



**COSMOSIL**

# Analysis of Saccharides

Technical Note

The major components of food include carbohydrates, lipids, proteins, vitamins and inorganic compounds. Carbohydrates are an important energy resource. The basic structure is made of long chains of saccharides. By using different separation modes, various types of saccharides can be separated by high-performance liquid chromatography (HPLC). Recommended HPLC columns for saccharide types are listed in this table.

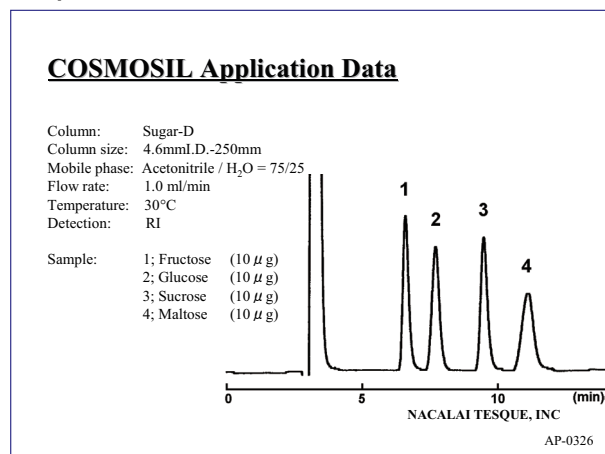


Classification 1	Classification 2	Example	Recommended HPLC columns
Monosaccharides · Disaccharides		Glucose	Sugar-D
Saccharide derivatives	With dissociating group	Phosphorylated saccharide	HILIC (Triazole)
	Without dissociating group	Sugar alcohol	Sugar-D
Oligosaccharides		Trehalose	Sugar-D
Polysaccharides		Hyaluronan	Diol-II series / CNT series
Hydrolysate of polysaccharide		Enzyme ecomposer	C18-MS-II etc. C18 series Cholester
Glycoside	Molecular weight under 2000	Catechins	C18-MS-II etc. C18 series Cholester
	Molecular weight above 2000	Glycoprotein	Column for Protein separation such as Protein-R

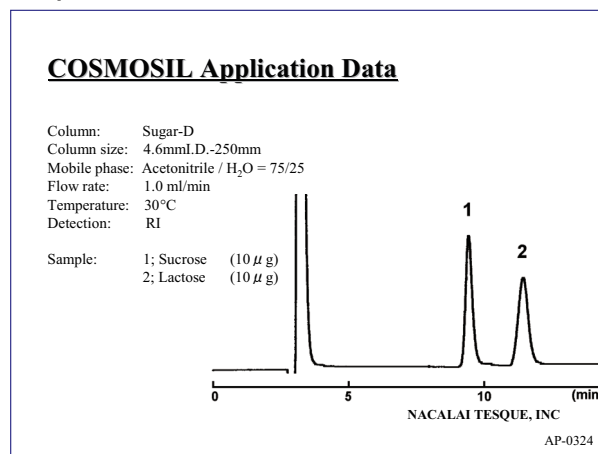
## Analysis of mono and oligosaccharides

Sugar-D is designed for separation of saccharides. It is the most suitable HPLC column for the analysis of mono- and oligosaccharides. Its quantitative capability and durability are excellent when comparing with conventional columns.

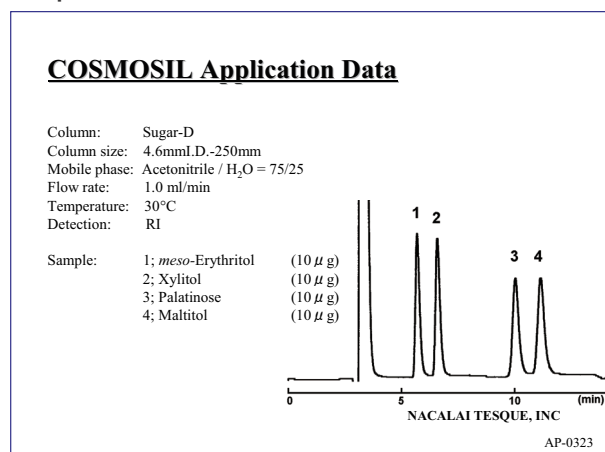
### Component of soft drink



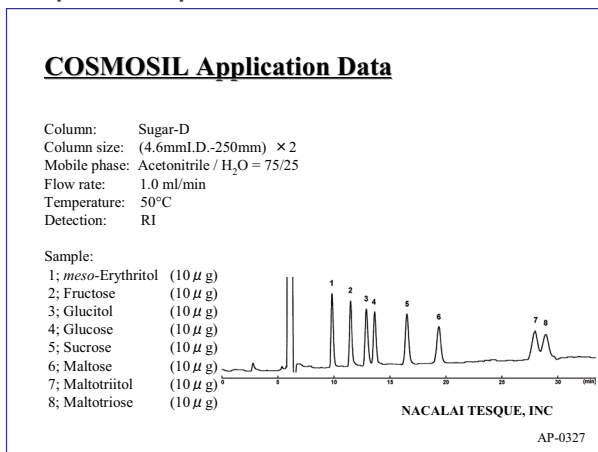
### Component of chocolate



### Component of GUM



### Component of sports drink



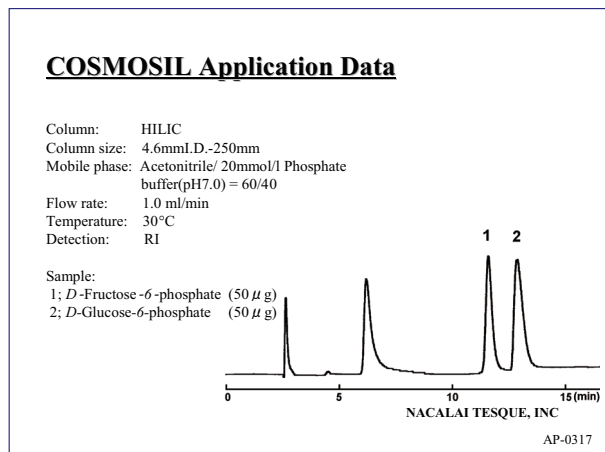
## Analysis of Saccharide Derivatives

There are two kinds of Saccharide derivatives. One has a dissociating group, such as Phosphorylated saccharide. The other one has none, such as sugar alcohol.

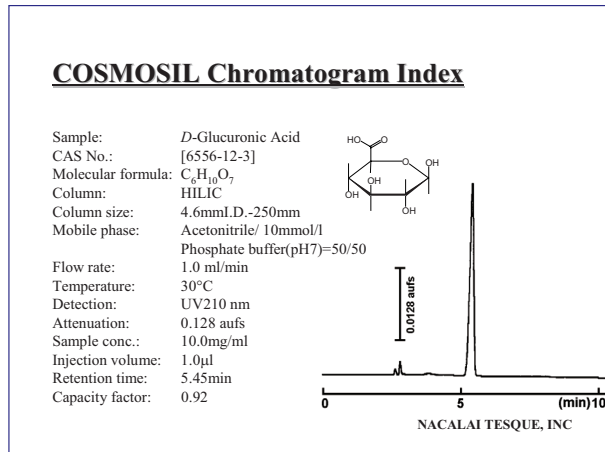
### (1) Saccharide derivatives with dissociating group (Phosphorylated saccharide etc.)

Saccharide derivatives with dissociating group can be well separated by COSMOSIL HILIC with high reproducibility. Sugar-D can be used but certain buffer may cause low reproducibility.

#### Phosphorylated saccharide



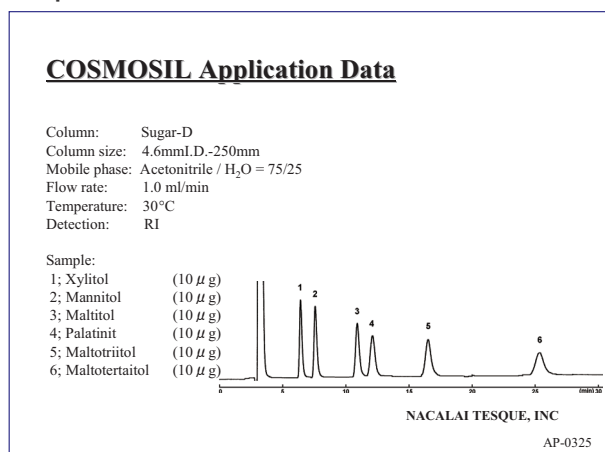
#### D-Glucuronic Acid



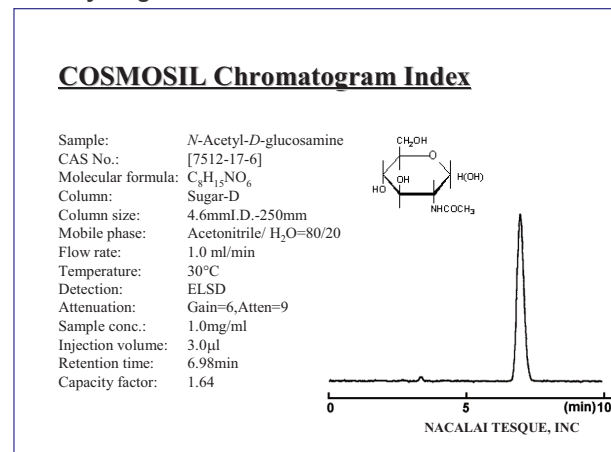
### (2) Saccharide derivatives without dissociating group (Sugar alcohol etc.)

Saccharide derivatives without dissociating group can be separated by Sugar-D.

#### Component of GUM



#### N-Acetyl-D-glucosamine



## Analysis of Oligosaccharides

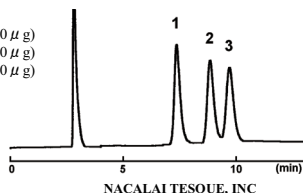
Oligosaccharides benefit human health in various ways, such as increasing beneficial intestinal bacteria...etc. Therefore, they are widely used in health food. Decasaccharide analysis can be conducted by COSMOSIL Sugar-D.

### Cyclodextrin

#### COSMOSIL Application Data

Column: Sugar-D  
 Column size: 4.6mmI.D.-250mm  
 Mobile phase: Acetonitrile / H<sub>2</sub>O = 65/35  
 Flow rate: 1.0 ml/min  
 Temperature: 30°C  
 Detection: RI

Sample: 1;  $\alpha$ -Cyclodextrin (10  $\mu$ g)  
 2;  $\beta$ -Cyclodextrin (10  $\mu$ g)  
 3;  $\gamma$ -Cyclodextrin (10  $\mu$ g)



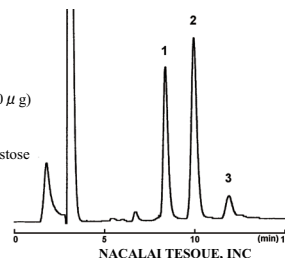
AP-0336

### Fructooligosaccharides

#### COSMOSIL Application Data

Column: Sugar-D  
 Column size: 4.6mmI.D.-250mm  
 Mobile phase: Acetonitrile / H<sub>2</sub>O = 70/30  
 Flow rate: 1.0 ml/min  
 Temperature: 30°C  
 Detection: RI

Sample: Fructooligosaccharides (50  $\mu$ g)  
 1; *J*-Kestose  
 2; Nystose  
 3; *J*-Fructofuranosyl-*D*-nystose



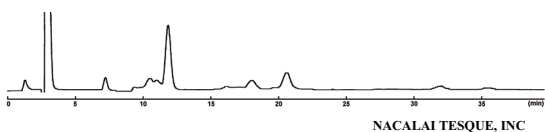
AP-0320

### Isomaltooligosaccharides

#### COSMOSIL Application Data

Column: Sugar-D  
 Column size: 4.6mmI.D.-250mm  
 Mobile phase: Acetonitrile / H<sub>2</sub>O = 75/25  
 Flow rate: 1.0 ml/min  
 Temperature: 30°C  
 Detection: RI

Sample: Isomaltooligosaccharides (50  $\mu$ g)  
 (Isomaltose, Isomaltotriose, Panose etc.)

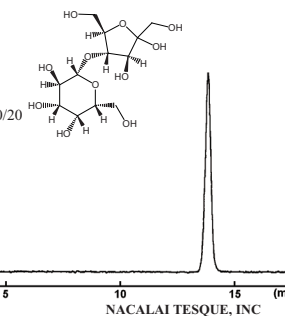


AP-0322

### Lactulose

#### COSMOSIL Chromatogram Index

Sample: Lactulose  
 CAS No.: [4618-18-2]  
 Molecular formula: C<sub>12</sub>H<sub>22</sub>O<sub>11</sub>  
 Column: Sugar-D  
 Column size: 4.6mmI.D.-250mm  
 Mobile phase: Acetonitrile / H<sub>2</sub>O=80/20  
 Flow rate: 1.0 ml/min  
 Temperature: 30°C  
 Detection: ELSD  
 Attenuation: Gain=6, Atten=9  
 Sample conc.: 1.0mg/ml  
 Injection volume: 5.0 $\mu$ l  
 Retention time: 13.96min  
 Capacity factor: 4.31



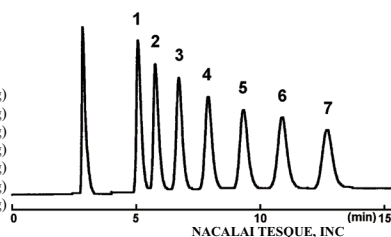
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### Maltooligosaccharide

#### COSMOSIL Application Data

Column: Sugar-D  
 Column size: 4.6mmI.D.-250mm  
 Mobile phase: Acetonitrile / H<sub>2</sub>O = 65/35  
 Flow rate: 1.0 ml/min  
 Temperature: 30°C  
 Detection: RI

Sample: 1; Glucose (10  $\mu$ g)  
 2; Maltose (10  $\mu$ g)  
 3; Maltotriose (10  $\mu$ g)  
 4; Maltotetraose (10  $\mu$ g)  
 5; Maltopentaose (10  $\mu$ g)  
 6; Malthexaose (10  $\mu$ g)  
 7; Maltoheptaose (10  $\mu$ g)



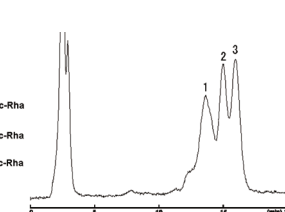
AP-0331

### Saponin

#### COSMOSIL Application Data

Column: Sugar-D  
 Column size: 4.6mmI.D.-150mm  
 Mobile phase: Acetonitrile / H<sub>2</sub>O = 75/25  
 Flow rate: 1.0 ml/min  
 Temperature: 30°C  
 Detection: UV210nm

Sample: 1; Glc-Rha-Glc-Glc-Glc-Rib-Rha-Ara-Hederagenin-Glc-Glc-Rha  
 Glc Isoferuloyl  
 2; Glc-Rha-Glc-Glc-Glc-Rib-Rha-Ara-Hederagenin-Glc-Glc-Rha  
 Glc Isoferuloyl  
 3; Glc-Rha-Glc-Glc-Glc-Rib-Rha-Ara-Hederagenin-Glc-Glc-Rha  
 Glc-Glc Isoferuloyl



〒提供 北陸大学薬学部 川田幸雄先生

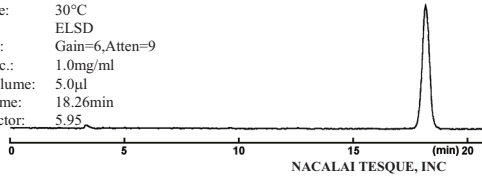
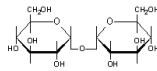
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AP-0451

## D-Trehalose

### COSMOSIL Chromatogram Index

Sample: D-(+)-Trehalose  
CAS No.: [6138-23-4]  
Molecular formula: C<sub>12</sub>H<sub>22</sub>O<sub>11</sub>  
Column: Sugar-D  
Column size: 4.6mm I.D.-250mm  
Mobile phase: Acetonitrile/ H<sub>2</sub>O=80/20  
Flow rate: 1.0 ml/min  
Temperature: 30°C  
Detection: ELSD  
Attenuation: Gain=6, Atten=9  
Sample conc.: 1.0mg/ml  
Injection volume: 5.0µl  
Retention time: 18.26min  
Capacity factor: 5.95

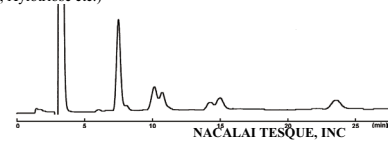


## Xylooligosaccharides

### COSMOSIL Application Data

Column: Sugar-D  
Column size: 4.6mm I.D.-250mm  
Mobile phase: Acetonitrile / H<sub>2</sub>O = 75/25  
Flow rate: 1.0 ml/min  
Temperature: 30°C  
Detection: RI

Sample: Xylooligosaccharides (50 µg)  
(Xylobiose, Xylotriose etc.)



AP-0321

## Size Exclusion Analysis of Polysaccharide

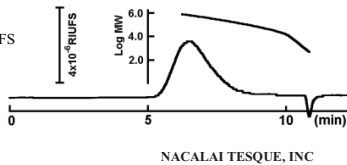
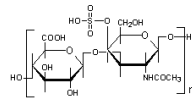
Polysaccharides such as hyaluronan and chondroitin are widely used in food, cosmetics and medicine.

COSMOSIL Diol-II is excellent for the analysis of polysaccharides in gel filtration mode. COSMOSIL CNT column is suitable for large molecular weight compounds (approximately one million) such as hyaluronan.

### Chondroitin Sulfate A

#### COSMOSIL Chromatogram Index

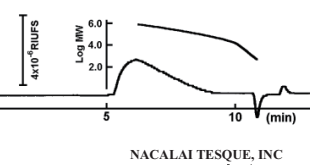
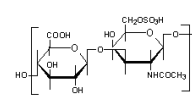
Sample: Chondroitin Sulfate A  
CAS No.: [39455-18-0]  
Column: 5Diol-300-II  
Column size: 7.5mm I.D.-300mm  
Mobile phase: 20mmol/l Phosphate buffer,  
100mmol/l Na<sub>2</sub>SO<sub>4</sub>(pH6.7)  
Flow rate: 1.0 ml/min  
Temperature: 30°C  
Detection: RI  
Attenuation: 4x10<sup>3</sup> RIU/FS  
Sample conc.: 20.0mg/ml  
Injection volume: 1.0µl  
Retention time: 6.45min



### Chondroitin Sulfate C

#### COSMOSIL Chromatogram Index

Sample: Chondroitin Sulfate C  
CAS No.: [12678-07-8]  
Column: 5Diol-300-II  
Column size: 7.5mm I.D.-300mm  
Mobile phase: 20mmol/l Phosphate buffer,  
100mmol/l Na<sub>2</sub>SO<sub>4</sub>(pH6.7)  
Flow rate: 1.0 ml/min  
Temperature: 30°C  
Detection: RI  
Attenuation: 4x10<sup>3</sup> RIU/FS  
Sample conc.: 20.0mg/ml  
Injection volume: 1.0µl  
Retention time: 6.14min

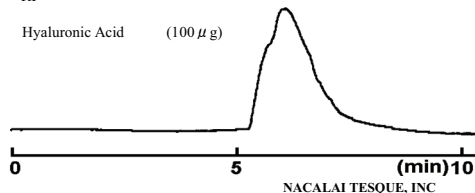


## Hyaluronic Acid

### COSMOSIL Application Data

Column: CNT-1000  
Column size: 7.5mm I.D.-300mm  
Mobile phase: 20mmol/l Phosphate buffer(pH7), 100mmol/l Na<sub>2</sub>SO<sub>4</sub>  
Flow rate: 1.0 ml/min  
Temperature: 30°C  
Detection: RI

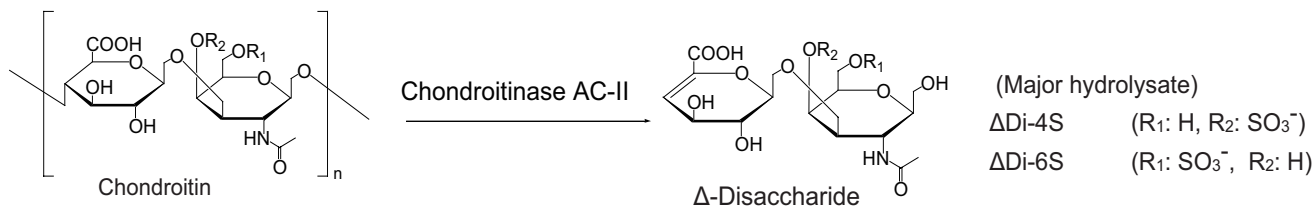
Sample: Hyaluronic Acid (100 µg)



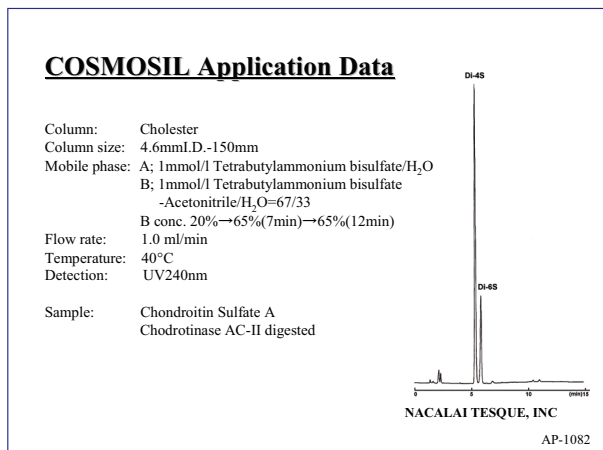
AP-1081

## (1) Chondroitin

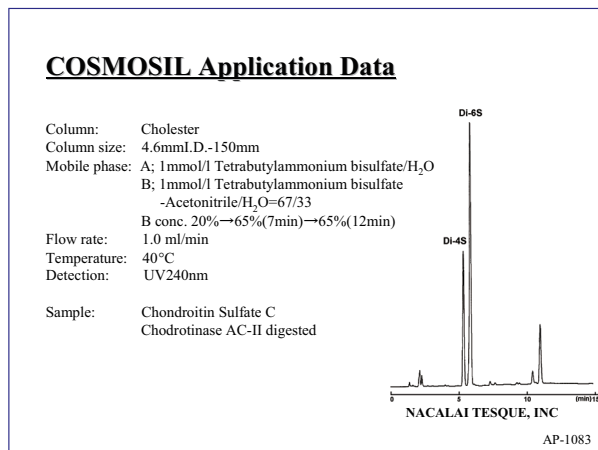
Although polysaccharides can be analyzed by gel filtration as described in 4, detection of hydrolysates is a commonly used enzymatic digestion technique.



### Enzyme digest of Chondroitin Sulfate A



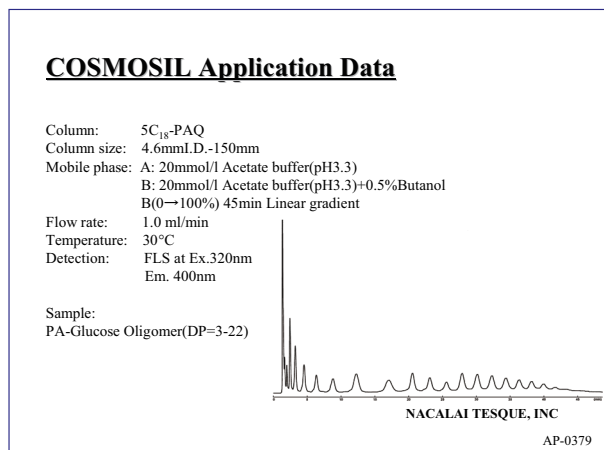
### Enzyme digest of Chondroitin Sulfate C



## (2) Derivatization of Sugar chain

Generally, Sugar chains have low ultraviolet absorption so that highly sensitive detection is difficult to achieve. In such cases, highly sensitive detection can be achieved by PA derivatization and fluorescence.

### PA-Glucose Oligomer



## (1) Molecular weight under 2000

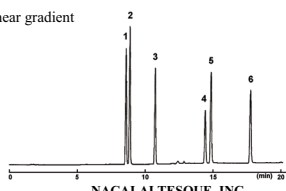
Most of glycosides, such as saponin and cardiac glycosides, are physiologically active substance. These substances are hydrophobic so they can be separated by C18 reversed phase columns.

### Isoflavone glucoside

#### COSMOSIL Application Data

Column: 5C<sub>18</sub>-MS-II  
 Column size: 4.6mmI.D.-150mm  
 Mobile phase: A: Acetonitrile/ 20mmol/l Phosphate buffer(pH2.5) = 10/90  
 B: Acetonitrile/ 20mmol/l Phosphate buffer(pH2.5) = 50/50  
 B conc. 0→100% 20min Linear gradient  
 Flow rate: 1.0 ml/min  
 Temperature: 30°C  
 Detection: UV260nm

Sample: 1; Daidzin (0.05 μg)  
 2; Glycitin (0.05 μg)  
 3; Genistin (0.05 μg)  
 4; Daidzein (0.05 μg)  
 5; Glycitein (0.075 μg)  
 6; Genistein (0.025 μg)



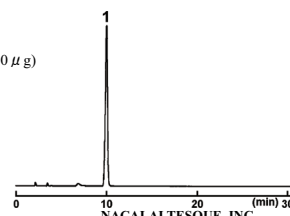
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AP-0275

### D- Amygdalin

#### COSMOSIL Application Data

Column: 5C<sub>18</sub>-MS-II  
 Column size: 4.6mmI.D.-150mm  
 Mobile phase: Methanol/ 50mmol/l NaH<sub>2</sub>PO<sub>4</sub> = 1/5  
 Flow rate: 0.8 ml/min  
 Temperature: 45°C  
 Detection: UV210nm

Sample: 1; D-(-)-Amygdalin (2.0 μg)



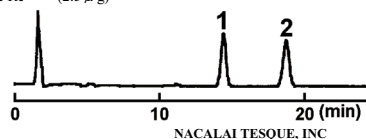
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AP-0944

### Ginsenosides

#### COSMOSIL Application Data

Column: 5C<sub>18</sub>-MS-II  
 Column size: 4.6mmI.D.-150mm  
 Mobile phase: Acetonitrile/ H<sub>2</sub>O = 30/70  
 Flow rate: 1.0 ml/min  
 Temperature: 40°C  
 Detection: UV203nm

Sample: 1; Ginsenoside Rb1 (2.5 μg)  
 2; Ginsenoside Rc (2.5 μg)



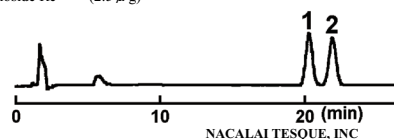
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AP-0923

### Ginsenosides

#### COSMOSIL Application Data

Column: 5C<sub>18</sub>-MS-II  
 Column size: 4.6mmI.D.-150mm  
 Mobile phase: Acetonitrile/ H<sub>2</sub>O = 20/80  
 Flow rate: 1.0 ml/min  
 Temperature: 30°C  
 Detection: UV203nm

Sample: 1; Ginsenoside Rg1 (2.5 μg)  
 2; Ginsenoside Re (2.5 μg)



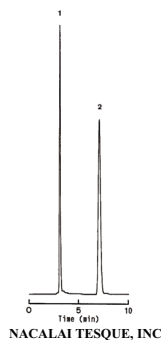
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AP-0920

### Cardiac glycosides

#### COSMOSIL Application Data

Column: 5C<sub>18</sub>-AR-II  
 Column size: 4.6mmI.D.-150mm  
 Mobile phase: Methanol/ H<sub>2</sub>O = 70/30  
 Flow rate: 1.0 ml/min  
 Temperature: 30°C  
 Detection: UV230nm, 0.32AUFS

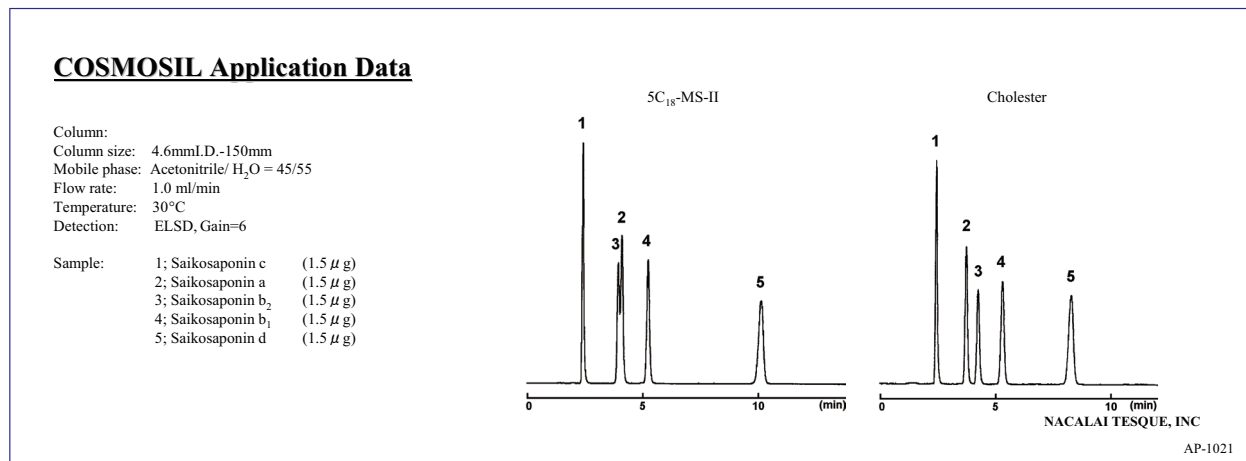
Sample: 1; Digitoxigenin (2.5 μg)  
 2; Digitoxin (5.0 μg)



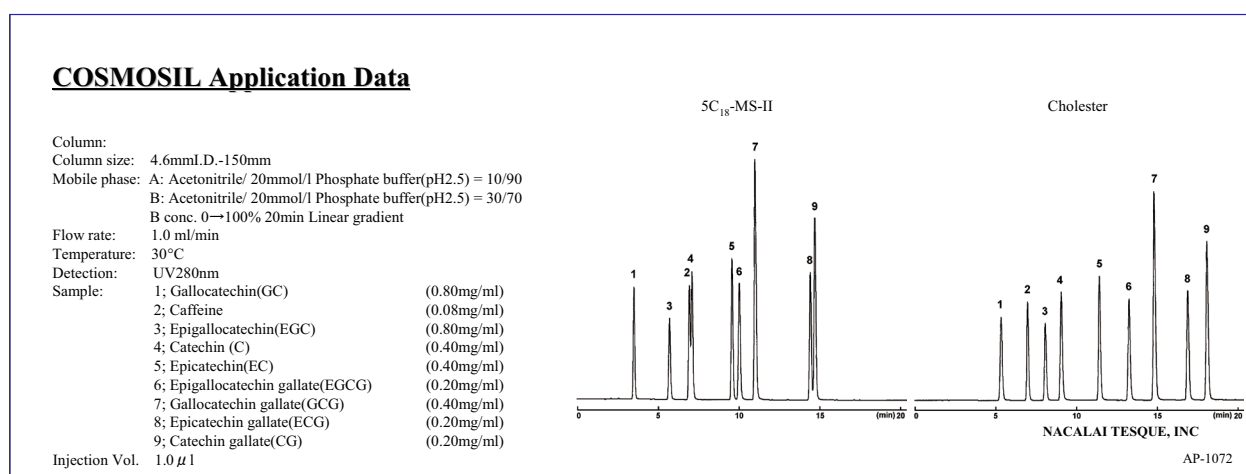
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AP-0083

Some analytes can not be separated adequately by C18, but they can be well separated using COSMOSIL Cholester column.

### Saikosaponins



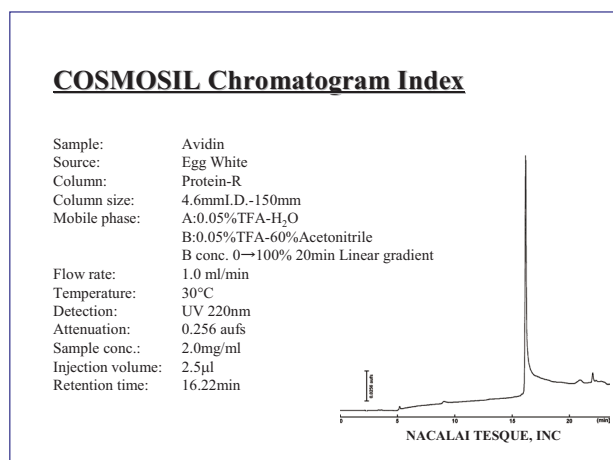
### Catechin



## (2) Molecular weight above 2000

Glycoproteins with high molecular weight can be separated by using Protein-R. Application data of avidin analysis by using Protein-R is shown below.

### Avidin

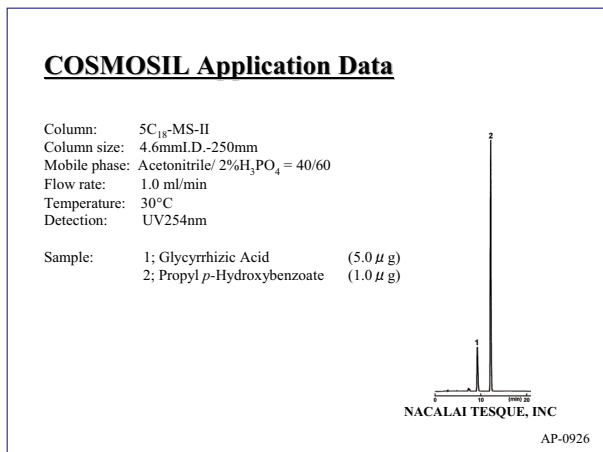


## <Reference> Analysis of food additives

Food additives are substances added to food to preserve flavor or enhance its taste and appearance. Saccharides are usually used in food additives

The ingredient standards of food additives are stated by "Specifications and Standards for Food additives". It indicated extensive amounts of analysis methods by using Gas Chromatography. However, many HPLC methods also apply to refractory substances and high temperature analysis condition required substances. Below is an analysis method for Glycyrrhizic Acid described in the "Specifications and Standards for Food additives".

### Glycyrrhizic Acid



### << Analysis method for crude and purified licorice >> (Described in "Specifications and Standards for Food additives")

**Detection:** UV254nm  
**Column:** Packed with octadecyl bonded silica gel for 5 ~ 10 μm LC.  
**Column Size:** 4 ~ 6mmI.D.- 150 ~ 300 mm  
**Temperature:** 40°C  
**Mobile Phase:** Acetic acid (1→50) / acetonitrile = 3: 2  
**Flow rate:** Adjust the retention time of glycyrrhizic acid to 10min.  
**Selection of Column:**  
 Dissolve reference standard of Glycyrrhizic Acid (5 mg) and Propyl p-Hydroxybenzoate (1 mg) in 20ml of 50% ethanol. A column which can completely separate Glycyrrhizic Acid and Propyl p-Hydroxybenzoate when inject 20 ul of the above solvent could be used.

## Price list

Product Name		Sugar-D	HILIC	Diol-120-II	Diol-300-II	C <sub>18</sub> -MS-II	Cholester	Protein-R
Separation mode		Hydrophilic		Gel filtration		Reversed phase		
2.0-150	Product No.		07054-71			38025-91	05971-11	06514-71
2.0-250	Product No.	05689-31	07489-91			05761-61	05972-01	
3.0-150	Product No.	05690-91	07871-61			34245-31	05973-91	
3.0-250	Product No.	05691-81	07872-51			34254-11	05974-81	
4.6-150	Product No.	05395-71	07056-51			38019-81	05976-61	06526-21
4.6-250	Product No.	05397-51	07057-41			38020-41	05977-51	06527-11
7.5-300	Product No.			38050-51	38053-21			
7.5-600	Product No.			38051-41	38054-11			
10-250	Product No.	05692-71	07059-21			38023-11	05979-31	06530-51
20-250	Product No.	05693-61	07060-81			38024-01	05982-71	06532-31

Other size may be available. Please enquire.

For research use only, not intended for diagnostic or drug use.