**Safety:**

This protocol involves the use of **Dichloromethane**, a hazardous chemical.

Wear gloves, closed toed shoes, a labcoat, and work under the vent hood while using it.

Discard gloves that have come in contact with **dichloromethane** immediately. If contacted with skin, wash thoroughly with water. **May cause chemical burns and is toxic.**

**Preparation:**

Done to ensure you have clean filters

First, you’ll need:

Two beakers (100ml is fine)

100microliter pipettes and Drummond syringe. If you contact the pipette with anything other than the filter or the beaker of dichloromethane, discard pipette and use new one. There is a metallic waste collection bin in the vent hood.

**Procedure:**

1. Use the “arm aperture” device to hold the filter in place under the vent hood.
2. Place a waste collection beaker under the filter.
3. Put a small amount of **dichloromethane** into second beaker and place under vent.
4. Using 100micro liter pipette / Drummond syringe, siphon **dichloromethane**.
5. Put the 100 micro liters of **dichloromethane** into the filter. Do this step 3 times for 300microliters total.
6. Purge filter using nitrogen gas.
7. Repeat from step 4. Total **dichloromethane** use will be 600 microliters.
8. Wrap the now clean filters in clean foil.
9. Cleanup. Once **dichloromethane** has evaporated, wash beakers in water, then sparkleen, then acetone (don’t rinse acetone). Place beakers in oven (50c).

Note: The vent hood will sound an alarm if it cannot maintain enough positive air pressure. If this happens, close the windows more and then press the button on the right side to turn alarm off. Also, be sure to remove arm aperture device and place Drummond and pipettes into appropriate storage, and close the vent hood completely after done.

**Volatile Extraction**

Will use a vacuum pump to collect the gasses off rose plants

First, you’ll want to purge the pump tank. There is a spigot valve on the bottom of the pump tank. Failure to purge tank can lead to long term damage.

**Procedure:**

1. Get 4 rose plants (2 pos, 2 neg) from the quarantine greenhouse and bring to the small greenhouse (where the volatile extraction device is located).
2. Put oven bag over a section of the rose plant. Ensure air hose (pumping air in) is also in the bag.
3. Use zip ties to seal the bag. Will need to ensure there is a good seal, or you’ll have to redo. (Bag will inflate with good seal).
4. Turn on air pressure. Usually a setting of 1 will be ok, but experiment. Make sure the bag remains inflated but is not bursting.
5. Insert filter into vacuum hose. Make sure it is properly fitted.
6. Cut a corner of the oven bag. Place corresponding vacuum hose into bag, and seal with zip ties. Turn vacuum on but ensure the suction power is lower than the air pushing into the bag (if the bag collapses, adjust).
7. Redo for all other plant specimens. Leave running for 24 hours.
8. Collect filters. Wrap them in foil carefully and store in a dark place at room temperature.

**Preparing Extractions**

Will wash filters and collect the volatiles into a vial, which will be ready for the **Gas Chromatograph/Mass Spectrometer.**

Note: You can keep the filters wrapped in foil in a safe place and they’ll be ok for a while. Once you start processing them, the end result will need to be stored carefully. Ensure you have enough time to complete.

First you’ll need:

75microliter pipettes and 5microliter pipettes plus Drummond Syringe. Be careful not to contact the pipettes with any contaminants.

Small vials and “mandrils”

A 100ml beaker

**Procedure:**

1. Place mandril into small vial, with its arms down. It will be a snug fit.
2. Label the small vial using pencil. Include plant ID, + or – and date of extraction.
3. Use the “arm aperture” device’s bottom arm to hold the small vial in place.
4. Use the “arm aperture” device’s top arm to hold the filter in place under the vent hood. Be sure the filter feeds into the small vial. Be careful, they are fragile.
5. Put small amount of **dichloromethane** into beaker.
6. Use 75microliter pipette to siphon **dichloromethane**. Feed into the filter, which will drip into the small vial. Do this twice for 150microliters total.
7. Use nitrogen gas to purge the filter. Be careful to use low pressure due to the fragility of the vial.
8. Remove vial from arm aperture device. Using 5 microliter pipette and Drummond, place 5microliters of internal standard (nonyl acetate) in vial.
9. Cap the vial and put in freezer located in Martini lab. Should be a blue tray, place negatives with negatives, positives with positives.