

Onion Waxes - GCMS

1. Using scissors and forceps cut onion leaf samples ~1.5 inches long. Wash tools in chloroform frequently.
2. In the greenhouse, samples can be weighed on filter paper and an aliquot of docosane dissolved in chloroform added to each at the rate of 0.1 μ g/0.3g of tissue. When docosane dries, drop samples into 16x100mm vials and cover with aluminum foil.
3. To sample in the field, label and pre-weigh all vials. Drop samples in vials, cover with foil and bring back to the lab for adding docosane. Weigh the vials and subtract the previous weight to calculate docosane amount. This can be done quickly with an excel formula.
4. Working in a fume hood, add ~7mL HPLC grade chloroform to each vial, or enough to submerge the leaf sample. Floating samples may need to be held down with forceps. Submerge each sample for 1 min, then remove and discard. Allow chloroform to evaporate in the fume hood. This will take a few days.
5. As soon as chloroform is dried off, follow the rest of this protocol. Dried samples may begin to oxidize if left in the hood. Also make sure that time is reserved on the GCMS, as derivatized samples may start to form precipitate after 2-3 weeks.
6. Add 500 μ L anhydrous chloroform, 600 μ L acetonitrile, and 210 μ L BSTFA to each vial and cap tightly. Bake samples in an oven at 80°C for 30 min. If GCMS is not available soon, do NOT add BSTFA or bake samples. Re-dissolved samples can be stored longer than 2-3 weeks in the refrigerator.
7. When samples are cooled, use 1mL disposable syringes with filters to filter samples into Supelco GCMS vials. If disposable syringe doesn't reach bottom of sample vial, try to get at least 0.5mL of sample to filter into GCMS vial. If GCMS vial is not 1/4 to 1/3 full, the GCMS Hamilton syringe may not reach the sample.
8. Run derivatized and filtered samples on GCMS as soon as possible. Set column temp to 300°C for 30 min to burn out. Tuning may be necessary. Make sure the Hamilton syringe plunger is clean and slides freely. If not, clean with chloroform (you will need to do this periodically between runs). Carefully insert into Auto-sampler. Prepare wash and waste vials. In CG consumables, check the number of runs on the septum and glass inlet liner to see if replacement is necessary. Build batch table using an old one as a template and save the new file. Maximum number of samples per batch is 98. A new tuning file may need to be cited in the batch table but the method file should be the same (MJH_test_ver_1).