Preparation of tBDMS derivatives of methanol/chloroform/water (MCW) extracts

Procedure

1. Label 2-mL GC vials with the code for each sample. It's best to write on the white window with a pencil, or else use an alcohol-proof marker

2. Pipette internal standard into each GC vial. (typically 5 µL of 0.2 mg/mL norleucine). Use the multipette and an appropriate combitip if you are doing > 5 samples

3. In a fume hood, pipette a know volume of MCW aqueous phase (that you've defrosted, if it was frozen) into a 2-mL glass GC vial. Use a Gilson pipette and fresh tip for each sample. 50 µL is the usual volume

4. Dry sample with N2 in mini-vap manifold, or in speed-vac or equivalent. It is incredibly important that the sample is dry, so don't rush this step. If using mini-vap you should clean the needles before use so as to prevent contamination (to clean use a tissue with acetone then a separate clean tissue with ethanol).

5. While samples are drying get the MTBSTFA+1% TBDMCS out of the fridge

6. Once you think the samples are dry add 50 µL of dichloromethane and evaporate again. This step ensures that your samples are absolutely dry. If you aren't going to derivatize immediately then samples have to be stored dry (preferably under N2). In most cases you are best to derivatize immediately

7. using a Gilson pipette or a multipette and combitip, add 100 µL of anhydrous acetonitrile to each of the vials – replacing their caps as you go (but don't crimp if you're using crimp-top vials)

8. Add 50 µL of MTBSTFA/TBDMCS to each vial using a Gilson Pipette or glass and stainless syringe. Don't worry about getting it exactly 50 µL, within 10% is OK[1]. Do this step quickly and try and keep lids on vials as much as possible. Mix thoroughly

9. Once you are finished return the remaining MTBSTFA/TBDMCS to the fridge.

10. Put samples in the oven (80C for 45 minutes). Shake twice during the 45 minute incubation

11. Remove samples from oven after 45 minutes

12. Let cool in the fume hood, remove cap and remove sample with Gilson pipette and transfer solution to a glass insert. Put the glass insert into the 2-mL GC vial and add cap. Try and do this step quickly so as to avoid exposure of sample to air (O2 and H2O)

13. Analyse samples ASAP. Preferably within 24 hours. Keep samples away from heat and direct light while awaiting analysis

14. If you used glass and stainless syrine, clean syringe with acetone, methanol and then hexane. Let air dry.

[1] The reason it's OK is because you've got an internal standard and all quantification is relative to the internal standard

Notes

1. It is best to work in batches of no more than 11 samples (+ 1 blank) because you have to work quickly to avoid sample and solvent being exposed to air (O2 and H2O). 12 is convenient because the mini-vap manifold holds 12 samples. Don't try and do more than 36 samples in a day because it's important that samples are analysed within 24 hours of derivatisation. This isn't possible if you have too many samples.

2. **Know your enemy!**

Your enemies are H2O and O2. Work fast. Ensure all reagents, solvents and samples are dry. Avoid exposing samples and solvents to air. Solvent should be stored over molecular sieve and every step should be taken to open lid as rarely as possible. Derivatisation reagent (MTBSTFA) is very hygroscopic. It is best stored in fridge in a glass ampoule or a vial with septum (remove it from the fridge and let warm to room temperature before use). Never open MTBSTFA to atmosphere. If necessary transfer it to a GC vial and remove it through septum with a syringe.

3. Always carry a blank through the extraction and derivatization procedure

4. Read the MSDS for MTBSTFA/TBDMCS and acetonitrile

5. Both are toxic. Wear gloves and work in the fumehood where necessary.

6. Both MTBSTFA and acetonitrile will remove marker pen, so it is best to label GC vials with pencil

7. Do not place sample vials on top of GCMS. Any acetonitrile or MTBSTFA on outside of vial will eat through the GCMS's plastic.

8. Use of the vial insert may not be necessary if the analytes of interest are sufficiently concentrated. However, if you don't use an insert you will need to dissolve sample in more acetonitrile so that there is enough sample to be taken up by the autosampler.