Measurement of volatiles

from ground tissues and solutions

Notes for all methods:

- These protocols are used to estimate the total volatile content of tissues or solutions (as exposed to measuring the volatiles emitted into the headspace).
- Should I use a tissue extraction or headspace sampling (SPME, other adsorbent like PDMS tubing, dynamic headspace sampling) to detect volatiles in tissues?
    - SPME is more sensitive, but less quantitative, more difficult and more time-consuming.
    - Dynamic headspace sampling of ground tissues is more quantitative than SPME/adsorbent methods but more difficult and more time-consuming than solvent extraction.
    - If an extract is sufficient, it is preferable.
    - Extraction and GC-MS analysis is most likely to work for lipophilic compounds of low volatility, e.g. sesquiterpenes. Compounds with higher volatility will have a higher percentage loss during extraction, and more polar volatiles are harder to separate from water and other hydrophilic compounds in plant material which should not be injected into a GC.
- Method 1 can be adapted for SPME or other adsorbents (e.g. PDMS tubing) (1 mL of CaCl2 solution in a 4 mL vial, no stirring, use vial with septum).
- You should always use an internal standard.
    - The best choice is a compound similar in structure to the compounds you want to analyze (e.g. a GLV or terpene not found in your headspace), or which elutes in the middle of your chromatogram (e.g. tetralin) if you are analyzing multiple types of compounds.
    - You should prepare a stock solution of your standard in a suitable solvent (such as hexane or methanol) at a concentration of 1 ug/uL, and add enough standard so that a 100% yield of standard would give you 10-20 ng/uL in your final extract.

Methods for highly volatile compounds

From G. Bonaventure, M. Kallenbach, S. Allmann, P. Gilardoni, M. Schuman

Method 1: $CaCl_2$ mixture

Based on Takabayashi/Matsui methods

Suitable for highly volatile as well as less volatile compounds

For dynamic headspace sampling (with Poropak or charcoal filters):

1. Harvest and flash-freeze ca. 300 mg tissue (more is also ok) and weigh; write down mass.

2. Grind frozen tissue thoroughly (GenoGrinder); do not allow to thaw before step 4.
3. Pipette 5 mL of di water saturated with $CaCl_2$ to inhibit enzyme activity into scintillation vial with a small stir bar.
4. Add frozen tissue with an internal standard; close tightly and mix thoroughly **WITHOUT CONTAMINATING THE LID**.
5. **MAKE SURE THE SCINTILLATION VIAL IS AIRTIGHT**. You may seal it with a PTFE O-ring inside, and PTFE tape around the outside. **DO NOT USE PARAFILM.** Evaporate volatiles in the scintillation vial under a stream of nitrogen and collect with filter (charcoal or poropak Q) while heating the vial at 40-60 deg C and stirring. You may simultaneously apply a light vaccuum to the filter.
6. For the first collection, make a kinetic in 20 min intervals to determine how long you must trap to collect most of the volatiles from the tissue.
7. Elute the volatile trap according to the dynamic headspace trapping protocol and analyze by GC.

For SPME or other adsorbent:

1. Harvest and flash-freeze ca. 300 mg tissue (more is also ok) and weigh; write down mass.
2. Grind frozen tissue thoroughly (GenoGrinder); do not allow to thaw before step 4.
3. Pipette 1 mL of di water saturated with $CaCl_2$ to inhibit enzyme activity (74.5 g/100 mL, or 98.69 g/100 mL for dihydrate) into a 4 mL scintillation vial. Optional, but recommended: add an internal standard.
4. Add frozen tissue; close tightly and mix thoroughly **WITHOUT CONTAMINATING THE LID**.
5. For the first collection, try exposing the fiber for 15 minutes; if the signal is too low, increase the exposure time.

Method 2: dichloromethane evaporative extract

Suitable for highly volatile compounds (e.g. GLVs)

Important: no plastic!! DCM will dissolve plastic and contaminate your sample.

1. Harvest and flash-freeze ca. 300 mg tissue (more is also ok) and weigh; write down mass.
2. Add 5 mL DCM and grind the tissue thoroughly in a 10 mL glass tube using the polytron.
3. Add 2 mL of di water and vortex thoroughly.
4. Centrifuge 15 min at 2,000 RPM (tubes break at higher speeds).
5. Using a glass Pasteur pipette, take the organic (lower) phase and transfer to a scintillation vial (or store at -20 deg C in a fresh 10 mL glass tube for later measurement).
6. **MAKE SURE THE SCINTILLATION VIAL IS AIRTIGHT**. You may seal it with a PTFE O-ring inside, and PTFE tape around the outside. **DO NOT USE PARAFILM.** Evaporate volatiles in the scintillation vial under a stream of nitrogen and collect with filter (charcoal or poropak Q) while heating the vial at 40 deg C. You may simultaneously apply a light vaccuum to the filter. Collect until all DCM is evaporated (ca. 20 min).
7. Elute the volatile trap according to the dynamic headspace trapping protocol and analyze by GC.

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Method for less volatile compounds: hexane/pentane extraction

T. Köllner, M. Kallenbach, M. Schuman September 2010

Important: no plastic!!

Should I use hexane or pentane?

- Pentane is more volatile and thus can be more safely evaporated to concentrate samples. However, because pentane is more volatile, the samples are less stable and will evaporate more quickly during storage.
- So hexane is safer, but if you know you will need to concentrate your samples, it is better to use pentane.
- Do not inject extracts without first asking GC instrument dads. The injector should be protected with glass wool and/or cleaned well after multiple injections.
- Methanol may also be used for more polar compounds, but only for injection on DB-wax column and not without permission from GC instrument dads (methanol will take up the water).

Protocol:

1. Harvest tissue, flash-freeze in liquid N2 and grind to a fine powder.
2. Weigh out ca. 100 mg of frozen tissue (record the exact mass) into a glass GC vial with a screw-on septum lid.
3. Spike tissue with ca. 3 ug of an internal standard (see **notes for all methods** at the top of this page).
4. Add 200-300 uL hexane (or pentane or methanol) to frozen tissue; minimize the volume to maximize extract concentration.
5. Incubate rotating overnight-24 h at RT.
6. Allow tissue to settle; alternatively, the Eppendorf Speed Vac in the analytical lab can be used for centrifugation without vacuum. The right insert you can usually find in the drawer below Celie’s lab bench. Take supernatant; avoid taking the water phase (in case of hexane and pentane).
7. Ask a GC instrument dad to prepare the GC injector for extract injection.
8. Inject 1 uL of extract for analysis.