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 20µL Daily Internal Standard Mix to each sample
3- Add 750 µL Methanol and 750 µL 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 µL of supernatant to an LC vial.

Data Collection:

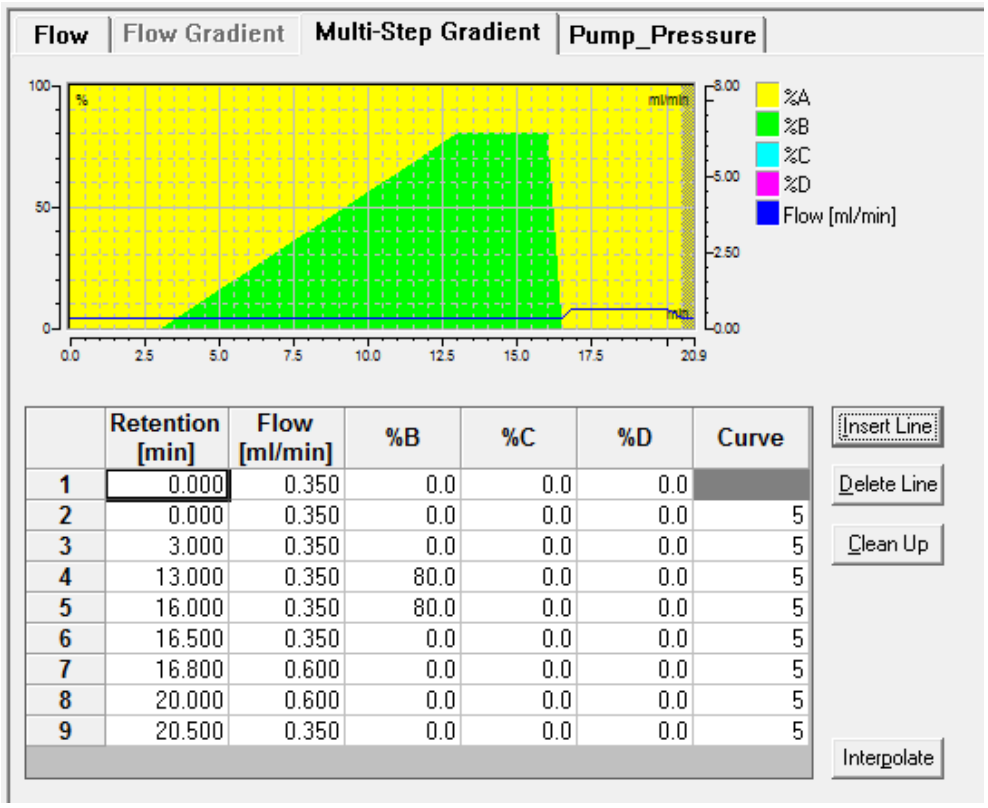
1- Ensure that Column is an ACE Excel 2 C18-PFP with dimensions of 100 x 2.1mm, 2.0 µm with a Halo C18-PFP guard attached
2- Check total injections on column and make note in read_me file.
3- Begin equilibration of the system by taking control through chromelean. Set flow rate to 350uL of 100% pump A.
4- Open tunefile "Metabolomics-Pos-Neg-30sLens.mstune" using tuner window. Once this tunefile has been opened set Mass Spectrometer to on.
    a. Steps 2 and 3 combined will allow the system to equilibrate before sequence begins. It is recommended to let system equilibrate ~10 minutes before start of run.
5- Create folder where all raw files will be saved and generate folder hierarchy following naming protocol. (see appendix B)
6-
7- Set up sequence starting with 3 blanks, 1 neat QC and 1 Pooled QC followed by unknown samples. After 10 unknown samples run another QC set consisting of one blank, one Neat QC and one Pooled QC.
8- Name samples following protocol, verify location of samples, ensure method is "PFP-metabolomics-pos-350-0-SID-17min-new-injector_sycWpump" or "PFP-metabolomics-neg-350-0-SID-17min-new-injector_sycWpump" and injection volume is 2uL for positive injections and 4uL for negative injections.

Gradient Information

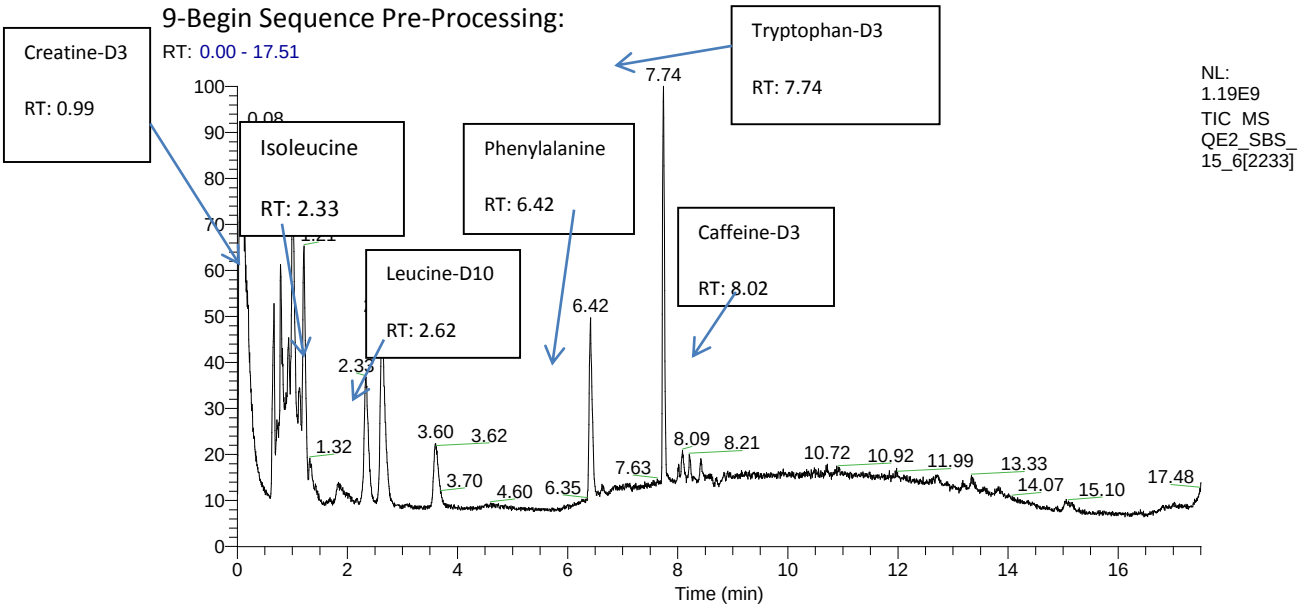
- Duration of run is 20.5 minutes
- Initial conditions are 100% Pump A (0.1% FA in Water)
- Flow rate is .350mL/min until run time 16.8
- Beginning at Run Time 3 minutes and ending at Run Time 13 minutes, begin a ramp gradient up to 80% pump B (Acetonitrile)

- Hold conditions at 80% pump B from Run Time 13 minutes to Run Time 16 minutes
- Beginning at Run Time 16 minutes, return to initial conditions at ending at Run Time 16.5 minutes
- At run time 16.8 increase flow rate to .600mL/min
- Continue until run time 20 and decrease flow rate back to 350 mL/min until Run Time 20.5 minutes
- pump curve=5

Instrument Parameters		
HESI Probe	Positive (+)	Negative (-)
Probe Temperature	350°C	350°C
Spray Voltage	3500 V	3500 V
Capillary Temperature	320°C	320°C
Sheath Gas	40	45
Auxillary Gas	10	10
Spare gas	1	1
Mass resolution 70,000 @ m/z 200		



	Retention [min]	Flow [ml/min]	%B	%C	%D	Curve
1	0.000	0.350	0.0	0.0	0.0	
2	0.000	0.350	0.0	0.0	0.0	5
3	3.000	0.350	0.0	0.0	0.0	5
4	13.000	0.350	80.0	0.0	0.0	5
5	16.000	0.350	80.0	0.0	0.0	5
6	16.500	0.350	0.0	0.0	0.0	5
7	16.800	0.600	0.0	0.0	0.0	5
8	20.000	0.600	0.0	0.0	0.0	5
9	20.500	0.350	0.0	0.0	0.0	5



1-Check to see presence of amino acids and standards within individual runs

2-Generate summary report and sample report.

3-Move all .raw files to server and open on processing computer

4- Convert .raw files to .MZxml files

5- process using MZmine

6-Process further with higher level statistics such as Metaboanalyst

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