FSP Title: **Methyl esterification GC/MS Protocol**

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1.0 Background

Fatty acids are important mediators of health maintenance and disease risk. Considering their fundamental importance for human health, optimal fatty acid identification and reliable quantification assays are of major interest as well as for the exploration of metabolic fluxes.

2.0 Scope

This facility specific protocol (FSP) describes the liver sample preparation steps for analysis of Methyl esterification via GC-MS.

3.0 Materials

3.1 Equipment

- Ice
- Homogenization tubes
- Acrylic Homogenization Beads
- Homogenizer
- Microcentrifuge tubes
- Vortexer
- Centrifuge
- SpeedVac
- Autosampler vials and Crimper

3.2 Reagents

- Methanol
- Hexane
- K2CO3

4.0 Procedure

This procedure assumes there is adequate amount or volume of sample available to perform all the described analysis. The minimum volume of all the study samples should be checked ahead of

time. If there are samples with low volume, then the volumes described in this protocol can be adjusted as long as the ratios of the reagents are kept constant.

Important: Prepare a blank. Follow all steps for blank along with samples. All steps are performed ideally at 4 °C.

4.1 Sample preparation

1. Weigh 10 mg of liver (or cell pellet) into 2 ml EP tube, add 500 µl methanol (HPLC grade) and beads for grinding using tissue lyser (20 Hz, 90 s), repeat three times, interval of 3 ~ 5 minutes.
2. Take 1/5 of the homogenate, i.e. 100 µl (containing 2 mg liver) and load into 5 ml glass centrifuge tube.
3. Add 20 µl internal standard. {1 mg/ml C17, 0.5 mg/ml C23, 2 mg/ml BHT (butyl hydroxytoluene, antioxidant), in n-hexane}.
4. Add 1 ml of methanol / n-hexane (4:1) mixture.
5. Vortex, be careful not to have deposits at the bottom. Be sure to unscrew the cap before placing it in the liquid nitrogen, otherwise it is not easy to open the cap and add acetyl chloride in the liquid nitrogen bath.
6. Liquid nitrogen bath for 10 min until the appearance of crystallization on the outer wall of glass tube.
7. Add 100 µl of acetyl chloride slowly. Reacting for 2 min in liquid nitrogen.
8. 25 °C in dark for 24 hours.
9. Then 2.5 ml 6% K_2CO_3 (prepared with double distilled water) was added and air bubbles were discharged.
10. Extract with 200 ul n-hexane four times, centrifuge for 10 min at 800 g to collect the supernatant.
11. Volatilization for 12 hours to get rid of organic solvent. Add 100 µl n-hexane to the sample.

5.0 References