Methods Optimization:

1. Does the methanolysis method w/o acidification actually result in methylation?
   1. Yes, comparing derivitized and un-derivatized runs of Tri-Mix, it is clear that this methanolysis method can derivatize TAG
      1. Compare JTB-E02-42-08 (Derivatized TAG) to JTB-E02-42-10 (underivatized Tri Mix)
2. Does the methanolysis method w/ acidification actually result in methylation?
   1. Yes, these derivatizations need to be confirmed during one single run between FAME’d and un-FAME’d but from my initial estimates this method does result in the derivatization of Tri-Mix TAGs
      1. Overlay: JTB-E02-42-10 and JTB-E02-46-09
3. How much TAG derivatization do you get with the method that uses acidification?
   1. File name: 20170208\_JTB-E02-46\_recovery\_ Tri Mix Deriv with Acid
4. How much TAG derizatization do you get with the method that does not require acidification?
   1. File name: 20170213\_JTB-E02-41\_Method Testing TriMix Standards in CH2Cl2\_inprocess

**Methanolysis Method**

1. Run derivatization methanolysis only at 15, 30, 60mins
   1. The samples at 60mins get better results
      1. Compare JTB-E02-52’s
2. Ice vs. no ice
   1. The iced samples have a better result
      1. Compare JTB-E02-52’s and JTB-E02-53’s
3. N2 drying vs. no drying
   1. Do not dry. Simply correct for large volume during calculation
4. Which initial hold temp and time work best: 30c 35c 40c
   1. 40 C works best and is easier on the instrument

**Methanolysis with Acidification:** Re-run trial under the higher acid conditions.

1. How much base is in the reaction? How much acid is needed to quench the base and push the reaction forward?
   1. After comparing the reactions mechanism for triglycerides under basic conditions and ensure there is enough acid to both quench the base in the reaction and push the rxn forward For every ***two*** mole of acid added into the solution, ***one*** mole of acid is used
      1. (Moles B/Vol B) = (moles A/Vol A)

**Lipid Layer Extraction: FOLCH**

1. Does hexanes Extract to spotless-ness?
   1. After 6 rinses 2:1 hexane:MeOH spots remain
   2. After 4 rinses 2:1 🡪 Hex spots remain
2. Does pether Extract to spotless-ness?
3. Does DCM extract to spotless-ness
   1. Checked on TLC plate
      1. 2:1 DCM and MeOH leaves spots at 4 rinses
4. SPE Clean up
   1. Cartridge rinse with 4mL of solvent DCM:MeOH(1:0,95:5,90:10,0:1)
      1. TAG rinse:
         1. Weight compared before and after compared
      2. Solvent only:
         1. Weight compared before and after compared
            1. ~40mg of weight extracted

Run Tri Mix Standard Curve

Run FAME standard curve

Run LOD and LOQ

Prepare standard matrix

Conduct power analysis