

Laboratory Methods Documentation

Dan Raftery Laboratory
Metabolomics
GC-GC-MS Analysis
LECO Agilent 6890

Sample Preparation Method
Submitted by Lingyan Liu 11/28/2007

For the GC-GC-MS analysis we will run 30 samples each day, but need to include 6 extra samples for standardization and monitoring of the instrument.

Sample preparation for GC:

1. Add 200 μ l Serum to eppendorf tube
2. Add 400 μ l methanol (MeOH), vortex briefly and place in -20 °C freezer for 30 minutes.
3. Remove the sample from the freezer, spin down while cold @14,000 RPM for 10 minutes using a bench top centrifuge.
4. Transfer the methanol supernatant to eppendorf tube.
5. Add 200 μ l Chloroform (CHCl_3) to protein pellet, vortex, spin down @14,000 RPM for 10 minutes.
6. Remove the CHCl_3 supernatant and combine with MeOH supernatant from step 4 so that total volume is ~ 600 μ l.
7. Dry the combined supernatant from step 6 using SpeedVac about 90 minutes and store at -80 °C.

TBDMS (ter-butyldimethylsilyl)Derivatization: Murthy

1. Add 50 μ l of anhydrous pyridine via syringe to the residue got after SpeedVac and vortex briefly.
2. Transfer 20 μ l to the fresh eppendorf tube and freeze the remaining 30 μ l for back-up use.
3. Add 20 μ l MTBSTFA to the 20 μ l pyridine solution from step 2 and heat @ 60 °C for 1 hour.
4. Transfer the content to a glass GC auto sampler vial and submit for GC-GC-MS

Sample preparation (30 samples, 6 control samples)	3 hours
Derivatization (30 samples, 6 control samples)	3 hours
GC-GC-MS run (36 total samples)	27 hours
GC-GC run goes as follows:	
2 blank samples	
1 composite sample	
10 real samples	
1 composite sample	
10 real samples	
1 composite sample	
10 real samples	
1 composite sample	
TOTAL: 36	