

Supporting Information

Using Micro Pillar Array Columns (μ PAC) for the Analysis of Permethylated Glycans

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


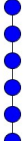
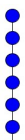
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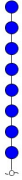
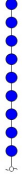
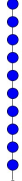
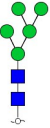
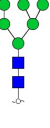
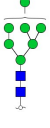
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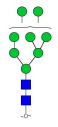
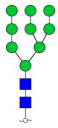
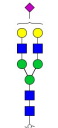
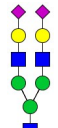
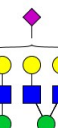
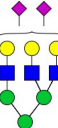
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Key words: Glycan, LC-MS, Micro Pillar Array Column, Permethylation, Isomeric
Seperation

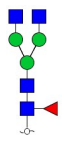
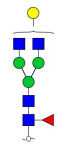
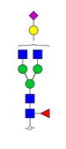
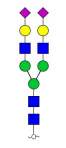
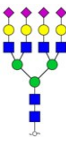
1 **Table S1.** Assessment of chromatographic performances of each glucose unit and
 2 glycans shown in Figure 1,2 and 3. Full width half max (FWHM), retention factor (k),
 3 theoretical plates (N), and plate height (H) have been calculated (n=3) for each column
 4 and conditions applied. It should be noted that the linear gradient was used.

Glycan/ GU	Sample	Column and Condition	FWHM	Retention Factor (k)	Theoretical Plates (N)	Theoretical Plate Height (H)
	Dextran	μPAC 50cm, 100% MeCN as Mobile Phase B	0.23±0.02	0.23±0.003	56637±9847	9.0E-4±1.7E-4
		μPAC 50cm, 80% MeCN as Mobile Phase B	0.30±0.02	0.41±0.003	35473±3733	1.4E-3±1.6E-4
	Dextran	μPAC 50cm, 100% MeCN as Mobile Phase B	0.23±0.02	0.34±0.01	65303±9820	7.8E-4±1.1E-4
		μPAC 50cm, 80% MeCN as Mobile Phase B	0.29±0.026	0.66±0.0034	53350±9147	9.6E-4±1.8E-4
		μPAC 200cm, 100% MeCN as Mobile Phase B	0.24±0.006	0.53±0.0005	326434±15493	6.1E-4±3.0E-5
		μPAC 200cm, 80% MeCN as Mobile Phase B	0.37±0.02	1.13±0.007	254923±23662	7.9E-4±6.9E-5
	Dextran	μPAC 50cm, 100% MeCN as Mobile Phase B	0.26±0.03	0.48±0.003	62719±13428	8.2E-4±1.6E-4
		μPAC 50cm, 80% MeCN as Mobile Phase B	0.28±0.01	0.97±0.003	79833±5934	6.3E-4±4.7E-5
		μPAC 200cm, 100% MeCN as Mobile Phase B	0.29±0.01	0.79±0.005	305941±23038	6.6E-4±4.7E-5
		μPAC 200cm, 80% MeCN as Mobile Phase B	0.44±0.006	1.83±0.01	323909±8970	6.2E-4±1.7E-5
	Dextran	μPAC 50cm, 100% MeCN as Mobile Phase B	0.24±0.02	0.65±0.003	87497±10415	5.8E-4±7.1E-5
		μPAC 50cm, 80% MeCN as Mobile Phase B	0.28±0.02	1.33±0.003	109430±16874	4.7E-4±7.9E-5
		μPAC 200cm, 100% MeCN as Mobile Phase B	0.32±0.01	1.12±0.007	360153±24686	5.6E-4±3.8E-5
		μPAC 200cm, 80% MeCN as Mobile Phase B	0.47±0.02	2.67±0.01	463651±41867	4.3E-4±4.1E-5
	Dextran	μPAC 50cm, 100% MeCN as Mobile Phase B	0.25±0.02	0.78±0.1	107637±21376	4.8E-4±8.5E-5
		μPAC 50cm, 80% MeCN as Mobile Phase B	0.24±0.03	1.69±0.003	211411±44126	2.4E-4±4.5E-5
		μPAC 200cm, 100% MeCN as Mobile Phase B	0.33±0.00	1.38±0.2	478003±3012	4.2E-4±2.6E-6
		μPAC 200cm, 80% MeCN as Mobile Phase B	0.47±0.02	3.54±0.02	743642±68557	2.7E-4±2.6E-5

Glycan/ GU	Sample	Column and Condition	FWHM	Retention Factor (k)	Theoretical Plates (N)	Theoretical Plate Height (H)
	Dextran	μPAC 50cm, 100% MeCN as Mobile Phase B	0.23±0.02	1.07±0.003	155163±24381	3.3E-4±4.7E-5
		μPAC 50cm, 80% MeCN as Mobile Phase B	0.26±0.03	2.05±0.003	218859±41191	2.3E-4±4.5E-5
		μPAC 200cm, 100% MeCN as Mobile Phase B	0.36±0.04	1.97±0.008	570850±103308	3.6E-4±7.3E-5
		μPAC 200cm, 80% MeCN as Mobile Phase B	0.50±0.03	4.40±0.0201	915491±90942	2.2E-4±2.1E-5
	Dextran	μPAC 50cm, 100% MeCN as Mobile Phase B	0.23±0.02	1.32±0.003	199958±27281	2.5E-4±3.3E-5
		μPAC 50cm, 80% MeCN as Mobile Phase B	0.27±0.01	2.40±0.003	254019±19286	2.0E-4±1.5E-5
		μPAC 200cm, 100% MeCN as Mobile Phase B	0.39±0.006	2.46±0.01	636863±18336	3.1E-4±9.2E-6
		μPAC 200cm, 80% MeCN as Mobile Phase B	0.49±0.02	5.22±0.02	1262185±122126	1.6E-4±1.5E-5
	Dextran	μPAC 50cm, 100% MeCN as Mobile Phase B	0.33±0.02	1.58±0.003	114186±14558	4.4E-4±5.3E-5
		μPAC 50cm, 80% MeCN as Mobile Phase B	0.35±0.03	2.72±0.000	180026±32439	2.8E-4±4.8E-5
		μPAC 200cm, 100% MeCN as Mobile Phase B	0.38±0.09	2.96±0.009	1000105±549898	2.4E-4±1.0E-4
		μPAC 200cm, 80% MeCN as Mobile Phase B	0.55±0.01	5.97±0.02	1252561±48146	1.6E-4±6.3E-6
GU11	Dextran	μPAC 50cm, 100% MeCN as Mobile Phase B	0.34±0.03	1.84±0.003	136067±20525	3.7E-4±5.4E-5
		μPAC 50cm, 80% MeCN as Mobile Phase B	0.37±0.03	3.02±0.0000	194830±31271	2.6E-4±4.4E-5
	Ribonuclease B	Thermo PepMap 15cm	0.45±0.03	0.68±0.001	17302±2397	8.8E-4±1.2E-4
		μPAC 50cm, 100% MeCN as Mobile Phase B	0.33±0.05	0.21±0.02	34606±9861	1.5E-3±4.1E-4
		μPAC 50cm, 80% MeCN as Mobile Phase B	0.42±0.01	0.47±0.003	30040±1413	1.7E-3±7.8E-5
	Ribonuclease B	Thermo PepMap 15cm	0.46±0.04	0.96±0.004	23017±3329	6.6E-4±1.0E-4
		μPAC 50cm, 100% MeCN as Mobile Phase B	0.33±0.01	0.32±0.02	37602±2766	1.3E-3±9.4E-5
		μPAC 50cm, 80% MeCN as Mobile Phase B	0.54±0.04	0.70±0.002	24311±3551	2.1E-3±3.2E-4
	Ribonuclease B	Thermo PepMap 15cm	0.82±0.6	1.29±0.001	9936±1548	1.5E-3±2.2E-4
		μPAC 50cm, 100% MeCN as Mobile Phase B	0.30±0.09	0.43±0.02	60986±27591	9.9E-4±5.6E-4

Glycan/ GU	Sample	Column and Condition	FWHM	Retention Factor (k)	Theoretical Plates (N)	Theoretical Plate Height (H)
	Ribonuclease B	μPAC 50cm, 80% MeCN as Mobile Phase B	0.47±0.01	0.93±0.005	41384±1764	1.2E-3±5.1E-5
		Thermo PepMap 15cm	0.44±0.02	1.60±0.003	43087±4366	3.5E-4±3.4E-5
		μPAC 50cm, 100% MeCN as Mobile Phase B	0.34±0.03	0.51±0.07	50492±8928	1.0E-3±1.8E-4
		μPAC 50cm, 80% MeCN as Mobile Phase B	0.38±0.04	1.14±0.002	81269±18913	6.4E-4±1.3E-4
	Ribonuclease B	Thermo PepMap 15cm	0.41±0.02	1.94±0.006	66151±1529	2.3E-4±5.3E-6
		μPAC 50cm, 100% MeCN as Mobile Phase B	0.31±0.006	0.69±0.02	73307±2868	6.8E-4±2.6E-5
		μPAC 50cm, 80% MeCN as Mobile Phase B	0.34±0.006	1.37±0.003	121965±4563	4.1E-4±1.5E-5
	Fetuin	Thermo PepMap 15cm	1.13±0.3	0.96±0.004	12648±7732	1.5E-3±7.0E-4
		μPAC 50cm, 100% MeCN as Mobile Phase B	0.9±0.05	0.57±0.000	15916±4421	3.3E-3±1.0E-3
		μPAC 50cm, 80% MeCN as Mobile Phase B	0.8±0.04	1.16±0.01	35461±3701	1.4E-3±1.4E-4
	Fetuin		0.75±0.2	1.20±0.01	33045±13297	5.0E-4±1.9E-4
		Thermo PepMap 15cm	(Isomer 1)	(Isomer 1)	(Isomer 1)	(Isomer 1)
			1.08±0.2	1.22±0.01	15017±1221	1.0E-3±7.8E-5
			(Isomer 2)	(Isomer 2)	(Isomer 2)	(Isomer 2)
		μPAC 50cm, 100% MeCN as Mobile Phase B	0.95±0.05	0.68±0.00	15714±1510	3.2E-3±3.0E-4
	μPAC 50cm, 80% MeCN as Mobile Phase B	1.05±0.05	1.4±0.007	24030±2199	2.1E-3±1.9E-4	
	Fetuin		1.01±0.2	1.07±0.00	15857±5981	1.0E-3±3.2E-4
			(Isomer 1)	(Isomer 1)	(Isomer 1)	(Isomer 1)
		Thermo PepMap 15cm	0.72±0.04	1.10±0.004	30519±3553	5.0E-4±6.2E-5
			(Isomer 2)	(Isomer 2)	(Isomer 2)	(Isomer 2)
			0.85±0.2	1.21±0.004	28689±17337	6.5E-4±3.2E-4
			(Isomer 3)	(Isomer 3)	(Isomer 3)	(Isomer 3)
		μPAC 50cm, 100% MeCN as Mobile Phase B	0.77±0.02	0.63±0.003	26324±1716	1.9E-3±1.2E-4
		μPAC 50cm, 80% MeCN as Mobile Phase B	0.91±0.1	1.27±0.003	30444±6952	1.7E-3±3.6E-4
	Fetuin		0.82±0.03	1.28±0.004	27477±2096	5.5E-4±4.2E-5
		Thermo PepMap 15cm	(Isomer 1)	(Isomer 1)	(Isomer 1)	(Isomer 1)
			1.12±0.03	1.32±0.004	15223±633	9.9E-4±4.1E-5

Glycan/ GU	Sample	Column and Condition	FWHM	Retention Factor (k)	Theoretical Plates (N)	Theoretical Plate Height (H)
	Fetuin	μPAC 50cm, 100% MeCN as Mobile Phase B	(Isomer 2)	(Isomer 2)	(Isomer 2)	(Isomer 2)
			0.80±0.1	1.38±0.004	32663±9199	4.9E-4±1.6E-4
			(Isomer 3)	(Isomer 3)	(Isomer 3)	(Isomer 3)
			0.68±0.02	0.73± 0.00	32033±1885	1.6E-3±9.2E-5
			(Isomer 1)	(Isomer 1)	(Isomer 1)	(Isomer 1)
			0.64±0.07	0.75±0.003	38616±9121	1.3E-3±3.0E-4
		(Isomer 2)	(Isomer 2)	(Isomer 2)	(Isomer 2)	
		1.17±0.2	0.81±0.003	12543±3731	4.2E-3±1.3E-3	
		(Isomer 3)	(Isomer 3)	(Isomer 3)	(Isomer 3)	
		0.68±0.04	1.46±0.009	62803±6729	8.0E-4±9.1E-5	
		(Isomer 1)	(Isomer 1)	(Isomer 1)	(Isomer 1)	
		0.84±0.05	1.49±0.006	42126±4527	1.2E-3±1.4E-4	
		(Isomer 2)	(Isomer 2)	(Isomer 2)	(Isomer 2)	
		0.90±0.1	1.60±0.01	40238±8629	1.3E-3±2.9E-4	
		(Isomer 3)	(Isomer 3)	(Isomer 3)	(Isomer 3)	
	Fetuin	Thermo PepMap 15cm	0.77±0.09	1.51±0.000	38600±9950	4E-4±9.4E-5
			(Isomer 1)	(Isomer 1)	(Isomer 1)	(Isomer 1)
			0.93±0.08	1.58±0.004	27706±4697	5.5E-4±9.0E-5
		(Isomer 2)	(Isomer 2)	(Isomer 2)	(Isomer 2)	
		μPAC 50cm, 100% MeCN as Mobile Phase B	0.62±0.05	0.82±0.006	43819±6119	1.2E-3±1.7E-4
			(Isomer 1)	(Isomer 1)	(Isomer 1)	(Isomer 1)
			0.94±0.1	0.84±0.003	19668±4066	2.6E-3±5.8E-4
		(Isomer 2)	(Isomer 2)	(Isomer 2)	(Isomer 2)	
		μPAC 50cm, 80% MeCN as Mobile Phase B	0.64±0.02	1.62±0.006	80521±6349	6.2E-4±4.7E-5
			(Isomer 1)	(Isomer 1)	(Isomer 1)	(Isomer 1)
			0.99±0.04	1.66±0.009	34145±2658	1.5E-3±1.2E-4
		(Isomer 2)	(Isomer 2)	(Isomer 2)	(Isomer 2)	
	Fetuin	Thermo PepMap 15cm	0.70±0.13	1.75±0.004	59092±25915	2.8E-4±1.0E-4
			(Isomer 1)	(Isomer 1)	(Isomer 1)	(Isomer 1)
			0.85±0.1	1.81±0.004	39856±9914	3.9E-4±8.5E-5
		(Isomer 2)	(Isomer 2)	(Isomer 2)	(Isomer 2)	
		μPAC 50cm, 100% MeCN as Mobile Phase B	1.06±0.15	0.95±0.003	17540±5512	3.0E-3±8.0E-4
			(Isomer 1)	(Isomer 1)	(Isomer 1)	(Isomer 1)

Glycan/ GU	Sample	Column and Condition	FWHM	Retention Factor (k)	Theoretical Plates (N)	Theoretical Plate Height (H)
			0.96±0.17	1.03±0.003	23557±7002	2.3E-3±8.2E-4
			(Isomer 2)	(Isomer 2)	(Isomer 2)	(Isomer 2)
			0.97±0.1	1.81±0.009	40657±8315	1.3E-3±2.7E-4
			(Isomer 1)	(Isomer 1)	(Isomer 1)	(Isomer 1)
		μPAC 50cm, 80% MeCN as Mobile Phase B	0.79±0.01	1.91±0.006	64365±1373	7.8E-4±1.7E-5
			(Isomer 2)	(Isomer 2)	(Isomer 2)	(Isomer 2)
	Human Blood Serum	μPAC 50cm, 80% MeCN as Mobile Phase B Flowrate = 0.3μL/min	0.26±0.006	0.74±0.0005	90817±3907	5.5E-4±2.4E-5
		μPAC 50cm, 80% MeCN as Mobile Phase B Flowrate = 0.6μL/min	0.22±0.006	1.17±0.02	52359±2362	9.6E-4±4.4E-5
	Human Blood Serum	μPAC 50cm, 80% MeCN as Mobile Phase B Flowrate = 0.3μL/min	0.29±0.010	0.89±0.0006	87970±6070	5.7E-4±3.9E-5
		μPAC 50cm, 80% MeCN as Mobile Phase B Flowrate = 0.6μL/min	0.30±0.02	1.42±0.02	36233±4616	1.4E-3±1.9E-4
	Human Blood Serum	μPAC 50cm, 80% MeCN as Mobile Phase B Flowrate = 0.3μL/min	0.39±0.02	1.17±0.004	65458±5092	7.7E-4±5.8E-5
		μPAC 50cm, 80% MeCN as Mobile Phase B Flowrate = 0.6μL/min	0.39±0.01	1.93±0.03	30843±1232	1.6E-3±6.3E-5
	Human Blood Serum	μPAC 50cm, 80% MeCN as Mobile Phase B Flowrate = 0.3μL/min	0.65±0.01	1.34±0.0007	27083±952	1.8E-3±6.6E-5
		μPAC 50cm, 80% MeCN as Mobile Phase B Flowrate = 0.6μL/min	0.64±0.02	2.28±0.03	14383±535	3.5E-3±1.3E-4
	Human Blood Serum	μPAC 50cm, 80% MeCN as Mobile Phase B Flowrate = 0.3μL/min	0.63±0.02	1.94±0.0009	43856±2429	1.1E-3±6.1E-5
		(Isomer 1)	(Isomer 1)	(Isomer 1)	(Isomer 1)	
		0.35±0.02	1.98±0.004	153213±14092	3.3E-4±2.9E-5	
		(Isomer 2)	(Isomer 2)	(Isomer 2)	(Isomer 2)	
			0.58±0.03	3.08±0.7	33512±3322	1.5E-3±1.4E-4
			(Isomer 1)	(Isomer 1)	(Isomer 1)	(Isomer 1)
			0.40±0.02	3.54±0.03	73123±7957	6.9E-4±7.1E-5

Glycan/ GU	Sample	Column and Condition	FWHM	Retention Factor (k)	Theoretical Plates (N)	Theoretical Plate Height (H)
			(Isomer 2)	(Isomer 2)	(Isomer 2)	(Isomer 2)

1

1 **Table S2.** Summary of Glucose Unit Index (GUI) using μ PAC columns with different
 2 concentration of acetonitrile in mobile phase B.

Column	Mobile Phase A	Mobile Phase B	GUI Equation	R ²
μ PAC 50cm	0.1% Formic Acid in 100% Water	0.1% Formic Acid in 80% Acetonitrile	$y=5.7x+5.8$	0.9986
		0.1% Formic Acid in 100% Acetonitrile	$y=3.8x+9.2$	0.9828
μ PAC 200cm		0.1% Formic Acid in 80% Acetonitrile	$y=29.6x-39.3$	0.9993
		0.1% Formic Acid in 100% Acetonitrile	$y=16.4x+12.9$	0.9887

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4

5

1 **Figure Legends**

2 **Figure S1.** Workflow used in this study.

3 **Figure S2.** Comparison of μ PAC trapping column against PepMap trapping column for the
4 online purification of permethylated glycans (N=5). μ PAC Trapping Column was
5 compared with PepMap 2cm Trap Column and various conditions were examined
6 with μ PAC Trapping Column. Error bars represent standard deviation. Astricks
7 indicate the significant improvement in abundance using μ PAC Trapping Column v.
8 PepMap 2cm Trap Column.

9 **Figure S3.** Assessment of the effects of increased flowrate in sensitivity (N=3). While there
10 was no obvious difference in high mannose structures, fucosylated and sialylated
11 glycans showed noticeable decrease in sensitivity. Error bars represent standard
12 deviation.

13 **Figure S4.** Analysis of permethylated N-glycans derived from mouse IgG using 200cm μ PAC.
14 Separation of terminal galactose isomers were shown in c) but not shown with core
15 fucose in d)

16 **Figure S5.** Separation of permethylated Man8 isomers derived from human blood serum using
17 200cm μ PAC. MS2 spectra of each peak is shown to validate the separation of
18 Man8 isomers.

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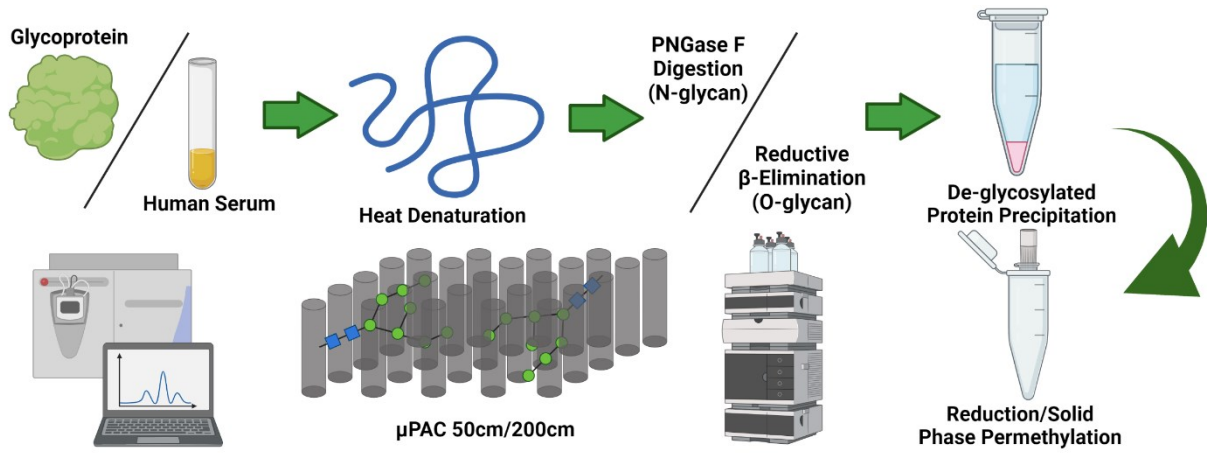
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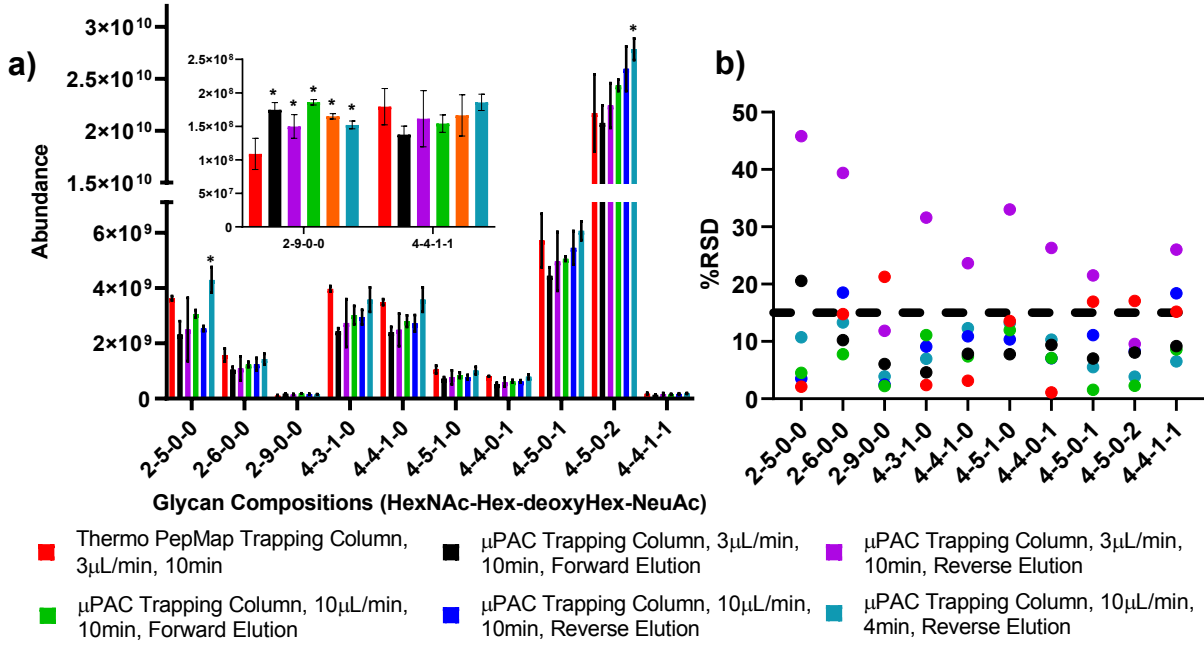
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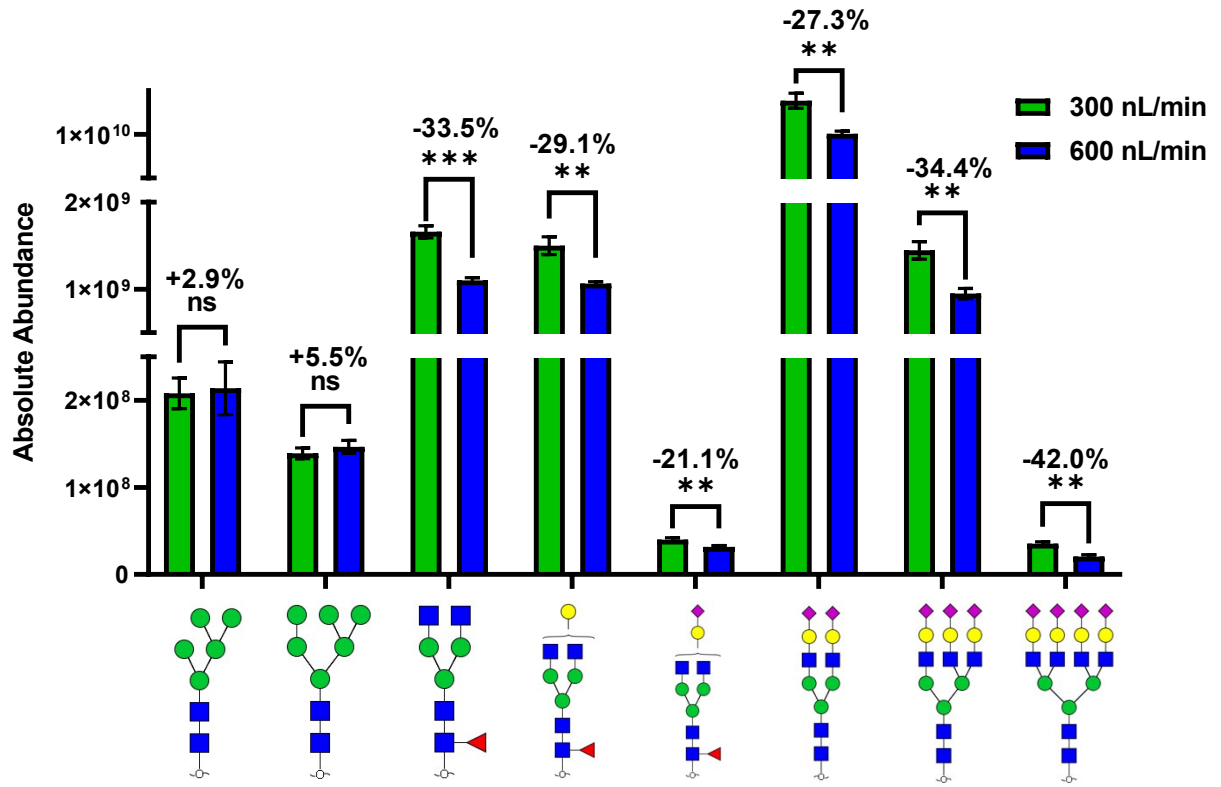
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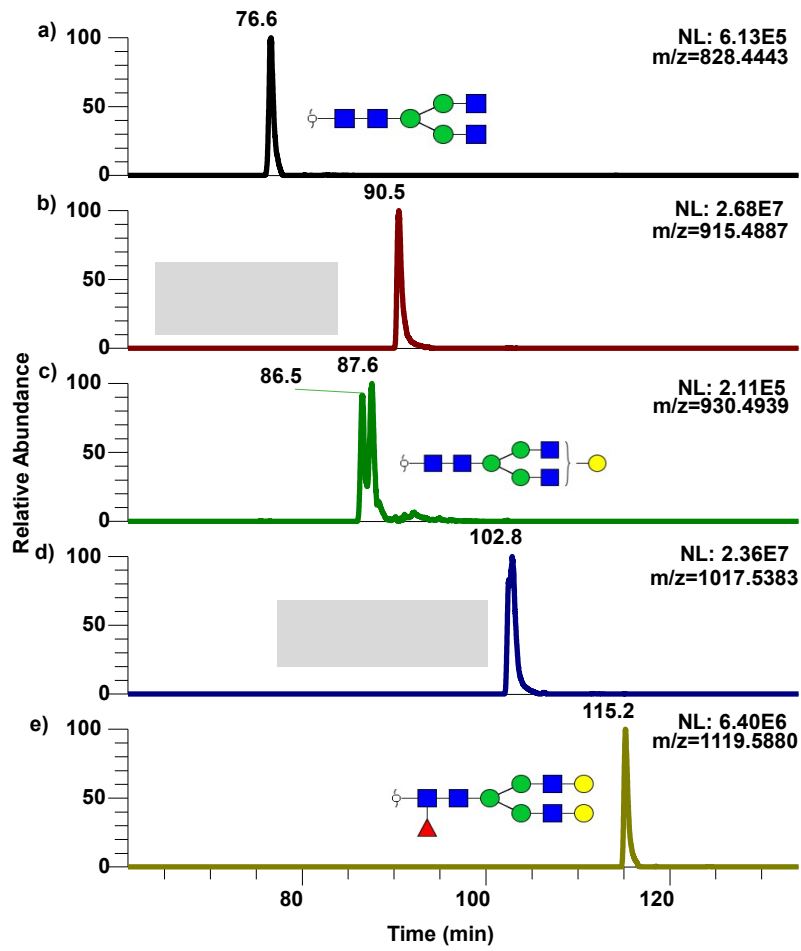
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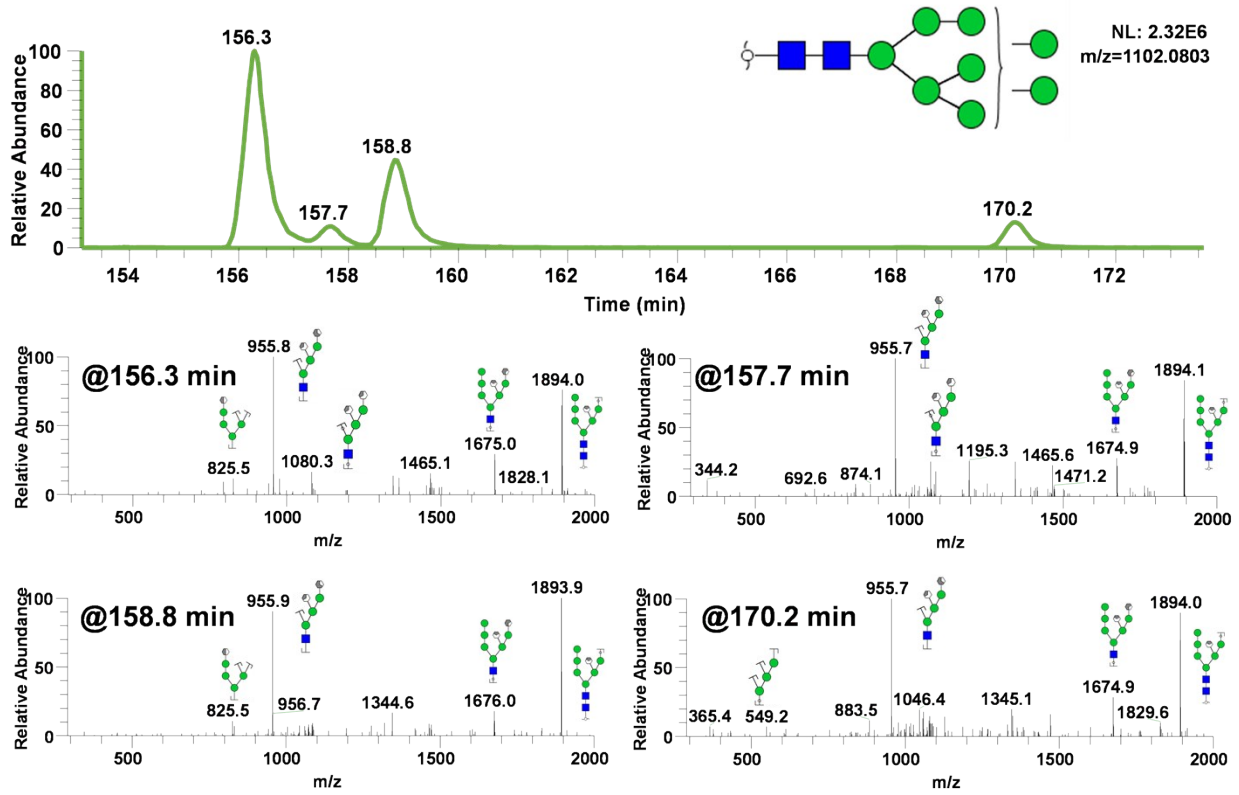
Cho *et al.* Figure S4



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Cho et al. Figure S5



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