

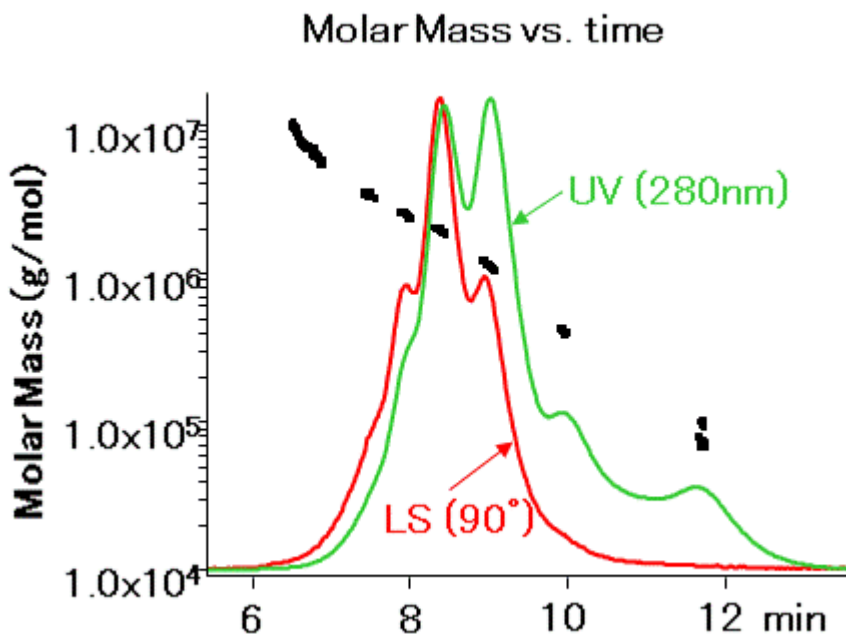
High Molecular Weight Protein Analysis of Hemocyanin (KW405-4F)

Hemocyanin is a respiratory pigment present in body fluids of mollusks and crustaceans, and is a high molecular weight (HMW) protein or macro-protein association composed of ten (or more) subunits which have the molecular weight on the several hundred kDa scale. In this application, Hemocyanin from keyhole limpet was analyzed using [KW405-4F](#), an aqueous SEC (GFC) column. The estimated exclusion limit of [KW405-4F](#) is 20,000, 000 Da, and it is suitable for the analysis of macro-proteins and other high molecular weight proteins.

Additional macro-protein and HMW applications using the KW405-4F:

[Determination of Particle Size Distribution of Hepatitis B Surface Antigen Virus-like Particle \(HBsAg VLP\)](#)

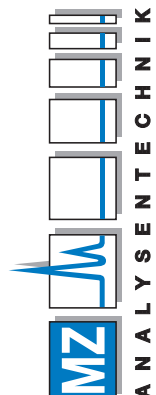
[Protein Standards on the KW405-4F](#)



KW405-4F

Sample : 10 μ L
Hemocyanin
(from keyhole limpet) 16mg/mL

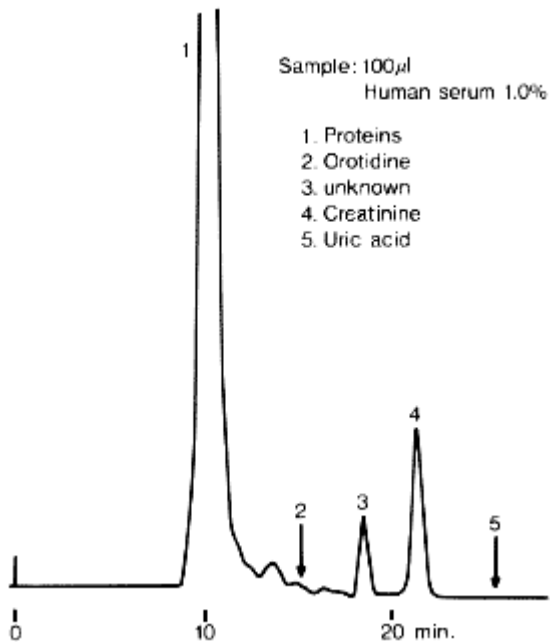
AUTHORIZED DISTRIBUTOR
MZ-Analyse-technik GmbH, Barcelona-Allee 17 • D-55129 Mainz
Tel +49 6131 880 96-0, Fax +49 6131 880 96-20
e-mail: info@mz-at.de, www.mz-at.de



Column : Shodex KW405-4F (4.6mmI.D. x 250mm)
Eluent : Phosphate buffered saline without magnesium and calcium
Flow rate : 0.35mL/min
Detector : UV(280nm) (small cell volume)
 : MALS (μ DAWN produced by Wyatt Technology Corp.)
Column temp. : 25°C

Human Serum (1) (SB-802 HQ)

Human serum was analyzed using OHPak SB-802 HQ (a column for GFC separation)



Sample : Human [serum](#)

1. Proteins
2. [Orotidine](#)
3. unknown
4. [Creatinine](#)
5. [Uric acid](#)

Column : Shodex OHPak SB-802 HQ (8.0mmID*300mm)

Eluent : 50mM Sodium phosphate buffer(pH7.0) + 0.3M NaCl

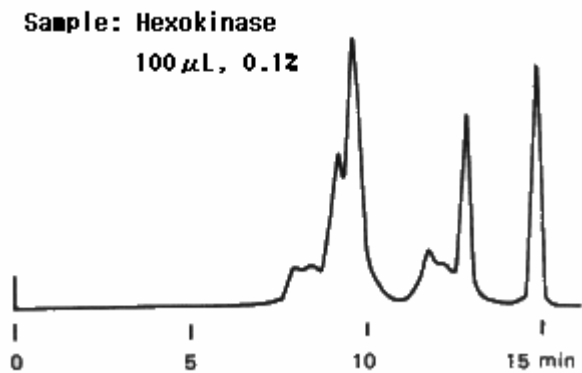
Flow rate : 0.5mL/min

Detector : UV(250nm)

Column temp. : Room temp.

Crude Hexokinase

Crude hexokinase was analyzed using PROTEIN KW-803 (a column for Aqueous SEC (GFC)).

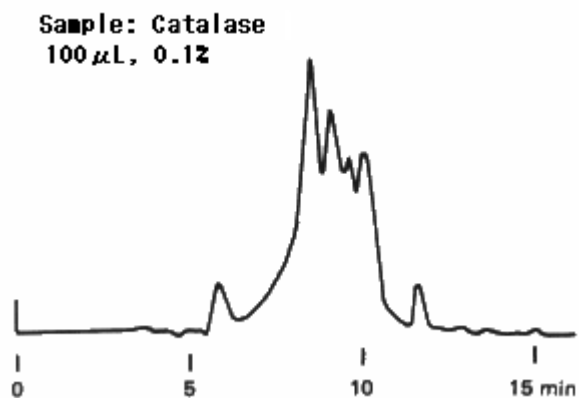


Sample: Crude Hexokinase

Column : Shodex PROTEIN KW-803 (8.0mmI.D. x 300mm)
Eluent : 50mM Phosphate buffer(pH7.0) + 0.3M NaCl
Flow rate : 1.0mL/min
Detector : UV(280nm)
Column temp. : Room temp.

Crude Catalase

Crude catalase was analyzed using PROTEIN KW-803 (a column for Aqueous SEC (GFC)).



Sample: Crude Catalase

Column : Shodex PROTEIN KW-803 (8.0mmI.D. x 300mm)
Eluent : 50mM Phosphate buffer(pH7.0) + 0.3M NaCl
Flow rate : 1.0mL/min
Detector : UV(280nm)
Column temp. : Room temp.

Analysis of Epoetin According to USP-NF Method (LW-803)

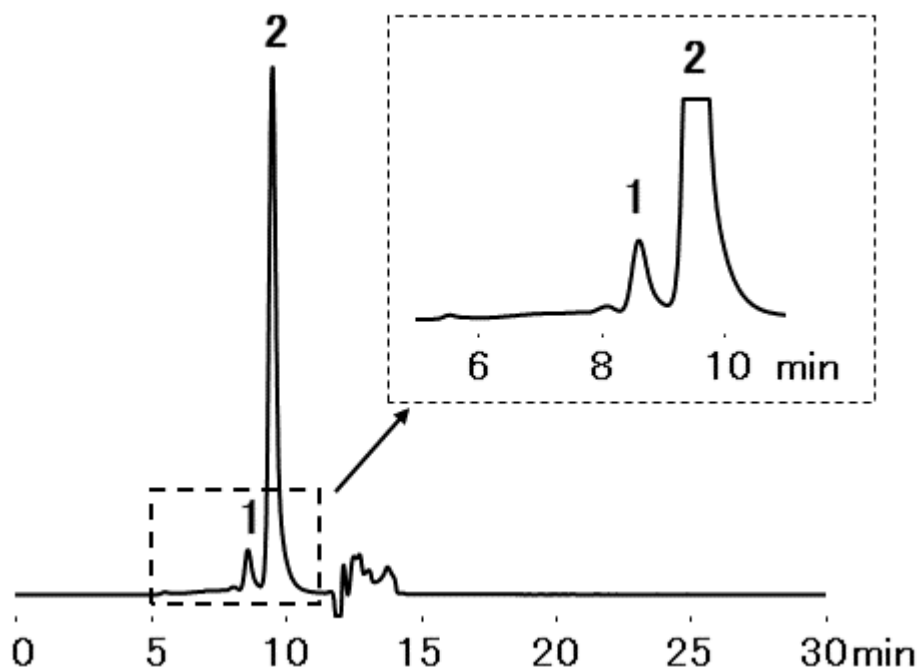
According to the United States Pharmacopeia and the National Formulary (USP 41-NF 36*), analysis of high molecular proteins in "Epoetin" (erythropoietin preparation) should be carried out with a column filled with L20 packing material and meets following requirements. The [PROTEIN LW-803](#) confirmed the requirements were met.

System suitability requirements Retention time: 8 to 10 min
Relative retention times: 0.9 (dimer) and 1.0 (monomer)
Relative standard deviation (RSD): $\leq 2.0\%$

*The version at the time of the application acquisition.

Sample: 40 μ L
Erythropoietin 2 mg/mL (in Eluent)
Heat at 80 $^{\circ}$ C for 30 min

1. Dimer
2. Monomer



Column \square Shodex [PROTEIN LW-803](#) (8.0 mm I.D. x 300 mm)
Eluent \square 20 mM Sodium citrate + 100 mM NaCl aq. (pH 6.9 adjust with Hydrochloric

acid)

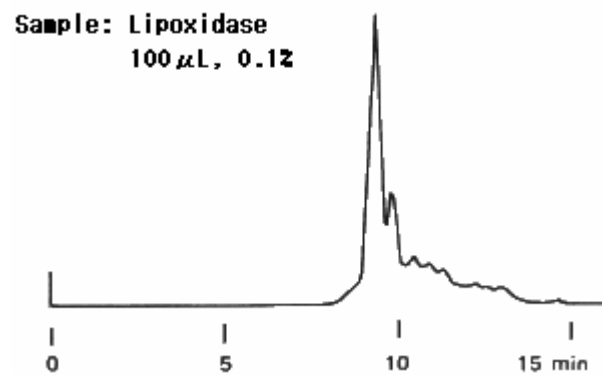
Flow rate 1.0 mL/min

Detector UV (230 nm)

Column temp. 25 °C

Crude Lipoxidase

Crude lipoxidase was analyzed using PROTEIN KW-803 (a column for Aqueous SEC (GFC)).

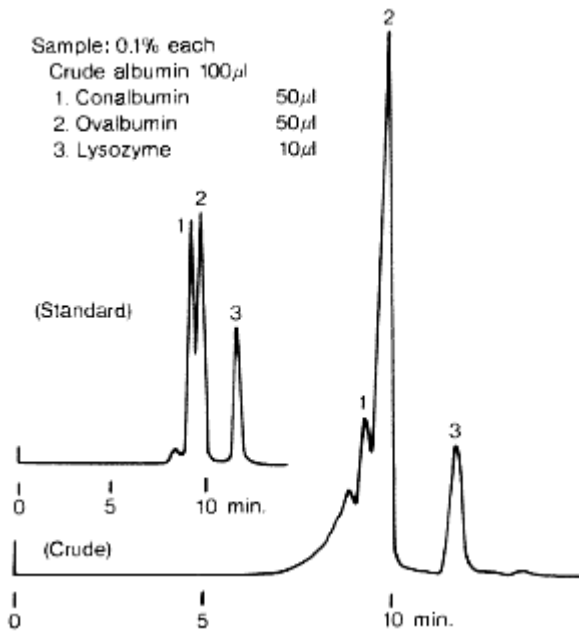


Sample: Crude Lipoxidase

Column : Shodex PROTEIN KW-803 (8.0mmI.D. x 300mm)
Eluent : 50mM Phosphate buffer(pH7.0) + 0.3M NaCl
Flow rate : 1.0mL/min
Detector : UV(280nm)
Column temp. : Room temp.

Crude Albumin (Chicken Egg) (1) (KW-803)

Crude albumin in the chicken egg was analyzed using PROTEIN KW-803 (a column for Aqueous SEC (GFC)).



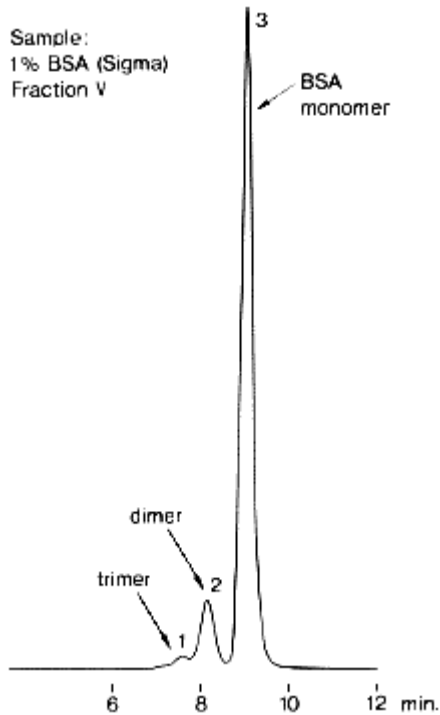
Sample; Crude Albumin

1. Conalbumin
2. Ovalbumin
3. Lysozyme

Column : Shodex PROTEIN KW-803 (8.0mmI.D. x 300mm)
Eluent : 50mM Phosphate buffer(pH7.0) + 0.3M NaCl
Flow rate : 1.0mL/min
Detector : UV(280nm)
Column temp. : Room temp.

Dimer and Trimer of BSA

Dimer and trimer of BSA were analyzed using PROTEIN KW-803 (a column for Aqueous SEC (GFC)).



Sample: BSA (Sigma) Fraction V

Column : Shodex PROTEIN KW-803 (8.0mmI.D. x 300mm)
Eluent : 50mM Phosphate buffer(pH7.0) + 0.3M NaCl
Flow rate : 1.0mL/min
Detector : UV(280nm)
Column temp. : Room temp.

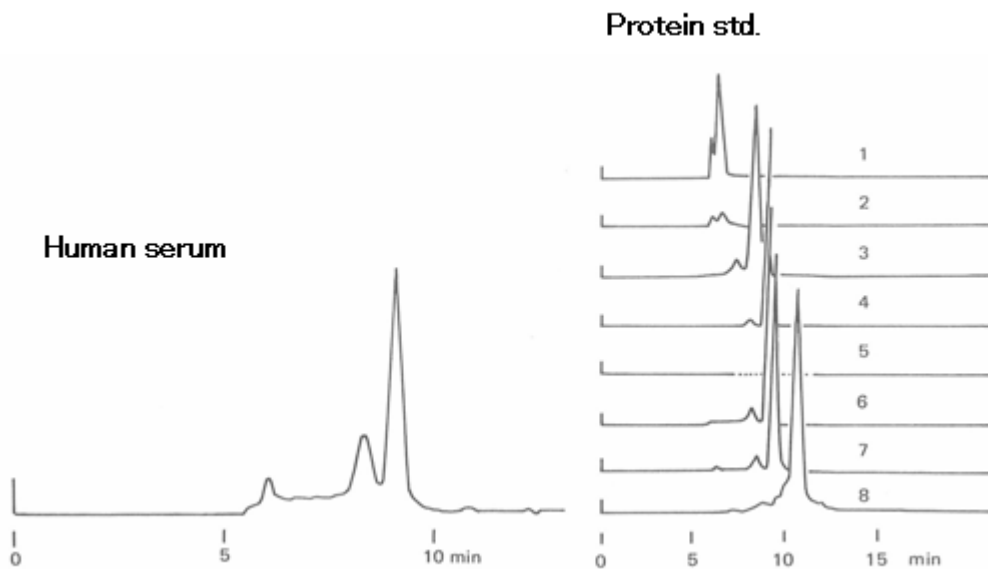
Proteins in Human Serum (3) (KW-803)

Proteins in human serum were analyzed using PROTEIN KW-803 (a column for Aqueous SEC (GFC)).

Sample: Human serum, 20 μ L.

Sample : Protein std. 0.1% each

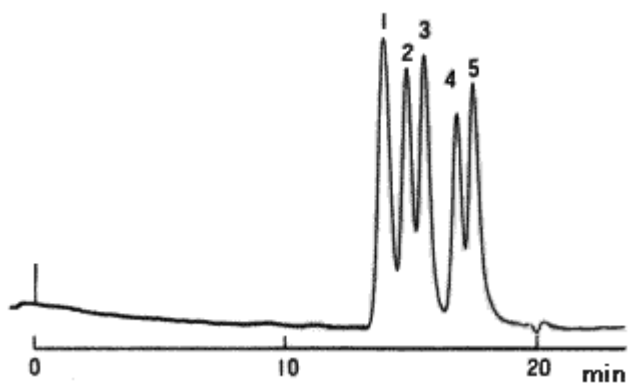
1. Fibrinogen 50 μ L
2. α 2 Macroglobulin 50 μ L
3. IgG 50 μ L
4. Transferrin 50 μ L
5. Plasminogen 50 μ L
6. Albumin 100 μ L
7. Antitrypsin 100 μ L
8. Hemoglobin 100 μ L.



Columns : Shodex PROTEIN KW-803 (8.0mmI.D. x 300mm)
Eluent : 50mM Phosphate buffer(pH7.0) + 0.3M NaCl
Flow rate : 1.0mL/min
Detector : UV(280nm)
Column temp. : Room temp.

Standard Proteins (23) (GF-510 HQ)

Protein standards were analyzed with Asahipak [GF-510 HQ](#).



Sample : Molecular weight marker

1. Glutamate dehydrogenase
2. Lactate dehydrogenase
3. Enolase
4. Adenylate kinase
5. Cytochrome c

Column : Shodex Asahipak GF-510 HQ (7.6mmID*300mm)

Eluent : 0.1M Sodium phosphate buffer(pH7.0) + 0.1M NaCl

Flow rate : 0.5mL/min

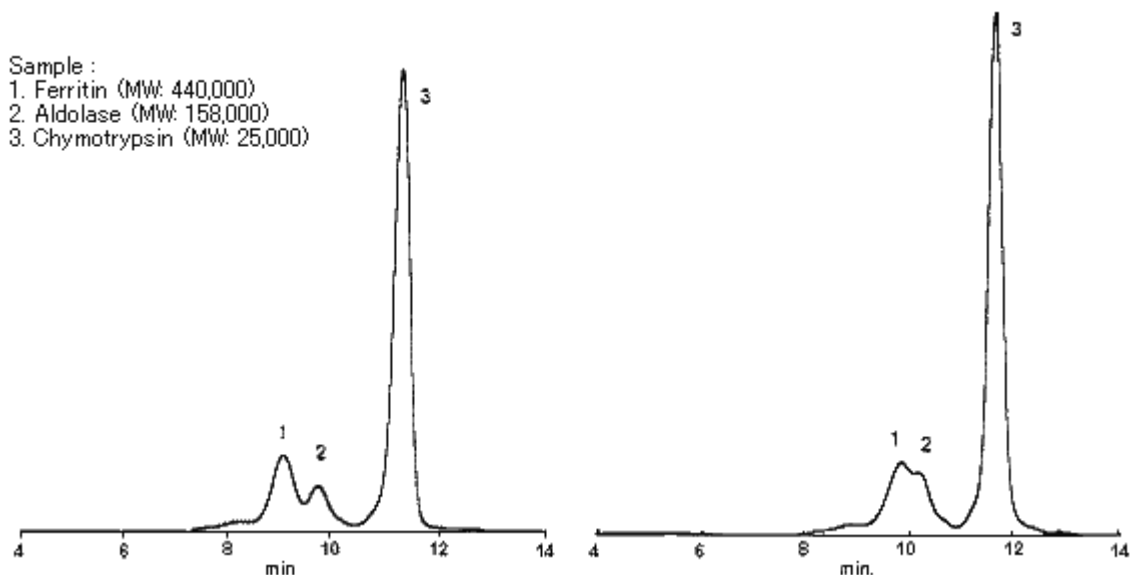
Detector : UV(280nm)

Column temp. : 30°C

Standard Proteins (19) (KW-804)

PROTEIN KW-804 (a column for aqueous SEC (GFC)) is suitable for the analysis of samples containing high molecular weight portions because the pore size of KW-804 is larger than that of the Company A column.

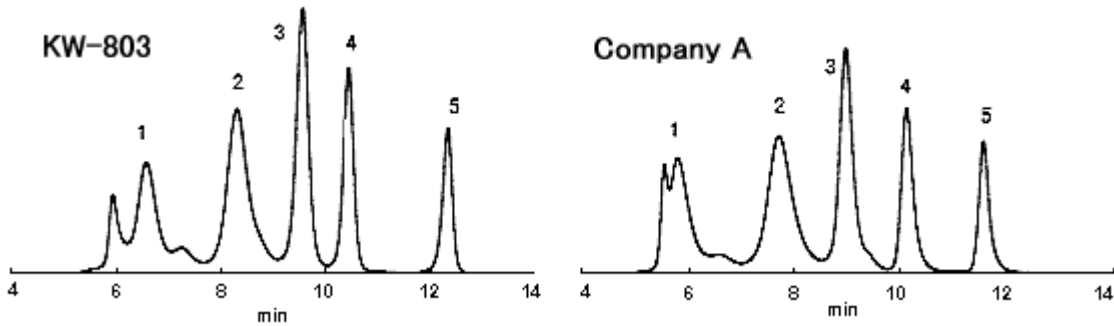
Sample: 1. Ferritin (MW: 440,000) , 2. Aldolase (MW: 158,000) , 3. Chymotrypsin (MW: 25,000).



Columns : (left); Shodex PROTEIN KW-804 (8.0mmI.D. x 300mm),
(right); Company A column
Eluent : 50mM Sodium phosphate buffer(pH7.0) + 0.3M NaCl
Flow rate : 1.0mL/min
Detector : UV(220nm)
Column temp. : Room temp.

Standard Proteins (18) (KW-803)

When a sample contains high molecular weight portions, PROTEIN KW-803 (a column for aqueous SEC (GFC)) will give a better result than Company A column.



Sample : Gel Filtration Standard (Bio Rad)

Peak	Protein	PROTEIN KW-803			Company A column		
		Rt(min)	Width(min)	Rs	Rt(min)	Width(min)	Rs
1	Thyroglobulin (MW: 670,000)	6.5657	0.7550	2.59	6.5657	0.7550	2.27
2	γ-Globulin (MW: 158,000)	8.2957	0.5825	2.33	7.7203	0.8450	1.84
3	Ovalbumin (MW: 44,000)	9.5547	0.5000	1.93	8.9840	0.5250	2.39
4	Myoglobin (MW: 17,000)	10.4380	0.4175	4.69	10.1290	0.4350	3.57
5	Vitamin B₁₂ (MW: 1,350)	12.3423	0.3950		11.5647	0.3700	

Columns : (Left); Shodex PROTEIN KW-803 (8.0mmI.D. x 300mm),
(Right); Company A column

Eluent : 50mM Sodium phosphate buffer(pH7.0) + 0.3M NaCl

Flow rate : 1.0mL/min

Detector : UV(220nm)

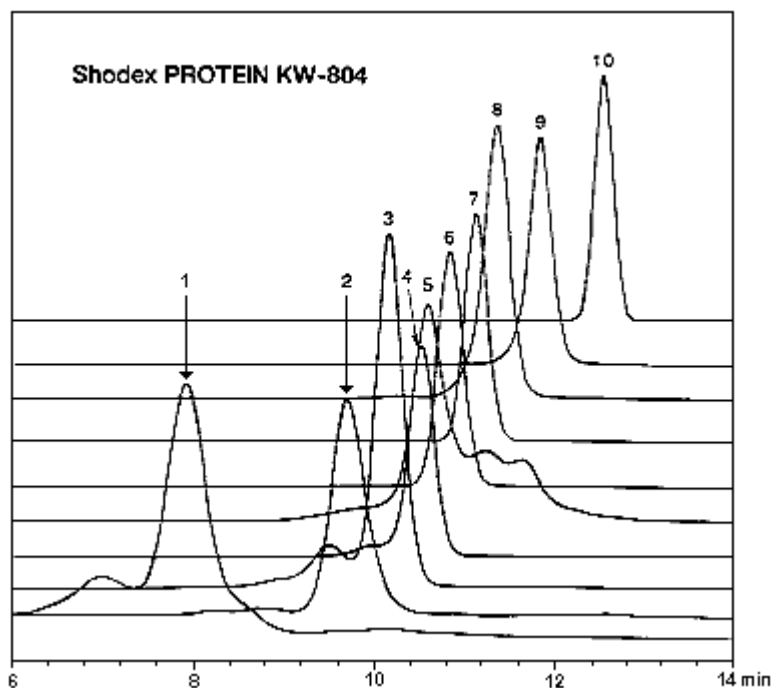
Column temp. : Room temp.

Standard Proteins (16) (KW-804)

Ten kinds of protein standards were analyzed using PROTEIN KW-804 (a column for aqueous SEC (GFC)).

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 B₁₂ (MW: 1,350).



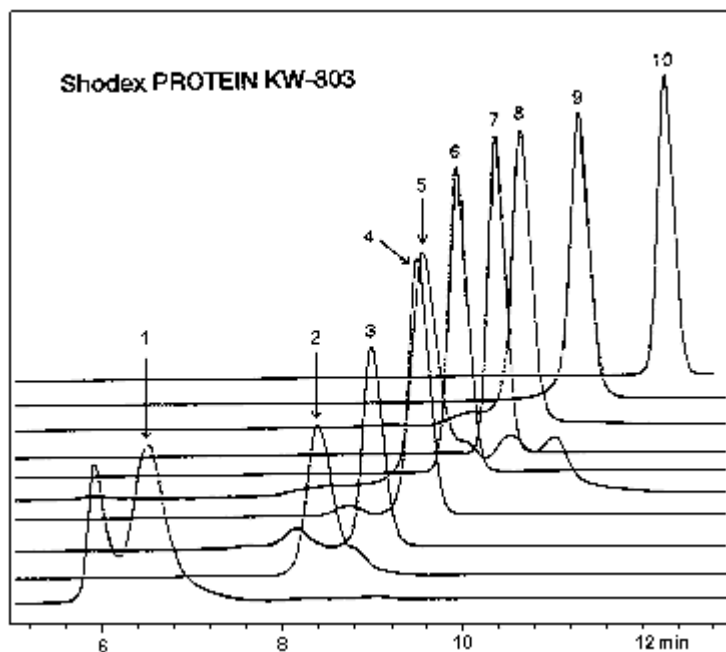
Column : Shodex PROTEIN KW-804 (8.0mmI.D. x 300mm)
Eluent : 50mM Sodium phosphate buffer(pH7.0) + 0.3M NaCl
Flow rate : 1.0mL/min
Detector : UV(220nm)
Column temp. : Room temp.

Standard Proteins (15) (KW-803)

Ten kinds of protein standards were analyzed using PROTEIN KW-803 (a column for aqueous SEC (GFC)).

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 B₁₂ (MW: 1,350).



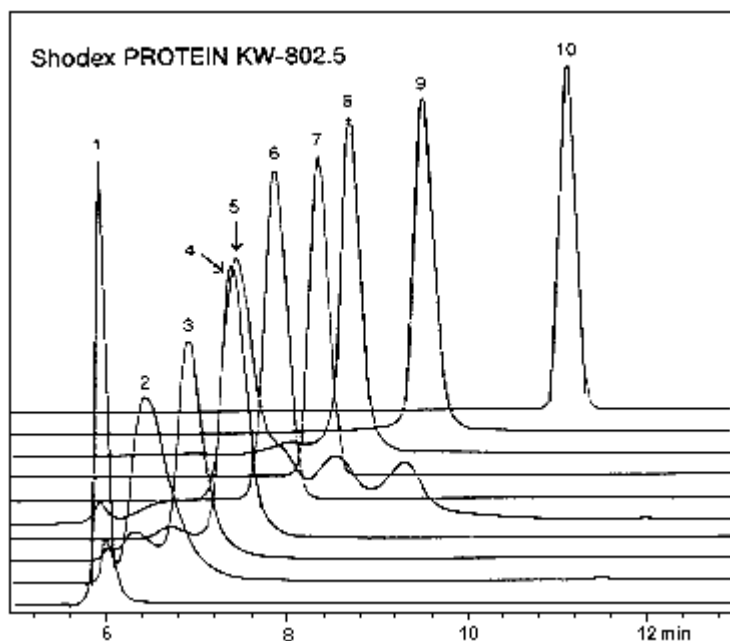
Column : Shodex PROTEIN KW-803 (8.0mmI.D. x 300mm)
Eluent : 50mM Sodium phosphate buffer(pH7.0) + 0.3M NaCl
Flow rate : 1.0mL/min
Detector : UV(220nm)
Column temp. : Room temp.

Standard Proteins (14) (KW-802.5)

Ten kinds of protein standards were analyzed using PROTEIN KW-802.5 (a column for aqueous SEC (GFC)).

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 B₁₂ (MW: 1,350).



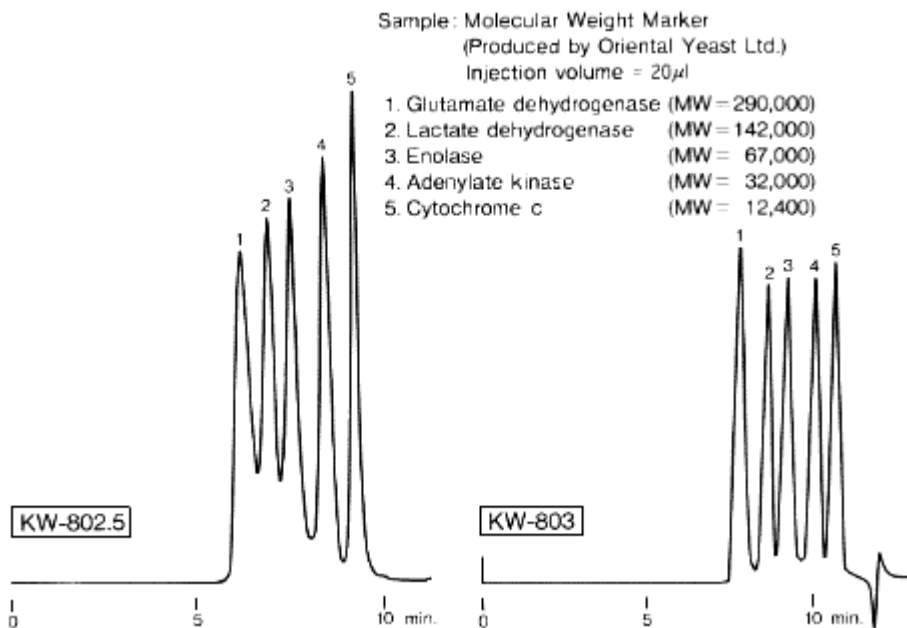
Column : Shodex PROTEIN KW-802.5 (8.0mmI.D. x 300mm)
Eluent : 50mM Sodium phosphate buffer(pH7.0) + 0.3M NaCl
Flow rate : 1.0mL/min
Detector : UV(220nm)
Column temp. : Room temp.

Standard Proteins (2) (KW-802.5 and KW-803)

This figure shows the separation of standard proteins using aqueous SEC (GFC) mode. Two columns for GFC separation, PROTEIN KW-802.5 and KW-803 were used. KW-802.5 is suitable for the analysis of proteins with molecular weights of less than 150,000, while KW-803, which has a larger pore size than KW-802.5, is suitable for the analysis of proteins with molecular weights of more than 150,000.

Sample: Molecular weight marker

1. Glutamate dehydrogenase
2. Lactate dehydrogenase
3. Enolase
4. Adenylate kinase
5. Cytochrome c.



Columns : Shodex PROTEIN KW-802.5, KW-803 (8.0 mm I.D. x 300 mm each)
Eluent : 50 mM Phosphate buffer(pH7.0) + 0.3 M NaCl
Flow rate : 1.0 mL/min
Detector : UV(280 nm)
Column temp. : Room temp.

Standard Proteins (1) (KW-802.5)

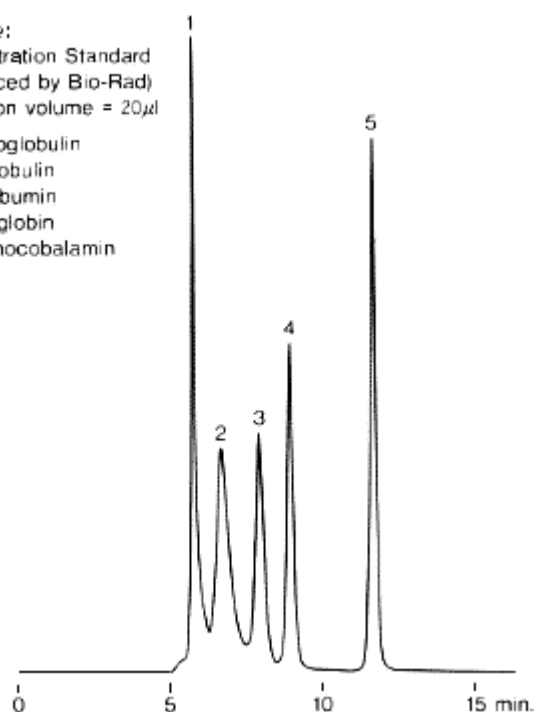
Five kinds of protein standards were analyzed using PROTEIN KW-802.5 (a column for aqueous SEC (GFC)).

Sample: Gel filtration standard

1. Thyroglobulin
2. γ -Globulin
3. Ovalbumin
4. Myoglobin
5. Cyanocobalamin.

Sample:
Gel Filtration Standard
(Produced by Bio-Rad)
Injection volume = 20 μ l

1. Thyroglobulin
2. γ Globulin
3. Ovalbumin
4. Myoglobin
5. Cyanocobalamin



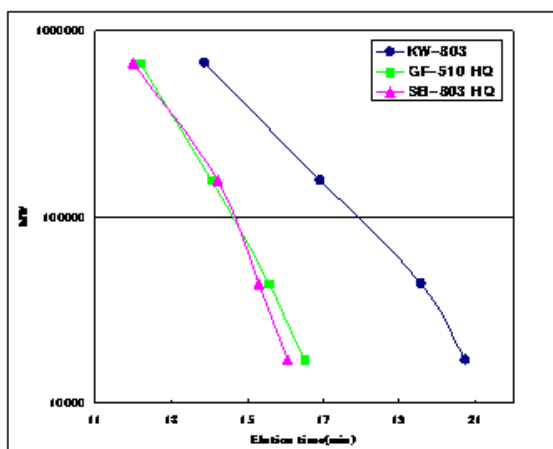
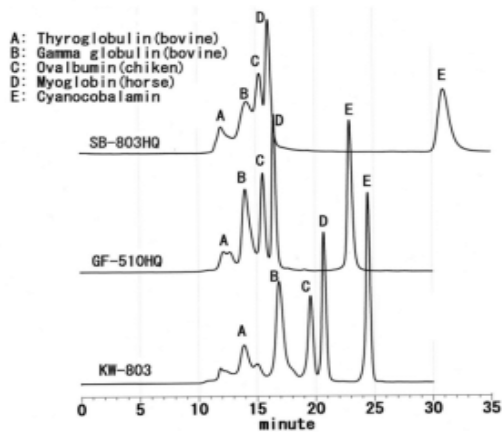
Column : Shodex PROTEIN KW-802.5 (8.0mmI.D. x 300mm)
Eluent : 50mM Phosphate buffer(pH7.0) + 0.3M NaCl
Flow rate : 1.0mL/min
Detector : UV(280nm)
Column temp. : Room temp.

Comparison of Chromatograms of Protein mixtures

This figure shows the chromatogram of protein mixtures analyzed with OHPak SB-803 HQ, Asahipak GF-510 HQ or PROTEIN KW-803. KW-803 is the best column for the separation of these proteins.

Sample :

- A: Thyroglobulin (bovine)
- B: γ -Globulin (bovine)
- C: Ovalbumin (chicken)
- D: Myoglobin (horse)
- E: Cyanocobalamin



Columns : Shodex OHpak SB-803 HQ (8.0mmI.D. x 300mm)
Shodex Asahipak GF-510 HQ (7.5mmI.D. x 300mm)
Shodex PROTEIN KW-803 (8.0mmI.D. x 300mm)
Eluent : 0.2M Phosphate buffer(pH6.9)
Flow rate : 0.5mL/min
Detector : UV(280nm)
Column temp. : 30°C

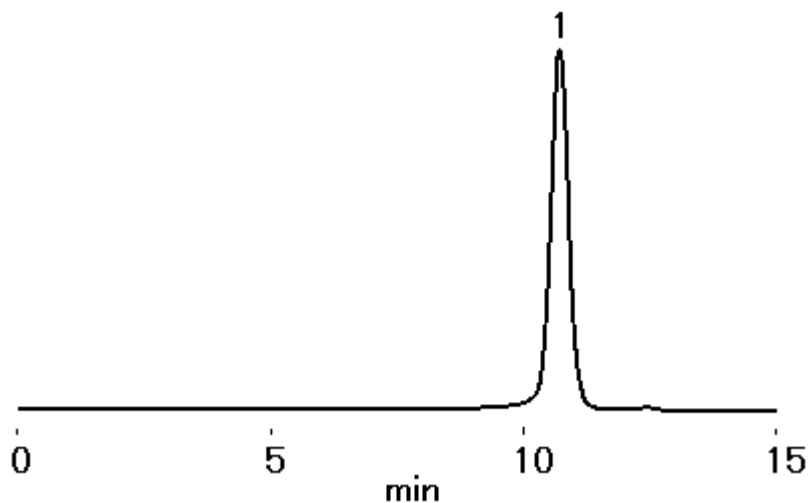
α_1 Acid glycoprotein, Orosomucoid

α_1 Acid glycoprotein from bovine plasma was analyzed using PROTEIN KW-804 (a column for GFC separation).

Sample: 50 μ L

1. α_1 Acid glycoprotein

from bovine plasma (Sigma-Aldrich) 0.1%.



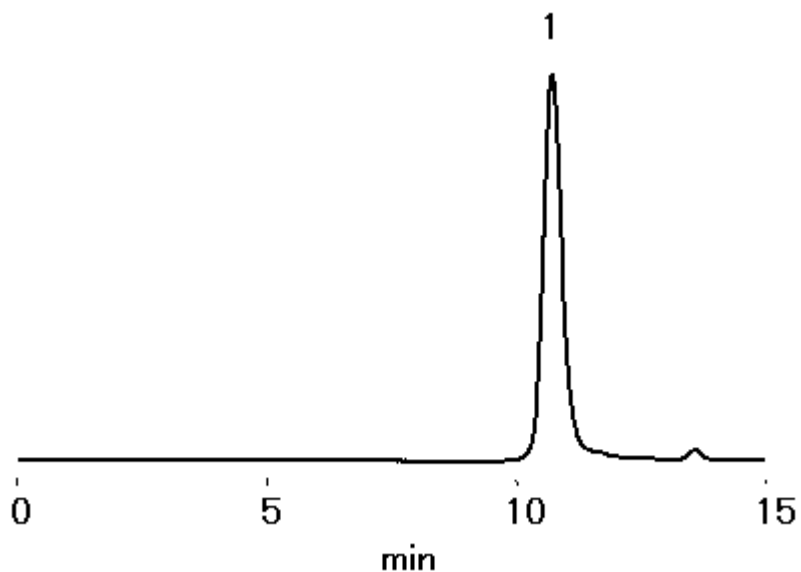
Column : Shodex PROTEIN KW-804 (8.0mmI.D. x 300mm)
Eluent : 100mM Sodium phosphate buffer(pH7.0) + 0.3M NaCl
Flow rate : 1.0mL/min
Detector : UV(254nm)
Column temp. : 30°C

Glycoprotein Avidin

Avidin, one of the saccharide-binding proteins, was analyzed using aqueous solvent SEC (GFC) column, PROTEIN KW-804.

Sample: 50 μ L

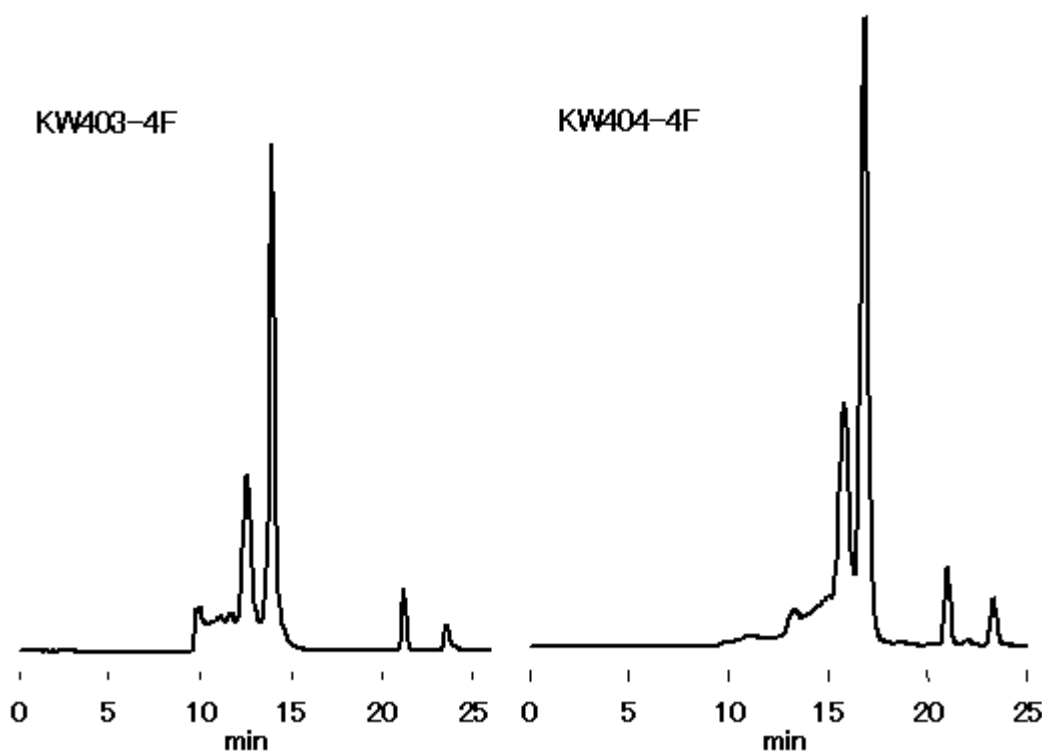
1. Avidin (Wako Pure Chemical Industries, Ltd.) 0.1%.



Column : Shodex PROTEIN KW-804 (8.0mmI.D. x 300mm)
Eluent : 100mM Sodium phosphate buffer(pH7.0) + 0.3M NaCl
Flow rate : 1.0mL/min
Detector : UV(254nm)
Column temp. : 30°C

Human Control Serum

Human control serum was analyzed using aqueous solvent SEC (GFC) high performance semi-micro columns, KW403-4F and KW404-4F. The bigger pore size (1,500Å compared to 800Å) of packed gel in KW404-4F is suitable for the analysis of large molecules that were excluded as void when using a KW403-4F column.



Sample : 1 μ L

Control [serum](#) wako/B

Column : Shodex KW403-4F, KW404-4F (4.6mmID*300mm each)

Eluent : 50mM Sodium phosphate buffer (pH7.0) + 0.3M NaCl

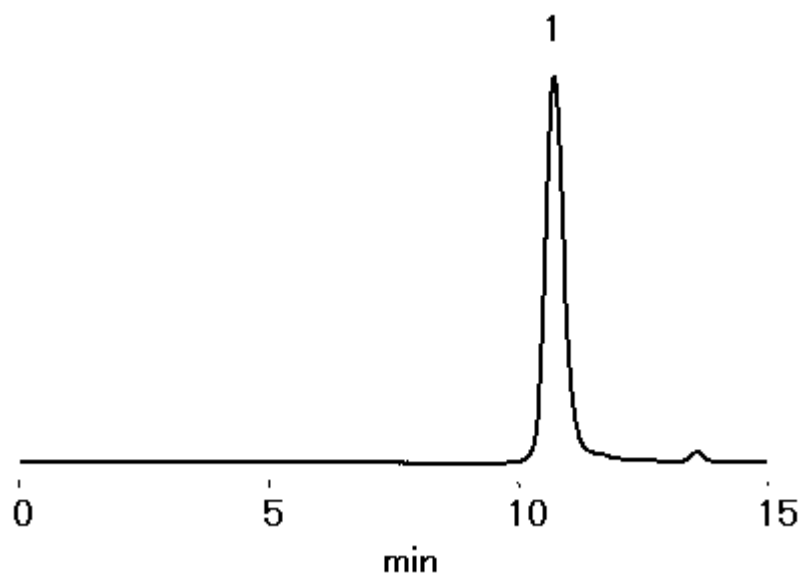
Flow rate : 0.20mL/min

Detector : UV(280nm)

Column temp. : 25° C

Avidin (2)

Avidin, one of the saccharide-binding proteins, was analyzed using aqueous solvent SEC (GFC) column, PROTEIN KW-804.



Sample : 50 μ L

1. Avidine

(Wako Pure Chemical Industries, Ltd.) 0.1%

Column : Shodex PROTEIN KW-804 (8.0mmID*300mm)

Eluent : 100mM Sodium phosphate buffer(pH7.0) + 0.3M NaCl

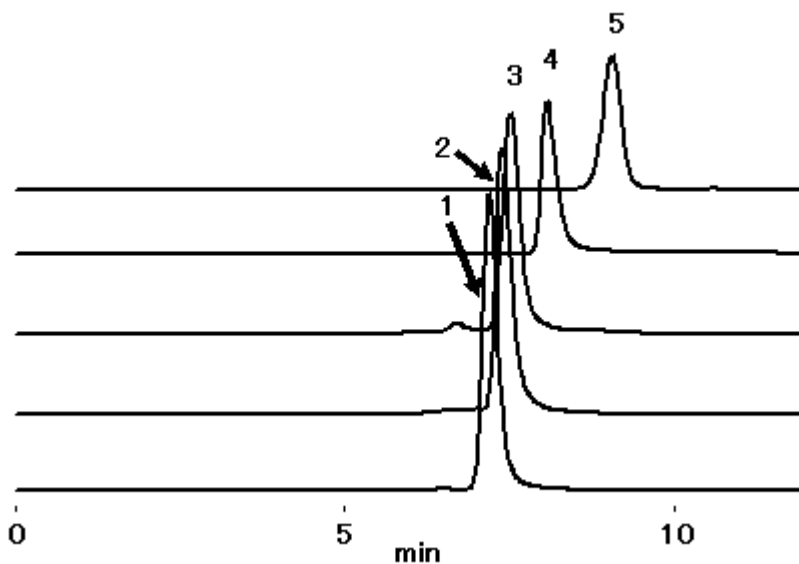
Flow rate : 1.0mL/min

Detector : UV(254nm)

Column temp. : 30° C

Lectin Protein

Lectin from various sources was analyzed using aqueous solvent SEC (GFC) high performance semi-micro column, KW402.5-4F. Lectin is a protein that has specific binding characteristic to saccharide and can exist as compounds of saccharide-binding protein lectin or metal-binding lectin.



Sample : 5 μ L

1. [Lectin](#) from Soybean
2. [Lectin](#) from *Arachis hypogaea*
3. [Lectin](#) from *Canavalia ensiformis* (Con A)
4. [Lectin](#) from *Lens culinaris*
5. [Lectin](#) from *Triticum vulgare*

Column : Shodex KW402.5-4F (4.6mmID*300mm)

Eluent : 50mM Sodium phosphate buffer (pH7.0) + 0.3M NaCl

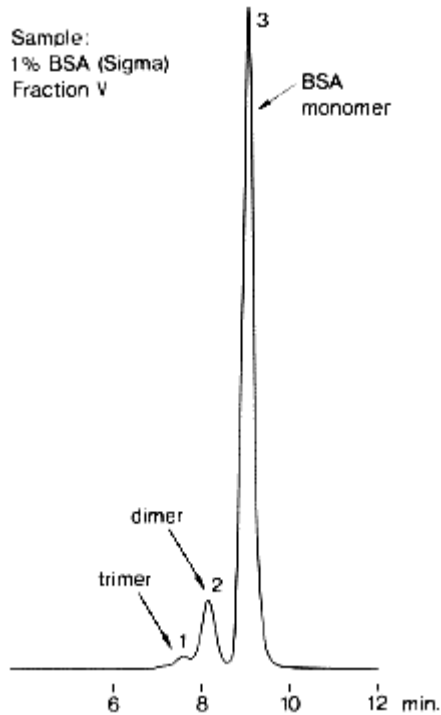
Flow rate : 0.33mL/min

Detector : UV(220nm)

Column temp. : 30° C

Dimer and Trimer of BSA

Dimer and trimer of BSA were analyzed using [PROTEIN KW-803](#) (a column for Aqueous SEC (GFC)).

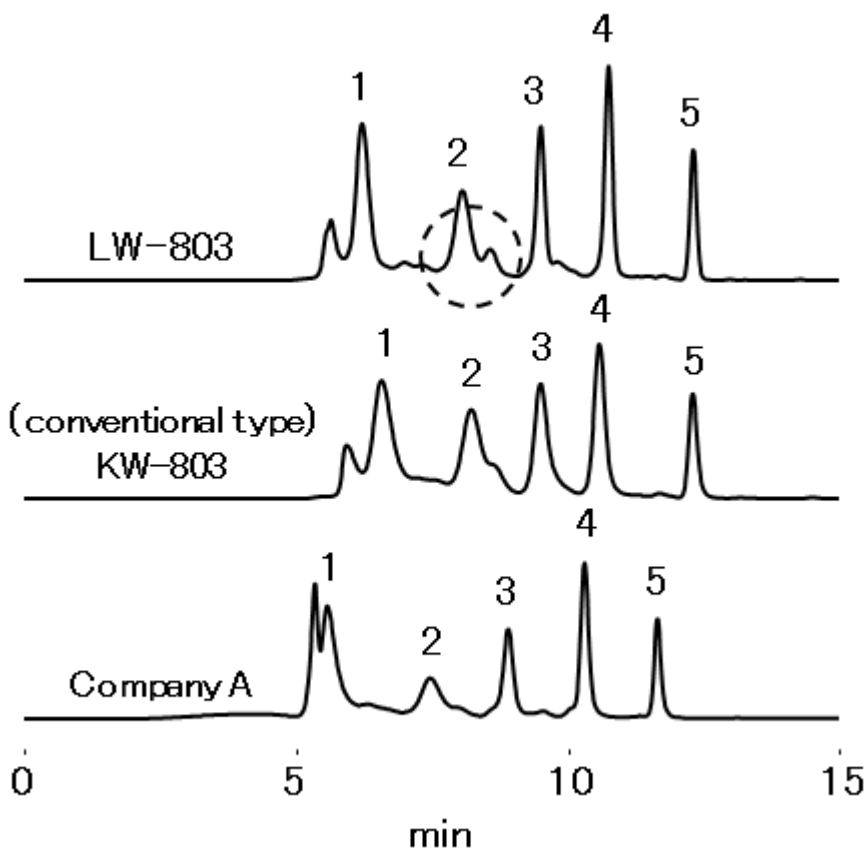


Sample : BSA (Sigma) Fraction V

Column : Shodex PROTEIN KW-803 (8.0mmI.D. x 300mm)
Eluent : 50mM Phosphate buffer(pH7.0) + 0.3M NaCl
Flow rate : 1.0mL/min
Detector : UV(280nm)
Column temp. : Room temp.

Comparison of SEC Separation of Standard Proteins

Standard proteins were analyzed with PROTEIN LW-803 which is a column for Aqueous SEC (GFC). [PROTEIN LW-803](#) is suitable for analyzing proteins with a molecular weight of several hundreds of thousands. Furthermore, this column is especially suitable for separating γ -Globulin with the molecular weight of 160,000 and vicinity. The samples are better separated with LW-803 than a conventional column ([KW-803](#)) or other manufacturer's columns. Improvement of separation performance for this molecular weight range is effective for separation of monomer and dimer of IgG which is the mainstream of antibody drugs.



- Sample : 5 μ L
1. Thyroglobulin
(MW : 670,000) 7mg/mL
 2. γ -Globulin
(MW : 160,000) 6mg/mL
 3. Ovalbumin

(MW : 44,300) 4.8mg/mL

4. Ribonuclease A

(MW : 13,700) 7mg/mL

5. Uridine

(MW : 244) 0.1mg/mL

Columns : Shodex PROTEIN LW-803 (8.0mmI.D. x 300mm)
Shodex PROTEIN KW-803 (8.0mmI.D. x 300mm)
Silica-based SEC column from other manufacturer (7.8mmI.D. x

300mm)

Eluent : 50mM Sodium phosphate buffer (pH7.0) + 0.3M NaCl

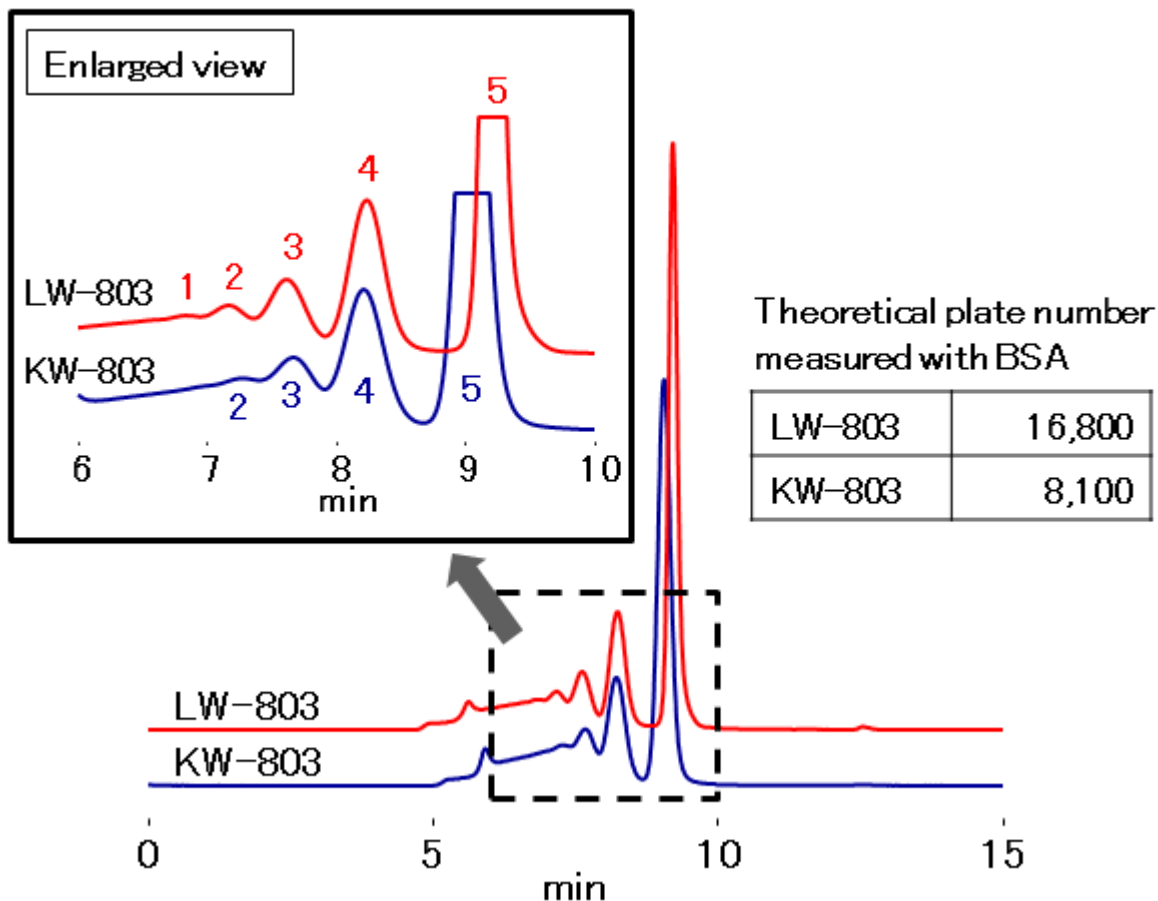
Flow rate : 1.0mL/min

Detector : UV(280nm)

Column temp. : Room temp.

Comparison of Separation of BSA Aggregates

BSA and its aggregates were separated with [PROTEIN LW-803](#) which is an aqueous SEC (GFC) column, and a conventional column (KW-803). With [KW-803](#), the tetramer can be detected, however, the pentamer can be detected with LW-803. It is confirmed that LW-803 provided a better separation of the monomer and dimer than KW-803.



Sample : 5 μ L
BSA 24mg/mL (SIGMA A1900 BSA monomer)
1. Pentamer
2. Tetramer
3. Trimer
4. Dimer
5. BSA monomer (MW 66,000)

Columns : Shodex PROTEIN LW-803 (8.0mmI.D. x 300mm)

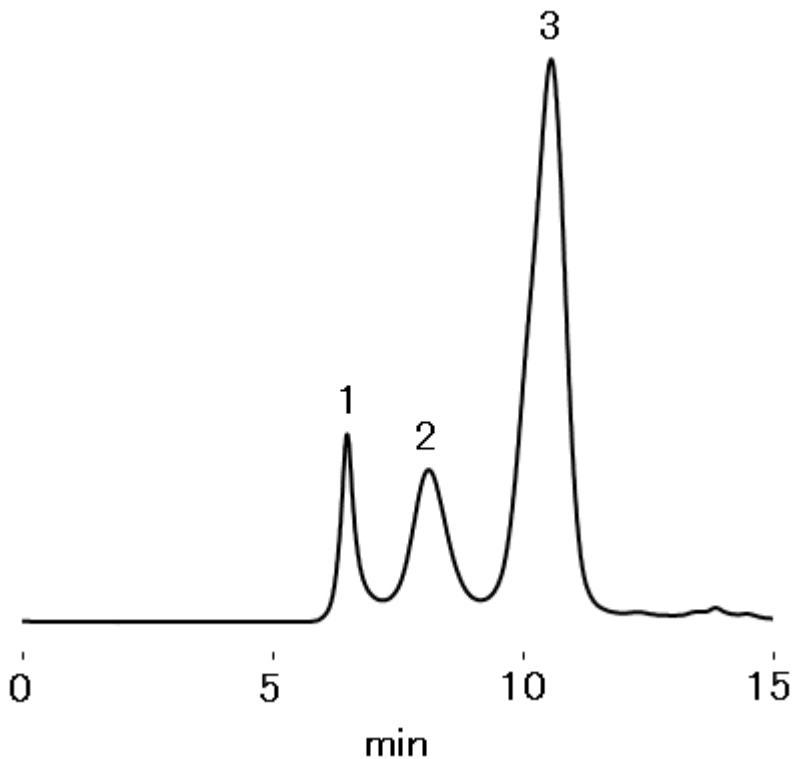
Shodex PROTEIN KW-803 (8.0mmI.D. x 300mm)
Eluent : 50mM Sodium phosphate buffer (pH7.0) + 0.3M NaCl
Flow rate : 1.0mL/min
Detector : UV(280nm)
Column temp. : 25°C

Analysis of Lipoproteins in Serum (KW-804)

Whole lipoproteins from serum of a healthy person were analyzed using [PROTEIN KW-804](#), an aqueous SEC (GFC) column. The result shows a good separation of the three kinds of lipoproteins (VLDL, LDL and HDL) in serum.

(Preparation of sample)

- Adjust specific gravity of serum of a healthy person to 1.210g/mL added potassium bromide followed by ultracentrifugation for 24 hours.
- After dialyzing the supernatant, solvent substitute by PBS*.
- Measure protein concentration by Lowry method and dilute with PBS* to 1.0mg/mL.



Sample : 40 μ L

Whole lipoproteins from serum of a healthy person 1.0mg/mL

1. VLDL
2. LDL
3. HDL

Columns : Shodex PROTEIN KW-G 6B (6.0mmI.D. x 50mm) + KW-804 (8.0mmI.D. x 300mm)
Eluent : 10-fold diluted x10 PBS* with H₂O
Flow rate : 1.0mL/min
Detector : UV(280nm)
Column temp. : 30°C

x10 PBS* : 80g NaCl + 29g Na₂HPO₄·12H₂O + 2g KCl + 2g KH₂PO₄ in 1000mL of H₂O

Data courtesy of Ohkawa Ryunosuke,
Graduate School of Health Care Sciences, Analytical Laboratory Chemistry, Tokyo
Medical and Dental University

Comparison of SEC Separation of Standard Proteins between LW-403 4D and LW-803

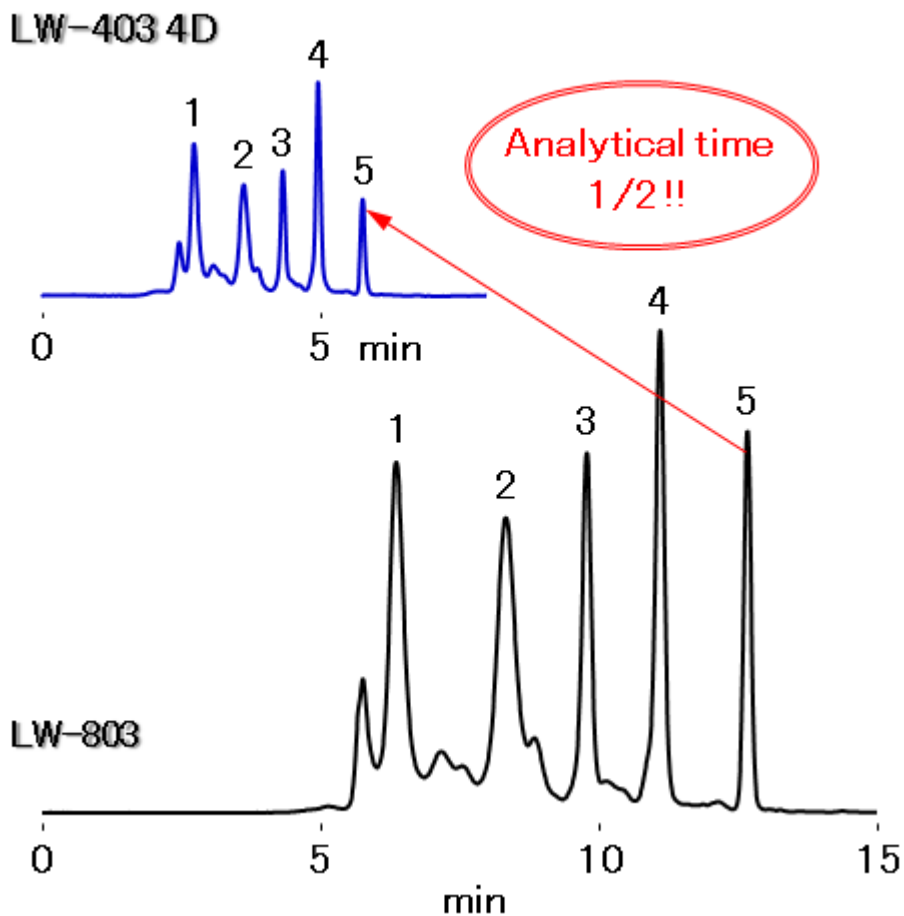
PROTEIN LW-403 4D is an aqueous SEC (GFC) semi-micro column for rapid analysis of antibody proteins. PROTEIN LW-403 4D and PROTEIN LW-803 (a standard column) were compared for the separation of standard proteins. Analysis using LW-403 4D can be completed in half the time compared to LW-803.

Sample:

(LW-403 4D) 0.5 μ L

(LW-803) 5 μ L

1. Thyroglobulin
(MW : 670,000) 7mg/mL
2. γ -Globulin
(MW : 160,000) 6mg/mL
3. Ovalbumin
(MW : 44,300) 4.8mg/mL
4. Ribonuclease A
(MW : 13,700) 7mg/mL
5. Uridine
(MW : 244) 0.1mg/mL



Columns : Shodex [PROTEIN LW-403](#) 4D (4.6mmI.D. x 150mm)
 Shodex [PROTEIN LW-803](#) (8.0mmI.D. x 300mm)
 Eluent : 50mM Sodium phosphate buffer (pH7.0) + 0.3M NaCl
 Flow rate : (LW-403 4D) 0.35mL/min
 (LW-803) 1.0mL/min
 Detector : (LW-403 4D) UV(280nm) (small cell volume)
 (LW-803) UV(280nm) (conventional type)
 Column temp. : Room temp.