Chemicals needed:

* Chloroform:methanol(1mM BHT) (2:1, v:v) on ice (Folch solvent)
* water on ice
* chloroform:methanol (2:1, v:v) on ice
* Reconstitution Solution: isopropanol (IPA)
* Mobile phases, 90:8:2 isopropanol:acetonitrile:water (IPA:ACN:water) with 10 mM ammonium formate and 0.1% Formic Acid and 60:40 ACN:Water with 10 mM ammonium formate and 0.1% Formic Acid LC/MS grade
* Internal standards dissolved in chloroform:methanol (2:1, v:v) as stock prior to mixing into Folch solvent
  + Cer(d18:1/17:0), 0.5 µM (Avanti Polar Lipids, Inc)
  + d5-DG mix I (Avanti Polar Lipids, Inc)



Materials needed:

* Labeled 2 mL Eppendorf tubes
* Homogenization tubes with zirconium oxide (1.0 mm diameter) beads
* Repeater Pipette
* Calibrated Micropipettes in various volumes\* (see table below)
* Appropriate Micropipette tips\* (see table below)
* Refrigerator
* Refrigerated Centrifuge
* Vortex
* N2 Dryer
* Labeled LC vials with appropriate caps or 96-well tray
* LC-MS
* Waters Acquity C18 BEH (50 x 2.1 mm, 1.7 µm) with guard
* Positive Calibration Solution
* Negative Calibration Solution
* Personal Protective Equipment

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| **Type** | **Volumes (μL)** | **Tip color** |
| P10 | 0.5 – 10 | white |
| P20 | 2 – 20 | yellow |
| P200 | 20 – 200 | yellow |
| P1000 | 200 – 1000 | blue |

Precise Micropipette Volume and Transfer capabilities



Procedure:

1. Mass out 15 mg of tissue sample to clean, homogenization tube with zirconium beads.
2. Record mass of tissue and place back in liquid nitrogen while other samples are weighed
3. Add internal standards: 200 µL of 0.5 µM Cer (d18:1/17:0), and 15 µL of 20 µM d5-DG mix I
4. Add Folch solvent based on the volume of the tissue (µL of solvent = 20 x mg of tissue, e.g., 15.0 mg of tissue = 300 µL solvent)
5. Homogenize for 120 seconds total (may need to include breaks depending on homogenizer)
6. Incubate on ice with occasional vortex for 20 mins
7. Add water at a volume of ¼ the Folch solvent volume (e.g., 75 µL for 15.0 mg tissue)
8. 10 minutes of incubation on ice with occasional vortex
9. Centrifuge at 20,000 rcf for 5 minutes at 6 °C
10. Collect 170 µL organic phase (bottom layer) from each tube into new, clean centrifuge tube
11. Re-extract remaining phases with chloroform:methanol (2:1, v:v) at 50% the volume added in step 4. Vortex and centrifuge.
12. Incubate on ice 10 mins
13. Remove 75 µL of new organic phase (bottom layer) and combine the two organic phases
14. Dry under nitrogen at 30 °C
15. Reconstitute sample by adding 500 µL IPA
16. Add 25 µL of this to corresponding LC vial containing injection standard (dried)
17. Add 975 µL isopropanol for injection
18. Load samples into auto sampler







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| Created By: | Antoine Ducrocq | Date:05/27/2014 |
| Reviewed By: | Rainey Patterson | Date:05/28/2014 |
| Approved By: |  | Date: |

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| --- | --- | --- | --- |
| Revision Number | Name | Reason for Revision | Effective Date |
| 01 | Antoine Ducrocq | Creation of SOP | 05/29/2014 |
| 02 | Rainey Patterson | Change from Matyash to Folch  Removed instrument info, just sample prep | 11/16/2015 |