Analytical and Bioanalytical Chemistry

Electronic Supplementary Material

Chip-based nLC-TOF-MS is a highly stable technology for large-scale high-throughput analyses

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Table S1. Intra- and inter- day variability of nLC-chip-TOF-MS analysis of serum-derived N-glycans. Glycan compositions that were observed in all 51 analyses over 3 days are listed together with their calculated masses; intra-day CV's are calculated within a day and the overall inter-day CV across 3 days. CV's were calculated based on the $\log_{10}$ transformed values

		Intra-Day CV (%)			
Mass	**Composition**	**Day 1**	**Day 2**	**Day 3**	**Inter-Day CV (%)**
910.328	H_3N_2	2%	9%	1%	6%
1072.38	H_4N_2	1%	5%	1%	3%
1113.41	H_3N_3	3%	2%	5%	5%
1234.43	H_5N_2	1%	2%	1%	2%
1259.47	$H_3N_3F_1$	4%	2%	1%	4%
1275.46	H_4N_3	3%	1%	1%	4%
1316.49	H_3N_4	1%	3%	1%	2%
1396.49	H_6N_2	1%	4%	1%	3%
1421.52	$H_4N_3F_1$	2%	1%	1%	3%
1437.51	H_5N_3	2%	6%	1%	4%
1462.54	$H_3N_4F_1$	1%	1%	1%	2%
1478.54	H_4N_4	2%	2%	1%	2%

1566.56	$H_4N_3S_1$	1%	1%	1%	3%
1599.57	H_6N_3	1%	3%	1%	3%
1624.6	$H_4N_4F_1$	1%	2%	1%	2%
1640.59	H_5N_4	1%	1%	1%	2%
1665.62	$H_3N_5F_1$	1%	6%	1%	4%
1681.62	H_4N_5	1%	9%	1%	6%
1712.61	$H_4N_3F_1S_1$	5%	2%	3%	6%
1720.59	H_8N_2	1%	8%	1%	5%
1728.61	$H_5N_3S_1$	1%	1%	1%	3%
1769.64	$H_4N_4S_1$	1%	1%	1%	3%
1786.65	$H_5N_4F_1$	3%	1%	1%	3%
1827.68	$H_4N_5F_1$	1%	4%	1%	3%
1843.67	H_5N_5	1%	9%	1%	6%
1882.65	H_9N_2	1%	8%	1%	5%
1890.66	$H_6N_3S_1$	2%	1%	2%	4%
1915.69	$H_4N_4F_1S_1$	2%	3%	3%	5%
1931.69	$H_5N_4S_1$	1%	1%	1%	3%
1972.71	$H_4N_5S_1$	2%	4%	2%	4%
1989.73	$H_5N_5F_1$	1%	3%	1%	2%
2005.72	H_6N_5	4%	3%	5%	7%
2044.7	$H_{10}N_2$	1%	6%	2%	4%
2077.75	$H_5N_4F_1S_1$	2%	1%	2%	4%
2093.74	$H_6N_4S_1$	2%	1%	3%	4%
2118.77	$H_4N_5F_1S_1$	2%	1%	2%	3%
2134.77	$H_5N_5S_1$	1%	1%	1%	2%
2222.78	$H_5N_4S_2$	1%	2%	1%	4%
2280.83	$H_5N_5F_1S_1$	1%	1%	1%	2%
2296.82	$H_6N_5S_1$	3%	1%	2%	4%
2368.84	$H_5N_4F_1S_2$	1%	1%	1%	4%
2384.84	$H_6N_4S_2$	4%	1%	3%	5%
2425.86	$H_5N_5S_2$	3%	3%	2%	5%
2442.88	$H_6N_5F_1S_1$	5%	2%	5%	7%
2571.92	$H_5N_5F_1S_2$	2%	3%	1%	4%
2587.92	$H_6N_5S_2$	2%	1%	1%	4%
2733.97	$H_6N_5F_1S_2$	2%	1%	1%	5%
2879.01	$H_6N_5S_3$	2%	2%	1%	4%
2953.05	$H_7N_6S_2$	2%	2%	3%	6%
3025.07	$H_6N_5F_1S_3$	1%	3%	1%	5%

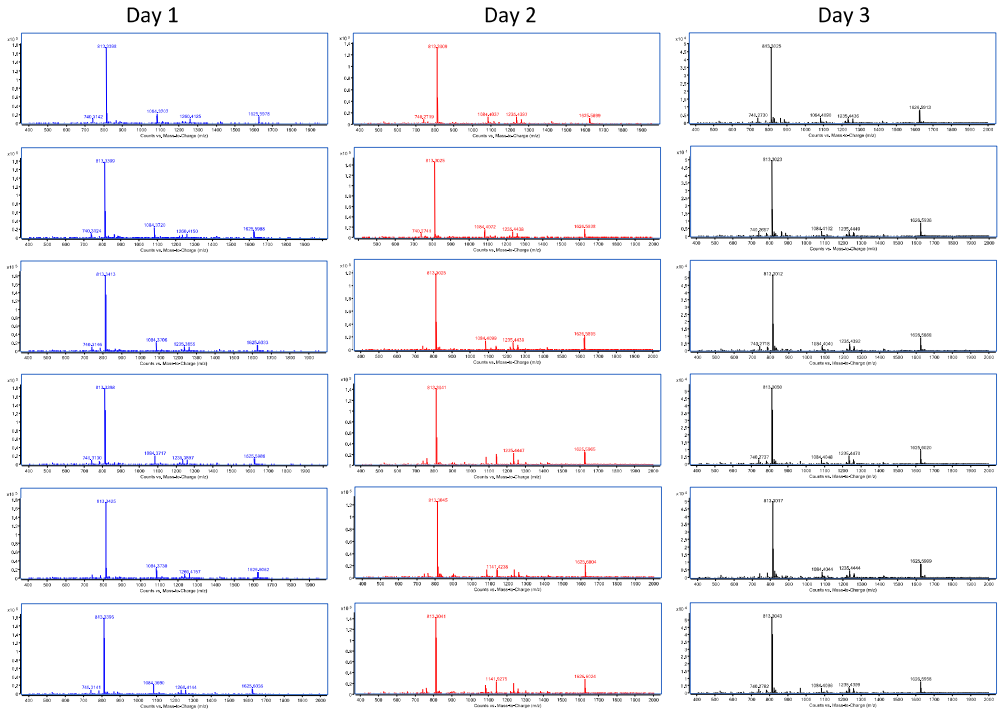
Table S2. Intraclass correlations for each glycan for Day 1 vs. Day 2, Day 1 vs. Day 3 and Day 2 vs. Day 3

Glycan	Day1 vs. Day2	Day1 vs. Day3	Day2 vs. Day3
2222.783	0.211	0.963	0.910
1931.688	0.032	0.933	0.964
2879.011	0.000	0.944	0.914
1624.597	0.177	0.947	0.833
2368.841	0.926	0.826	0.974
1462.544	0.000	0.886	0.778
2077.745	0.393	0.821	0.942
3025.068	0.026	0.970	0.900
1827.677	0.000	0.797	0.000
1786.65	0.235	0.740	0.954
1665.624	0.066	0.478	0.000
2587.915	0.053	0.913	0.961
2571.92	0.087	0.887	0.667
2280.825	0.434	0.900	0.895
1640.592	0.392	0.855	0.857
1566.555	0.772	0.926	0.962
1234.433	0.333	0.459	0.704
1989.729	0.000	0.186	0.150
1882.645	0.087	0.858	0.000
1396.486	0.000	0.000	0.000
2733.973	0.000	0.961	0.976
1478.539	0.025	0.573	0.616
1720.592	0.068	0.647	0.000
1681.619	0.059	0.000	0.070
1769.635	0.921	0.750	0.950
1316.487	0.000	0.898	0.404
2296.82	0.377	0.802	0.962
1728.608	0.572	0.803	0.913
1072.381	0.079	0.842	0.000
2134.767	0.692	0.725	0.880
910.3278	0.000	0.109	0.077
1259.465	0.510	0.173	0.877
1275.46	0.587	0.527	0.946
1421.518	0.594	0.868	0.955
1843.672	0.000	0.000	0.000
2118.772	0.630	0.595	0.868
1113.407	0.045	0.615	0.732
1890.661	0.458	0.793	0.902
2953.047	0.172	0.924	0.947
1915.693	0.000	0.871	0.839
2425.862	0.038	0.816	0.888
1599.566	0.377	0.000	0.347

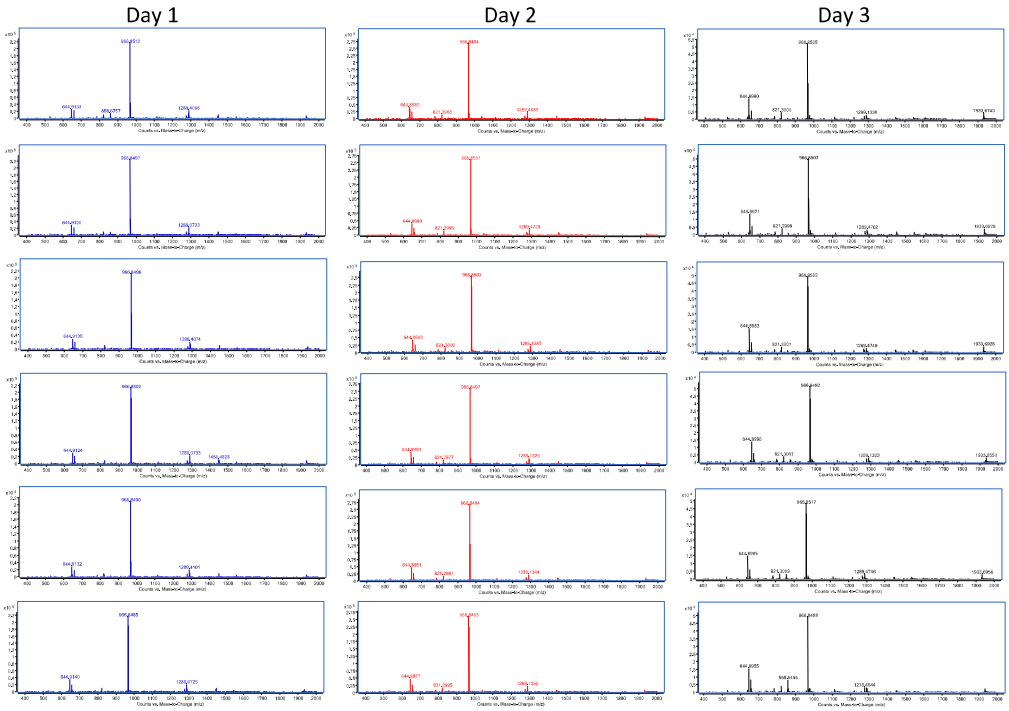
1712.613	0.763	0.186	0.917
1437.513	0.124	0.833	0.000
2384.836	0.378	0.743	0.927
2093.74	0.683	0.650	0.902
1972.714	0.300	0.277	0.034
2044.697	0.000	0.461	0.000
2442.878	0.135	0.676	0.848
2005.724	0.213	0.696	0.848

Fig. S1. MS spectra for the two glycan structures $H_4N_4F_1$ and $H_5N_4S_1$ at m/z 813.31 and m/z 966.85 and 8.2 min and 9.5 min, respectively. MS spectra are shown for six runs on the three different days

$H_4N_4F_1$ at m/z 813.31 and 8.2 min.



$H_5N_4S_1$ at m/z 966.85 and 9.5 min.



Materials and methods

Study design

N-glycan analysis from one standard serum sample was performed repeatedly with intermittent blanks on three non-consecutive days. Twelve duplicate analyses were performed on Day 1, twenty-two analyses were performed on Day 2 and seventeen analyses were performed on Day 3; the analyses on days 1 and 2 were performed on a previously used chip with approximately 300 hours of operation, while for the analyses on Day 3 a new analytical chip was used.

N-glycan sample preparation

To evaluate the stability of the nLC-PGC-chip-TOF-MS instrument for profiling of N-glycans from serum, N-glycans were released from the proteins in 50 μl of serum standard (Sigma-aldrich, St.Louis, MO) as previously described [1]. Briefly, proteins were denatured using 100mM dithiothreitol (DTT, Promega, Madison, WI), and subsequent heating. Two μL of PNGaseF (New England Biolabs, Ipswich, MA) were added to the samples, and enzymatic glycan release was performed in a CEM (Matthews, NC) microwave at 20W for 10 min [1]. Deglycosylated proteins were precipitated using 400 μL of ice-cold ethanol. After centrifugation, the supernatant containing the glycans was brought to dryness *in vacuo*.

N-linked glycans released by PNGaseF were purified using graphitized carbon SPE (Grace, Deerfield, IL) [2-4]. Briefly, cartridges were conditioned using 4 mL of 80% ACN containing 0.05% TFA (EMD chemicals, Gibbstown, NJ), followed by 4 mL of

water containing 0.05% TFA. Glycan samples were reconstituted in 500 µL of water and subsequently loaded onto the cartridges. Cartridges were washed using 3 x 4 mL of water and N-glycans were eluted using 4 mL of 40% ACN containing 0.05% TFA. Samples were dried *in vacuo* prior to analysis.

nHPLC-chip-TOF-MS analysis

N-glycans were analyzed using an Agilent (Santa Clara, CA) 6200 series nanoHPLC-chip-TOF-MS, consisting of an autosampler, which was maintained at 8°C, a capillary loading pump, a nanopump, HPLC-chip-MS interface and an Agilent 6210 Time Of Flight mass spectrometer. The chip (Glycan Chip II, Agilent) contained a 9 x 0.075 mm i.d. enrichment column coupled to a 43 x 0.075 mm i.d. analytical column; both packed with 5 µm porous graphitized carbon (PGC). N-glycan samples were reconstituted in 45 µL of water and diluted 1:5 with water prior to analysis; 1 µL of sample was used for injection. Upon injection, the sample was loaded onto the enrichment column using 3% ACN containing 0.1% formic acid (FA, Fluka, St. Louis, MO). Then the analytical column was switched in-line so that the nano-pump delivered a gradient of 3%ACN with 0.5% FA (solvent A) to 90% ACN with 0.5% FA (solvent B). The mass spectrometer was operated in positive ionization mode.

Data analysis

Data analysis was performed using Masshunter® qualitative analysis (version B.03.01, Agilent) and Microsoft® Excel® for Mac 2011 (version 14.1.3, Microsoft), according to Hua et al. [4] with modifications. Data was loaded into Masshunter® qualitative analysis, and glycan features were identified and integrated using the Molecular Feature Extractor

algorithm. First, signals above a signal to noise threshold of 5.0 were considered. Then, signals were deconvoluted using a tolerance of 0.0025 *m/z* ± 10 ppm. The resulting deconvoluted masses were subsequently annotated using a retrosynthetic theoretical glycan library which was previously developed [5] and contained 331 possible N-glycan compositions. A 15 ppm mass error was allowed. Glycan compositions, retention times and peak area were exported to csv-format for further evaluation.

Statistics

For statistical evaluation, the integral values were $\log_{10}$ transformed to reduce the influence of extreme values. Patterns in total glycan area and the proportion of non-detectables over time were evaluated graphically as was the relationship with the proportion of non-detectables and area values. Coefficients of variance (CVs) were calculated to assess inter- and intra-day variability.

References

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