Supporting Information

Comparison of different HILIC stationary phases in the separation of hemopexin and immunoglobulin G glycopeptides and their isomers

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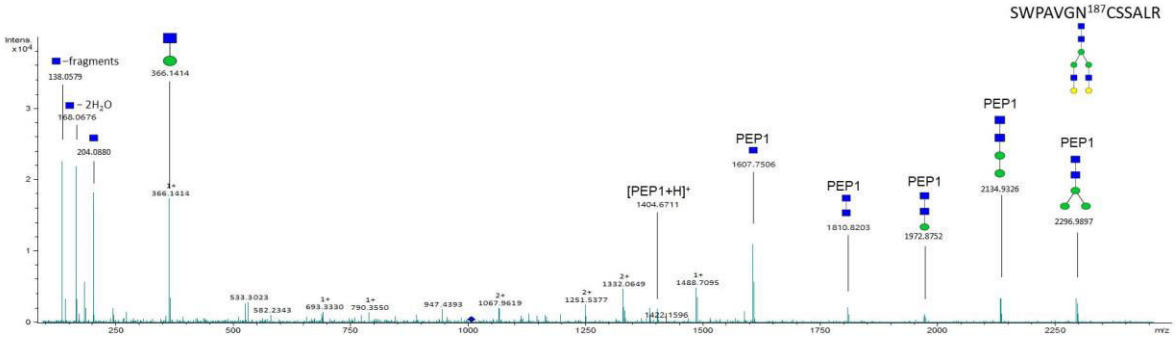
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x10^4
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138.0579
- 2H2O
168.0676
204.0880
366.1414
2+
366.1414
[PEP1+H]+
1404.6711
PEP1
1607.7506
PEP1
1810.8203
PEP1
1972.8752
PEP1
2134.9326
PEP1
2296.9897
533.3023
582.2343
1+
693.3330
1+
790.3550
947.4393
2+
1067.9619
2+
1251.5377
2+
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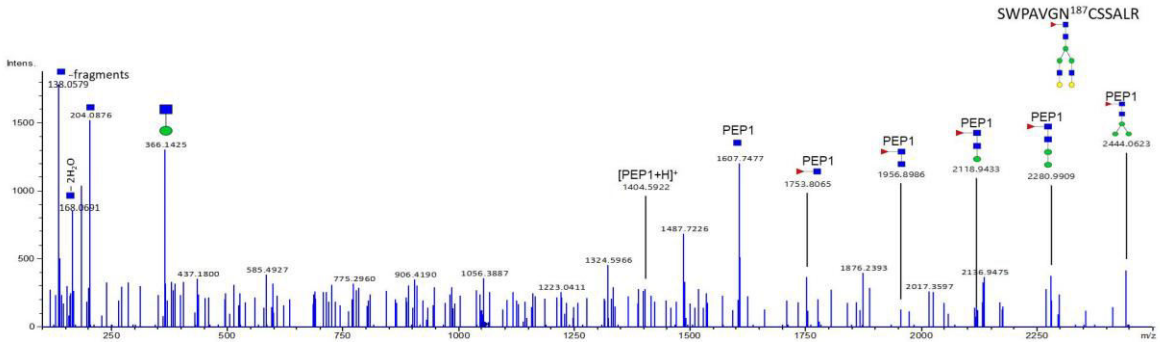
m/z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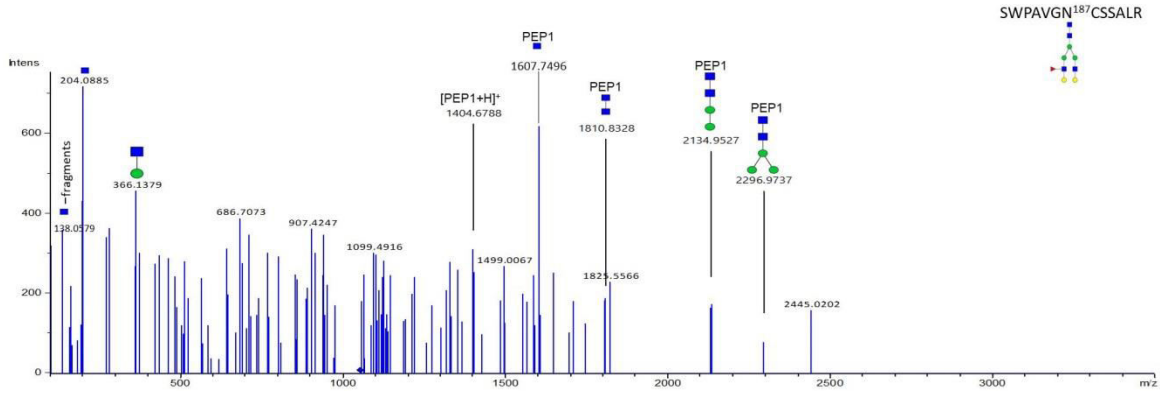
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3000
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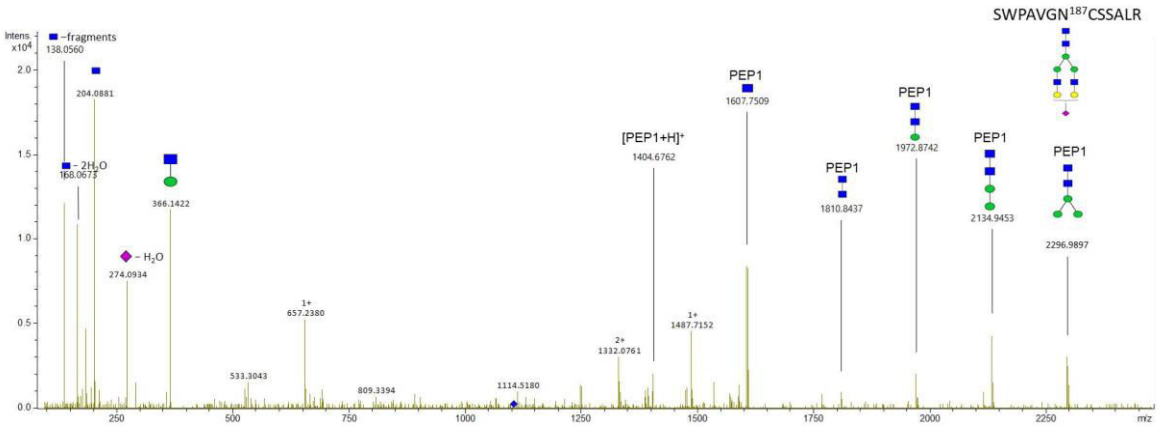
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366.1422
- H2O
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[PEP1+H]+
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PEP1
1607.7509
PEP1
1810.8437
PEP1
1972.8742
PEP1
2134.9453
PEP1
2296.9897
1+
657.2380
533.3043
809.3394
1114.5180
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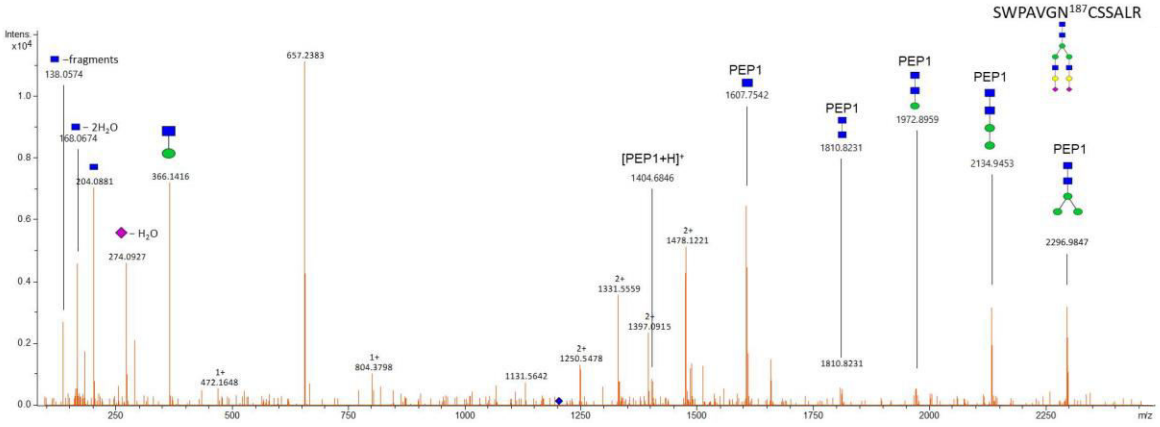
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− H2O
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657.2383
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PEP1
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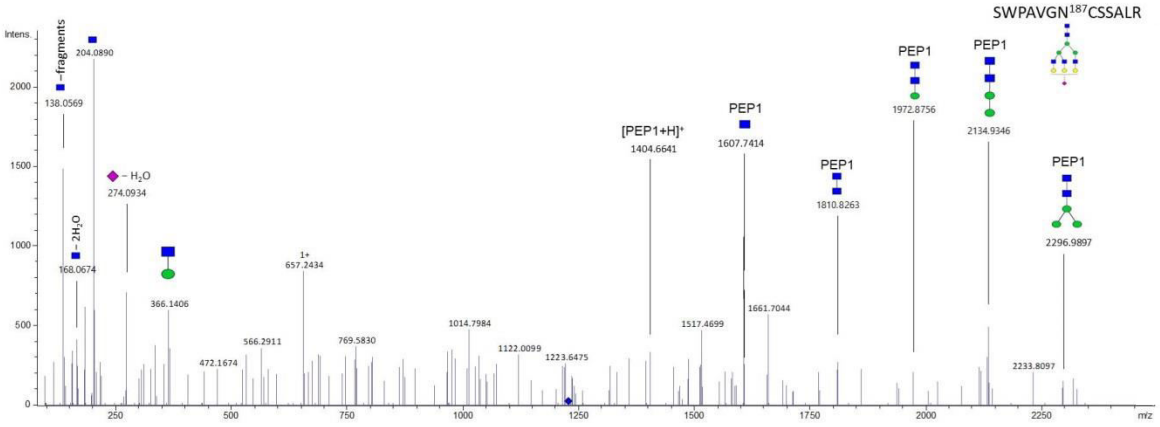
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274.0934
− 2H2O
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1+
657.2434
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PEP1
1810.8263
PEP1
1972.8756
PEP1
2134.9346
PEP1
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566.2911
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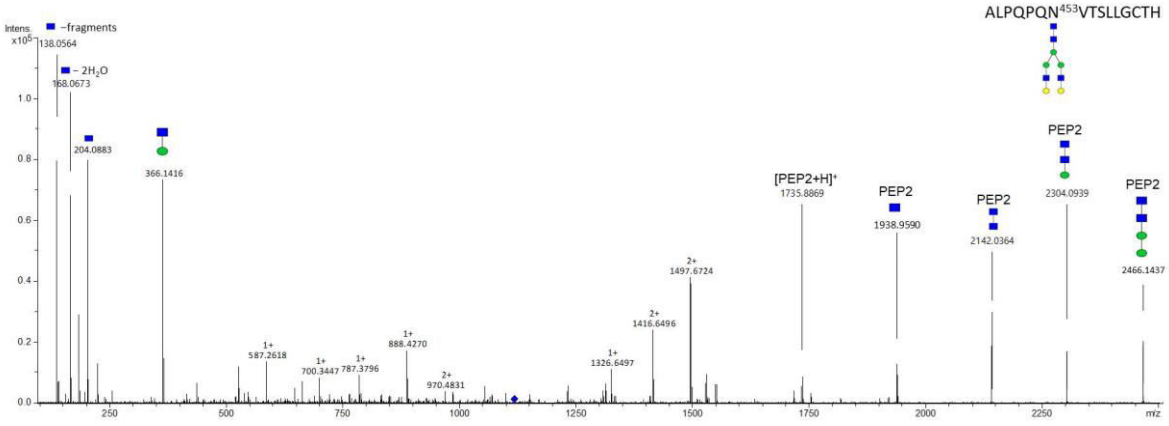
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PEP2
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[PEP2+H]+
1735.8869
PEP2
1938.9590
PEP2
2142.0364
2+
1497.6724
2+
1416.6496
1+
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1+
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1+
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2+
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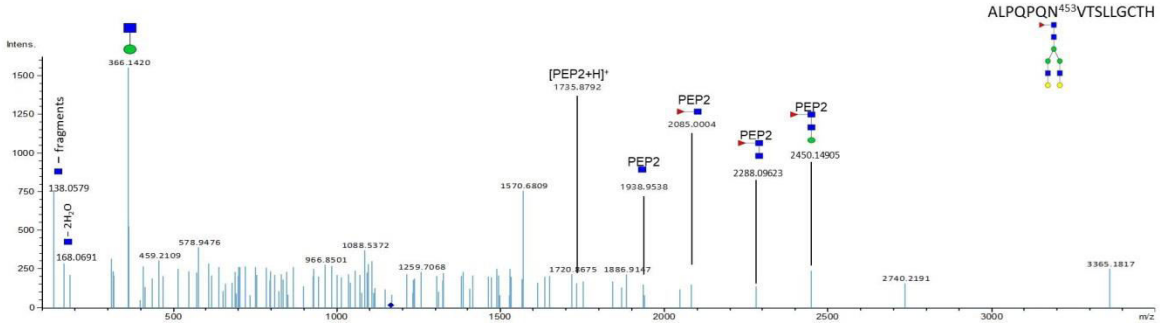
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− fragments
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− 2H2O
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PEP2
2085.0004
PEP2
2450.14905
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578.9476
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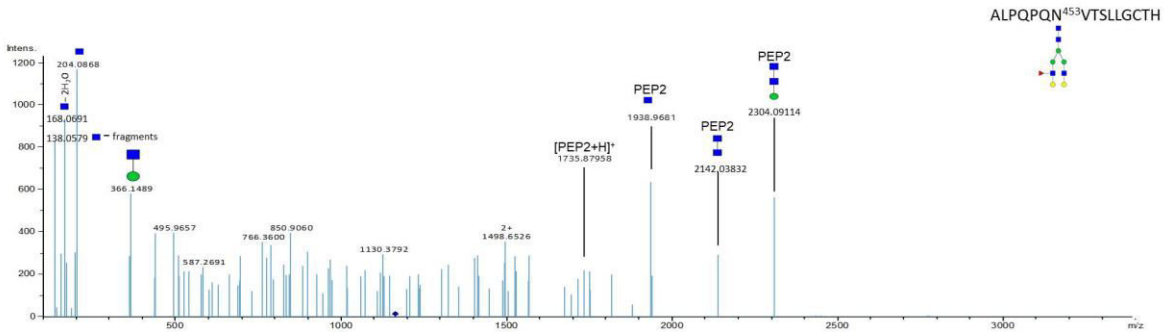
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Intens.
204.0868
– 2H2O
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138.0579
– fragments
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587.2691
1130.3792
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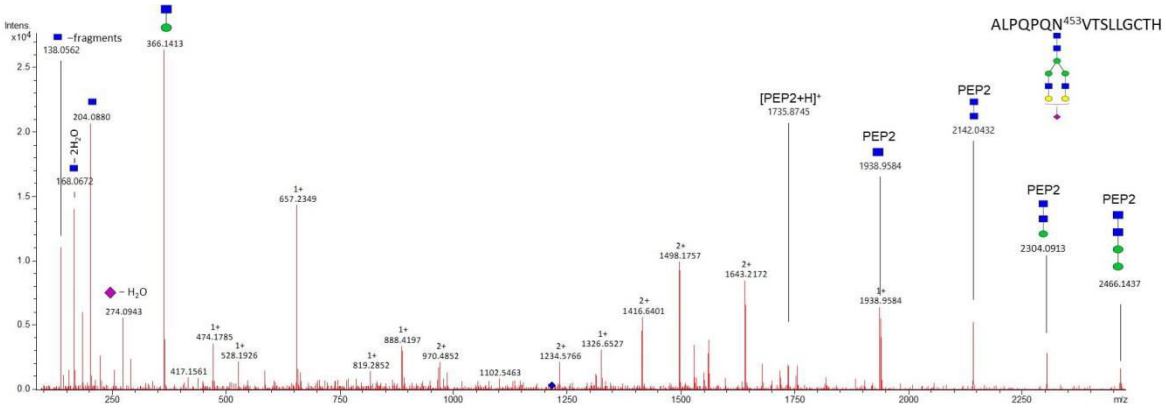
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– H2O
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[PEP2+H]+
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PEP2
1938.9584
PEP2
2304.0913
PEP2
2466.1437
2+
1498.1757
2+
1643.2172
2+
1416.6401
1+
1938.9584
1+
474.1785
1+
528.1926
417.1561
1+
888.4197
2+
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1+
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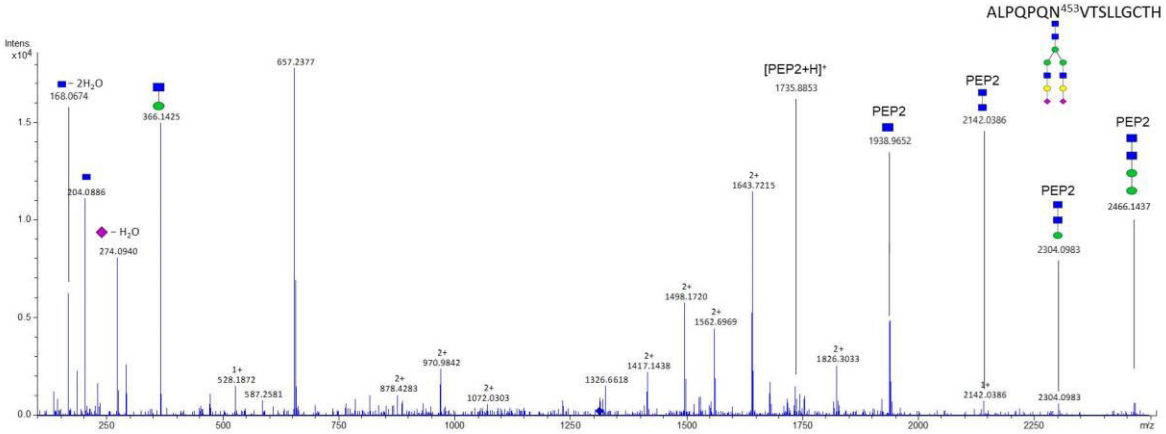
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– H2O
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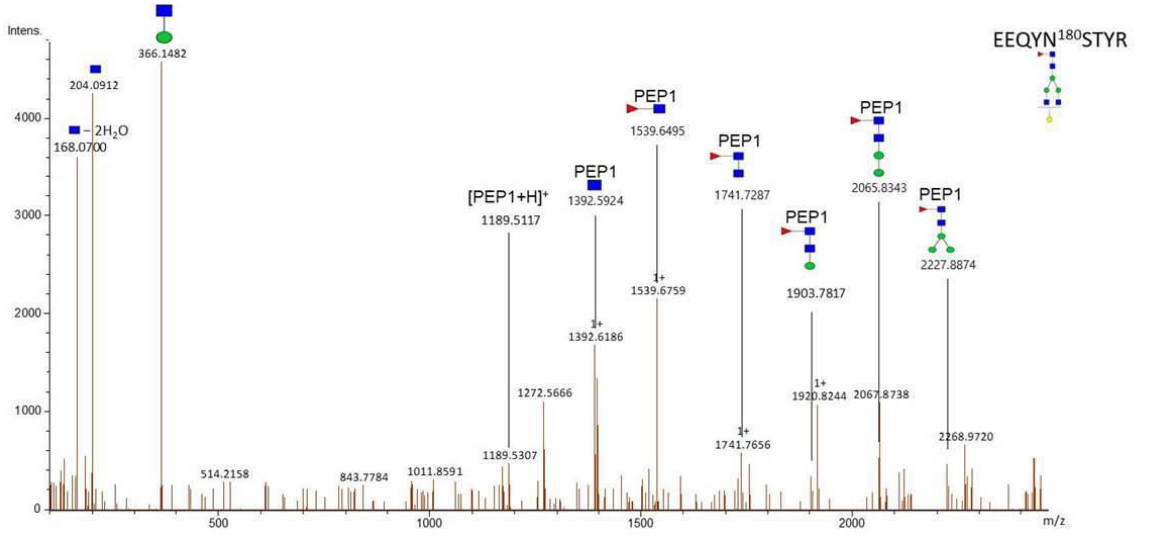
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– 2H2O
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PEP1
2227.8874
[PEP1+H]+
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PEP1
1392.5924
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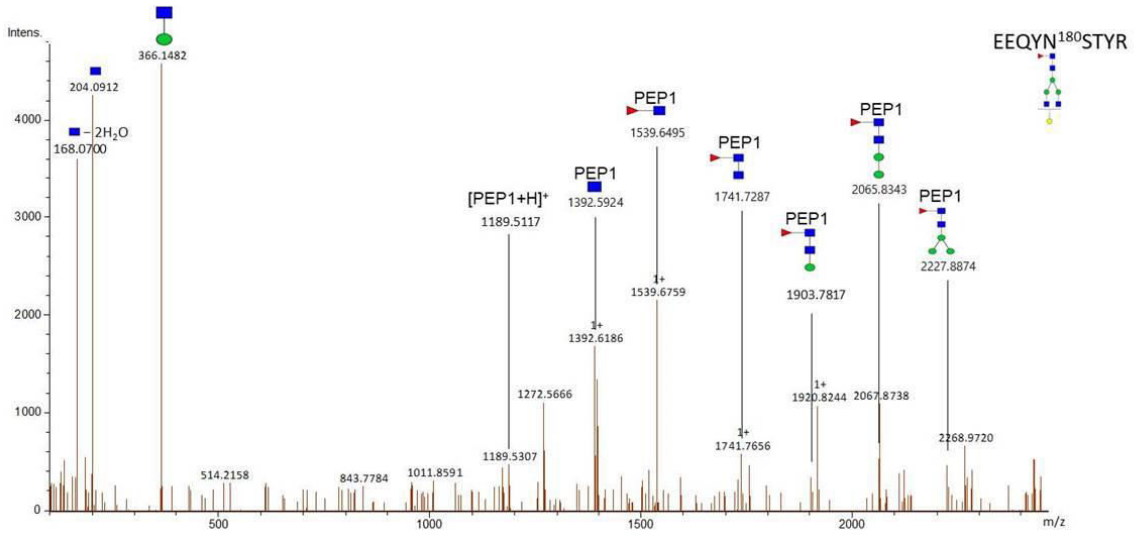
Intens.
EEQYN180STYR
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204.0912
−2H2O
168.0700
PEP1
1539.6495
PEP1
1741.7287
PEP1
2065.8343
PEP1
1392.5924
[PEP1+H]+
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PEP1
1903.7817
PEP1
2227.8874
1+
1539.6759
1+
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1+
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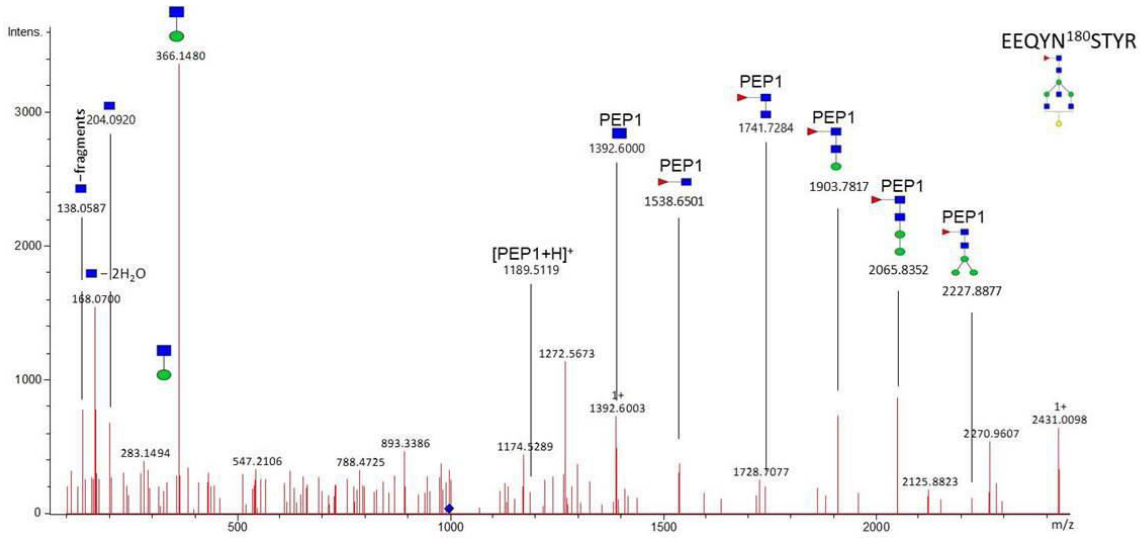
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PEP1
1392.6000
PEP1
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PEP1
1538.6501
PEP1
2065.8352
PEP1
2227.8877
[PEP1+H]+
1189.5119
1272.5673
1+
1392.6003
1+
2431.0098
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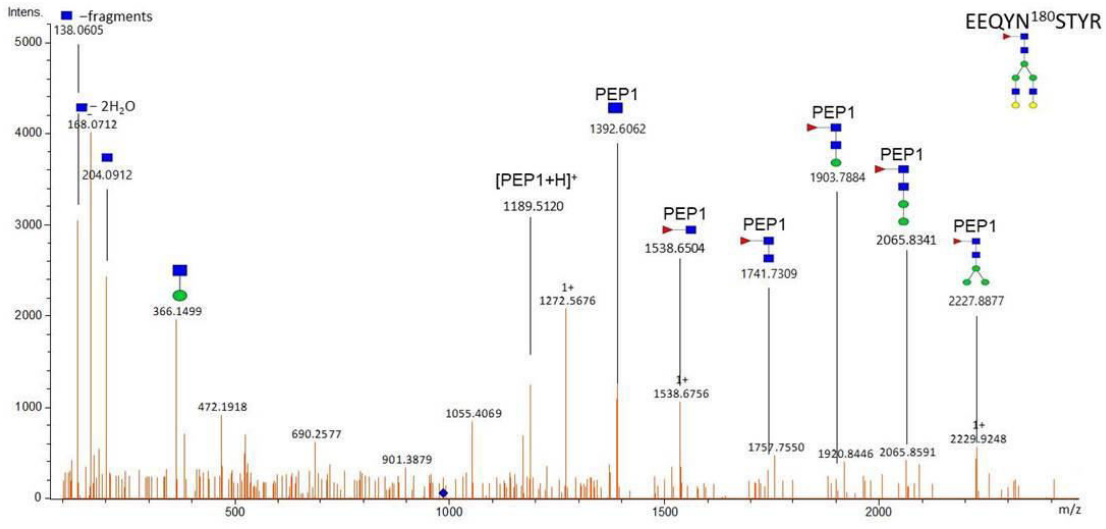
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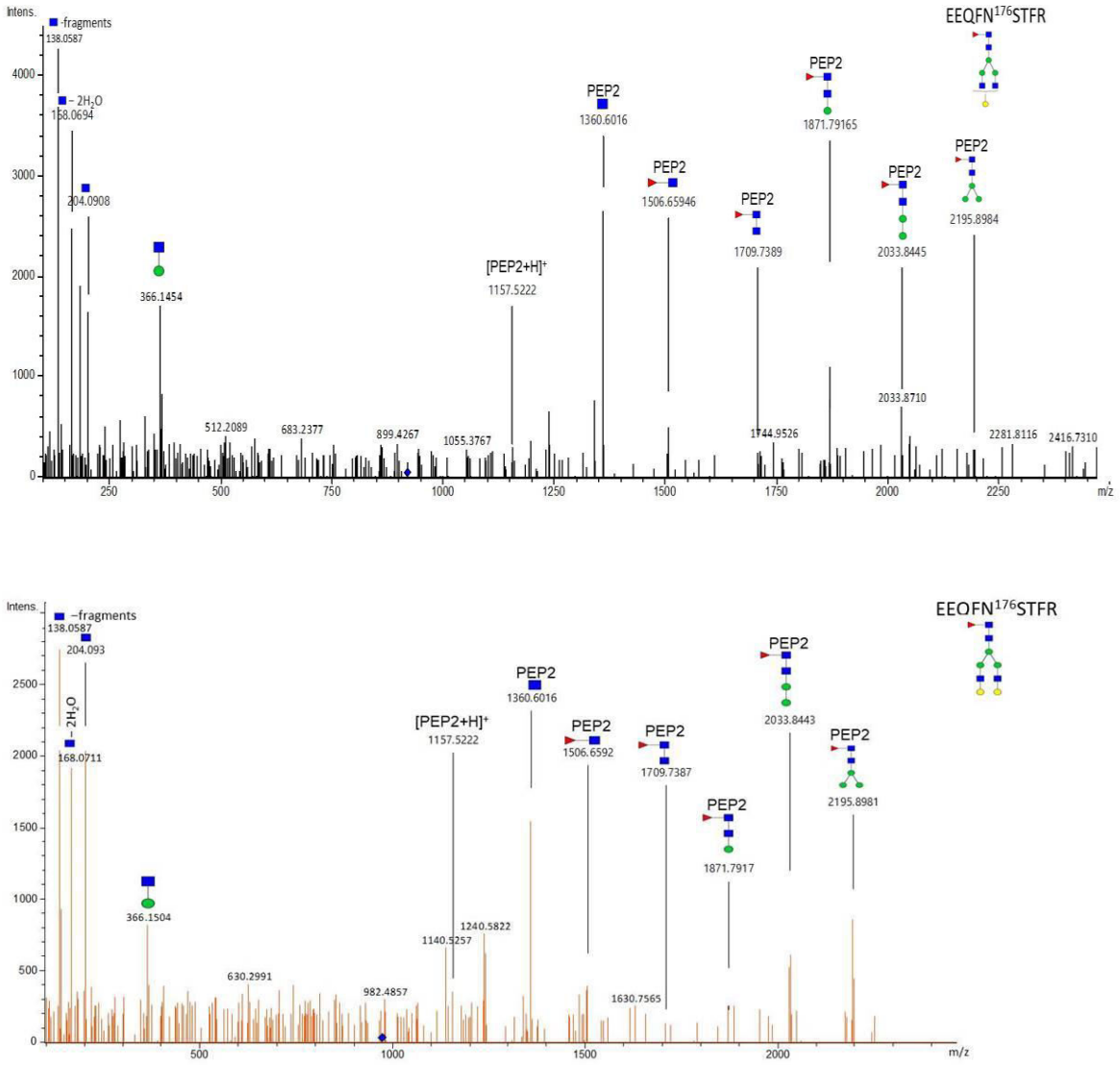

Figure S1 Fragmentation spectra of all studied glycopeptides in HALO® penta-HILIC column.

Table S1 Observed monoisotopic *m/z* for all studied analytes and their rentention times, t_R, in different HILIC columns. ND means not detected analyte.

Analyte		Column		
Hemopexin SWPAVGN[187]CSSALR	Observed *m/z* (Charge)	HALO® penta-HILIC t_R	Glycan BEH Amide t_R	ZIC-HILIC t_R
A2G2	1009.7550 (3^+)	17.2	30.4	43.3
A2G2F1 (core)	1058.4409 (3^+)	17.8	30.6	43.5
A2G2F1 (outer arm)		18.2	30.9	
A2G2S1 (isomer 1)	1106.7868 (3^+)	24.7	30.8	41.3
A2G2S1 (isomer 2)		25.2	30.9	41.6
A2G2S2	1203.8186 (3^+)	31.6	31.4	40.2
A3G3S1 (isomer 1)	1228.4975 (3^+)	25.9	31.3	41.9
A3G3S1 (isomer 2)		26.2	31.5	ND
A3G3S1 (isomer 3)		26.4	ND	ND
ALPQPQN[453]VTSLLGCTH				
A2G2	1120.1586 (3^+)	19.0	30.1	42.5
A2G2F1 (core)	1168.8445 (3^+)	19.7	30.6	42.7
A2G2F1 (outer arm)		19.9		
A2G2S1 (isomer 1)	1217.1904 (3^+)	26.9	30.5	40.8
A2G2S1 (isomer 2)		27.3	30.6	40.9
A2G2S2	1314.2222 (3^+)	33.0	31.1	39.8
IgG1 EEQYN[180]STYR				
A2F1	878.6868 (3^+)	19.9	31.1	44.9
A2G1F	932.7044 (3^+)	20.8	31.6	44.9
G1A3F1	1000.3975 (3^+)	21.2	31.7	ND
A2G2F1	986.7220 (3^+)	21.7	32.1	45
IgG2 EEQFN[176]STFR				
A2G1F (isomer 1)	922.0411 (3^+)	19.5	30.8	43.9
A2G1F (isomer 2)		19.7	30.9	
A2G2F1	976.0588 (3^+)	20.6	31.3	44.0

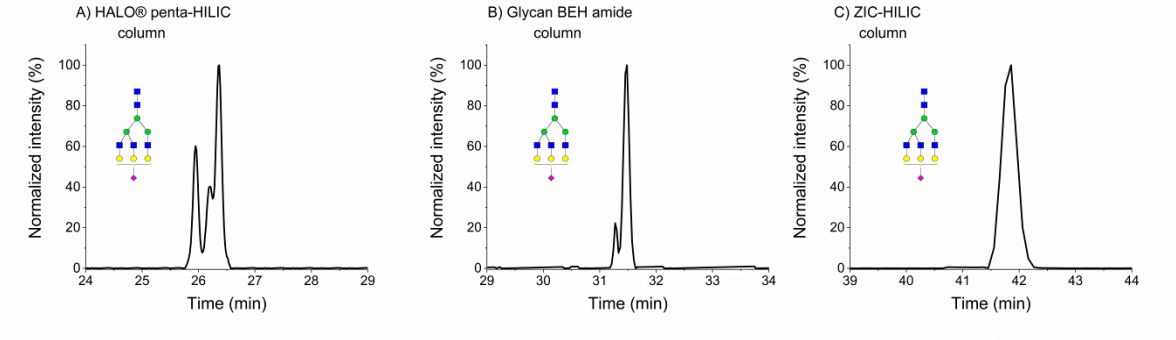

Figure S2 Normalized EIC chromatograms of A3G3S1 glycoform of the SWPAVGN[187]CSSALR peptide of hemopexin in HALO® penta-HILIC (A), Glycan BEH amide (B) and ZIC-HILIC (C) column.

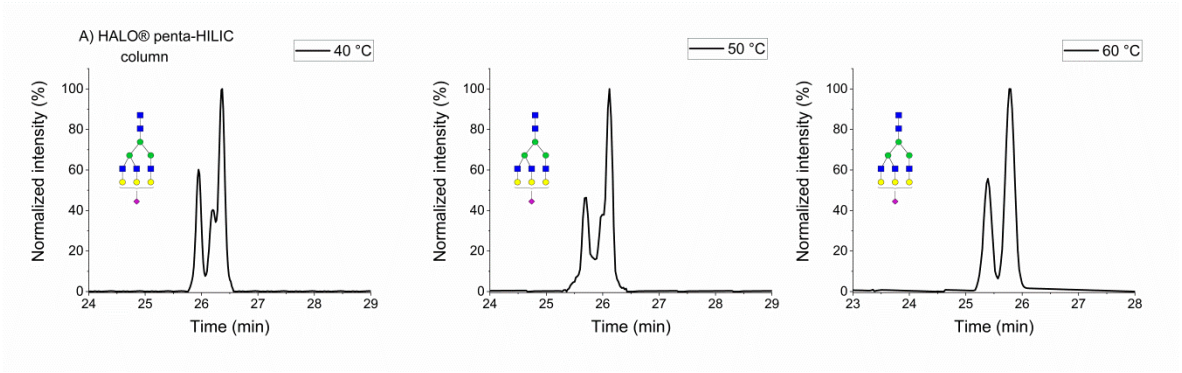

Figure S3 Separation of A3G3S1 glycoform of the of the SWPAVGN[187]CSSALR peptide of hemopexin in HALO® penta-HILIC at different column temperatures.

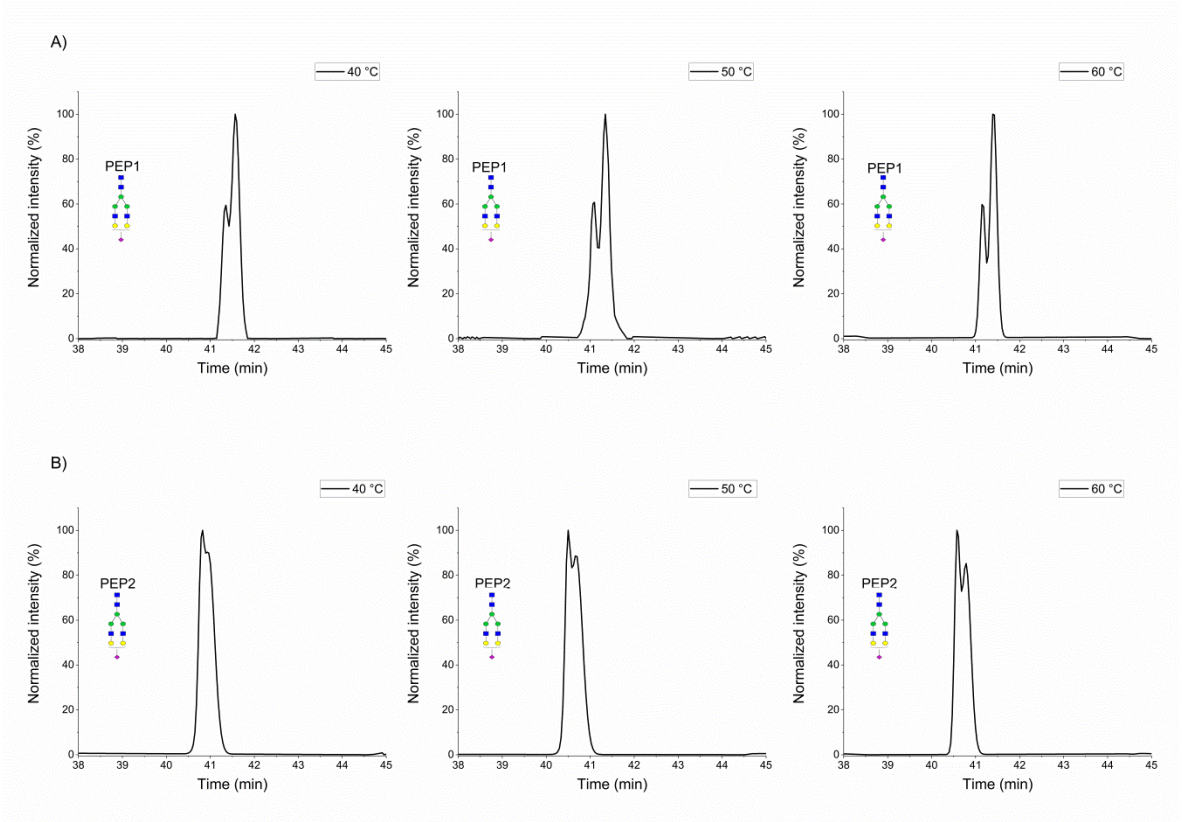

Figure S4 Separation of A2G2S1 glycoform of the of the SWPAVGN[187]CSSALR (A) and ALPQPQN[453]VTSLLGCTH (B) peptide of hemopexin in ZIC-HILIC column at different column temperatures. PEP1 refers to SWPAVGN[187]CSSALR and PEP2 to ALPQPQN[453]VTSLLGCTH.