Chemicals needed:

- 10mM Ammonium Formate
- Ice-cold Methanol (Optima grade)
- 0.1% Formic Acid in Water (HPLC-MS grade) as Reconstitution Solution
- 0.1% Formic Acid in Water(HPLC-MS grade) and Acetonitrile (HPLC-MS grade) as Mobile Phases
- Internal Standard Mixes

Materials needed:

- 2mL Bead Beater tubes, labeled and clean
- Protein Kit
- Protein Quantification tubes
- 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)
- Sonicator
- Ice
- Refrigerated Centrifuge
- Nitrogen Dryer
- Labeled LC vials with appropriate caps or 96-well tray
- LC-MS
- ACE PFP column
- Positive Calibration Solution
- Negative Calibration Solution
- 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:

Bead Beater,

Qubit Protein Quantification,

Sonicator,

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.

UHPLC,

Thermo Scientific-Dionex Ultimate 3000: While setting up sequence, ensure that these initial conditions for analysis are as follows: 5 uL injection, 0.300mL/min flow rate, gradient of 100% pump B. Always purge lines if solvent bottles are replaced.

Mass Spectrometer, Thermo Scientific- Q Exactive: Divert valve set to position 2

To calibrate in positive ion mode: Correctly set up positive ion syringe filled with positive ion calibration solution and attach to MS using positive ion calibration tubing. Open Tuner→ File→ Load Tune File→ Click on StabilityTestMStune. Go to Instrument control tab and ensure conditions are as follows:

For Scan Parameter

Scan Type	Full MS
Scan Range	120-1,800
Fragmentation	In-Source CID 2 eV
Resolution	35,000
Polarity	Positive

Microscans	1
Lock Masses	Off
AGC target	1e6
Max Inject time	30

Go to calibrate tab → source auto → default settings. Ensure settings are as follow

Set syringe to dispense positive ion calibration solution of a 500uL volume at 3µL /min. Begin dispensing. Turn MS on. Begin calibration. Negative calibration completed in similar fashion using negative ion calibration solution. Calibration should only be performed by trained staff.

Procedure:

1- Add 500 µL of pre-chilled 10mM Ammonium Formate to each pre-weighed sample aiming for 100 mg.
2- Add 20µL Daily Working Internal Standard solution.
3- Homogenize sample using bead beater. Beat samples for 30 seconds. Allow instrument to cool, beat for another 30 seconds.
4- Centrifuge samples at 10C and 20,000 RCF for 10 minutes.
5- Add 20µL of supernatant to protein quantification vial. Add Protein buffer. Allow to sit for 15 minutes before quantifying.
6- Pull off 400 µL of supernatant and transfer to a new, labeled microcentrifuge tube.
7- Add 400 µL ice cold methanol each sample.
8- Place in -20 freezer for 30 minutes to further precipitate proteins
9- Centrifuge samples at 10C and 20,000 RCF for 10 minutes
10- Transfer supernatant to a new, labeled microcentrifuge tube
11- Dry down with clean nitrogen.
12- Reconstitute sample in 200 µL 0.1% FA in water.
13- Vortex
14- Centrifuge to separate any further solid from sample
15- Transfer liquid portion to labeled, glass LC vial.
16- Samples are now ready for metabolomic profiling.

Data Collection:

1- Turn on UHPLC and MS and set to starting conditions by loading method. Calibrate MS if not yet performed.

-Ensure that Column is a Waters Cortecs HILIC with dimensions of 150 x 2.1mm, 1.7um with guard

2- Create file and name it following protocol.

3- Set up sequence starting with 4 blanks, 1 neat QC and enter samples with one blank and one QC following every 10 samples.

4- Name samples following protocol, Double check location of samples in auto sampler, verify method is correct, and ensure injection volume is set to 5uL.

5- Check MS settings to make sure they are correct.

For Scan Parameter

Scan Type	Full MS
Scan Range	70-1,000
Fragmentation	In-Source CID 2 eV
Resolution	70,000
Polarity	Positive
Microscans	1
Lock Masses	On
AGC target	3e6
Max Inject time	100

For HESI Source (Orbitrap)

Sheath Gas Flow Rate	40
Aux Gas Flow Rate	5
Sweep Gas Flow Rate	1
Spray Voltage (kV)	3.0
Spray Current (uA)	(Blank)
Capillary Temp (°C)	300
S-Lens RF Level (%)	40.0
Heater Temp (°C)	200

Gradient Information

- Initial conditions are 100% Pump a (0.1% Formic Acid in Water)
- Beginning at Run Time 1 minute and ending at Run Time 11 minutes, begin a ramp gradient up to 50% pump B (Acetonitrile)

- Hold conditions at 80% pump B from Run Time 11 minutes to Run Time 14 minutes
- Beginning at Run Time 14 minutes, return to initial conditions at ending at Run Time 14.5 minutes
- Equilibrate until Run Time 17.5 minutes
- Flow= 350µL/min, pump curve=5

6- Begin Sequence

Created By:	Sandi Batson	Date: 01/12/15
Reviewed By:	Tim Garrett	Date: 01/12/15
Approved By:		

Revision Number	Name	Reason for Revision	Effective Date
01	Sandi Batson	Creation of SOP	01/12/15