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 We use this protocol and it's working

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Case - Lipid Analysis Assay by GC-mass spectrometry

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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-d₇. 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.

Keywords: Lipid Analysis, GC-mass spectrometry

MATERIALS

MATERIALS

⊗ Heptadecanoic acid and cholesterol-d7 **Sigma Aldrich**

⊗ *TMS **Regis**

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 INSTRUCTIONS

• **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 85°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°C
- 10 React with 60µl of TMS, cover, heat at 75°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 µm).

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