

9. ANHANG

Tabelle 24: Übersicht der molekularen Massen für die Kalkulationen mit GlycoProtMass

Molekül	m/z frei	m/z im Oligomer	m/z permethyliert im Oligomer
Desoxy-pentose	134,0579046	116,0473400	130,0629900
Pentose	150,0528230	132,0422584	160,0735584
Desoxy-hexose	164,0684730	146,0579084	174,0892084
Hexosamin	179,0793720	161,0688074	217,1314074
Hexose	180,0633876	162,0528230	204,0997730
Hexuronäure	194,0426546	176,0320900	218,0790400
Heptose	210,0739546	192,0633900	248,1259900
N-Acetyl-hexosamin	221,0899366	203,0793720	245,1263220
KDO	238,0688646	220,0583000	276,1209000
KDN	268,0794546	250,0688900	320,1471400
Mureinsäure	293,1110646	275,1005000	317,1474500
N-Acetyl-neuraminsäure	309,1059804	291,0954158	361,1736658
N-Glycolyl-neuraminsäure	325,1008946	307,0903300	391,1842300
Alanin	89,0476785	71,0371138	
Asparaginsäure	133,0375077	115,0269430	
Arginin	174,1116757	156,1011110	
Asparagin	132,0534921	114,0429274	
Cystein	121,0197492	103,0091845	
Glutamin	146,0691422	128,0585775	
Glutaminsäure	147,0531578	129,0425931	
Glycin	75,0320284	57,0214637	
Histidin	155,0694765	137,0589119	
Isoleucin	131,0946287	113,0840640	
Leucin	131,0946287	113,0840640	
Lysin	146,1055277	128,0949630	
Methionin	149,0510493	131,0404846	
Phenylalanin	165,0789786	147,0684139	
Prolin	115,0633285	97,0527639	
Selenocystein	168,9642003	150,9536356	
Serin	105,0425931	87,0320284	
Threonin	119,0582432	101,0476785	
Tryptophan	204,0898776	186,0793130	
Tyrosin	181,0738932	163,0633285	
Sulfatierung		79,9568156	65,9411656

Phosphorylierung		79,9663322	93,9819822
Acetylierung		42,0105646	42,0105646
H	1,0078250	P	30,9737634
C	12,0000000	S	31,9720718
N	14,0030740	Se	79,9165000
O	15,9949146		

Tabelle 25: Übersicht zu molekularen Massen von typischen sialylierten N-Glykanen

Glykan	HexNAc	Hex	DHex	Neu5Ac	m/z bei Ladung [M-H] ⁻			
					1x	2x	3x	4x
Bi 1Neu5Ac	4	5		1	1930,680	964,836	642,888	481,914
Bi Fuc 1Neu5Ac	4	5	1	1	2076,738	1037,865	691,574	518,429
Bi Bisec 1Neu5Ac	5	5		1	2133,759	1066,376	710,581	532,684
Bi 2Neu5Ac	4	5		2	2221,775	1110,384	739,920	554,688
Bi 2Fuc 1Neu5Ac	4	5	2	1	2222,796	1110,894	740,260	554,943
Bi Fuc Bisec 1Neu5Ac	5	5	1	1	2279,817	1139,405	759,267	569,198
Tri 1Neu5Ac	5	6		1	2295,812	1147,402	764,599	573,197
Bi Fuc 2Neu5Ac	4	5	1	2	2367,833	1183,413	788,606	591,202
Bi Bisec 2Neu5Ac	5	5		2	2424,855	1211,923	807,613	605,458
Bi 2Fuc Bisec 1Neu5Ac	5	5	2	1	2425,875	1212,434	807,953	605,713
Tri Fuc 1Neu5Ac	5	6	1	1	2441,870	1220,431	813,285	609,712
Tri Bisec 1Neu5Ac	6	6		1	2498,891	1248,942	832,292	623,967
Bi 2Fuc 2Neu5Ac	4	5	2	2	2513,891	1256,442	837,292	627,717
Bi Fuc Bisec 2Neu5Ac	5	5	1	2	2570,912	1284,952	856,299	641,972
Tri 2Neu5Ac	5	6		2	2586,907	1292,950	861,631	645,971
Tri 2Fuc 1Neu5Ac	5	6	2	1	2587,928	1293,460	861,971	646,226
Tri Fuc Bisec 1Neu5Ac	6	6	1	1	2644,949	1321,971	880,978	660,481
Tetra 1Neu5Ac	6	7		1	2660,944	1329,968	886,309	664,480
Bi 2Fuc Bisec 2Neu5Ac	5	5	2	2	2716,970	1357,981	904,985	678,487
Tri Fuc 2Neu5Ac	5	6	1	2	2732,965	1365,979	910,317	682,485
Tri Bisec 2Neu5Ac	6	6		2	2789,987	1394,489	929,324	696,741
Tri 2Fuc Bisec 1Neu5Ac	6	6	2	1	2791,007	1395,000	929,664	696,996
Tetra Fuc 1Neu5Ac	6	7	1	1	2807,002	1402,997	934,995	700,995
Tetra Bisec 1Neu5Ac	7	7		1	2864,024	1431,508	954,003	715,250
Tri 3Neu5Ac	5	6		3	2878,003	1438,497	958,662	718,745

Tri 2Fuc 2Neu5Ac	5	6	2	2	2879,023	1439,008	959,003	719,000
Tri Fuc Bisec 2Neu5Ac	6	6	1	2	2936,045	1467,518	978,010	733,255
Tetra 2Neu5Ac	6	7		2	2952,040	1475,516	983,341	737,254
Tetra 2Fuc 1Neu5Ac	6	7	2	1	2953,060	1476,026	983,681	737,509
Tetra Fuc Bisec 1Neu5Ac	7	7	1	1	3010,081	1504,537	1002,689	751,764
Tri Fuc 3Neu5Ac	5	6	1	3	3024,061	1511,526	1007,348	755,259
Tri Bisec 3Neu5Ac	6	6		3	3081,082	1540,037	1026,356	769,515
Tri 2Fuc Bisec 2Neu5Ac	6	6	2	2	3082,103	1540,547	1026,696	769,770
Tetra Fuc 2Neu5Ac	6	7	1	2	3098,097	1548,545	1032,027	773,768
Tetra Bisec 2Neu5Ac	7	7		2	3155,119	1577,056	1051,034	788,024
Tetra 2Fuc Bisec 1Neu5Ac	7	7	2	1	3156,139	1577,566	1051,375	788,279
Tri 2Fuc 3Neu5Ac	5	6	2	3	3170,119	1584,555	1056,034	791,774
Tri Fuc Bisec 3Neu5Ac	6	6	1	3	3227,140	1613,066	1075,041	806,029
Tetra 3Neu5Ac	6	7		3	3243,135	1621,064	1080,373	810,028
Tetra 2Fuc 2Neu5Ac	6	7	2	2	3244,155	1621,574	1080,713	810,283
Tetra Fuc Bisec 2Neu5Ac	7	7	1	2	3301,177	1650,085	1099,720	824,538
Tri 2Fuc Bisec 3Neu5Ac	6	6	2	3	3373,198	1686,095	1123,727	842,544
Tetra Fuc 3Neu5Ac	6	7	1	3	3389,193	1694,093	1129,059	846,542
Tetra Bisec 3Neu5Ac	7	7		3	3446,214	1722,603	1148,066	860,798
Tetra 2Fuc Bisec 2Neu5Ac	7	7	2	2	3447,235	1723,113	1148,406	861,053
Tetra 4Neu5Ac	6	7		4	3534,230	1766,611	1177,405	882,802
Tetra 2Fuc 3Neu5Ac	6	7	2	3	3535,251	1767,121	1177,745	883,057
Tetra Fuc Bisec 3Neu5Ac	7	7	1	3	3592,272	1795,632	1196,752	897,312
Tetra Fuc 4Neu5Ac	6	7	1	4	3680,288	1839,640	1226,091	919,316
Tetra Bisec 4Neu5Ac	7	7		4	3737,310	1868,151	1245,098	933,572
Tetra 2Fuc Bisec 3Neu5Ac	7	7	2	3	3738,330	1868,661	1245,438	933,827
Tetra 2Fuc 4Neu5Ac	6	7	2	4	3826,346	1912,669	1274,777	955,831
Tetra Fuc Bisec 4Neu5Ac	7	7	1	4	3883,368	1941,180	1293,784	970,086
Tetra 2Fuc Bisec 4Neu5Ac	7	7	2	4	4029,426	2014,209	1342,470	1006,601

Tabelle 26: Übersicht zu molekularen Massen von typischen nicht-sialylierten N-Glykanen

Glykan	HexNAc	Hex	dHex	m/z bei Ladung [M+Na] ⁺			
				1x	2x	3x	4x
Core	2	3		933,318	466,659	311,106	233,329
Core 1Man	2	4		1095,370	547,685	365,123	273,843
Core 2Man	2	5		1257,423	628,712	419,141	314,356
Core 3Man	2	6		1419,476	709,738	473,159	354,869
Core 4Man	2	7		1581,529	790,764	527,176	395,382
Bi	4	5		1663,582	831,791	554,527	415,895
Core 5Man	2	8		1743,582	871,791	581,194	435,895
Bi Fuc	4	5	1	1809,640	904,820	603,213	452,410
Bi Bisec	5	5		1866,661	933,331	622,220	466,665
Core 6Man	2	9		1905,634	952,817	635,211	476,409
Bi 2Fuc	4	5	2	1955,698	977,849	651,899	488,924
Bi Fuc Bisec	5	5	1	2012,719	1006,360	670,906	503,180
Tri	5	6		2028,714	1014,357	676,238	507,179
Core 7Man	2	10		2067,687	1033,844	689,229	516,922
Bi 2Fuc Bisec	5	5	2	2158,777	1079,389	719,592	539,694
Tri Fuc	5	6	1	2174,772	1087,386	724,924	543,693
Core 8Man	2	11		2229,740	1114,870	743,247	557,435
Tri Bisec	6	6		2231,794	1115,897	743,931	557,948
Tri 2Fuc	5	6	2	2320,830	1160,415	773,610	580,207
Tri Fuc Bisec	6	6	1	2377,851	1188,926	792,617	594,463
Core 9Man	2	12		2391,793	1195,896	797,264	597,948
Tetra	6	7		2393,846	1196,923	797,949	598,462
Tri 2Fuc Bisec	6	6	2	2523,909	1261,955	841,303	630,977
Tetra Fuc	6	7	1	2539,904	1269,952	846,635	634,976
Tetra Bisec	7	7		2596,926	1298,463	865,642	649,231
Tetra 2Fuc	6	7	2	2685,962	1342,981	895,321	671,491
Tetra Fuc Bisec	7	7	1	2742,984	1371,492	914,328	685,746
Tetra 2Fuc Bisec	7	7	2	2889,042	1444,521	963,014	722,260

Massen-Differenzen

Ion n	1x			2x			3x			4x			5x			6x								
	m/z	H	Na	K	NH ₄	m/z	H	Na	K	NH ₄	m/z	H	Na	K	NH ₄	m/z	H	Na	K	NH ₄				
H 1	1,0	22,0	38,0	17,0	0,5	11,0	19,0	8,5	0,3	7,3	12,7	5,7	0,3	5,5	9,5	4,3	0,2	4,4	7,6	3,4	0,2	3,7	6,3	2,8
Na 1	23,0	22,0	16,0	5,0	11,5	11,0	8,0	2,5	7,7	7,3	5,3	1,7	5,5	4,0	1,2	4,6	4,4	3,2	1,0	3,8	3,7	2,7	0,8	0,8
K 1	39,0	38,0	16,0	20,9	19,5	19,0	8,0	10,5	13,0	12,7	5,3	7,0	9,7	9,5	4,0	5,2	7,8	7,6	3,2	4,2	6,5	6,3	2,7	3,5
NH₄ 1	18,0	17,0	5,0	20,9	9,0	8,5	2,5	10,5	6,0	5,7	1,7	7,0	4,5	4,3	1,2	5,2	3,6	3,4	1,0	4,2	3,0	2,8	0,8	3,5

Abbildung 87: Kationen-abhängige Massendifferenzen

Tabelle 27: Massen des Dextran-Hydolysats bzw. eines Hexose-Polymers

Anzahl Hexosen	m/z	m/z permethyliert [M+Na] ⁺
1 Hex	203,053	273,131
2 Hex	365,106	477,231
3 Hex	527,159	681,331
4 Hex	689,212	885,431
5 Hex	851,264	1089,530
6 Hex	1013,317	1293,630
7 Hex	1175,370	1497,730
8 Hex	1337,423	1701,830
9 Hex	1499,476	1905,930
10 Hex	1661,529	2110,029
11 Hex	1823,581	2314,129
12 Hex	1985,634	2518,229
13 Hex	2147,687	2722,329
14 Hex	2309,740	2926,428
15 Hex	2471,793	3130,528
16 Hex	2633,846	3334,628
17 Hex	2795,898	3538,728
18 Hex	2957,951	3742,828
19 Hex	3120,004	3946,927
20 Hex	3282,057	4151,027
21 Hex	3444,110	4355,127
22 Hex	3606,162	4559,227
23 Hex	3768,215	4763,326
24 Hex	3930,268	4967,426
25 Hex	4092,321	5171,526
26 Hex	4254,374	5375,626
27 Hex	4416,427	5579,726
28 Hex	4578,479	5783,825
29 Hex	4740,532	5987,925
30 Hex	4902,585	6192,025

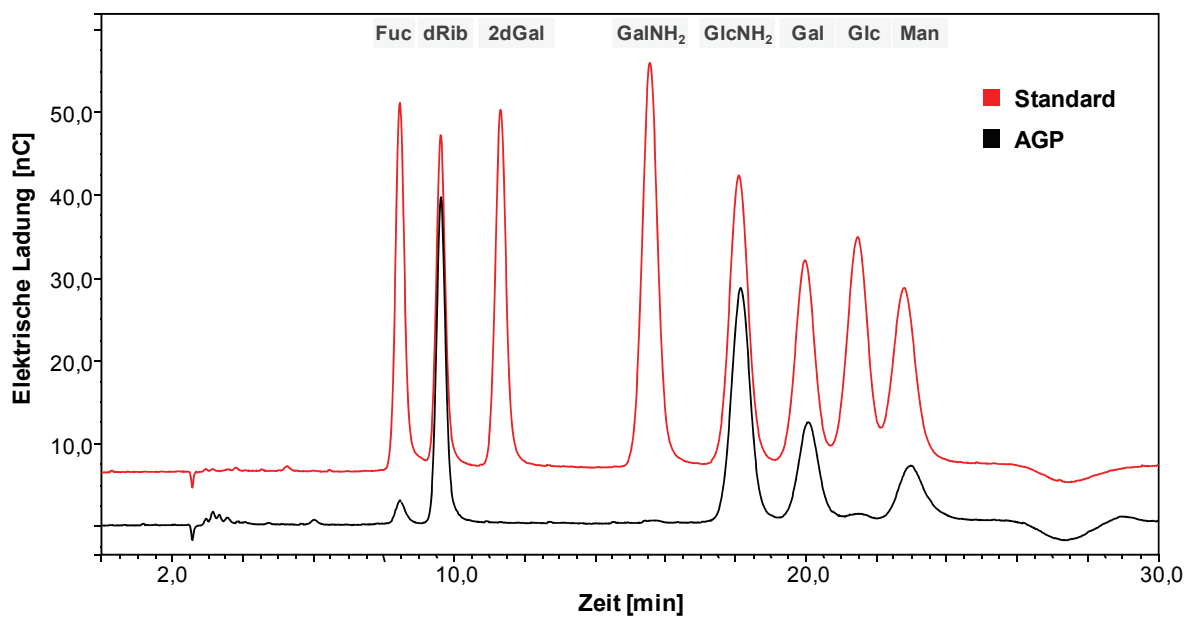


Abbildung 88: HPAEC-PAD-Chromatogramme des Monosaccharid-Standard und des Glykoprotein AGP
Standard=Gemisch aus definierten Monosaccharid-Konzentrationen

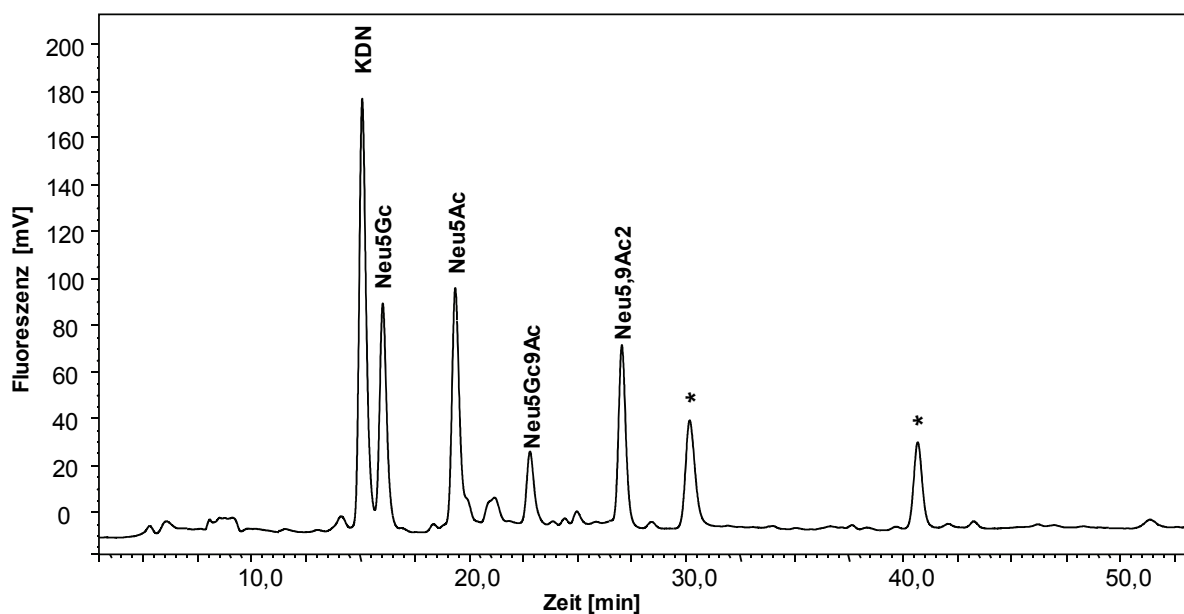


Abbildung 89: Chromatogramm der Standards zur DMB-Markierung von Sialinsäuren (* = DMB-Signal)

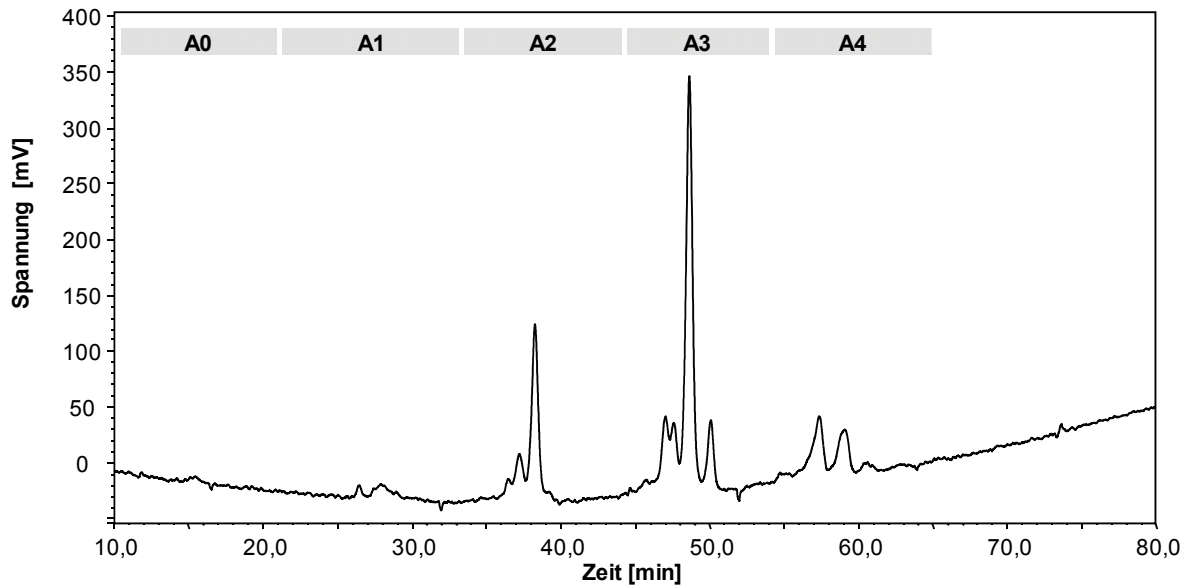


Abbildung 90: Chromatogramm zur Auftrennung der 2AB-markierten N-Glykane aus AGP mittels Asahipak-HPLC zur Bestimmung des Sialylierung dieses Glykoprotein zeigt eine fast vollständige Sialylierung, der größte Anteil sind Strukturen mit 3 Sialinsäuren (A0, A1, A2, A3, A4 = 0, 1, 2, 3, 4 Sialinsäure(n))

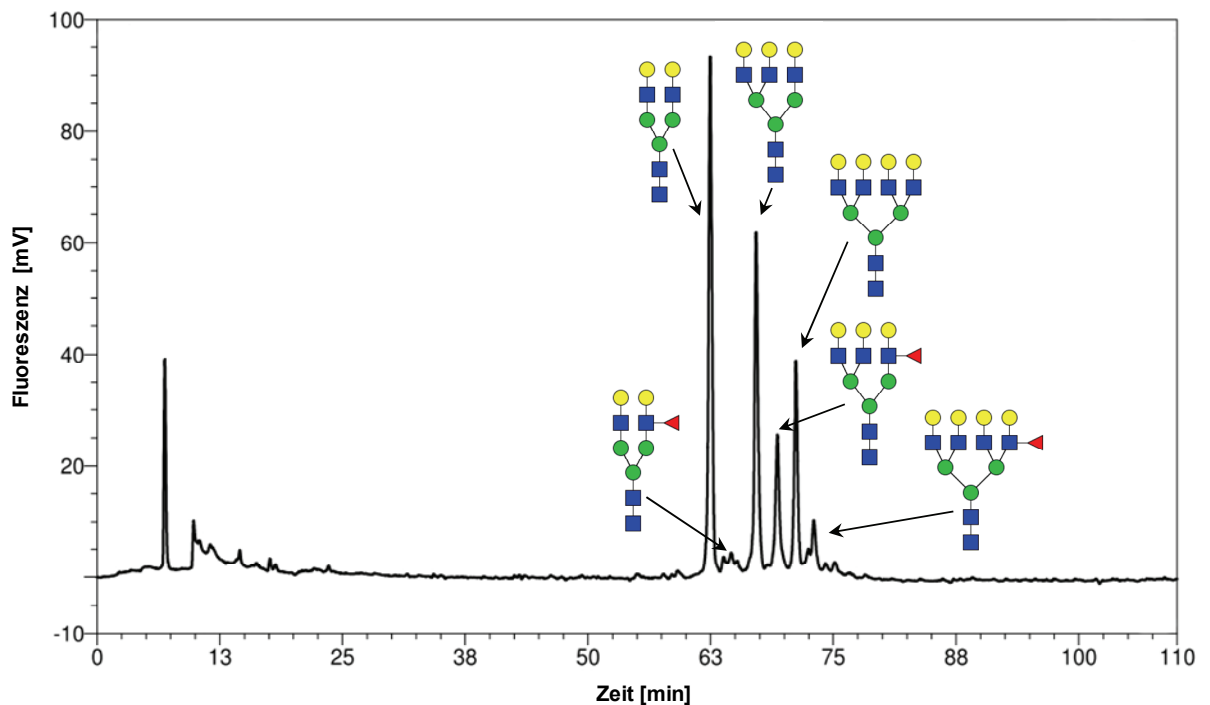


Abbildung 91: Chromatogramm der Auftrennung von desialylierten 2AB-markierten Glykanen aus AGP zur Bestimmung der Antennaritäten mittels Luna-HPLC

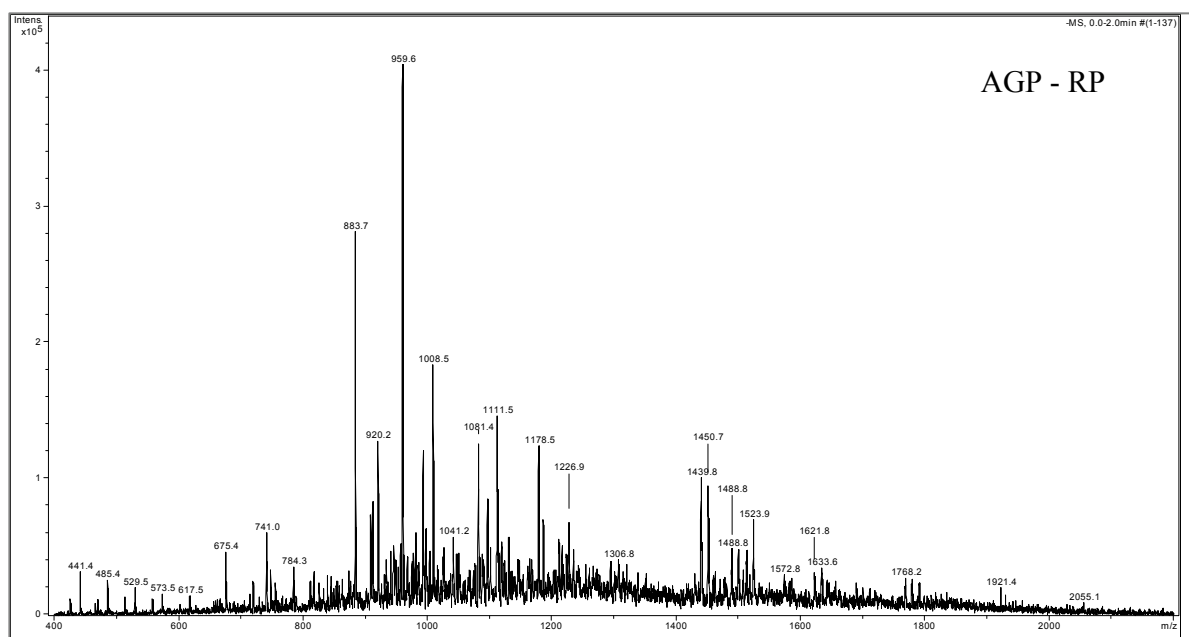
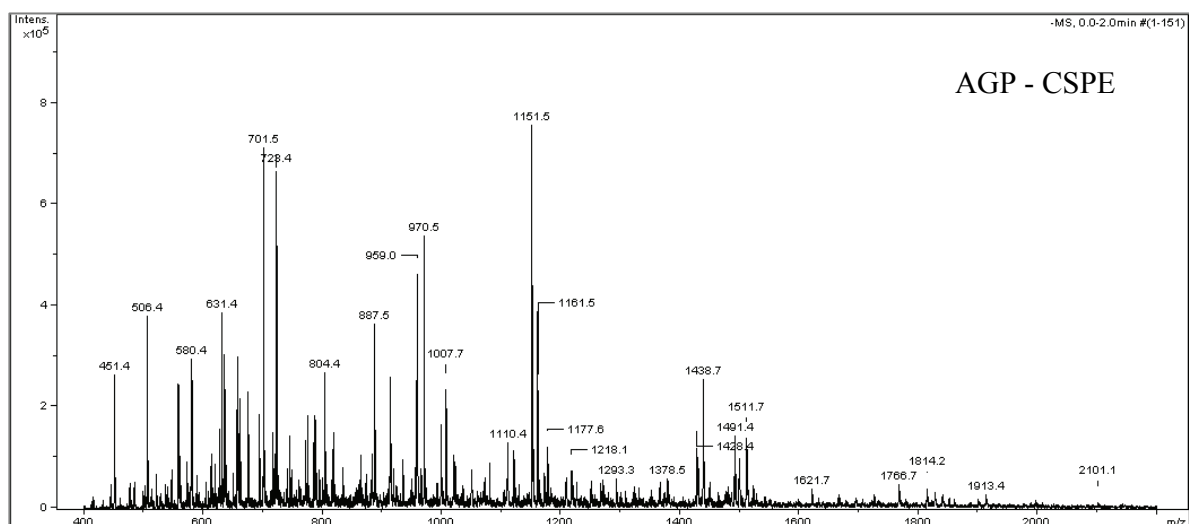
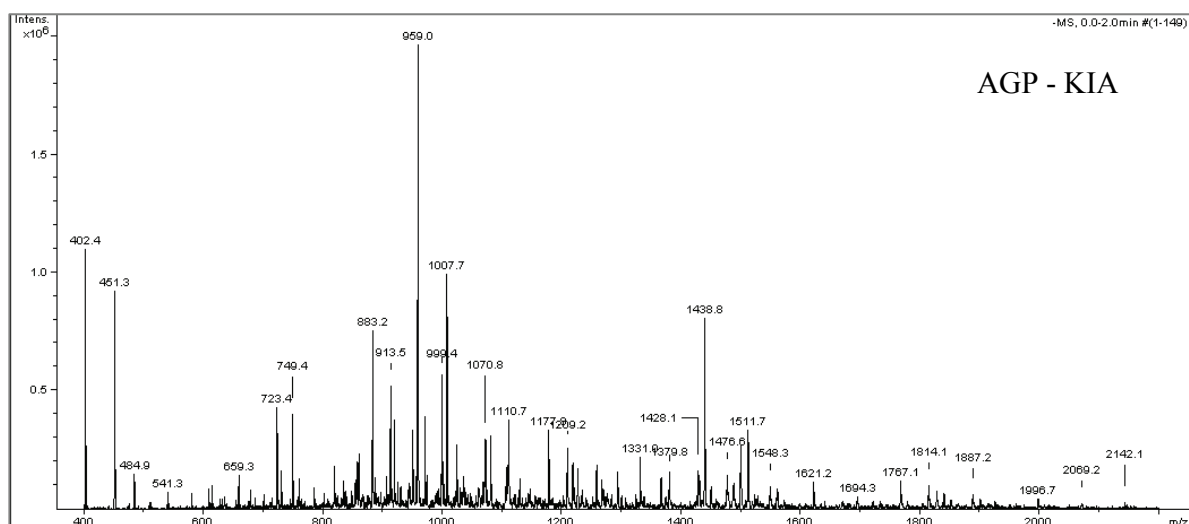


Abbildung 92: Messung verschiedener Glykan-Aufreinigungen von AGP in der ESI-IonTrap-MS
KIA=Kationenaustauscher; CSPE=Graphit-Material; RP=C18-Umkehrphase

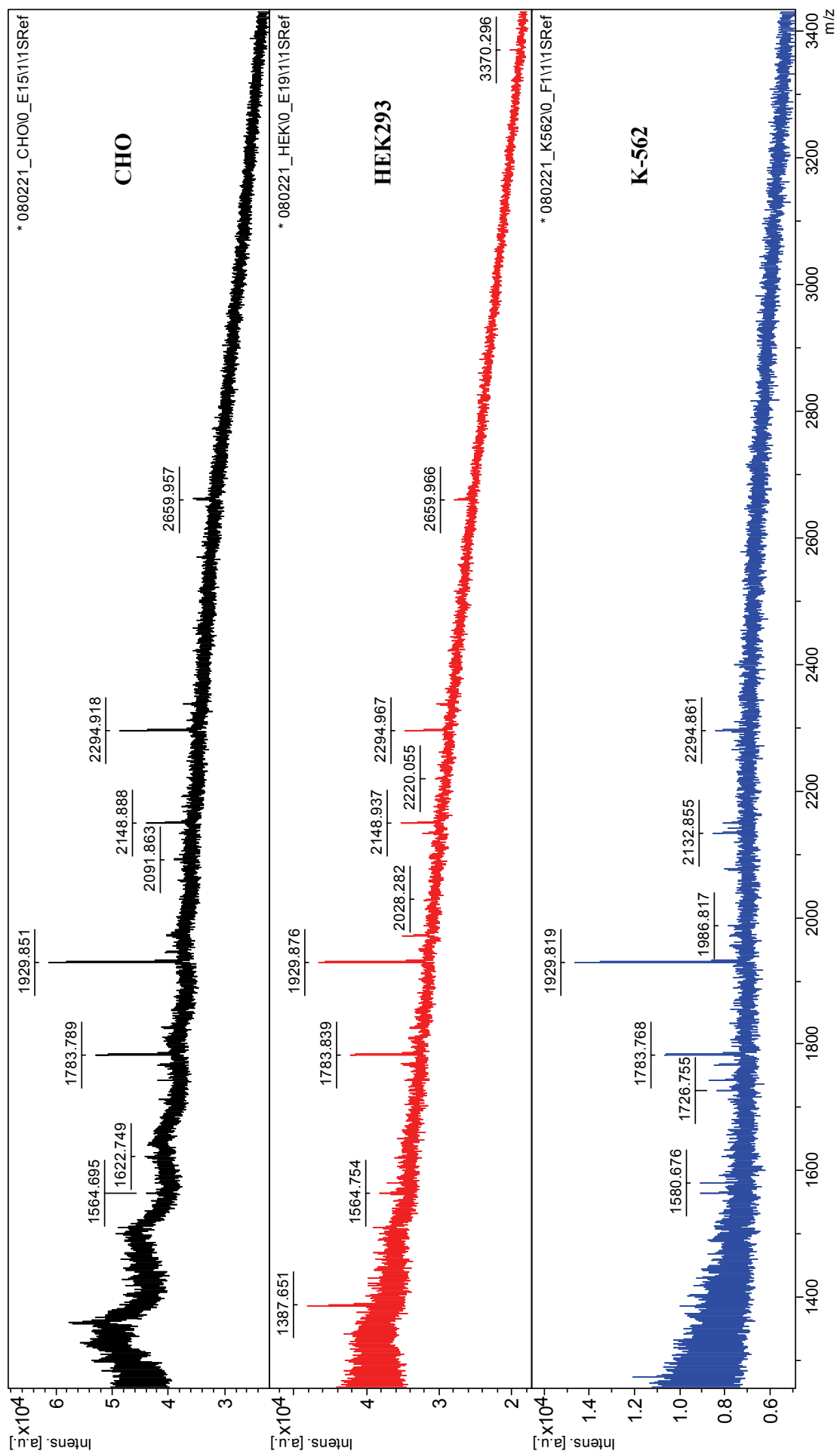


Abbildung 93: Vergleich von 2AB-markierten desialylierten Glykanen aus CHO-, HEK293- und K-562-Zellen mittels MALDI-TOF-MS Matrix Arabinosazon

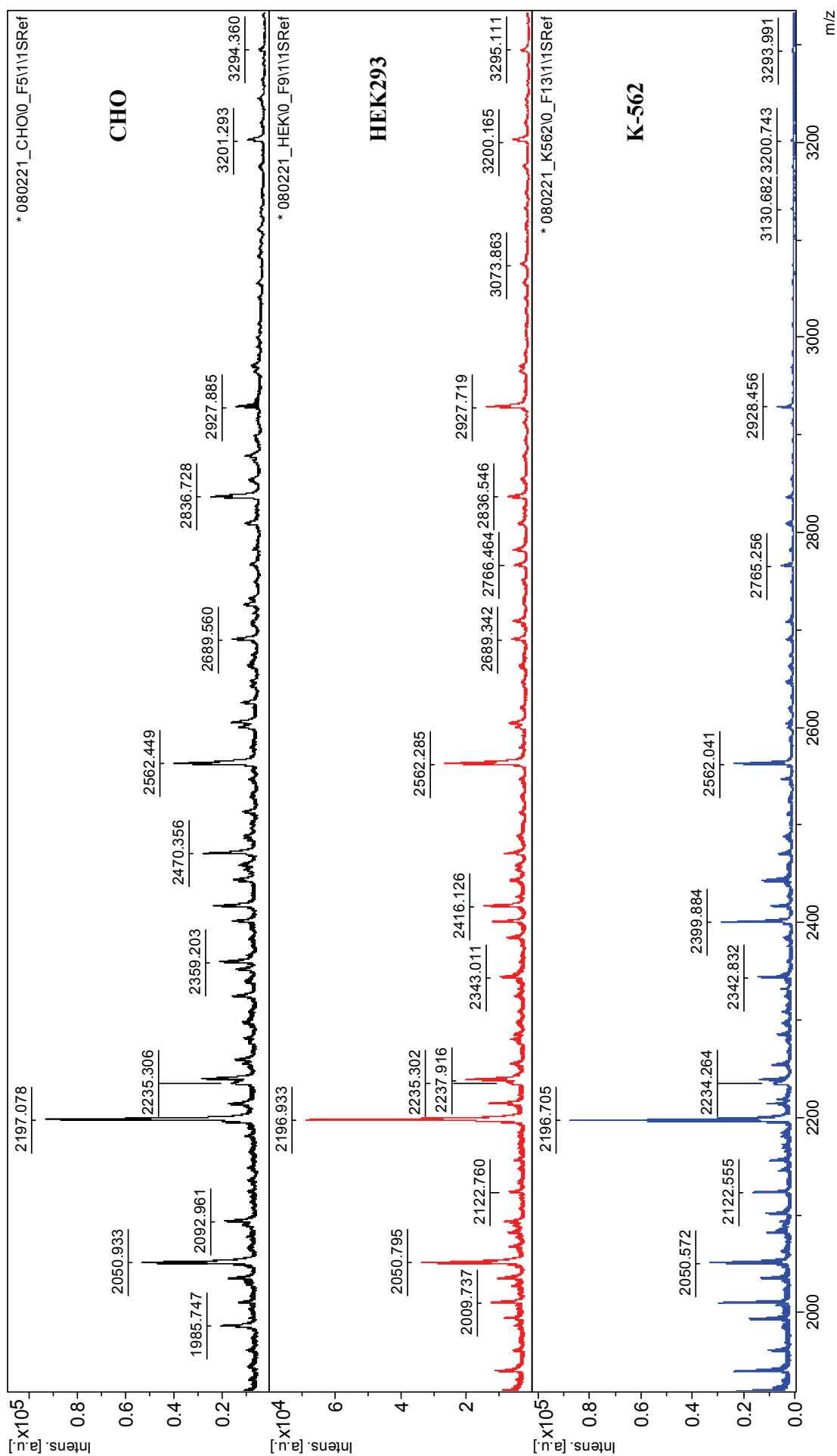


Abbildung 94: Vergleich von 2AB-markierten sialylierten Glykanen aus CHO-, HEK293- und K-562-Zellen mittels MALDI-TOF-MS Matrix ATT

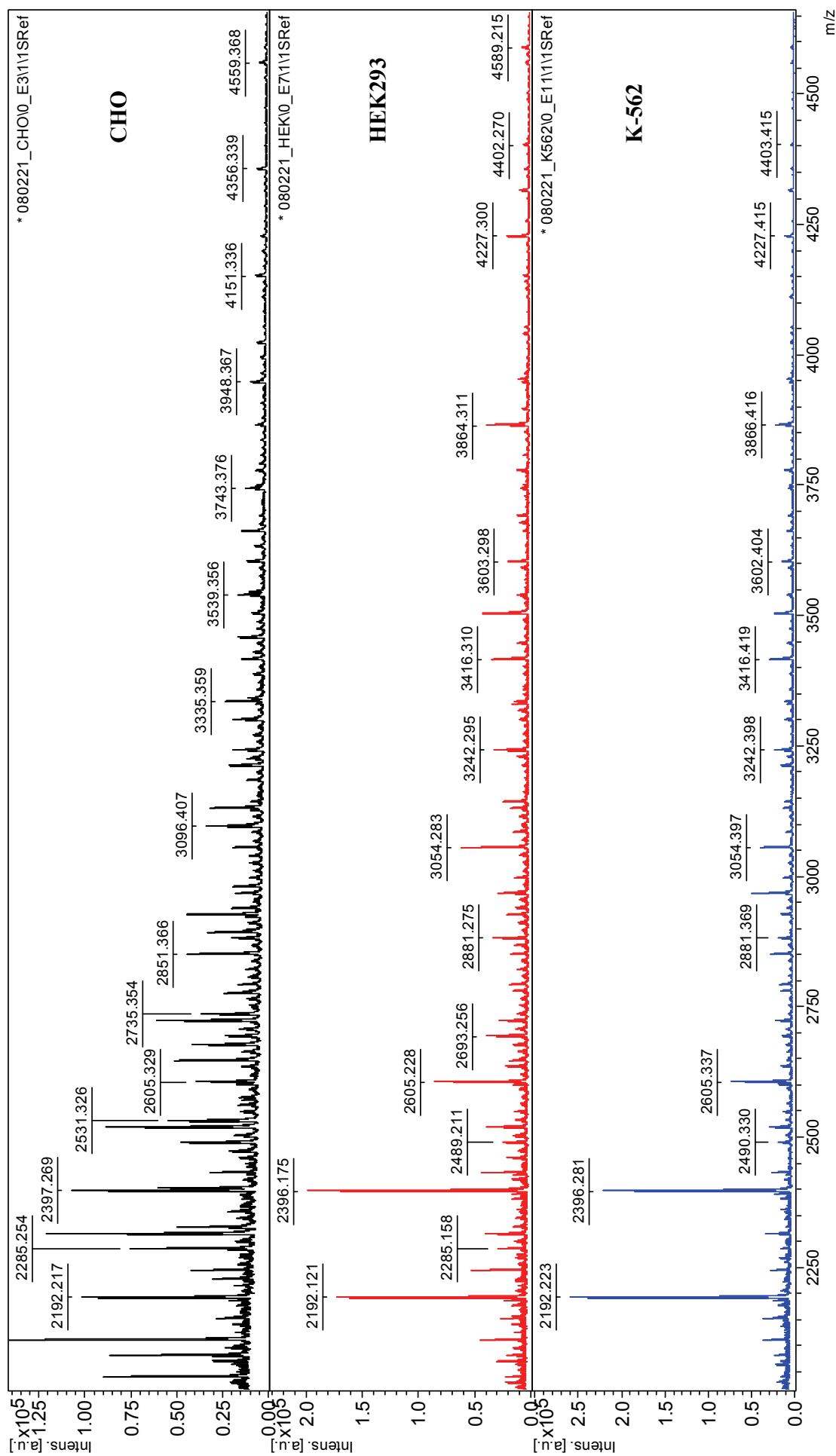


Abbildung 95: Vergleich von permethylierten Glykanen aus CHO-, HEK293- und K-562-Zellen mittels MALDI-TOF-MS Matrix Arabinosazon

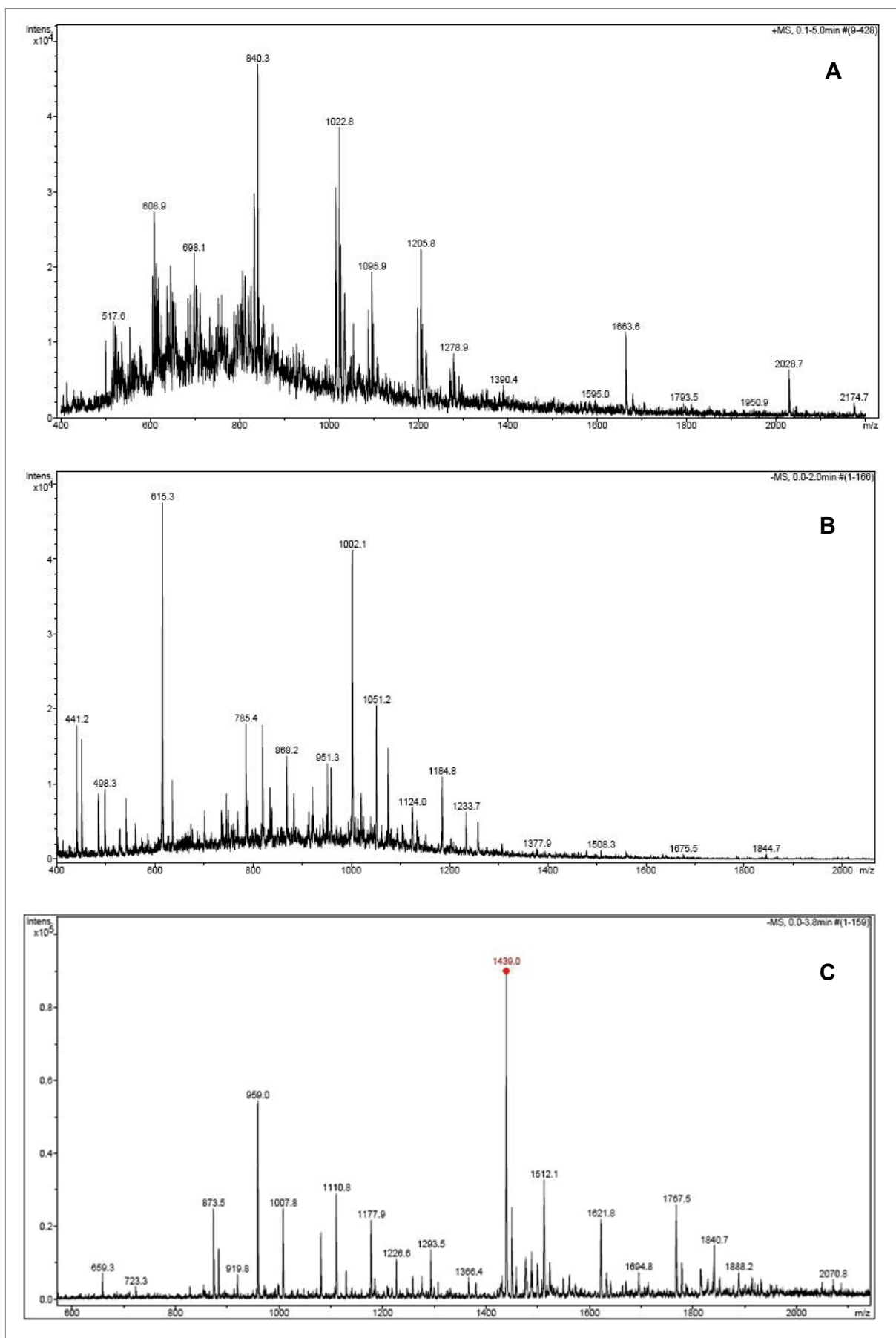


Abbildung 96: ESI-IonTrap Spektren vom AGP A: desialylierte Glykane im positiven Modus; B: desialylierte Glykane im negativen Modus; C: sialylierte Glykane im negativen Modus



















Name_KH	Mode Glykan s / as	redEnd	HexNAc	Hex	desHex	Pent	NeuAc	NeuGc	SO3	HPO3	m/z 1x			H	K	NH ₂	Na
											Permethyliert						
Core 10Man	+ N as	1	2	13							2553,846	3121,581		0	1		
Core Fuc 9Man	+ N as	1	2	12	1						2537,851	3182,571		0	1		
Core Fuc Bisec 8Man	+ N as	1	3	11	1						2578,877	3223,597		0	1		
Tri 2Fuc Bisec	+ N as	1	6	6	2						2523,909	3112,567		0	1		
Tri 2Fuc Bisec	+ N as	1	6	6	2						2523,909	3112,567		0	1		
Tetra Fuc	+ N as	1	6	7	1						2539,904	3142,577		0	1		
Tri Fuc Bisec	+ N as	1	6	6	2						2523,909	3112,567		0	1		
Tri Fuc Bisec	+ N as	1	6	6	2						2523,909	3112,567		0	1		
Tri 2Fuc Bisec	+ N as	1	6	6	2						2523,909	3112,567		0	1		
Tetra Fuc	+ N as	1	6	7	1						2539,904	3142,577		0	1		
Bi 2Fuc 2NANA	+ N s	1	4	5	2	2					2537,889	3140,562		0	1		
Bi 2Fuc 2NANA	+ N s	1	4	5	2	2					2537,889	3140,562		0	1		
Tri Fuc 1NANA	+ N s	1	5	6	1	1					2465,867	3054,525		0	1		
Tri Fuc 1NANA	+ N s	1	5	6	1	1					2465,867	3054,525		0	1		
Tri Fuc 1NANA	+ N s	1	5	6	1	1					2465,867	3054,525		0	1		
Tri Fuc 1NANA	+ N s	1	5	6	1	1					2465,867	3054,525		0	1		
Tri Bisec 1NANA	+ N s	1	6	6		1					2522,889	3125,562		0	1		
Tri Bisec 1NANA	+ N s	1	6	6		1					2522,889	3125,562		0	1		

Abbildung 98: Layout „Glykane x PM Bild“ der Datei *Glykane.fp7* von GlycoProtMass 3.0

Name	Glykan N O	AA	Basic	Peptid	Protein 1x	pI							
						2x	3x	4x	5x	6x	7x	8x	
a1GP ORM2-06	+	12	1417,644	1417,644	1417,644	708,822	472,548	354,411	283,529	236,274	202,521	177,205	
a1GP ORM1-07	+	12	1444,654	1444,654	1444,654	722,327	481,551	361,164	288,931	240,776	206,379	180,582	
a1GP ORM1-06	+	14	1684,777	1684,777	1684,777	842,388	561,592	421,194	336,955	280,796	240,682	210,597	
a1GP ORM1-05	+	15	1707,847	1707,847	1707,847	853,923	569,282	426,962	341,569	284,641	243,978	213,481	
a1GP ORM1-04	+	15	1751,947	1751,947	1751,947	875,974	583,982	437,987	350,389	291,991	250,278	218,993	
a1GP ORM2-05	+	15	1755,847	1755,847	1778,837	889,418	592,946	444,709	355,767	296,473	254,120	222,355	
a1GP ORM2-04	+	15	1862,837	1862,837	1862,837	931,418	620,946	465,709	372,567	310,473	266,120	232,855	
a1GP ORM1-02	+	16	1918,947	1918,947	1918,947	959,473	639,649	479,737	383,789	319,824	274,135	239,868	
a1GP ORM2-03	+	16	1918,947	1918,947	1918,947	959,473	639,649	479,737	383,789	319,824	274,135	239,868	
a1GP ORM1-03	+	15	1857,868	1937,834	1960,824	980,412	653,608	490,206	392,165	326,804	280,118	245,103	
a1GP ORM1-03	+	15	1857,868	1937,834	1960,824	980,412	653,608	490,206	392,165	326,804	280,118	245,103	
a1GP ORM2-02	+	17	1997,923	1997,923	1997,923	998,961	665,974	499,481	399,585	332,987	285,418	249,740	
a1GP ORM2-01	+	20	2147,177	2147,177	2147,177	1073,589	715,726	536,794	429,435	357,863	306,740	268,397	
a1GP ORM1 ohne leader	+	183	21546,633	21546,633	21546,633	10773,316	7182,211	5386,658	4309,327	3591,105	3078,090	2693,329	
a1GP ORM2 ohne leader	+	183	21637,505	21637,505	21637,505	10818,753	7212,502	5409,376	4327,501	3606,251	3091,072	2704,688	
a1GP ORM1 mit leader	+	201	23496,759	23496,759	23496,759	11748,380	7832,253	5874,190	4699,352	3916,127	3356,680	2937,095	
a1GP ORM2 mit leader	+	201	23587,632	23587,632	23587,632	11793,816	7862,544	5896,908	4717,526	3931,272	3369,662	2948,454	

Abbildung 99: Layout „Peptide“ der Datei *Peptide.fp7* von GlycoProtMass 3.0

Name	n H	n Na	n NH ₄	n K	n SO ₃	n HPO ₃	AA	Summe KH	stättig	Glykan 1	Peptid	GlycoPeptid	2x	3x	4x	5x	6x	7x	8x
N II mit Bi+1 NeuAc	+						16	1913,677	+	N	1998,913	3935,580	1967,790	1311,860	983,895	787,116	655,930	562,226	491,947
GP 2	+						7	2408,872	+	N	775,382	3207,244	1603,622	1069,081	801,811	641,449	534,541	458,178	400,906
GP 3	+						7	3721,327	+	N	841,333	4585,650	2292,825	1528,550	1146,413	917,130	764,275	655,093	573,206
GP 4	+						7	2643,941	+	N	775,382	3442,314	1721,157	1147,438	860,578	688,463	573,719	491,759	430,289
GP 4	+						7	1622,582	-	N	935,315	2580,886	1290,443	860,295	645,222	516,177	430,148	368,698	322,611

Abbildung 100: Layout „Glykopeptid“ der Datei *Glykoproteine.fp7* von GlycoProtMass 3.0