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Thermo Scientific Dionex CarboPac PA300-4µm column

Dionex CarboPac PA300-4µm column – A new column for glycan analysis

Keywords

Pulsed amperometry, derivatization-free, direct detection, gold electrodes, oligosaccharide, glycome, glycomics, glycan, mucin, HPAE-MS, glycosylation, carbohydrate, ion chromatography

Achieve high-efficiency and high-resolution separations of complex neutral and charged glycans of different sizes using the Thermo Scientific™ Dionex™ CarboPac™ PA300-4µm column. This high-efficiency column is designed for isomer level resolution analysis of complex oligosaccharides in heterogeneous biological and food samples.

Benefits

- Total glycome analysis with isomer resolution
- Resolution of N-linked and O-linked released glycans
- Free glycan analysis
- Oligosaccharide analysis of therapeutic and food samples

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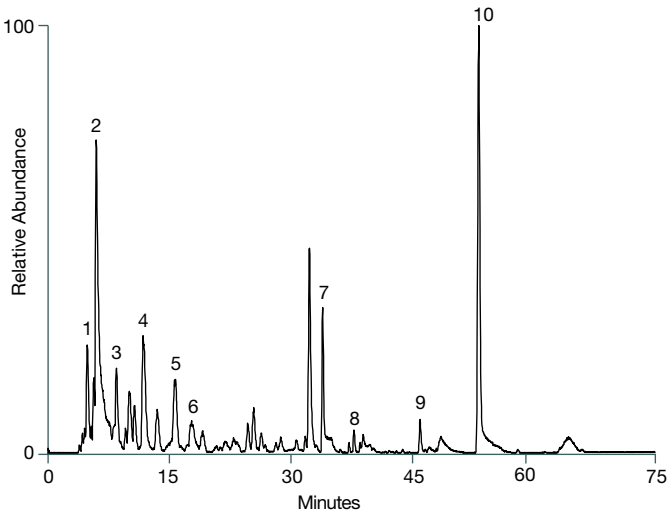
Isomer resolution of neutral and sulfated O-glycans from porcine gastric mucin

Glycosylation in mammals represents a very diverse set of pre- and post-translational modifications. One of the most diverse and complex sets of glycans is found on mammalian mucins. When used in an HPAE-MS workflow, the CarboPac PA300-4µm column takes advantage of the improved chromatographic separation, as well as, the high resolution and mass accuracy of the Thermo Scientific™ Orbitrap™ mass analyzer, therefore enabling the identification of glycan structures.



Column:	Dionex CarboPac PA300-4µm (Guard + Analytical)
Flow Rate:	0.25 mL/min
Temperature:	30 ºC
Detection:	Thermo Scientific™ Q Exactive™ HF-X Hybrid Quadrupole-Orbitrap™ MS
Injection Volume:	20 µL
Eluents:	A - Milli Q Water B - 200 mM NaOH C - 50 mM NaOH 25 mM NaOAc D - 100 mM NaOH 250 mM NaOAc

Eluent Program:

Time	%A	%B	%C	%D	Comments
-1.0	78.5	19.5	2	0	Equilibration
0.0	78.5	19.5	2	0	Load/inject
4.0	78.5	19.5	2	0	
20.0	20	20	60	0	
50.0	0	0	0	100	Regeneration
59.9	0	0	0	100	
60.0	78.5	19.5	2	0	Equilibration
75.0	78.5	19.5	2	0	End

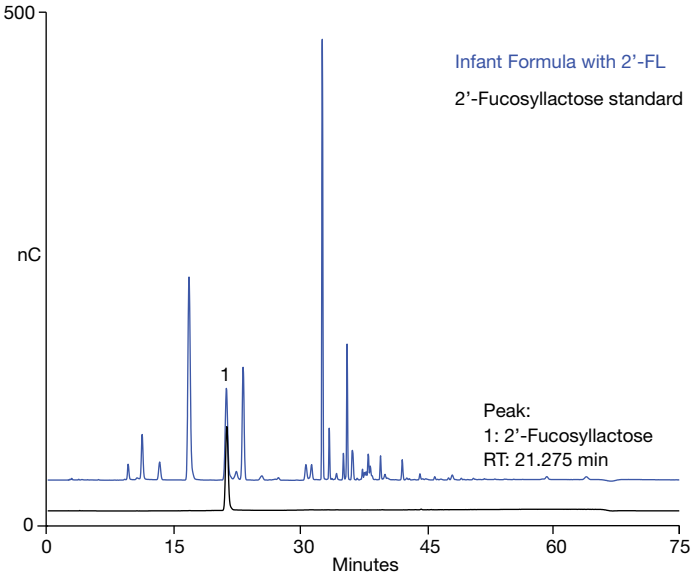
Figure 1. Base peak chromatogram of mucin type III O-glycan.

Peak	RT (min)	Observed *m/z*	Theoretical *m/z*	Mass accuracy (ppm)	Composition	Ion
1	4.85	733.2896	733. 2884	1.6465	HexFuc(HexNAc)2	$[M-H]^-$
2	6.08	530.2080	530.2090	1.8860	HexFucHexNAc	$[M-H]^-$
3	8.46	733.2892	733. 2884	1.0910	HexFuc(HexNAc)2	$[M-H]^-$
4	11.97	1041.4006	1041.3992	1.4403	(Hex)2(Fuc)2(HexNAc)2	$[M-H]^-$
5	15.58	1098.4233	1098.4206	2.4581	(Hex)2Fuc(HexNAc)3	$[M-H]^-$
6	17.66	1203.4506	1203.4520	1.1633	(Hex)3(Fuc)2(HexNAc)2	$[M-H]^-$
7	34.01	821.3063	821.3045	1.8264	NeuAcHexFucHexNAc	$[M-H]^-$
8	38.19	852.2811	852.2848	4.3413	N-glycan, hybrid, sulfated	$[M-2H]^{2-}$
9	45.92	1121.3582	1121.3560	1.9619	(Hex)2(Fuc)2(HexNAc)2-S	$[M-H]^-$
10	53.19	813.2474	813.2452	2.7052	HexFuc(HexNAc)2-S	$[M-H]^-$

Table 1. Compositional annotation of peaks in Figure 1. The CarboPac PA300-4µm column provides isomer resolution of both neutral and sulfated O-glycans from a porcine gastric mucin type III sample with detection by a Q Exactive HF-X Hybrid Quadrupole-Orbitrap mass spectrometer.

O-Linked Glycan Alditol Profiling of Bovine Fetuin

Glycans present on proteins can have a profound effect on the protein structure and on their biological function. Therefore, the study of glycans is an important part of protein characterization. The high resolution of the CarboPac PA300-4µm column is demonstrated in Figure 3 showing the separation O-linked alditols.



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Temperature:	30 °C
Detection:	Integrated Amperometry, Quadruple Pulse Waveform
Working Electrode:	PTFE Gold (Disposable)
Ref Electrode:	Ag/AgCl
Injection Volume:	2.5 µL
Eluents:	A - Milli Q Water B - 200 mM NaOH C - 50 mM NaOH 25 mM NaOAc D - 100 mM NaOH 250 mM NaOAc

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Figure 3. Infant formula supplemented with 2'-FL.

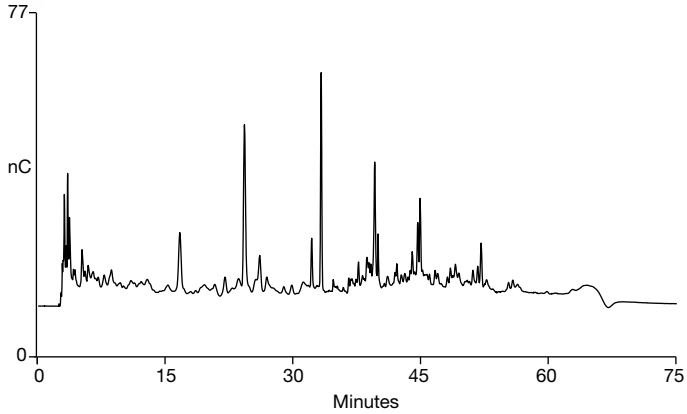

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Figure 2. Fetuin O-glycan alditol.

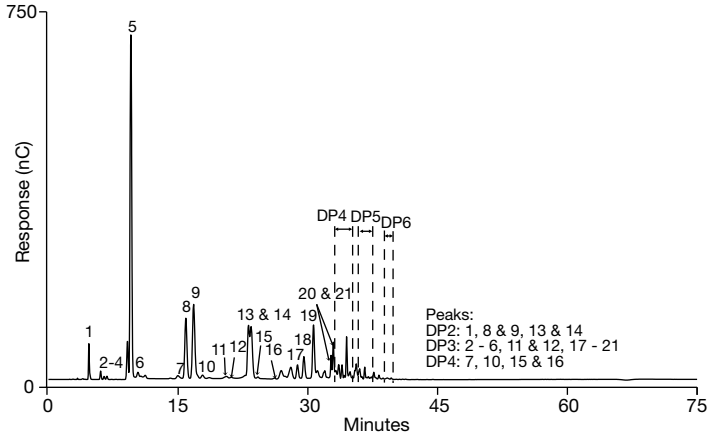
Free and Released Glycan Analysis

Human milk oligosaccharides (HMOs) are a family of free glycans that contains diverse structures and are highly abundant in human milk. Numerous publications have demonstrated the importance of HMOs not only in enhancing the development of the intestinal microbiota of newborns, but also bolstering the immune system in breastfed infants. Therefore, HMOs are a vital component of infant nutrition. The most abundant HMO in most mothers' breast milk is 2′-fucosyllactose (2'-FL). Preclinical data suggests that the addition of HMOs to infant formula is safe, resulting in the addition of 2′-FL HMO to some commercial infant formulas. Figure 4 shows the oligosaccharide profile in a commercial infant formula product with 2'-FL.

Oligosaccharide analysis of therapeutic and food samples

Galacto-oligosaccharides (GOS) are a group of non-digestible carbohydrates that are increasingly being used as functional food ingredients. The GOS fractions comprise galactose oligosaccharides with a terminal glucose, varying in type of glycosidic linkages between monosaccharide units and in the degree of polymerization (DP). The differences in GOS structures can affect their roles on gut microbiota.

Figure 4 demonstrates the separation of oligosaccharides in Bimuno GOS syrup with a CarboPac PA300-4µm column. The mixture of GOS is separated mainly based on their size (DP). The early eluting peaks (RT earlier than 33 min) are mainly DP2 and DP3 oligosaccharides, with a presence of low-abundant DP4 molecules. Peaks eluting between 33 and 35 min are mainly DP4. Peaks present between 35 and 37 min are mainly DP5. Peaks eluting near 39 min are mainly DP6. The size of the oligosaccharides was identified by the HPAE-MS platform.



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75.0	78.5	19.5	2	0	End

Figure 4. Galacto-oligosaccharides syrup.

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