**Notes on Extractions for KM1906, FK180310, and KM1910**

Amounts of Internal Standards added for *all* samples.

Before extraction:

Extraction IS – 80 uL

Organic IS – 20 uL

After extraction:

Injection IS #1 – 40 uL

Injection IS #2 – 40 uL

320 uL of H2O is added so the total reconstitution volume is 400 uL for all Aqueous samples.

400 uL of 90/10 MeOH/Toluene is added to all Organic samples.

Aliquots for KM1906 samples:

HILIC Aq – 20uL of H2O + 20 uL of sample

RP Aq – 30 uL of sample

CoA Aq – 30 uL of sample

30 uL of each sample was added to the Pooled Aq sample.

25 uL of each sample was added to the Pooled Org sample.

Notes:

~HILIC samples are being diluted 1 to 1. RP and CoA samples are being fun at full strength.

~The KM1906 Pooled Aq sample was **not** diluted 1 to 1!

~For HILIC and Cyano std mixes and matrix in water samples, the matrix was from the FatAs Aq B sample.

Aliquots for FK180310 samples:

HILIC Aq – 20uL of H2O + 20 uL of sample

RP Aq – 30 uL of sample

CoA Aq – 30 uL of sample

20 uL of each sample was added to the Pooled Aq sample.

50 uL of each sample was added to the Pooled Org sample.

Notes:

~HILIC samples are being diluted 1 to 1. RP and CoA samples are being fun at full strength.

~The FK180310 Pooled Aq sample was diluted 1 to 1 to match all the samples.

~For HILIC and Cyano std mixes and matrix in water samples, the matrix was from the FatAs Aq B sample.

Aliquots for KM1906 samples:

HILIC Aq – 20uL of H2O + 20 uL of sample

RP Aq – 30 uL of sample

CoA Aq – 30 uL of sample

20 uL of each sample was added to the Pooled Aq sample.

25 uL of each sample was added to the Pooled Org sample.

Notes:

~HILIC samples are being diluted 1 to 1. RP and CoA samples are being fun at full strength.

~The KM1910 Pooled Aq sample was diluted 1 to 1 to match all the samples.

~For HILIC and Cyano std mixes and matrix in water samples, the matrix was from the FatAs Aq B sample.

Notes on HILIC analysis:

Tried to set-up a continuous run but had lots of issues with tubing getting clogged so the run stopped and started a few times.

Notes on RP (Cyano) analysis:

No issues with clogged tubing. Set up KM1906 run first and then shutdown over the weekend. Calibrated on Monday and set up run for FK180310 and KM1910.

Notes on CoA analysis:

Notes on Lipid LCMS analysis:

Approx. 400 mL of solvent A and B are used in 24 hours. Run had to stop periodically to calibrate QE. Stopping the run, changing solvent bottles, and topping off the solvent bottles all caused the retention times to shift. Organized samples into batches depending on when the run was stopped. Decided that the best thing was to calibrate during a blank run (have a blank running but not going to the mass spec) and topping off solvents during the blank. This seemed to minimize retention time shifts.

**Internal Standard Concentrations**

Concentrations (uM) in full strength vial!

Extraction Standards

|  |  |
| --- | --- |
| IAA | 10 |
| Taurine | 2 |
| Cysteic Acid | 2 |
| Sulfolactic Acid | 2 |
| Sulfoacetic Acid | 5 |
| Isethionic Acid | 1 |
| Tryptamine | 5 |

Injection Standards #1

|  |  |
| --- | --- |
| Tryptophan | 0.2 |
| histidine | 5 |
| alanine | 10 |
| succinic acid | 20 |
| isoluecine | 1 |
| Phenylalanine | 0.2 |
| Acetyl CoA | 2 |
| Vitamin B2 | 0.2 |
| Methionine | 2 |
| Vitamin B1 | 0.4 |
| Pyridoxal | 0.4 |
| Valine | 2 |
| Proline | 2 |
| AMP | 10 |
| GMP | 4 |
| Trehalose | 10 |
| Sucrose | 10 |
| Arsenobetaine | 2 |

Injection Standards #2

|  |  |
| --- | --- |
| Betaine, 13C5-15N | 2 |
| Adenine, 15N2 | 2 |
| Cytosine, 13C2-15N3 | 4 |
| Guanine, 13C-15N2 | 4 |
| Thymine, D4 | 12 |
| Uracil, 15N2-D2 | 16 |