Supporting Information

Glucose Unit Index (GUI) of Permethylated Glycans For Effective Identification of Glycans and Glycan Isomers

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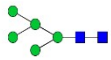
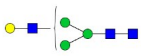
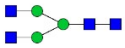
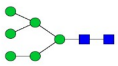
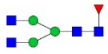
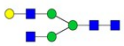
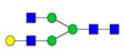
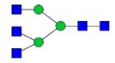
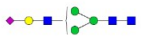
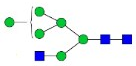
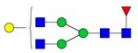
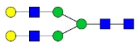
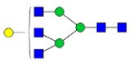
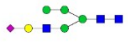
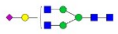
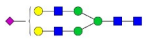
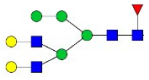
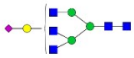
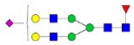
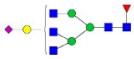
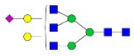
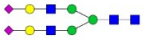
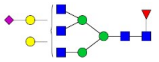
Key words: Glucose unit index, Permethylated glycans, LC-MS/MS, C18 column, Porous graphitic carbon

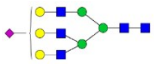
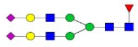
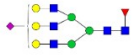
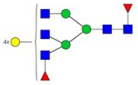
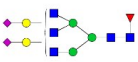
Table-1 GUI of permethylated glycans on C18 Column

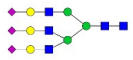
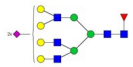
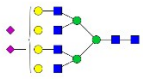
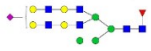
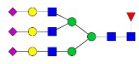
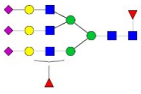
Glycan Structure	Glycan code	Theoretical *m/z*	Observed *m/z*	Mass accuracy (in ppm)	MS2 ions for structural confirmation	Average GUI	SD
	2500	787.4203	787.4177	3.3019	627.2 755.5 1062.6 1355.7	4.2	0.1
	3400	807.9336	807.9313	2.7849	464.3 903.1 1103.6 1321.8 1396.8	3.5	0.1
			807.9312	2.9087	464.2 1103.7 1321.7 1396.6	3.9	0.2
	4300	828.4468	828.4444	2.8970	636.2 1117.6 1137.6 1192.5 1362.7	3.4	0.2
	2600	889.4702	889.4672	3.3728	391.3 876.2 915.2 1062.7 1266.6 1355.2 1559.8	5.2	0.1
	4310	915.4914	915.4891	2.5123	280.3 468.2 899.6 1103.7 1178.6 1362.7 1570.8	4.0	0.2
	4400	930.4967	930.4930	3.9764	464.2 917.3 1103.5 1142.6 1348.7 1396.6 1566.8	3.8	0.2
			930.4938	3.1166	436.2 468.3 899.6 1103.6 1600.8 1641.9	4.2	0.2
Glycan Structure	**Glycan code**	**Theoretical *m/z***	**Observed *m/z***	**Mass accuracy (in**	**MS2 ions for structural**	**Average GUI**	**SD**

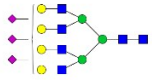
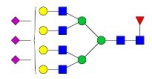
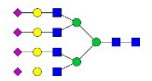
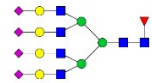
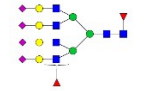
				ppm)	confirmation		
	5300	951.0100	951.0070	3.1545	903.6 933.5 1089.6 1144.7 1348.7 1607.7	4.5	0.2
	3401	988.5204	988.5178	2.6302	825.7 858.6 1103.6 1307.6 1396.7	4.3	0.2
	3600	1020.5467	1020.5432	3.4638	No MS^2	5.1	0.1
	4410	1017.5413	1017.5384	2.8500	432.2 468.2 899.7 1103.7 1275.7 1307.7 1366.6	4.5	0.2
	4500	1032.5466	1032.5437	2.8086	432.3 464.3 903.1 1307.8 1600.9	4.3	0.2
			1032.5427	3.7771	464.3 808.1 885.4 1307.6 1378.6	4.7	0.2
	5400	1053.0599	1053.0568	2.9438	432.4 464.3 1089.8 1144.7 1334.8 1348.7 1552.8 1641.8 1845.9	4.9	0.2
			1053.0562	3.5136	464.4 885.6 1089.7 1144.8 1307.7 1348.8 1552.9	5.4	0.2
Glycan	**Glycan**	**Theoretical**	**Observed**	**Mass**	**MS2 ions for**	**Average**	**SD**

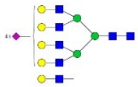
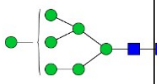
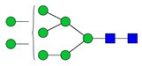
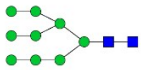
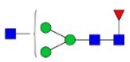
Structure	code	*m/z*	*m/z*	accuracy (in ppm)	structural confirmation	GUI	
	3501	1090.5703	1090.5668	3.2093	376.4 436.3 825.6 826.4 1030.7 1089.9 1307.8 1355.6 1805.0	5.3	0.2
	4401	741.0583	741.0558	3.4185	376.2 450.3 825.6 876.3 1103.6 1293.8 1396.6 1552.8 1668.9	4.7	0.2
	4510	746.7301	746.7281	2.6783	464.3 844.5 1089.7 1552.6	5.0	0.2
	4411	799.0881	799.0853	3.4623	377.5 418.5 436.3 468.2 489.0 525.4 581.4 622.1 885.6 1178.7 1348.7 1669.0 1761.1	5.3	0.2
	4501	809.0916	809.0889	3.3371	622.4 808.4 826.6 844.5 1066.9 1089.1 1103.6	5.1	0.2
	4610	814.7634	814.7641	0.8591	622.3 658.3 785.4 1066.8 1307.7	5.1	0.2
Glycan Structure	**Glycan code**	**Theoretical *m/z***	**Observed *m/z***	**Mass accuracy (in ppm)**	**MS2 ions for structural confirmation**	**Average GUI**	**SD**

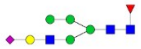
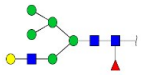
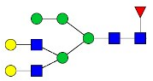
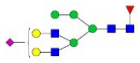
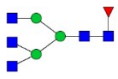
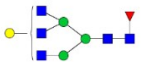
	5401	822.7671	822.7639	3.8893	849.7 883.6 899.7 1334.7 1348.7 1538.7	5.6	0.2
	4511	867.1213	867.1182	3.6135	432.3 1066.8 1089.7 1307.9 1441.7 1482.6 1538.8 1552.8 1913.9 1932.2	5.6	0.2
	5411	880.7968	880.7948	2.3085	468.2 808.5 867.1 899.8 1121.4 1144.7 1334.8 1368.5 1593.9 1710.8	6.2	0.3
	5501	890.8004	890.7980	2.6568	432.2 464.4 468.0 1029.7 1089.9 1148.4 1334.7 1552.8	5.9	0.3
	4502	929.4828	929.4798	3.2635	450.3 622.4 825.6 885.5 1029.6 1334.7 1538.9 1831.8 1831.8 1914.0	5.7	0.2
Glycan Structure	**Glycan code**	**Theoretical *m/z***	**Observed *m/z***	**Mass accuracy (in ppm)**	**MS2 ions for structural confirmation**	**Average GUI**	**SD**
	5511	948.8301	948.8268	3.4780	432.3 677.4	6.4	0.3

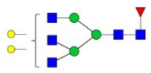
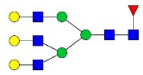
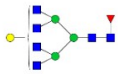
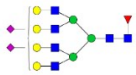
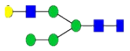
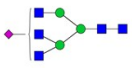
					808.6 825.6 826.6 858.2 881.5 1538.9 1552.9		
	5601	958.8336	958.8306	3.1636	432.2 464.3 668.2 843.4 1103.8 1396.7 1538.8 1757.0	5.4	0.2
			958.8303	3.4764	464.2 825.4 1334.4 1538.9 1800.0 1899.8 1989.2	5.8	0.2
	4512	987.5126	987.5096	3.0042	468.3 580.6 825.5 1293.6 1914.1	6.3	0.3
	5611	1016.8634	1016.8597	3.6059	No MS^2	6.0	0.3
			1016.8595	3.8025	464.3 468.4 826.4 1089.7 1103.7 1743.0 1784.0	6.4	0.2
			1016.8604	2.9175	826.5 919.2 1015.8 1103.7 1468.6 1554.8	7.0	0.3
	5720	1022.5351	1022.5362	1.0432	464.2 636.4 668.5 1030.6 1048.7 1942.7 1988.4	6.5	0.3
Glycan Structure	**Glycan code**	**Theoretical *m/z***	**Observed *m/z***	**Mass accuracy (in ppm)**	**MS2 ions for structural confirmation**	**Average GUI**	**SD**
	5512	802.1680	802.1648	3.9580	413.3 468.4	6.9	0.3

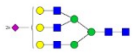
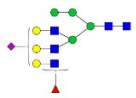
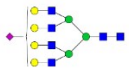
					825.5 1130.7 1988.3		
	5603	1199.6161	1199.6121	3.3344	450.3 580.4 825.4 1464.7 1900.0 1948.2	6.5	0.3
			1199.6110	4.2514	450.3 580.3 825.5 997.6 1465.0 1900.0	7.0	0.0
	5712	904.2179	904.2173	0.6359	867.1 881.0 903.6 1284.8 1297.8 1298.9 1320.9 1840.6	6.5	0.3
	6702	921.9772	921.9742	3.1996	464.4 793.6 825.4 1015.6 1164.6 1468.7	6.7	0.3
	6811	926.2310	926.2308	0.1889	432.1 767.1 1053.5 1537.2 1561.4 1636.5	6.7	0.3
	5613	943.4864	943.4834	3.1267	450.3 580.4 825.6 1464.6 1899.8	6.9	0.3
			943.4839	2.5968	825.6 1427.8 1900.0 1975.1 1991.7	7.6	0.3
Glycan Structure	**Glycan code**	**Theoretical *m/z***	**Observed *m/z***	**Mass accuracy (in ppm)**	**MS2 ions for structural confirmation**	**Average GUI**	**SD**
	5623	987.0087	943.4839	2.5968	468.2 699.0 999.5 1000.6	7.4	0.3

			987.0056	3.0901	418.2 624.4 793.4 811.4 825.5 999.6 1242.7 1638.8 1714.0	7.5	0.3
	6703	1012.2706	1012.2666	3.9268	450.3 729.1 825.7 826.6 899.4 1478.5 1557.0 1659.8 1763.8	7.2	0.4
	6713	1055.7929	1055.7898	2.9125	523.6 825.6 867.5 899.4 1057.7 1350.9	7.4	0.3
			1055.7915	1.3023	825.5 1015.6 1075.8 1636.7 1675.5 1974.8	7.7	0.3
			1055.7902	2.5336	825.7 844.6 1015.3 1033.5 1039.8 1057.7 1446.7	8.2	0.3
	6704	1102.5640	1102.5603	3.3558	450.4 580.3 825.5 1879.6 1900.1	7.7	0.4
Glycan Structure	**Glycan code**	**Theoretical *m/z***	**Observed *m/z***	**Mass accuracy (in ppm)**	**MS2 ions for structural confirmation**	**Average GUI**	**SD**
	6714	1146.0863	1146.0822	3.5774	450.2 825.5 6193.9 1864.0 1957.3	8.1	0.4
	6724	1189.6086	1189.6056	2.5218	420.5	8.5	0.4

					450.2 580.3 598.5 793.5 811.4 999.6 1660.0		
			1189.6053	2.7740	450.2 793.4 825.7 999.7 1826.0	8.7	0.4
	7804	1214.8705	1214.8670	2.9015	450.3 825.5 1094.3 1557.0	8.0	0.4
			1214.8684	1.7492	450.4 825.5 1278.3 1414.2 1953.1	8.3	0.4
	2400	1369.7329	1369.7295	2.4822	No MS^2	3.5	0.1
	2700	1000.0335	1000.0303	3.1849	785.2 840.4 901.9 1101.2 1689.0 1764.0	6.0	0.1
	2800	1110.5968	1110.5927	3.6917	622.3 1035.1 1252.7 1528.1 1674.9 1713.1 1893.0	6.8	0.1
	2900	1204.1333	808.7661	0.8408	599.7 729.4 826.5 1123.4 1338.0 1411.9 1750.4	7.5	0.1
Glycan Structure	**Glycan code**	**Theoretical *m/z***	**Observed *m/z***	**Mass accuracy (in ppm)**	**MS2 ions for structural confirmation**	**Average GUI**	**SD**
	3310	792.9283	792.9260	2.8376	464.3 654.2 867.3 899.5 903.6 1121.5 1366.7	3.5	0.1

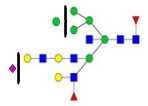
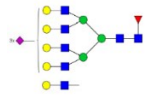
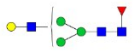
					1396.7		
			792.9268	1.8287	464.2 468.3 654.3 679.2 840.2 858.5 899.6 903.2 1117.6 1325.7 1366.7	3.7	0.1
	3411	1075.5650	1075.5624	2.4173	432.4 450.4 507.5 580.5 843.7 1103.7 1571.0	4.9	0.2
	3511	1177.6149	1177.6097	4.4157	No MS^2	5.8	0.2
	3610	1107.5913	1107.5863	4.5143	418.2 464.4 489.2 507.3 826.6 1048.6 1352.8	5.3	0.2
	4610	814.7634	814.7641	0.8591	No MS^2	5.1	0.2
	4611	935.1546	935.1545	0.1069	450.2 695.2 825.3 843.6 885.5 1075.5 1205.6 1609.8 1947.4	5.7	0.3
Glycan Structure	**Glycan code**	**Theoretical *m/z***	**Observed *m/z***	**Mass accuracy (in ppm)**	**MS2 ions for structural confirmation**	**Average GUI**	**SD**
	5310	1038.0546	1038.0516	2.8419	468.2 1144.6 1316.8 1348.6 1607.8	5.1	0.2
	5410	1140.1045	1140.1019	2.2805	464.2 604.2 885.4	5.5	0.2

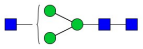
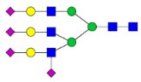
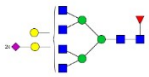
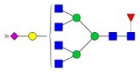
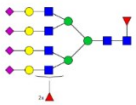
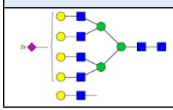
					1089.6 1294.0 1348.8 1593.9 1628.0		
	5510	828.4389	828.4363	3.0982	858.7 844.5 858.7 885.4 1103.6	5.7	0.2
	5610	1344.2043	1344.2064	1.5995	No MS^2	5.9	0.3
	6410	1262.6676	1262.6647	2.2967	No MS^2	5.9	0.0
	6712	1286.9966	1286.9926	3.1339	825.4 1223.7 1260.5 1728.4 1859.0	7.2	0.3
			1286.9934	2.5123	681.3 825.4 1039.8 1668.7	7.6	0.4
	3500	909.9835	909.9807	3.0770	No MS^2	4.6	0.1
	5301	760.4092	760.4059	4.3398	418.3 432.3 711.4 729.4 826.5 897.2 1334.6 1348.6	5.6	0.2
Glycan Structure	**Glycan code**	**Theoretical *m/z***	**Observed *m/z***	**Mass accuracy (in ppm)**	**MS2 ions for structural confirmation**	**Average GUI**	**SD**
	5500	770.4091	770.4061	3.9373	No MS^2	5.3	0.2

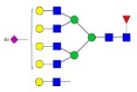
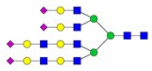
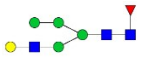
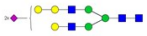
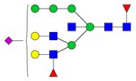
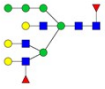
	5602	1079.2249	1079.2209	3.6755	432.3 645.2 825.4 1103.6 1314.3 1464.8 1744.0	6.3	0.1
			1079.2213	3.3048	464.3 580.3 825.5 1123.2 1586.9 1600.1 1743.0 1783.7	6.6	0.3
			1079.2201	4.4168	450.2 464.1 825.5 1029.3 1538.8 1586.7 1743.8 1783.9	7.1	0.3
	5711	813.9245	813.9243	0.2457	No MS^2	6.2	0.3
	6701	831.6837	831.6815	2.6753	450.1 521.1 604.0 1089.7 1293.6 1538.7 1742.9	6.2	0.2
Glycan Structure	**Glycan code**	**Theoretical *m/z***	**Observed *m/z***	**Mass accuracy (in ppm)**	**MS2 ions for structural confirmation**	**Average GUI**	**SD**

	5612	1137.2546	1137.2509	3.2534	432.3 464.3 825.6 1040.0 1103.6 1243.5 1538.8 1654.8 1714.0 1783.8	6.6	0.4
			1137.2511	3.0776	432.3 464.2 825.6 997.2 1089.6 1293.9 1538.9 1572.8 1713.6	6.7	0.4
			1137.2507	3.4293	No MS^2	7.0	0.4
			1137.2504	3.6931	No MS^2	7.1	0.3
			1137.2517	2.5500	432.2 825.5 1053.3 1089.6 1284.8 1293.8 1742.9 1899.9	7.6	0.3
	6711	1166.6054	1166.6022	2.7430	No MS^2	6.7	0.3
			1166.6034	1.7144		6.9	0.3
			1166.6005	4.2002		7.1	0.4

Glycan Structure	Glycan code	Theoretical *m/z*	Observed *m/z*	Mass accuracy (in ppm)	MS2 ions for structural confirmation	Average GUI	SD

	6921	1360.7017	1360.7047	2.2292	450.4 825.3 1007.1 1161.5 1215.6	7.2	0.4
	7813	1168.0994	1168.0973	1.7978	450.4 681.4 695.5 825.7 1169.7 1279.9 1432.4 1465.7 1520.4 1924.2	8.1	0.3
			1168.0952	3.5956	464.4 825.4 1043.4 1075.9 1432.7 1475.0 1914.5	8.3	0.3
			1168.0947	4.0236	825.5 1103.4 1300.5 1478.8 1511.3 1565.4 1692.7	8.6	0.3
	3410	894.9782	894.9820	4.3018	885.4 1085.8 1334.6 1552.9 1600.5	3.7	0.1
			894.9754	3.0727	840.4 885.8 1076.1 1103.4 1325.5	4.2	0.1
Glycan Structure	**Glycan code**	**Theoretical *m/z***	**Observed *m/z***	**Mass accuracy (in ppm)**	**MS2 ions for structural confirmation**	**Average GUI**	**SD**

	3300	705.8837	705.8821	2.1958	294.3 613.1 654.1 858.6 876.3 890.4 1117.5 1151.6	3.0	0.1
	5502	1516.2835	1516.2778	3.7262	No MS^2	5.7	0.3
	5604	1320.0073	1320.0024	3.7374	450.3 589.2 654.1 794.7 825.4 1057.8 1409.9	7.4	0.1
			1320.0035	2.9040	811.4 825.5 871.3 1053.6 1250.8	7.5	0.0
	6612	1218.9634	1218.9628	0.4649	No MS^2	7.2	0.4
	6613	1339.3546	1339.3517	2.1652	450.4 468.4 715.5 825.5 1062.5 1323.4 1377.2 1906.2	7.6	0.4
	6734	1233.1309	1233.1298	0.8920	No MS^2	9.2	0.3
Glycan Structure	**Glycan code**	**Theoretical *m/z***	**Observed *m/z***	**Mass accuracy (in ppm)**	**MS2 ions for structural confirmation**	**Average GUI**	**SD**
	7803	1124.5771	1124.5756	1.3338	No MS^2	7.5	0.2

			1124.5732	3.4680	418.4 450.3 464.3 825.6 899.6 1098.8 1107.4 1464.7 1502.4 1669.0 1927.0	7.7	0.2
			1124.5729	3.7347	450.3 464.2 825.5 919.4 1057.7 1107.9 1547.9 1914.6	7.9	0.2
	7814	1258.3928	1258.3900	2.2449	No MS^2	8.5	0.3
			1258.3895	2.6423		8.8	0.3
	8904	1327.1771	1327.1730	3.0516	899.7 1110.6 1193.1 1260.1 1270.8	8.4	0.3
			1327.1724	3.5037	No MS^2	8.7	0.3
	3510	997.0281	997.0311	3.0591	No MS^2	4.4	0.1
	4602	997.5161	997.5121	4.0100	418.2 468.1 654.0 826.6 1075.6 1293.9	6.5	0.3
	5721	1142.9264	1142.9258	0.4958	No MS^2	6.6	0.3
			1142.9253	0.9333		6.7	0.3
			1142.9257	0.5833		6.9	0.3
Glycan Structure	**Glycan code**	**Theoretical *m/z***	**Observed *m/z***	**Mass accuracy (in ppm)**	**MS2 ions for structural confirmation**	**Average GUI**	**SD**
	5820	1090.5684	1090.5739	5.0432	No MS^2	6.1	0.3

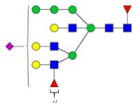
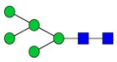
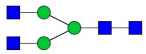
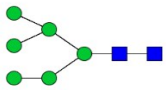
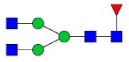
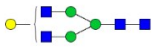
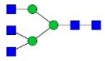
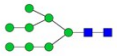
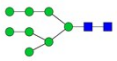
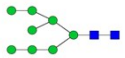
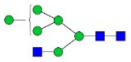
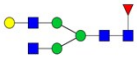
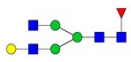
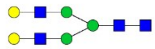
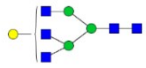
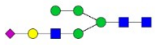
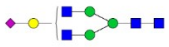
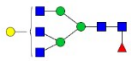
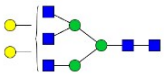
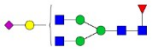
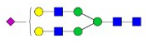
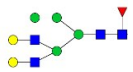
	5831	1268.9894	1268.9923	2.3116	665.7 826.6 1043.6 1063.6 1077.7 1184.8 1449.4 1612.9 1717.0 1821.9	7.0	0.3

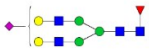
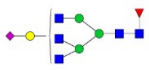
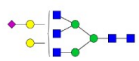
Table-2 GUI of permethylated glycans on PGC Column

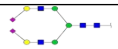
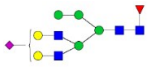
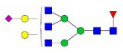
Glycan Structure	Glycan code	Theoretical *m/z*	Observed *m/z*	Mass accuracy (in ppm)	MS2 ions for structural confirmation	Average GUI	SD
	2500	787.4203	787.4191	1.5240	636.7 844.6 1062.5 1137.6 1355.7	5.1	0.1
	3300	705.8837	705.8831	0.8500	858.5 899.6 947.5 1117.7 1151.6 1192.7	4.3	0.1
	3400	807.9336	807.9332	0.4951	464.5 858.6 1321.7 1378.8	4.9	0.1
	4300	828.4468	828.4461	0.8450	654.5 899.6 1103.6 1192.7 1362.7 1396.7	4.9	0.2
	2600	889.4702	889.4693	1.0118	604.5 858.7 1355.8 1559.8	4.8	0.2
			889.4695	0.7870	1062.6 1119.9 1151.6 1266.9 1341.4 1559.8	5.1	0.2
	4310	915.4914	915.4921	0.7646	468.4 844.5 899.5 1071.6 1103.6 1362.7 1570.7	6.5	0.2
	4400	930.4967	930.4961	0.6448	464.4 1307.8 1396.8 1566.8	5.2	0.1
	5300	951.0100	951.0101	0.1052	432.4 794.2 1089.6 1144.6 1348.7	6.2	0.2

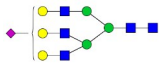
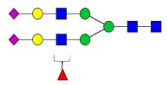
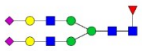
Glycan Structure	Glycan code	Theoretical *m/z*	Observed *m/z*	Mass accuracy (in ppm)	MS2 ions for structural confirmation	Average GUI	SD
	3401	988.5204	988.5192	1.2139	825.5 858.5 1103.6 1151.7 1307.7 1600.9 1682.9 1739.9	5.8	0.3
			988.5203	0.1012	826.4 1103.4 1151.7 1219.8 1307.7 1396.5 1600.8 1682.8	6.2	0.3
	2800	1110.5968	1110.5942	2.3411	No MS^2	4.8	0.1
			1110.6000	2.8813		5.0	0.1
			1110.6014	4.1419		5.4	0.2
	3600	1020.5467	1020.5464	0.2940	No MS^2	5.7	0.1
	4410	1017.5413	1017.5422	0.8845	464.4 604.3 899.6 1071.5 1103.6 1307.7 1348.6 1366.7 1566.8	6.7	0.2
			1017.5417	0.3931	464.3 899.6 1103.6 1307.6 1348.7 1566.9 1775.1 1816.1	7.0	0.1

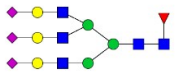
Glycan Structure	Glycan code	Theoretical *m/z*	Observed *m/z*	Mass accuracy (in ppm)	MS2 ions for structural confirmation	Average GUI	SD
	4500	1032.5466	1032.5461	0.4842	432.3 885.0 1071.6 1307.8 1382.9 1552.7 1770.8	5.3	0.2
			1032.5459	0.6779	432.3 464.2 1089.5 1103.7 1307.6 1552.7 1600.8 1770.9	6.0	0.3
			1032.5470	0.3874	No MS^2	7.0	0.1
	5400	1053.0599	1053.0596	0.2849	464.5 826.4 1089.5 1348.7 1382.8 1552.8 1642.0	6.4	0.2
	3501	1090.5703	1090.5703	0.0000	825.5 1062.6 1307.7 1511.7 1600.8	6.5	0.2
	4401	741.0583	741.0574	1.2145	No MS^2	6.3	0.2
			741.0580	0.4048	598.4 825.5 899.7 1103.4 1192.6 1348.6 1396.7 1552.8	6.6	0.2
	5301	1140.1102	1140.1044	5.0872	468.3 636.1 862.4 1334.6 1316.7 1348.6 1350.9	8.5	0.2

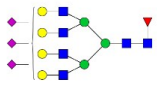
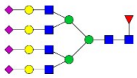
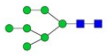
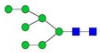
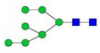
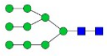
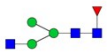
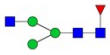
Glycan Structure	Glycan code	Theoretical *m/z*	Observed *m/z*	Mass accuracy (in ppm)	MS2 ions for structural confirmation	Average GUI	SD
	5410	1140.1045	1140.1044	0.0877	464.4 885.9 1089.5 1144.8 1348.7 1552.7 1554.8 1556.7 1593.9	8.7	0.1
			1140.1036	0.7894	464.2 1089.6 1144.6 1316.6 1348.7 1552.8 1784.0 1812.0	9.1	0.2
	5500	770.4091	770.4093	0.2596	No MS^2	7.0	0.2
	4411	799.0881	799.0877	0.5006	No MS^2	7.8	0.5
			799.0884	0.3754	418.4 468.3 825.4 1348.7 1552.8 1760.7	8.2	0.2
	4501	809.0916	809.0914	0.2472	464.5 711.6 1066.9 1334.8 1552.9	6.3	0.4
			809.0914	0.2472	464.3 1067.0 1293.6 1552.7 1668.7 1757.9	6.9	0.3
	4610	814.7634	814.7664	3.6821	525.2 636.4 645.1 699.0 785.1 794.7 1307.8	6.9	0.2

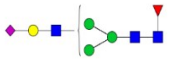
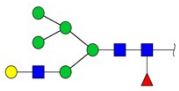
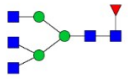
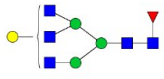
			814.7661	3.3138	No MS^2	7.4	0.3
Glycan Structure	**Glycan code**	**Theoretical *m/z***	**Observed *m/z***	**Mass accuracy (in ppm)**	**MS2 ions for structural confirmation**	**Average GUI**	**SD**
	5401	822.7671	822.7676	0.6077	450.2 899.7 1087.7 1348.7 1593.8	7.3	0.3
			822.7663	0.9723	No MS^2	7.5	0.1
	4511	867.1213	867.1209	0.4613	No MS^2	7.0	0.4
			867.1218	0.5766	468.2 654.5 1066.7 1307.7 1552.8 1756.9 1832.0 1948.0	7.5	0.3
			867.1215	0.2306	468.4 825.5 1066.9 1334.7 1552.9 1586.9 1668.9 1914.2	8.5	0.2
	5411	880.7968	880.7965	0.3406	436.3 468.4 825.4 899.8 1334.6 1538.8 1797.9 1914.0	9.0	0.2
			880.7972	0.4541	794.6 825.4 1348.6 1593.7 1710.9 1797.9 1815.7 1988.2	9.6	0.2
	5501	890.8004	890.8008	0.4490	464.4 849.8	7.7	0.2

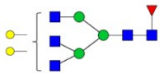
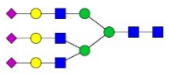
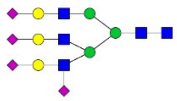
					1148.3 1348.8 1464.8 1552.7		
Glycan Structure	**Glycan code**	**Theoretical *m/z***	**Observed *m/z***	**Mass accuracy (in ppm)**	**MS2 ions for structural confirmation**	**Average GUI**	**SD**
	4502	929.4828	929.4826	0.2152	598.5 825.6 844.6 1293.7 1334.8 1668.6 1962.6	6.3	0.2
			929.4843	1.6138	450.1 825.5 1089.5 1293.5 1334.5 1538.8	7.1	0.3
			929.4831	0.3228	No MS^2	7.8	0.3
			929.4824	0.4303	450.3 825.5 1293.7 1334.8 1538.8 1914.1	8.3	0.0
	4611	935.1546	935.1587	4.3843	432.2 622.3 672.0 825.3 833.7 889.2 899.4 1293.7 1609.9	7.1	0.3
			935.1591	4.8120	636.4 803.7 825.5 876.4 997.3 1015.5 1205.9 1293.5 1334.7	7.9	0.2
	5511	948.8301	948.8307	0.6324	464.3 826.4 905.4 1030.2 1091.7 1314.6 1334.7 1524.7	9.9	0.3

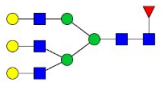
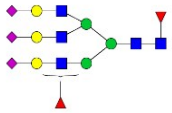
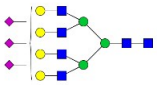
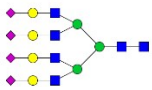
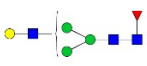
					1540.9 1552.9 1580.1 1913.8		
Glycan Structure	**Glycan code**	**Theoretical *m/z***	**Observed *m/z***	**Mass accuracy (in ppm)**	**MS2 ions for structural confirmation**	**Average GUI**	**SD**
	5601	958.8336	958.8345	0.9386	464.2 645.5 825.6 844.6 1069.4 1128.6 1219.2 1307.9 1538.7 1757.9 1783.9	9.2	0.2
	4512	987.5126	987.5134	0.8101	No MS^2	6.7	0.2
			987.5134	0.8101	793.4 811.6 825.7 1000.5 1089.6 1240.4 1263.7 1293.8 1334.6	7.2	0.1
	4512	987.5126	987.5128	0.2025	468.3 599.4 825.5 1293.1 1311.4 1668.7 1743.2	7.7	0.2
			987.5122	0.4051	468.7 825.6 1069.3 1089.5 1293.6 1334.6 1668.9 1914.0	8.6	0.2
			987.5135	0.9114	450.3 468.2 825.5 844.6 1069.1 1089.7 1293.4 1668.9 1743.9	9.4	0.2

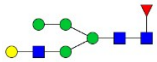
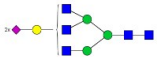
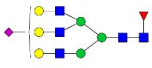
					1801.8 1932.2		
Glycan Structure	**Glycan code**	**Theoretical *m/z***	**Observed *m/z***	**Mass accuracy (in ppm)**	**MS2 ions for structural confirmation**	**Average GUI**	**SD**
	5712	904.2179	904.2211	3.5390	418.2 464.3 825.7 903.5 1079.9 1169.5 1334.6 1555.0 1730.0 1914.0	8.8	0.3
			904.2212	3.6496	848.8 871.0 887.4 903.6 1103.1	9.5	0.2
	5613	943.4864	943.4860	0.4240	No MS^2	8.9	0.5
			943.4886	2.3318	450.4 793.4 984.5 1038.5 1132.9 1423.9 1451.3 1474.3 1510.9 1551.9	9.6	0.6
			943.4869	0.5299	826.5 844.0 867.8 881.7 899.2 1242.6 1650.9 1684.0 1696.8 1858.9 1988.2	10.0	0.2

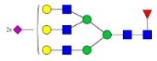
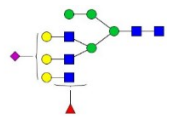
			943.4853	1.1659	418.2 531.6 640.1 661.4 695.4 825.5 1334.7 1447.9 1526.8 1683.9 1900.4 1948.0	10.3	0.4
Glycan Structure	**Glycan code**	**Theoretical *m/z***	**Observed *m/z***	**Mass accuracy (in ppm)**	**MS2 ions for structural confirmation**	**Average GUI**	**SD**
	6713	1055.7929	1055.7922	0.6630	825.6 1026.0 1057.6 1282.7 1446.5 1604.6	10.3	0.2
			1055.7940	1.0419	No MS^2	11.7	0.0
			1055.7944	1.4207	No MS^2	12.4	0.0
	6714	1146.0863	1146.0851	1.0470	517.4 798.6 1071.6 1446.7 1696.9 1887.9	10.2	0.2
			1146.0879	1.3961	No MS^2	11.4	0.0
			1146.0864	0.0873	No MS^2	12.3	0.0
	2700	1000.0335	1000.0325	1.0000	No MS^2	4.8	0.3
			1000.0317	1.7999	711.2 821.5 1470.7 1688.8 1764.0	5.1	0.3
			1000.0317	1.7999	No MS^2	5.6	0.6
	2900	1204.1333	1204.1332	0.0830	400.2 794.7 915.6 1879.1	5.2	0.4
	3310	792.9283	792.9275	1.0089	436.3 654.4 858.5 899.5 1117.5	6.0	0.1
			792.9290	0.8828	No MS^2	6.6	0.2

	3411	1075.5650	1075.5674	2.2314	468.4 636.6 825.5 858.5 1103.6 1307.6 1570.8 1682.8 1774.7	7.4	0.3
	3610	1107.5913	1107.5967	4.8754	450.4 432.5 1089.8 1107.6	6.6	0.1
Glycan Structure	**Glycan code**	**Theoretical *m/z***	**Observed *m/z***	**Mass accuracy (in ppm)**	**MS2 ions for structural confirmation**	**Average GUI**	**SD**
	4510	1119.5912	1119.5933	1.8757	No MS^2	6.6	0.3
			1119.5920	0.7145	844.8 885.8 903.6 1071.3 1087.9 1121.5 1771.0	7.1	0.4
			1119.5939	2.4116	432.2 464.3 1087.4 1103.6 1121.4 1307.7 1552.8 1770.9	8.7	0.2
	5310	1038.0546	1038.0543	0.2890	468.3 899.4 1089.6 1144.6 1316.7 1348.7 1557.0 1607.8 1816.0	8.5	0.2
			1038.0569	2.2157	468.2 885.4 1037.3 1144.6 1348.7 1815.8	9.0	0.2
	5410	1140.1045	1140.1039	0.5263	436.3 885.6 1091.8 1350.8 1609.9	8.7	0.1
			1140.1033	1.0525	432.2	9.1	0.2

					1146.7 1350.7 1609.9		
	5510	1242.1544	1242.1545	0.0805	No MS^2	7.4	0.3
			1242.1552	0.6440	464.3 776.8 1089.4 1348.7 1520.7 1552.7 1580.0	9.2	0.2
Glycan Structure	**Glycan code**	**Theoretical *m/z***	**Observed *m/z***	**Mass accuracy (in ppm)**	**MS2 ions for structural confirmation**	**Average GUI**	**SD**
	5603	1199.6161	1199.6157	0.3334	825.5 1052.6 1201.6 1320.8	8.3	0.1
			1199.6215	4.5014	450.3 825.4 1201.4 1260.5 1465.8	8.8	0.3
			1199.6189	2.3341	450.5 645.8 825.4 1465.4 1899.9 1948.4	9.3	0.4
			1199.6168	0.5835	450.4 825.7 1039.7 1465.7 1948.2	9.8	0.3
	5604	1320.0073	1320.0127	4.0909	825.6 997.5 1087.0 1186.6 1543.2 1646.0 1986.1	8.0	0.1
			1320.0116	3.2575	825.6 830.7 998.6 1646.0 1882.8	8.2	0.1
			1320.0115	3.1818	825.5 829.7 997.7 1187.6 1817.7 1948.0	8.5	0.1

			1320.0131	4.3939	825.7 1186.8 1320.1 1645.9 1901.2	8.9	0.2
			1320.0131	4.3939	825.6 1029.7 1186.8 1605.0 1646.0 1758.0 1886.2	9.7	0.1
Glycan Structure	**Glycan code**	**Theoretical *m/z***	**Observed *m/z***	**Mass accuracy (in ppm)**	**MS2 ions for structural confirmation**	**Average GUI**	**SD**
	5610	1344.2043	1344.2104	4.5380	No MS^2	7.8	0.1
	5623	1321.3510	1321.3520	0.7568	468.3 793.5 825.4 875.4 903.3 1017.6 1107.8 1300.7	10.5	0.4
	6703	1012.2706	1012.2713	0.6915	825.5 1164.4 1225.3 1355.8 1464.8 1613.5	10.7	0.4
			1012.2695	1.0867	No MS^2	11.8	0.0
			1012.2720	1.3830	No MS^2	12.7	0.0
	6704	1102.5640	1102.5664	2.1767	825.5 1037.7 1205.9 1270.2 1604.6 1767.6	10.4	0.2
			1102.5648	0.7256	450.3 825.5 871.7 1605.1 1660.8	11.8	0.0
			1102.5665	2.2674	No MS^2	12.5	0.0
	3410	894.9782	894.9786	0.4469	No MS^2	6.1	0.3
			894.9783	0.1117		6.6	0.1

	3500	909.9835	909.9831	0.4396	432.3 817.3 1062.5 1307.8 1525.9	5.5	0.1
	3510	997.0281	997.0317	3.6107	817.5 826.5 1103.8 1307.7 1787.0	6.1	0.1
			997.0309	2.8083	No MS^2	6.5	0.2
			997.0266	1.5045	468.3 825.5 1307.7 1525.8 1774.8	6.7	0.1
Glycan Structure	**Glycan code**	**Theoretical *m/z***	**Observed *m/z***	**Mass accuracy (in ppm)**	**MS2 ions for structural confirmation**	**Average GUI**	**SD**
	4602	997.5161	997.5156	0.5012	432.2 657.9 843.8 1251.8 1719.9	7.3	0.1
			997.5156	0.5012	432.3 450.1 844.2 858.4 915.7 1119.7	7.5	0.2
			997.5145	1.6040	No MS^2	8.5	0.5
	5502	1011.1916	1011.1926	0.9889	No MS^2	8.7	0.1
			1011.1952	3.5602		9.4	0.1
			1011.1923	0.6923		10.3	0.2
	5611	1016.8634	1016.8619	1.4751	432.2 464.3 1016.6 1146.3 1164.1 1307.7	9.7	0.1

			1016.8641	0.6884	432.3 464.3 468.3 843.6 1016.7	11.2	0.0
			1016.8636	0.1967	464.3 980.3 1016.7 1802.0	11.9	0.0
			1016.8647	1.2784	464..4 697.5 1016.7 1039.6	12.5	0.1
Glycan Structure	**Glycan code**	**Theoretical *m/z***	**Observed *m/z***	**Mass accuracy (in ppm)**	**MS2 ions for structural confirmation**	**Average GUI**	**SD**
	5612	1137.2546	1137.2545	0.0879	No MS^2	8.4	0.0
			1137.2546	0.0000		9.1	0.1
			1137.2565	1.6707		9.3	0.0
			1137.2551	0.4397		10.1	0.0
			1137.2549	0.2638	464.3 793.5 1116.5 1284.5 1456.3 1471.8 1518.9 1559.8	10.2	0.3
			1137.2546	0.0000	464.3 1284.4 1742.9 1972.0	11.3	0.0
	5711	1084.8966	1084.9011	4.1479	No MS^2	8.7	0.0
			1084.8995	2.6731	450.3 590.2 1066.5 1068.0 1431.2 1472.8 1699.8 1858.9	9.7	0.2
			1084.9014	4.4244	464.3 991.6	10.0	0.1

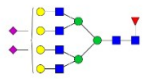
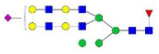
					1049.7 1052.5 1062.7 1272.3 1490.9 1900.2		
	5720	1022.5351	1022.5393	4.1074	No MS^2	9.8	0.3
	5721	1142.9264	1142.9301	3.2373	No MS^2	8.3	0.1
			1142.9307	3.7623		9.2	0.1
			1142.9310	4.0248		10.6	0.0
Glycan Structure	**Glycan code**	**Theoretical *m/z***	**Observed *m/z***	**Mass accuracy (in ppm)**	**MS2 ions for structural confirmation**	**Average GUI**	**SD**
	6702	921.9772	921.9789	1.8439	No MS^2	11.3	0.1
			921.9782	1.0846	464.3 825.5 1282.8 1415.0 1679.1 1988.1	12.0	0.0
	6712	1286.9966	1286.9972	0.4662	No MS^2	11.8	0.0
	6811	926.2310	926.2353	4.6425	No MS^2	12.0	0.0

Figure Legend

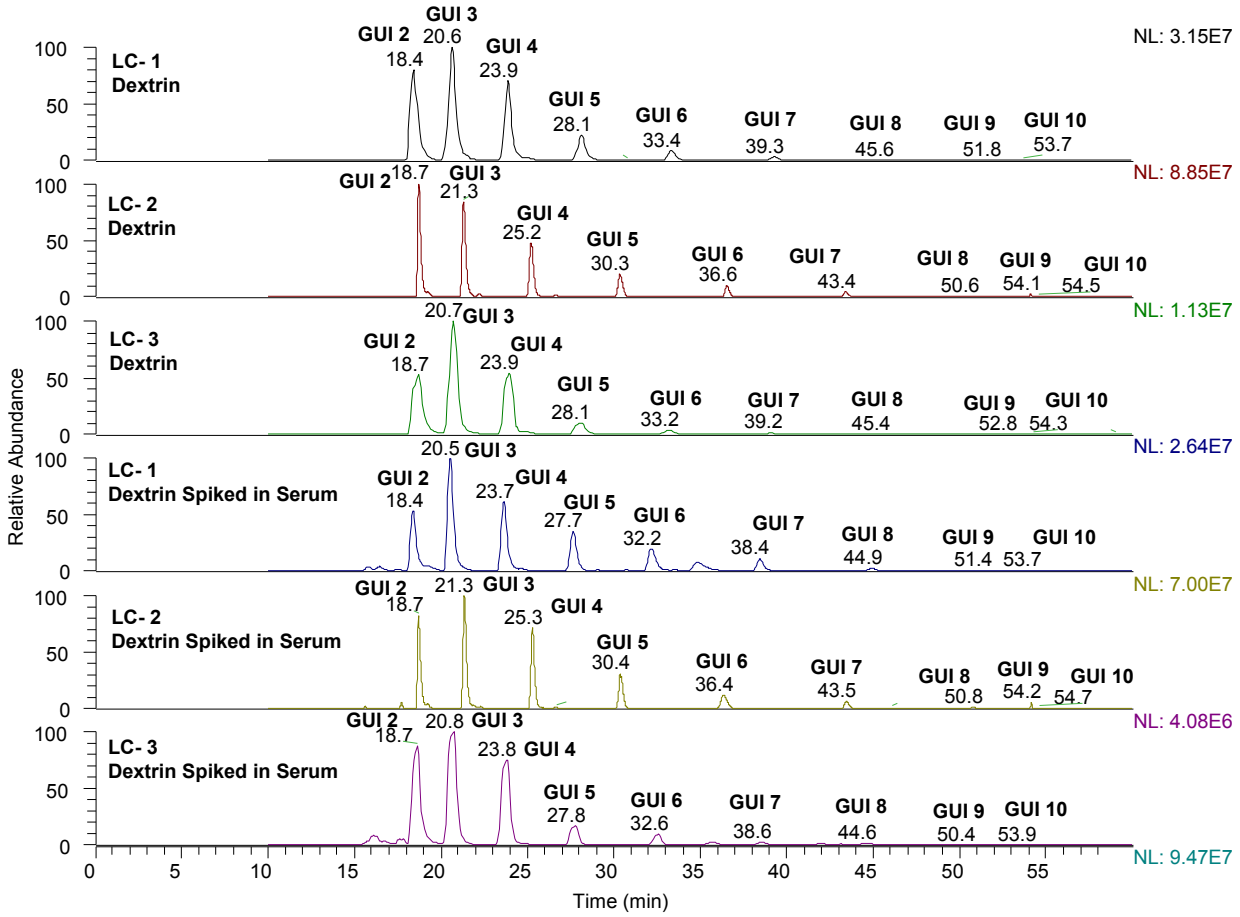
Figure S1. Representation of dextrin ladders alone as well as spiked into serum, separated on three different LC systems. The ladder shows the retention times of GUIs 2 to 10 on different systems.

Figure S2. Scatter plot showing reproducibility of GUI values on two different days on **A**. C-18 column **B.** PGC column

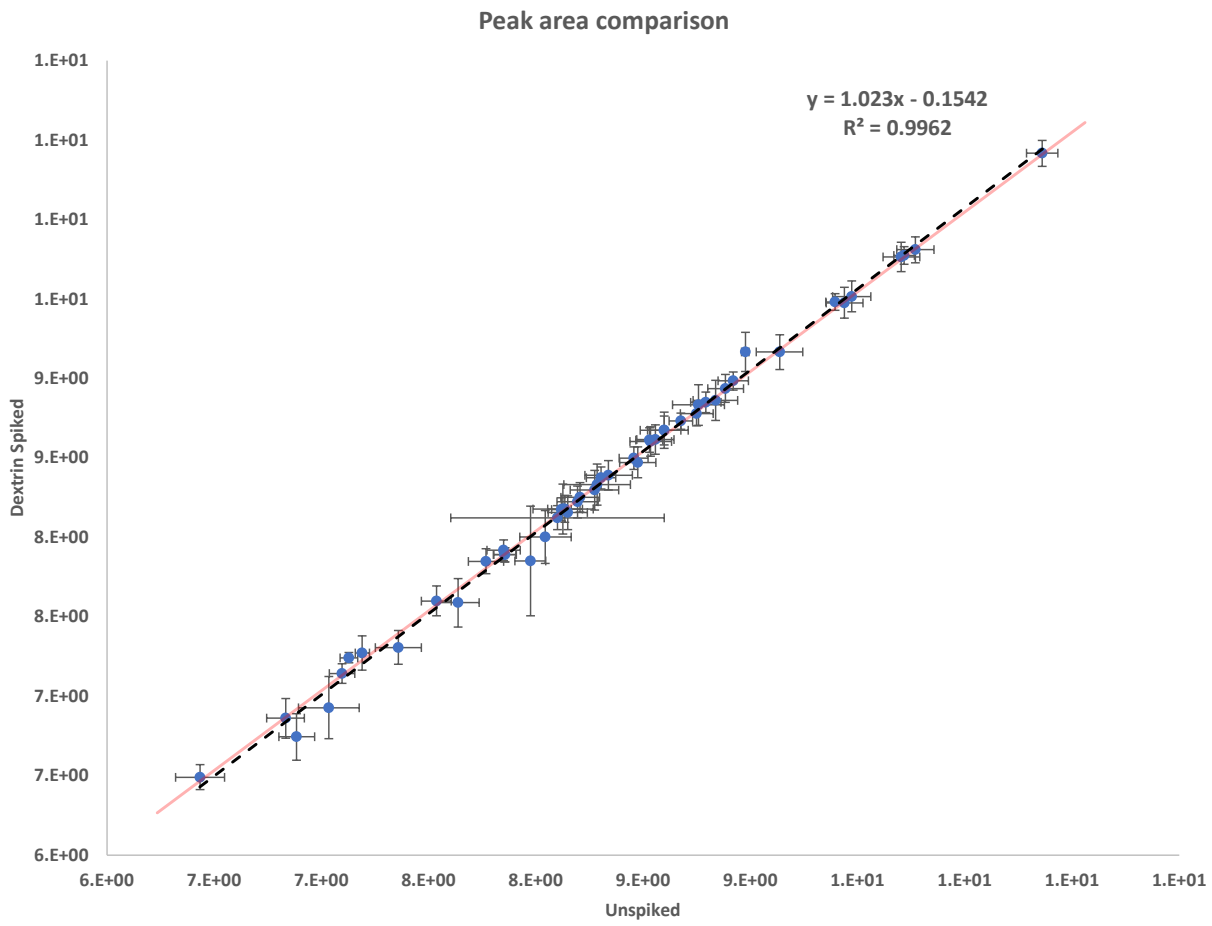
Figure S3. Scatter plot showing comparison of peak areas between spiked and unspiked samples. The figure shows that the peak areas are comparable, indicating there is no suppression in ionization efficiencies of the permethylated glycans derived from the spiked samples.

Figure S4. Scatter plot showing the comparison of GUI values from spiked and unspiked samples. The R^2 value of 0.999 shows the reproducibility of the values between both and unspiked samples, depicting that the samples with the internal standard are not showing variations in GUI.

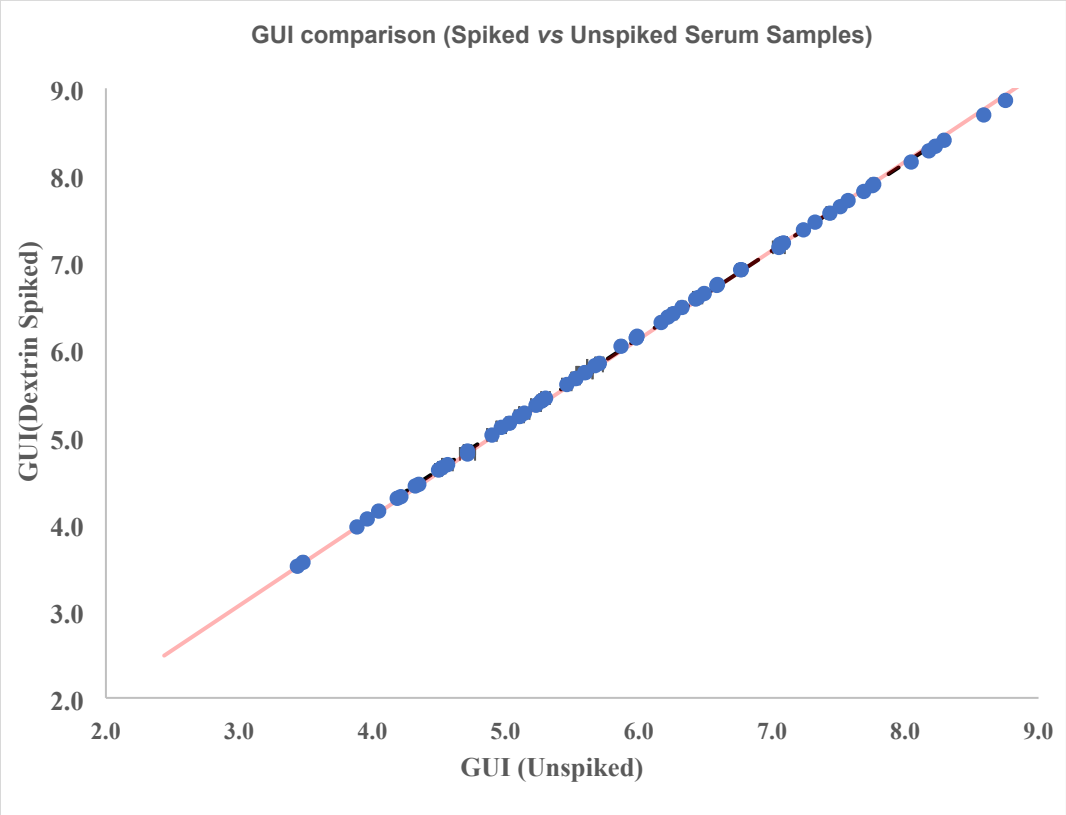
Gautam *et al.* Figure S1



Gautam *et al.* Figure S2

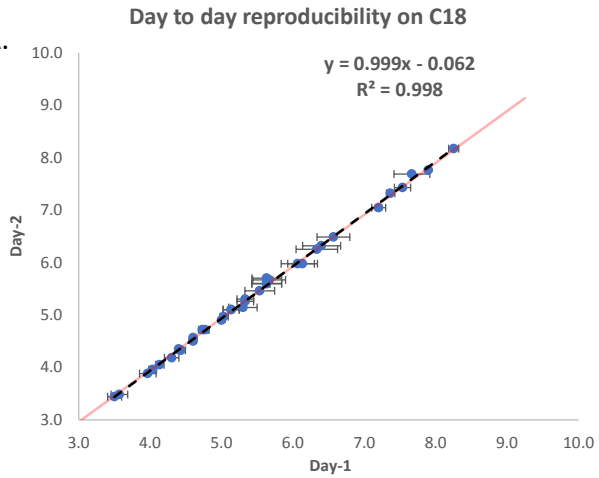


Gautam *et al.* figure S3

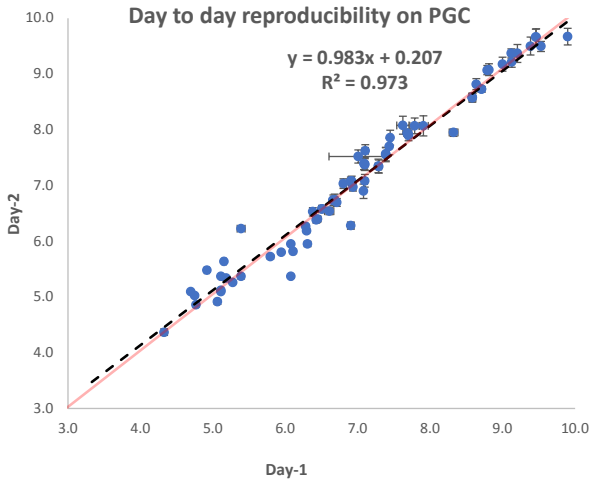


Gautam *et al.* figure S4

A.



B.



C18 LC-MS Method for Velos

Sampler.TempCtrl = On
ColumnOven.TempCtrl = On
ColumnOven.Temperature.Nominal = 55.0 [°C]
ColumnOven.Temperature.LowerLimit = 20.0 [°C]
ColumnOven.Temperature.UpperLimit = 60.0 [°C]
EquilibrationTime = 1.0 [min]
ColumnOven.ReadyTempDelta = 5.0 [°C]
Sampler.Temperature.Nominal = 5.0 [°C]
Sampler.Temperature.LowerLimit = 4.0 [°C]
Sampler.Temperature.UpperLimit = 45.0 [°C]
Sampler.ReadyTempDelta = 2.0 [°C]
LoadingPump.Pressure.LowerLimit = 0 [psi]
LoadingPump.Pressure.UpperLimit = 6498 [psi]
LoadingPump.MaximumFlowRampDown = 1001 [µl/min²]
LoadingPump.MaximumFlowRampUp = 1001 [µl/min²]
LoadingPump.%A.Equate = "%A"
LoadingPump.%B.Equate = "%B"
%C.Equate = "%C"
NC_Pump.Pressure.LowerLimit = 0 [psi]
NC_Pump.Pressure.UpperLimit = 11603 [psi]
NC_Pump.MaximumFlowRampDown = 999.996 [µl/min²]
NC_Pump.MaximumFlowRampUp = 999.996 [µl/min²]
NC_Pump.%A.Equate = "%A"
NC_Pump.%B.Equate = "%B"
DrawSpeed = 1000 [nl/s]
DrawDelay = 20000 [ms]
DispSpeed = 1000 [nl/s]
DispenseDelay = 20000 [ms]
WasteSpeed = 5000 [nl/s]
WashSpeed = 8000 [nl/s]
LoopWashFactor = 2.000
SampleHeight = 1.000 [mm]
PunctureDepth = 11.000 [mm]
WashVolume = 50.000 [µl]
RinseBetweenReinjections = No
LowDispersionMode = Off
InjectMode = ulPickUp
FirstTransportVial = R1
LastTransportVial = R1
TransportVialCapacity = 10000
TransLiquidHeight = 10.000 [mm]
TransVialPunctureDepth = 11.000 [mm]
FlushVolume = 5.000 [µl]
LoadingPump.Flow = 3.000 [µl/min]
LoadingPump.%B = 0.0 [%]
%C = 0.0 [%]
LoadingPump.Curve = 5
MSInject.State Off

```
    ValveRight =              1_2

 0.000 Wait                   LoadingPump.Ready and NC_Pump.Ready and ColumnOven.Ready and
          Sampler.Ready and PumpModule.Ready
    ;Chromeleon sets this property to signal to Xcalibur that it is ready to start a run.
    ReadyToRun =              1
    ;Xcalibur sets this property to start the run or injection.
    Wait                      StartRun
    NC_Pump.Flow =            0.350 [µl/min]
    NC_Pump.%B =              20.0 [%]
    Wait                      LoadingPump.Ready and NC_Pump.Ready and ColumnOven.Ready and
          Sampler.Ready and PumpModule.Ready
    Inject
    InjectResponse =          1
    ;Chromeleon sets this property to signal the injection to Xcalibur.
    ;Depending on your system configuration it might be necessary to manually insert
    ;a "Relay" command below in order to send the start signal to the MS.
    ;Typical syntaxes:
    ;Pump_Relay_1.Closed  Duration = 2.00
    ;UM3PUMP_Relay1.On    Duration = 2.00
    MSInject.On Duration=     0.01
    ;Pump_Relay_1.Closed Duration =2.00
    ;UM3PUMP_Relay1.On Duration = 2.00
    NC_Pump.Flow =            0.350 [µl/min]
    NC_Pump.%B =              20.0 [%]
    WASH

 0.100 MSInject.Off Duration=     0.01

10.000 NC_Pump.Flow =            0.350 [µl/min]
    NC_Pump.%B =              20.0 [%]
    ValveRight =              10_1

11.000 NC_Pump.Flow =            0.350 [µl/min]
    NC_Pump.%B =              42.0 [%]

48.000 NC_Pump.Flow =            0.350 [µl/min]
    NC_Pump.%B =              55.0 [%]

49.000 NC_Pump.Flow =            0.350 [µl/min]
    NC_Pump.%B =              90.0 [%]

54.000 NC_Pump.Flow =            0.350 [µl/min]
    NC_Pump.%B =              90.0 [%]

55.000 NC_Pump.Flow =            0.350 [µl/min]
    NC_Pump.%B =              20.0 [%]
    ValveRight =              1_2
```

60.000
NC_Pump.Flow = 0.350 [µl/min]
NC_Pump.%B = 20.0 [%]
InjectResponse = 0
End

MS Method

Creator: LTQ Orbitrap Velos

MS Run Time (min): 60.00

Sequence override of method parameters not enabled.

Divert Valve: not used during run

Contact Closure: not used during run

Syringe Pump: not used during run

MS Detector Settings:

Acquisition Start Delay (min): 10.00

Real-time modifications to method disabled

Stepped collision energy not enabled

Additional Microscans:

MS2	0	0
MS3	0	0
MS4	0	0
MS5	0	0
MS6	0	0
MS7	0	0
MS8	0	0
MS9	0	0
MS10	0	0

Experiment Type: Nth Order Double Play With ETD

Tune Method: nanoESI_08242017

Scan Event Details:

1: FTMS + p norm !corona !pi o(200.0-2000.0)

CV = 0.0V

2: ITMS + c norm Dep MS/MS Most intense ion from (1)

Activation Type: CID

Min. Signal Required: 5000.0

Isolation Width: 3.00

Normalized Coll. Energy: 30.0

Default Charge State: 2

Activation Q: 0.250

Activation Time: 15.000

CV = 0.0V

3: FTMS + c norm Dep MS/MS Most intense ion from (1)

Activation Type: HCD

Min. Signal Required: 1000.0

Isolation Width: 3.00

Normalized Coll. Energy: 45.0

Default Charge State: 2

Activation Time: 0.100

FT first mass mode: fixed at m/z

FT first mass value: 100.00

CV = 0.0V

Scan Event 2 and 3 repeated for top 4 peaks.

Lock Masses:

Pos List Name: N/A

Source: API Source

Mass List: (none)

Neg List Name: N/A

Source: API Source

Mass List: (none)

Data Dependent Settings:

Use separate polarity settings disabled

Parent Mass List: (none)

Reject Mass List: (none)

Neutral Loss Mass List: (none)

Product Mass List: (none)

Neutral loss in top: 3

Product in top: 3

Most intense if no parent masses found not enabled

Add/subtract mass not enabled

FT master scan preview mode enabled

Charge state screening enabled

Charge state dependent ETD time not enabled

Monoisotopic precursor selection enabled

Non-peptide monoisotopic recognition enabled

Charge state rejection enabled

Unassigned charge states : rejected

Charge state 1 : rejected

Charge state 2 : not rejected

Charge state 3 : not rejected

Charge states 4+ : not rejected

Chromatography mode is disabled

Global Data Dependent Settings:

Predict ion injection time enabled

Use global parent and reject mass lists not enabled

Exclude parent mass from data dependent selection not enabled

Exclusion mass width by mass

Exclusion mass width low: 1.50

Exclusion mass width high: 1.50

Parent mass width by mass

Parent mass width low: 1.50

Parent mass width high: 1.50

Reject mass width by mass

Reject mass width low: 1.50

Reject mass width high: 1.50

Zoom/UltraZoom scan mass width by mass

Zoom/UltraZoom scan mass width low: 5.00

Zoom/UltraZoom scan mass width high: 5.00

FT SIM scan mass width low: 5.00

FT SIM scan mass width high: 5.00

Neutral Loss candidates processed by decreasing intensity

Neutral Loss mass width by mass

Neutral Loss mass width low: 0.50

Neutral Loss mass width high: 0.50

Product candidates processed by decreasing intensity

Product mass width by mass

Product mass width low: 0.50

Product mass width high: 0.50

MS mass range: 0.00-1000000.00

MSn mass range by mass

MSn mass range: 0.00-1000000.00

Use m/z values as masses not enabled

Analog UV data dep. not enabled

Dynamic exclusion enabled

Repeat Count: 2

Repeat Duration: 30.00

Exclusion List Size: 50

Exclusion Duration: 60.00

Exclusion mass width by mass

Exclusion mass width low: 1.50

Exclusion mass width high: 1.50

Expiration: disabled

Isotopic data dependence not enabled

Custom Data Dependent Settings:

Not enabled

C18 LC-MS Method for Exactive

LoadingPump.Pressure.LowerLimit =0 [psi]
ColumnOven.TempCtrl = On
ColumnOven.Temperature.Nominal =55.0 [°C]
ColumnOven.Temperature.LowerLimit =20.0 [°C]
ColumnOven.Temperature.UpperLimit =60.0 [°C]
EquilibrationTime = 1.0 [min]
ColumnOven.ReadyTempDelta = 5.0 [°C]
LoadingPump.Pressure.UpperLimit =5000 [psi]
LoadingPump.MaximumFlowRampDown =1001 [µl/min²]
LoadingPump.MaximumFlowRampUp =1001 [µl/min²]
LoadingPump.%A.Equate = "%A"
LoadingPump.%B.Equate = "%B"
%C.Equate = "%C"
NC_Pump.Pressure.LowerLimit = 0 [psi]
NC_Pump.Pressure.UpperLimit = 7000 [psi]
NC_Pump.MaximumFlowRampDown = 999.996 [µl/min²]
NC_Pump.MaximumFlowRampUp = 999.996 [µl/min²]
NC_Pump.%A.Equate = "%A"
NC_Pump.%B.Equate = "%B"
DrawSpeed = 1000 [nl/s]
DrawDelay = 20000 [ms]
DispSpeed = 1000 [nl/s]
DispenseDelay = 20000 [ms]
WasteSpeed = 5000 [nl/s]
WashSpeed = 8000 [nl/s]
LoopWashFactor = 2.000
SampleHeight = 2.000 [mm]
PunctureDepth = 11.000 [mm]
WashVolume = 50.000 [µl]
RinseBetweenReinjections = No
LowDispersionMode = Off
InjectMode = ulPickUp
FirstTransportVial = R1
LastTransportVial = R1
TransportVialCapacity = 10000
TransLiquidHeight = 10.000 [mm]
TransVialPunctureDepth = 10.000 [mm]
FlushVolume = 5.000 [µl]
LoadingPump.Flow = 3.000 [µl/min]
LoadingPump.%B = 0.0 [%]
%C = 0.0 [%]
LoadingPump.Curve = 5
ValveRight = 1_2
Relay_4.State Off

0.000 Wait LoadingPump.Ready and NC_Pump.Ready and ColumnOven.Ready and Sampler.Ready and PumpModule.Ready

```
      ;Chromeleon sets this property to signal to Xcalibur that it is ready to start a run.
      ReadyToRun =              1
      ;Xcalibur sets this property to start the run or injection.
      Wait                  StartRun
      NC_Pump.Flow =            0.350 [µl/min]
      NC_Pump.%B =              20.0 [%]
      Wait                  LoadingPump.Ready and NC_Pump.Ready and ColumnOven.Ready
and Sampler.Ready and PumpModule.Ready
      Inject
      InjectResponse =          1
      ;Chromeleon sets this property to signal the injection to Xcalibur.
      ;Depending on your system configuration it might be necessary to manually insert
      ;a "Relay" command below in order to send the start signal to the MS.
      ;Typical syntaxes:
      ;Pump_Relay_1.Closed Duration =2.00
      ;UM3PUMP_Relay1.On Duration = 2.00
      Relay_4.On Duration=      0.01
      ;Pump_Relay_1.Closed Duration =2.00
      ;UM3PUMP_Relay1.On Duration = 2.00
      NC_Pump.Flow =            0.350 [µl/min]
      ValveRight =          1_2
      NC_Pump.%B =              20.0 [%]

 0.001 Relay_4.Off Duration=      0.01

10.000 NC_Pump.Flow =           0.350 [µl/min]
      NC_Pump.%B =              20.0 [%]
      ValveRight =          10_1

11.000 NC_Pump.Flow =           0.350 [µl/min]
      NC_Pump.%B =              42.0 [%]

48.000 NC_Pump.Flow =           0.350 [µl/min]
      NC_Pump.%B =              55.0 [%]

49.000 NC_Pump.Flow =           0.350 [µl/min]
      NC_Pump.%B =              90.0 [%]

54.000 NC_Pump.Flow =           0.350 [µl/min]
      NC_Pump.%B =              90.0 [%]

55.000 ValveRight =             1_2
      NC_Pump.Flow =            0.350 [µl/min]
      NC_Pump.%B =              20.0 [%]

60.000 NC_Pump.Flow =           0.350 [µl/min]
      NC_Pump.%B =              20.0 [%]
      InjectResponse =          0
      End
```

MS Method

Run settings
Start delay 10.00 min

==========

Orbitrap MS Detector
Acquisition time 50.00 min

Segment 1 50.00 min duration at 10.00
Tune file C:\Xcalibur\methods\NanoESI Positve Cal 1022018.ms tune
Lock masses Off

Event 1: Positive; 1 scans; R=50,000; AGC=1,000,000; Inject=100.00 ms; [300.0 - 2,000.0]; In-Source CID disabled

==========

Syringe pump not driven

==========

Divert valves
Divert valve 1 not driven
Divert valve 2 not driven

==========

Contact closure not provided

PGC LC-MS Method for Velos

Sampler.TempCtrl = On
ColumnOven.TempCtrl = On
ColumnOven.Temperature.Nominal = 75.0 [°C]
ColumnOven.Temperature.LowerLimit = 20.0 [°C]
ColumnOven.Temperature.UpperLimit = 75.0 [°C]
EquilibrationTime = 1.0 [min]
ColumnOven.ReadyTempDelta = 5.0 [°C]
Sampler.Temperature.Nominal = 5.0 [°C]
Sampler.Temperature.LowerLimit = 4.0 [°C]
Sampler.Temperature.UpperLimit = 45.0 [°C]
Sampler.ReadyTempDelta = 2.0 [°C]
LoadingPump.Pressure.LowerLimit = 0 [psi]
LoadingPump.Pressure.UpperLimit = 6498 [psi]
LoadingPump.MaximumFlowRampDown = 1001 [µl/min²]
LoadingPump.MaximumFlowRampUp = 1001 [µl/min²]
LoadingPump.%A.Equate = "%A"
LoadingPump.%B.Equate = "%B"
%C.Equate = "%C"
NC_Pump.Pressure.LowerLimit = 0 [psi]
NC_Pump.Pressure.UpperLimit = 11603 [psi]
NC_Pump.MaximumFlowRampDown = 999.996 [µl/min²]
NC_Pump.MaximumFlowRampUp = 999.996 [µl/min²]
NC_Pump.%A.Equate = "%A"
NC_Pump.%B.Equate = "%B"
DrawSpeed = 1000 [nl/s]
DrawDelay = 20000 [ms]
DispSpeed = 1000 [nl/s]
DispenseDelay = 20000 [ms]
WasteSpeed = 5000 [nl/s]
WashSpeed = 8000 [nl/s]
LoopWashFactor = 2.000
SampleHeight = 1.000 [mm]
PunctureDepth = 11.000 [mm]
WashVolume = 50.000 [µl]
RinseBetweenReinjections = No
LowDispersionMode = Off
InjectMode = ulPickUp
FirstTransportVial = R1
LastTransportVial = R1
TransportVialCapacity = 10000
TransLiquidHeight = 10.000 [mm]
TransVialPunctureDepth = 11.000 [mm]
FlushVolume = 5.000 [µl]
LoadingPump.Flow = 3.000 [µl/min]
LoadingPump.%B = 0.0 [%]
%C = 0.0 [%]
LoadingPump.Curve = 5
ValveRight = 1_2

```
 0.000 Wait                    LoadingPump.Ready and NC_Pump.Ready and ColumnOven.Ready
and Sampler.Ready and PumpModule.Ready
      ;Chromeleon sets this property to signal to Xcalibur that it is ready to start a run.
      ReadyToRun =              1
      ;Xcalibur sets this property to start the run or injection.
      Wait                  StartRun
      NC_Pump.Flow =            0.600 [µl/min]
      NC_Pump.%B =              20.0 [%]
      Wait                  LoadingPump.Ready and NC_Pump.Ready and ColumnOven.Ready
and Sampler.Ready and PumpModule.Ready
      Inject
      InjectResponse =          1
      ;Chromeleon sets this property to signal the injection to Xcalibur.
      ;Depending on your system configuration it might be necessary to manually insert
      ;a "Relay" command below in order to send the start signal to the MS.
      ;Typical syntaxes:
      ;Pump_Relay_1.Closed  Duration = 2.00
      ;UM3PUMP_Relay1.On    Duration = 2.00

      MSInject.On Duration=     0.10
      ;Pump_Relay_1.Closed Duration =2.00
      ;UM3PUMP_Relay1.On Duration = 2.00
      NC_Pump.Flow =            0.600 [µl/min]
      NC_Pump.%B =              20.0 [%]
      WASH

10.000 NC_Pump.Flow =           0.600 [µl/min]
      NC_Pump.%B =              20.0 [%]
      ValveRight =            10_1

20.000 NC_Pump.Flow =           0.600 [µl/min]
      NC_Pump.%B =              60.0 [%]

40.000 NC_Pump.Flow =           0.600 [µl/min]
      NC_Pump.%B =              85.0 [%]

60.000 NC_Pump.Flow =           0.600 [µl/min]
      NC_Pump.%B =              95.0 [%]

87.000 NC_Pump.Flow =           0.600 [µl/min]
      NC_Pump.%B =              95.0 [%]
      ValveRight =            1_2

88.000 NC_Pump.Flow =           0.600 [µl/min]
      NC_Pump.%B =              20.0 [%]

90.000 NC_Pump.Flow =           0.600 [µl/min]
      NC_Pump.%B =              20.0 [%]
```

InjectResponse = 0
End

MS Method

Creator: LTQ Orbitrap Velos

MS Run Time (min): 90.00

Sequence override of method parameters not enabled.

Divert Valve: not used during run

Contact Closure: not used during run

Syringe Pump: not used during run

MS Detector Settings:

Acquisition Start Delay (min): 10.00

Real-time modifications to method disabled

Stepped collision energy not enabled

Additional Microscans:

MS2	0	0
MS3	0	0
MS4	0	0
MS5	0	0
MS6	0	0
MS7	0	0
MS8	0	0
MS9	0	0
MS10	0	0

Experiment Type: Nth Order Double Play With ETD

Tune Method: nanoESI_08242017

Scan Event Details:

1: FTMS + p norm !corona !pi o(200.0-2000.0)

CV = 0.0V

2: ITMS + c norm Dep MS/MS Most intense ion from (1)

Activation Type: CID

Min. Signal Required: 5000.0

Isolation Width: 3.00

Normalized Coll. Energy: 30.0

Default Charge State: 2

Activation Q: 0.250

Activation Time: 15.000

CV = 0.0V

3: FTMS + c norm Dep MS/MS Most intense ion from (1)

Activation Type: HCD

Min. Signal Required: 1000.0

Isolation Width: 3.00

Normalized Coll. Energy: 45.0

Default Charge State: 2

Activation Time: 0.100

FT first mass mode: fixed at m/z

FT first mass value: 100.00

CV = 0.0V

Scan Event 2 and 3 repeated for top 6 peaks.

Lock Masses:

Pos List Name: N/A

Source: API Source

Mass List: (none)

Neg List Name: N/A

Source: API Source

Mass List: (none)

Data Dependent Settings:

Use separate polarity settings disabled

Parent Mass List: (none)

Reject Mass List: (none)

Neutral Loss Mass List: (none)

Product Mass List: (none)

Neutral loss in top: 3

Product in top: 3

Most intense if no parent masses found not enabled

Add/subtract mass not enabled

FT master scan preview mode enabled

Charge state screening not enabled

Charge state dependent ETD time not enabled

Monoisotopic precursor selection not enabled

Non-peptide monoisotopic recognition not enabled

Charge state rejection enabled

Unassigned charge states : rejected

Charge state 1 : rejected

Charge state 2 : not rejected

Charge state 3 : not rejected

Charge states 4+ : not rejected

Chromatography mode is disabled

Global Data Dependent Settings:

Predict ion injection time enabled

Use global parent and reject mass lists not enabled

Exclude parent mass from data dependent selection not enabled

Exclusion mass width by mass

Exclusion mass width low: 1.50

Exclusion mass width high: 1.50

Parent mass width by mass

Parent mass width low: 1.50

Parent mass width high: 1.50

Reject mass width by mass

Reject mass width low: 1.50

Reject mass width high: 1.50

Zoom/UltraZoom scan mass width by mass

Zoom/UltraZoom scan mass width low: 5.00

Zoom/UltraZoom scan mass width high: 5.00

FT SIM scan mass width low: 5.00

FT SIM scan mass width high: 5.00

Neutral Loss candidates processed by decreasing intensity

Neutral Loss mass width by mass

Neutral Loss mass width low: 0.50

Neutral Loss mass width high: 0.50

Product candidates processed by decreasing intensity

Product mass width by mass

Product mass width low: 0.50

Product mass width high: 0.50

MS mass range: 0.00-1000000.00

MSn mass range by mass

MSn mass range: 0.00-1000000.00

Use m/z values as masses not enabled

Analog UV data dep. not enabled

Dynamic exclusion enabled

Repeat Count: 2

Repeat Duration: 10.00

Exclusion List Size: 50

Exclusion Duration: 10.00

Exclusion mass width by mass

Exclusion mass width low: 1.50

Exclusion mass width high: 1.50

Expiration: disabled

Isotopic data dependence not enabled

Custom Data Dependent Settings:

Not enabled

PGC LC-MS Method for Exactive

LoadingPump.Pressure.LowerLimit =0 [psi]
ColumnOven.TempCtrl = On
ColumnOven.Temperature.Nominal =75.0 [°C]
ColumnOven.Temperature.LowerLimit =20.0 [°C]
ColumnOven.Temperature.UpperLimit =75.0 [°C]
EquilibrationTime = 1.0 [min]
ColumnOven.ReadyTempDelta = 5.0 [°C]
LoadingPump.Pressure.UpperLimit =5000 [psi]
LoadingPump.MaximumFlowRampDown =1001 [µl/min²]
LoadingPump.MaximumFlowRampUp =1001 [µl/min²]
LoadingPump.%A.Equate = "%A"
LoadingPump.%B.Equate = "%B"
%C.Equate = "%C"
NC_Pump.Pressure.LowerLimit = 0 [psi]
NC_Pump.Pressure.UpperLimit = 7000 [psi]
NC_Pump.MaximumFlowRampDown = 999.996 [µl/min²]
NC_Pump.MaximumFlowRampUp = 999.996 [µl/min²]
NC_Pump.%A.Equate = "%A"
NC_Pump.%B.Equate = "%B"
DrawSpeed = 1000 [nl/s]
DrawDelay = 20000 [ms]
DispSpeed = 1000 [nl/s]
DispenseDelay = 20000 [ms]
WasteSpeed = 5000 [nl/s]
WashSpeed = 8000 [nl/s]
LoopWashFactor = 2.000
SampleHeight = 2.000 [mm]
PunctureDepth = 11.000 [mm]
WashVolume = 50.000 [µl]
RinseBetweenReinjections = No
LowDispersionMode = Off
InjectMode = ulPickUp
FirstTransportVial = R1
LastTransportVial = R1
TransportVialCapacity = 10000
TransLiquidHeight = 10.000 [mm]
TransVialPunctureDepth = 10.000 [mm]
FlushVolume = 5.000 [µl]
LoadingPump.Flow = 3.000 [µl/min]
LoadingPump.%B = 0.0 [%]
%C = 0.0 [%]
LoadingPump.Curve = 5
ValveRight = 1_2
Relay_4.State Off

0.000 Wait LoadingPump.Ready and NC_Pump.Ready and ColumnOven.Ready and Sampler.Ready and PumpModule.Ready

```
      ;Chromeleon sets this property to signal to Xcalibur that it is ready to start a run.
      ReadyToRun =              1
      ;Xcalibur sets this property to start the run or injection.
      Wait                  StartRun
      NC_Pump.Flow =            0.600 [µl/min]
      NC_Pump.%B =              20.0 [%]
      Wait                  LoadingPump.Ready and NC_Pump.Ready and ColumnOven.Ready
and Sampler.Ready and PumpModule.Ready
      Inject
      InjectResponse =          1
      ;Chromeleon sets this property to signal the injection to Xcalibur.
      ;Depending on your system configuration it might be necessary to manually insert
      ;a "Relay" command below in order to send the start signal to the MS.
      ;Typical syntaxes:
      ;Pump_Relay_1.Closed Duration =2.00
      ;UM3PUMP_Relay1.On Duration = 2.00
      Relay_4.On Duration=      0.01
      ;Pump_Relay_1.Closed Duration =2.00
      ;UM3PUMP_Relay1.On Duration = 2.00
      NC_Pump.Flow =            0.600 [µl/min]
      ValveRight =            1_2
      NC_Pump.%B =              20.0 [%]

 0.001 Relay_4.Off Duration=      0.01

10.000 NC_Pump.Flow =           0.600 [µl/min]
      NC_Pump.%B =              20.0 [%]
      ValveRight =            10_1

20.000 NC_Pump.Flow =           0.600 [µl/min]
      NC_Pump.%B =              50.0 [%]

40.000 NC_Pump.Flow =           0.600 [µl/min]
      NC_Pump.%B =              80.0 [%]

60.000 NC_Pump.Flow =           0.600 [µl/min]
      NC_Pump.%B =              95.0 [%]

87.000 NC_Pump.Flow =           0.600 [µl/min]
      NC_Pump.%B =              95.0 [%]

88.000 NC_Pump.Flow =           0.600 [µl/min]
      NC_Pump.%B =              20.0 [%]

89.000 ValveRight =             1_2

90.000 NC_Pump.Flow =           0.600 [µl/min]
      NC_Pump.%B =              20.0 [%]
      InjectResponse =          0
```

End

MS Method

Run settings
Start delay 10.00 min

==========

Orbitrap MS Detector
Acquisition time 90.00 min

Segment 1 90.00 min duration at 10.00
Tune file C:\Xcalibur\methods\nanoESI_positive_20181116.mstune
Lock masses Off

Event 1: Positive; 1 scans; R=100,000; AGC=1,000,000; Inject=100.00 ms; [150.0 - 2,000.0]; In-Source CID disabled

==========

Syringe pump not driven

==========

Divert valves
Divert valve 1 not driven
Divert valve 2 not driven

==========

Contact closure not provided

Instrumental Software:

- LTQ Tune Plus Version 2.6.0.1065 SP3 for LTQ Orbitrap Velos™ (Thermo Scientific). Xcalibur 2.1.0 SP1.1160 (Thermo Scientific). Chromeleon® chromatography management systems; Version 6.80 SR13 Build 3818. Manufactured by Dionex Corporation (Thermo Fisher Scientific).
- Exactive Software Version 1.1 SP6 Build 1360 (Thermo Scientific). Xcalibur 2.2 SP1.48 (Thermo Scientific), Chromeleon® chromatography management systems. Version 6.80 SR13 Build 3818. Manufactured by Dionex Corporation (Thermo Fisher Scientific).