TOSOH

Application Note

UHPLC Glycosylation Analysis with TSKgel Amide-80 2 µm HILIC Columns

Glycosylation is one of the most common forms of post-translational modification of proteins. The polysaccharide side chains (Glycans) play critical roles in physiological and pathological reactions ranging from immunity to cell signaling. Besides the interest in characterizing glycosylation pattern of proteins for structure/function analysis, the thorough characterization of glycosylation is also a major quality parameter in the production of biotherapeutics. Hydrophilic interaction liquid chromatography (HILIC) is a well-recognized technique that effectively separates and quantifies isolated glycans.

Glycoprotein analysis involves characterizing complex N- and O-linked structures composed of sugar moieties. Besides mass spectrometric techniques, HILIC using amide-based stationary phases is a well-established, robust technique used by many laboratories to obtain high-resolution separation of N-linked glycans released from glycoproteins. Tagging the glycans with a fluorescent label such as 2-aminobenzamide (2AB) or aminopyridin (PA) allows the sugars to be detected at femtomole levels.

TSKgel Amide-80 column chemistry is ideally suited for the separation of charged and neutral fractions of glycan pools in one run. The retention of labelled polysaccharides by TSKgel Amide-80 enables the identification of glycan structures by comparison to a labelled dextran ladder that is used to normalize retention times in order to calculate the number of glucose units (GU values) of the separated glycans. The GU values obtained after separation of sequential exoglycosidase digests can be used to predict the glycan structure by database query (Glycobase, autoGU).

Packed with 2 micrometer spherical silica particles that are covalently bonded with non-ionic carbamoyl groups, TSKgel Amide-80 provides the same unique selectivity as TSKgel Amide-80 3 µm or 5 µm that are applied for glycan analysis in many QC labs for years. The new 2 µm material improves peak capacity and sensitivity for both, (U) HPLC and LC-MS analysis and allows a smooth transfer of established methods form HPLC to UHPLC. The columns are especially suited for use in UHPLC systems, as their reduced system volume and optimized detector specifications help to maintain the high resolution that can be achieved with 2 micron stationary phase.

RESULTS AND CONCLUSION

The new TSKgel Amide-80 2 µm phase shows a 1.4 fold higher resolution of PA-glycan peaks (Figure 1). Maximum pressure drops of TSKgel Amide-80 2 µm do not exceed 55 MPa during gradient at the conditions used (flow rate 0.5 mL/min). The suitability of the new 2 micron material for glycosylation analysis of labelled glycans by both fluorescence detection (Figure 2) and mass spectrometric detection (Figure 3) are demonstrated for various antibody samples.

COMPARISON OF TSKgel AMIDE-80 2 µm AND 3 µm

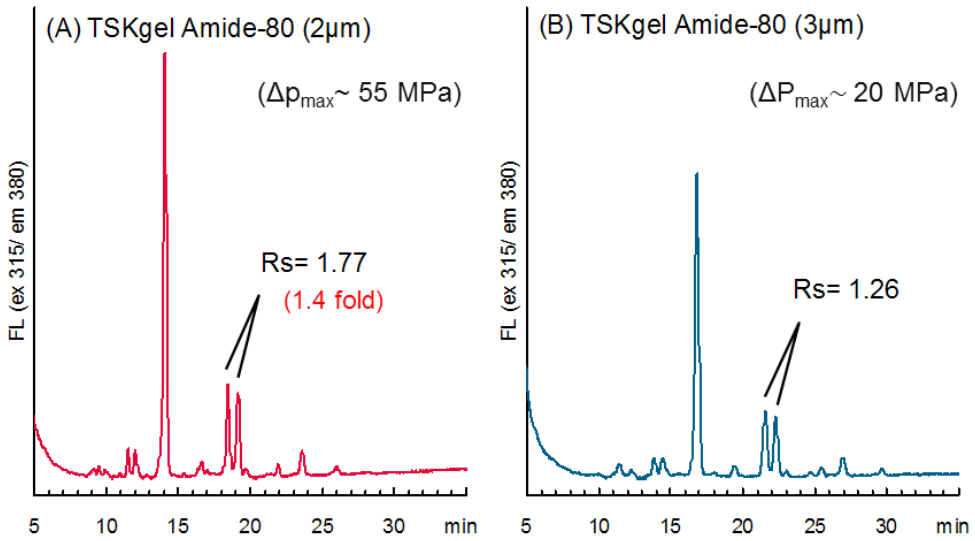

Figure 1

MATERIAL AND METHODS

UHPLC Analysis:

Columns:	TSKgel Amide-80 2 µm (2.0 mm ID x 15 cm) TSKgel Amide-80 3 µm (2.0 mm ID x 15 cm)
Mobile phase:	A: 200 mmol/L acetic acid + triethylamine (pH 7.3) B: acetonitrile
Gradient:	75%B (0-5min), 75-50%B (5-80 min, linear)
Flow rate:	0.5 mL/min
Temperature:	40 °C
Detection:	fluorescence (EX @ 315 nm, EM @ 380 nm Injection vol.: 50 µL
Sample:	Figure 1: pyridylaminated oligosaccharides released from mAb-1 (mouse) Figure2: (A) pyr dylaminated oligosaccharides released from mAb-1 (mouse) (B) pyridylaminated oligosaccharides released from mAb-2 (human) (C) PA-glucose ladder (3-22 mer) (TaKaRa Bio)

LC-MS Analysis:

Column:	TSKgel Amide-80 2 µm (2.0 mm ID x 15 cm)
Mobile phase:	A: 50 mmol/L HCOONH4, pH 7.5 B: acetonitrile
Gradient:	75 %B (0-5 min), 75-50 %B (5-30 min, linear)
Flow rate:	0.3 mL/min
Temperature:	40 °C
Detection:	(a) fluorescence (EX @ 315 nm, EM @ 380 nm) (b) LC/MS, ESI positive, SIM (Shimadzu LCMS-8030)
Injection vol.:	50 µL
Sample:	2-AB labelled N-glycans released from human IgG (Ludger, cat.# CLIBN-IGG-01)

GLYCOSYLATION ANALYSIS OF ANTIBODIES ON TSKgel AMIDE-80 2 µm

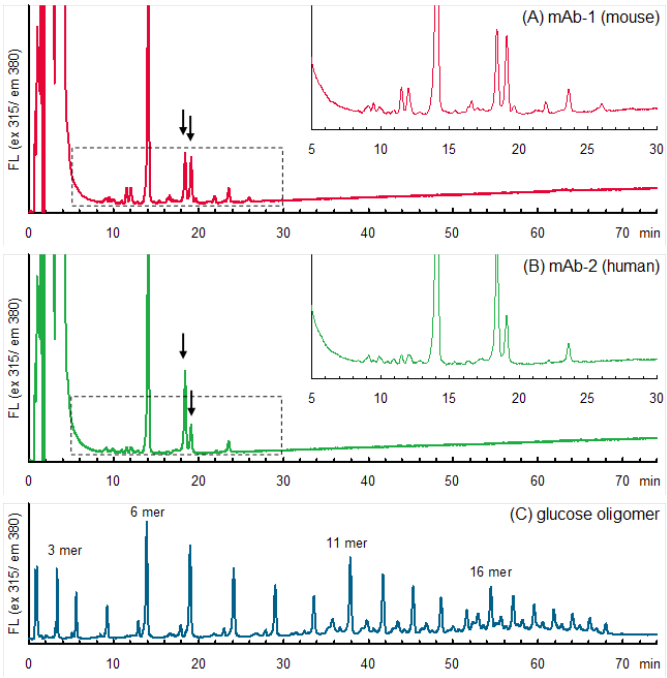

FIGURE 2

UHPLC-MS ANALYSIS OF 2-AB GLYCANS ON TSKgel AMIDE-80 2 µm

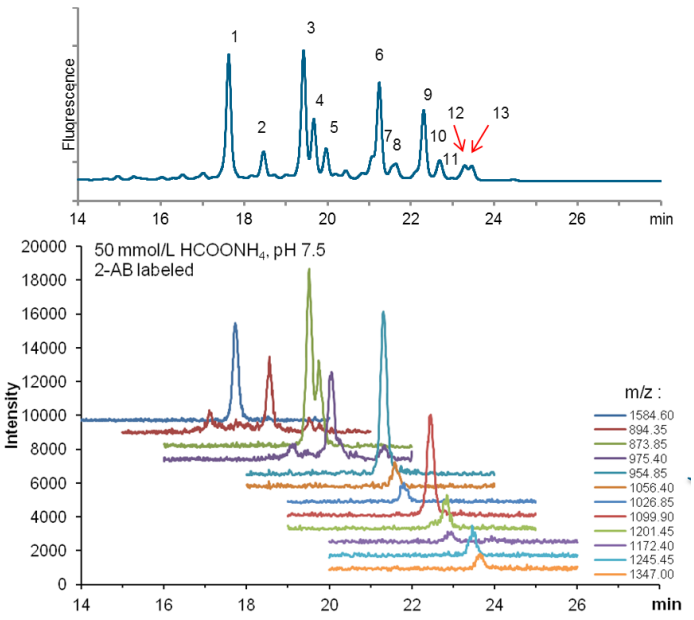

FIGURE 3

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