

A Distinctly Improved Stationary Phase for Saccharide Separation

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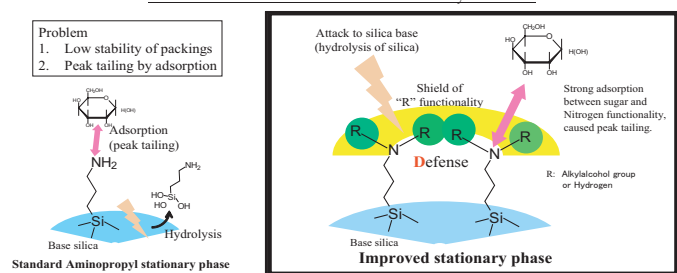
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Abstract :

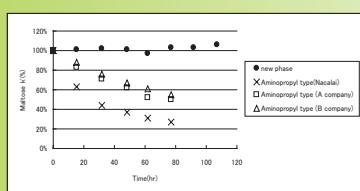
The most challenging issues facing chromatographers in the analysis of saccharides using aminopropyl bonded silica packing materials in normal phase liquid chromatography are the short column life and poor resolution. The bonded aminopropyl group easily strips from the silica surface exposing active silanols which in turn cause peak tailing, peak broadening, in some cases complete loss sample. In response to the great need for saccharide separation, we have developed the new stationary phase that specifically addresses the shortcomings of classic aminopropyl bonded packing material. By using a proprietary ligand bonding process to attach the amino group to the silica surface, we have succeeded in creating a powerful amino group bonded packing material that has significantly longer lifetime, no undesirable interactions with the sample and excellent retention of saccharides and related compounds.

Structure of the Novel Stationary Phase



Durability

The decrease of retention time was compared between conventional Aminopropyl bonded stationary phase and new stationary phase under severe condition of 100% water as eluent. More consistent capacity factors and longer column lifetimes are observed with new stationary phase.



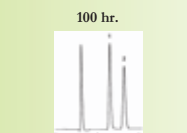
<Eluent Condition>

Eluent : water
Flow rate : 1ml/min
Temp. : RT

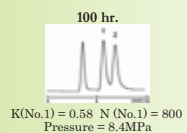
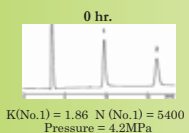
<Test Condition>

Mobile Phase : acetonitrile:water = 70:30
Flow rate : 1ml/min
Temp. : 30°C
Detection : RI
Sample : Maltose

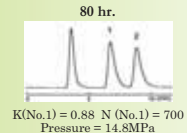
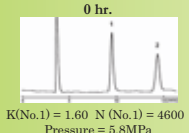
Improved type



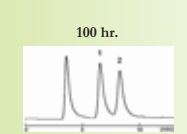
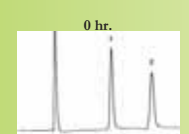
Aminopropyl type (Nacalai)



Aminopropyl type (A company)



Aminopropyl type (B company)

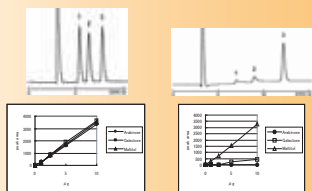


Test sample : 1. Glucose 2. Maltose

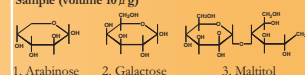
Adsorption Characteristic

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 this improved stationary phase.

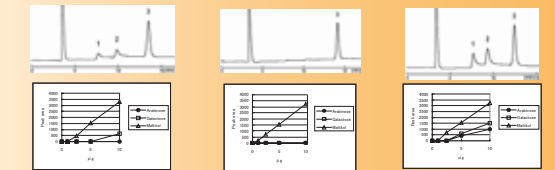
Improved type (Nacalai) Aminopropyl type (Nacalai)



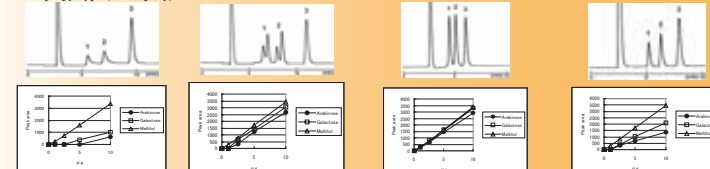
Separation Condition
Column size : 4.6mm I.D. x 250mm
Mobile phase : acetonitrile:water=70:30
Flow rate : 1ml/min
Temperature : 30°C
Detection : RI
Sample (volume 10 μg)



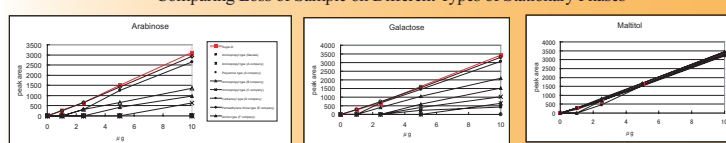
Aminopropyl type (A company) Polyamine type (A company) Aminopropyl type (B company)



Aminopropyl type (C company) Carbamoyl type (D company) Pentaethylene Imine type (E company) Amino type (F company)



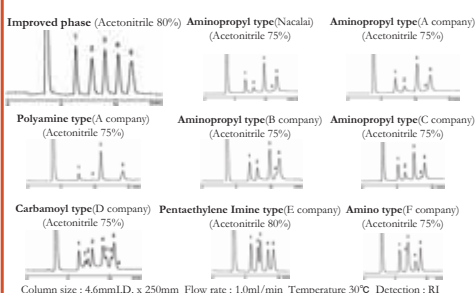
Comparing Loss of Sample on Different Types of Stationary Phases



<Separation> Saccharides

Mono- and oligo-saccharides

1. Rhamnose(10 μg) 2. Xylose(10 μg) 3. Fructose(10 μg) 4. Mannose(10 μg) 5. Glucose(10 μg)



<Separation> Saccharides

acetonitrile : water = 75 : 25

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

acetonitrile : water = 65 : 35

Cyclodextrin
1. α-Cyclodextrin (10 μg)
2. β-Cyclodextrin (10 μg)
3. γ-Cyclodextrin (10 μg)

acetonitrile : water = 65 : 35

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

acetonitrile : water = 75 : 25

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

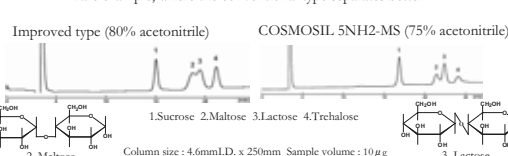
acetonitrile : water = 65 : 35

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

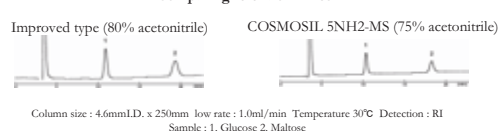
Column size : 4.6mm I.D. x 250mm Flow rate : 1.0ml/min Temperature 30°C Detection : RI

Differences between Aminopropyl Types and Improved type

A rare example, where the conventional type separates better



Comparing retention times



Conclusion :

Most chromatographers have experienced problems, such as short column life and poor resolution, separating saccharides using conventional aminopropyl bonded stationary phase. Considering the results of our research about Durability, Adsorption Characteristic, and Separation capacity, the improved stationary phase stand at the very high level. There may be the necessity of further improvement in this phase, however, it is one of the most excellent stationary phases for saccharide separation.