

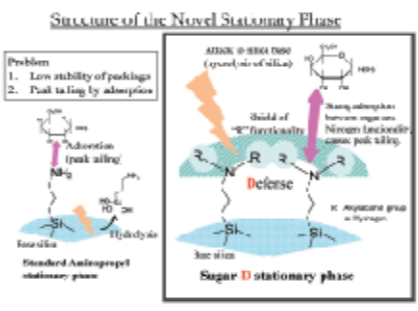
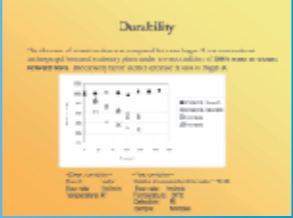
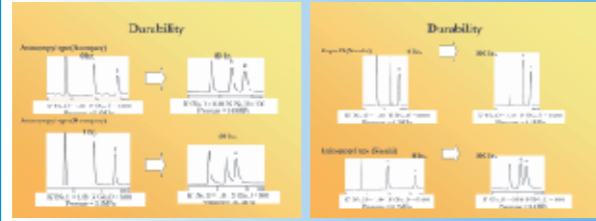
Sugar-D: A Distinctly Improved Stationary Phase for Saccharide Separation

T. SENO¹, T. ONO², T. HIROSE¹, K. KIMATA¹

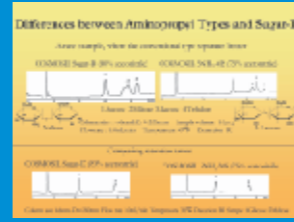
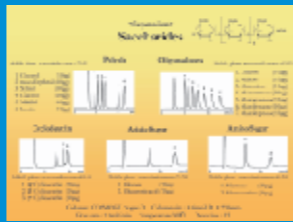
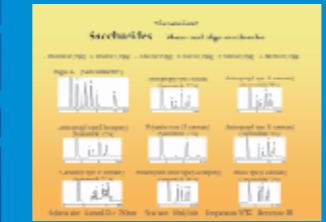
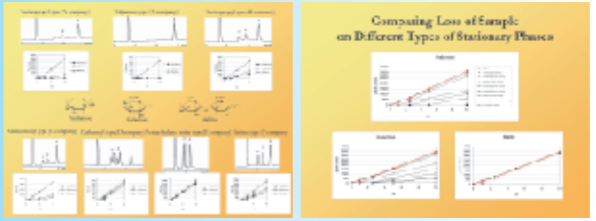
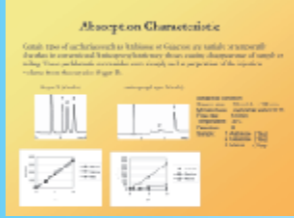
¹ Nacalai Tesque, Inc. 498 Higashitamaya-cho, Nakagyo-ku, Kyoto 604-0855 JAPAN
² Nacalai U.S.A., Inc. 6640 Lusk Blvd, Suite A 200, San Diego, CA 92121 USA

Abstract: The most challenging issues facing chromatographers in the analysis of sugars using conventional normal phase amino columns are the short column life and poor resolution. The bonded Aminopropyl easily strips from the silica surface exposing active silanols which in turn cause peak tailing, peak broadening, in some cases complete loss of sample. In response to the need for better saccharide separation, we have developed a novel type of stationary phase -Sugar-D- that specifically addresses the shortcomings of classic Aminopropyl columns. By using our new proprietary ligand bonding process to attach the active chemical group to the silica surface and creating a "defense shield" around, we have succeeded in creating a new, powerful HPLC column that has significantly longer active life, no undesirable interactions with the sample and excellent retention of saccharides and related compounds.

Durability



Absorption



Considering the capacity of **DURABILITY**, **ABSORPTION** and **SEPARATION**,
Sugar-D is one of the greatest column for saccharide separation!

Abstract

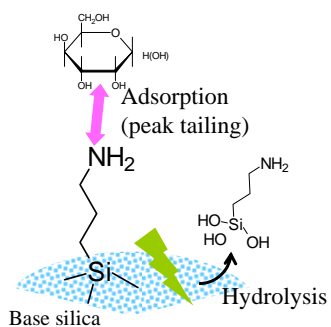
The most challenging issues facing chromatographers in the analysis of sugars using conventional normal phase amino columns are the short column life and poor resolution. The bonded Aminopropyl easily strips from the silica surface exposing active silanols which in turn cause peak tailing, peak broadening, in some cases complete loss of sample.

In response to the need for better saccharide separation, we have developed a novel type of stationary phase -Sugar-D- that specifically addresses the shortcomings of classic Aminopropyl columns. By using our new proprietary ligand bonding process to attach the active chemical group to the silica surface and creating a "defense shield" around, we have succeeded in creating a new, powerful HPLC column that has significantly longer active life, no undesirable interactions with the sample and excellent retention of saccharides and related compounds.

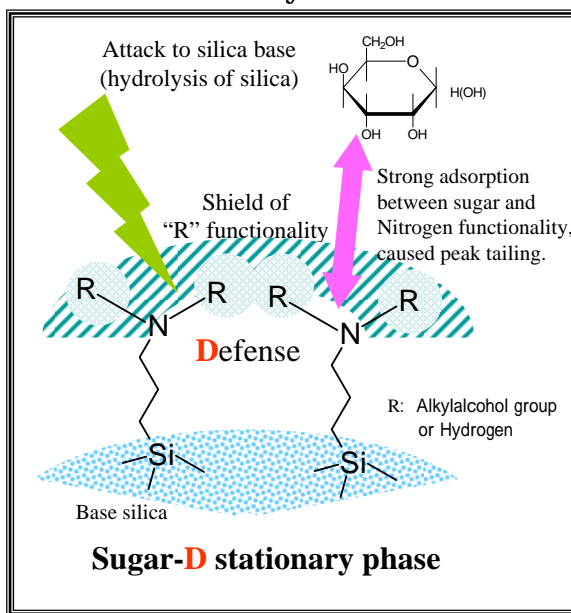
Structure of the Novel Stationary Phase

Problem

1. Low stability of packings
2. Peak tailing by adsorption



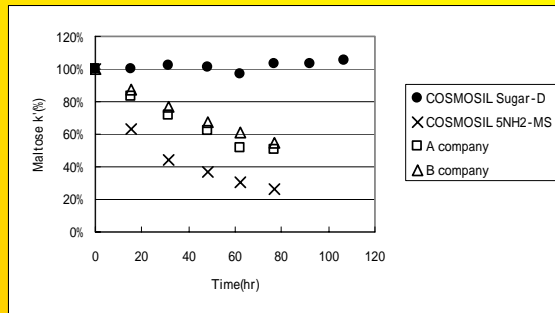
Standard Aminopropyl stationary phase



Sugar-D stationary phase

Durability

The decrease of retention time was compared between Sugar-D and conventional Aminopropyl bounded stationary phase under severe condition of **100% water as eluent between tests**. The capacity factor did not decrease in case of Sugar-D.



<Eluent condition>
 Eluent : water
 Flow rate : 1ml/min
 Temperature: RT

<Test condition>
 Mobile phase: acetonitrile:water=70:30
 Flow rate: 1ml/min
 Temperature: 30
 Detection: RI
 Sample: Maltose

Durability

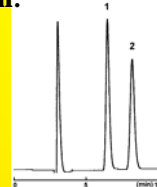
Sugar-D (Nacalai)

0 hr.



K' (No.1) = 1.08 N (No.1) = 3900
 Pressure = 4.3MPa

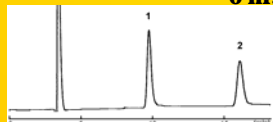
100 hr.



K' (No.1) = 1.17 N (No.1) = 4000
 Pressure = 4.5MPa

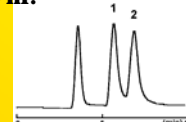
Aminopropyl type (Nacalai)

0 hr.



K' (No.1) = 1.86 N (No.1) = 5400
 Pressure = 4.2MPa

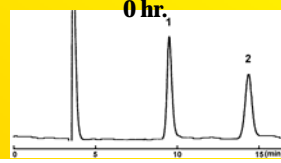
100 hr.



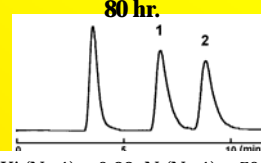
K' (No.1) = 0.58 N (No.1) = 800
 Pressure = 8.4MPa

Durability

Aminopropyl type (A company)

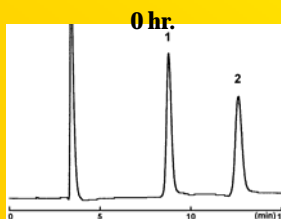


K' (No.1) = 1.60 N (No.1) = 4600
Pressure = 5.8MPa

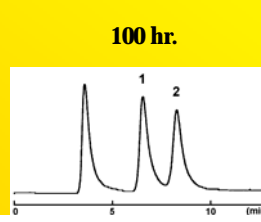


K' (No.1) = 0.88 N (No.1) = 700
Pressure = 14.8MPa

Aminopropyl type (B company)



K' (No.1) = 1.58 N (No.1) = 5000
Pressure = 3.3MPa

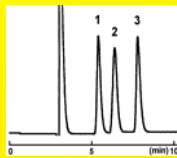


K' (No.1) = 1.81 N (No.1) = 900
Pressure = 10.1MPa

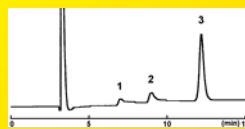
Absorption Characteristic

Certain types of saccharides such as Arabinose or Galactose are partially or temporarily absorbed in conventional Aminopropyl stationary phases causing disappearance of sample or tailing. These problematic saccharides elute sharply and in proportion of the injection volume from the superior Sugar-D.

Sugar-D (Nacalai)



Aminopropyl type (Nacalai)



Separation condition

Column size: 4.6mm I.D. x 150 mm

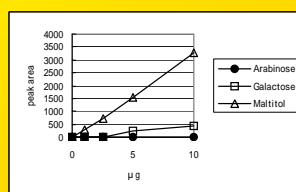
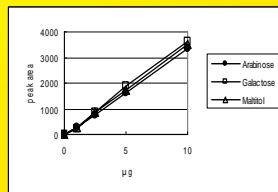
Mobile phase: acetonitrile:water=70:30

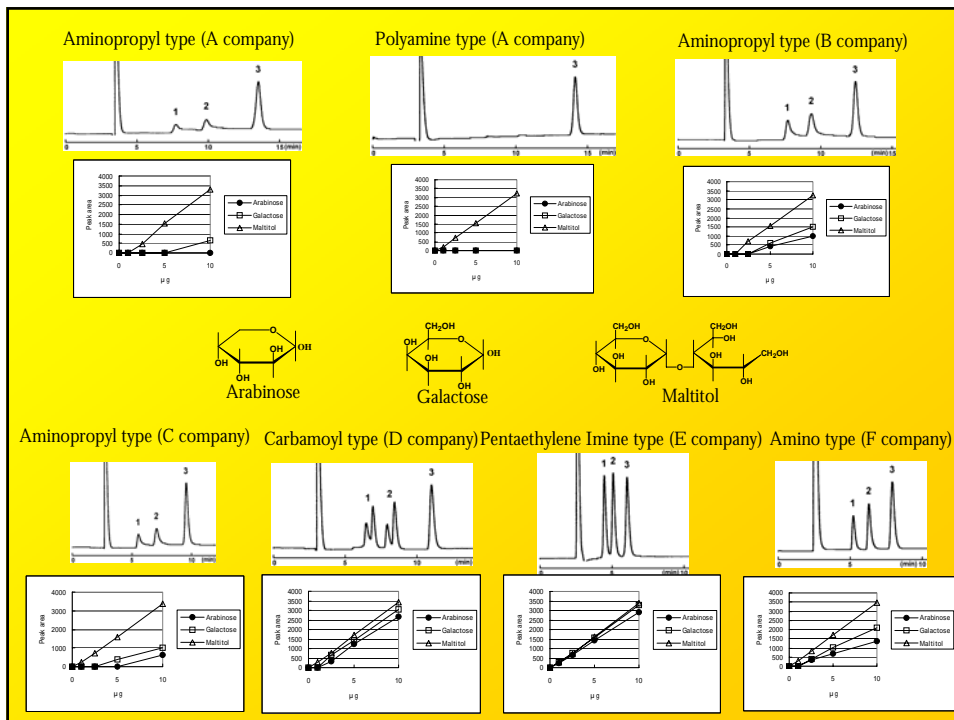
Flow rate: 1ml/min

Temperature: 30

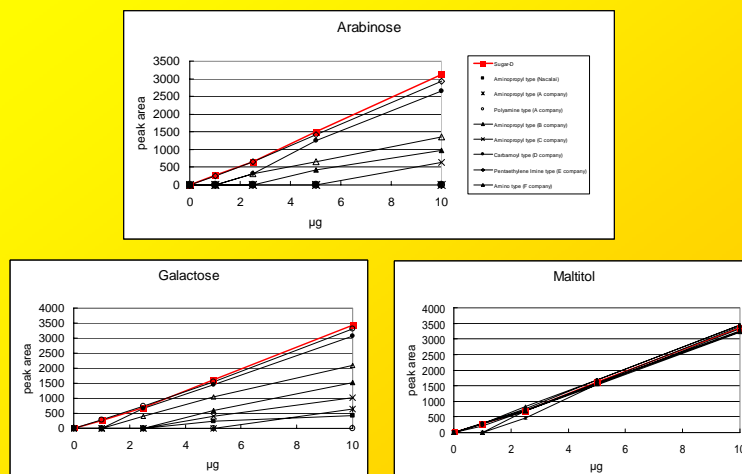
Detection: RI

Sample: 1. Arabinose (10 μ g)
2. Galactose (10 μ g)
3. Maltitol (10 μ g)





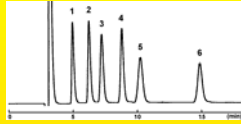
Comparing Loss of Sample on Different Types of Stationary Phases



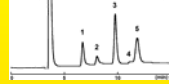
<Separation>
Saccharides Mono- and oligo-saccharides

1. Rhamnose(10µg) 2. Fructose (10µg) 3. Glucose(10µg) 4. Sucrose(10µg) 5. Maltose(10µg) 6. Raffinose(10µg)

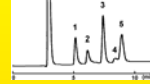
Sugar-D (Acetonitrile80%)



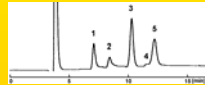
Aminopropyl type (Nacalai)
(Acetonitrile 75%)



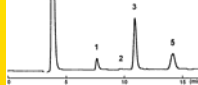
Aminopropyl type (C company)
(Acetonitrile 75%)



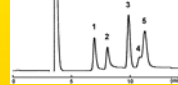
Aminopropyl type (D company)
(Acetonitrile 75%)



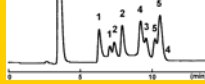
Polyamine type (D company)
(Acetonitrile 75%)



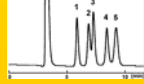
Aminopropyl type (E company)
(Acetonitrile 75%)



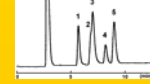
Carbamoyl type (F company)
(Acetonitrile 75%)



Pentaethylene Imine type (G company)
(Acetonitrile 80%)

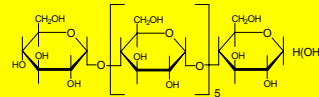


Amino type (I company)
(Acetonitrile 75%)



Column size : 4.6mmI.D. x 250mm Flow rate : 1.0ml/min Temperature 30 Detection : RI

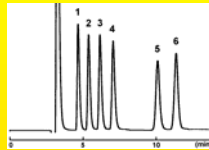
<Separation>
Saccharides



Mobile phase acetonitrile:water=75:25

Polyols

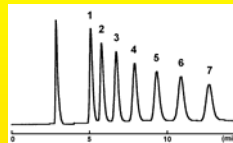
1. Glycerol (10µg)
2. meso-Erythriol (10µg)
3. Xylitol (10µg)
4. Glucitol (10µg)
5. Maltitol (10µg)
6. Inositol (10µg)



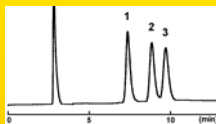
Oligomaltoses

Mobile phase acetonitrile:water=65:35

1. Glucose (10µg)
2. Maltose (10µg)
3. Maltotriose (10µg)
4. Maltotetraose (10µg)
5. Maltopentaose (10µg)
6. Maltohexaose (10µg)
7. Maltoheptaose (10µg)



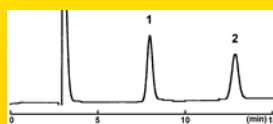
Cyclodextrin



Mobile phase acetonitrile:water=65:35

1. -Cyclodextrin (10µg)
2. -Cyclodextrin (10µg)
3. -Cyclodextrin (10µg)

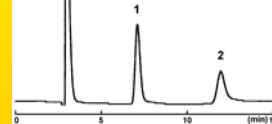
Acidic Sugar



Mobile phase acetonitrile:water=75:25

1. Glucose (10µg)
2. Glucuronic acid (10µg)

Amino Sugar



Mobile phase acetonitrile:water=65:35

1. Mannose (10µg)
2. Mannosamine (10µg)

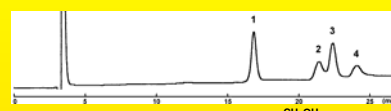
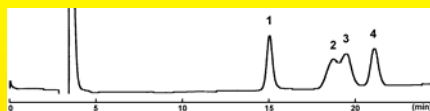
Column : COSMOSIL Sugar-D Column size : 4.6mmI.D. x 250mm
 Flow rate : 1.0ml/min Temperature 30 Detection : RI

Differences between Aminopropyl Types and Sugar-D

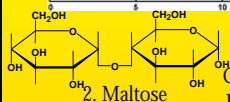
A rare example, where the conventional type separates better

COSMOSIL Sugar-D (80% acetonitrile)

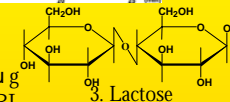
COSMOSIL 5NH₂-MS (75% acetonitrile)



1.Sucrose 2.Maltose 3.Lactose 4.Trehalose



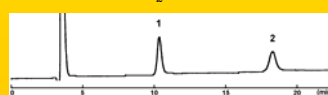
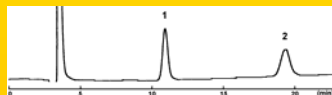
Column size : 4.6mm I.D. x 250mm Sample volume : 10 μg
Flow rate : 1.0ml/min Temperature 30 Detection : RI



Comparing retention times

COSMOSIL Sugar-D (80% acetonitrile)

COSMOSIL 5NH₂-MS (75% acetonitrile)



Column size 4.6mm I.D. x 250mm Flow rate 1.0ml/min Temperature: 30 Detection: RI Sample 1:Glucose 2:Maltose