

Chemicals needed:

- Methanol, Fisher Optima Grade
- 10 mM Ammonium Acetate (made fresh)
- Daily Internal Standard Mix\*

\*See Appendix A

Materials needed:

- Labeled 1.5 mL or 2 mL Eppendorf tubes
- Repeater Pipette
- Calibrated Micropipettes in various volumes\* (see table below)
- Appropriate Micropipette tips\* (see table below)
- Vortex
- Sonicator
- Centrifuge
- Labeled LC vials with appropriate caps or 96-well tray
- Personal Protective Equipment

| Type  | Volumes (µL) | Tip color |
|-------|--------------|-----------|
| P10   | 0.5 – 10     | white     |
| P20   | 2 – 20       | yellow    |
| P200  | 20 – 200     | yellow    |
| P1000 | 200 – 1000   | blue      |

Precise Micropipette Volume and Transfer capabilities

Instrumentation:

- Vortex, Fisher Brand- Vortex Genie 2:12-812: Ensure switch is set to touch mode and shake dial set to 8.
- Sonicator, Fisher Scientific- FS30: Turn heat switch to off and turn dial to desired time.
- Centrifuge, Eppendorf- 5417R: Open by pressing blue “open” button on bottom left of display. Check to be sure loading dock is cool. If not cool, close, press fast cool and wait until temperature is <10°C. When temperature is <10°C, press stop, wait for centrifuge to stop spinning, and open. Load samples making sure samples and/or weights are evenly distributed among the wheel.

Procedure:

- 1- Weigh 30mg of the freeze dried sample into clean Eppendorf tube.
- 2- Add 20 $\mu$ L Daily Internal Standard Mix to each sample
- 3- Add 750  $\mu$ L Methanol and 750  $\mu$ L 10mM Ammonium Acetate to sample.
- 4- Vortex each sample for 1 minute at room temperature (20-25 C).
- 5- Ultrasonicate for 10-20 minutes at room temperature.
- 6- Centrifuge at room temperature for 5-10 minutes at 17,000 G.
- 7- Transfer more than 1 mL of supernatant to a 1.5 mL eppendorf.
- 8- Transfer 800  $\mu$ L of supernatant to an LC vial.

|              |              |                |
|--------------|--------------|----------------|
| Created By:  | Sandi Batson | Date: 10/04/14 |
| Reviewed By: | Tim Garrett  | Date: 10/04/14 |
| Approved By: | Art Edison   | Date: 11/12/14 |

| Revision Number | Name               | Reason for Revision   | Effective Date |
|-----------------|--------------------|---|----------------|
| 01              | Sandi B. Sternberg | Creation of SOP   | 10/04/14       |
| 02              | Sandi B. Sternberg | Update Chemicals needed to reflect 10 mM Ammonium Acetate being replaced with water. Ammonium Acetate is not compatible with NMR. | 10/27/14       |
| 03              | Sandi B. Sternberg | Revert chemicals needed back to 10 mM Ammonium Acetate. Data analysis showed better features with Ammonium Acetate                | 11/04/14       |
| 04              | Sandi B. Sternberg | Added Vortex and Sonicator information to Instrumentation section.  | 11/05/14       |
| 05              | Sandi B. Sternberg | Added step in procedure to accommodate Internal standards.  | 11/07/14       |
| 06              | Sandi B. Sternberg | Added information for Appendix A  | 01/29/15       |