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Glucosylceramide and glucosylsphingosine analysis V.2

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We use this protocol and it's working

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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-20AHT 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. 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)

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 brain homogenates are 100 μ L and 5 μ L, 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-20AHT 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 $\times$ 50 mm, 2.7 μ m, Supelco, Bellefonte, PA) protected with a HILIC Securityguard[™] column (4 $\times$ 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.

Protocol references

Mol Cell Neurosci. 2020 Jan;102:103451, doi: 10.1016