**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](http://www.ncbi.nlm.nih.gov/pubmed/?term=Kasumov%20T%5Bauth%5D), [Hazel Huang](http://www.ncbi.nlm.nih.gov/pubmed/?term=Huang%20H%5Bauth%5D), [Yoon-Mi Chung](http://www.ncbi.nlm.nih.gov/pubmed/?term=Chung%20YM%5Bauth%5D), [Renliang Zhang](http://www.ncbi.nlm.nih.gov/pubmed/?term=Zhang%20R%5Bauth%5D), [Arthur J. McCullough](http://www.ncbi.nlm.nih.gov/pubmed/?term=McCullough%20AJ%5Bauth%5D), and [John P. Kirwan](http://www.ncbi.nlm.nih.gov/pubmed/?term=Kirwan%20JP%5Bauth%5D) (2010) "QUANTIFICATION OF CERAMIDE SPECIES IN BIOLOGICAL SAMPLES BY LIQUID CHROMATOGRAPHY-ELECTROSPRAY TANDEM MASS SPECTROMETRY", [Anal Biochem 401(1): 154–161.](http://www.ncbi.nlm.nih.gov/entrez/eutils/elink.fcgi?dbfrom=pubmed&retmode=ref&cmd=prlinks&id=20178771)

**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. N2 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 H2O

**Tissue Sample Preparation**

1. Samples already weighted in labeled eppendorf tube
2. Add 550 uL of H2O-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:CHCl3, vortex to mix, incubate 5min,vortex again.
5. Add 300 uL of CHCl3, 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 N2 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.