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

1. Prepare methoxyamination reagent (or buy pre-prepared MOX reagent)
    - Dissolve methoxyamine hydrochloride (stored in desiccator) at 20 mg ml^{-1} in (anhydrous) pyridine.
    - This reagent needs to be prepared fresh each day. It will require some shaking to dissolve
    - Don't forget to return methoxyamine hydrochloride to desiccator, preferably after purging glass bottle with dry N_2.

    Reagents are extremely toxic and should be handled in the fume hood

2. Prepare MSTFA with 1% TMCS (or buy pre-prepared MSTFA/TMCS mix)
    - Take an ampoule of MSTFA and large GC vial of TMCS from fridge and let warm for 15 minutes.
    - Label a 2-mL glass GC vial "MSTFA/TMCS". Break ampoule of MSTFA and decant into the GC vial. Place cap on top of vial but do not tighten or crimp.
    - Using a 50 µL glass and stainless syringe withdraw 10-13 µL of TMCS from GC vial and add to MSTFA vial under the surface. Cap mixture of MSTFA/TMCS and use within 48 hours. Wash syringe with acetone, then methanol then hexane.

    Reagents are extremely toxic and should be handled in the fume hood

3. Label 2-mL eppendorf tubes with the code for each sample (and the blank). It's best to use an alcohol-proof marker to write on side of tube

4. Pipette internal standard into each tube. (typically 5 µL of 0.2 mg/mL ribitol).

5. In a fume hood, pipette 10-50 µL of MCW aqueous phase (that you've defrosted, if it was frozen) into the tube (or a MCW blank into the blank).

6. Centrifuge for 5 seconds to ensure liquids are at bottom of tubes

7. Dry sample with N2 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 wipe with a tissue with water then a separate clean tissue with ethanol).

8. 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.

9. In a fume hood add 40 µL of methoxyamination reagent to each of the tubes and close cap. Incubate at 37ºC for 90 minutes (use the shaking incubator at 40 Hz)

10. Briefly centrifuge tubes (~ 5 seconds) so that reagents (which had condensed inside lid) are brought into contact with sample

11. In a fumehood add 70 µL of MSTFA/TMCS to each of the tubes. Cap securely, centrifuge for 5 seconds, and incubate at 37ºC for 30 minutes (use the shaking incubator at 40 Hz)

12. Briefly centrifuge tubes (~ 5 seconds)

13. Let tubes cool in the fume hood for at least 5 minutes, and then transfer (with a Gilson pipette) to a labelled 2-mL glass GC vial that has a glass insert. Cap securely

14. Analyse samples ASAP. Preferably within 24 hours. It's best to analyse samples in a random order. Keep samples away from heat and direct light while awaiting analysis

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). 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 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 reagents (MSTFA and TMCS) are very hygroscopic. They are 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 MSTFA or TMCS to atmosphere for prolonged periods. If necessary transfer 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 MSTFA, TMCS, pyridine and methoxyamine hydrochloride

5. Samples, reagents and solvents are toxic. Wear gloves and work in the fumehood

6. Samples, solvents and reagents will remove marker pen, so it is best to label Eppendorf tubes with ethanol-proof marker (GC vials can be marked with pencil if they have white windows)

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