Shodex™

HPLC Columns

Protein & peptide Shodex™ columns

Technical notebook
No. 7

SHOWA DENKO EUROPE

Contents

1. Introduction

The analysis of proteins and peptides is essential to food science, biomolecular, and pharmaceutical studies. One of the most useful tools for this purpose is HPLC, which relies on wide variety of separation techniques. The main separation modes used for the analysis of proteins by HPLC are: Size exclusion chromatography, Reversed phase chromatography, Ion exchange chromatography, Hydrophobic interaction chromatography and Affinity chromatography. In addition, Multi mode chromatography, which combines Size exclusion and Reversed phase chromatography (or Ion exchange chromatograpgy) can also be used. In this technical notebook, we will first review each separation mechanism, and then introduce the corresponding Shodex columns along with relevant application data.

2. Separation of Protein and Peptide by HPLC

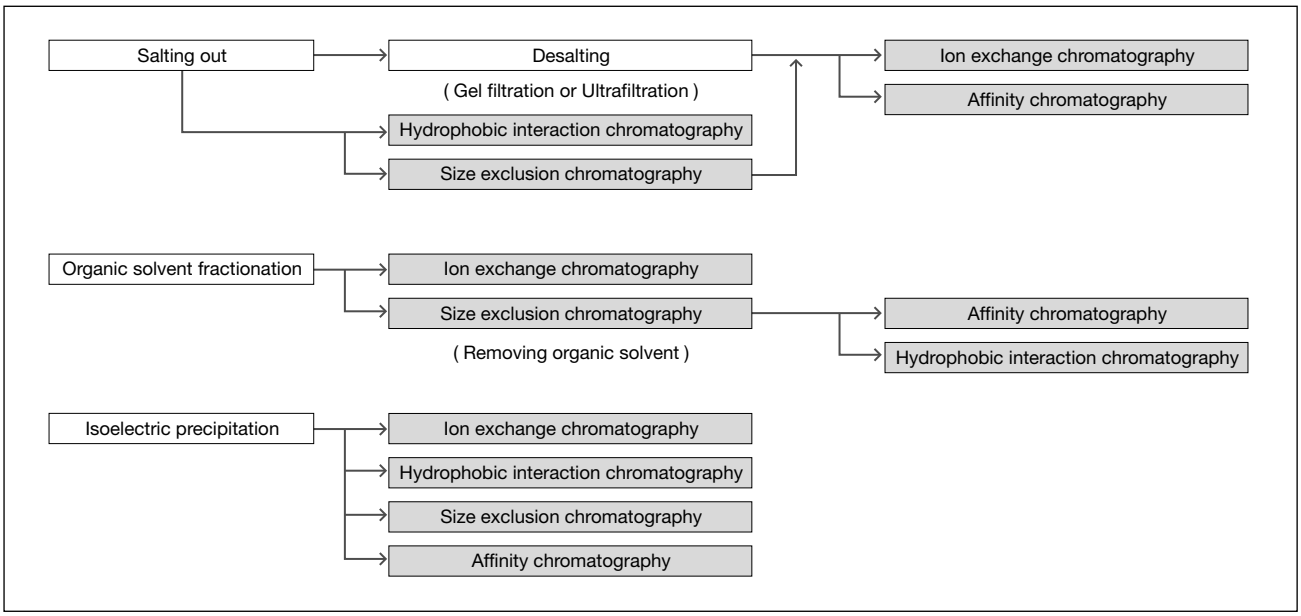
In order to plan the separation and purification of proteins or peptides, it is necessary to study the character of the target substances and select a suitable separation mode. Accordingly to choose a suitable separation mode, one must first consider which of the following case best describes the analytes:

(1) The case where there is large difference of molecular weight between the target substance and the matrix
➔ Size exclusion chromatography
(2) The case where the difference in isoelectric points between the target substance and the matrix is greater than 1
➔ Ion exchange chromatography
(3) The case where there is the difference in hydrophobicity between the target substance and the matrix
➔ Reversed phase chromatography, Hydrophobic interaction chromatography
(4) The case where a resin with affinity to the target substance is available
➔ Affinity chromatography
(5) The pH, salt concentration, and thermostability of the target substance

When analysis is the main purpose, studying points (1) – (4) is enough for the selection of a suitable separation mode. If purification is the main purpose, studying aspect (5) is also necessary in order to prevent protein denaturation.

Usually, a combination of several separation modes is used for separation and purification. A model purification shceme is shown in Figure 1. For an unknown sample, Size exclusion chromatography is usually suitable because all substances are eluted within a limited time in order of molecular size.

Fig. 1 Model separation & purification scheme



Shodex provides columns corresponding to each of the above mentioned-separation modes for the analysis of proteins and peptides. Figures 2-1 & 2-2 can be used for the selection of a suitable column, and each separation mode is explained in chapter 3.

Fig. 2-1 Shodex column selection guide (Size exclusion, Reversed phase)

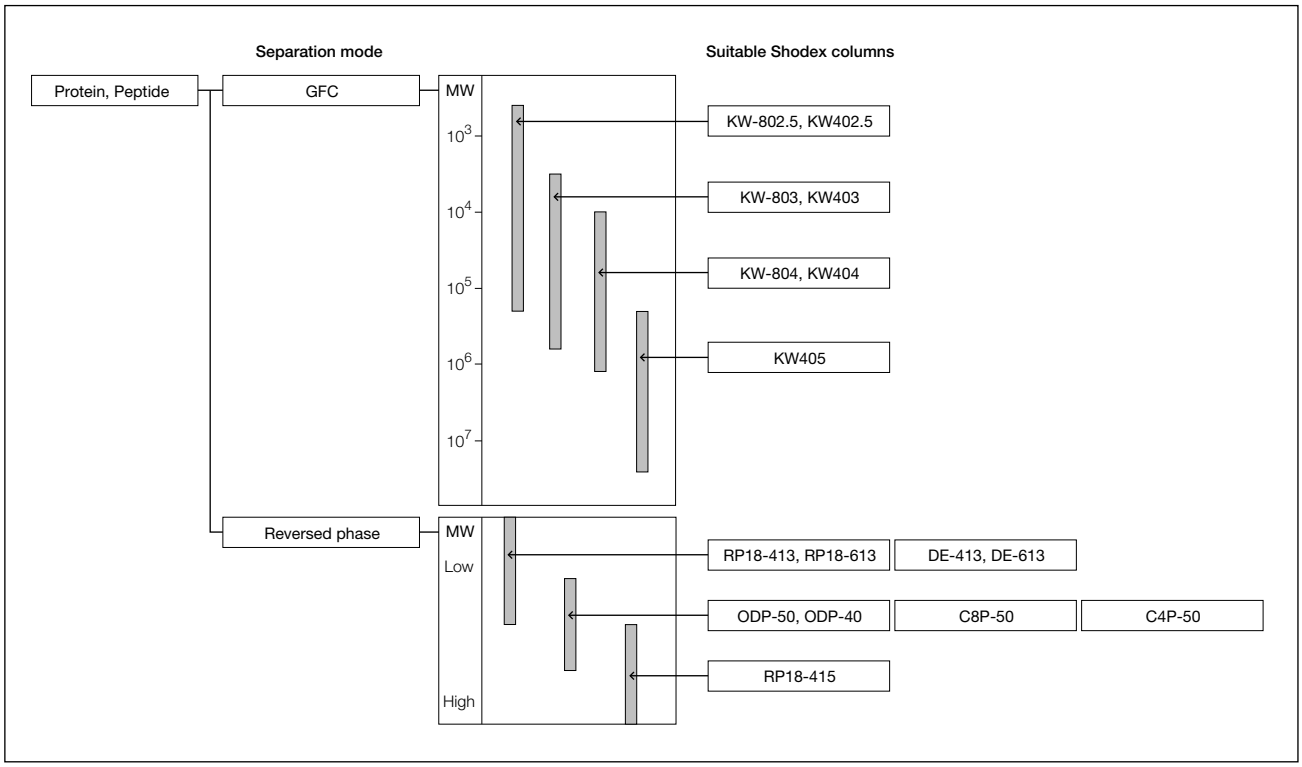
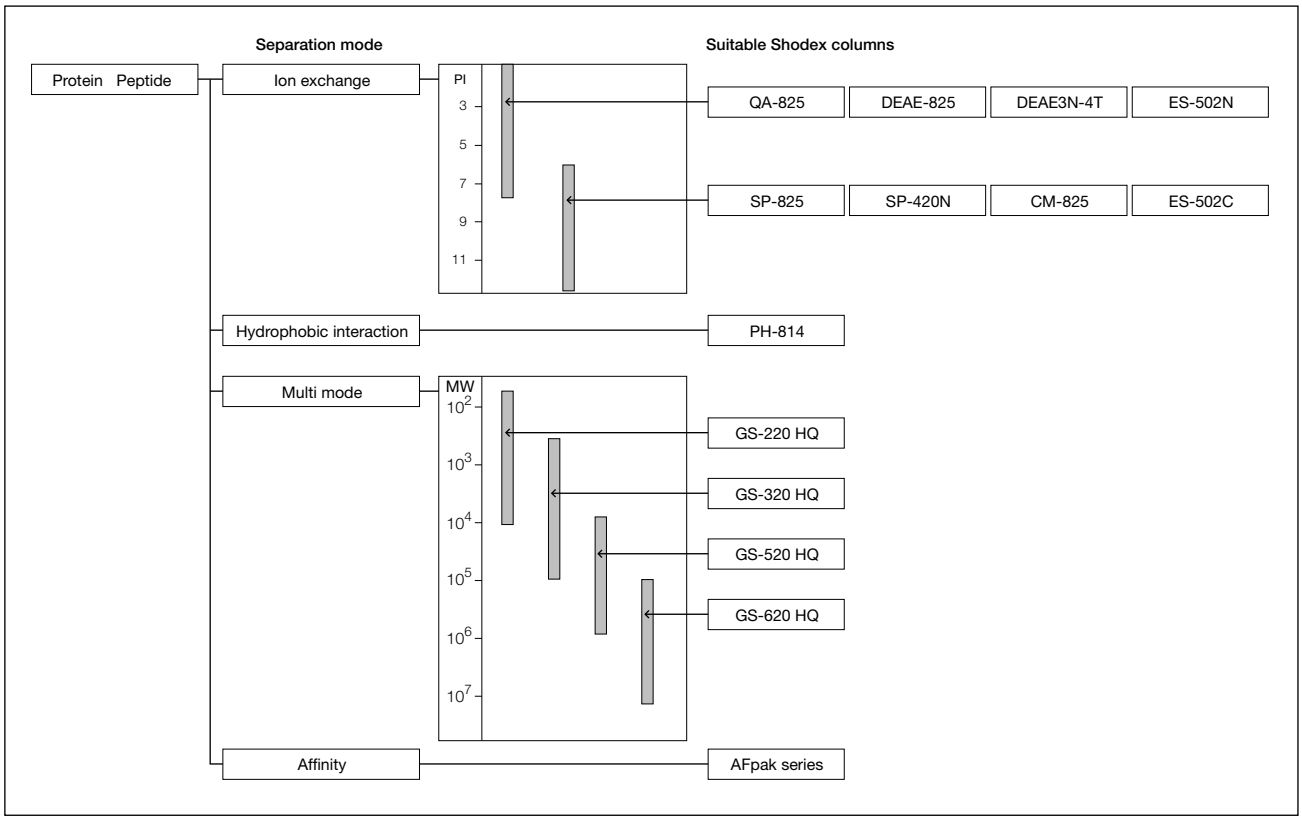


Fig. 2-2 Shodex column selection guide (Ion exchange, Hydrophobic interaction, Multi mode, Affinity)



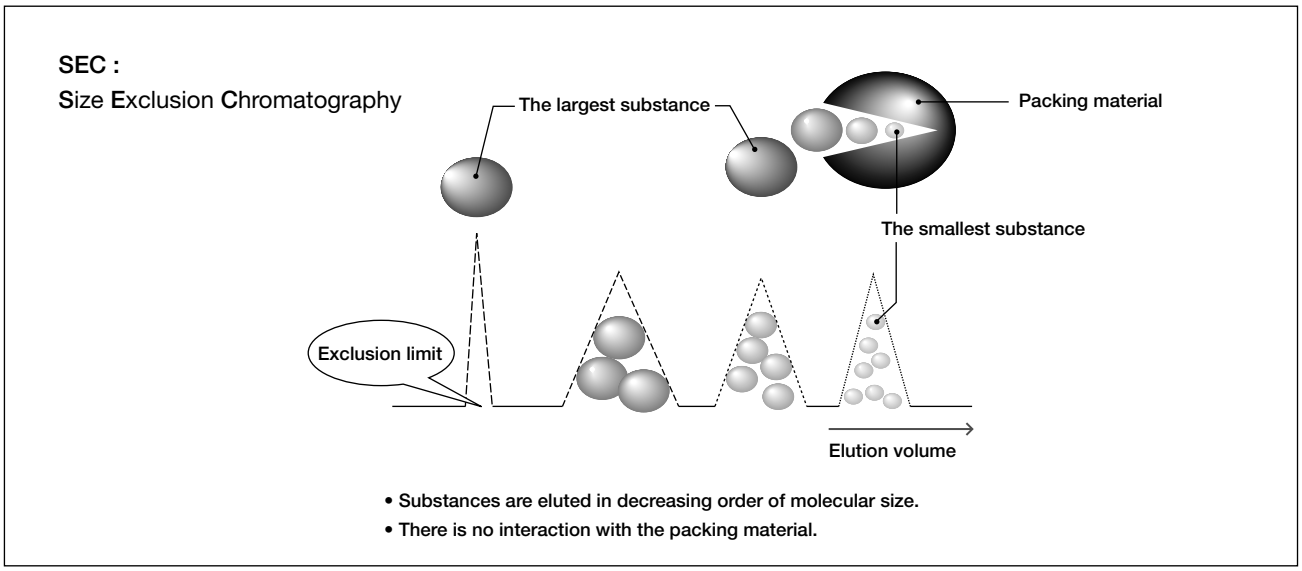
3. Separation modes

3-1. Size exclusion chromatography

3-1-1. Separation mechanism

Size exclusion chromatography (SEC) relies on the separation of molecules based on their size whereby larger molecules, which have a larger 3-dimensional volume in the eluent, elute faster. The principle of size exclusion chromatography is shown in Figure 3.

Fig. 3 The principle of SEC



The packing material has a network of small wedged holes called pores. When proteins are introduced into a column, they diffuse into pores by capillary action. Proteins which are larger than the pore size cannot penetrate into the pore and move forward with eluent, therefore eluting from the column earlier. On the other hand, proteins smaller than the pore size can get into the pore and elute from the column later. With SEC, proteins are eluted from the largest one on.

SEC has a disadvantage, which is a low loading capacity and the impossibility to increase the capacity factor (k') above 1.0. SEC is therefore not suitable for the separation of complex samples. In spite of that, SEC is frequently used as the first screening method for the separation of unknown proteins, because of the following three reasons: (1) The eluent can be easily selected. (2) It is possible to separate by molecular size. (3) The purification ratio is effective.

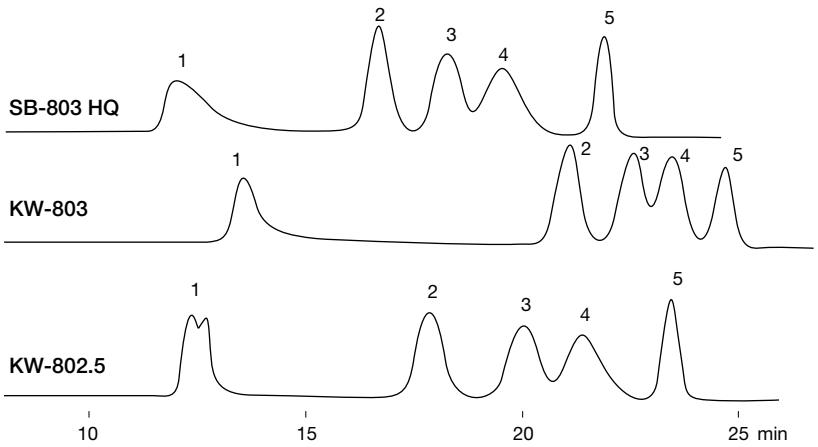
3-1-2. Introduction of Shodex columns

Two types of base materials can be packed into a column, i.e. silica-based, and polymer-basedmaterials. Generally speaking for proteins and peptides analysis, silica-based stationary phases show the best separation performance because the base material has a smaller pore size distribution. On the other hand, polymer-based stationary phases are at an advantage in alkaline eluents of pH higher than 7.5, because silica gel dissolves in alkaline eluents. Figure 4 shows the comparison chromatograms of a Polyethylene glycol (PEG) mixture between the polymer-based column OHpak SB-803 HQ and the silica-based PROTEIN KW-803 & KW-802.5. OHpak SB-803 HQ is suitable for the analysis of synthetic polymers with a wider molecular range thanks to its wider pore size distribution. On the other hand, the PROTEIN KW-803 & KW-802.5 columns are suitable for the analysis of proteins thanks to their narrower pore size distribution.

Fig. 4 Comparison between silica-based columns KW-803 & KW-802.5 and polymer-based column SB-803 HQ

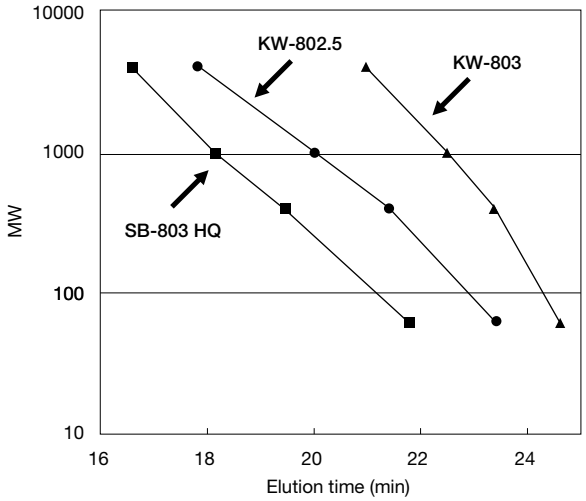
Fig. 4-1 Comparison of chromatograms

Sample :
1. Poly(ethylene glycol) (MW: 20,000)
2. Poly(ethylene glycol) (MW: 4,000)
3. Poly(ethylene glycol) (MW: 1,000)
4. Poly(ethylene glycol) (MW: 400)
5. EG, Ethylene glycol (MW: 62)



Columns : Shodex OHpak SB-803 HQ or PROTEIN KW-802.5 or KW-803 (8.0mmID x 300mm each)
Eluent : H_2O
Flow rate : 0.5mL/min
Detector : Shodex RI
Column temp. : 30°C

Fig. 4-2 Comparison of calibration curves

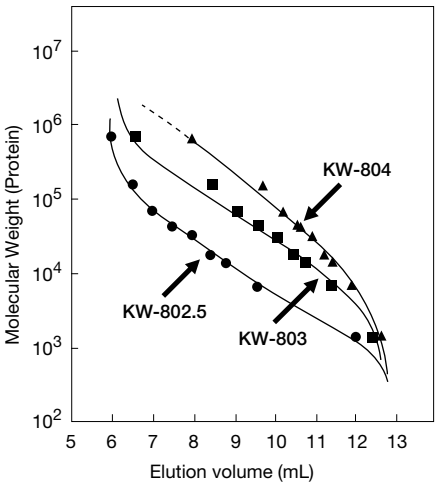


● PROTEIN KW-800 series (Silica-based)

The packing material of the Shodex PROTEIN KW-800 series is based on silica particles whose surface is coated with a hydrophilic polymer. This column is for aqueous SEC, and indexed in the Shodex catalogue under Gel filtration chromatography (GFC). It enjoys a positive reputation thanks to its high recovery ratio of proteins and peptide. The PROTEIN KW-800 series comprises three kinds of columns, differing in pore and particle size.

Figure 5 shows the analysis of a mixture of 10 standard proteins with the PROTEIN KW-800 series.

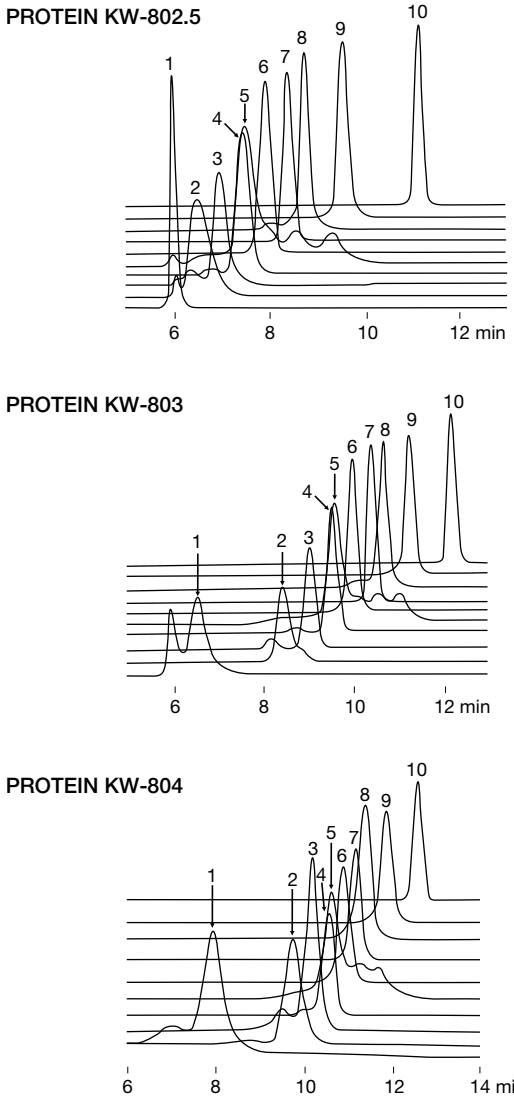
Fig. 5 Calibration curve of the PROTEIN KW-800 series



Sample

1.	Thyroglobulin	(Mw: 669,000)
2.	Aldolase	(Mw: 158,000)
3.	BSA	(Mw: 67,000)
4.	Ovalbumin	(Mw: 43,000)
5.	Peroxidase	(Mw: 40,200)
6.	Adenylate kinase	(Mw: 32,000)
7.	Myoglobin	(Mw: 17,000)
8.	Ribonuclease A	(Mw: 13,700)
9.	Aprotinin	(Mw: 6,500)
10.	Vitamin B_{12}	(Mw: 1,350)

Column : Shodex PROTEIN KW-800 series (8.0mmID x 300mm)
Eluent : 50mM Sodium phosphate buffer (pH7.0) + 0.3M NaCl
Flow rate : 1.0mL/min
Detector : UV (220nm)
Column temp. : Room temp.



The PROTEIN KW-800 series line-up

PROTEIN KW-800 series	Size (mm) I.D. x L	Plate Number (TP/column)	Exclusion Limit		Particle Size (μm)	Pore Size (Å)	
			Pullulan	Protein		Max.	Avg.
KW-802.5	8.0 x 300	> 21,000	60,000	150,000	5	400	150
KW-803	8.0 x 300	> 21,000	170,000	700,000	5	1,000	300
KW-804	8.0 x 300	> 16,000	500,000	1,000,000	7	1,500	500
KW-G	6.0 x 50	(Guard column)	—	—	7	—	—

● KW400 series (Silica-based)

The Shodex KW400-4F series is the high-performance semi-micro column version of the KW-800 series. The theoretical plate number and the sensitivity are improved by reducing the diameter of the packed particle to 3 micrometers.

KW405-4F, the newest addition to the KW400 line-up, has a larger pore size than any columns in the existing KW-800 series, and can analyze proteins whose molecular weight is larger than 1,000,000. It enables the scientist to carry out the analysis of protein complexes. Figure 6 shows the calibration curves of the KW400 series with proteins.

Fig. 6 Calibration curves of the KW400-4F series

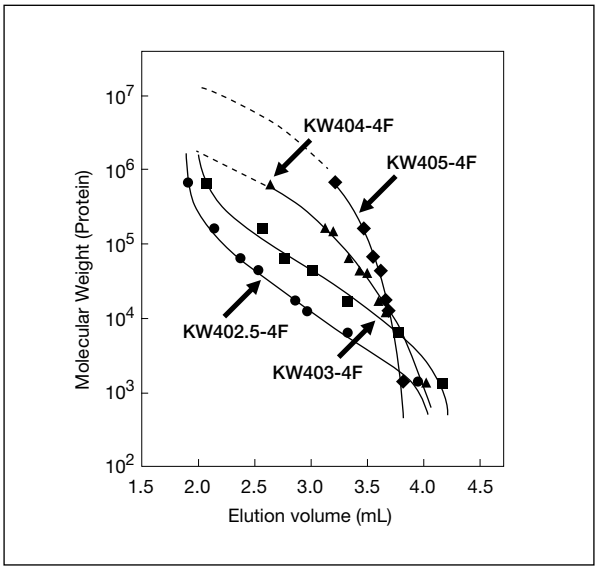
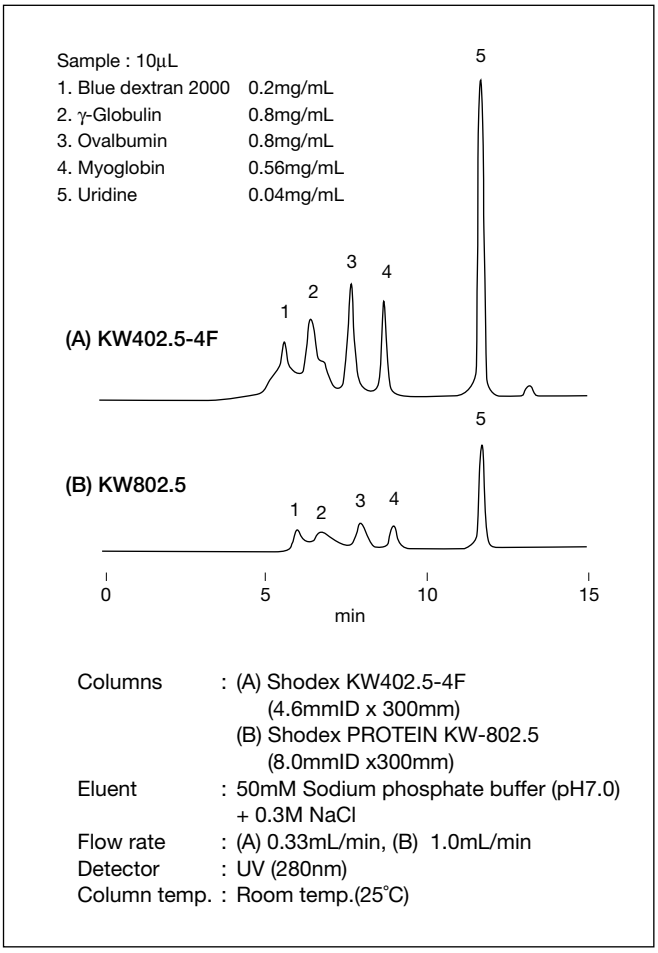


Figure 7 shows the comparison between the high-performance semi-micro column KW402.5-4F and the original PROTEIN KW-802.5 column for protein analysis. The KW400-4F series exhibits approximately a 1.5 times higher theoretical plate number and 4 times better sensitivity compared to the KW-800 series.

Fig. 7
Comparison between KW402.5-4F and KW-802.5 columns



The KW400 series line-up

KW400 series	Size (mm) I.D. x L	Plate Number (TP/column)	Exclusion Limit		Particle Size (μm)	Pore Size (Å)	
			Pullulan	Protein		Max.	Avg.
KW402.5-4F	4.6 x 300	> 35,000	60,000	150,000	3	400	150
KW403-4F	4.6 x 300	> 35,000	150,000	600,000	3	800	250
KW404-4F	4.6 x 300	> 25,000	500,000	1,000,000	5	1,500	500
KW405-4F	4.6 x 300	> 25,000	1,300,000	20,000,000	5	2,000	1,000
KW400G-4A	4.6 x 10	(Guard column)	—	—	5	—	—

The following semi-micro and micro columns can be prepared.
• Inner diameter : 2.0mm, 1.0mm • Length : 250mm, 150mm, 50mm

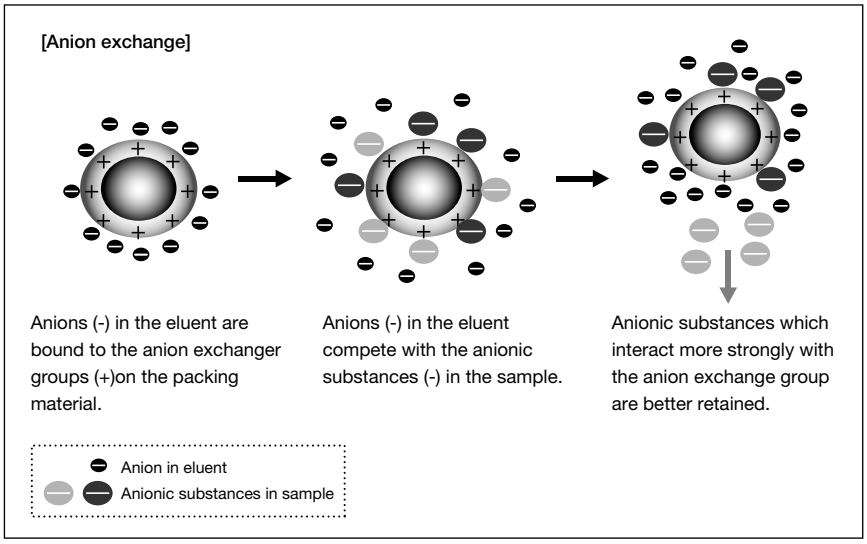
3-2. Ion exchange chromatography

3-2-1. Separation mechanism

Ion exchange chromatography is an analytical method relying on the interaction of ion-charges between the surface of proteins and the surface of the packing material. Packing materials with a positive surface charge are called “anion exchangers” and those with a negative surface charge are called “cation exchangers”.

Obtaining some information about the isoelectric point (pI) of proteins is useful for selecting suitable analytical conditions. Proteins consist of amino acids, and are amphoteric compounds, so there is pH at which the total sum of electrical charges becomes zero in the solution. That pH is called isoelectric point (pI). Around the pI, the electrical charge on the surface of proteins is neutralized and such proteins cannot easily adsorb on the surface of the packing material. In some cases, proteins precipitate around the pI, a phenomenon known as “isoelectric precipitation”. In order to adsorb the target protein, the pH of the eluent must be adjusted at more than 1.0 pH unit from pI. When the pH is more acidic than the pI, a cation exchanger should be used, and when the pH is more alkaline than the pI, an anion exchanger should be selected. Figure 8 shows the mechanism of an anion exchanger.

Fig. 8 The mechanism of anion exchange separations



Hereinafter, we describe the mechanism of an ion exchange separation through the example of a Diethylaminoethyl (DEAE)-based anion exchange resin. In the case of the separation of a protein whose pI is 7, the pH of the eluent should be adjusted to 8, to make the protein anionic. The DEAE ion exchanger should be equilibrated with a buffer solution with chloride (Cl^-) as the counter ion. When the protein, which has negative charge as an anion, is introduced into the column, it binds to the DEAE ion exchanger while removing Cl^-. If a protein with a pI of 9 is mixed in the sample, that protein exists as cation and goes through the DEAE ion exchanger without adsorption.

In order to elute the adsorbed proteins, there are two methods. One elution method is the gradual concentration increase of salts such as sodium chloride (NaCl) in the eluent, and another method is the gradual change of pH in the eluent. The first method is used more prevalently. Hereinafter we will discuss the salt concentration gradient method. When the concentration of NaCl increases in the eluent, the concentration of Cl^- increases and competes for adsorption against previously adsorbed negatively charged proteins,thus removing proteins from the anion exchanger.

The situation of the separation also depends on the type of ion exchanger. An Ion exchanger is classified not only by anion or cation exchanger type, but also by the strength of the exchanger resulting from differences in functional groups. The difference between a strong and a weak exchanger is not a difference in the binding strength for proteins but the difference in the effect of pH change in the eluent, i.e. the difference in buffering capacity. Strong ion exchangers can keep the same electrical charge and adsorb proteins in a wider pH range, while week ion exchangers are affected by pH changes in the eluent, readily changing the ion exchange capacity.

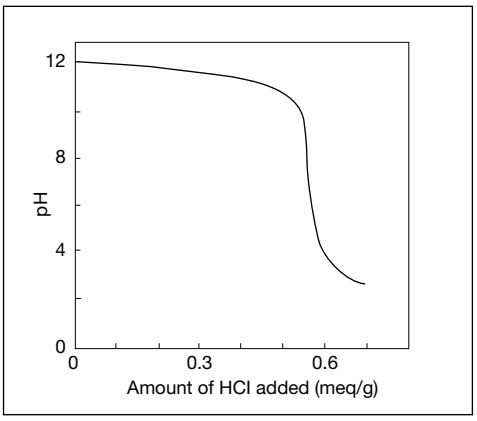
In the next chapter, we show how to optimize analytical conditions using Shodex ion exchange chromatography columns.

3-2-2. Introduction of Shodex columns

3-2-2-1. Anion exchange

- Strong anion exchange **IEC QA-825**
- Weak anion exchange **IEC DEAE-825, Asahipak ES-502N 7C**

Fig. 9 IEC QA-825 (Quaternary ammonium)



A strong anion exchange resin with a quaternary ammonium functional group is packed into the IEC QA-825 column. This column can be used at a wider pH range because the pKa of the ion exchange base is 11.7 (Figure 9).

Figure 10 shows the separation of five kinds of proteins with IEC QA-825. Conalbumin is eluted earlier at a pH higher than 6.0 because the pI of Conalbumin is between 6.0 and 6.8. On the other hand, the isoelectric points of other proteins, namely Transferin, Ovalbumin and Trypsin inhibitor are lower than 6.0 so these elute consecutively throughout the gradual the salt concentration increase.

Fig. 10 QA-825

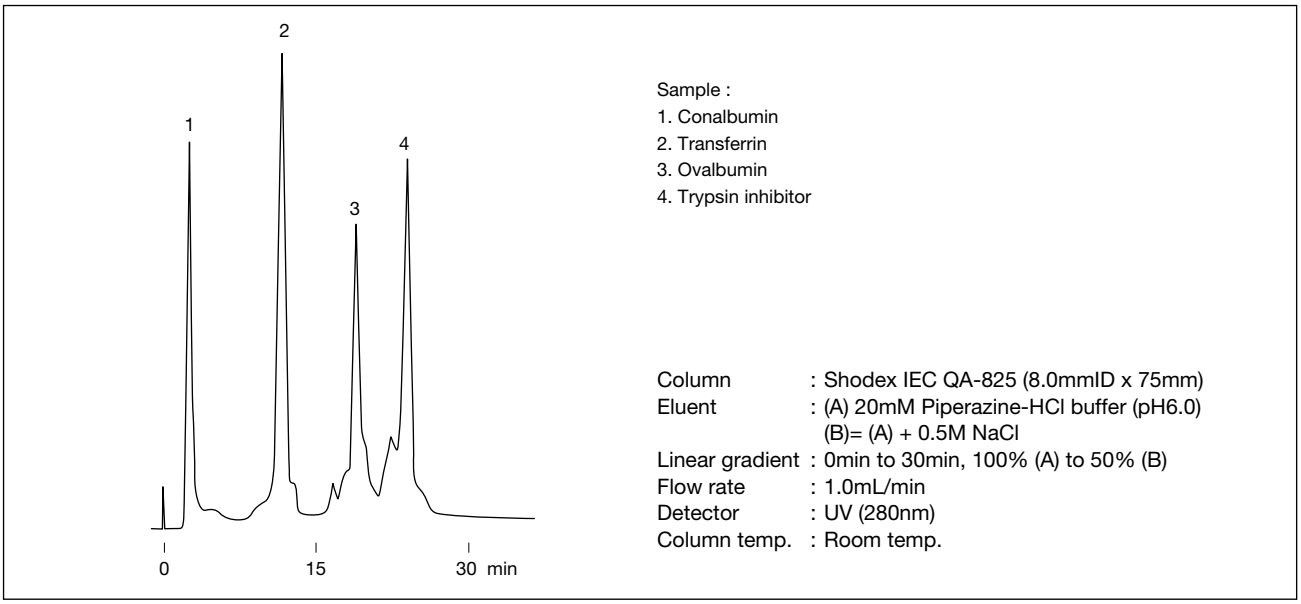
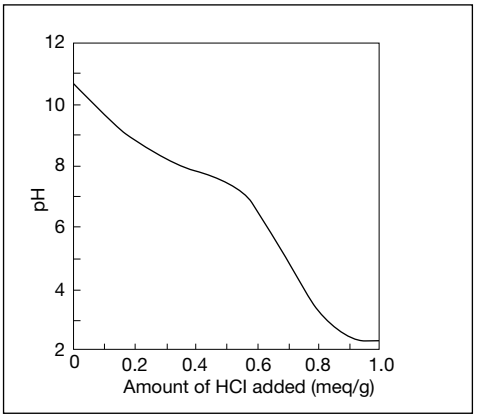


Fig. 11 IEC DEAE-825/Asahipak ES-502N 7C (DEAE base)

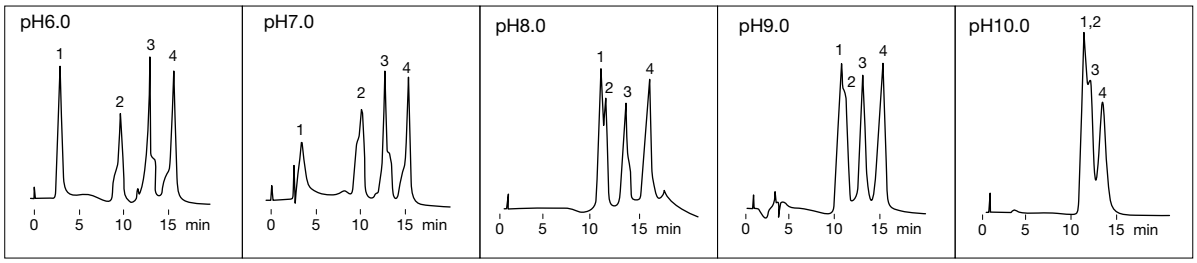


A weak anion exchange resin with a diethylaminoethyl (DEAE) functional group is packed into IEC DEAE-825 and Asahipak ES-502N columns. These columns are suitable for the separation of acidic proteins because the pKa of the ion exchanger is about 7.8 (Figure 11).

Figure 12 shows the effect of pH on the separation of proteins on weak anion exchange chromatography column IEC DEAE-825. Under acidic eluents, the four kinds of protein can be separated well but under alkaline eluents, the separation worsens with increasing pH. It is due to the ion exchanger not being properly ionized at alkali pH.

Weak anion exchangers (DEAE) are preferably used for protein analysis, even though the pH range for protein retention is narrower than the range for a strong anion exchanger, because milder analytical conditions can be used.

Fig. 12

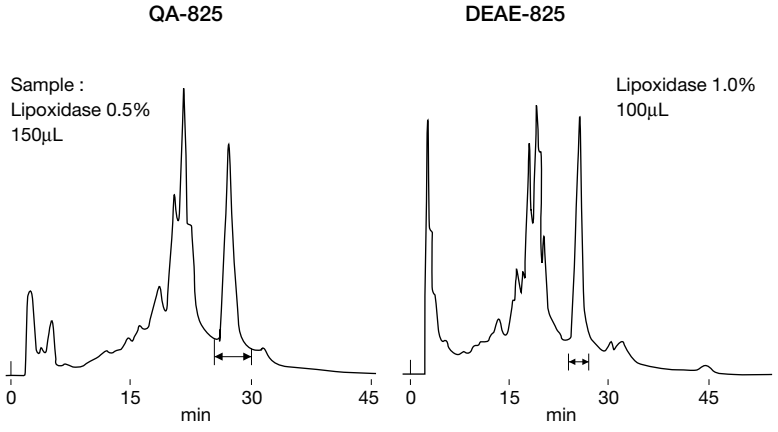


Column : Shodex IEC DEAE-825 (8.0mmID x 75mm)
Eluent : (A) 20mM Piperazine-HCl buffer (pH6.0) 20mM Tris-Trispropanol-HCl buffer (pH7.0)
20mM Tris-HCl buffer (pH8.0) 20mM Ethanolamine-HCl buffer (pH9.0)
20mM 1,3-Diaminopropane-HCl buffer (pH10.0)
(B)=(A) + 0.5M NaCl
Linear gradient : 0min to 20min, (A) to (B)
Flow rate : 1.0mL/min

Sample:
1. Conalbumin
2. Transferrin
3. Ovalbumin
4. Trypsin inhibitor

Figure 13 shows the analysis of Lipoxidase (reagent grade) with the IEC QA-825 (strong anion exchange resin) and IEC DEAE-825 (week anion exchange resin). Enzymatic activity was detected in the range delimited by double arrows.

Fig. 13 Comparison between QA-825 and DEAE-825



Sample : Lipoxidase from soybean

Columns : Shodex IEC QA-825 (8.0mmID x 75mm)
Eluent : (A); 20mM Ethanolamine-HCl buffer (pH9.0)
(B); (A) + 0.5M NaCl
Linear gradient : 0min to 60min, (A) to (B)
Flow rate : 1.0mL/min
Detector : UV(280nm)
Column temp. : Room temp.

Columns : Shodex IEC DEAE-825 (8.0mmID x 75mm)
Eluent : (A); 20mM Tris-HCl buffer (pH8.0)
(B); (A) + 0.5M NaCl
Linear gradient : 0min to 60min, (A) to (B)
Flow rate : 1.0mL/min
Detector : UV(280nm)
Column temp. : Room temp.

Anion exchange

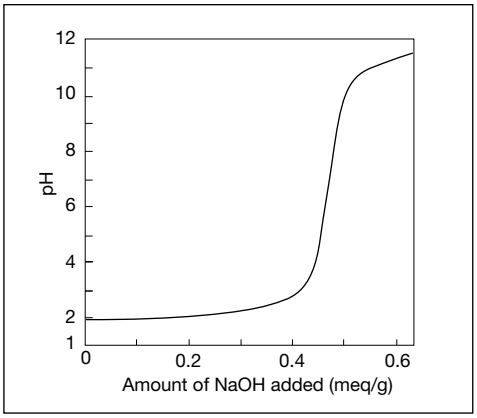
Product	Type	Size (mm) ID x L	Base material	Ion Exchange Capacity (meq/g)	Pore size (Å)	Particle size (µm)
IEC QA-825	Strong	8.0 x 75	Polyhydroxymethacrylate	0.45	5,000	12
IEC DEAE-825	Weak	8.0 x 75	Polyhydroxymethacrylate	0.6	5,000	8
Asahipak ES-502N 7C	Weak	7.5 x 100	Polyvinyl alcohol	0.55	2,000	9

The following semi-micro and micro columns can be prepared.
• Inner diameter : 2.0mm, 1.0mm, 0.8mm, 0.5mm, 0.3mm • Length : 150mm, 50mm, 35mm

3-2-2-2. Cation exchanger

- Strong cation exchange **IEC SP-825**
- Weak cation exchange **IEC CM-825, Asahipak ES-502C 7C**

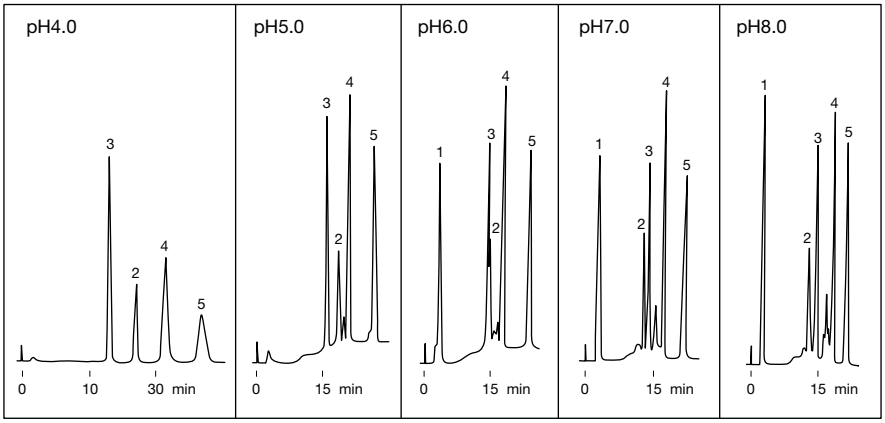
Fig. 14 IEC SP-825 (Sulfopropyl base)



A strong cation exchange resin with a sulfopropyl functional group is packed into the IEC SP-825 column. This column can be used at a wider pH range because the pKa of the ion exchange base is 2.3 (Figure 14).

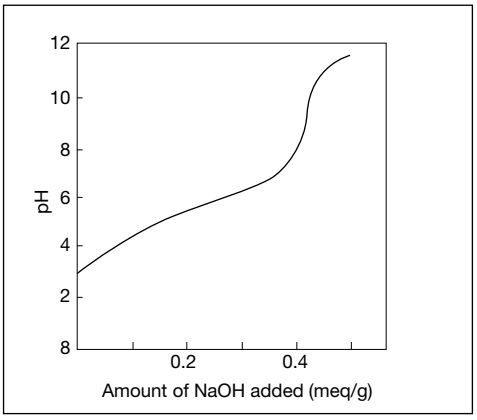
Figure 15 shows the effect of pH on the separation of proteins with IEC SP-825. The proteins can be retained at a wide pH range, and the five kinds of proteins were separated particularly well at pH 7.0 and pH 8.0. The retention time of Ribonuclease A increases at lower pH and it is eluted after α Chymotrypsinogen A under pH 6.0. At pH 6.0 the separation between Ribonuclease A and α Chymotrypsinogen A is insufficient, while at pH 4.0 and pH 5.0, the peak shape of Myoglobin is not sharp.

Fig. 15 Effect of pH (SP-825)



Column : Shodex IEC SP-825 (8.0mmID x 75mm)
Eluent : (A) 20mM Sodium formate buffer (pH4.0) 20mM Sodium malonate buffer (pH5.0 and 6.0)
20mM Sodium phosphate buffer (pH7.0) 20mM HEPES (pH8.0)
(B)= (A) + 0.5M NaCl
Linear gradient : 0min to 20min, (A) to (B)
Flow rate : 1.0mL/min

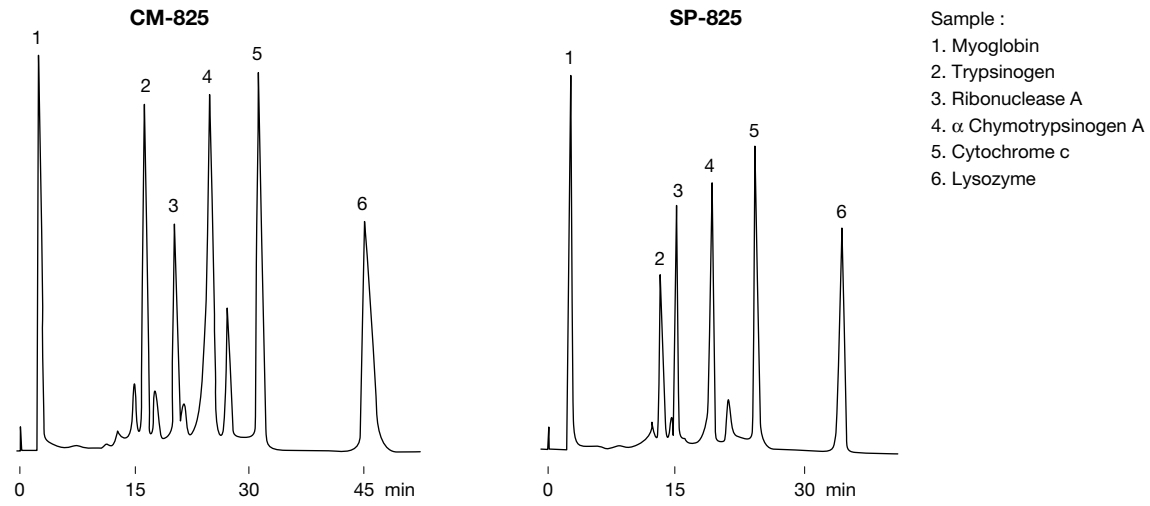
Fig. 16 IEC CM-825/Asahipak ES-502C 7C (Carboxymethyl base)



IEC CM-825 and Asahipak ES-502C are packed with a weak cation exchange resin with Carboxymethyl as the functional group. These columns are suitable for the separation of basic proteins because the pKa of the ion exchange base is 5.7 (Figure 16).

Figure 17 shows the separation of six kinds of proteins including Trypsinogen with the IEC CM-825 (week cation exchange resin) and IEC SP-825 (strong cation exchange) columns. The elution patterns are similar but the elution is faster with SP-825 than with CM-825.

Fig. 17 Comparison between CM-825 and SP-825



Columns : (Left) Shodex IEC CM-825 (8.0mmID x 75mm)
(Right) Shodex IEC SP-825 (8.0mmID x 75mm)
Eluent : (A) 20mM Sodium phosphate buffer (pH7.0)
(B)= (A) + 0.5M NaCl
Linear gradient : 0min to 60min, (A) to (B)
Flow rate : 1.0mL/min
Detector : UV (280nm)
Column temp. : Room temp.

Cation exchange

Product	Type	Size (mm) ID x L	Base material	Ion Exchange Capacity (meq/g)	Pore size (Å)	Particle size (μm)
IEC SP-825	Strong	8.0 x 75	Polyhydroxymethacrylate	0.4	5,000	8
IEC CM-825	Weak	8.0 x 75	Polyhydroxymethacrylate	0.4	5,000	8
Asahipak ES-502C 7C	Weak	7.5 x 100	Polyvinyl alcohol	0.55	2,000	9

The following semi-micro and micro columns can be prepared.
• Inner diameter : 2.0mm, 1.0mm, 0.8mm, 0.5mm, 0.3mm • Length : 150mm, 50mm, 35mm

3-2-2-3. Non-porous gel (Fast analysis)

● Anion exchange **DEAE3N-4T**

● Cation exchange **SP-420N**

For fast analysis, columns packed with a non-porous gel are effective. A non-porous gel is made of packing material that does not have pores. The particle diameter of a non-poous gel can be shortened and the compressive strength of the gel is high, so it can be used for fast analysis with increased eluent flow rates.

<Maximum flow rate>

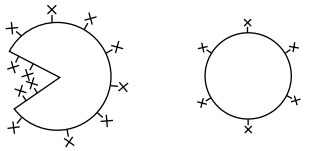
DEAE-825, SP-825 (porous resin) : 1.5ml/min
DEAE3N-4T, SP-420N (non-porous resin) : 2.0ml/mim

A disadvantage though, is that the maximum protein loading volume of non-porous gel is reduced.

<Maximum protein loading volume>

DEAE-825, SP-825 (porous resin) : 1,000μg/column
DEAE3N-4T, SP-420N (non-porous resin) : 20μg/column

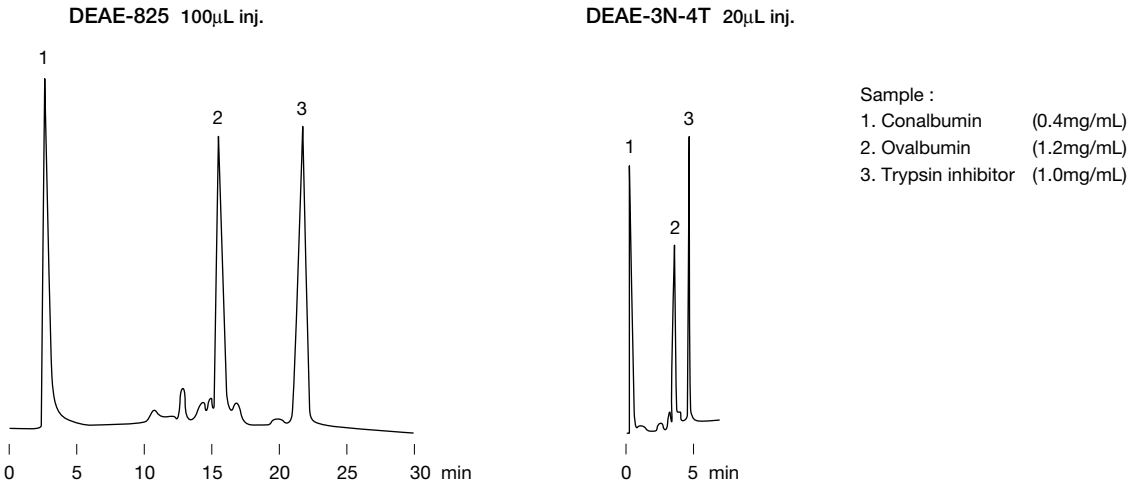
[Packing material schematic]



Porous gel Non porous gel

Figure 18 shows the separation of proteins with IEC DEAE3N-4T (non-porous weak anion exchange resin). The analytical time with DEAE3N-4T is shorter than the time with IEC DEAE-825, which is packed with a porous weak anion exchange resin.

Fig. 18 Comparison between DEAE-825 and DEAE3N-4T (Non-porous)



Columns	: Shodex IEC DEAE-825 (8.0mmID x 75mm)
Eluent	: (A) 20mM Piperazine-HCl buffer (pH6.0)
	(B)= (A) + 0.5M NaCl
Linear gradient	: 0min to 60min, (A) to (B)
Flow rate	: 1.0mL/min
Detector	: UV (280nm)
Column temp.	: Room temp.

Columns	: Shodex IEC DEAE3N-4T (4.6mmID x 35mm)
Eluent	: (A) 25mM Piperazine-HCl buffer (pH6.0)
	(B) (B) = (A) + 0.5M NaCl
Linear gradient	: 0min to 10min, (A) to (B)
Flow rate	: 1.5mL/min
Detector	: UV (280nm)
Column temp.	: Room temp.

Non-porous

Product	Type	Size (mm) ID x L	Base material	Ion Exchange Capacity (meq/g)	Pore size (Å)	Particle size (μm)
IEC DEAE3N-4T	Strong anion	4.6 x 35	Polyhydroxymethacrylate	0.4	—	2.5
IEC SP-420N	Strong cation	4.6 x 35	Polyhydroxymethacrylate	0.3	—	2.5

The following semi-micro and micro columns can be prepared.

• Inner diameter : 2.0mm, 1.0mm, 0.8mm, 0.5mm, 0.3mm • Length : 50mm, 35mm

(Appendix) Selection of a suitable buffer for the eluent

A buffer with a sufficient buffering power at the pH of the eluent and with an ion of identical electrical charge to that of the ion exchanger should be selected for the eluent. The table below shows some examples. We recommend the use of a salt solution of NaCl, KCl, Na_2SO_4 or K_2SO_4, possibly in combination with a buffer solution. The total salt concentration should remain within the 20mM to 600mM range.

PH	Cation Exchange Resin
3.8 ~ 4.3	Sodium formate
4.3	Sodium succinate
4.8 ~ 5.2	Sodium acetate
5.0 ~ 6.0	Sodium malonate
5.5 ~ 6.7	MES
6.7 ~ 7.6	Sodium phosphate
7.6 ~ 8.2	HEPES
8.2 ~ 8.7	BICINE

PH	Anion Exchange Resin
4.8 ~ 5.0	N-Methylpiperazine HCl
5.0 ~ 6.0	Piperazine HCl
5.8 ~ 6.4	Bis Tris HCl
6.4 ~ 7.3	Bis Tris Propane HCl
7.3 ~ 7.7	Triethanolamine HCl
7.5 ~ 8.0	Tris HCl
8.0 ~ 8.5	N-Methyldiethanolamine HCl
8.4 ~ 8.8	Diethanolamine HCl
8.5 ~ 9.0	1,3-Diaminopropane HCl
9.0 ~ 9.5	Ethanolamine HCl

MES : 2-(N-morpholino)ethanesulfonic acid

HEPES : 4-(2-hydroxymethyl)-1-piperazine ethanesulfonic acid

BICINE : N,N-bis(2-hydroxyethyl)glycine

Tris : tris(hydroxymethyl)aminomethane

Bis Tris : Bis(2-hydroxyethyl)iminotris(hydroxymethyl)methane

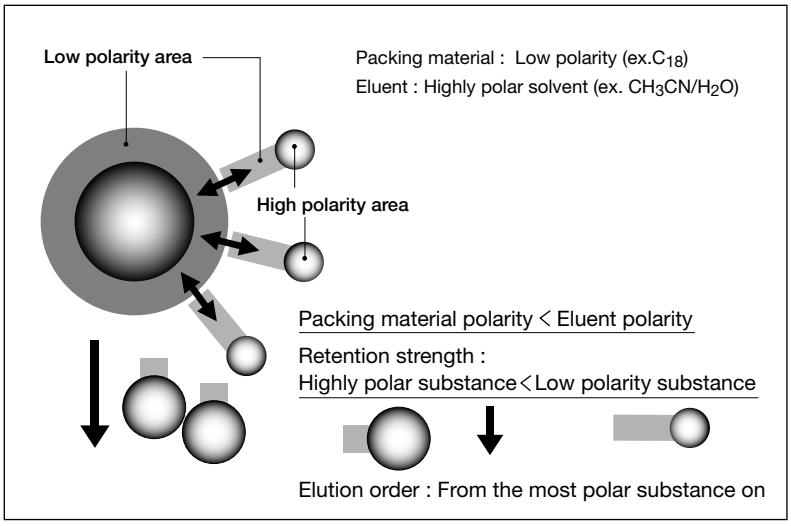
Bis Tris propane : 1,3-Bis[tris(hydroxymethyl)methylamino]propane

3-3. Reversed phase chromatography

3-3-1. Separation mechanism

The separation mode of reversed phase chromatography is based on the hydrophobic interactions governing the distribution/adsorption equilibrium between nonpolar amino acids in proteins and the surface of the packing material (See Figure 19). The surface of the packing material's polymer- or silica-based gel is bound with a hydrophobic functional group such as octadecyl (C_{18}). Some types of polymer gels can be used for reversed phase chromatography without the need for a functional group because the base material in itself is hydrophobic. Proteins with a higher hydrophobicity are retained more strongly, so proteins are eluted from the most hydrophilic one on.

Fig. 19 Separation mechanism of reversed phase chromatography



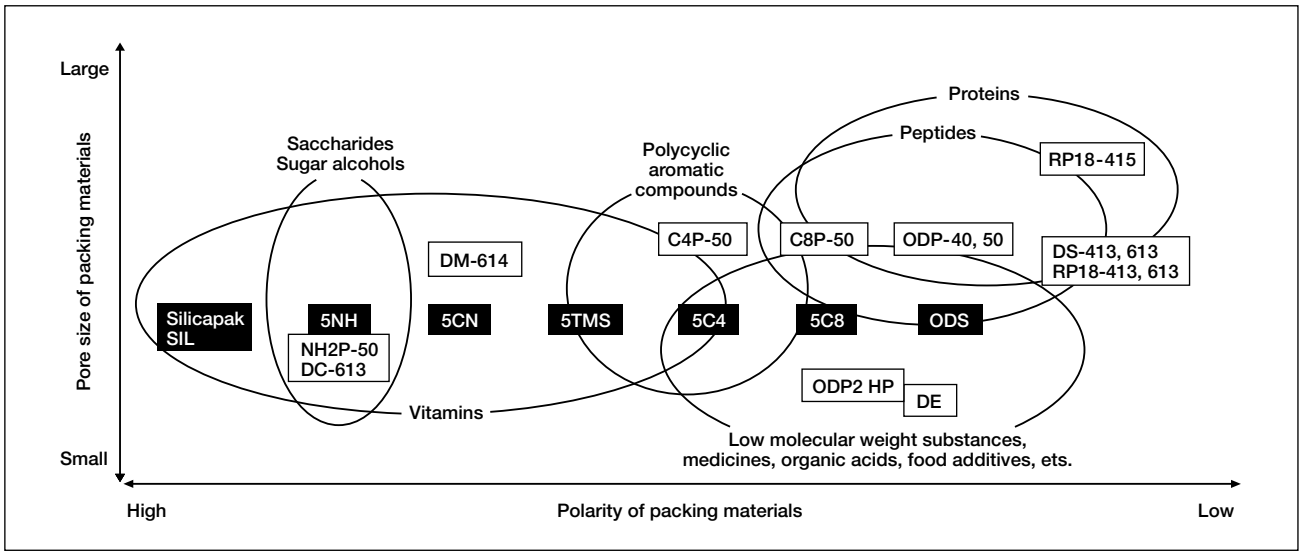
3-3-2. Introduction of Shodex columns

● Asahipak ODP, C8P, C4P series ● ODP2 HP series ● RSpak RP18, DS, DE series

In order to separate the target substance well, several kinds of columns are available with varying polarity and gel pore sizes (Figure 20). A smaller pore size is suitable for peptides, and larger pore size is suitable for proteins.

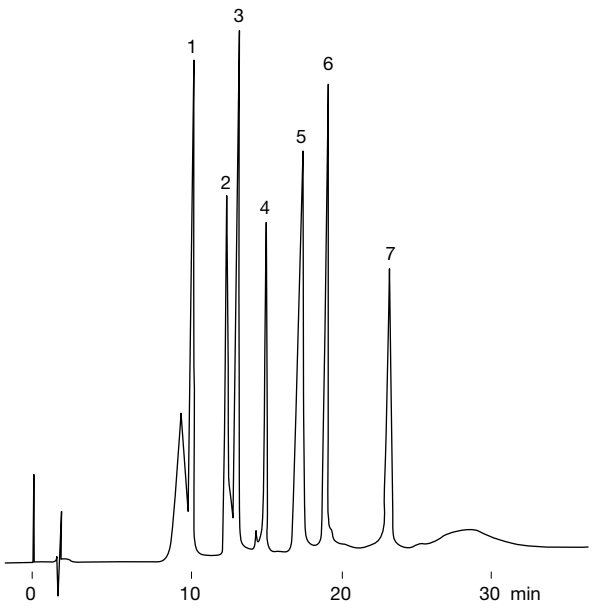
The RSpak RP18-415 column has the largest pore size (about 450 Å) in the Shodex lineup and is suitable for protein analysis. The pore size of Asahipak ODP, C8P and C4P columns is about 250 Å and these can be used for analyzing both proteins and peptides. The RSpak DE column has a smaller pore size (about 25 Å) and is suitable for analyzing smaller molecules like oligopeptides. The above-mentioned columns are polymer-based but can be used with similar analytical condition as silica-based ODS columns. In case the adsorption of the target substance is not sufficient with DE-613, DE-413 or ODS columns, RSpak RP18-413 and RP18-613 are recommended.

Fig. 20 Characteristics and applications of packing materials for normal & reversed phase columns



The chromatogram and recovery rate of proteins with the polymer-based reversed phase chromatography column RSpak RP18-415 are shown in Figure 21. The figure shows that the proteins and peptides are analyzed in 25 minutes with a high recovery rate. The maximum durable pressure is 22 MPa, so the flow rate can be increased for faster analysis.

Fig. 21

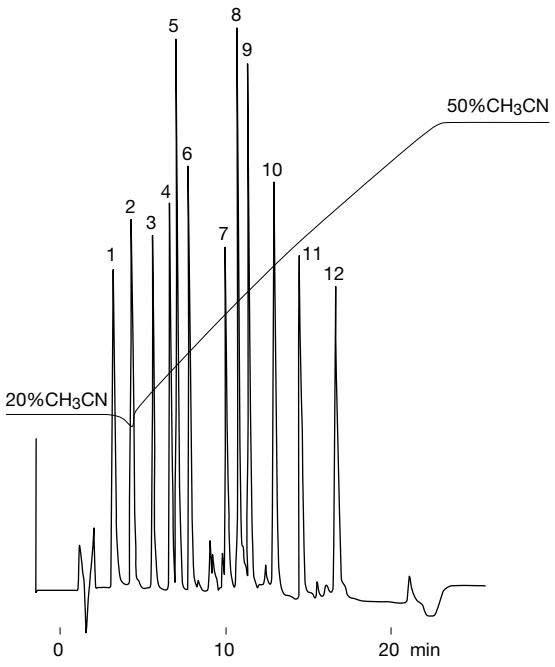


Recovery of proteins (%)	
1. Ribonuclease A	93
2. Insulin	98
3. Cytochrome c	100
4. Lysozyme	100
5. BSA	98
6. Myoglobin	108
7. Ovalbumin	—

Column : Shodex RSpak RP18-415 (4.6mmID x 150mm)
Eluent : (A) 0.1% TFA aq./CH_3CN=99/1 (B) 0.1% TFA aq./CH_3CN=5/95
Linear gradient : 0 to 25min, 20% (B) to 60% (B)
Flow rate : 1.0mL/min
Detector : UV (220nm)
Column temp. : Room temp.

Figure 22 shows the chromatogram of a mixed sample including proteins and peptides with gradient elution. The polymer-based Asahipak ODP-50 6D column is used for its excellent elution volume reproducibility for proteins and peptides. In addition, the results show a high recovery rate.

Fig. 22

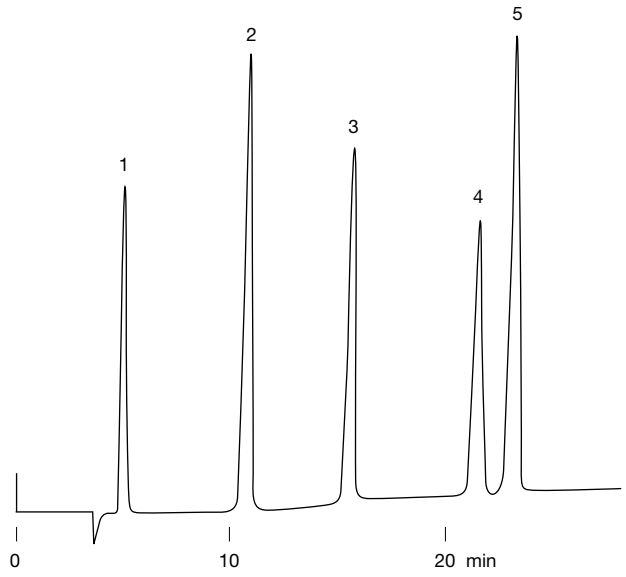


Proteins Peptides	Mw	VR(mL) First Analysis	VR(mL) Second Analysis	Recovery (%)
1. Lys-Bradykinin	1188	4.63	4.64	97
2. Bradykinin	1060	5.73	5.70	92
3. Met-Enkephalin	574	7.00	7.00	97
4. Neurotensin	1673	8.04	8.05	99
5. Leu-Enkephalin	556	8.47	8.48	100
6. Substance P	1348	9.24	9.26	93
7. Bacitracin	1450	11.47	11.48	81
8. Insulin	5750	12.24	12.25	95
9. Insulin B chain	3476	12.87	12.89	91
10. Lysozyme	14300	14.43	14.44	96
11. Mastoparan	1479	15.97	15.98	96
12. Myoglobin	17500	18.17	18.18	83

Column : Shodex Asahipak ODP-50 6D (6.0mm x 150mm)
Eluent : 0.05% TFA aq./CH_3CN
Linear gradient : 80/20 to 50/50, 20min
Flow rate : 1.0mL/min
Detector : UV (220nm)
Column temp. : 30℃

Figure 23 shows the separation of dipeptides with the polymer-based RSpak DE-613.

Fig. 23

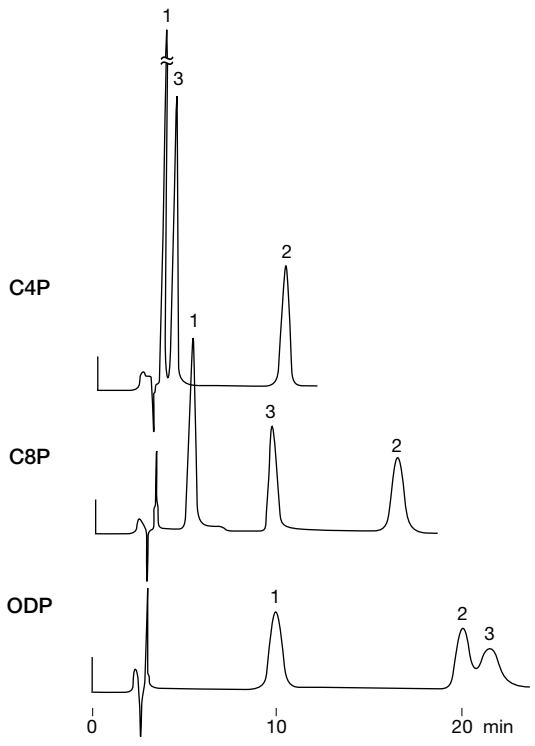


Sample : Dipeptides
1. Gly-val
2. Phe-Gly
3. Ala-Gly
4. Val-Phe
5. Tyr-Leu

Column	: Shodex RSpak DE-613 (6.0mmID x 150mm)
Eluent	: (A) 0.05% TFA aq./CH_3CN=96/4 (B) 0.05% TFA aq./CH_3CN=75/25
Linear gradient	: 0 to 30min, (A) to (B)
Flow rate	: 1.0mL/min
Detector	: UV (220nm)

Figure 24 shows the comparison of the retention of 3 kinds proteins with the polymer-based reversed phase chromatography columns Asahipak ODP-50, C8P-50 and C4P-50. The Octadecyl functional group (C_{18}) is bonded onto the base material of ODP-50, the Octyl group (C_8) is on C8P-50, and the Butyl group (C_4) is on C4P-50. The shorter alkyl chain has the weakest hydrophobic interaction. If the retention of the ODP-50 column is too strong, it is recommended to use C8P-50 or C4P-50 because the ratio of organic solvent in the eluent can be reduced and the analytical conditions for proteins and peptides can be milder.

Fig. 24



Sample :
1. β-Endorphin
2. Big gastrin
3. Insulin

Columns	: Shodex Asahipak ODP-50, C8P-50, C4P-50 (4.6mmID x 150mm each)
Eluent	: 0.05% TFA aq./CH_3CN=72/28
Flow rate	: 0.6mL/min
Detector	: UV (220 nm)
Column temp.	: 30℃

The Shodex Asahipak series line-up

Product	Size (mm) ID x L	Functional group	Base material	Pore size (Å)	Plate number (TPN/column)	Particle size (μm)
ODP-40 4D	4.6 x 150	Octadecyl	Polyvinyl alcohol	250	> 11,000	4
ODP-40 4E	4.6 x 250	Octadecyl	Polyvinyl alcohol	250	> 17,000	4
ODP-50 6D	6.0 x 150	Octadecyl	Polyvinyl alcohol	250	> 9,000	5
ODP-50 6E	6.0 x 250	Octadecyl	Polyvinyl alcohol	250	> 14,000	5
ODP-50G 6A	6.0 x 10	Octadecyl	Polyvinyl alcohol	—	(Guard column)	5
ODP-50 4B	4.6 x 50	Octadecyl	Polyvinyl alcohol	250	> 2,500	5
ODP-50 4D	4.6 x 150	Octadecyl	Polyvinyl alcohol	250	> 9,000	5
ODP-50 4E	4.6 x 250	Octadecyl	Polyvinyl alcohol	250	> 14,000	5
ODP-50G 4A	4.6 x 10	Octadecyl	Polyvinyl alcohol	—	(Guard column)	5
ODP-50 2D	2.0 x 150	Octadecyl	Polyvinyl alcohol	250	> 5,000	5
ODP-50G 2A	2.0 x 10	Octadecyl	Polyvinyl alcohol	—	(Guard column)	5
C8P-50 4D	4.6 x 150	Octyl	Polyvinyl alcohol	250	> 7,000	5
C8P-50 4E	4.6 x 250	Octyl	Polyvinyl alcohol	250	> 11,000	5
C8P-50G 4A	4.6 x 10	Octyl	Polyvinyl alcohol	—	(Guard column)	5
C4P-50 4D	4.6 x 150	Butyl	Polyvinyl alcohol	250	> 6,000	5
C4P-50 4E	4.6 x 250	Butyl	Polyvinyl alcohol	250	> 9,000	5
C4P-50G 4A	4.6 x 10	Butyl	Polyvinyl alcohol	—	(Guard column)	5

The following semi-micro and micro columns can be prepared for ODP-40.
• Inner diameter : 2.0mm, 1.0mm, 0.8mm, 0.5mm, 0.3mm • Length : 250mm, 150mm, 50mm

The Shodex RSpak series line-up

Product	Size (mm) ID x L	Functional group	Base material	Pore size (Å)	Plate number (TPN/column)	Particle size (μm)
RP18-415	4.6 x 150	—	Styrene divinylbenzene copolymer	450	> 5,000	6
RP18-613	6.0 x 150	—	Styrene divinylbenzene copolymer	200	> 13,000	3.5
RP18-413	4.6 x 150	—	Styrene divinylbenzene copolymer	200	> 11,000	3.5
RP18-G	4.6 x 10	—	Styrene divinylbenzene copolymer	—	(Guard column)	6
DS-613	6.0 x 150	—	Styrene divinylbenzene copolymer	200	> 6,500	6
DS-413	4.6 x 150	—	Styrene divinylbenzene copolymer	200	> 11,000	4
DS-G	4.6 x 10	—	Styrene divinylbenzene copolymer	—	(Guard column)	10
DE-613	6.0 x 150	—	Polymethacrylate	25	> 7,000	6
DE-413	4.6 x 150	—	Polymethacrylate	25	> 11,000	4
DE-413L	4.6 x 250	—	Polymethacrylate	25	> 17,000	4
DE-413S	4.6 x 50	—	Polymethacrylate	25	> 3,000	4
DE-G	4.6 x 150	—	Polymethacrylate	—	(Guard column)	10
DE-213	2.0 x 150	—	Polymethacrylate	25	> 3,000	4
DE-SG	2.0 x 10	—	Polymethacrylate	—	(Guard column)	6

The following semi-micro and micro columns can be prepared for DE-413.
• Inner diameter : 2.0mm, 1.0mm, 0.8mm, 0.5mm, 0.3mm • Length : 250mm, 150mm, 50mm

3-4. Hydrophobic interaction chromatography

3-4-1. Separation mechanism

The mechanism of hydrophobic interaction chromatography is based on the hydrophobic interaction between proteins and the surface of the packing material. It is like reversed phase chromatography, however eluent conditions differ. As the salt concentration increases, the hydrophobicity of the protein surface also increases and the protein adsorbs onto the packed material. This effect is similar to the “salting out” of proteins, a procedure usually carried out with ammonium sulfate. After adsorption, the adsorbed protein is eluted by decreasing the salt concentration thus weakening the hydrophobic interaction with the packing material.

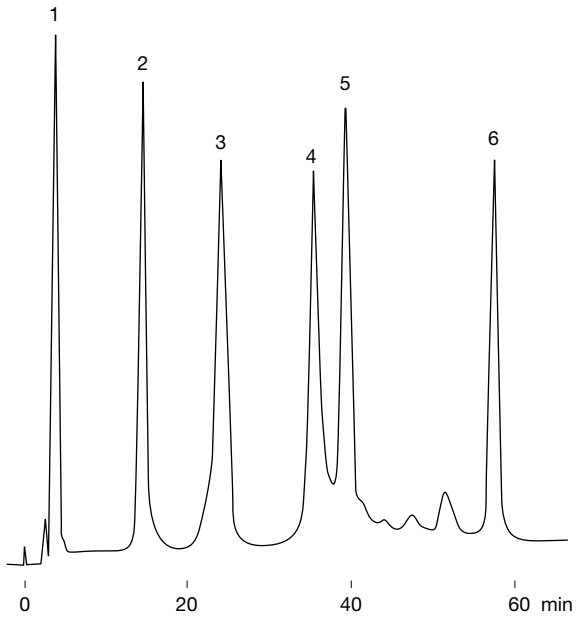
The protein is adsorbed under high salt concentration, so this separation mode is usable after ammonium sulfate precipitation or ion exchange chromatography. Moreover a less hydrophobic base like phenyl is used as the functional group, hence the analytical conditions for proteins are milder than the conditions of reversed phase chromatography, preserving the bio-activity of protein.

3-4-2. Introduction of Shodex columns

● HIC PH-814

Shodex HIC PH-814 is a column for hydrophobic interaction chromatography, with a phenyl group bound to the packing material as a hydrophobic ligand. Figure 25 shows the separation of 6 kinds of proteins with HIC PH-814.

Fig. 25

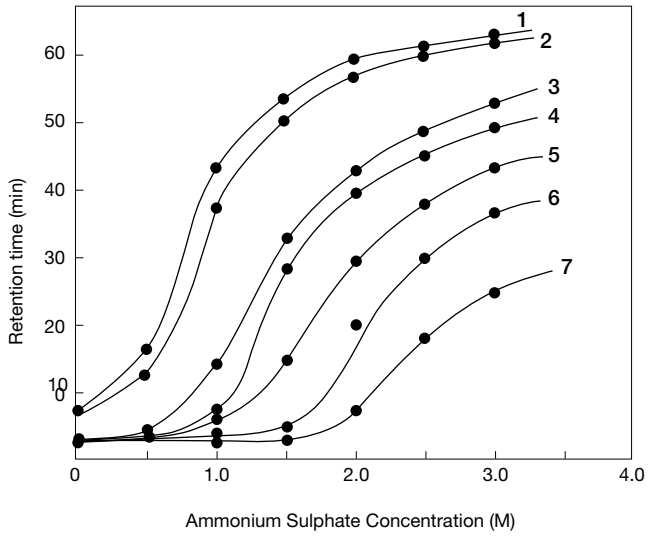


Sample :
1. Cytochrome c
2. Myoglobin
3. Ribonuclease A
4. Ovalbumin
5. Lysozyme
6. α Chymotrypsinogen

Column	: Shodex HIC PH-814 (8.0mmID x 75mm)
Eluent	: (A)=(B) + 1.8M Ammonium sulfate
	(B) 0.1M Phosphate buffer (pH7.0)
Linear gradient	: 0min to 60min, (A) to (B)
Flow rate	: 1.0mL/min
Detector	: UV (280nm)
Column temp.	: Room temp.

The effect of the ammonium sulfate concentration in the eluent on protein separation with HIC PH-814 is illustrated in Figure 26. The retention of proteins is controlled not only by the salt concentration but also by pH and temperature. The retention is stronger at higher pH or temperatures and weaker under lower pH and temperatures.

Fig. 26



Sample :
1. α Chymotrypsinogen A
2. α Chymotrypsin
3. Lysozyme
4. Ovalbumin
5. Ribonuclease A
6. Myoglobin
7. Cytochrome c

Column : Shodex HIC PH-814 (8.0mmID x 75mm)
Eluent : (A)=(B) + Ammonium sulfate
(B) 0.1M Phosphate buffer (pH7.0)
Linear gradient : (A) to (B)
Flow rate : 1.0mL/min

The Shodex HIC PH-814 column

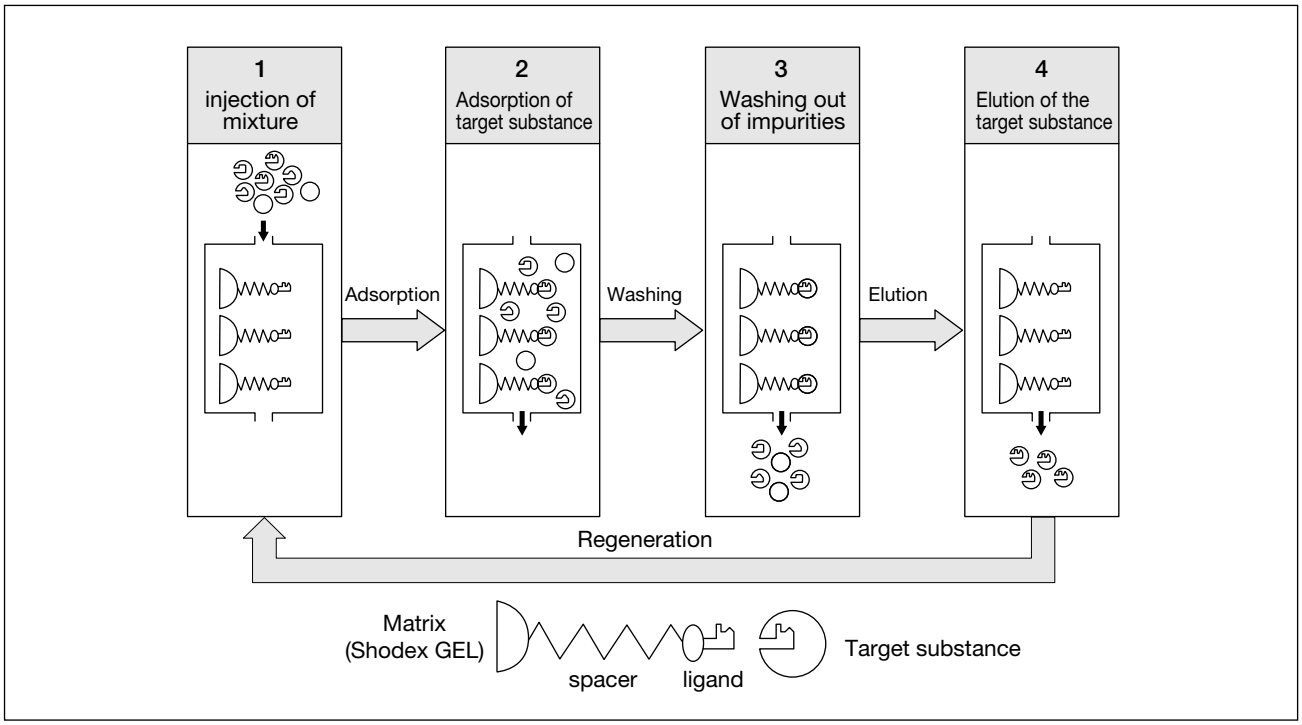
Product	Size (mm) ID x L	Functional group	Base material	Pore size (Å)	Particle size (μm)
HIC PH-814	8.0 x 75	Phenyl	Polyhydroxymethacrylate	2,000	10

3-5. Affinity chromatography

3-5-1. Separation mechanism

Affinity chromatography is a highly selective separation method relying on biochemical interaction.
The mechanism is explained in Figure 27.

Fig. 27



1) Inject sample into an initially equilibrated affinity chromatography column (AFpak).
2) Only the substances with affinity for the ligand are retained in the column.
3) Substances with no affinity for the ligand are eluted from the column without interacting with the stationary phase.
4) The substances retained in the column can be eluted by changing the pH or salt or organic solvent concentrations of the eluent.

3-5-2. Introduction of Shodex columns

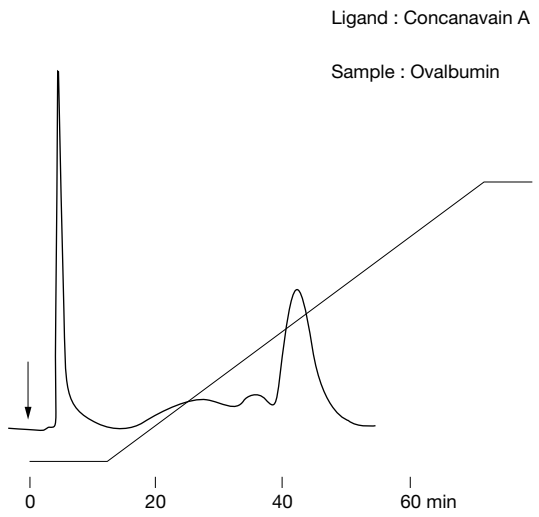
●AFpak series

The rigid polymer-based packing material used with the various ligands for Shodex AFpak columns, has the following advantages.

1) High-speed and high-pressure (5~10 MPa) analysis
2) Minimizes the detachment of ligands, ensuring highly reproducible analyses

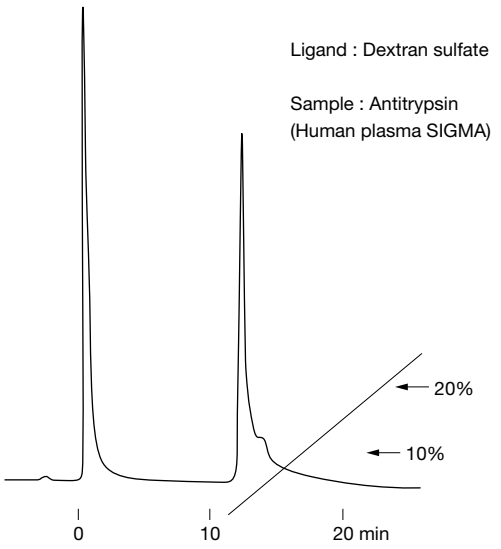
Application data with each AFpak column is shown below.

Fig. 28 Ovalbumin



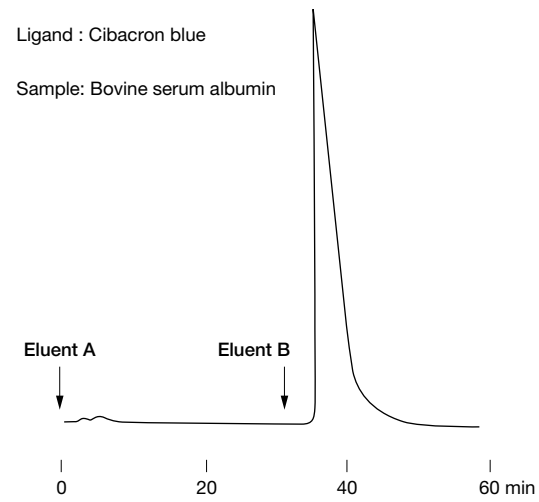
Column : Shodex AFpak ACA-894
(8.0mmID x 50mm)
Eluent : (A) 0.02M Tris-HCl buffer (pH7.4)
+ 0.5M NaCl
(B)= (A) + 0.05M α-Methyl-D(+)-glucoside
Linear gradient : (A) to (B) for 60min
Flow rate : 0.3mL/min
Detector : UV (280nm)
Column temp. : Room temp.

Fig. 29 α1 Antitrypsin



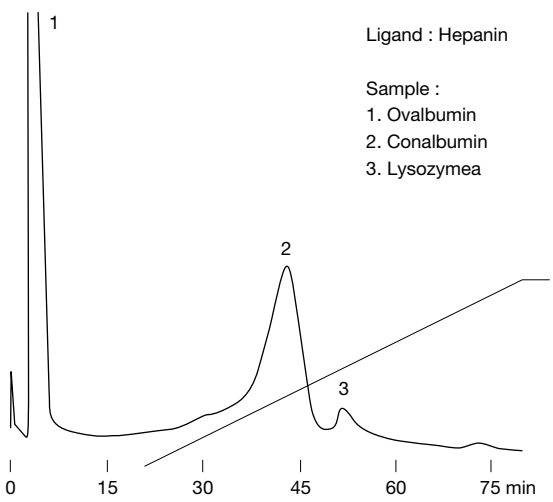
Column : Shodex AFpak ADS-894
(8.0mmID x 50mm)
Eluent : (A) 0.02M Tris-HCl (pH7.4)
(B) = (A) + 1.0M NaCl
Linear gradient : (A) to (B) for 60min
Flow rate : 1.0mL/min
Detector : UV (280nm)
Column temp. : Room temp.

Fig. 30 Bovine serum albumin



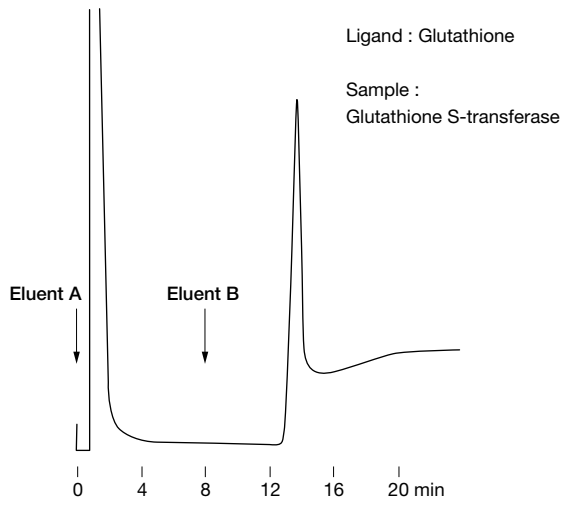
Column : Shodex AFpak ACB-894 (8.0mmID x 50mm)
Eluent : (A) 0.1M Potassium phosphate buffer (pH5.0)
(B) 0.1M Potassium phosphate buffer (pH7.5)
+ 1.5M KCl
Step gradient : (A) to (B)
Flow rate : 1.0mL/min
Detector : UV (280nm)
Column temp. : Room temp.

Fig. 31 Ovalbumin / Conalbumin / Lysozyme



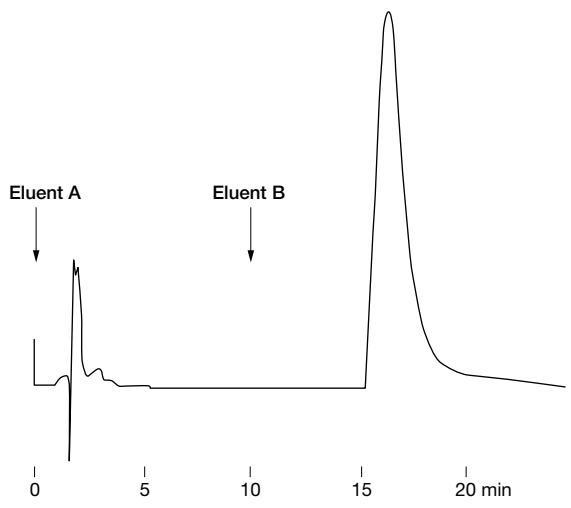
Column : Shodex AFpak AHR-894
(8.0mmID x 50mm)
Eluent : (A) 0.01M Tris-HCl (pH7.5) + 0.01M NaCl
(B) = (A) + 0.3 M NaCl
Linear gradient : (A) to (B)
Flow rate : 0.5mL/min
Detector : UV (280nm)
Column temp. : 25℃

Fig. 32 Glutathione S-transferase



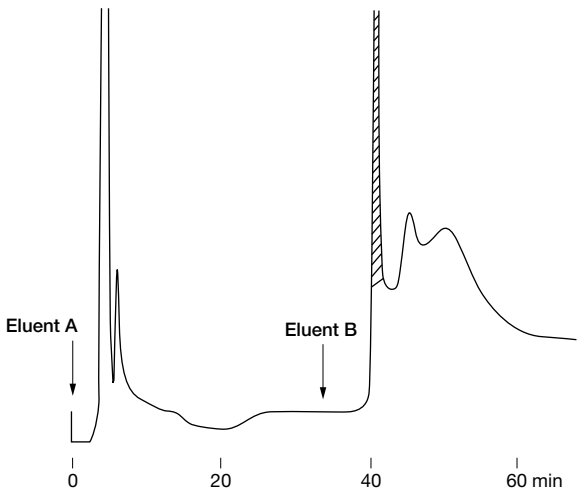
Column	: Shodex AFpak AGT-894 (8.0mm ID x 50mm)
Eluent	: (A) 22mM Phosphate buffer (pH7.0) (B) 50mM Tris buffer (pH9.6) + 5mM Glutathione
Step gradient	: (A) to (B)
Flow rate	: 1.0mL/min
Detector	: UV (280nm)
Column temp.	: Room temp.

Fig. 33 L-lactate dehydrogenasepsin



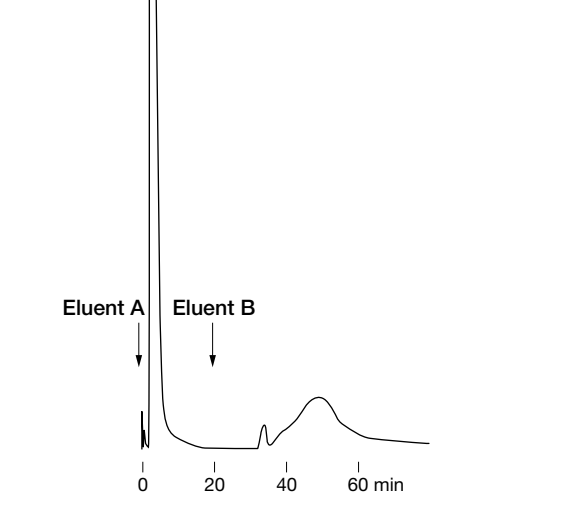
Column	: Shodex AFpak ANA-894 (8.0mmID x 50mm)
Eluent	: (A) 0.01M Potassium phosphate buffer (pH7.0) (B) = (A) + 0.5M KCl
Step gradient	: (A) to (B)
Flow rate	: 1.0mL/min
Detector	: UV (280nm)
Column temp.	: Room temp.

Fig. 34 Super oxide dismutase



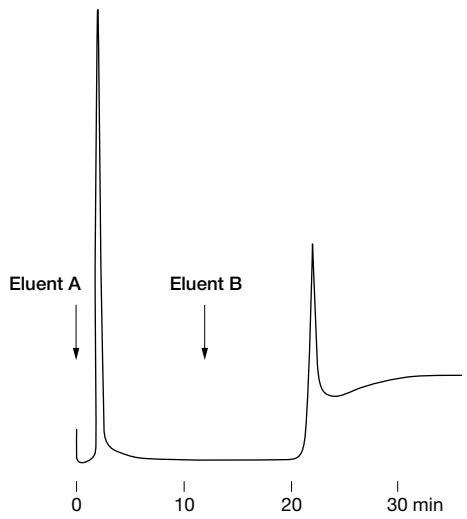
Column	: Shodex AFpak AIA-894 (8.0mmID x 50mm)
Eluent	: (A) 0.1M Sodium acetate buffer (pH7.7) + 0.5M NaCl (B) 0.05M Tris-HCl (pH8.0) + 0.15M NH_4Cl
Step gradient	: (A) to (B)
Flow rate	: 0.5mL/min
Detector	: UV (280nm)
Column temp.	: Room temp.

Fig. 35 Fibrinogen (Sigma)



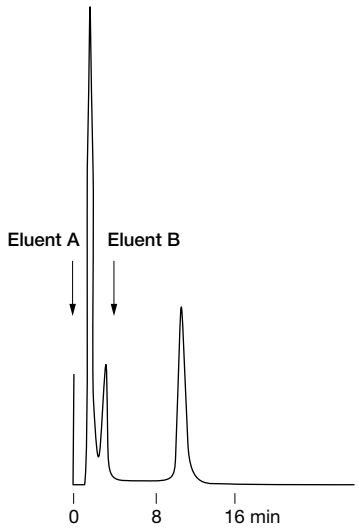
Column	: Shodex AFpak ALC-894 (8.0mmID x 50mm)
Eluent	: (A) 0.05M Tris-HCl (pH7.4) + 0.15M NaCl + 0.5mM $CaCl_2$ + 0.5mM $MnCl_2$ (B) = (A) + 1M0.1 M Methyl mannoside
Step gradient	: (A) to (B)
Flow rate	: 0.5mL/min
Detector	: UV (280nm)
Column temp.	: Room temp.

Fig. 36 Plasminogen (from bovine plasma, Sigma)



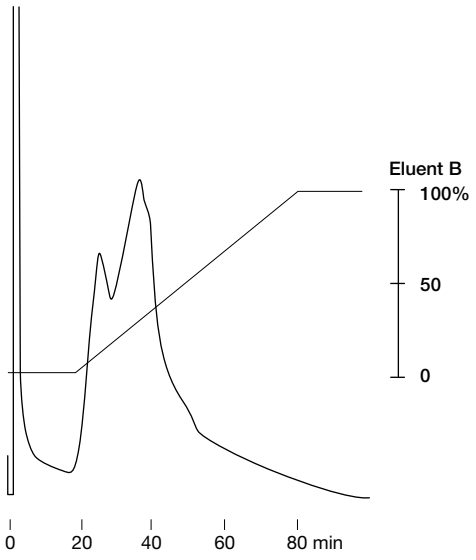
Column : Shodex AFpak ALS-894 (8.0mmID x 50mm)
Eluent : (A) 0.1M Sodium acetate (pH7.4)
(B) 0.1M Sodium acetate +
0.2M 6-aminohexanoic acid (pH4.0)
Step gradient : (A) to (B)
Flow rate : 1.0mL/min
Detector : UV (280nm)
Column temp. : Room temp.

Fig. 37 Human serum



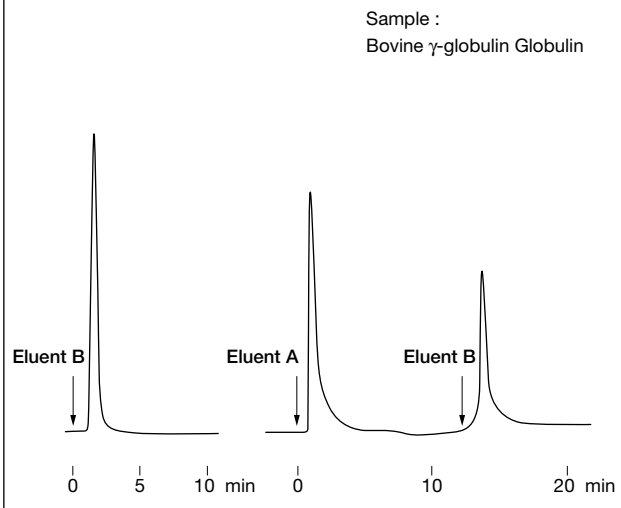
Column : Shodex AFpak APG-894
(8.0mmID x 50mm)
Eluent : (A) 0.2M Sodium phosphate buffer (pH4.0)
(B) 0.1M Sodium citrate buffer (pH2.5)
Step gradient : (A) to (B)
Flow rate : 1.0mL/min
Detector : UV (280nm)
Column temp. : Room temp.

Fig. 38 Globulin Bovine γ-globulin (Sigma)



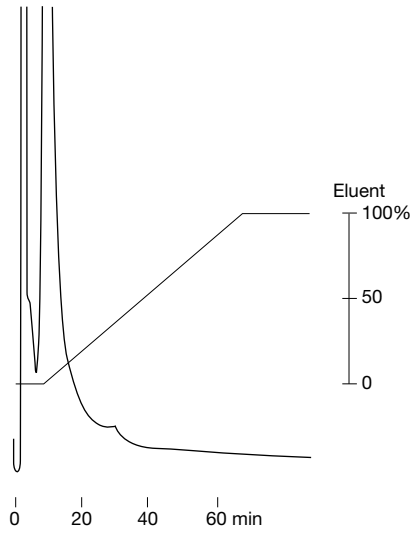
Column : Shodex AFpak APR-894 (8.0mmID x 50mm)
Eluent : (A) 0.01M Sodium phosphate buffer (pH6.5)
(B) = (A) + 1.0M NaCl
Flow rate : 1.0 mL/min
Detector : UV (280nm)
Column temp. : Room temp.

Fig. 39 Bovine γ-globulin Globulin



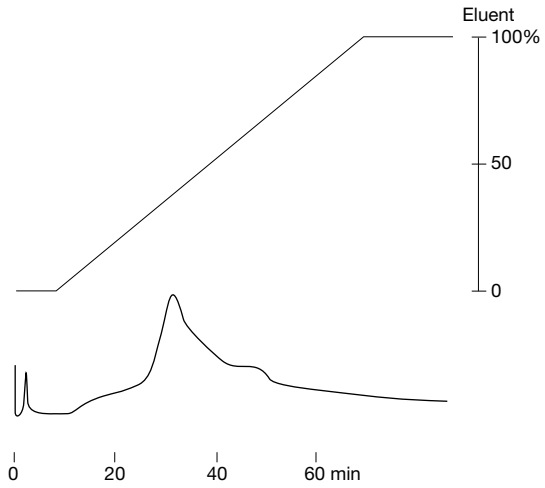
Column : Shodex AFpak APA-894 (8.0mmID x 50mm)
Eluent : (A) 0.1M Sodium phosphate buffer (pH4.5)
(B) 0.1M Sodium phosphate buffer (pH3.0)
Flow rate : 1.0mL/min
Detector : UV (280nm)
Column temp. : Room temp.

Fig. 40 Globulin Human γ-globulin (Sigma)



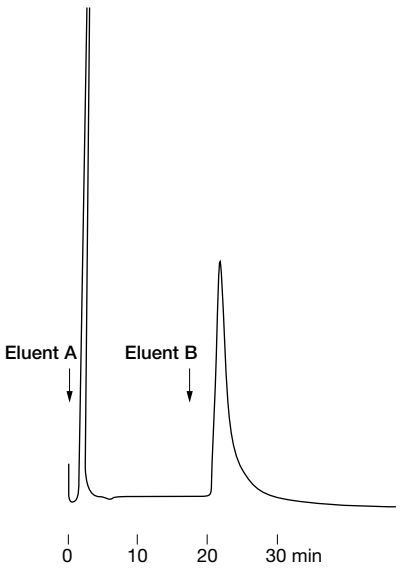
Column	: Shodex AFpak APR-894 (8.0mmID x 50mm)
Eluent	: (A) 0.01M Sodium phosphate buffer (pH6.5) (B) = (A) + 1.0M NaCl
Linear gradient	: (A) to (B)
Flow rate	: 1.0mL/min
Detector	: UV (280nm)
Column temp.	: Room temp.

Fig. 41 Human IgM (Sigma)



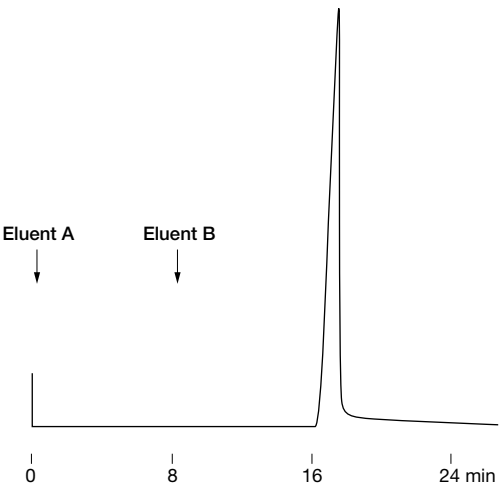
Column	: Shodex AFpak APR-894 (8.0mmID x 50mm)
Eluent	: (A) 0.02M Sodium phosphate buffer (pH6.5) (B) (B) = (A) + 1.0M NaCl
Linear gradient	: (A) to (B)
Flow rate	: 1.0mL/min
Detector	: UV (280nm)
Column temp.	: Room temp.

Fig. 42 Human serum albumin (Sigma)



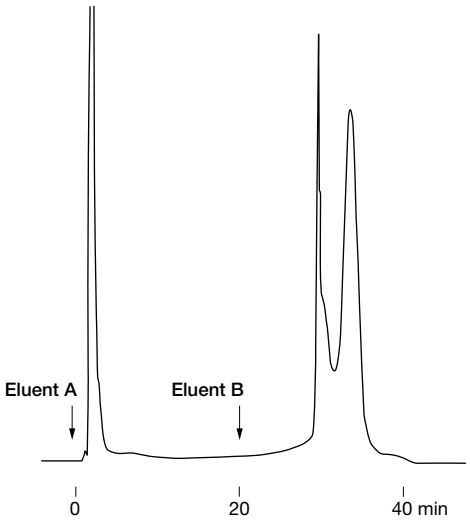
Column	: Shodex AFpak APD-894 (8.0mmID x 50mm)
Eluent	: (A) 0.02M Tris-HCl buffer (pH5.0) (B) = (A) + 2.0M KCl
Step gradient	: (A) to (B)
Flow rate	: 1.0mL/min
Detector	: UV (280nm)
Column temp.	: Room temp.

Fig. 43 C-Reactive protein (Sigma)



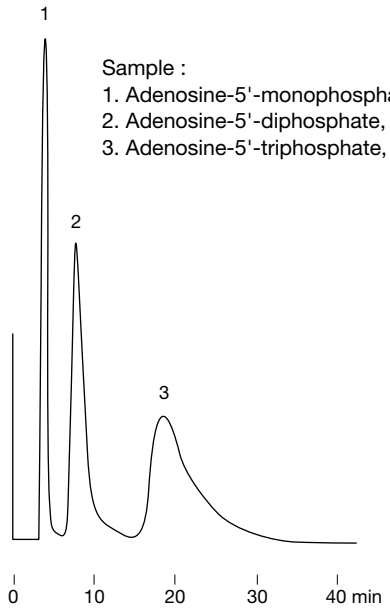
Column	: Shodex AFpak APE-894 (8.0mmID x 50mm)
Eluent	: (A) Tris-HCl (pH7.0) + 0.15M NaCl + 1mM $CaCl_2$ (B) = (A) + 0.2M Phosphorylcholine
Step gradient	: (A) to (B)
Flow rate	: 1.0mL/min
Detector	: UV (280nm)
Column temp.	: Room temp.

Fig. 44 Trypsin



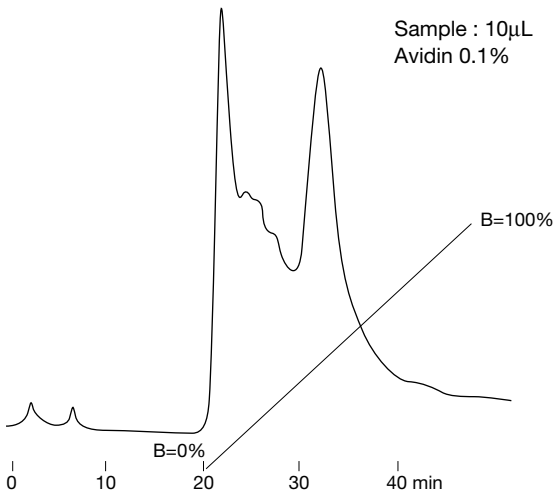
Column : Shodex AFpak AAB-894 (8.0mmID x 50mm)
Eluent : (A) 0.1 M Sodium acetate buffer (pH7.0)
+ 0.5M NaCl
(B) 0.1 M Sodium acetate buffer (pH2.8)
+ 0.5M NaCl
Step gradient : (A) to (B)
Flow rate : 1.0 mL/min
Detector : UV (280 nm)
Column temp. : Room temp.

Fig. 45 Adenosine phosphate



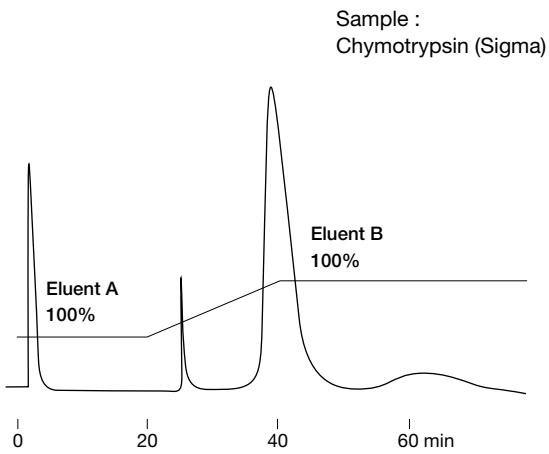
Column : Shodex AFpak AAF-894 (8.0mmID x 50mm)
Eluent : 0.1M Ethylmorpholine-acetate buffer (pH7.0)
Flow rate : 1.0mL/min
Detector : UV (260nm)
Column temp. : Room temp.

Fig. 46 0.1% Avidin, 10μL



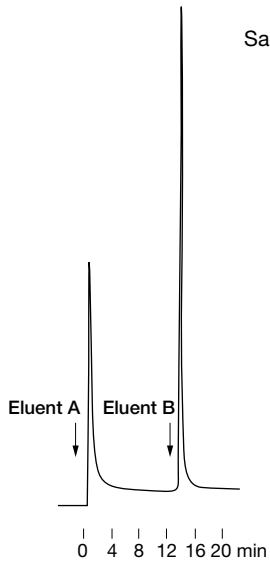
Column : Shodex AFpak ABT-894 (8.0mmID x 50mm)
Eluent : (A) 0.01M Sodium phosphate buffer (pH7.0)
(B) = (A) + 1.0M NaCl
Linear gradient : 0min to 20min, (A), 20min to 50min,
(A) to (B)
Flow rate : 0.5mL/min
Detector : UV (230nm)
Column temp. : Room temp.

Fig. 47 Chymotrypsin



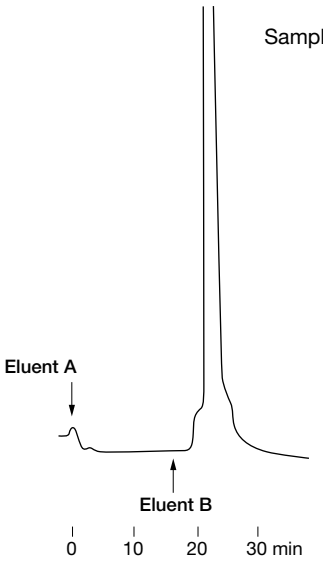
Column : Shodex AFpak AST-894 (8.0mmID x 50mm)
Eluent : (A) 0.1M Sodium acetate buffer (pH7.7)
+ 0.5M NaCl
(B) 0.1M Sodium acetate buffer (pH2.8)
+ 0.5M NaCl
Linear gradient : 0min to 20min, (A), 20min to 40min,
(A) to (B), after 40min, (B)
Flow rate : 1.0 mL/min
Detector : UV (280 nm)
Column temp. : Room temp.

Fig. 48 Lysozyme



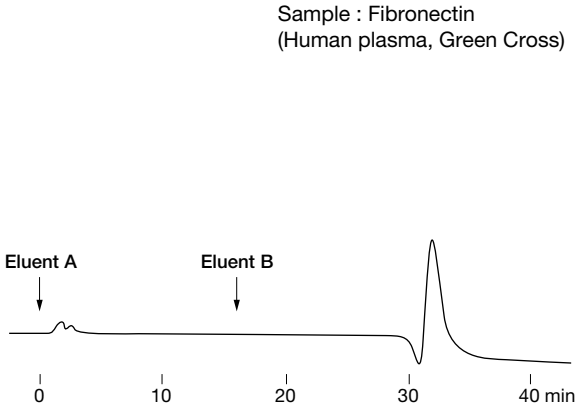
Column : Shodex AFpak AGA-894 (8.0mmID x 50mm)
Eluent : (A): 0.01M Tris-HCl buffer (pH7.5) + 0.01M NaCl
(B) 0.01M Tris-HCl buffer (pH7.5) + 0.5M NaCl
Step gradient : (A) to (B)
Flow rate : 0.5mL/min
Detector : UV (280nm)
Column temp. : Room temp.

Fig. 49 Lysozyme



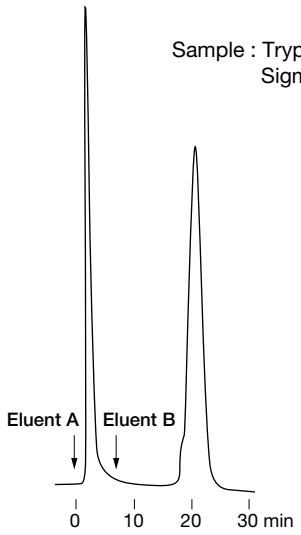
Column : Shodex AFpak AHR-894 (8.0mmID x 50mm)
Eluent : (A) 0.01M Tris-HCl buffer (pH7.5) + 0.01M NaCl
(B) 0.01M Tris-HCl buffer (pH7.5) + 0.3M NaCl
Step gradient : (A) to (B)
Flow rate : 1.0 mL/min
Detector : UV (280nm)
Column temp. : Room temp.

Fig. 50 Fibronectin



Column : Shodex AFpak AGE-894 (8.0mmID x50mm)
Eluent : (A) 50mM Tris-HCl buffer (pH7.4) + 0.01M NaCl
(B) = (A) + 4M Urea
Step gradient : (A) to (B)
Flow rate : 0.3mL/min
Detector : UV (280nm)
Column temp. : Room temp.

Fig. 51 Trypsin (type I)



Column : Shodex AFpak AOV-894 (8.0mmID x 50mm)
Eluent : (A) 0.1M Sodium acetate buffer (pH7.7)
+ 0.5M NaCl
(B) 0.1M Sodium acetate buffer (pH2.8)
+ 0.5M NaCl
Step gradient : (A) to (B)
Flow rate : 1.0mL/min
Detector : UV (280nm)
Column temp. : Room temp.

The Shodex AFpak (Column size : I.D. 8.0mm x L 50mm)

Product AFpak	Ligand	Application	Ligand load /gel(g)	Capacity	Particle size (μm)
AAB-894	Aminobenzamidine	Serine protease	100μmol	—	18
AAM-894	5'AMP	NAD, ATP dependent enzymes	10μmol	Lactic dehydronase 1.5mg/g	18
AAP-894	Aprotinin	Serine proteases	5mg	Trypsin 3mg/g	18
ABT-894	Biotin	Avidin	—	Avidin 9mg/g	18
ACA-894	Concanavalin A (ConA)	Glycoprotein, polysaccharides	15mg	—	18
ACB-894	Cibacron Blue	Albumin, NDA dependent enzymes	40μmol	BSA 20mg/column	18
ADS-894	Dextran sulfate	Lipoproteins, blood coagulation factors	30mg	LDL 5mg/g	18
AED-894	Ethylenediamine diacetic acid	Nucleic acids, serum proteins	30μmol	—	18
AGA-894	N-acetyl-glucosamine	Lectins, carbohydrate metabolizing enzymes	—	Lysozyme 0.6mg	18
AGE-894	Gelatin	Fibronectin	30mg	Fibronectin 150mg/g	18
AGT-894	Glutathione	Enzymes related to glutathione	50μmol	—	18
AHR-894	Heparin	Lipoproteins, blood coagulation factors	5mg	Lysozyme 4mg/column	18
AIA-894	Iminodiacetic acid	Interferon, serum proteins	70μmol	BSA 70mg/column	18
ALC-894	Lentil lectin	Glycoproteins, polysaccharides	6-7mg	—	18
ALS-894	Lysine (LCA)	Plasminogen, plasminogen activator, RNA, DNA	—	Plasminogen 54μg/g	18
ANA-894	NAD	NAD-Dependent dehydrogenase	10μmol	Lactic dehydronase 1.4mg/g	18
AOV-894	Ovomucoid	Trypsin-like protease	10mg	Trypin 100mg/g	18
APA-894	Protein A	Human IgG, immune complexes	4mg	IgG Human 20mg/g	18
APB-894	Aminophenyl boronic acid	Nucleic acids and cathecholamines	800μmol	Sorbitol 0.2mg/column	10
APD-894	Procion red	NAD, NADP, interferon enzymes	40-60μmol	BSA 72mg/g	18
APE-894	Phosphorylethanolamine	C-reactive protein, enzymes	—	C-reactive protein 0.91mg/g	18
APG-894	Protein G	IgG immune complex	4-5mg	IgG Humnan 10mg/g	18
APH-894	Phenyl alanine	Subtilisin Carlsberg	50μmol	Subtilisin Carlsberg 9mg/g	18
APR-894	Proteamine	IgM	4-5mg	IgM Human 1.9mg/column	18
ARC-894	RCA-I	Glycoproteins, polysaccharides	20mg	—	18
AST-894	Soybean trypsin inhibitor	Trypsin-like proteases	20mg	Trypsin 100mg/g	18
AWG-894	Wheat germ agglutinin (WGA)	Glycoproteins, polysaccharides	14mg	—	18
ACH-494	Choline oxydase, acetylcholine esterase	Choline, acetylcholine	—	—	18

The following semi-micro and micro columns can be prepared.

• Inner diameter : 2.0mm, 1.0mm, 0.8mm, 0.5mm, 0.3mm • Length : 50mm, 35mm

3-6. Multi mode chromatography

3-6-1. Separation mechanism

Multi mode chromatography is an analytical method combining several separation modes. When a single separation mode is not enough for separating the target substance, an improvement can be expected by using multi mode chromatography. The balance between the separation modes involved can be controlled by changing the eluent composition.

3-6-2. Introduction of Shodex columns

●Asahipak GS series

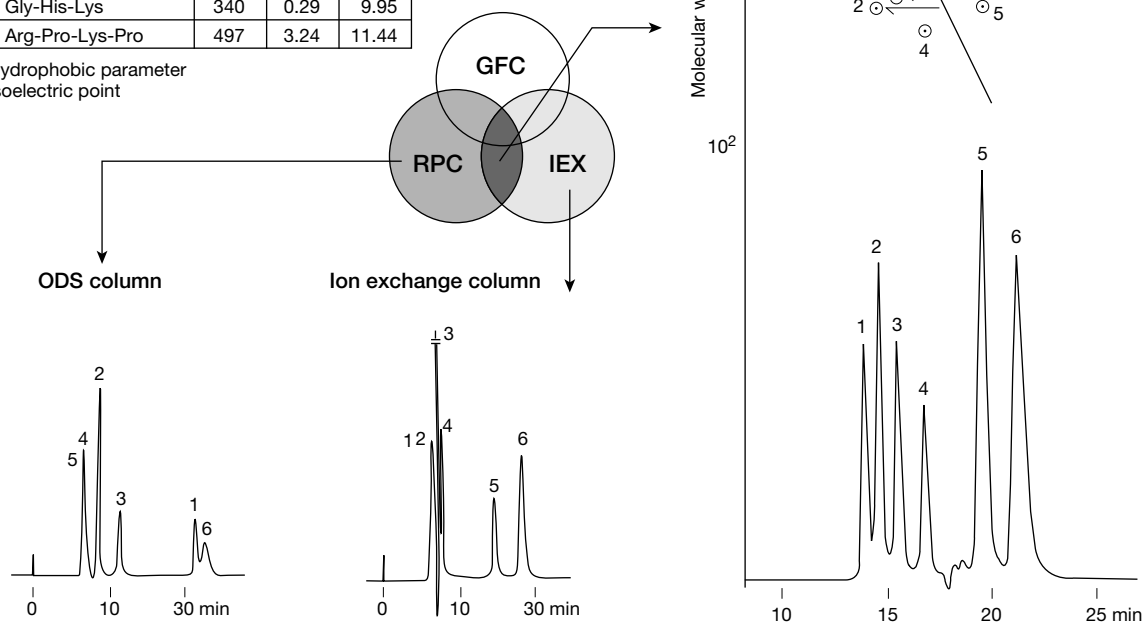
The Shodex Asahipak GS-HQ series mainly relies on size exclusion chromatography but also works in multi mode with the combination of reversed phase and ion exchange modes One can therefore expect different separation patterns from those obtained by standard size exclusion chromatography. The GS-HQ series is particularly suited for the separation of hydrophilic peptides, especially acidic peptides and basic peptides (See Figure 52). The base material is a polymer, resulting in the following advantages; 1) usable at a wide pH range, 2) washable with alkaline solutions, 3) column durability.

Fig. 52

Sample :

No.		MW	Σf	pI
1	Val-Glu-Glu-Ala-Glu	576	1.78	3.02
2	Glu-Ala-Glu	347	0.39	3.12
3	Val-Glu-Ser-Glu	390	0.83	3.12
4	Arg-Asp	289	0.68	6.75
5	Gly-His-Lys	340	0.29	9.95
6	Arg-Pro-Lys-Pro	497	3.24	11.44

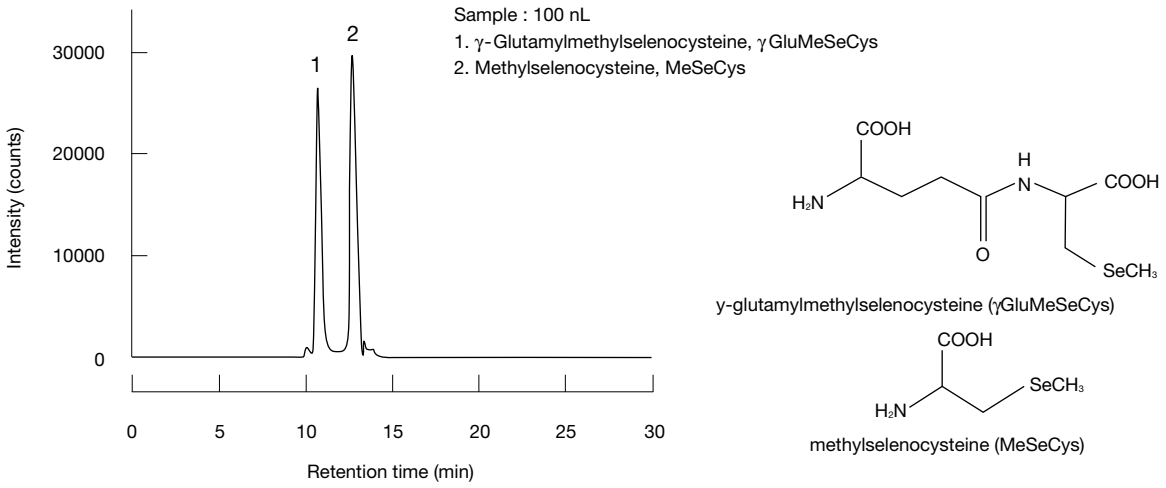
Σf : Hydrophobic parameter
pI : Isoelectric point



Column : Shodex Asahipak GS-320 HQ (7.5mmID x 300mM)
Eluent : 50mM Ammonium acetate buffer (pH6.7)
Flow rate : 0.5mL/min
Detector : UV (220nm)
Column temp. : 30˚C

Metal-bonded amino acids can be analyzed by multi mode chromatography. Figure 53 shows the investigation of metal-bonded amino acids which is important for the study of how metals are metabolized in the human body. Selenized amino acids are analyzed using the micro column GS320A-M5D (0.5 mm ID x 150 mm) and ICP/MS. The sensitivity of the analysis using this micro column is higher than with a conventional type column.

Fig. 53



Column : Shodex GS320A-M5D (0.5mmID x 150mm)
Eluent : 50mM CH_3COONH_4 buffer (pH6.5)
Flow rate : 2.0μL/min
Detector : ICP-MS (Se at m/z=82)
Courtesy of Dr. Yasumitsu Ogra, Graduate School of Pharmaceutical Sciences, Chiba University

The Shodex Asahipak GS series

Product	Size (mm) ID x L	Plate Number (TP/column)	Exclusion Limit (Pullulan)	Particle size (μm)	Pore size (Å)		Usable pH range
					Max.	Avg	
GS-220 HQ	7.5 x 300	> 19,000	3,000	6	150		2 to 9
GS-320 HQ	7.5 x 300	> 19,000	40,000	6	400		2 to 12
GS-520 HQ	7.5 x 300	> 18,000	300,000	7	2,000		2 to 12
GS-620 HQ	7.5 x 300	>18,000	2,000,000	7	7,000		2 to 12
GS-2G 7B	7.5 x 50	(Guard column)	—	9	—		2 to 12

Max. usable organic solvent concentrations:
GS-320 HQ, GS-520 HQ, GS-620 HQ : 100% Methanol, 50% Acetonitrile
GS-220 HQ : 30% Methanol, 50% Acetonitrile

The following semi-micro and micro columns can be prepared.
• Inner diameter : 4.6mm, 2.0mm, 1.0mm, 0.8mm, 0.5mm, 0.3mm • Length : 250mm, 150mm, 50mm

Shodex™

HPLC Columns

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