Shodex Technical Training

NEW Columns

2017

SHOWA DENKO EUROPE

MZ ANALYSENTECHNIK

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Shodex™

HPLC Columns

New

New Products

HILIC Columns

- HILICpak VN Series — Polymer-based
- HILICpak VC Series — Polymer-based

SEC Columns

- PROTEIN LW-803 — Silica-based
- OHpak LB-800 Series — Polymer-based
- GPC HK-404L — Polymer-based

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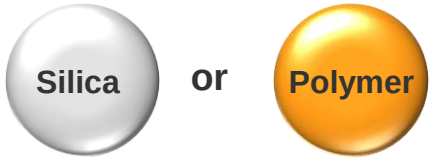
Background - Materials

Housing Materials

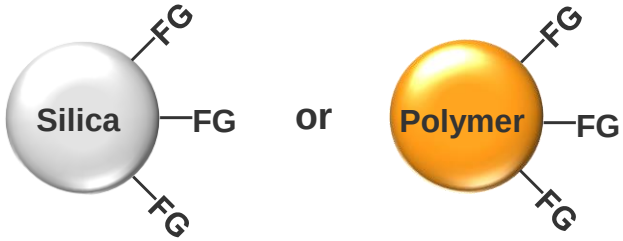
- **Steel** (SUS)
- **PEEK** (**P**oly**e**ther **E**ther **K**eton)

Base Materials (Stationary Phase)

unmodified



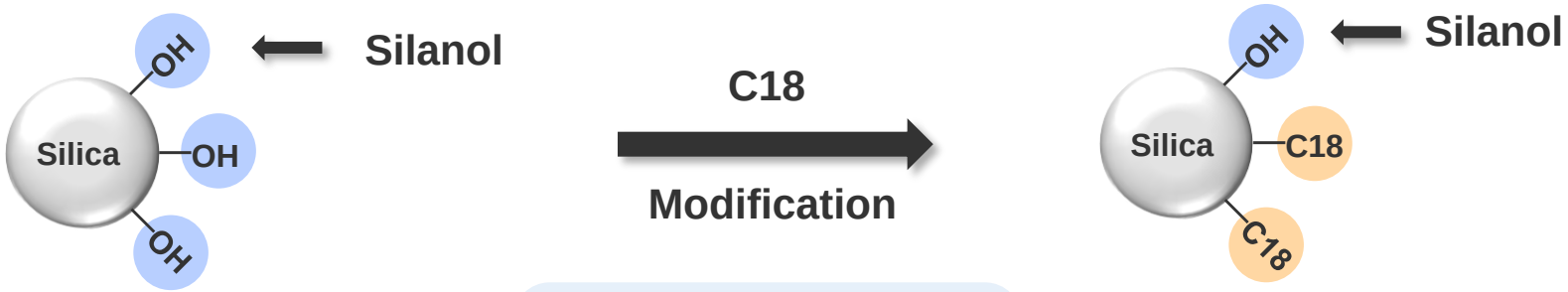
modified



FG (**F**unctional **G**roup):
e.g. -OH, -NH_2, -CN, -SO_3^-, -NR_3^+, -C18, -C8, etc.

Background – Comparison Silica vs. Polymer

Base Materials (Stationary Phase)

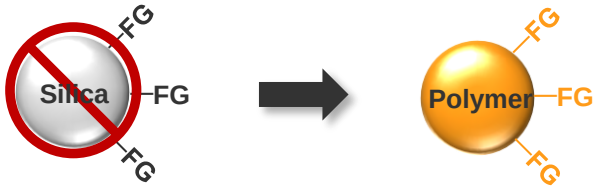


- Monomeric alkylsilane
- Polymeric alkylsilane
- Endcapping

→

- Unstable under basic conditions (pH > 7)
- Basic compounds (e.g. amines) are absorbed
- Highly polar compounds are absorbed

Background – Comparison Silica vs. Polymer



Polymer-based

Advantages

- Extended **pH range** (2-13)
- 2-3 times **longer lifetime** (chemical resistance)
- Almost **no bleeding** (MS, LS & CAD detection)
- **Low salt** concentrations (MS detection)
- Higher **resolution**
- Better **retention** of highly polar compounds
- Simple analysis of **basic compounds**

FG = Functional Group

Polymer = Polyvinyl alchohl, Polymethacrylate, Polyhdroxymethacrylate or Polystyrene divinylbenzene copolymer

2. HILIC

Product: **Asahipak NH2P HILICpak VG-50** & **VT-50**

Application: small polar molecules, saccharides

Fields: pharma, food, beverages, life science

Technical Notebook No. 2 HILIC

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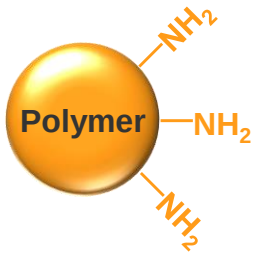
HILIC Chromatography - Background

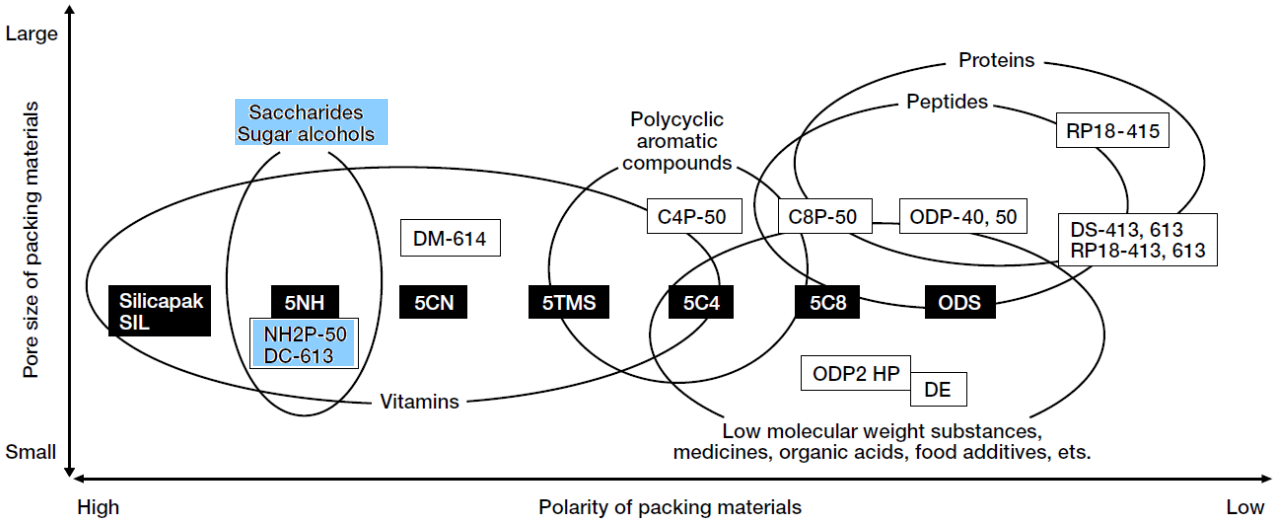
HILIC: **H**ydrophilic **I**nteraction **Li**quid **C**hromatography

- Suitable for polar analytes (like in **IEC** chromatography)
- Polar stationary phase (like in **NP** chromatography)
- Polar mobile phase, e.g. water/ACN mixtures
  (like in **RP** chromatography, but with higher ACN content (> 95%))

Functional groups:

- **Amino (-NH_2)**
- Diol
- Zwitterionic
- Ionic FG
- Pure silica

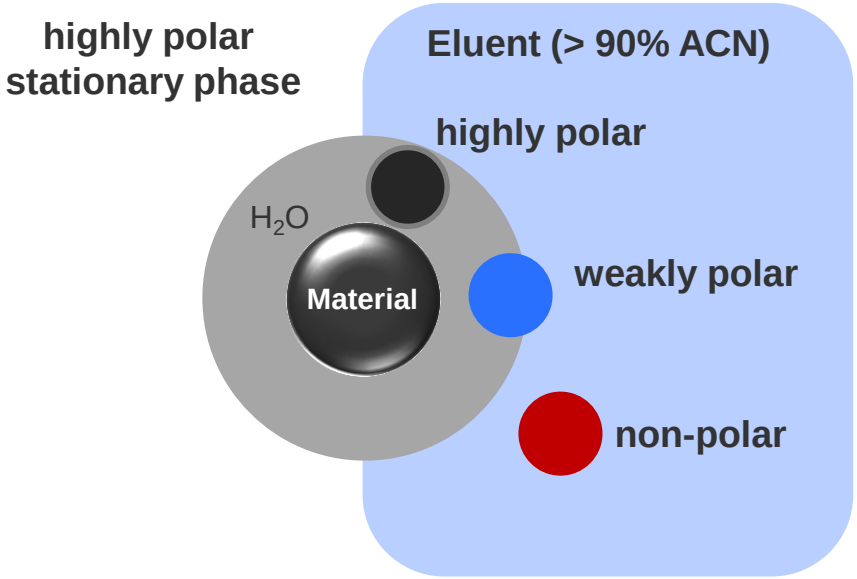




HILIC Chromatography - Background

When **HILIC** should be used?

- Analysis of highly polar compounds, saccharides, etc.
- LogP < 0



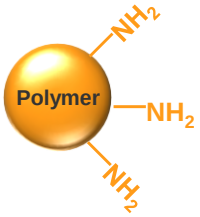
Interactions:

- Hydrophilic partitioning
- Electrostatic interaction
- Hydrogen bonding

HILIC Chromatography – Stationary Phases

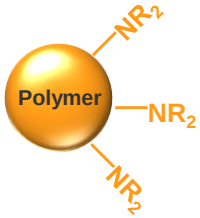
Asahipak NH2P Serie:

Polyvinyl alcohol



HILICpak VG Serie:

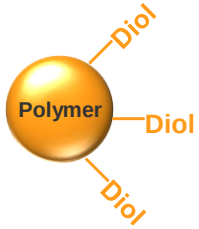
Polyvinyl alcohol



New

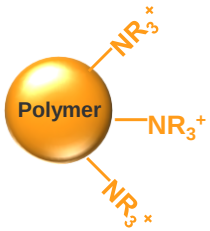
HILICpak VN Serie:

Polyvinyl alcohol



HILICpak VT Serie:

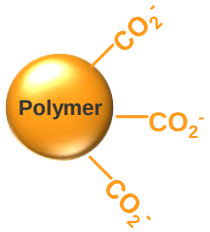
Polyvinyl alcohol



New

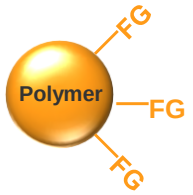
HILICpak VC Serie:

Polyvinyl alcohol



New

HILICpak VC and VN Series



FG: -COO^-, Diol

VC-50
(Housing Material: PEEK)

Product Code	Product Name	Plate Number (TP/column)	Functional Group	Particle Size (µm)	Pore Size (Å)	Column Size (mm) I.D.x Length	Shipping Solvent
F7630700	**HILICpak VC-50 2D**	≥ 3,500	Carboxyl	5	100	**2.0 x 150**	H_2O
F6711600	**HILICpak VC-50G 2A**	Guard column	Carboxyl	5	-	**2.0 x 10**	H_2O

Base Material : Polyvinyl alcohol

VN-50
(Housing Material: PEEK)

Product Code	Product Name	Plate Number (TP/column)	Functional Group	Particle Size (µm)	Pore Size (Å)	Column Size (mm) I.D.x Length	Shipping Solvent
F7630500	**HILICpak VN-50 4D**	≥ 10,000	Diol	5	100	**4.6 x 150**	H_2O/CH_3CN=25/75
F6711400	**HILICpak VN-50G 4A**	-	Diol	5	-	**4.6 x 10**	H_2O/CH_3CN=25/75
F7630600	**HILICpak VN-50 2D**	≥ 3,500	Diol	5	100	**2.0 x 150**	H_2O/CH_3CN=25/75
F6711500	**HILICpak VN-50G 2A**	-	Diol	5	-	**2.0 x 10**	H_2O/CH_3CN=25/75

Base Material : Polyvinyl alcohol

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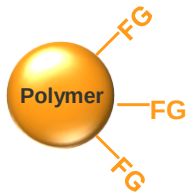
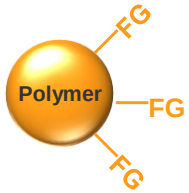
HILIC Chromatraphy - Properties of polymeric HILIC Columns



FG: -NH_2, -NR_2, NR_3^+, -COO^-, Diol

Polymeric HILIC Columns
• pH 2 - 13 (depends on column series)
• Regeneration with NaOH Solution
• suitable for ELSD, CAD and LC/MS
• USP L82 (Asahipak NH2P)
• combination of HILIC and IEC possible
• max. pressure up to 15 MPa (150 bar)
• Gradient is possible

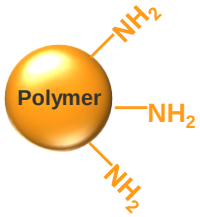
HILIC Chromatraphy - Properties of polymeric HILIC Columns

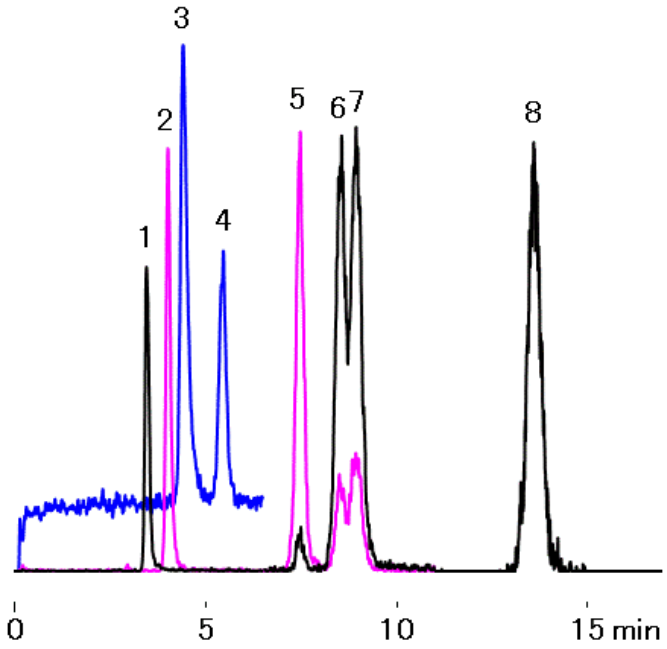


FG: $-NH_2$, $-NR_2$, NR_3^+, $-COO^-$, Diol

Polymeric HILIC Columns	Application Overview
Asahipak NH2P Series (FG: polyamine, $-NH_2$)	**Saccharide analysis** by HILIC
HILICpak VG Series (FG: $-NR_2$; R: hydrophilic rest)	**Saccharide analysis** (improved recovery of reducing sugars)
HILICpak VT Series (FG: $-NR_3^+$; R: hydrophilic rest)	high-sensitivity analysis of **anionic compounds**
HILICpak VC Series New (FG: $-COO^-$)	high-sensitvity analysis of **cationic compounds** (e.g. choline, acetylcholine, amino acids and amino-based neurotransmitters)
HILICpak VN Series New (FG: Diol)	high-sensitivity analysis of **oligosaccharides** (e.g. hydrolysis fragments of cellulose)

Asahipak NH2P Series - Applications



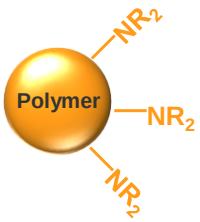


Sample : 50 ng/mL each, 5 µL

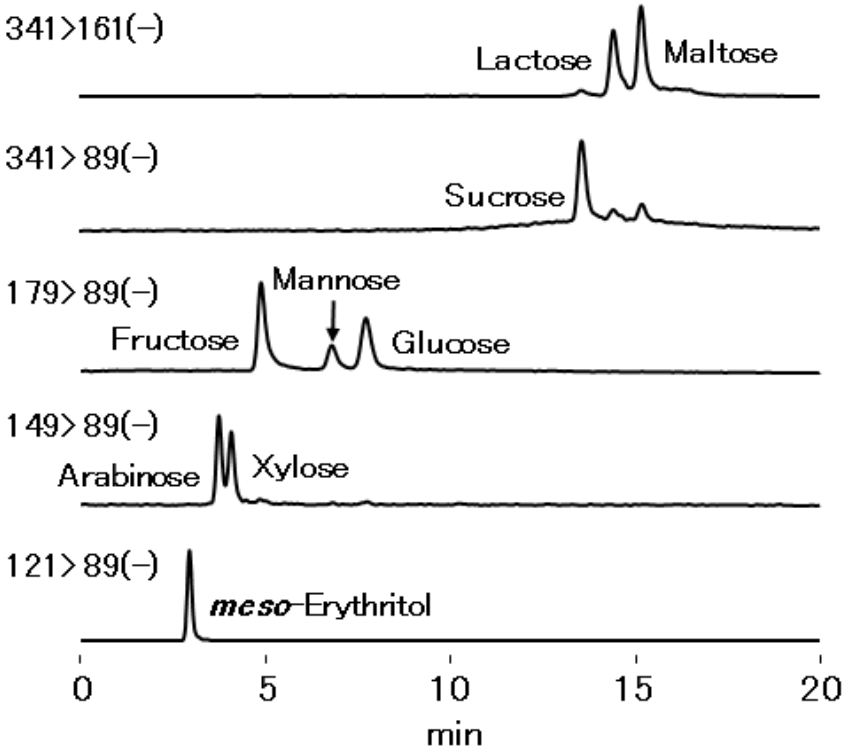
1. *meso*-Erythritol;
2. Xylitol;
3. Fructose;
4. Glucose;
5. Sucrose;
6. Lactose;
7. Maltose;
8. Raffinose

Column: Shodex **Asahipak NH2P-40 2D** (2.0 mm I.D. x 150 mm)
Eluent: (A) 0.05% NH_3 aq. /(B) CH_3CN Isocratic ; (B%) 75%
Flow rate: 0.2 mL/min
Detector: ESI-MS/MS (MRM Negative)
(*m/z*) 121→82(-) for *meso*-Erythritol, 151→59(-) for Xylitol,
179→89(-) for Fructose and Glucose, 341→59(-) for Sucrose,
341→161(-) for Lactose and Maltose, 503→89(-) for Raffinose
Column temp. : 40 °C

HILICpak VG Series - Applications

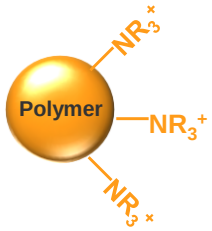


Sample: 50 ng/mL each
(in H_2O/CH_3CN=1/9), 5 µL

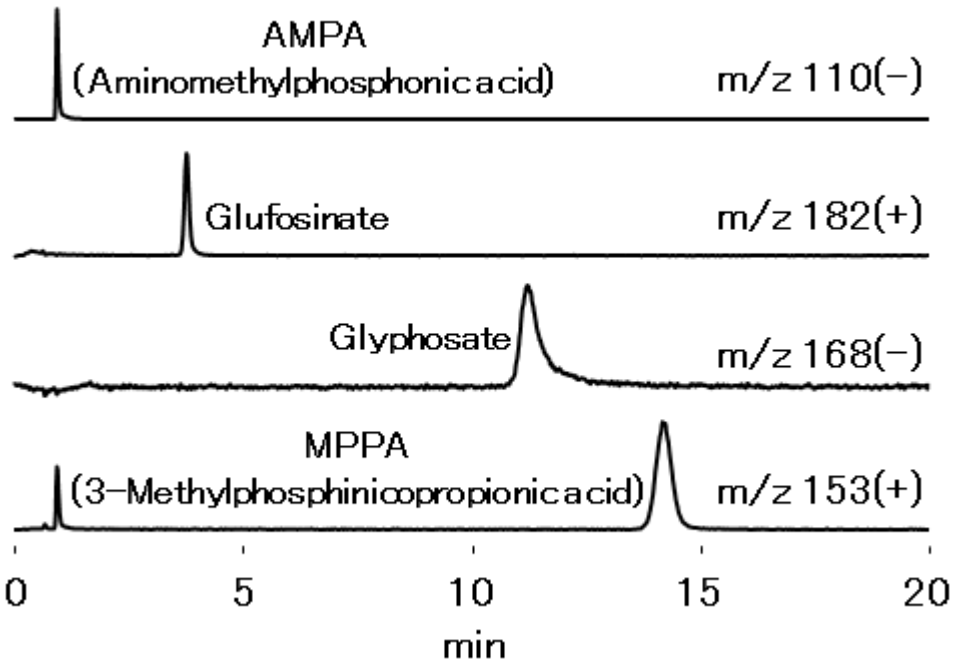


Column : Shodex **HILICpak VG-50 2D** (2.0 mm I.D. x 150 mm)
Eluent : (A); 0.1% NH_3 aq. (B); CH_3CN
Linear gradient ; (B%) 95% (0 to 5min), 95% to 80% (5 to 15min), 80% (15 to 20min)
Flow rate : 0.3 mL/min
Detector : ESI-MS/MS (MRM Negative)
Column temp. : 60 °C

HILICpak VT Series - Applications



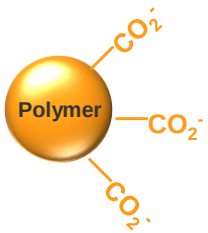
Sample: 5 µL
1 µg/mL each



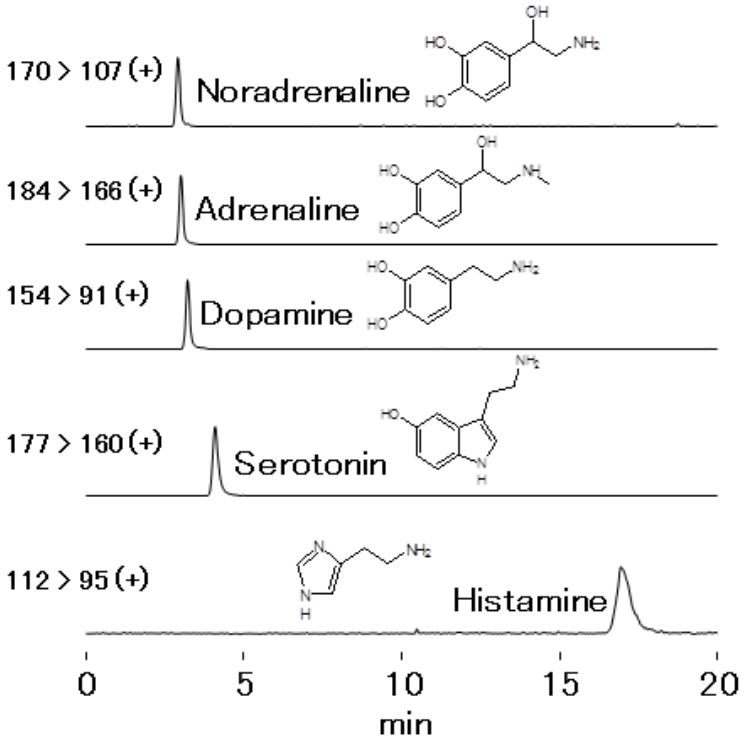
Column : Shodex **HILICpak VT-50 2D** (2.0 mm I.D. x 150 mm)
Eluent : H_2O/1% HCOOH aq./CH_3CN = 70/20/10
Flow rate : 0.3 mL/min
Detector : ESI-MS (SIM)
Column temp. : 40 °C

New

HILICpak VC Series - Applications



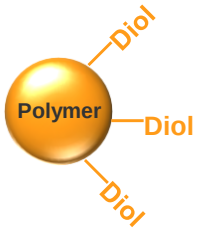
Sample:
0.1 µM each (in H_2O), 20 µL

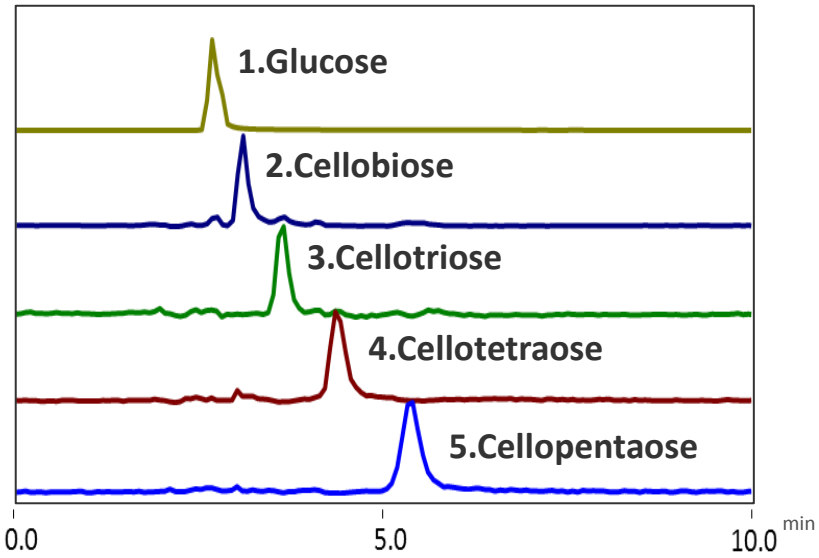


Column : **Shodex HILICpak VC-50 2D** (2.0 mm I.D. x 150 mm)
Eluent : (A) 200 mM HCOOH aq, (B) CH_3CN
Linear gradient (High pressure) ; B% = 60% (0 to 5min) 60% to 10% (5 to 6min) 10% (6 to 20min)
Flow rate : 0.3 mL/min
Detector : ESI-MS/MS (MRM Positive)
Column temp. : 40℃

New

HILICpak VN Series - Applications



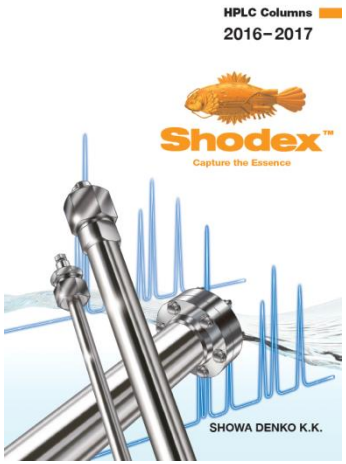


Sample: 100 µg/mL , 1 µL
Column: Shodex Asahipak **VN-50 2D**
Eluent: 100 mM $HCOONH_4$ aq. 30% / Acetonitrile 70% Isocratic
Flow rate: 0.2 mL/min
Column temp.: 40 ℃
Detector ESI-MS (SIM)

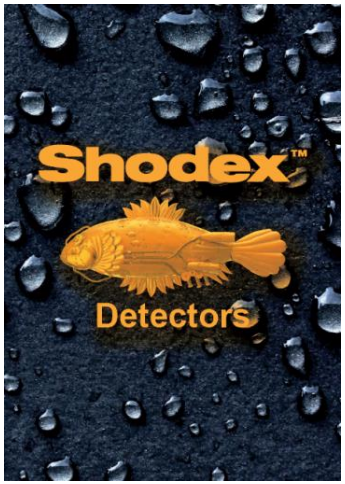
Summary

Application	Shodex Column Name	Separation Technique
Saccharides, organic acids, polar compounds	• **Asahipak NH2P Series**	HILIC
	• **HILICpak VG and VT Series**	
	• **HILICpak VC and VN Series** New	
Proteins & peptides	• **PROTEIN KW and LW Series** New	size exclusion (SEC)
	• **OHpak SB and LB Series** New	size exclusion (SEC)
Synthetic polymers, plastics	• **GPC KF and HK Series** New	size exclusion (SEC)

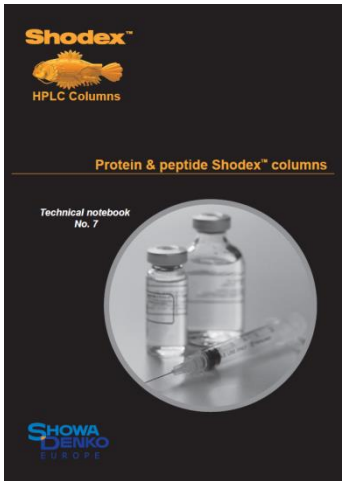
Shodex Information



Column Catalogue



Detector Catalogue



Technical Notebooks

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Applications

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