagents and Solvents

- Ethanol (EtOH), analytical grade – for extraction (prepare 75:25 EtOH:H₂O, v/v)
- Acetonitrile (CH₃CN), LC-MS grade – for resuspension (prepare 50:50 H₂O:CH₃CN, v/v)
- Deionized or Milli-Q water
- 96 % Ethanol – for surface sterilization

Plant Sampling Supplies

- 50 mL Falcon tubes (sterile)
- Sterile knife or scalpel
- Sterile scissors
- Fine mesh fabric (e.g., nylon or gauze)
- Rubber bands (for securing mesh)
- Disposable nitrile gloves
- Box with dry ice

Extraction and Processing Supplies

- 2.0 mL round-bottom Eppendorf tubes
- Stainless steel grinding beads (suitable for 2.0 mL tubes)
- Labels (for plant ID, extraction tube, and LC-MS vials)
- Pipette (100–1000 µL) and sterile filter tips
- LC-MS glass or polypropylene vials with caps (and inserts if applicable)

Instruments and Equipment

- Analytical balance (precision ± 0.1 mg)
- TissueLyser (rpm-based, compatible with 2.0 mL tubes)
- IKA mill (for powdering lyophilized material)
- Lyophilizer (freeze dryer with compatible flasks)
- Centrifuge (capable of 14 000 rpm, with tube rotor)
- Vortex mixer
- Ultrasonic bath
- SpeedVac concentrator
- Heat block or water bath (settable to 40 °C)

Safety warnings

- Ethanol (EtOH) and acetonitrile (CH₃CN) are flammable solvents—keep away from open flames or heat sources. Always wear appropriate personal protective equipment (PPE), including lab coat, safety goggles, and nitrile gloves.
- During TissueLyser and IKA mill operation, ensure the device is secured and the samples are balanced to prevent mechanical accidents.
- Handle dry ice with insulated gloves and avoid direct skin contact to prevent frostbite.
- Dispose of solvent waste according to your institution's chemical safety regulations.

Before start

- Sampling kit assembled (sterile instruments, gloves, ethanol, dry ice, labeled tubes).
- All solvents freshly prepared or verified.
- Instruments powered, clean, and pre-calibrated (balance, vortex, centrifuge, SpeedVac, etc.)
- Extraction workspace is clear, ventilated, and equipped with proper waste containers.

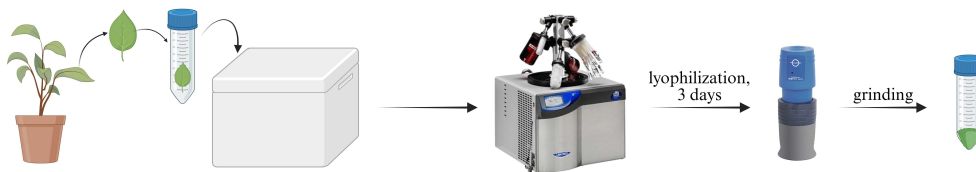
Sampling Procedure and Plant Material Processing

3d 0h 22m 30s

1 Preparation of Sampling Materials and Equipment



- 50 mL Falcon tubes,
- a scalpel or knife,
- scissors,
- 96 % ethanol for surface sterilization,
- disposable gloves for handling,
- a transport box containing dry ice.



- 2 Cut a defined portion of fresh plant tissue (e.g., leaf, root, stem, flower, or fruit) and immediately transfer it into a sterile 50 mL conical centrifuge tube. 5m
- 3 Immediately place the tubes on dry ice to preserve sample integrity during transport to the laboratory. 30s
- 4 Cover the opening of the tube with a single piece of fine mesh fabric to allow airflow while preventing contamination. 30s
- 5 Secure the mesh fabric over the tube opening using a rubber band to ensure it remains firmly in place. 30s
- 6 Place the prepared tubes into the lyophilizer flasks, ensuring a stable and upright position to facilitate efficient drying. 10m
- 7 Perform lyophilization for a duration of 3 days, following the manufacturer's operating guidelines for optimal drying efficiency. 3d
- 8 Transfer the lyophilized plant material into an analytical mill and grind to a fine, homogeneous powder. 1m



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| 14 | Add 2 000 µL of EtOH-H ₂ O solution (75:25, v/v) to each tube and vortex briefly to ensure thorough mixing. | 1m |
| 15 | Incubate the samples at 40 °C for 3 minutes to enhance extraction efficiency. | 3m |
| | |  |
| 16 | Homogenize the samples using a TissueLyser at 25 rpm for 60 seconds . | 1m |
| 17 | Centrifuge the samples at 14 000 rpm for 5 minutes to pellet debris. | 5m |
| | |  |
| 18 | Carefully transfer 500 µL of the supernatant into a new 2.0 mL Eppendorf tube, avoiding any particulate matter. | 1m |
| 19 | Evaporate the solvent by drying the samples in a SpeedVac concentrator until completely dry. | 4h |
| 20 | Resuspend the dried extract in 2 000 µL of CH ₃ CN-H ₂ O solution (50:50, v/v) | 30s |
| 21 | Sonicate the samples for 15 seconds , followed by vortexing for 30 seconds to ensure complete dissolution. | 15s |
| 22 | Centrifuge the samples at 14 000 rpm for 5 minutes to clarify the extract. | 5m |
| | |  |
| 23 | Carefully transfer the clarified supernatant into LC-MS vials, avoiding any residual particulates. | 1m |