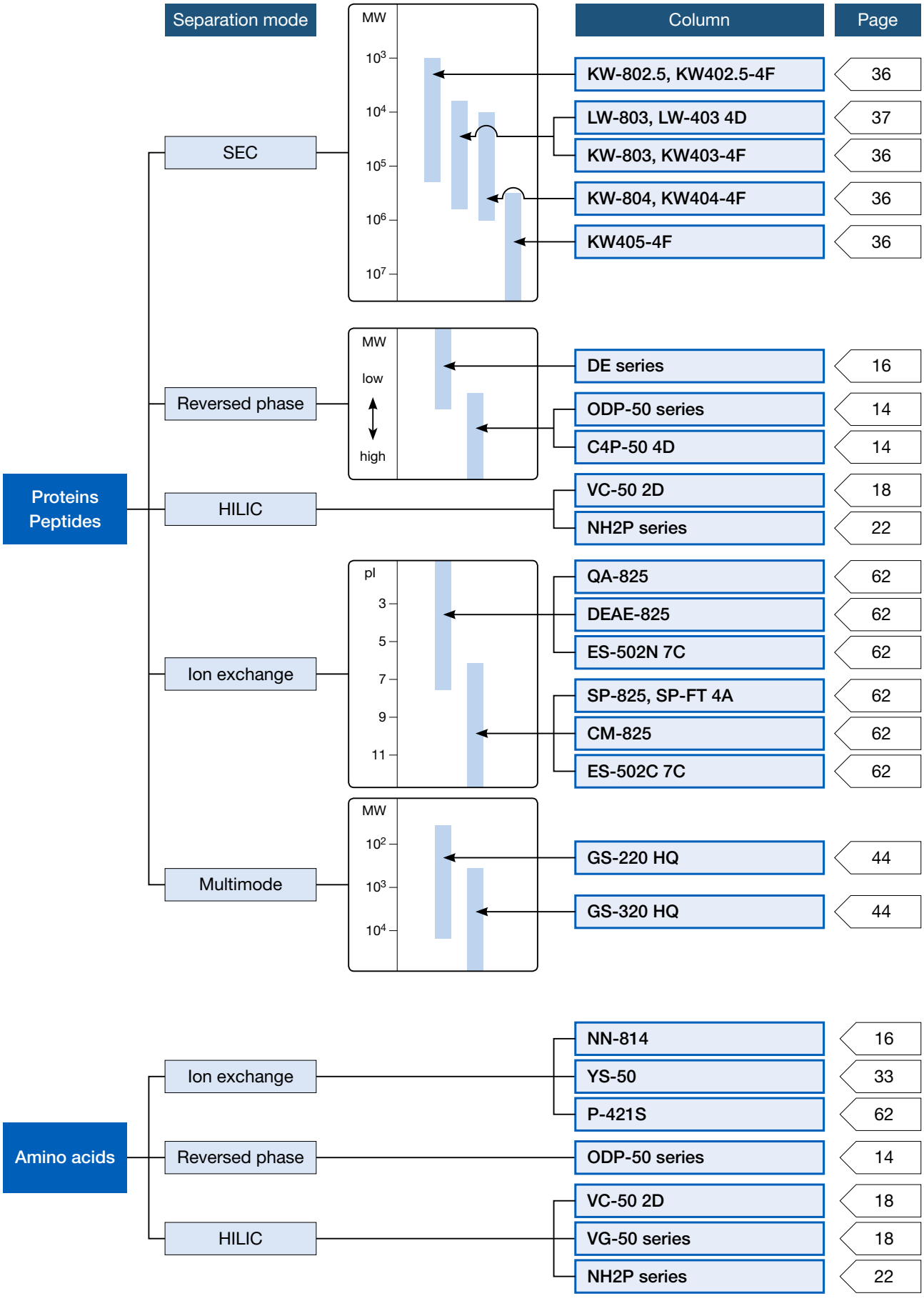
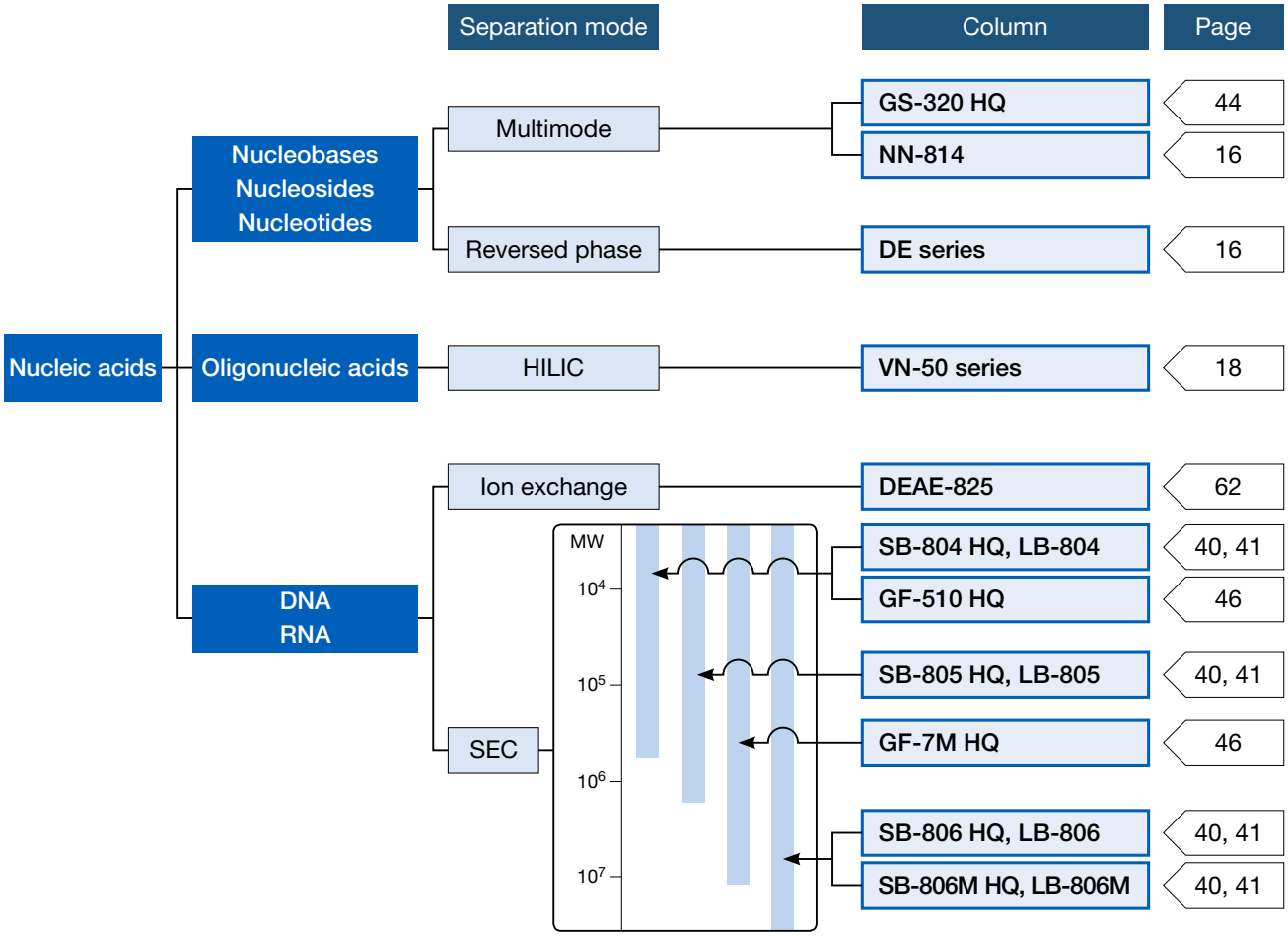
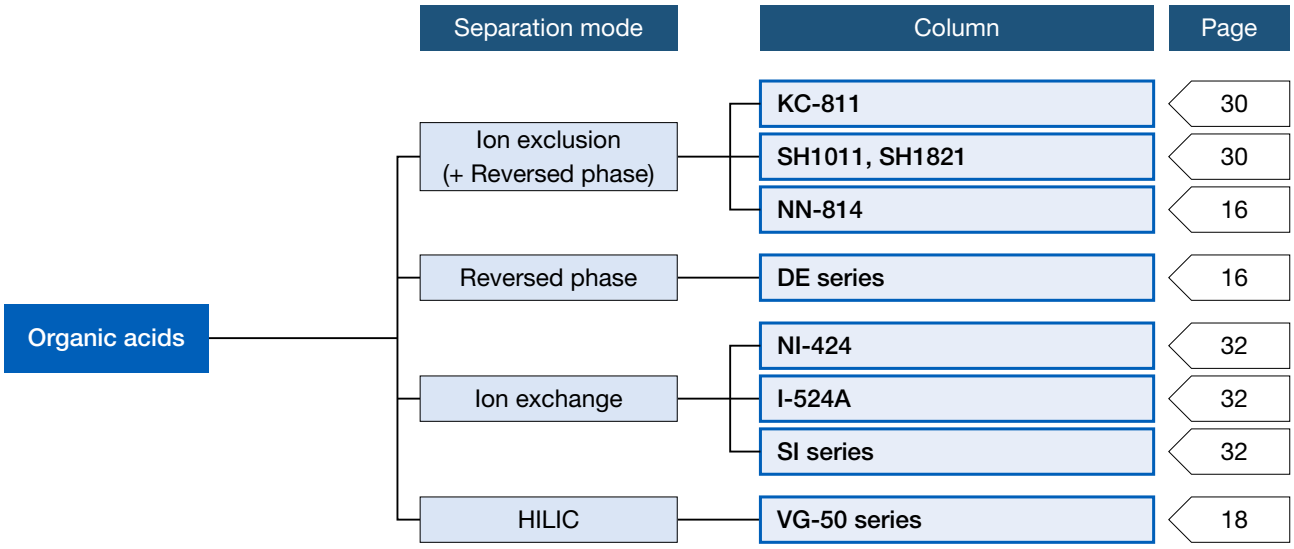
Column Selection (Proteins, Peptides, and Amino Acids)



Column Selection (Nucleic Acids)

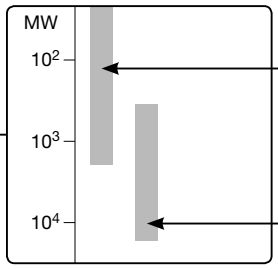


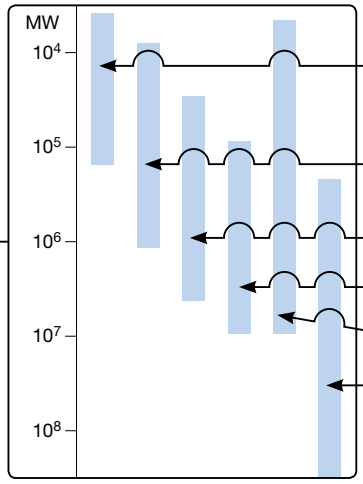
Column Selection (Organic Acids)



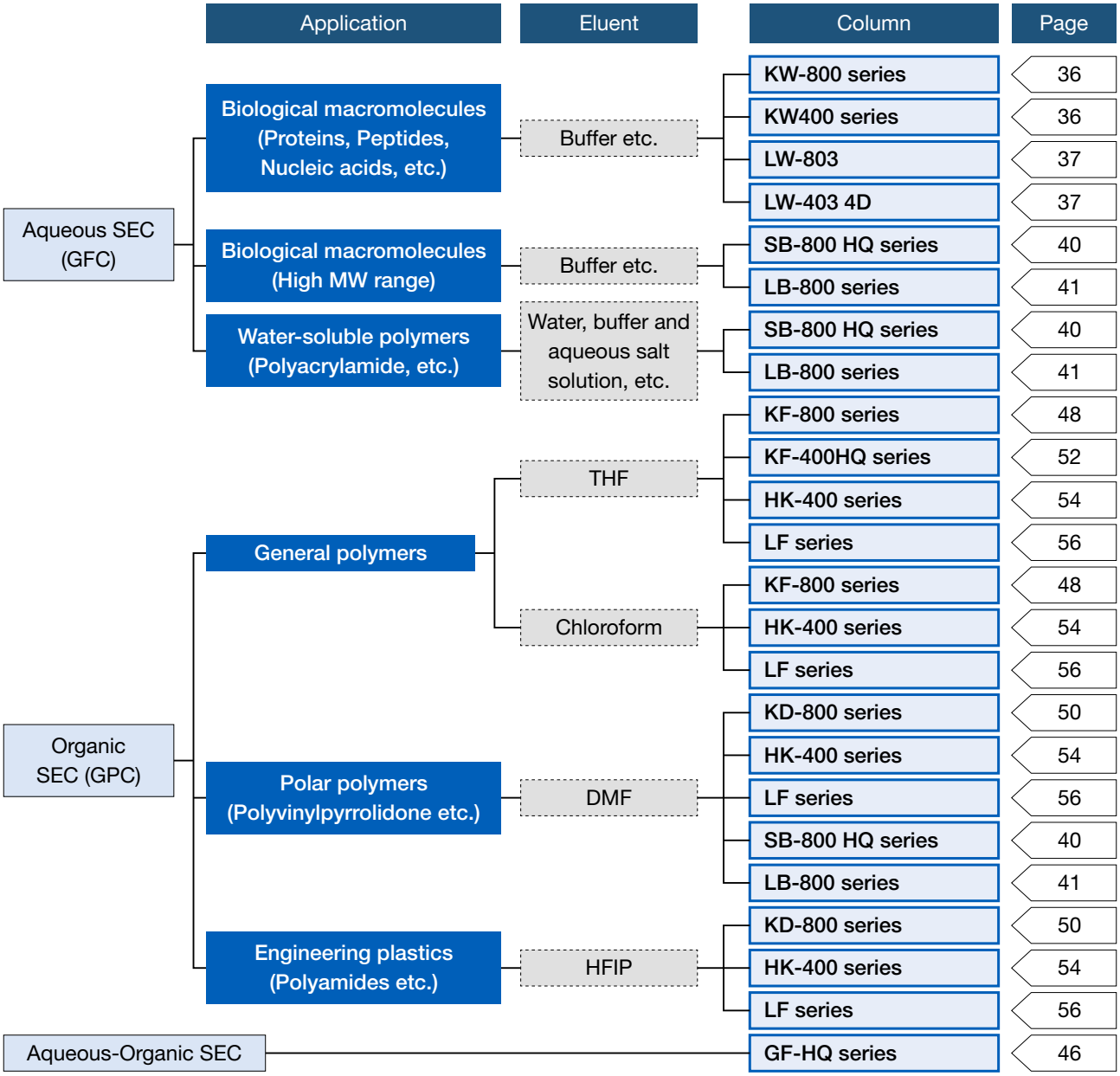
Column Selection (Saccharides)

	Separation mode	Column	Page
Mono-, di-saccharides, and sugar alcohols Saccharides and sugar alcohols	Ligand exchange + SEC	SP0810 (Pb^{2+})	26
		SC1011 (Ca^{2+})	26
		KS-801 (Na^{+})	26
	Ligand exchange + HILIC	SZ5532 (Zn^{2+})	26
		DC-613 (Na^{+})	26
	HILIC	VG-50 series	18
		NH2P series	22
Sugar alcohols	Ligand exchange + HILIC	SC1211 (Ca^{2+})	26
Oligosaccharides and sugar alcohols	Ligand exchange + SEC	KS-801 (Na^{+}) + KS-802 (Na^{+})	26
Amino sugars	HILIC	VG-50 series	18
		NH2P series	22
	Ion exchange	SC1011 (Ca^{2+})	26
Acidic sugars	Ion exclusion	SH1011 (H^{+})	30
		KC-811	30
	Ion exchange	VT-50 2D	18
		NH2P series	22
Saccharides and organic acids	Ion exclusion + SEC	SH1011 (H^{+}), SH1821 (H^{+})	30
Oligosaccharides	SEC	KS-801 (Na^{+})	26
		SB-802 HQ	40
		GS-220 HQ	44
		KS-802 (Na^{+})	26
		SB-802.5 HQ, LB-802.5	40, 41
		GS-320 HQ	44
	HILIC	VN-50 series	18
		NH2P series	22
Polysaccharides	SEC	KS-803 (Na^{+})	26
		SB-803 HQ, LB-803	40, 41
		KS-804 (Na^{+})	26
		SB-804 HQ, LB-804	40, 41
		SB-805 HQ, LB-805	40, 41
		SB-806 HQ, LB-806	40, 41
		SB-806M HQ, LB-806M	40, 41
		SB-807 HQ	40

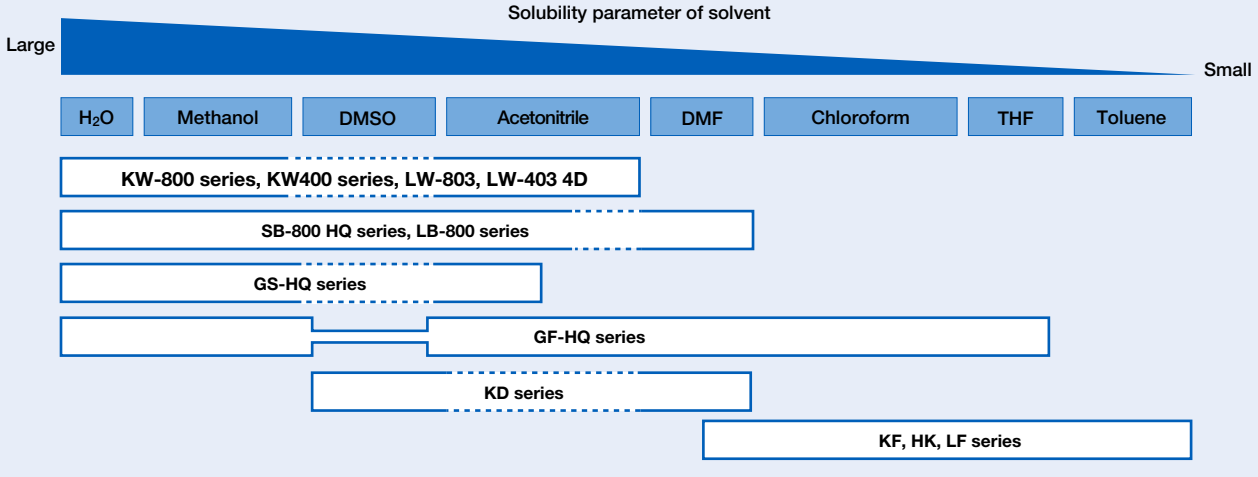




Column Selection (Polymers)



Guideline for SEC column selection by solvent usability



See page 60 for the solvent replaceability of organic solvent SEC (GPC) packed columns.

Precautions for Polar Polymer Analysis

Unexpected interactions in the column can affect the size exclusion chromatography analysis of polar polymers.
These interactions may change elution patterns and results in an invalid molecular weight calculation.
It is important to reduce these interfering interactions in order to obtain the accurate molecular weight distribution.

~ Interfering interactions likely to be observed ~

Interactions between the analyte and the packing materials

◆ Hydrophobic interaction
→ The analyte is adsorbed on the packing material.
This delays the analyte elution and results in under estimating the analyte's molecular weight. See (B) and (D).

◆ Ionic interaction
(1) Ion Exclusion
→ The analyte is repelled from the packing material.
This accelerates the analyte elution and results in over estimating the analyte's molecular weight. See (A) and (C).
(2) Ion Exchange
→ The analyte is adsorbed onto the packing material.
This delays the analyte elution and results in under estimating the analyte's molecular weight. See (B) and (D).

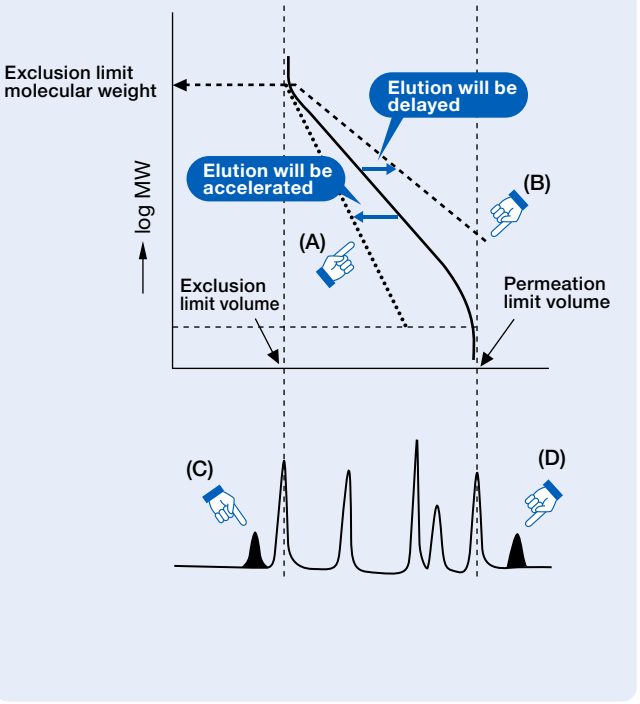
Interaction within and between the analyte

◆ Ionic repulsion effects observed within the multivalent macromolecules causes structure expansion
→ This accelerates the analyte elution and results in over estimating the analyte's molecular weight. See (A).

◆ Association between the molecules
→ This accelerates the analyte elution and results in over estimating the analyte's molecular weight. See (A).

Interactions between the analyte and the solvent

◆ The multivalent ion in the solvent works as a bridge to bind ionic molecules (analyte).



Methods to reduce interactions

Aqueous SEC (GFC)

Ionic interaction

◆ Add salt into the eluent

Hydrophobic interaction

◆ Increase the analyte dissociation
Cationic polymer → Lower the eluent pH
Anionic polymer → Higher the eluent pH

◆ Lower the eluent polarity
e.g. Add acetonitrile or methanol

Organic SEC (GPC)

Ionic interaction

◆ Add salt into the eluent
e.g. Add LiBr to DMF
Add CF_3COONa to HFIP

Hydrophobic interaction

◆ Lower the eluent polarity
e.g. Change the eluent from DMF to THF

Hydrophilic interaction

◆ Increase the eluent polarity
e.g. Change the eluent from THF to DMF

Multimode Columns

https://www.shodex.de/asahipak-gs-columns

Features

GS-HQ

- SEC is the main separation mode
- With the choice of eluent, the column provides multimode features of reversed phase, HILIC, and ion exchange modes to SEC
- Suitable for the separation of peptides or nucleic acids with similar molecular weights
- Suitable for desalting samples or substituting buffer in protein analysis

Standard columns

Product Code	Product Name	Plate Number (TP/column)	Particle Size (µm)	Pore Size (Å)	Column Size (mm) I.D. x Length	Shipping Solvent
F7600005	**Asahipak GS-220 HQ**	≥ 19,000	6	150	**7.5 x 300**	H_2O/CH_3OH = 70/30
F7600006	**Asahipak GS-320 HQ**	≥ 19,000	6	400	**7.5 x 300**	H_2O/CH_3OH = 70/30
F6710019	**Asahipak GS-2G 7B**	(guard column)	9	—	**7.5 x 50**	H_2O/CH_3OH = 70/30

Base Material: Polyvinyl alcohol
Usable pH Range: pH 2 - 9 (GS-220 HQ)
pH 2 - 12 (GS-320 HQ)

Preparative columns [Preparative columns are made to order.]

Product Code	Product Name	Plate Number (TP/column)	Particle Size (µm)	Column Size (mm) I.D. x Length	Shipping Solvent	Standard Column
F6810034	**Asahipak GS-220 20G**	≥ 14,000	13	**20.0 x 500**	H_2O/CH_3OH = 70/30	GS-220 HQ
F6810035	**Asahipak GS-320 20G**	≥ 14,000	13	**20.0 x 500**	H_2O/CH_3OH = 70/30	GS-320 HQ

Base Material: Polyvinyl alcohol

Usable solvents

Product Name	Maximum Usable Concentration (%)	
	Methanol	Acetonitrile
GS-220 HQ	30	50
GS-320 HQ	100	50

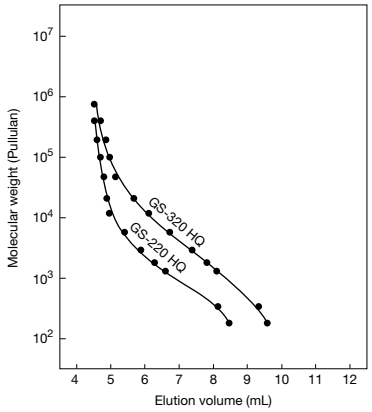
Target molecular weight range and exclusion limit

Measured with pullulan (eluent: ultrapure water)

Product Name	Target Molecular Weight Range	Exclusion Limit
GS-220 HQ	300 - 3,000	7,000
GS-320 HQ	300 - 20,000	40,000

Please use the above table for reference purposes only when selecting columns.

Calibration curves for GS-HQ series using pullulan



Column : **Shodex Asahipak GS-HQ series**
Eluent : H_2O
Flow rate : 0.6 mL/min
Detector : RI
Column temp. : 30 ˚C

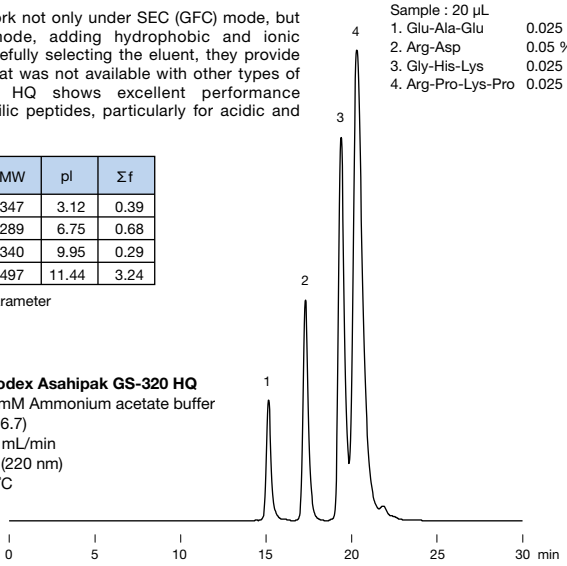
Peptides

GS-HQ columns work not only under SEC (GFC) mode, but also under multimode, adding hydrophobic and ionic interactions. By carefully selecting the eluent, they provide separation mode that was not available with other types of columns. GS-320 HQ shows excellent performance separating hydrophilic peptides, particularly for acidic and basic peptides.

	MW	pI	Σf
Glu-Ala-Glu	347	3.12	0.39
Arg-Asp	289	6.75	0.68
Gly-His-Lys	340	9.95	0.29
Arg-Pro-Lys-Pro	497	11.44	3.24

Σf : Hydrophobic parameter
pI : Isoelectric point

Sample : 20 µL
1. Glu-Ala-Glu 0.025 %
2. Arg-Asp 0.05 %
3. Gly-His-Lys 0.025 %
4. Arg-Pro-Lys-Pro 0.025 %

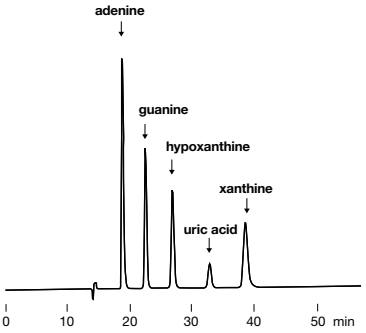


Column : **Shodex Asahipak GS-320 HQ**
Eluent : 30 mM Ammonium acetate buffer (pH6.7)
Flow rate : 0.5 mL/min
Detector : UV (220 nm)
Column temp. : 30 ˚C

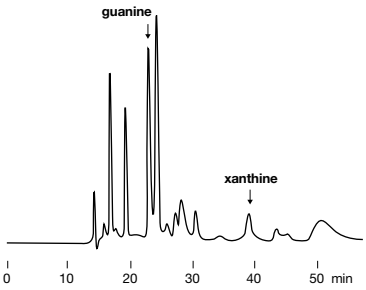
Purine bases in beer

Purine in food is analyzed as purine base after steps of sample preparation; homogenization, freeze drying, hydolyzation with 70 % perchloric acid, and neutralization. Example below shows the analysis of purine in regular beer and beer treated with guanase (an enzyme that degrades guanine to xanthine). The following data indicate that guanine was decreased and xanthine was increased by guanase.

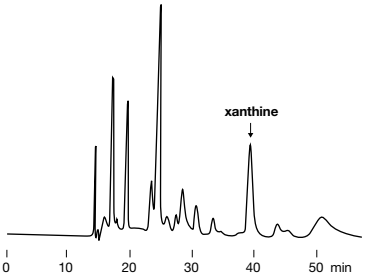
Purine base standards



Normal beer



Guanase treated beer



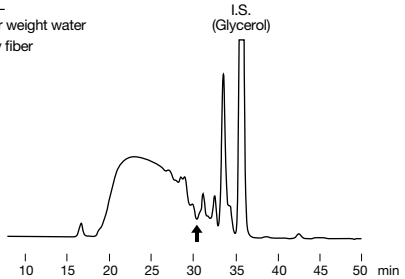
Column : **Shodex Asahipak GS-320 HQ**
Eluent : 150 mM Sodium phosphate buffer (pH2.5)
Flow rate : 0.6 mL/min
Detector : UV (260 nm)
Column temp. : 35 ˚C

Data provided by Kiyoko Kaneko Ph.D.,
Faculty of Pharmaceutical Sciences, Teikyo University

Low molecular weight water-soluble dietary fiber

GS-220 HQ allows to elute monosaccharides, disaccharides, and sugar alcohols after the indigestible component fraction (indicated by an arrow on the chromatogram).
This separation makes the method preferable for the quantification of low molecular weight water-soluble dietary fiber.

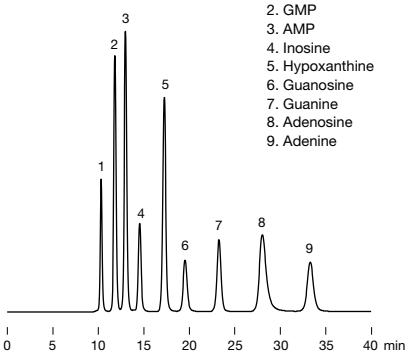
Sample : 20 µL
Low molecular weight water soluble dietary fiber



Column : **Shodex Asahipak GS-220 HQ x 2**
Eluent : H_2O
Flow rate : 0.5 mL/min
Detector : RI
Column temp. : 60 ˚C

"Umami"

Sample : 50 µg/mL each, 20 µL
1. IMP
2. GMP
3. AMP
4. Inosine
5. Hypoxanthine
6. Guanosine
7. Guanine
8. Adenosine
9. Adenine



Column : **Shodex Asahipak GS-320 HQ**
Eluent : 10 mM NaH_2PO_4 aq./10 mM Na_2HPO_4 aq. = 1000/31
Flow rate : 1.0 mL/min
Detector : UV (260 nm)
Column temp. : 40 ˚C