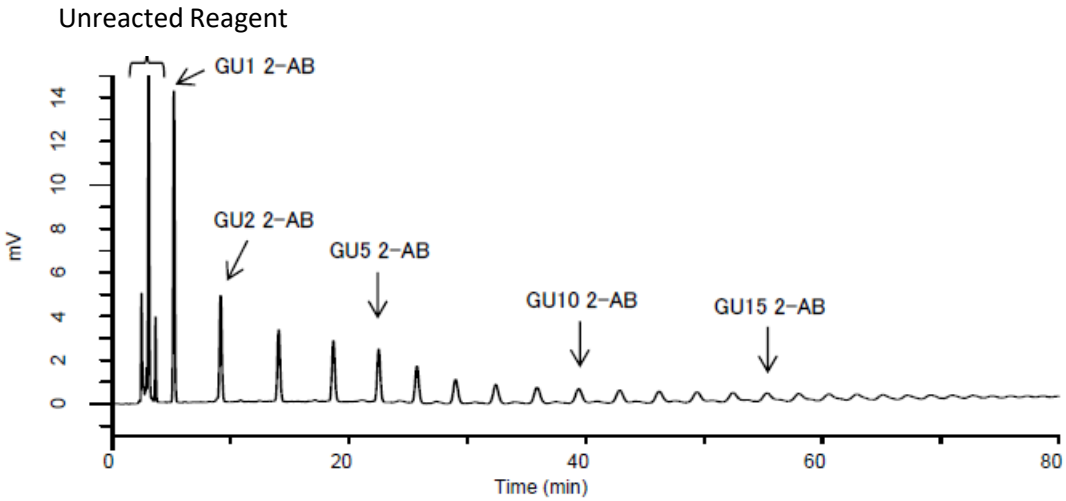
Purification of 2-AB Derivatized Glycans Using MonoSpin

When analyzing glycans, Fluorescent derivatization method is often used before measurement by HPLC. Although large amounts of unreacted derivatizing reagents remain in the solution immediately after the derivatization reaction, only unreacted reagents can be removed by performing solid-phase extractions prior to injecting them into the HPLC.

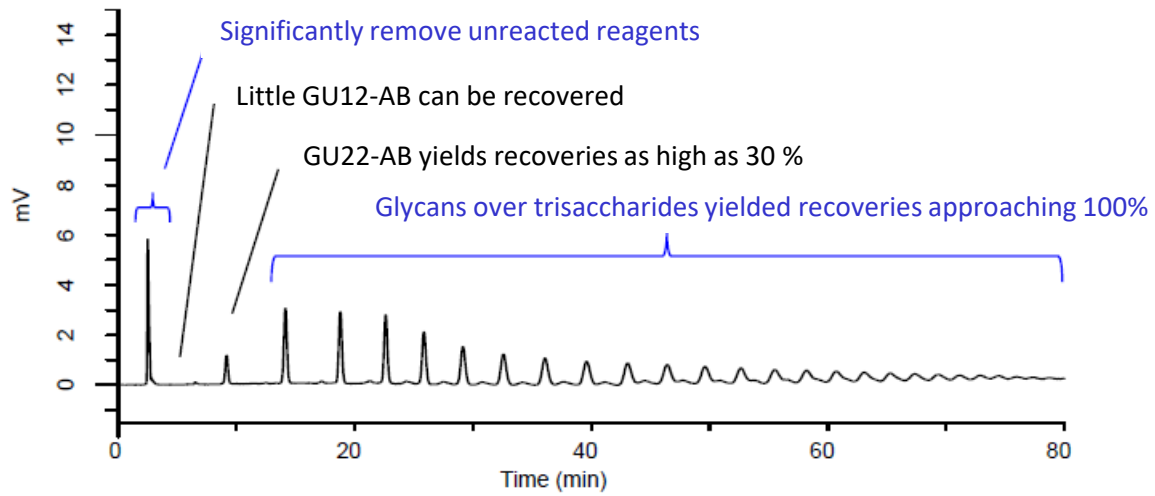
Using MonoSpin, a spin-type solid-state column, separate sugar chains derivatized with 2-aminobenzamide (2-Aminobenzamide,2-AB) from unreacted reagents. As a result of evaluating the two types of MonoSpin Amide and MonoSpin NH2, both of them recover sugar chains of more than trisaccharide.

(Y. Yui, C. Aoyama, S. Ota)

Sample Solution Measurement Without Purification



Sample Solution Measurement After Purification using MonoSpin Amide



HPLC Condition

System : GL7700 HPLC System
Column : InertSustain Amide (5 µm, 250 X4.6 mm I.D.)
Eluent : A) CH_3CN
B) 50nM $HCOONH_4$ in H_2O (pH 4.4, HCOOH)

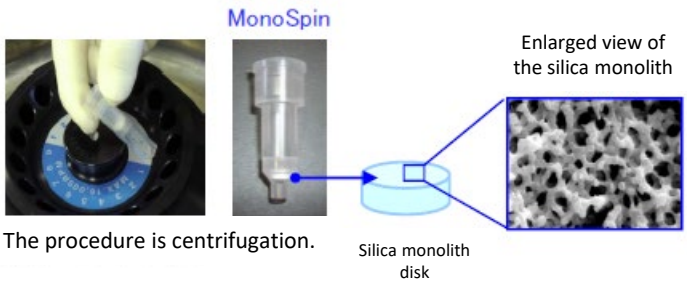
Time [min]	A (vol%)	B (vol%)
0	80	20
20	65	35
80	50	50
90	50	50

Flow Rate : 1.0 mL/min
Column Temp. : 50 ℃
Eluent : FL Ex 330 nm Em 420 nm
(FL7753 FL Detector)
Injection Volume: 25 µL
Sample : 2-AB Labeled Glucose Homopolymer Ladder

GL Sciences

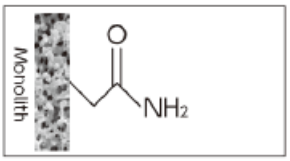
◆MonoSpin

The MonoSpin series is a spin-column using silica monoliths with uniform continuum pores. Silica monoliths with high porosity are used as carriers, so they can be passed through by centrifugal manipulation alone. Therefore, it is possible to purify and concentrate samples by a simple operation in a short time. It is also suitable when the sample volume is small because the bed volume is small and the liquid can be cut off.



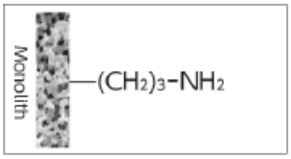
◆Purification using MonoSpin

MonoSpin Amide



A column modified amid group. Mainly used as HILIC mode and is optimal for extracting wide range of hydrophilic compounds.

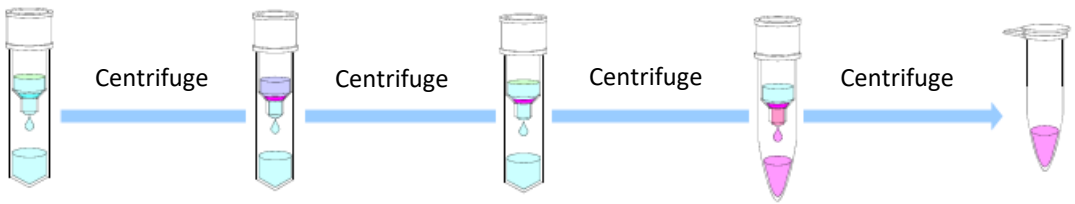
MonoSpin NH2



A column modified aminopropyl group on its surface of silica monolith. It is used as not only HILIC mode but also as weak anion-exchange columns

Purification Procedure

Centrifuge: 9,000 x g 1 min



1. Conditioning
Solution A 200 µL
↓
Solution B 200 µL

2. Adsorption
Sample Solution 200 µL

3. Rinsing
Solution B 200 µL

4. Eluent
Solution A 200 µL

Purified Sample

Solution A
Water : Acetonitril : Formic acid = 50:50:0.1 (v/v/v)

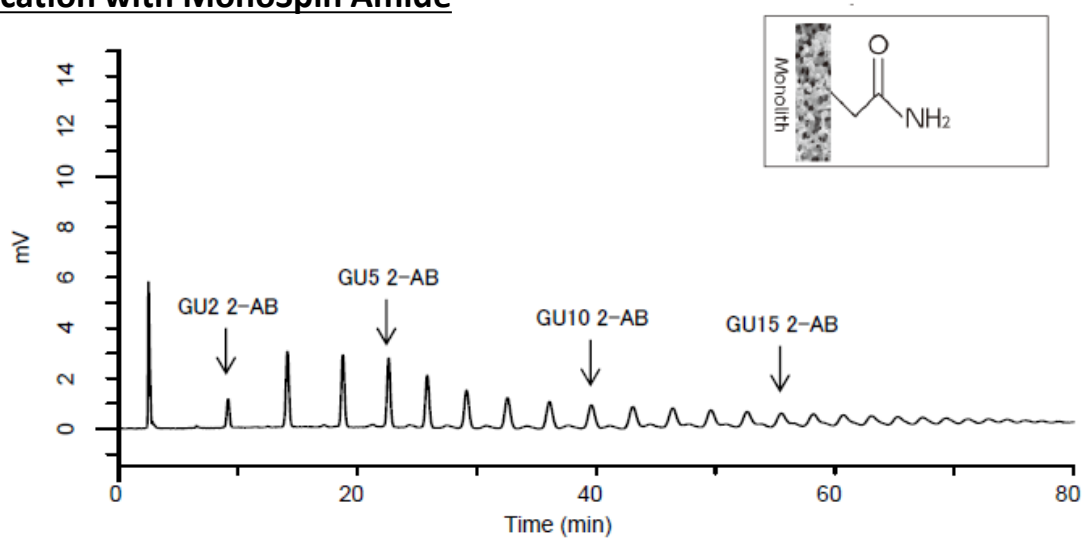
Solution B
Water : Acetonitril : Formic acid = 10:90:0.2 (v/v/v)

Sample Solution
In this study, 10µL of water and 180µL of acetonitrile were mixed with 10µL of a solution containing 2-AB sugar chains dissolved in DMSO:acetic acid=70:30 (v/v).

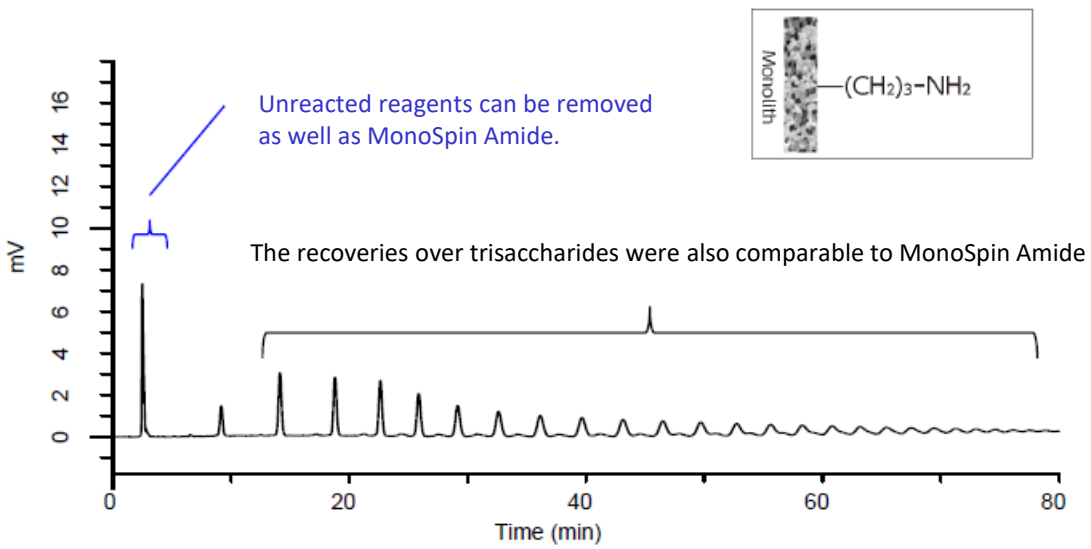
If MpnoSpin NH2 is Used Instead of MonoSpin Amide

MonoSpin NH2 works not only in the HILIC mode but also in the negative ion-exchange mode, but the effects of this study were almost equivalent to those of MonoSpin Amide.

Purification with MonoSpin Amide



Purification with MonoSpin NH2



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