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We use this protocol and it's working

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# Glucosylceramide and glucosylsphingosine analysis V.1

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)

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## Tissue preparation

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

2 The homogenates were 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 glucosylsphingosine and glucosylceramide in homogenate (50 µL) were extracted and analyzed

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

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

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

7 The crude extracts were directly injected to LC–MS/MS system for analysis of GluSph and GluCer. CSF and GluSph in brain homogenates and the injection volumes were 100 µL and 5 µL, respectively.

8 LC–MS/MS analysis was 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)

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

10 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)

11 The compartment of the autosampler was set at 4 oC.

12 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)) were 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.

13 The HPLC flow was diverted to waste except for 2.0–6.5 min to mass spectrometer.

14 The ESI source temperature was 600 °C; the ESI needle was 5000 V; the declustering potentials for GluSph and d5-GluSph were 76, 76, 76, 76, and 80 V, respectively; both the entrance potential and the collision cell exit potential were 10 V for all compounds.

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

16 Both desolvation gas and nebulizing gas were 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 were 31, 26, 38, and 31 V, respectively.

17 The dwell time was set at 50 ms for each mass transition

18 For analysis of GluCer, the chromatography was 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).

19 Mobile phase A (5 mM ammonium acetate in water) and mobile phase B (5 mM ammonium acetate in acetonitrilemethanol-acetic acid (97:2:1)) were 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.

20 The HPLC flow was diverted to waste except for 2.0–6.5 min to mass spectrometer

21 The ESI source temperature was 550 °C; the ESI needle was 5000 V; the declustering potentials for GluCer, GalCer, and DihexCer were 75, 75, and 80 V, respectively; both the entrance potential and the collision cell exit potential was 10 V for all the compounds.

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

23 The desolvation gas and nebulizing gas were set at 35 and 55, respectively.

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

862.7 to 264.3 for DihexCer(16:0), m/z 890.7 to 264.3 for DihexCer (18:0), m/z 918.7 to 264.3 for DihexCer(20:0), m/z 946.7 to 264.3 for DihexCer(22:0), m/z 974.8 to 264.3 for DihexCer(24:0), m/z 972.7 to 264.3 for DihexCer(24:1), m/z 733.5 to 269.3 for d5-GluCer(18:0), and m/z 865.7 to 264.3 for d3- LacCer(16:0) were 50, 50, 50, 50, 50, 50, 61, 67, 67, 67, 67, 50 and 61 V, respectively.

25 The dwell time was set at 50 ms for each mass transition.

26 Data processing was conducted with Analyst 1.5.1 (Applied Biosystems).

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

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