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]. Step 8 from that document is detailed here.

1. Record the polar 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 1/10th 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. Split the remaining extract into 2 equal parts to 1.5 ml microfuge tubes for NMR.
4. Lyophilize all aliquots with a liquid N_2 pretrap.

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.

5. When GCMS aliquots are removed from the freezedrier, seal with parafilm unless acidifying immediately.
6. Acidify with 5 nmole of internal standard (50 µl of 0.1 mM Norleucine) in 10% trichloroacetic acid (TCA).
7. 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).

8. Lyophilize the extract with a liq. N2 trap. When removed from freezedrier, vials can be sealed with parafilm if not derivitizing immediately.
9. Derivatize one lyophilized GCMS aliquot, 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.

Note: MTBSTFA:acetonitrile should be added within a chemical safety hood. Crimp cap vials immediately after adding derivatizing agent.

10. Transfer the derivatized extract to a 200 µl polyspring glass insert and put insert into a low profile screw-cap vial (Fisher Scientific Cat. # 03376492) and cap with Teflon lined cap. This operation should be done in a chemical safety hood.
11. 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

12. Reconstitute one of the NMR aliquots (step 3, in 1.5 ml microfuge) in 50μL of 50:50 H_2O:D_2O with 30 nmole DSS (0 ppm standard)
13. Vortex to resuspend the sample and centrifuge at 4ºC and 20,800 rcf (14,000 rpm) for 5 minutes to remove particulates.
14. Transfer the supernatant into a 1.7 mm NMR tube with a microloader pipet tips (USA Scientific cat. # 4093-1007Q).