

6. Hydrophilic Interaction Columns

COSMOSIL HILIC



- Triazole-bonded stationary phase
- Unique anion-exchange mechanism (Hydrophilic interaction + Anion-exchange)

Suitable Samples

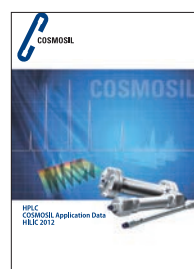
- Hydrophilic compounds that would not be retained in reversed phase chromatography
- Melamine, water-soluble vitamins, organic acids, free amino acids, peptides, nucleotides and natural compounds

Specifications

Packing Material	HILIC	
Silica Gel	High purity porous spherical silica	
Average Particle Size	2.5 μm	5 μm
Average Pore Size	130 \AA	120 \AA
Specific Surface Area	330 m^2/g	300 m^2/g
Bonded Phase	Triazole	
Main Interaction	Hydrophilic interaction, Anion-exchange	
Features	Suitable for compounds not retained by C_{18}	

COSMOSIL HILIC Application Data

COSMOSIL HILIC Application Notebook contains about 200 chromatograms for the separation of polar compounds using COSMOSIL HILIC column. It also describes how the mobile phase conditions, such as buffer pH and salt concentration influence the separation in HILIC mode.

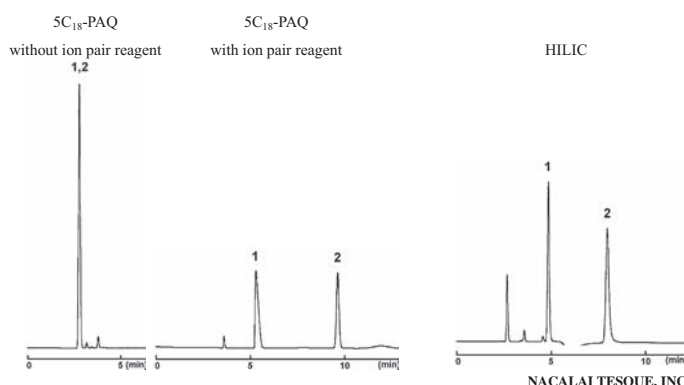


Comparison with Reversed Phase (C_{18}) Columns

The hydrophilic interaction chromatography is a variation of normal phase chromatography where a polar stationary phase is used with a mobile phase which contains a high concentration of water miscible organic solvent and a low concentration of aqueous eluent. The main retention mechanism is the partitioning of the polar analytes between the polar stationary and the non-polar mobile phase. As it is also called "aqueous normal phase", the elution order is similar to that of normal phase, and the sample elution is in the order of increasing hydrophilicity. Without using ion-pair reagent COSMOSIL HILIC retains highly polar analytes that would not be retained in reversed phase chromatography. It also shows a weak anion-exchange mechanism with the positively charged stationary phase, thus acidic compounds are strongly retained.

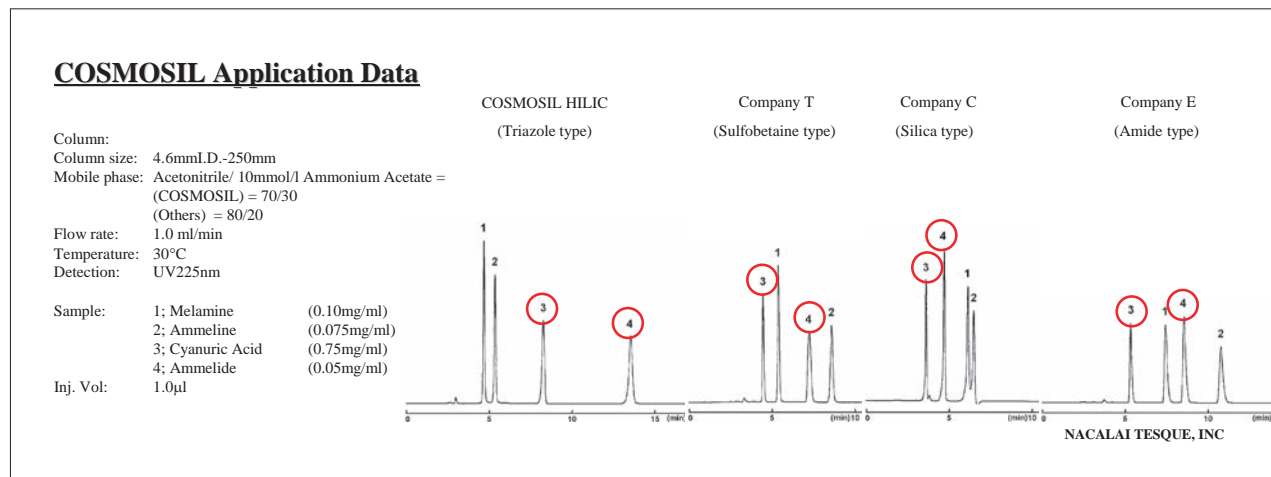
Comparison with C_{18}

Column: 4.6mm I.D.-250mm
 Column size: 4.6mm I.D.-250mm
 Mobile phase: (HILIC) Acetonitrile/10mmol/l Ammonium Acetate = 60/40
 (C_{18} -PAQ) 20mmol/l Phosphate buffer(pH2.5)
 -(Ion pair) 5mmol/l Sodium *I*-Octanesulfonate
 Flow rate: 1.0 ml/min
 Temperature: 30°C
 Detection: UV210nm
 Sample: 1; Glycine (5.0mg/ml)
 2; Glycylglycine (0.125mg/ml)
 Inj. Vol 2.0 μl



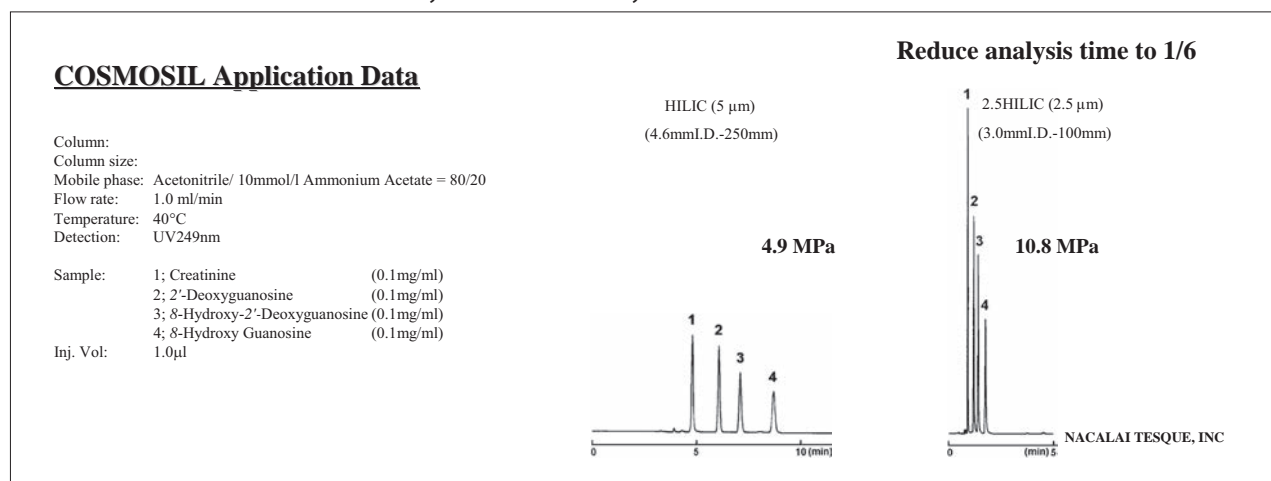
Alternative Selectivity

Anionic compounds were used to evaluate the anion-exchange capability. The only COSMOSIL HILIC showed strong selectivity of anionic compounds. The positively charged triazole stationary phase shows anion-exchange mechanism, thus acidic compounds (peak 3, 4) can be more strongly retained than with competitors' columns



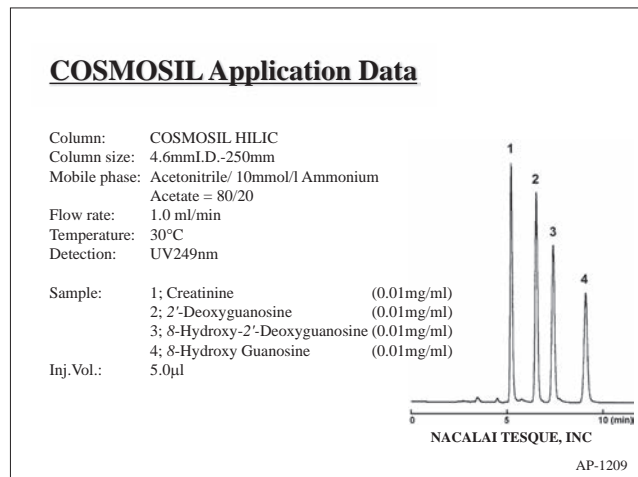
Fast LC Analysis (COSMOSIL 2.5HILIC)

COSMOSIL 2.5HILIC can be used with any conventional LC system.

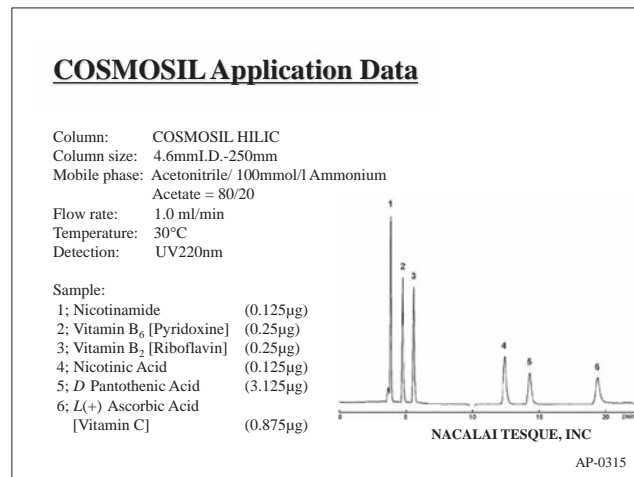


Applications

● Oxidative Stress Markers



● Water-Soluble Vitamins



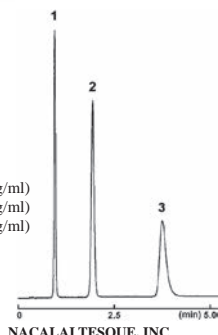
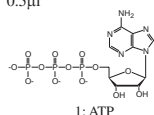
Applications

● Nucleotides

COSMOSIL Application Data

Column: COSMOSIL 2.5HILIC
Column size: 2.0mm I.D.-50mm
Mobile phase: Acetonitrile/ 20mmol/l Phosphate buffer(pH7.0) = 50/50
Flow rate: 0.4 ml/min
Temperature: 40°C
Detection: UV260nm

Sample: 1; Adenosine-5'-monophosphate (0.25mg/ml)
2; Adenosine-5'-diphosphate (0.50mg/ml)
3; Adenosine-5'-triphosphate (0.50mg/ml)
Inj. Vol.: 0.5μl



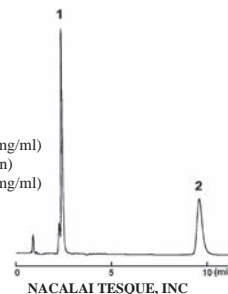
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AP-1275

● Phosphopeptide

COSMOSIL Application Data

Column: COSMOSIL 2.5HILIC
Column size: 2.0mm I.D.-150mm
Mobile phase: Acetonitrile/ 20mmol/l Phosphate buffer(pH7.0) = 70/30
Flow rate: 0.4 ml/min
Temperature: 40°C
Detection: UV220nm

Sample: 1; Angiotensin II(Human) (0.5mg/ml)
2; [Tyr(PO₃H₂)₄]-Angiotensin II(Human) (0.5mg/ml)
Inj. Vol.: 2.0μl



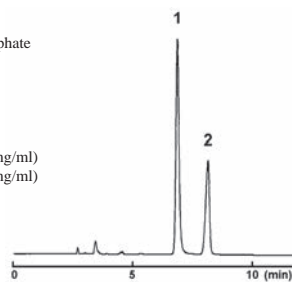
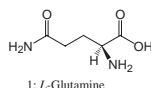
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● Glutamine, Glutamic acid

COSMOSIL Application Data

Column: COSMOSIL HILIC
Column size: 4.6mm I.D.-250mm
Mobile phase: Acetonitrile/ 10mmol/l Phosphate buffer(pH7.0) = 70/30
Flow rate: 1.0 ml/min
Temperature: 30°C
Detection: UV210nm

Sample: 1; L-Glutamine (10mg/ml)
2; L-Glutamic Acid (10mg/ml)
Inj. Vol.: 1.0μl



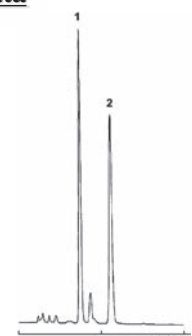
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● Sweeteners

COSMOSIL Application Data

Column: COSMOSIL 2.5HILIC
Column size: 2.0mm I.D.-100mm
Mobile phase: Acetonitrile/ H₂O = 80/20
Flow rate: 0.4 ml/min
Temperature: 40°C
Detection: UV210nm

Sample: 1; Stevioside
2; Rebaudioside A



NACALAI TESQUE, INC
AP-1281

Ordering Information

● COSMOSIL HILIC Analytical / Preparative Columns (Particle Size: 5 μm)

Packed Column

I.D. x Length (mm)	Product Number
1.0×150	07869-11
1.0×250	07870-71
2.0× 30	08568-21
2.0× 50	07052-91
2.0×100	08569-11
2.0×150	07054-71
2.0×250	07489-91
3.0×150	07871-61
3.0×250	07872-51

I.D. x Length (mm)	Product Number
4.6×150*	07056-51
4.6×150 3 Lots Set*	09385-23
4.6×250*	07057-41
10×150	05564-51
10×250	07059-21
20×250	07060-81
28×250	07875-21

* Columns for validation

Guard Column / Guard Cartridge

I.D. x Length (mm)	Product Number
2.0×10	07569-41
4.6×10	07055-61
4.6×10 Cartridge†	19184-14
10×20	07058-31
20×20	07854-91
20×50	07873-41
28×50	07874-31

† 2 cartridges included. Guard cartridge holder required; refer to page 78.

● COSMOSIL 2.5HILIC Fast LC columns (Particle Size: 2.5 μm)

Packed Column

I.D. x Length (mm)	Product Number
2.0× 50	11766-21
2.0× 75	11768-01
2.0×100	11769-91

I.D. x Length (mm)	Product Number
2.0×150	11770-51
3.0× 50	11771-41
3.0× 75	11772-31

I.D. x Length (mm)	Product Number
3.0×100	11773-21
3.0×150	11774-11