VERSION 2

DEC 20, 2023

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.261gedk8wv47/v2

Protocol Citation: Tae-Un Han, Carley Corado 2023. Glucosylceramide and glucosylsphingosine analysis. **protocols.io** https://dx.doi.org/10.17504/protocols.io.261gedk8wv47/v2 Version created by Tae-Un Han

Protocol status: Working
We use this protocol and it's working

Glucosylceramide and glucosylsphingosine analysis V.2

Tae-Un Han[1], Carley Corado[2]

[1]National Institute of Health; [2]BioMarin Pharmaceutical, Inc.

Tae-Un Han

ABSTRACT

This protocol was used to analysis glucosylceramide and glucosylsphingosine levels in mouse brain and liver.
It was also described in previous publication (Mol Cell Neurosci. 2020 Jan:102:103451, doi: 10.1016)

MATERIALS

1. Bead Ruptor 24 (Omni International, Kennesaw, GA)
2. Shimadzu (Columbia, MD) Prominence HPLC system coupled with an Applied Biosystems/MDS Sciex (Ontario, Canada) 4000QTRAP mass spectrometer using multiple reaction monitoring (MRM)
3. The HPLC system consists of a CBM-20A system controller, 2 LC-20 CE pumps, a SIL-20ACHT autosampler, and a DGU-20A5R degasser.
4. Ascentis® Express HILIC (4.6 × 50 mm, 2.7 µm, Supelco, Bellefonte, PA) protected with a HILIC Securityguard™ column (4 × 3.0 mm, Phenomenex, Torrance, CA)
5. SupelcosilTM LC-Si (2.1 × 250 mm, 5 µm, Supelco, Bellefonte, PA) protected with a HILIC Securityguard™ column (4 × 3.0 mm, Phenomenex, Torrance, CA)

Created: Dec 20, 2023

Last Modified: Dec 20, 2023

PROTOCOL integer ID: 92584

Keywords: ASAPCRN

Funders Acknowledgement:
ASAP
Grant ID: ASAP-000458

Tissue preparation

1 The mouse brain and liver tissues (100-300 mg) are homogenized in 2% CHAPS solution (4 mL/g wet tissue) in 2 mL Omni homogenization tubes containing 8 mm ceramic beads

2 The homogenates are processed on the Bead Ruptor 24 (Omni International, Kennesaw, GA) for two 30 second cycles at 5.65 m/s with a 45 second pause time.

liquid chromatography-tandem mass spectrometry

3 The brain internal standards including d5-GluCer(18:0) (250 ng/mL) for GluCer and d5-GluSph (200 ng/mL) for GluSph are prepared in acetonitrile solution.

4 The samples are vortexed for approximately 3 min and then centrifuged at 10,000 rpm for 10 min. The supernatants are transferred to 1.2 mL glass inserts (VWR, West Chester, PA) in 96 well plates.

5 A quality control (QC) sample is prepared by pooling 20% of extracts from study samples and used to monitor the instrument performance.

6 The crude extracts are directly injected to LC–MS/MS system for analysis of GluSph and GluCer.

Injection volumes for CSF and and brain homogenates are 100 µL and 5 µL, respectively.

7 LC–MS/MS analysis is conducted on a Shimadzu (Columbia, MD) Prominence HPLC system coupled with an Applied Biosystems/MDS Sciex (Ontario, Canada) 4000QTRAP mass spectrometer using multiple reaction monitoring (MRM).

8 The HPLC system consists of CBM-20A system controller, 2 LC-20 CE pumps, a SIL-20ACHT autosampler, and a DGU-20A5R degasser. The compartment of the autosampler is set at 4 °C.

9 For analysis of GluSph, the chromatography was performed at ambient temperature using Ascentis® Express HILIC (4.6 × 50 mm, 2.7 µm, Supelco, Bellefonte, PA) protected with a HILIC Securityguard™ column (4 × 3.0 mm, Phenomenex, Torrance, CA)

10 Mobile phase A (0.1% formic acid and 1 mM ammonium formate in water) and mobile phase B (0.1% formic acid and 1 mM ammonium formate in acetonitrile-water (95:5)) are operated with a gradient elution as follows: 0–0.2 min 100–95% B, 0.2–3.5 min 95% B, 3.5–3.9 min 95–90% B, 3.9–5.8 min 90% B, 5.8–5.9 min 90–10% B, 5.9–6.9 min 10% B, 6.9–7.0 min 10–100% B, and 7.0–8.8 min 100% B at a flow rate of 1.5 mL/min.

11 The HPLC flow is diverted to waste except for 2.0–6.5 min to mass spectrometer.

12 The ESI source temperature is 600 °C; the ESI needle is 5000 V; the declustering potentials for GluSph is 76 V, respectively; both the entrance potential and the collision cell exit potential are 10 V for all compounds.

13 The collision and curtain gas are set at medium and 20, respectively.

14 Both desolvation gas and nebulizing gas are set at 45. For MRM, the collision energies for mass transitions of m/z 462.3 to 282.3 for GluSph and that for d5-GluSph are 31, 26, 38, and 31 V,

respectively.

15 The dwell time is set at 50 ms for each mass transition

16 For analysis of GluCer, the chromatography is performed at ambient temperature using Supelcosil™ LC-Si (2.1 × 250 mm, 5 µm, Supelco, Bellefonte, PA) protected with a HILIC Securityguard™ column (4 × 3.0 mm, Phenomenex, Torrance, CA).

17 Mobile phase A (5 mM ammonium acetate in water) and mobile phase B (5 mM ammonium acetate in acetonitrilemethanol-acetic acid (97:2:1)) are operated with a gradient elution as follows: 0–4.0 min 100% B, 4.0–4.1 min 100–95% B, 4.1–6.5 min 95% B, 6.5–6.6 min 95–10% B, 6.6–7.6 min 10% B, 7.6–7.7 min 10–100% B, 6.9–7.0 min 10–100% B, and 7.0–10 min 100% B at a flow rate of 1.5 mL/min.

18 The HPLC flow is diverted to waste except for 2.0–6.5 min to mass spectrometer

19 The ESI source temperature is 550 °C; the ESI needle is 5000 V; the declustering potentials for GluCer is 75 V; both the entrance potential and the collision cell exit potential is 10 V for all the compounds.

20 The collision and curtain gas are set at medium and 20, respectively.

21 The desolvation gas and nebulizing gas are set at 35 and 55, respectively.

22 For MRM, the collision energies for mass transitions of m/z 700.5 to 264.3 for GluCer(16:0), m/z 728.5 to 264.3 for GluCer(18:0), m/z 756.5 to 264.3 for GluCer(20:0), m/z 784.6 to 264.3 for GluCer(22:0), m/z 812.7 to 264.3 for GluCer (24:0), m/z 810.7 to 264.3 for GluCer(24:1).

23 The dwell time is set at 50 ms for each mass transition.

24 Data processing is conducted with Analyst 1.5.1 (Applied Biosystems).

25 The signal of noise ratio of analyte < 3 is defined as below limit of detection (LOD).

26 The relative quantification data are obtained as peak area ratios of analytes to their internal standards, which are converted to concentrations.