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Case - Lipid Analysis Assay by GC-mass spectrometry

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Protocol status: Working
We use this protocol and it's working

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Abstract

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).

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.

Materials

MATERIALS

Heptadecanoic acid and cholesterol-d7 **Merck MilliporeSigma (Sigma-Aldrich)**

*TMS **Regis Technologies, Inc.**

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)

Note:
Sigma-Aldrich, RRID:SCR_008988

Before start

• 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-d7 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 85ºC

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 75ºC

10 React with 60µl of TMS, cover, heat at 75ºC 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