An LC-SRM Approach for the Separation and Quantitation of Sialylated N-glycans Linkage Isomers

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Supporting Information

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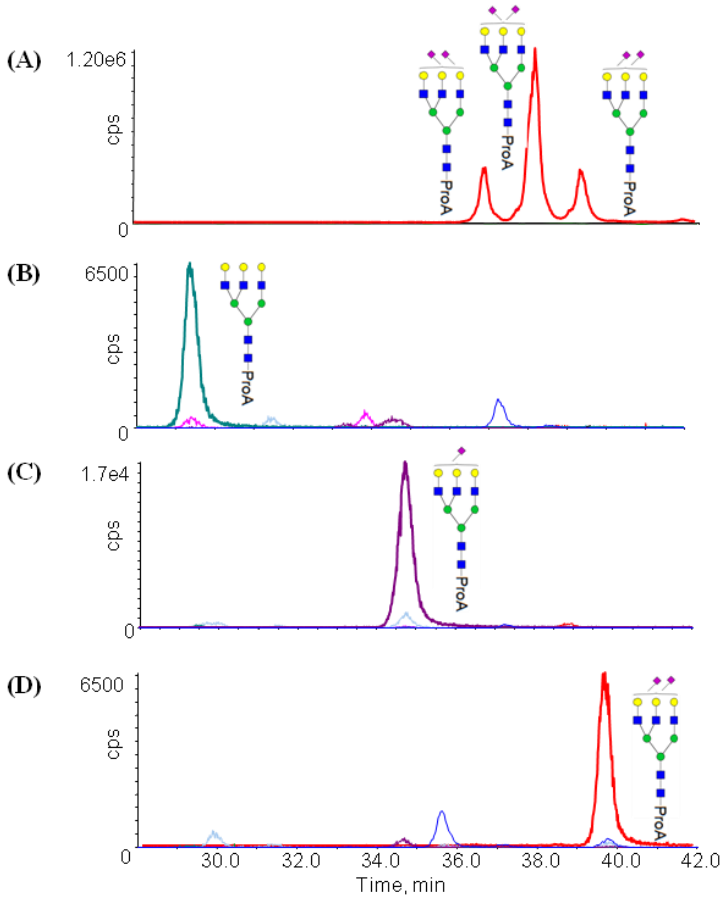

Figure S-1. Sialidase S digestions for Tri-2SA fractions by SRM detection. **(A)** LC-SRM trace for Tri-2SA(m/z at 936.9) from analysis of the fetuin N-glycans. LC-SRM analysis illustrates **(B)** the disappearance of the red peak (Tri-2SA) and the appearance of the dark blue peak (Tri-0SA) after Sialidase S digestion for the first fraction; **(C)** the disappearance of the red peak (Tri-2SA) and the appearance of the purple peak (Tri-SA) after Sialidase digestion for the second fraction; and **(D)** no change of the red peak (Tri-2SA) after Sialidase S digestion for the third fraction. **Figures B-D** contain SRM traces for all three possible glycans resulting from this treatment, i.e., the Tri-0SA, Tri-SA and Tri-2SA, however the low levels make these difficult to see in all of the figures.

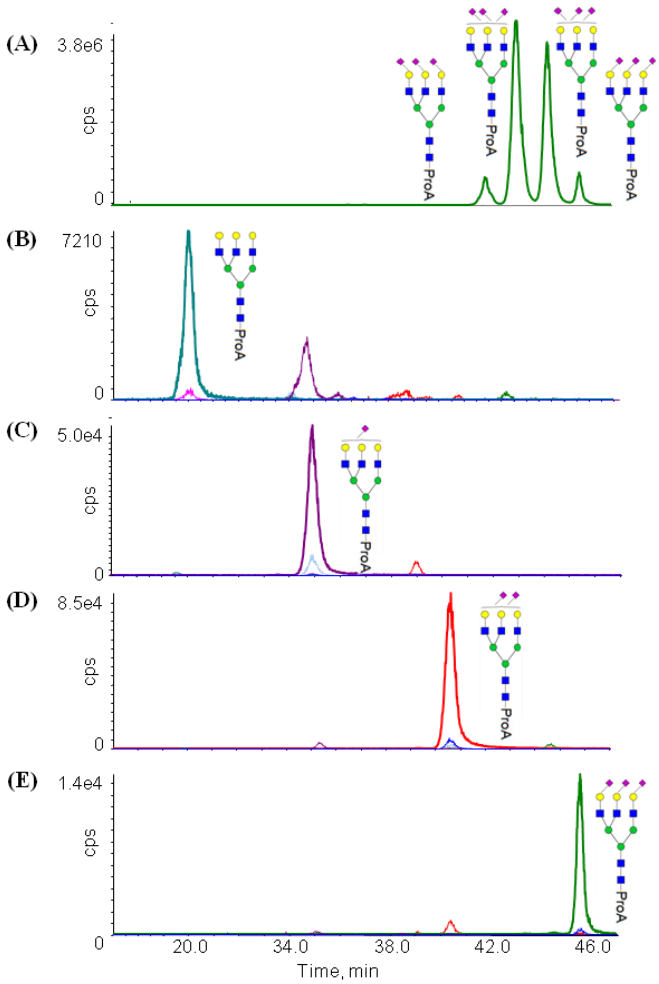

Figure S-2. Sialidase S digestions for Tri-3SA fractions by SRM detection. **(A)** LC-SRM trace for Tri-3SA (m/z at 1033.9) from analysis of the fetuin N-glycans. LC-SRM analysis illustrates **(B)** the disappearance of the green peak (Tri-3SA) and the appearance of the dark blue peak (Tri-0SA) after Sialidase S digestion for the first fraction; **(C)** the disappearance of the green peak (Tri-3SA) and the appearance of the purple peak (Tri-SA) after Sialidase S digestion for the second fraction; and **(D)** the disappearance of the green peak (Tri-3SA) and the appearance of the red peak (Tri-2SA) after Sialidase S digestion for the third fraction; and **(E)** no change of the green peak (Tri-3SA) after Sialidase S digestion for the fourth fraction. **Figures B-E** contain SRM traces for all four possible glycans resulting from this treatment, i.e., the Tri-0SA, Tri-SA, Tri-2SA and Tri-3SA, however the low levels make these difficult to see in all of the figures.

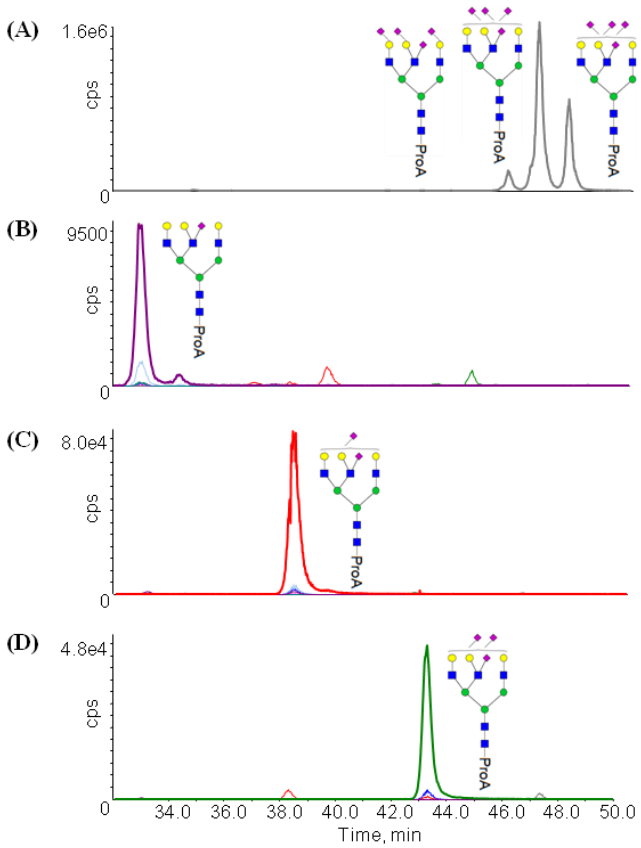

Figure S-3. Sialidase S digestions for Tri-4SA fractions by SRM detection. **(A)** LC-SRM trace for Tri-4SA(m/z at 1130.9) from analysis of the fetuin N-glycans. LC-SRM analysis illustrates **(B)** the disappearance of the grey peak (Tri-4SA) and the appearance of the purple peak (Tri-SA) after Sialidase S digestion for the first fraction; **(C)** the disappearance of the grey peak (Tri-4SA) and the appearance of the red peak (Tri-2SA) after Sialidase S digestion for the second fraction; and **(D)** the disappearance of the grey peak (Tri-4SA) and the appearance of the green peak (Tri-3SA) after Sialidase S digestion for the third fraction. Figures B-D contain SRM traces for all five possible glycans resulting from this treatment, i.e., the Tri-0SA, Tri-SA, Tri-2SA, Tri-3SA and Tri-4SA, however the low levels make these difficult to see in all of the figures.

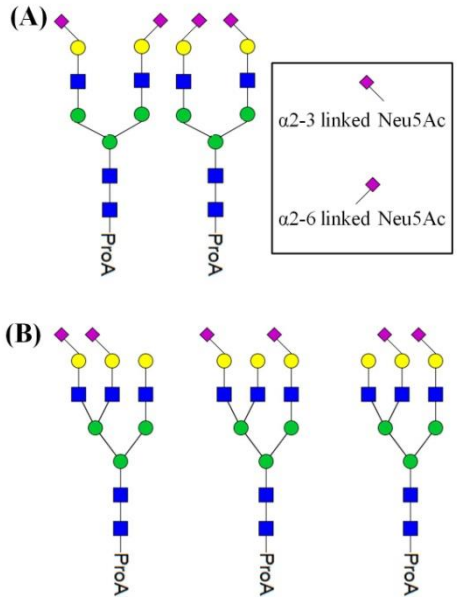

Figure S-4. Branching isoforms. **(A)** Two isomeric structures for Bi-2SA(3,6) glycans; **(B)** Three isomeric structures for Tri-2SA(3,3) glycans.

Table S-1. N-glycans Detected in the Fetuin Sample.

Group	Observed *m/z* (charge)	Theoretical *m/z* (charge)	Composition	Peak No.
I	1222.1 (+2)	1222.06 (+2)	Bi-2SA	1, 2, 4
II	1259.2 (+2)	1259.08 (+2)	Tri-SA	3
III	936.9 (+3)	936.75 (+3)	Tri-2SA	5, 6, 8
IV	1367.8 (+2)	1367.60 (+2)	Bi-3SA	7, 9
V	1033.9 (+3)	1033.78 (+3)	Tri-3SA	10, 11, 13, 15
VI	1155.9 (+3)	1155.49 (+3)	Tetra-3SA	12, 14
VII	1130.9 (+3)	1130.81(+3)	Tri-4SA	16, 17, 19
VIII	1252.9 (+3)	1252.52 (+3)	Tetra-4SA	18, 20, 21

Table S-2. SRM transitions for Sialidase S digestion of fetuin N-glycan studies.

Composition	Q1 (charge)	Q3 (charge)	CE (V)	Dwell (msec)
Bi-0SA	931.1 (+2)	441.4 (+1)	70	100
Bi-SA	1076.5 (+2)	441.4 (+1)	70	100
Bi-2SA	1222.1 (+2)	441.4 (+1)	70	100
Tri-0SA	1113.7 (+2)	441.4 (+1)	70	100
Tri-SA	1259.2 (+2)	441.4 (+1)	70	100
Tri-2SA	936.9 (+3)	441.4 (+1)	70	100
Tri-3SA	1033.9 (+3)	441.4 (+1)	70	100
Tri-4SA	1130.9 (+3)	441.4 (+1)	70	100

Table S-3. P-Values obtained from Independent Two-Tailed Students T-test of the difference between the relative quantitation using UV and SRM detection when **(I)** the response for each glycoform is calculated relative to the summed response for all identified glycans and **(II)** the response for each glycoform is calculated relative to the summed response for all glycoforms with the same composition.

	P-Value	**P-Value**
Bi-2SA(3,3)	**1.0E-05**	0.5113
Bi-2SA(3,6)	**8.7E-05**	0.3489
Bi-2SA(6,6)	**3.9E-05**	0.0916
Tri-2SA(3,3)	**5.5E-04**	0.3409
Tri-2SA(3,6)	**8.1E-04**	0.4050
Tri-2SA(6,6)	**0.0021**	0.6826
Tri-3SA(3,3,3)	0.2697	0.9177
Tri-3SA(3,3,6)	**6.0E-05**	0.5113
Tri-3SA(3,6,6)	0.0146	0.0519
Tri-3SA(6,6,6)	**0.0044**	0.2180
Tri-4SA(3,3,3,*6*)	**2.8E-05**	0.0428
Tri-4SA(3,3,6,*6*)	**2.2E-05**	0.0594
Tri-4SA(3,6,6,*6*)	**3.2E-05**	0.0065

Bold values are significant at P≤ 0.001.