

Methanol/Chloroform/Water (MCW) extraction of freeze dried leaves/roots

Procedure

1. Weigh 20 ± 1 mg of freeze-dried tissue into a 2-ml eppendorf tube. (record weight to nearest 0.1 mg). You may not need to weigh samples if you are using a known number of leaf discs of known area and mass (e.g. SLA or LMA are measured on another subsample). For example, with leaves of *Eucalyptus* spp. it's common to use four number 3 or 4 leaf discs, then determine SLA on another subsample of leaf discs
2. Using a multipette add 700 μ L of hot (65 °C) methanol and incubate in a water bath @ 65 °C for 30 minutes, agitating from time to time. It's also possible to use the shaking incubator for samples (set it at 65 °C, 50 Hz, 30 minutes). Either way, it may be necessary to release built-up pressure after a few minutes (otherwise the lids can pop open and lots of the methanol will evaporate)
3. Using a multipette add 400 μ L of chloroform (at room temperature).
4. Using a multipette add 800 μ L of ultra-pure water (at room temperature)
5. Mix thoroughly using a vortex mixer, centrifuge in a benchtop microfuge (5 min at max speed) and let stand for 10 minutes.
6. Use a Gilson pipette to remove 700 μ l of the alcohol/aqueous phase (top) into a clean, labelled eppendorf tube.
7. Freeze samples (-80°C), or else dry and derivatize

Notes

1. Freeze dried material and high temperatures may not be the most appropriate. Many studies use fresh or fresh-frozen material and extract at low temperatures.
2. Some people advocate sparging solvents with N₂ so as to remove O₂ before doing any extraction. If you experience oxidation problems this may be worthwhile. However, this is not the only place that O₂ can contact sample so sparging is by no means a panacea.
3. Read the MSDS for methanol and chloroform.
4. Methanol and chloroform are toxic. Wear gloves and work in the fumehood where necessary.
5. Methanol is also flammable, so don't use it near a naked flame.
6. Methanol will remove marker pen, so be careful you don't inadvertently lose all your labels!
7. Use a multipette for pipetting methanol, chloroform and water. Devote one combitip to methanol, another to chloroform and one to water. Wash the combitips when finished (they can, and should, be reused)