Sample preparation for Permethylated GSL profiling by MALDI/TOF-MS and NSI-MSn

Date written: 10/8/18

Authors: Kazuhiro Aoki* and Mayumi Ishihara
Complex Carbohydrate Research Center, University of Georgia, Athens, GA 30602
*To whom correspondence should be addressed; Kazuhiro Aoki (kaoki@ccrc.uga.edu)

Reagents:
- Methanol (MeOH)
- α-dihyroxybenzoic acid (DHBA)
- HPLC Water
- Sodium Hydroxide Solution, 50% w/w
- Iso-propanol (2-PrOH)
- n-propanol (1-PrOH)

Equipment/Apparatuses:
- Glass culture tubes (round bottom)
- 500 uL Microcentrifuge tube or glass inserts for GC autosampler vial
- GC glass autosampler vial (conical bottom)
- 1.5 mL Microcentrifuge tube
- Glass pipette
- Plastic pipette
- Glass syringe
- Centrifuge
- Vortex

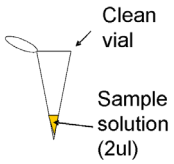
Procedures:

MALDI/TOF-MS analysis

MALDI/TOF-MS analysis of permethylated glycosphingolipids is performed in the linear or reflector positive ion mode using α-dihyroxybenzoic acid (DHBA, 20mg/mL solution in 50%methanol: water) as a matrix. The procedures are described in detail below.

1. Prepare Matrix solution; 20mg α-dihyroxybenzoic acid (DHBA) in 1mL of 50% methanol: water in a 1.5 mL Microcentrifuge tube

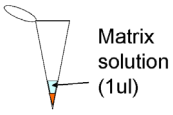
2. Dissolve the permethylated sample with 20~µL (Adjust the volume depends on your sample amount) of methanol using a clean glass micro syringe (rinsed with Methanol).



3. Take 1~2 µL of sample solution using a clean glass micro syringe (rinsed with Methanol), and then transfer into the bottom of a small vial.

4. Wait until the solution volume becomes approximately 1µL (~3 min).

5. Take 1µL of matrix solution using a micro pipet and then add into the sample solution.



6. Immediately mix them by pipetting the solution several times.

7. Take 1µL of mixture and then put on a well of a MALDI plate. Immediately, take another 1ul of mixture then put on another well.

8. Wait until the mixtures on the plate dry.

Note:

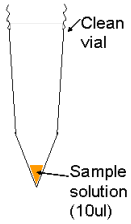
- The matrix solution is preservable at -20^{o}C. Defrost the matrix solution before use.
- A good crystallized sample is necessary to get a strong signal. However, making a good crystal is not always possible, hence, we recommend making at least two crystals per sample.

<u>NSI-MSn analysis</u>

Mass analysis by NSI-MS are performed by direct infusion of permethylated glycosphingolipids dissolved in infusion buffer the instrument at a constant flow rate of 0.4~1 µL/min via a syringe pump. The procedures are desrcibed in detail below.

1. Prepare infusion buffer solution: Methanol/2-Propanol/1-Propanol/1 mM NaOH (16:3:3:2 by volume)

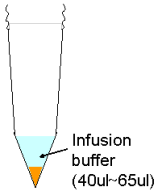
2. Dissolve the permethylated GSLs with 20~µL (Adjust the volume depends on your sample amount) of methanol using a clean glass micro syringe (rinsed with methanol).



3. Take 10 µL of sample solution using a clean glass micro syringe (rinsed with methanol), and then transfer into a small vial (rinsed with methanol).

4. Add known concentration of heavy MeI permethylated Dp4 as a reference standard if quantification is desired (Optional).

5. Take 40~65µL of the infusion buffer and then add to the sample solution.



6. Screw the cap, mix them by vortexing, and briefly centrifuge the vial.