

## 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  $\mu\text{L}$  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 known 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  $\mu\text{L}$  is the usual volume
4. Dry sample with  $\text{N}_2$  in mini-vap manifold, or in speed-vac or equivalent. 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). It is incredibly important that the sample is dry, so don't rush this step.
5. Once you think the samples are dry add 50  $\mu\text{L}$  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  $\text{N}_2$ ). In most cases you are best to derivatize immediately
6. Get a multipette and combitip. Rinse once with a full volume of DMF, then add 100  $\mu\text{L}$  to each of the vials – replacing their caps as you go (but don't crimp)
7. The next step involves adding MTBSTFA. This is best done in lots of 3. Take a 250  $\mu\text{L}$  glass and SS syringe and fill with ~150  $\mu\text{L}$  of air. Insert into MTBSTFA vial and squirt in air. Then remove 150  $\mu\text{L}$  of MTBSTFA. Squirt ~ 50  $\mu\text{L}$  down the inside of each of the three vials. Don't worry about getting it exactly 50  $\mu\text{L}$ , within 10% is OK<sup>1</sup>. As soon as you've added MTBSTFA to the three vials you should crimp the tops securely. Mix thoroughly
8. The next step is to repeat the addition of MTBSTFA with the next batch of samples
9. When all samples have DMF, MTBSTFA, are crimped and have been mixed you can put them in the oven (80C for 45 minutes)
10. Once you are finished return the remaining MTBSTFA to the fridge.
11. Remove samples from oven after 45 minutes
12. Let cool in the fume hood, remove cap with de-capper tool, remove sample with Gilson pipette and transfer solution to a glass insert. Put the glass insert back into the 2-mL GC vial and re-crimp with a new cap. Try and do this step quickly so as to avoid exposure of sample to air ( $\text{O}_2$  and  $\text{H}_2\text{O}$ )
13. Analyse samples ASAP. Preferably within 24 hours. Keep samples away from heat and direct light while awaiting analysis
14. Clean syringe with acetone, methanol and then hexane. Let air dry.

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<sup>1</sup> 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 (O<sub>2</sub> and H<sub>2</sub>O). The other reason to work with a small number of samples is that samples should be analysed within 24 hours of derivatization.

### **2. Know your enemy!**

Your enemies are H<sub>2</sub>O and O<sub>2</sub>. 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 and DMF
5. Both are toxic. Wear gloves and work in the fumehood where necessary.
6. Both MTBSTFA and DMF 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 DMF 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 DMF so that there is enough sample to be taken up by the autosampler.