SOP: CASE MMPC

Glycerol and Glucose Assays:

by GC-mass spectrometry

Chemical Ionization (CI) Mode

#CA2015

Also CA2020 / CA2022 / CA2024 / CA2024CT

Summary:

Total glycerol is determined by first hydrolyzing glycerides (in plasma or tissues) in 1 N KOH-ethanol + heat and then the hydrolysate extracted following acidification ~pH 1.0. A two phase extract is created using chloroform. The aqueous phase contains the total glycerol and the organic phase contains total fatty acids. An aliquot of aqueous phase in dried down by nitrogen gas and the concentration and/or ^{2}H-labelled glycerol is then determined by GC-MS following conversion of glycerol to its triacetate derivative.

Glucose assay involves extraction of glucose from blood, plasma or tissues and the concentration and/or $^{2}H/^{13}C$-labelled glucose is also determined by GC-MS following conversion to its pentaacetate derivative. Since the derivatizing methods are the same for glucose as glycerol, the can be assayed from a single sample preparation under the same GC-MS conditions and single run-time.

Total / **Free Glycerol Assay

Reagents/Materials:

Reagent/Material	Quantity Required	Vendor
*internal standard (IS): [$^{2}H_5$]glycerol	20 µL [0.5 mM]	Sigma Aldrich
HCl	50µL	stock
Chloroform	300µL	stock
Pyridine	50µL	stock
Acetic Anhydride	50µL	stock

Protocol:

1. Place 20µl of plasma sample or 50 mg tissue into glass, screw-top tube
2. Add ~20µl of IS [$^{2}H_{5}$]glycerol, for concentration measurements
3. For total glycerol: add 200µl of KOH-EtOH (1M KOH in 70% ethanol)
4. Heat at 80^{o}C for 3 hours on a heating block to hydrolyze glyceride esters
5. Then add 50µl of 6N HCl
6. Add 300µl of chloroform, vortex for 2 min and then transfer to Eppendorf
7. Centrifuge for 2 min
8. Remove top aqueous layer and place in test tube and dry under nitrogen gas
9. React the dry residue with 50µl pyridine and 50µl acetic anhydride (100 µL of pyridine: acetic anhydride, 1:1) on heating block for until dry (don't over dry)
10. Then add 70 µL ethyl-acetate, as running solvent for GC-MS
11. Transfer to GC insert, follow GC parameters as outlined below (1)
12. **For free glycerol: omit steps #3,#4 and ~20µl 6N HCL

Glucose Assay

Reagents/Materials:

Reagent/Material	Quantity Required	Vendor
Internal standard (IS): U^{13}C or ^{2}H labelled glucose	10µL [5mM]	Sigma Aldrich
Methanol	250µL	stock
Pyridine	50µL	stock
Acetic Anhydride	50µL	stock

Protocol:

1. Place 10µl of blood sample in Eppendorf
2. Add 10µl of a 1mg/ml of IS U^{13}C or ^{2}H labelled glucose for concentration
    a. may use ribose as an internal standard for 13C glucose labelling assays
3. Add 250µl of methanol to Eppendorf
4. Centrifuge for ~2 minutes
5. Place supernatant in test tube and dry under nitrogen gas

6. React the dry residue with 50µl pyridine and 50µl acetic anhydride (100 µL of pyridine: acetic anhydride, 1:1)

7. Heat on heating block at 80^{o}C, until dry (do not over dry)

8. Add 70 µL ethyl-acetate, as running solvent for GC-MS

9. Transfer to GC insert, follow GC parameters as outlined below (1)

GC-MS Analysis: glycerol and/or/ glucose 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 under ammonia chemical ionization mode ionization, and selective ion monitoring (SIM) of NH_4 adducts are performed: For glycerol-triacetate, SIM *m*/*z* 236, 237, and 238 (M_0,M_1 and M_2) and for glucose-pentaacetate, SIM, *m*/*z* 408–414 (M_0 to M_6)

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.;284(10):6101-8 (2009)