Chemicals needed:

- Mobile phases, H_20 with 0.1% Formic Acid LC/MS grade and Acetonitrile LC/MS grade

Materials needed:

- Labeled LC vials with appropriate caps or 96-well tray
- LC-HRMS
- ACE Excel 2 C18-PFP Column (100 x 2.1mm) 2.0 µm
- Halo C18 PFP guard Column
- Positive Calibration Solution
- Negative Calibration Solution
- Personal Protective Equipment

UHPLC, Thermo Scientific-Dionex Ultimate 3000: While setting up sequence, ensure that method to be utilized is PFP-metabolomics-neg350-NoBuffer-0-SID and PFP-metabolomics-neg-350-dd-MS2 for negative sequences. For positive sequences utilize PFP-metabolomics-pos-350-0-SID and PFP-metabolomics-pos-350-dd-MS2. Check the lines for air bubbles and purge line if present. Set injection volume to 2uL for positive samples and 4uL for negative samples.

Mass Spectrometer, Thermo Scientific- Q Exactive: Divert valve set to position 2. Calibrations should be performed every Monday by a trained staff member and before 24 hour (+) runs. Refer to calibration SOP if needed. The HESI II probe should be installed at position D.

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.

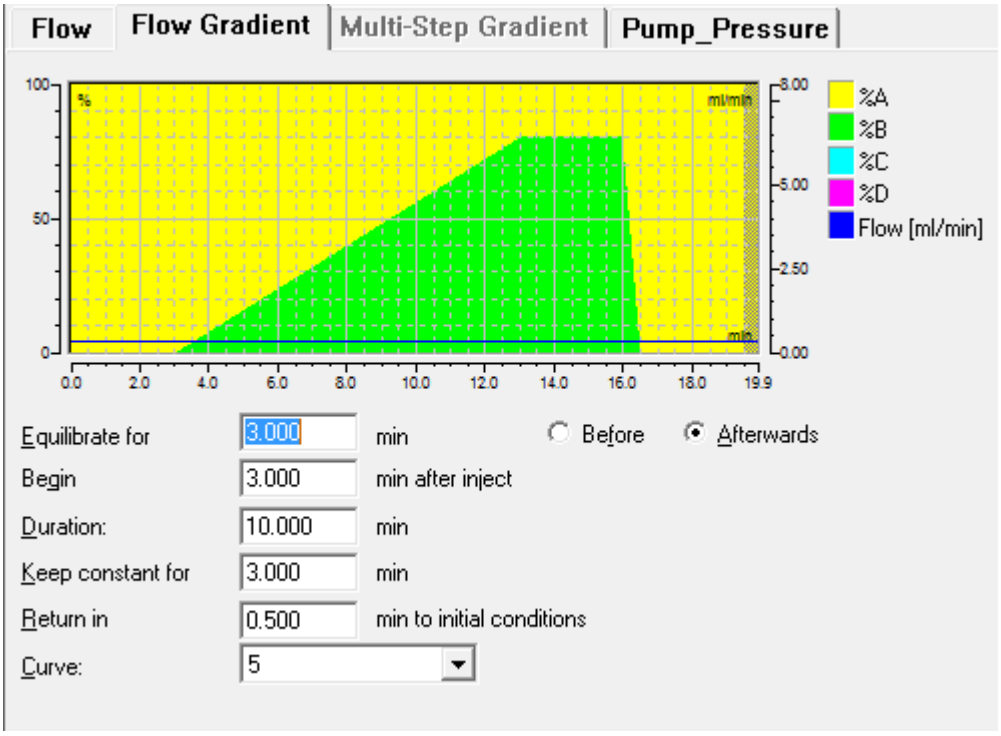
6- Set up sequence starting with 3 blanks, 1 neat QC and 1 Pooled QC followed by unknown samples. After 10 unknown samples run another QC set consisting of one blank, one Neat QC and one Pooled QC.

7- Name samples following protocol, verify location of samples, ensure method is "PFP-metabolomics-pos-350-0-SID" or "PFP-metabolomics-neg-350-NoBuffer-0-SID" and injection volume is 2uL for positive injections and 4uL for negative injections.

Gradient Information

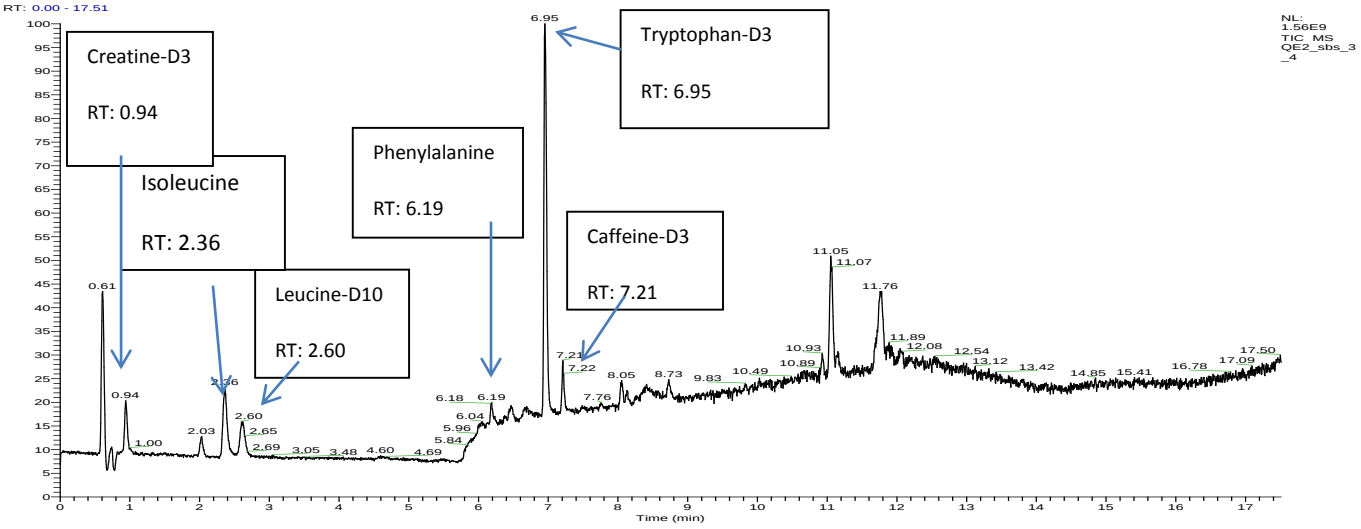
- Duration of run is 16.5 minutes
- Initial conditions are 100% Pump A (0.1% FA in Water)
- Beginning at Run Time 3 minutes and ending at Run Time 13 minutes, begin a ramp gradient up to 80% pump B (Acetonitrile)
- Hold conditions at 80% pump B from Run Time 13 minutes to Run Time 16 minutes
- Beginning at Run Time 16 minutes, return to initial conditions at ending at Run Time 16.5 minutes
- Equilibrate until Run Time 19 minutes
- Flow= 350µL/min, 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		



8- Begin Sequence

Pre-Processing:



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: 02/02/15
Reviewed By:	Tim Garrett	Date: 02/02/15
Approved By:	Rick Yost	Date: 02/02/15

Revision Number	Name	Reason for Revision	Effective Date
01	Sandi Batson	Creation of SOP	02/02/15