SOP: CASE MMPC

Lipid Analysis Assay

by GC-mass spectrometry

#CA2016 / CA2038 / CA2040

Summary:

A known quantity of tissue / plasma is hydrolyzed and extracted after adding known amounts of internal standards: eg. heptadecanoic acid and cholesterol-d_7. Fatty acids / cholesterol are analyzed as their trimethylsilyl derivatives using gas chromatography-electron impact ionization mass spectrometry (GCMS) (note: this protocol outlines the processing for palmitate and cholesterol; other fatty acids and sterols can be assayed using this preparation, see refs 1,2).

Reagents/Materials:

Reagent/Material	Quantity Required	Vendor
KOH / Ethanol	1mL	stock
Heptadecanoic acid and cholesterol-d_7	25µL 25µL	Sigma Aldrich
HCl	50µL	stock
Chloroform	300µL	stock
*TMS	60µL	Regis

*bis(trimethylsilyl) trifluoroacetamide+ 1% trimethylchlorosilane (Regis, Morton Grove, IL) (TMS)

Protocol:

- For total bound lipids/cholesterol follow steps 1-12
- For free lipids/cholesterol weigh tissue as in step 1 (use 1ml HCL 6N in place of KOH ethanol solution- omit step 4, *do not heat*), then proceed to step 6-12

1. Pipette 1ml KOH ethanol solution (1N KOH in 70% EtOH) for every 100mg of tissue or 100 µl of plasma use glass screw top tubes (may use less tissue/plasma)

2. Internals standards (IS): add 25 µl of 1mg/ml heptadecanoic acid (C17:0) and cholesterol-d_7 for every 100µl of tissue (note: adjust added amount of internal standards by testing a representative sample)

3. Homogenize on ice with polytron homogenizer (tissue only)

4. Cover and heat for 3 hours at 85^{o}C

5. Pipette 50-100µl of solution into an Eppendorf tube

6. Add 50µl of 6N HCl

7. Add 300µl of Chloroform

8. Vortex and centrifuge for 2 minutes

9. Take 200µl of chloroform phase (bottom layer) and dry in GC vial at 75^{o}C

10. React with 60µl of TMS, cover, heat at 75^{o}C for 20 minutes

11. Transfer to GC insert and cap

GC-MS Analysis: Lipid TMS derivatives are analyzed using an Agilent 5973N-MSD equipped with an Agilent 6890 GC system, and a DB-17MS capillary column (30 m x 0.25 mm x 0.25 um). The mass spectrometer is operated in the electron impact mode (EI; 70 eV).

12. Selective ion monitoring of mass-to-charge ratios (m/z)
    a. Palmitate: M0=313-M+; IS, C:17=327
    b. Cholesterol: M0=368-M+; IS, Cholesterol-d_7=375
    c. For other lipids: see references

References:

1. Triglyceride synthesis in epididymal adipose tissue: contribution of glucose and non-glucose carbon sources. Bederman IR, Foy S, Chandramouli V, Alexander JC, Previs SF. J Biol Chem. 2009, 284(10):6101-8.

2. Influence of diet on the modeling of adipose tissue triglycerides during growth. Brunengraber DZ, McCabe BJ, Kasumov T, Alexander JC, Chandramouli V, Previs SF. Am J Physiol Endocrinol Metab. 2003, 285(4):E917-25.