

Chemicals needed:

- Mobile phases, H₂O 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:

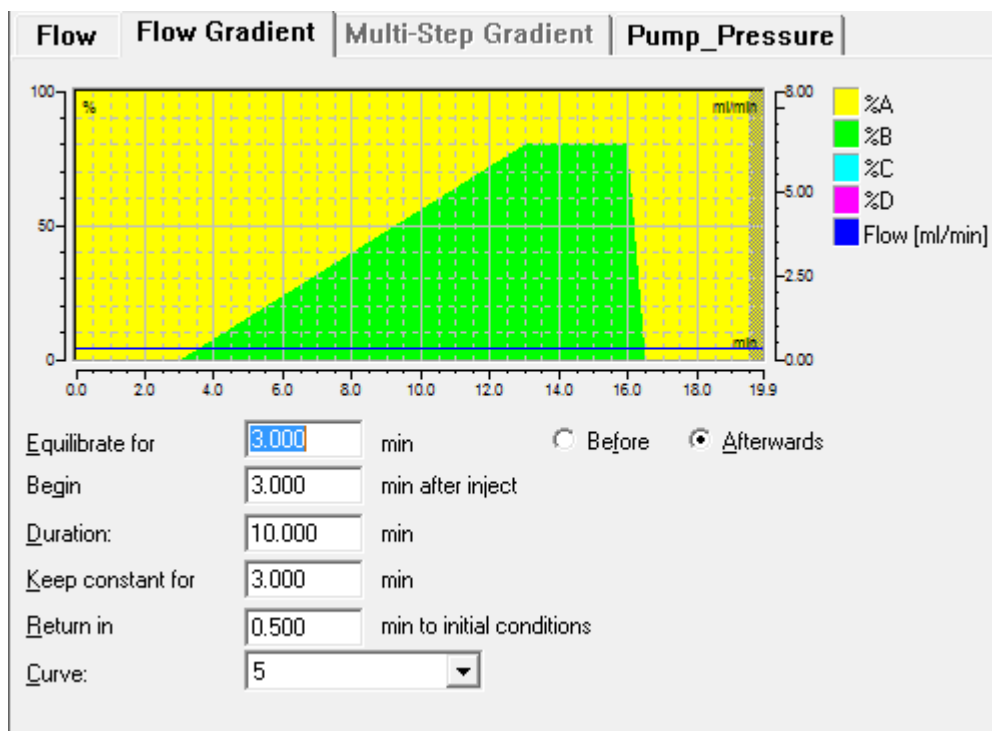
- 1- Ensure that Column is an ACE Excel 2 C18-PFP with dimensions of 100 x 2.1mm, 2.0 µm with a Halo C18-PFP guard attached
- 2- Check total injections on column and make note in read_me file.
- 3- Begin equilibration of the system by taking control through chromelean. Set flow rate to 350uL of 100% pump A.
- 4- Open 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.

- 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

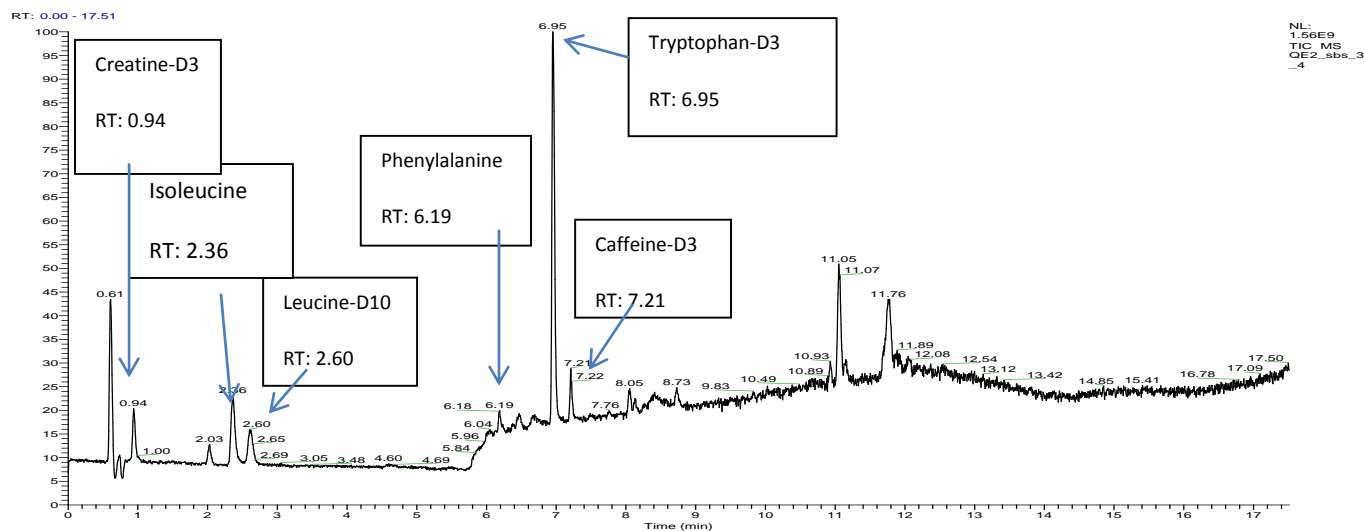
- Duration of run is 16.5 minutes
- Initial conditions are 100% Pump A (0.1% FA in Water)
- Beginning at Run Time 3 minutes and ending at Run Time 13 minutes, begin a ramp gradient up to 80% pump B (Acetonitrile)
- Hold conditions at 80% pump B from Run Time 13 minutes to Run Time 16 minutes
- Beginning 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 |