Supplementary Materials

Comparison of Four Rapid N-glycan Analytical Methods and Great Application Potential in Cell Line Development

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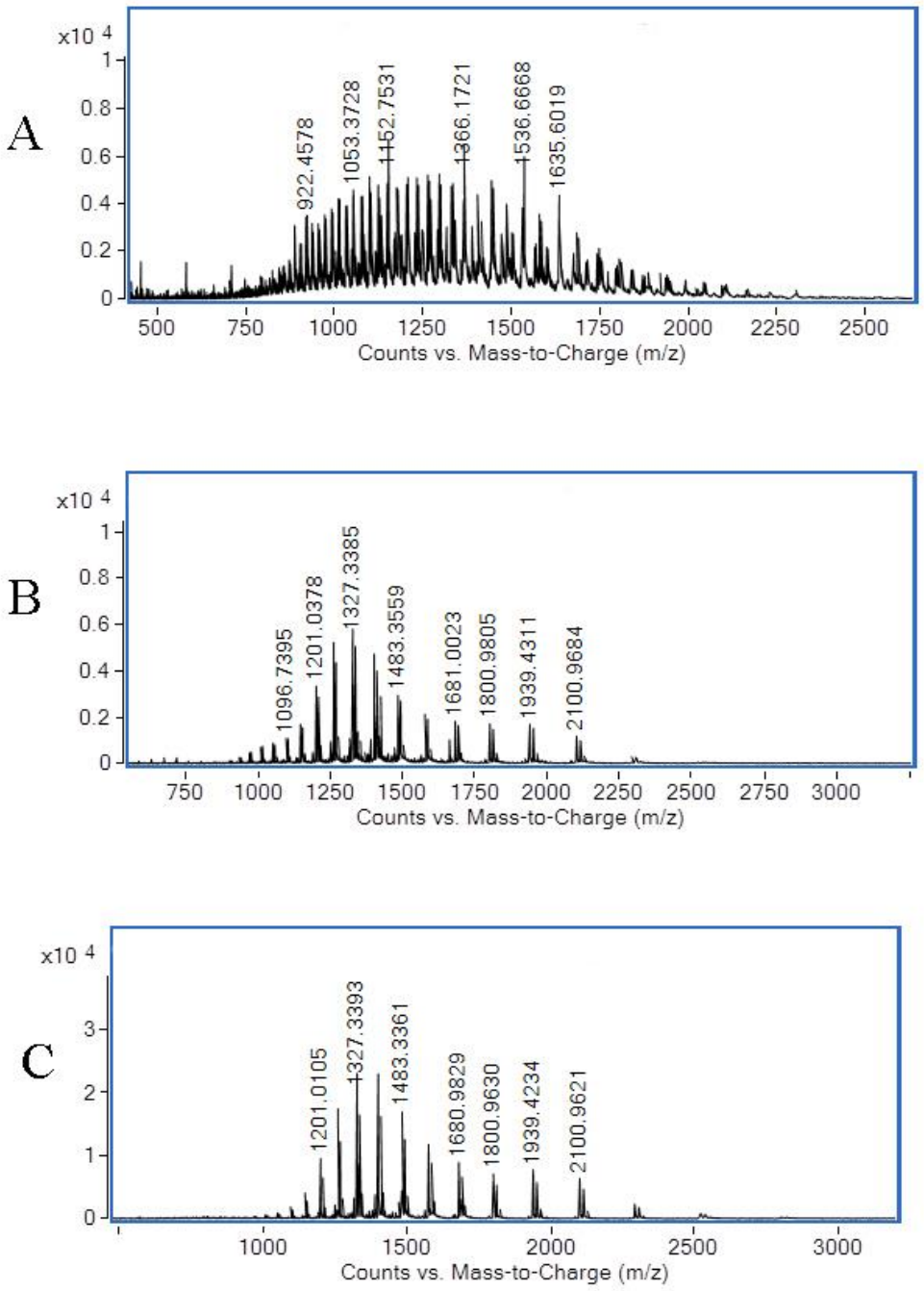


Figure S1. Raw MS spectra of mAb A.

(A) Raw MS spectra by reduction method. (B) Raw MS spectra by off-line IdeS digestion method. (C) Raw MS spectra by 2D-LC-MS method with on-line immobilized IdeS digestion.

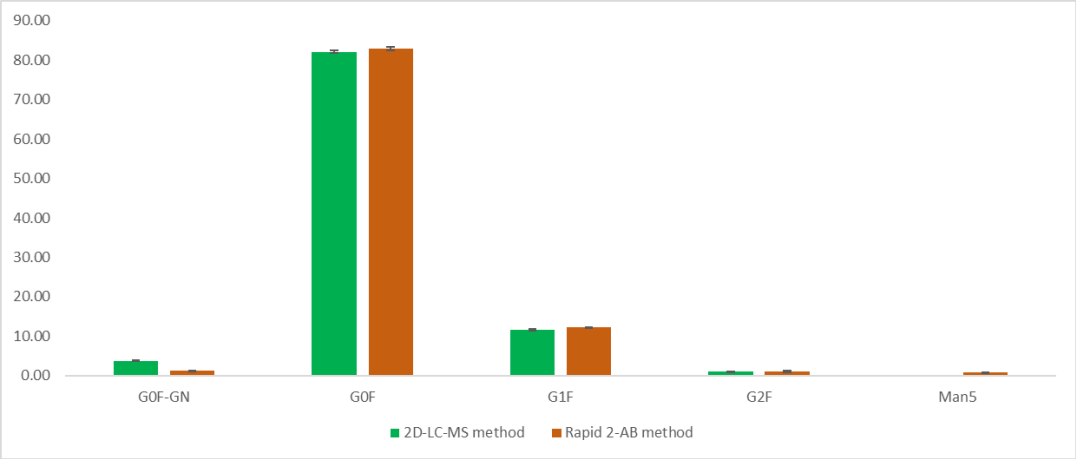


Figure S2. Comparison of five major glycans of mAb B by 2D-LC-MS method (n=3) and rapid 2-AB method (n=3).

Table S1. The reproducibility of the major N-glycans data by reduction method in triplicate.

Reduction method	Theoretical molecular weight Da	Detected molecular weight Da	Mass error Da	Glycan name	Intensity	Relative ratio %
Run 1	50311.8821	50308.9437	-2.9384	G0F-GN	180.00	1.97
	50474.0253	50471.5355	-2.4898	G1F-GN	190.14	2.08
	50515.0782	50511.7552	-3.3230	G0F	3934.62	43.08
	50677.2214	50674.3045	-2.9169	G1F	3852.09	42.18
	50839.3647	50836.2658	-3.0989	G2F	811.92	8.89
	50286.8286	50283.3109	-3.5177	Man5	164.79	1.80
Run 2	50311.8821	50309.3052	-2.5769	G0F-GN	169.33	1.54
	50474.0253	50472.0673	-1.9580	G1F-GN	200.45	1.82
	50515.0782	50511.8324	-3.2458	G0F	4905.80	44.64
	50677.2214	50674.3904	-2.8310	G1F	4515.03	41.08
	50839.3647	50836.4891	-2.8756	G2F	1034.29	9.41
	50286.8286	50283.5905	-3.2381	Man5	164.71	1.50
Run 3	50311.8821	50308.9517	-2.9304	G0F-GN	187.56	1.73
	50474.0253	50472.0891	-1.9362	G1F-GN	217.29	2.01
	50515.0782	50511.9925	-3.0857	G0F	4887.98	45.21
	50677.2214	50674.3222	-2.8992	G1F	4266.23	39.46
	50839.3647	50836.6135	-2.7512	G2F	1050.53	9.72
	50286.8286	50283.2146	-3.6140	Man5	202.54	1.87

Table S2. The reproducibility of the major N-glycans data by off-line IdeS digestion method in triplicate.

Off-line IdeS digestion method	Theoretical molecular weight Da	Detected molecular weight Da	Mass error Da	Glycan name	Intensity	Relative ratio %
Run 1	24997.1953	24996.8610	-0.3343	G0F-GN	3353.79	4.85
	25159.3386	25158.9868	-0.3518	G1F-GN	1743.02	2.52
	25200.3914	25200.0450	-0.3464	G0F	31134.18	45.05
	25362.5347	25362.3501	-0.1846	G1F	24960.74	36.11
	25524.6779	25524.6932	0.0153	G2F	5434.21	7.86
	24972.1419	24972.0641	-0.0778	Man5	2491.93	3.61
Run 2	24997.1953	24996.7611	-0.4342	G0F-GN	18275.65	5.52
	25159.3386	25158.8765	-0.4621	G1F-GN	10013.80	3.02
	25200.3914	25199.9155	-0.4759	G0F	141609.26	42.77
	25362.5347	25362.1672	-0.3675	G1F	122975.63	37.14
	25524.6779	25524.4118	-0.2661	G2F	27675.81	8.36
	24972.1419	24971.7778	-0.3641	Man5	10583.13	3.20
Run 3	24997.1953	24996.6513	-0.5440	G0F-GN	19089.97	5.49
	25159.3386	25158.8859	-0.4527	G1F-GN	9872.44	2.84
	25200.3914	25199.8719	-0.5195	G0F	149930.85	43.12
	25362.5347	25362.1132	-0.4215	G1F	129413.93	37.22
	25524.6779	25524.3492	-0.3287	G2F	27518.06	7.91
	24972.1419	24971.8301	-0.3118	Man5	11896.00	3.42

Table S3. The reproducibility of the major N-glycans data by 2D-LC-MS method in triplicate.

2D-LC-MS method	Theoretical molecular weight Da	Detected molecular weight Da	Mass error Da	Glycan name	Intensity	Relative ratio %
Run 1	24997.1953	24996.6405	-0.5548	G0F-GN	13376.21	4.74
	25159.3386	25158.8615	-0.4771	G1F-GN	8696.06	3.08
	25200.3914	25199.8534	-0.5380	G0F	140422.66	49.76
	25362.5347	25362.0678	-0.4669	G1F	97396.24	34.51
	25524.6779	25524.1630	-0.5149	G2F	21403.79	7.58
	24972.1419	24972.9000	0.7581	Man5	900.00	0.32
Run 2	24997.1953	24996.6951	-0.5002	G0F-GN	13036.12	4.90
	25159.3386	25158.8537	-0.4849	G1F-GN	7933.34	2.98
	25200.3914	25199.7830	-0.6084	G0F	131773.91	49.57
	25362.5347	25362.0927	-0.4420	G1F	91512.80	34.42
	25524.6779	25524.2075	-0.4704	G2F	20839.10	7.84
	24972.1419	24971.2000	-0.9419	Man5	750.00	0.28
Run 3	24997.1953	24996.4288	-0.7665	G0F-GN	11239.90	4.59
	25159.3386	25158.7643	-0.5743	G1F-GN	6987.22	2.85
	25200.3914	25199.8996	-0.4918	G0F	120099.49	49.04
	25362.5347	25362.0717	-0.4630	G1F	86483.22	35.31
	25524.6779	25524.4143	-0.2636	G2F	19329.98	7.89
	24972.1419	24972.3500	0.2081	Man5	775.00	0.32

Table S4. The cosine value of the six major glycans of mAb A between 2D-LC-MS method and other methods.

Glycan	2D-LC-MS method	Rapid 2-AB method	2D-LC-MS method	Reduction method	2D-LC-MS method	Off-line IdeS digestion method
G0F-GN	4.74	1.85	4.74	1.75	4.74	5.29
G1F-GN	2.97	0.00	2.97	1.97	2.97	2.80
G0F	49.46	41.22	49.46	44.31	49.46	43.64
G1F	34.75	38.48	34.75	40.91	34.75	36.82
G2F	7.77	9.53	7.77	9.34	7.77	8.04
Man5	0.31	4.30	0.31	1.73	0.31	3.41
Cosine value	**0.9853**		**0.9895**		**0.9946**	

Note: the cosine value was calculated by the below equation.

$$\text{similarity} = \cos(\theta) = \frac{A \cdot B}{\|A\|\|B\|} = \frac{\sum_{i=1}^{n} A_i \times B_i}{\sqrt{\sum_{i=1}^{n} (A_i)^2} \times \sqrt{\sum_{i=1}^{n} (B_i)^2}}.$$

Table S5. The cosine value of the five major glycans of mAb B between 2D-LC-MS method and rapid 2-AB method.

Glycan	2D-LC-MS method	Rapid 2-AB method
G0F-GN	3.72	1.18
G0F	82.13	82.94
G1F	11.58	12.22
G2F	1.01	1.02
Man5	0.00	0.79
Cosine value	**0.9995**	

Note: the cosine value was calculated by the below equation.

$$\text{similarity} = \cos(\theta) = \frac{A \cdot B}{\|A\|\|B\|} = \frac{\sum_{i=1}^{n} A_i \times B_i}{\sqrt{\sum_{i=1}^{n} (A_i)^2} \times \sqrt{\sum_{i=1}^{n} (B_i)^2}}.$$