



Article

High-throughput Liquid Chromatography-Tandem Mass Spectrometry Quantification of Glycosaminoglycans as Biomarkers of Mucopolysaccharidosis II

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Appendix A – Supplemental data

Table S1. Acquisition parameters and retention time (RT) for GAG species on QTRAP 6500+.

Figure S1. HS and DS disaccharides by acid hydrolysis.

Figure S2. Methanolysis and Butanolysis LC-MS/MS of HS and DS in standard and CSF.

Figure S3. LC-MS and high-resolution MS/MS of HS by butanolysis using both butanol (B) and deuterated butanol (B-d10).

Figure S4. LC-MS and high-resolution MS/MS of DS by methanolysis using methanol-d4.

Figure S5. LC-MS/MS of DS (D0a4), HS (D0A0 and D0S0) in 40,000 FACS isolated neurons.

Figure S6. HS and DS released by Enzymatic digestion using Heparitinase or chondroitinase B.

Table S1. Acquisition parameters and retention time (RT) for GAG species on QTRAP 6500+.

GAG Name	Internal Standard	RT (min)	Q1 m/z	Q3 m/z	Time (msec)	CE	DP
D0A0	4UA-2S-GlcNCOEt-6S	4.02	378.1	87.0	50	-30	-80
D0S0	4UA-2S-GlcNCOEt-6S	4.70	416.1	138.0	50	-30	-80
D0a4	4UA-2S-GlcNCOEt-6S	4.16	458.1	300.0	50	-30	-80
4UA-2S-GlcNCOEt-6S	N/A	4.3	472.0	97.0	50	-28	-80

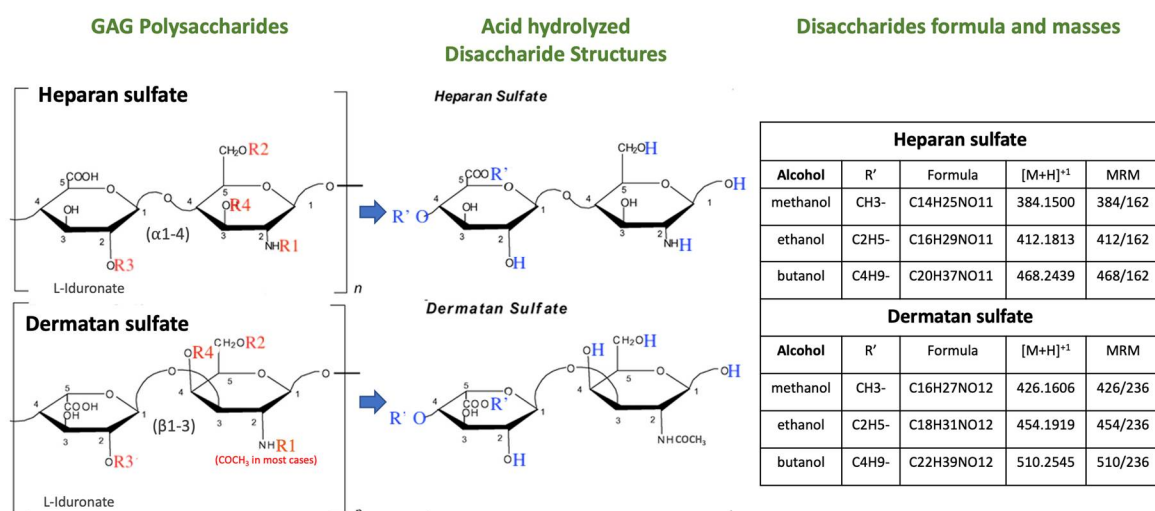


Figure S1. HS and DS disaccharides by acid hydrolysis. The hydrolysis with either methanol, ethanol or butanol depolymerization could reduce the chemical diversity of GAG species, for example, HS or CS/DS to a corresponding single small molecule disaccharide through the desulfation/deacetylation and dialkylated reactions. The structure, formula, detection mass in positive mode and the MRM are listed. R1-R4 in red are one of the following H, AC or SO³⁻ groups; R' in blue corresponds to the alcohol group that is used with acid for the hydrolysis reaction, and could be Me, Et, or Bu. After hydrolysis, the R is eliminated and R' is generated depending on the alcohol, the diversity is reduced to uniform product in this way.

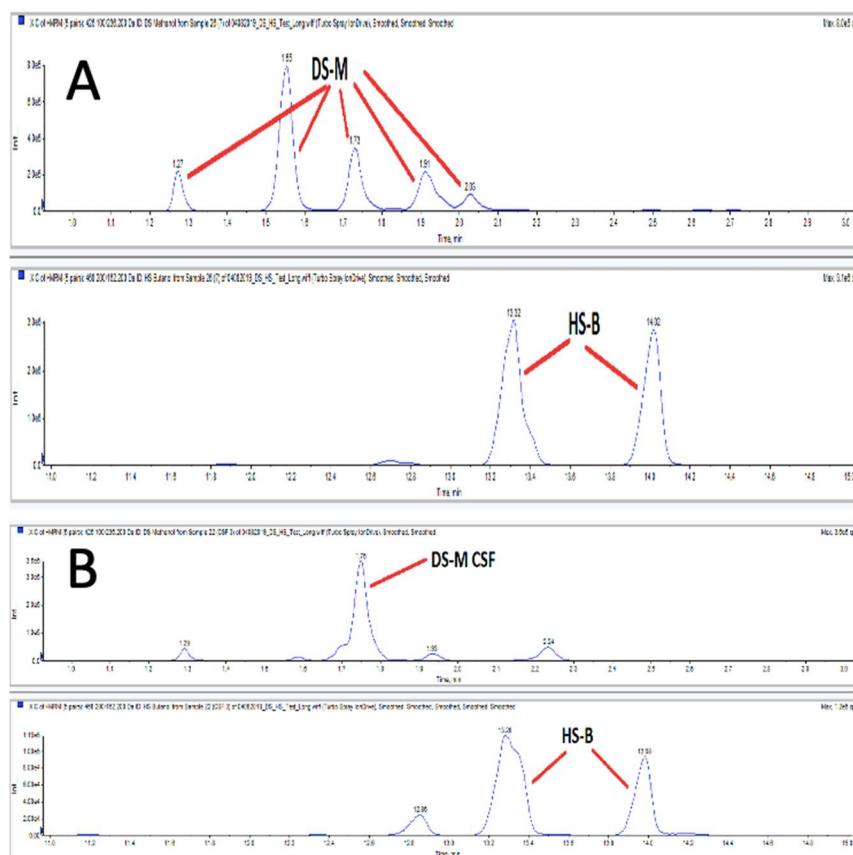


Figure S2. Methanolysis and Butanolysis LC-MS/MS of HS and DS in standard and CSF. (A). Standard. (B) CSF. Methanolysis Method: Sample preparation: 10 μ L of fluid was evaporated to dryness. 200 μ L methanolic HCL and 10 μ L 2,2-dimethoxypropane was added to each well. Incubated at 65 $^{\circ}$ C for 75 min, evaporated to dryness and reconstituted with water 0.1% formic acid. Butanolysis Method: Sample preparation: 10 μ L of fluid was evaporated to dryness. 100 μ L of 3 N HCL in N-Butanol was added to each tube. Incubated at 65 $^{\circ}$ C for 25 min (HS) and 90 $^{\circ}$ C for 60 min (DS), evaporated to dryness and reconstituted with water/acetonitrile 1/1. LC-MS/MS Method: Mobil Phase A: Water 20 mM Ammonium formate, Mobil Phase B: Acetonitrile 0.1% formic acid. Column-Supelco Ascentis RP-Amide (10cm x 4.6 mm, 3 μ m). Gradient- 5% B hold 30 seconds, ramp to 95% over 4 minutes. Mass Spec: Sciex QTRAP 5500. MRM: for HS-B: m/z 468 > 162 (positive ion), for DS-M: m/z 426 > 236 (positive ion).

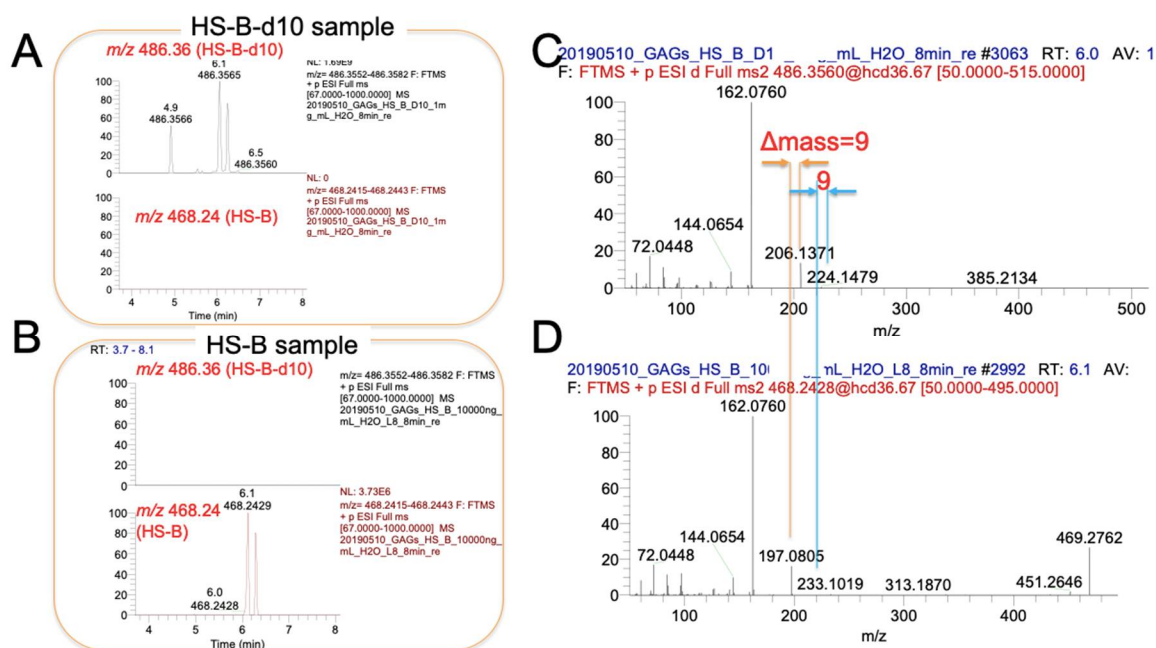


Figure S3. LC-MS and high resolution MS/MS of HS by butanolysis using both butanol (B) and deuterated butanol (B-d10). A) HS-B-d10 and B) HS-B peaks. Two peaks were detected for each sample. C) MS/MS for HS-B-d10, and D) MS/MS of HS-B. Both spectra confirmed the identities of the reactants. The HS-B spectrum verified the parent/dominant fragment masses are 468/162, which matches to the listing in Figure S3. LC-MS/MS Method: Mobil Phase A: Water 20 mM Ammonium formate, Mobil Phase B: Acetonitrile 0.1% formic acid. Column-Supelco Ascentis RP-Amide (10cm x 4.6 mm, 3 μ m). Gradient- 5% B hold 30 seconds, ramp to 95% over 4 minutes. Mass Spec: Thermo Scientific. Q Exactive operated under positive ion mode.

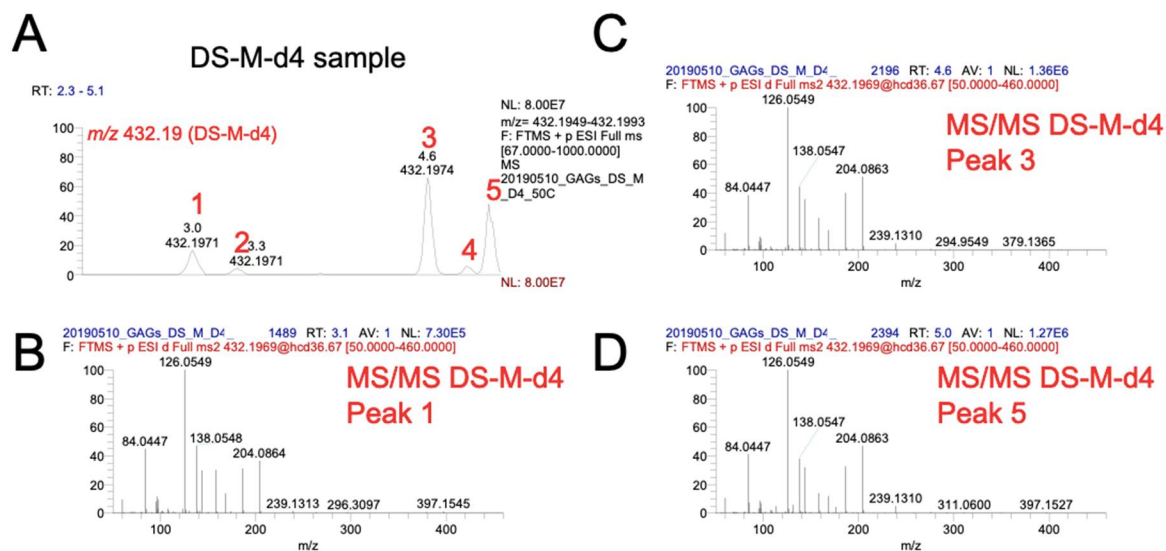


Figure S4. LC-MS and high resolution MS/MS of DS by methanolysis using methanol-d4. A) Five peaks observed for the DS-M-d4. B-D) MS/MS for the DS-B-d4 peak 1, 3, 5. The spectra are identical and they confirmed the identities of the reactants. LC-MS/MS Method: Mobil Phase A: Water 20 mM Ammonium formate, Mobil Phase B: Acetonitrile 0.1% formic acid. Column-Supelco Ascentis RP-Amide (10cm x 4.6 mm, 3 μ m). Gradient- 5% B hold 30 seconds, ramp to 95% over 4 minutes. Mass Spec: Thermo Scientific. Q Exactive operated under positive ion mode.

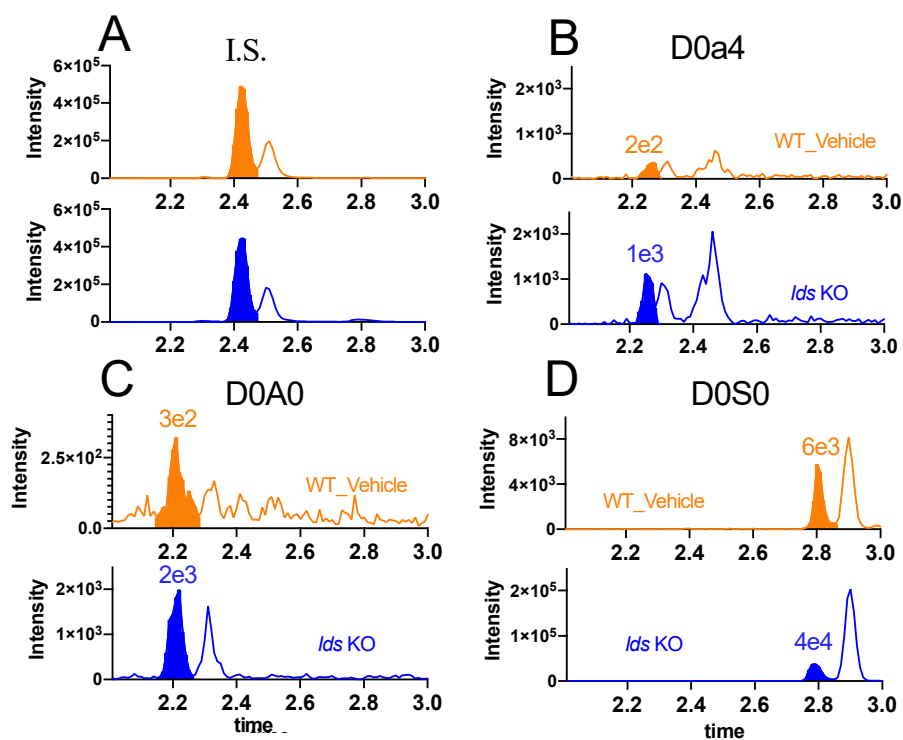


Figure S5. LC-MS/MS of DS (D0a4), HS (D0A0 and D0S0) in 40,000 FACS isolated neurons. D0A0 and D0S0 were quantifiable in 40,000 neurons and D0a4 signal was below LLOQ. Orange trace = WT sample and blue trace = *Ids*:KO sample. LC was performed using a 4.5-minute gradient programmed as follows: 0.0–0.5 minutes at 80%B, 0.5–3.5 minutes from 80%B to 50%B, 3.5–4.0 minutes 50%B to 80%B, 4.0–4.5 minutes hold at 80%B. LC flow rate of 0.55 mL/min, column temp at 55°C. Column: ACQUITY UPLC BEH Amide 1.7mm, 2.1×150 mm (Catalog # 186004802, Waters Corp., Milford, MA USA). Mass Spec: Sciex QTRAP 6500+.

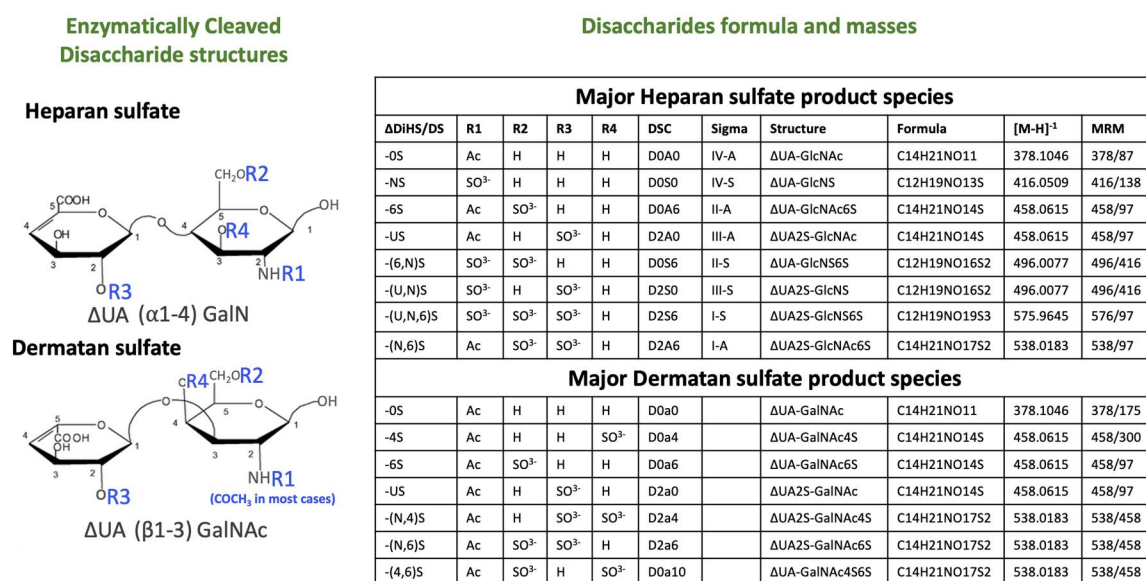


Figure S6. HS and DS released by Enzymatic digestion using Heparinase or Chondroitinase B. The disaccharide code (DSC) (according to Lawrence [1]), structure nomenclature, formula, accurate mass and ion transition (MRM) are listed for the major HS and DS species. Products maintain both HS and DS original chemical modifications and the structural diversities.

Reference:

[1] Lawrence, R.; Lu, H.; Rosenberg, R.D.; Esko, J.D.; Zhang, L. Disaccharide structure code for the easy representation of constituent oligosaccharides from glycosaminoglycans. *Nat. Methods* **2008**, *5*, 291-292