Chromatographic Performance of an Amine/amino-bonded Column and a Monolithic Reversed-Phase Column for the Separation of Fluorescently Labeled Glycoprotein Glycans

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Abstract
For the HPLC analysis of glycoprotein glycans, oligosaccharides released from glycoproteins are often derivatized with fluorescent tags to achieve quantitative separation. However, the influence of such fluorescent tags on the structure and retention of glycans is not completely understood. Here, to investigate the separation of labeled glycans, we prepared four types of derivatives (2-aminopyridine, 2-aminobenzoic acid, 2-aminobenzamide, and ethyl 4-aminobenzoate) of three model glycans (high-mannose oligosaccharides from ribonuclease B, complex oligosaccharides from bovine fetuin, and an isomaltooligosaccharide mixture). The glycan derivatives were separated on newly developed stationary phases, namely, an amino- and amide-bonded hydrophilic (AAH) phase and a capillary-packed monolithic ODS phase without end-capping treatment. The performance of these phases was compared with typical amide hydrophilic interaction chromatography (HILIC) and end-capped C18 packed columns. In the HILIC separation mode, the AAH column exhibited superior resolution for isomaltooligosaccharides, compared to those obtained on a conventional amide column. Nevertheless, the plate numbers were the same for glycoprotein glycans and the three linkage isomers of Man7GlcNAc2 could not be resolved, whereas they could be separated on the amide column. However, the cationic amino groups of the AAH column enhanced the resolution of sialylated complex glycans that could not be separated on the amide HILIC column. In the reversed-phase separation mode, the retention of glycan derivatives was mainly dependent on the hydrophobicity of the labeling groups. In contrast to the conventional packed ODS column, the monolithic capillary ODS column showed good resolution for neutral glycans.

Keywords: Hydrophilic interaction chromatography; Reversed-phase liquid chromatography; N-Glycans; Derivatization

1. Introduction
Glycosylation is a common posttranslational modification in protein biosynthesis. Many membrane and secretory proteins are glycosylated while passing through the endoplasmic reticulum to the Golgi apparatus [1]. In particular, more than 60% of human proteins are glycosylated.[2]. The addition of glycans induces structural and conformational changes in proteins, which may influence protein activity and protein interactions including cell signaling, recognition, immune defenses, and infections [3,4].

Protein glycosylation can be analyzed using intact glycoproteins [5-7], glycopeptides [8-10], or released glycans [11]. The analysis of glycoproteins is the most straightforward strategy for determining the distribution of glycans, and ESI- or MALDI-MS is commonly used for this purpose. Disadvantageously, intact glycoprotein analysis relies on separation according to the differences between glycoforms, which is especially difficult for proteins with many glycosylation sites with various glycan structures. The analysis of glycopeptides as a mixture of protease-digested glycoproteins is advantageous because glycosylation can be assigned to specific locations in the protein sequence. However, the sensitivity of LC-MS for glycopeptides is generally low [12,13]. Furthermore, the molecular ion polarity is dependent on the charge of the glycan. Anionic oligosaccharides tend to give better signals in the negative ion mode owing to deprotonation, whereas...
neutral oligosaccharides tend to be cationized with sodium ions in the positive ion mode. Therefore, most LC-MS methods require the enrichment of glycopeptides [14]. In contrast to MS analysis, fluorimetric LC analysis following derivatization with fluorescent tags is a simpler method for obtaining quantitative profiles of glycoprotein glycans. The reducing ends of Asn-linked glycans commonly have a trimannosyl-chitobiose core. Therefore, labeling the reducing end of N-glycans may produce the same molar response, which would allow glycan profiles to be directly determined from the HPLC chromatogram.

The fluorochemically labeled oligosaccharides from glycoproteins are often separated on hydrophilic interaction chromatography (HILIC) or reversed-phase (RP) columns. On HILIC phases, which have become popular for the separation of labeled glycans, separation is primarily governed by the hydrophilic nature of the glycan derivatives [15]. The recent development of 1.7 µm particles has reduced the analysis time and increased the separation efficiency for the linkage isomers of glycans [16]. High-performance anion exchange (HPAE) chromatography is another technique available for glycan analysis [17]. As the HPAE separation mechanism mainly depends on the negative charge of analytes, acidic glycans such as sialylated glycans are separated based on the number of acidic groups. This feature enables glycan separation based on the number of sialic acid residues.

The reversed-phase liquid chromatography (RPLC) separation of glycans is usually conducted after derivatization with fluorescent tags [18]. The hydrophobicity of the labeling groups may affect the retention and resolution of the glycan derivatives. However, the relationship between the type of labeling group and glycan structures in RP separation remains unknown. Retention in the RP mode depends on the hydrophobicity of the derivatives. Therefore, the retention of glycan derivatives is highly dependent on the properties of the labeling groups. Glycan derivatives are separated using a shallow organic phase concentration gradient, which may impair separation based on differences in glycan structure.

Here, we chose 2-aminopyridine (AP), 2-aminobenzoic acid (AA), 2-aminobenzamidine (AB), and ethyl 4-aminobenzoate (ABEE) as commonly used for labeling glycan analysis (Fig. 1), and prepared those derivatives of three glycan mixtures (high-mannose glycans, triantennary complex glycans, and isomaltooligosaccharides). The separation of these labeled glycans was performed on an amino- and amide-bonded hydrophilic (AAH) phase as a mixed-mode HILIC column and a monolithic RP column, and the resolution of these phases was compared with that of conventional amide HILIC and ODS packed columns, respectively.

2. Experimental

2.1. Reagents and materials

AA, AB, ABEE, AP, sodium cyanoborohydride, and pyridine-borane were purchased from Fujifilm Wako Pure Chemicals (Tokyo, Japan). AP was further purified by crystallization from hexane three times. A mixture of isomaltooligosaccharides (~20mers) was purchased from Seikagaku Kogyo (Tokyo, Japan). Bovine pancreas ribonuclease B and fetal calf serum fetuin were purchased from Sigma-Aldrich, Japan (Tokyo, Japan). Peptide N-glycosidase F (PNGase F) was purchased from F. Hoffmann-La Roche (Mannheim, Germany). Sephadex G15 fine and Sephadex LH-20 were obtained from GE Healthcare, Japan (Tokyo, Japan). All other solvents and reagents were of analytical grade.

2.2. PNGase F digestion of glycoproteins

An aqueous solution (210 µL) containing 0.1 mg of a glycoprotein was mixed with 2-mercaptoethanol (2.4 µL) and 10% sodium dodecyl sulfate (24 µL), and the solution was heated at 100°C for 5 min. Subsequently, 24 µL of 10% NP-40, 29 µL of 1 M sodium phosphate buffer (pH 7.5), and 2 µL of PNGase F (2 U) were added, and the solution was incubated at 37°C overnight. After heating the resultant solution at 100°C for 5 min, the proteins were precipitated by the addition of 695 µL of ethanol and then removed by centrifugation at 20,000 × g for 10 min. The supernatant was evaporated to dryness using a centrifugal evaporator.

2.3. Labeling of oligosaccharides with AA

The labeling procedure has been described previously [19]. Crude oligosaccharides corresponding to 0.1 mg of a glycoprotein or 0.1 mg of isomaltooligosaccharides dissolved in 5 µL of water were mixed with 20 µL of a reagent solution (15 mg of AA and 15 mg of NaBH₄CN in 1.5 mL of 4% sodium acetate–2% boric acid–water, 1:4:141, v/v), and the mixture was heated at 80°C for 1 h. The resultant solution was mixed with 120 µL of water, applied to a Sephadex LH-20 column (1 cm i.d., 30 cm), and eluted with methanol–water (1:1, v/v). The separation profile was monitored using an online-connected fluorescence detector.

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**Fig. 1.** Structures of labeling reagents and reductive amination reaction with saccharides.
(340 nm/410 nm). The first peak corresponding to \(\text{AA-oligosaccharides} \) was collected and evaporated to dryness. The resultant residue was redissolved in 100 µL of water, and a 5 µL portion was injected for analysis.

2.4. Labeling of oligosaccharides with \(\text{AB and ABEE} \)

The labeling procedure has been described previously [20]. Oligosaccharides (0.1 mg) were dissolved in 20 µL of AB or ABEE solution (136 mg of AB or 165 mg of ABEE in 40 µL of acetic acid and 250 µL of methanol) and 20 µL of NaBH₃CN solution (35 mg in 200 µL of methanol), and the mixture was heated at 80°C for 50 min. After evaporating to dryness, the residue was redissolved in 100 µL of water and excess reagents were removed by extraction with ethyl acetate (3 × 100 µL). The saccharide mixture was separated on a Sephadex G15 column (1 cm i.d., 30 cm) with fluorimetric monitoring at 330 nm/420 nm for AB derivatives and 305 nm/360 nm for ABEE derivatives.

2.5. Labeling of oligosaccharides with \(\text{AP} \)

The labeling procedure has been described previously [21]. Crude oligosaccharides (0.1 mg) were dissolved in 20 µL of AP solution (552 mg in 200 µL of acetic acid) and 20 µL of dimethylamine borane solution (39 mg in 200 µL of acetic acid) was added and the solution was heated at 80°C for 50 min. After evaporating to dryness, the residue was redissolved in 100 µL of water and excess reagents were removed by extraction with chloroform–phenol (1:1, v/v, 100 µL). Following evaporation for a few minutes to remove any remaining chloroform, the aqueous phase was separated on a Sephadex G15 column and eluted with 10 mM acetic acid. The first eluting fluorescent fraction (320 /390 nm) was collected and evaporated to dryness.

2.6. Instruments of HPLC on AAH, amide, and RP packed columns

The HPLC system was equipped with two Shimadzu LC-10AD pumps, a Shimadzu RF-10AXL fluorimetric detector, a GL Science C0631A column oven, a Gastor BG34 degasser, and a Rheodyne 7125 injector with a 5 µL sample loop. The excitation/emission wavelengths for fluorimetric detection of AA, AP, AB, and ABEE derivatives were 340 nm/410 nm, 320 nm/390 nm, 330 nm/420 nm, and 305 nm/360 nm, respectively.

An AAH column (Luna Omega Sugar100, 3 µm, 4.6 mm i.d., 150 mm) or an amide column (InertSustain Amide, 5 µm, 4.6 mm i.d., 250 mm) thermostated at 40°C was used for HILIC separation at a flow rate of 0.7 or 1.0 mL/min, respectively. Mobile phase A was 200 mM ammonium formate (pH 7.0) and mobile phase B was acetonitrile. A linear gradient of 20:80 (A/B) to 50:50 (A/B) for 100 min was used for the amide column, whereas 20:80 (A/B) to 70:30 (A/B) for 10 min and then 70:30 (A/B) to 50:50 (A/B) for 50 min was used for the AAH column.

A 5C18-ARII packed column (4.6 mm i.d., 250 mm) thermostated at 40°C was eluted with acetonitrile (A) and 0.1% trifluoroacetic acid (TFA) (B). Isocratic elution with 3:97 (A/B) was used for AA and AB derivatives, whereas 2:98 (A/B) was used for AP derivatives. ABEE derivatives were eluted using a linear gradient from 2:98 to 155:85 (A/B) for 40 min.

2.7. Instruments of \(\text{RP-HPLC} \) on monolithic C18-silica capillary column

A Thermo Scientific UltiMate 3000 RSLCnano system was used. A C18 monolithic capillary column (Shinwa Kako, Ultron HF-ODS(N), 0.1 mm i.d., 250 mm) was thermostated at 40°C. All derivatives were detected by absorbance at 214 nm. Mobile phases A and B were 0.1% trifluoroacetic acid (B). Isocratic elution with 2:98 (A/B) for 40 min was used. A C18 monolithic capillary column was used. A C18 monolithic capillary column was used. Acid was used. A 5C18-ARII packed column (4.6 mm i.d., 250 mm) thermostated at 40°C was eluted with acetonitrile (A) and 0.1% trifluoroacetic acid (TFA) (B). Isocratic elution with 3:97 (A/B) was used for AA and AB derivatives, whereas 2:98 (A/B) was used for AP derivatives. ABEE derivatives were eluted using a linear gradient from 2:98 to 155:85 (A/B) for 40 min.

Table 1. Structures of isomaltooligosaccharides and the glycans derived from ribonuclease B and fetuin.

| Structure | Abbrev. |
|-----------|---------|
| Glcα1(-6Manα1-)3Glc | M5 |
| Manα1-6 | M6 |
| Manα1-3 | M7a |
| Manα1-3 | M7b |
| Manα1-3 | M7c |
| Manα1-3 | M8 |
| Manα1-3 | M9 |
| Manα1-3 | Di3 |
| Manα1-3 | Di6 |
| Manα1-3 | Tr3 |
| Manα1-3 | Tr6 |
| Manα1-3 | Tet3 |
| Manα1-3 | Tetra6 |
TFA and acetonitrile, respectively, and the flow rate was 0.5 µL/min. The AP and AB derivatives were eluted using a linear gradient from 99:1 to 93:7 (A/B) for 60 min. The AA derivatives were eluted using a linear gradient from 98:2 to 95:5 (A/B) for 60 min. The ABEE derivatives were eluted using a linear gradient from 97:3 to 91:9 (A/B) for 60 min.

3. Results and discussion

3.1. Saccharide specimens

A few hundred species of glycans are present in glycoproteins, with each glycoprotein containing up to a dozen glycan species. Therefore, it is necessary to select glycoproteins with an appropriate set of glycans to evaluate the separation performance of various columns. Here, we chose an isomaltose series of oligosaccharides, glycans derived from bovine ribonuclease B, and glycans derived from calf fetal serum fetuin, the structures of which are shown in Table 1.

Isomaltooligosaccharides, which are prepared by the acid hydrolysis of starch, consist of a mixture of α1,6-linked glucose oligomers. This mixture is commonly used as a retention marker for various glycoprotein glycans. Therefore, it is important to determine the extent to which a column can separate oligosaccharides with a high degree of polymerization.

Ribonuclease B contains a series of high-mannose glycans. M5, M6, M8, and M9 have almost no structural isomers, whereas M7 has three linkage isomers (M7a–c). [22]. In the HILIC mode, high-mannose glycans are separated according to their size, and the resolution of the three isomers of M7 acts as an index for the resolving power of a column.

Fetuin contains complex glycans with di-, tri- and tetraantennary branches containing two to four sialic acids. Tetrasialylated triantennary glycans have one sialyl Lewis X-type sequence. Sialic acids have both α-2,3- and α-2,6-linkages [23]. Therefore, the resolution of fetuin glycans is an indicator of the resolving power of a column for the linkage isomers of terminal D-galactose and sialic acids.

3.2. Separation of glycan derivatives on HILIC columns

For the HILIC separation mode, we chose a conventional amide-bonded phase with 5 µm particles and an AAH phase with 3 µm particles with primary amine and amide groups. In the separation of glycan derivatives on the HILIC mode columns, the peak capacities and theoretical plate numbers showed no apparent dependence on the eluent. Therefore, we used a linear gradient of 200 mM ammonium formate (pH 7.0)–acetonitrile for both columns.

The separations of the AA, AP, AB, and ABBEE derivatives of isomaltooligosaccharides on the amide and AAH columns are shown in Fig. 2. Minor components of isomaltooligosaccharides were observed as the tailing peaks on the amide column and a series of minor peaks just before the main peaks on AAH column. On both columns, the derivatives were eluted in the order of their polymerization degree. However, the resolution was apparently higher on the AAH column, with a high number of theoretical plates (up to 130,000) and peak capacities of ~120, as calculated.
from the peak widths for the ABEE-labeled isomaltooligosaccharides. Since the particle size is 3 µm for the AAH column and 5 µm for the amide column, it is difficult to compare the column performance from the number of theoretical plates. The amide column indicated more than 20 times lower theoretical plates numbers (~5,500) and the peak capacities were ~60. Owing to this difference in the number of theoretical plates, 30mers could be detected with the AAH column, whereas up to ~20mers could be detected with the amide column. The retention order for the derivatives was AB = ABEE < AA < AP on the amide column owing to the difference in hydrophilicity of the labeling groups, but the AAH column showed decreased retention of AP derivatives. Repulsion between the cationic

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**Fig. 3.** Separation of AA, AP, AB, and ABEE derivatives of high-mannose glycans from ribonuclease B on (a) amide and (b) AAH columns.

**Fig. 4.** Separation of AA, AP, AB, and ABEE derivatives of fetuin-derived complex glycans from ribonuclease B on (a) amide and (b) AAH columns.
moiety of AP and the amino groups of the AAH column may limit the retention of AP-labeled saccharides in the HILIC mode.

Figure 3 shows the separation of the AA, AP, AB, and ABEE derivatives of high-mannose glycans from ribonuclease B on the amide and AAH columns. On both columns, all the labeled high-mannose glycans were eluted in the order of increasing molecular size. Compared with that of the isomaltooligosaccharides, the peak sharpness of the high-mannose glycans was enhanced on the amide column. Therefore, the separation efficiencies of high-mannose glycans were similar for both columns. The peak capacities and numbers of theoretical plates were ~90 and 29,000 for the amide column and ~150 and 40,000 for the AAH column.

Surprisingly, there was a considerable difference between the columns for the separation of the three M7 isomers. The M7 isomers of all the derivatives were successfully separated on the amide column but could not be separated on the AAH column. Thus, the presence of amino groups in the AAH column may influence the separation of glycan isomers in the HILIC mode.

Fetuin contains a series of sialylated glycans consisting of biantennary and triantennary glycans with two to four sialic acids. The contents of these six glycans decrease in the order of Di3 > Di6 > Tri3 ≈ Tet3 > Tri6 > Tet6, and the ratio of Di, Tri and Tet glycans has been reported to be 14:68:14 [23]. The separation of the fetuin glycans on the amide and AAH columns is shown in Fig. 4. Sialylated glycans are unstable and thus were partially hydrolyzed during the preparation process. This phenomenon complicated the separation profiles owing to the appearance of many unassignable peaks corresponding to the partial removal of sialic acids. The elution profiles on both columns were closely related. The amide column provided a narrow separation windows, with the glycans separated within 20 min. In contrast, the AAH column exhibited increased resolution with wider elution ranges than the amide column. Thus, the AAH column provides better separation performance based on differences in the number of sialic acids, likely due to interactions between the sialic acids and the amino groups of AAH, which enhances the separation of sialic acid-containing glycans.

3.3. Separations of glycan derivatives on particle-packed and capillary-packed monolithic ODS columns

As glycans are diverse and highly hydrophilic, RP separation of glycan derivatives based on their structural differences is difficult. Therefore, compared with HILIC separation, there are few examples of the RP separation of labeled glycans. Nevertheless, glycans have been separated using a relatively large column (4.6 × 250 mm) with a shallow gradient of organic solvent [24]. Here, we chose an ODS monolith without end-capping because silanol groups remaining on the column are expected aid in the effective separation of hydrophilic glycans. As fluorescent tags are more hydrophobic than glycans, the tags must be carefully chosen to avoid inhibiting the separation based on the structural differences between glycans. The RP mode has been reported to achieve separation based on the

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Fig. 5. RP separation of AA, AP, AB, and ABEE derivatives of isomaltooligosaccharides on (a) particle-packed and (b) monolithic capillary ODS columns.
microheterogeneity of glycans [25]. This feature enables the separation of complicated mixtures of glycans using two-dimensional separation with normal phase/RP or RP/ion exchange modes [26].

The RP separation of the isomaltooligosaccharide derivatives on particle-packed and monolithic capillary columns is shown in Fig. 5. An eluent of 0.1% TFA–acetonitrile was used for both columns, but the elution conditions were optimized for each derivative. The AA derivatives show moderate hydrophobicity under acidic conditions and were eluted in the order of increasing glucose units, with similar elution profiles observed for both columns. However, increased peak sharpness was observed for the monolithic column, with a resolution of up to 32mers for isomaltooligosaccharides. In contrast, the particle-packed column exhibited broad peaks and only achieved the separation of 20mers or less.

AP is highly hydrophilic and exists as a cation under the investigated separation conditions. Therefore, the AP-labeled saccharides were eluted near the void volume for both columns. Elution on the particle-packed column was completed within 35 min with broadened peaks owing to the poor separation of larger oligosaccharides under isocratic elution with 2% acetonitrile. In contrast, the capillary column indicates provided better resolution for larger oligosaccharides, and the peak of 24mers could be detected, which must be due to the presence of silanol groups.

The performance of the RP columns for the separation of AB derivatives was relatively poor. These derivatives were eluted within 20 min by isocratic elution with 3% acetonitrile on the particle-packed column and within 30 min by gradient elution with 1% to 7% acetonitrile on the capillary column. The enhanced retention on the monolith column may be due to the presence of silanol groups.

In contrast to the AA, AP, and AB derivatives, a different elution profile was observed for the ABEE derivatives, which were eluted in order of size, from large to small saccharides. In addition, the separation on the particle-packed column was superior to that on the capillary column. Presumably, hydrophobic ABEE-labeled oligosaccharides favor the larger stationary phase of the packed column.

Figure 6 shows the separation profiles of the AA-labeled isomaltooligosaccharides on the particle packed column, under isocratic elution with 3%, 4%, and 5% acetonitrile in 0.1% TFA. At 4% acetonitrile, the AA-labeled isomaltooligosaccharides were not resolved and eluted as a large fused peak. By reducing the acetonitrile concentration to 3%, elution from smaller to larger oligosaccharides was observed, whereas using 5% acetonitrile, elution occurred in the opposite order. The elution order of a series of compounds is rarely reversed by varying the concentration of the organic solvent in the eluent. This phenomenon may be due to the introduction of ABEE as a hydrophobic tag on saccharides. A comparison of the two columns suggests that the monolithic RP column is superior to the particle-packed column for the size separation of labeled isomaltooligosaccharides, with the exception of ABEE derivatives.

In comparison to the HILIC separation mode, unique retention orders were observed for the high-mannose glycans in the RP mode. For example, the AP-labeled high-mannose glycans were eluted in the order of M7a, M9, M7c, M6, M7b, M8, and M5 in RP separation [24]. The contents of M5 and M6 are abundant in ribonuclease B, with the M5 content being slightly higher than that of M6. Fig. 7 shows the RP separation of the AA, AP, AB, and ABEE derivatives of high-mannose glycans on the particle-packed and capillary columns. Although retention was dependent on the type of tag, the elution profiles of the four derivatives were similar. Compared with that on the particle-packed column, the retention of AP and AB was reduced on the monolithic capillary column. However, the elution profiles were maintained regardless of the labeling groups, and there were no significant differences between the monolithic and packed columns. The retention of fetuin-derived glycans is dependent on the number of branches, linkages, and sialic acids. AP-labeled fetuin glycans have been reported to elute in the order of Di3, Di6, Tri3, Tri6, Tetra3, and Tetra6 on an RP column [24]. Fig. 8 shows the RP separation of the AA, AP, AB, and ABEE derivatives of fetuin-derived complex glycans on the particle-packed and monolithic capillary columns. Regarding the separation of sialylated glycans, there was almost no difference between particle packed and monolithic columns. However, the resolution for Tri glycans was similar on both columns. Furthermore, the separation of ABEE-labeled glycans on the packed column

![Figure 6](https://example.com/figure6.png)

**Fig. 6.** Separation profiles of AA-labeled isomaltooligosaccharides under isocratic elution with 3%, 4%, and 5% acetonitrile.
was superior to that on the monolithic column. Although characteristic separation behavior was observed for each derivative, we could not assign all the peaks owing to the partial removal of sialic acids from glycans, which occurs because the terminal sialic acids are labile in highly sialylated glycans [27]. Therefore, the elution profiles were complicated depending on the labeling reagent. Low-resolution profiles were observed for the separation of the AP and AB derivatives. In contrast, somewhat enhanced separation was achieved for the ABEE derivatives, which indicates that the RP mode is suitable for the separation of ABEE-labeled glycans.

4. Conclusions
Here, we compared the performance of an AAH column as an amine-/amide-mixed phase and a capillary-packed column.
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monolithic column without end-capping with that of widely used columns for the separation of three types of oligosaccharides (isomaltooligosaccharides, high-mannose glycans, and complex glycans) labeled with AA, AB, ABEE, and AP. The resolution in the HILIC mode were not dependent on the type of labeling groups, but hydrophobic labeling groups seem preferable in RP mode separation. The AAH column exhibited superior resolution for isomaltooligosaccharides and sialylated complex glycans. However, for all the derivatives, the three M7 were separated on the conventional amide column but not on the AAH column. The separation of glycan derivatives on the ODS columns was strongly dependent on the type of labeling group. ABEE labeled glycans indicated somewhat increased retention and resolution due to the hydrophobicity of ABEE groups. The monolithic column showed good resolution for the labeled isomaltooligosaccharides and neutral glycoprotein glycans. The results indicate that the capillary column is an alternative for the RP separation of glycans and expands the potential for RP separation of labeled oligosaccharides using LC-MS.

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References

[1] Varki, A. Glycobiology 2017, 27, 3-49.
[2] Kalay, H.; Ambrosini, M.; van Berkel, P. H. C.; Parren, P. W. H. I.; van Kooyk, Y.; Garcia Vallejo, J. J. Anal. Biochem. 2012, 423, 153-162.
[3] Varki, A. Glycobiology 1993, 3, 97-130.
[4] Bondt, A.; Selman, M. H. J.; Deelder, A. M.; Hazes, J. M. W.; Willemsen, S. P.; Wuhrer, M. J. Proteome Res. 2013, 12, 4522-4531.
[5] Haselberg, R.; de Jong, G. J.; Somsen, G. W. Electrophoresis 2013, 34, 99-112.
[6] Jacobs, J. F. M.; Wevers, R. A.; Lefebvre, D. J.; van Scherpenzeel, M. Clin. Chim. Acta 2016, 461, 90-97.
[7] Donnely, D. P.; Rawlins, C. M.; DeHart, C. J. Nat. Methods 2019, 16, 587-594.
[8] Desaire, H. Mol. Cell Proteomics 2013, 12, 893-901.
[9] Dalpathado, D. S. I.; Desaire, H. Analyst 2008, 133, 731-738.
[10] Wuhrer, M.; Catalina, M. I.; Deelder, A. M.; Hokke, C. H. Chromatogr. B 2007, 849, 115-128.
[11] Harvey, D. J. Mass Spectrom. Rev. 2017, 36, 255-422.
[12] Tharmalingam, T.; Adamczyk, B.; Doherty, M. A.; Royle, L.; Rudd, P. M. Glycoconj. J. 2013, 30, 137-146.
[13] Pabst, M.; Altmann, F. Proteomics 2011, 11, 631-643.