

**Chemicals needed:**

- Sodium Azide
- 98:2 Water:Acetonitrile with 0.1% Sodium Azide
- Mobile phases, H<sub>2</sub>O with 0.1% Formic Acid LC/MS grade and Acetonitrile LC/MS grade
- Internal Standard Solution containing Creatine-D3, L-Leucine-D10, L-Tryptophan-D3, Caffeine-D3 and Salicylic Acid.

**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)
- 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

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: Flip switch should be set to touch. Turn dial set to 8. When ready to use place sample(s) on black stage and apply pressure.

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.

#### Extraction Procedure:

- 1- Transfer 50uL of thawed urine to clean, labeled microcentrifuge-filter tube.
- 2- Add 20µL isotopically labeled Internal standard mixture
- 3- Dilute urine with 400uL 98:2 water/acetonitrile with 0.1% sodium azide. Vortex to ensure mixing.
- 4- Centrifuge filter tube and discard filter.
- 5- Transfer sample to glass vial and load into autosampler.

#### Data Collection:

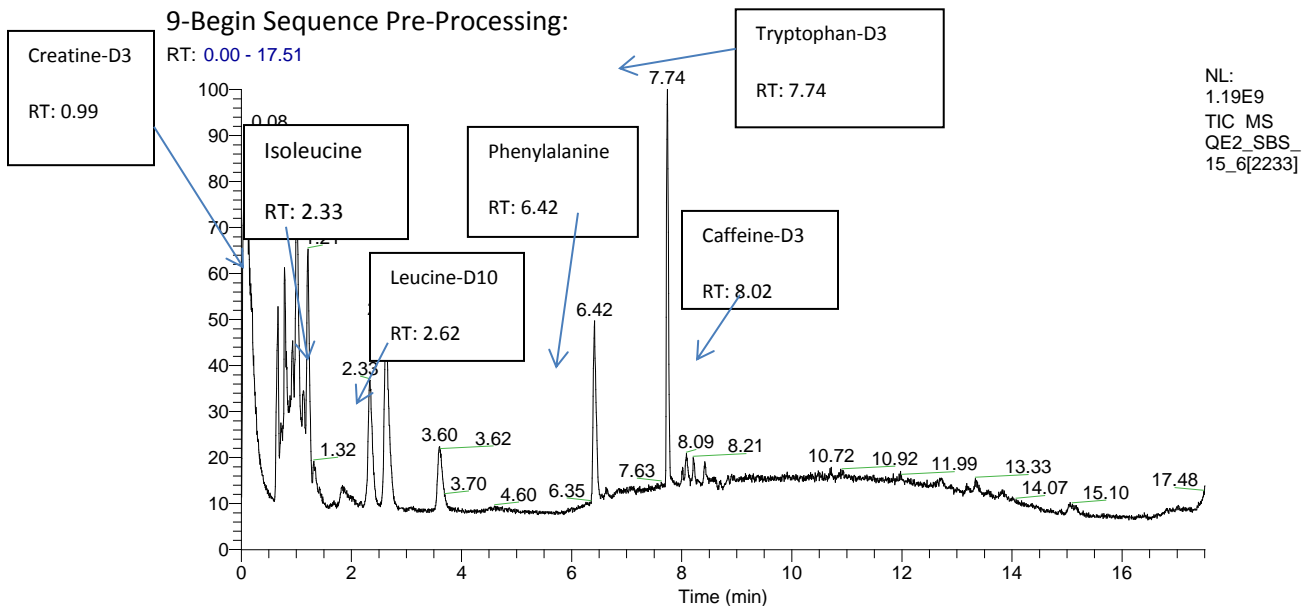
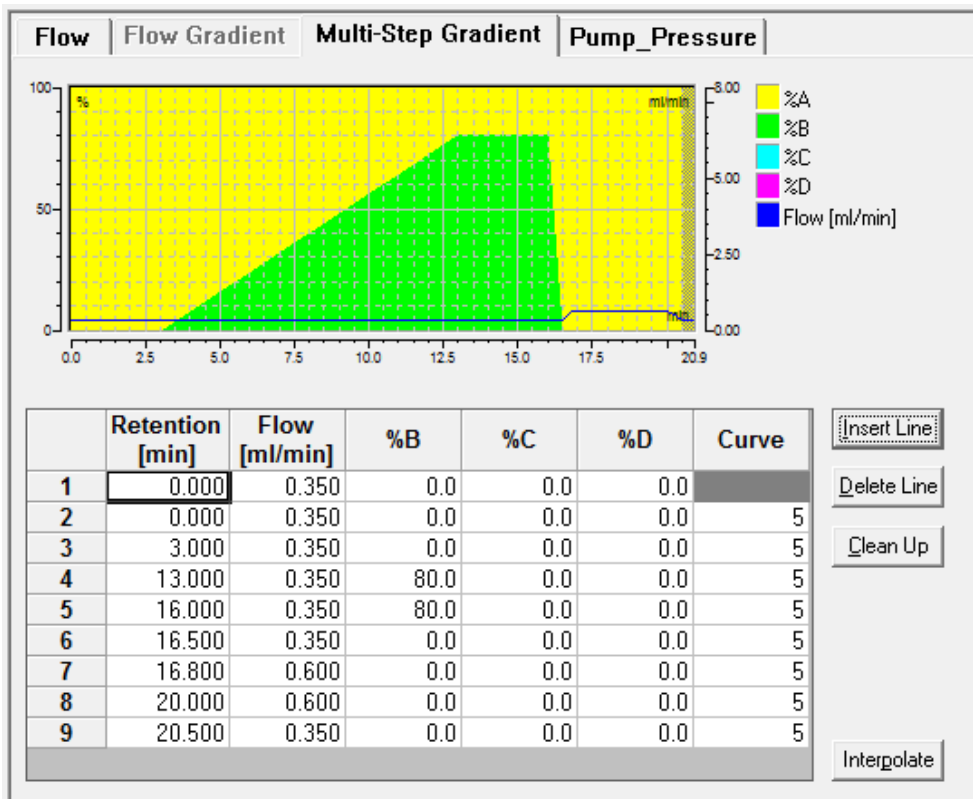
- 1- Ensure that Column is an ACE Excel 2 C18-PFP with dimensions of 100 x 2.1mm, 2.0 µm with a Halo C18-PFP guard attached
- 2- Check total injections on column and make note in read\_me file.
- 3- Begin equilibration of the system by taking control through chromelean. Set flow rate to 350uL of 100% pump A.
- 4- Open tune file "Metabolomics-Pos-Neg-30sLens.mstune" using tuner window. Once this tune file 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- 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-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		



- 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

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Revision Number	Name	Reason for Revision	Effective Date
01	Sandi Batson	Creation of SOP	04/14/15