Ceramide Profile
Service Code: Ceramides

Summary: Profile 8 ceramide species by LLE of tissue samples, separated on a 2.1mm x50mm Biphenyl column in a 20 min cycle. All analytes and Internal Standards are measured by ESI^+ ionization on a LC-QQQ mass spectrometer using MRM methods and reported as ng/ul and normalized to wet tissue weight. CV's are generally 15%.

Container: Eppendorf tube
Normal Volume: 20-50mg
Minimal Volume: 5mg
Special Handling: If human or primate, note any known presence of infectious agents
Sample Collection: Resect and snap-freeze as soon as practical in tared centrifuge tube. Provide both sample weight and tared vial weight on sample submission

Reference: Takhar Kasumov, Hazel Huang, Yoon-Mi Chung, Renliang Zhang, Arthur J. McCullough, and John P. Kirwan (2010) "QUANTIFICATION OF CERAMIDE SPECIES IN BIOLOGICAL SAMPLES BY LIQUID CHROMATOGRAPHY-ELECTROSPRAY TANDEM MASS SPECTROMETRY", Anal Biochem 401(1): 154–161.

Table I: Analytes reported. Others such as glucosylceramides on special request:

Analyte	Abbr.	MRM	LOQ (ng/ul)
C-14 ceramide	C14	492.4→264.2	0.1
C-16 ceramide	C16	520.4→264.2	0.1
C-18:0 ceramide	C18	548.4→264.2	0.1
C-18:1 ceramide	C18:1	546.4→264.2	0.1
C-20 ceramide	C20	576.4→264.2	0.1
C-22 ceramide	C22	604.4→264.2	0.1
C-24 ceramide	C24	632.5→264.2	0.1
C-24:1 ceramide	C24:1	630.5→264.2	0.1

Table II: Internal standards and corresponding analytes

Internal Standards	Source	Cat#	Analytes quantified	ug/ml
Ceramide (C25)	Avanti	LM-2225	C20, C22, C24, C24:1 ceramide	2
Ceramide (C17)	Avanti	860517P	C14, C16, C18:1, C18:0 ceramide	2

Materials

1. Ceramide authentic standards and stable-isotope labeled internal standards (see Tables I & II)
2. LC/MS grade water, acetonitrile (ACN), isopropanol (iPOH)

3. ACS grade methanol, chloroform, ammonium acetate, ammonium hydroxide
4. N_2 drying/heating block
5. Bullet Blender GOLD with appropriate beads and protocol for tissues to be analyzed OR: Branson Sonifier 450 probe sonicator (narrow tip) using 20% duty cycle
6. Benchtop Centrifuge
7. Accurate pipettors (1 uL-1000 uL)
8. Microbalance
9. Vortex mixer
10. Agilent 6410 triple quad mass spectrometer
11. Agilent 1260 LC System

PROCEDURES:

Extraction solvent preparation:

1. Mix 1 volume of chloroform and 2 volumes of methanol in a glass vial, sufficient to extract all samples
2. Add 1.5ml of IS mix to 15ml of H_2O

Tissue Sample Preparation

1. Samples already weighted in labeled eppendorf tube
2. Add 550 uL of H_2O-IS mixture, vortex to mix, homogenize samples using proper method.
3. Do the following steps for all samples and standards
4. Transfer to autosampler vial, add 800 uL of 2:1 MeOH:$CHCl_3$, vortex to mix, incubate 5min,vortex again.
5. Add 300 uL of $CHCl_3$, vortex to mix, incubate 5min
6. Collect the bottom layer with a Pasteur pipette and transfer to a clean glass vial.
7. Dry under UHP N_2 at room temperature
8. Re-constitute in 100 uL of Mobile Phase Buffer B

LC-MS procedure

1. LC column: Xbridge C18 2mm x 50mm; 40 °C
2. Mobile phase A: 5 mM ammonium acetate in water, adjust to pH 9.9 with ammonium hydroxide
3. Mobile phase B: 3 volumes acetonitrile (ACN): 2 volumes isopropanol (iPOH)
4. Gradient: 0min, 50%B, 5min, 100%B, 25min, 100%B, 25.1min, 50%B, 35min, 50%B; flow rate: 200ul/min
5. Autosampler: 4°C, 2 uL injection
6. Agilent 6410 QQQ: ESI^+, Method: **QM-0002** or equivalent
   Collect standard curve data first, then sample data if system is suitable.

Quantification:

Internal standard mixture is spiked in samples and calibration standards. External calibration curve is constructed from calibration standards and it is used to calculate metabolite concentrations in biological samples.