Preparation of polar EXTRACTS for GCMS and NMR analysis by Teresa Fan, University of Kentucky

GCMS SAMPLE PREPARATION

Note: This procedure follows [Fan_Extract_Polar_Lipid_Prot_SOP]. Step 8 from that SOP is detailed here.

1. Record the extract weight. (Polar + tare)
2. Centrifuge the 5 ml centrifuge tubes with pulse by pressing 'pulse' button and holding it until the rate reaches ~2,000 – 2,400 rpm in order to let any particulate on the tube wall and cap go down.
3. On a 4-place balance, weigh two aliquots (g polar GCMS A and B) of approximately 1/8th the total volume of the polar extract into each of (2) 1.5 ml GC glass vials (vial Fisher 03-375-11BA (National Scientific C4010-1W) with crimp top caps Fisher 03-375-29A (National Scientific C4010-40A)) for GC-MS and weigh two aliquots (g polar FTMS A and B) of 1/16th of the total volume of the polar extract into each of two in small volume screw top microfuge tubes (USA Scientific 1405-9300) for FT-ICR-MS. Divide the remaining extract in two equal portions into tared screw-top microfuge tubes, recording the weights (g polar NMR A and B). Lyophilize, with a liquid N_2 pretrap, all six aliquots and store at -80 degrees C. If preparing GCMS samples immediately, proceed to step 4 – do not lyophilize.

Note: a 4-place balance weighing is more accurate than volumetric pipetting; aliquot weight can be converted to volume based on the water density of 1 g/ml.

Note: two aliquots are prepared in case of loss during subsequent steps. The second aliquot for GC-MS is optional.

4. Add 5 nmole of internal standard (50 µl of 0.1 mM Norleucine) and 40% trichloroacetic acid (TCA) to make the final TCA concentration of 10%; also prepare a blank containing 5 nmole NorLeu and a GCMS standard containing various amino acids and organic acids.

Note: TCA should be added with sample on ice and acidified sample is immediately frozen in liq. N2 to minimize acid hydrolysis).

Note: If polar extract has already been lyophilized (at the end of step 3), add 20 µl 10% TCA containing 5 µl 1 mM NorLeu then freeze quickly in LN2 and lyophilize overnight.

5. Lyophilize the extract with a liq. N2 trap.
6. Derivatize the lyophilized extract, blank and standards with 50 µl MTBSTFA:acetonitrile (1:1, v/v) mixture by sonication for 3 hr and let stand overnight in sonic bath.
7. Transfer the derivatized extract to a 200 µl polyspring glass insert and put insert back to the same glass vial before crimping with a Teflon-faced cap
8. Centrifuge the capped glass vial with insert in vacuum centrifuge for 10-15 min to remove insoluble materials

Note: Acidification, derivatization, and GC-MS analysis should be performed without any delay to minimize degradation of metabolites such as Gln.

NMR PREPARATION

9. Reconstitute one of the aliquots of the polar fraction set aside for NMR (step 3, in 2 ml screw-top tube) in 50μL of D_2O in nanopure water with 30 nmole DSS (0 ppm standard).
10. Vortex to resuspend the sample and centrifuge at 4ºC and 20,800 rcf (14,000 rpm) for 5 minutes to remove particulates.
11. Transfer the supernatant into a capillary NMR tube using a gel loading pipet tip.
    a. Label 15 ml centrifuge tubes to hold each capillary NMR tube.
    b. The gel-loading tip can be placed within the capillary tube. Dispense the extract as the tip is moved up and out of the capillary. Flick the capillary (as with a traditional thermometer) to move the extract down, and then continue to fill.
    c. Cap the tube.

Note: Hanging the pipet vertically will keep the extract in the tip while the capillary is shaken down.

12. Rinse the tip with 50 μL 18 mega Ohm (nanopure) H_2O, pushing the water through the tip from the top, and store wash in the 2 ml screw-top centrifuge tube at -20ºC.
13. Centrifuge the capillary NMR tubes inside the 15 ml tubes one pulse, not exceding 200 rpm.
14. Label the capillary NMR tubes with labeling tape on the caps.
15. After NMR analysis, the extract is removed from the capillary NMR tube by centrifuging inverted within the original 2-ml screw cap microfuge tube.
16. The extract can then be lyophilized and stored at -80°C.