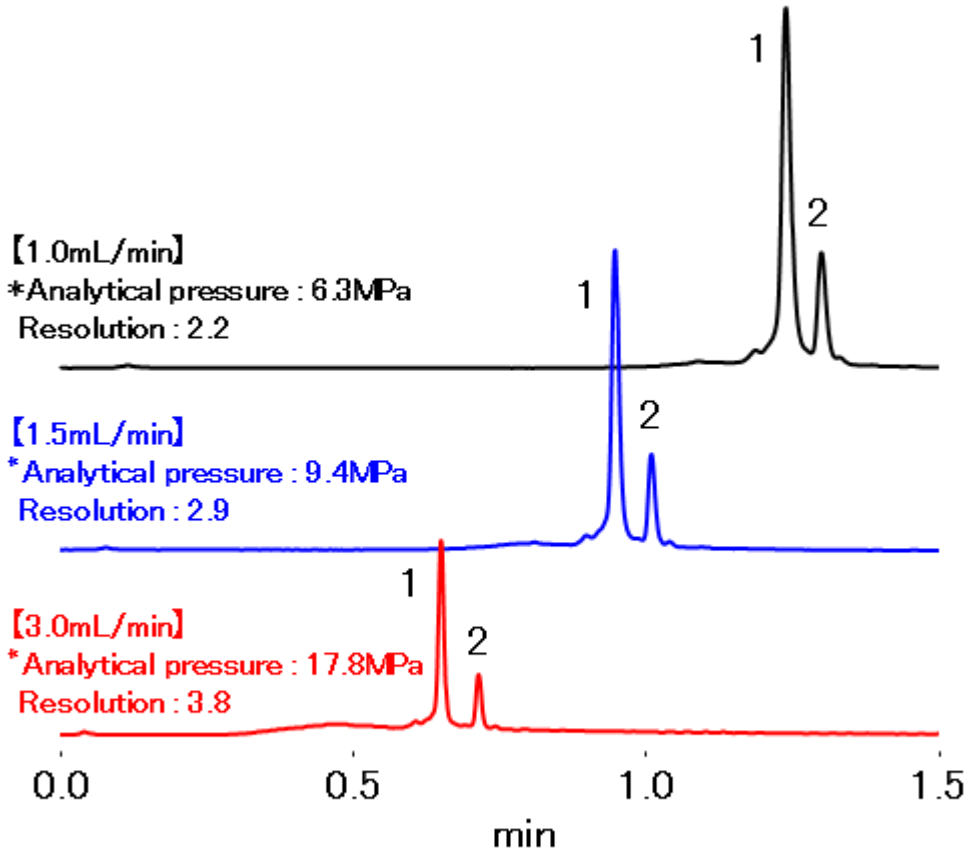
Effects of Flow Rate for Protein Analysis (SP-FT 4A)

The effects of flow rate for protein analysis with IEC SP-FT 4A, a column for cation exchange chromatography, were investigated. The particle size of SP-FT 4A is small (2.7µm) but the pressure of the column can be suppressed by advanced control for particle size. Since the pressure is under 20MPa at 3.0mL/min flow rate, SP-FT 4A can provide ultra-rapid and high-resolution analysis using conventional devices as same as when using UHPLC.
(Caution) Backpressure will differ depends on the LC system used and the analytical conditions such as eluent compositions and column temperature. Please set the system pressure (sum of column and system pressures) less than 20 MPa.



*Analytical pressure :
Total of instrument pressure and column pressure

Sample : 5µL
1mg/mL each
1. α-Chymotrypsinogen A
2. Ribonuclease A

AUTHORIZED DISTRIBUTOR
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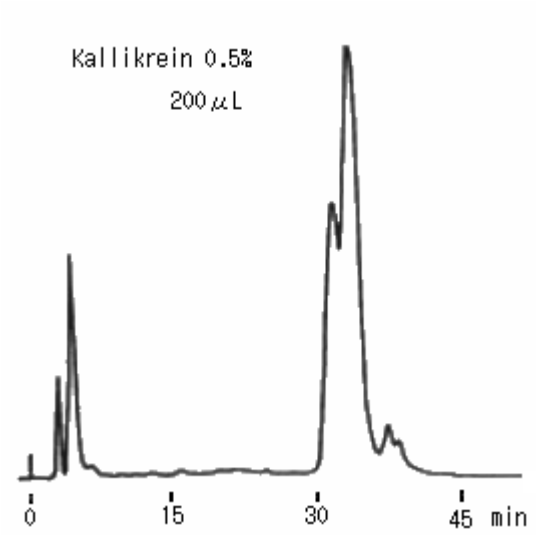
e-mail: info@mz-at.de, www.mz-at.de
ANALYSENTECHNIK

Column : Shodex IEC SP-FT 4A (4.6mm I.D. x 10mm)
Eluent : (A); 20mM *MES buffer (pH5.6)
(B); (A) + 0.5M Na_2SO_4
Linear gradient: (A) to (B) (2min) □□□□
Flow rate : 1.0, 1.5, 3.0mL/min
Detector : UV(280nm)
Column temp. : Room temp.

*MES : 2-(N-Morpholino) ethanesulfonic acid

Purified Kallikrein from Pig Pancreas

Purified Kallikrein from Pig Pancreas was analyzed using IEC QA-825 (a column for strong anion exchange chromatography). Enzyme activity was found at the large peak which has two peak tops after 30 minutes. The recovery of enzyme activity was 95%.

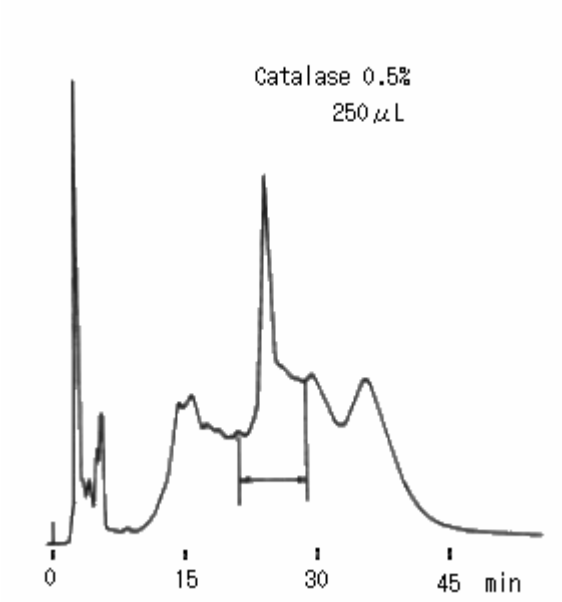


Sample : 0.5% Kallikrein, 200micro-L

Column : Shodex IEC QA-825 (8.0mmID*75mm)
Eluent : (A); 20mM Tris-HCl buffer(pH7.5)
(B); (A) + 0.5M NaCl
Linear gradient: 0min to 60min, (A) to (B)
Flow rate : 1.0mL/min
Detector : UV (280nm)
Column temp. : 25°C

Bovine Liver Catalase

Commercially available catalase was analyzed using IEC QA-825 (a column for strong anion exchange chromatography). Enzyme activity was found at about 18 min at the position of arrow mark.

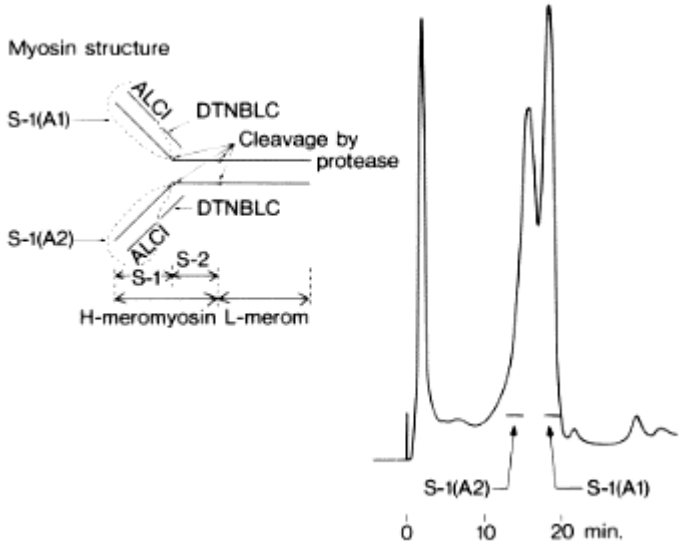


Sample : 0.5% Catalase, 250micro-L

Column : Shodex IEC QA-825 (8.0mmID*50mm)
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. : 25°C

Myosin Subfragments (ES-502C 7C)

Myosin subfragments were analyzed using Asahipak ES-502C 7C (a column for weak cation exchange chromatography).



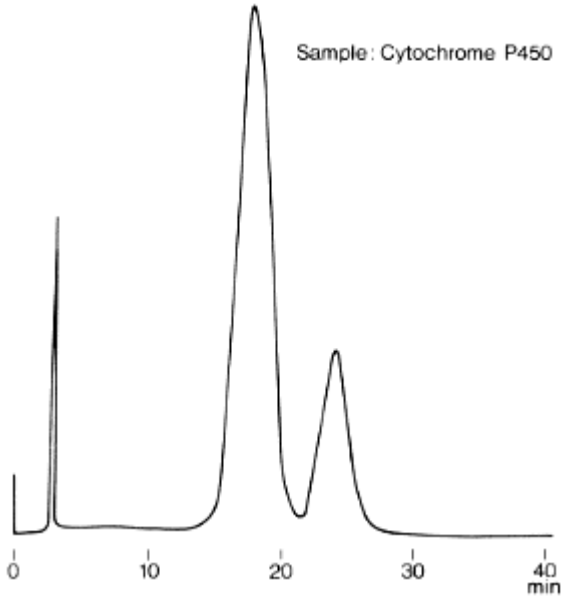
Sample : Myosin subfragment

Column : Shodex Asahipak ES-502C 7C (7.6mmID*100mm)
Eluent : (A); 5mM Sodium phosphate buffer(pH7.0)
(B); (A) + 0.1M NaCl
Linear gradient: 0min to 25min, (A) to (B)
Flow rate : 1.0mL/min
Detector : UV(220nm)
Column temp. : 10°C

Courtesy of Dr. Muno, Juntendo University

Cytochrome P450 in Rat Liver Microsome

Chytochrome P450 in rat liver microsome was analyzed using Asahipak ES-502C 7C (a column for weak cation exchange chromatography).



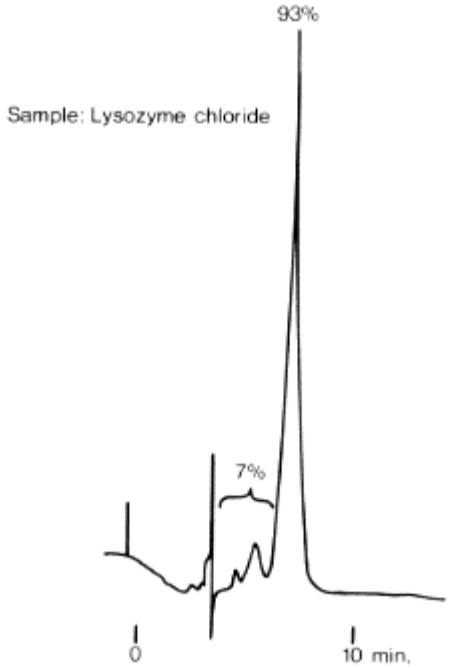
Sample : Cytochrome P450 in rat liver microsome

Column : Shodex Asahipak ES-502C 7C (7.6mmID*100mm)
Eluent : (A); 20mM Sodium phosphate buffer(pH6.5) + 20% Glycerol + 0.4% Emulgen 911
(B); (A) + 1.0M Sodium Acetate
Linear gradient: 0min to 50min, (A) to (B)
Flow rate : 1.0mL/min
Detector : VIS(417nm)
Column temp. : Room temp.

Courtesy of Dr. Funae, Osaka City University

Lysozyme Chloride (ES-502 7C)

Weak cation exchange columns bonded with carboxy methyl groups such as Asahipak ES-502C 7C are suitable for the analysis of basic proteins.

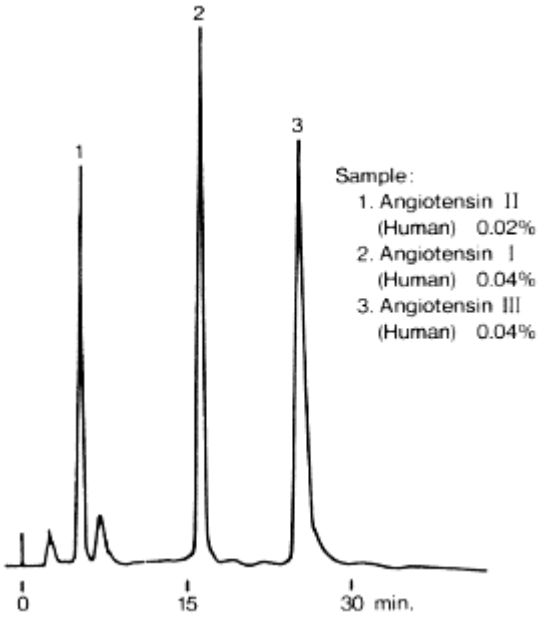


Sample : Lysozyme chloride

Column : Shodex Asahipak ES-502C 7C (7.6mmID*100mm)
Eluent : 50mM Sodium phosphate buffer(pH7.0) + 300mM NaCl
Flow rate : 1.0mL/min
Detector : UV(280nm)
Column temp. : 30°C

Angiotensin I, II, III

Angiotensins were analyzed using IEC SP-825 (a column for strong cation exchange chromatography). Angiotensins are physically active peptides and cause blood pressure to rise. Angiotensin I is a decapeptide, Angiotensin II an octapeptide and Angiotensis III a hexapeptide. These three components separated very well by gradient elution with increasing salt concentration.

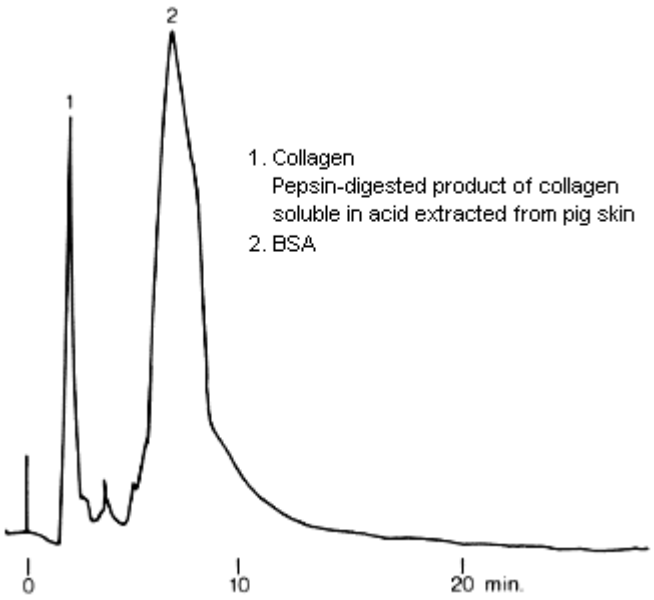


Sample : 250µL
1. Angiotensin II
2. Angiotensin I
3. Angiotensin III

Column : Shodex IEC SP-825 (8.0mmID*75mm)
Eluent : (A); 0mM 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.

Collagen and BSA

Collagen and BSA were analyzed using Asahipak ES-502N 7C (a column for weak anion exchange chromatography).

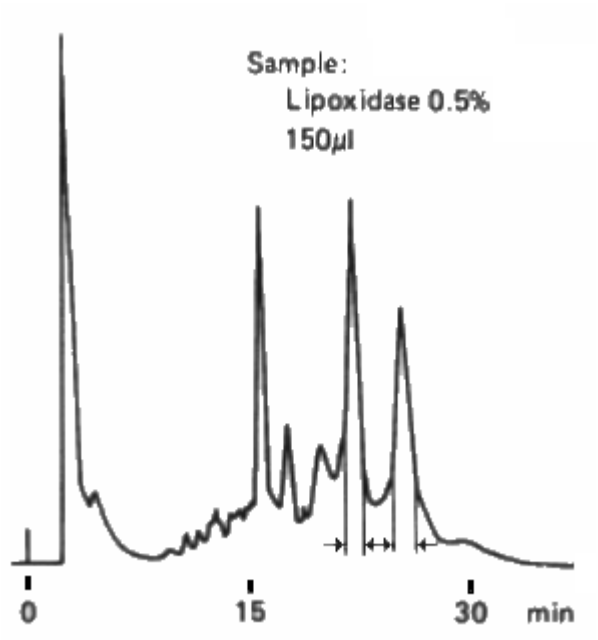


Column : Shodex Asahipak ES-502N 7C (7.6mmID*100mm)
Eluent : 20mM Monoethanolamine buffer(pH9.5) + 500mM NaCl
Flow rate : 1.0mL/min
Detector : UV(280nm)
Column temp. : 30°C

Courtesy of Dr. Shirai, Tokyo University of Agriculture and Technology

Lipoxidase (1) (SP-825)

Lipoxidase, an oxidoreductase was analyzed using IEC SP-825 (a column for strong anion exchange chromatography). It is a glycoprotein of MW 80,000. Lipoxidase from soybean consists of three fractions. Enzyme activity was found at two peaks around 25 minutes.



Sample: 0.5% Lipoxidase, 150 µL

Column : Shodex IEC SP-825 (8.0mmID*75mm)
Eluent : (A); 20mM Citric acid-NaOH buffer(pH4.3)
(B); (A) + 0.5M Na_2SO_4
Linear gradient: 0min to 60min, (A) to (B)
Flow rate : 1.0mL/min
Detector : UV(280nm)
Column temp. : 25°C

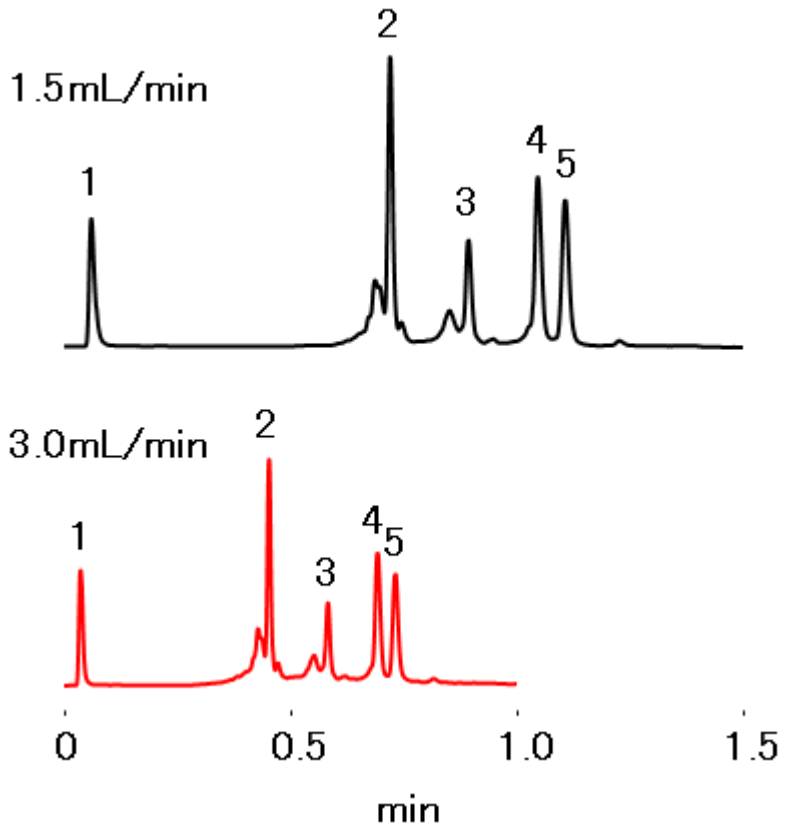
Ultra-rapid Analysis of Standard Proteins (SP-FT 4A)

IEC SP-FT 4A, a non-porous type column for cation exchange chromatography, analyzed five standard proteins. These proteins can be separated well at a 3.0mL/min flow rate while maintaining the same separation at a 1.5mL/min flow rate. This analysis can be completed, within a mere 50 seconds.

(Caution) Backpressure will differ depending on the LC system used and the analytical conditions such as eluent compositions and column temperature. Please set the system pressure (sum of column and system pressures) to less than 20 MPa.

Sample: 5µL

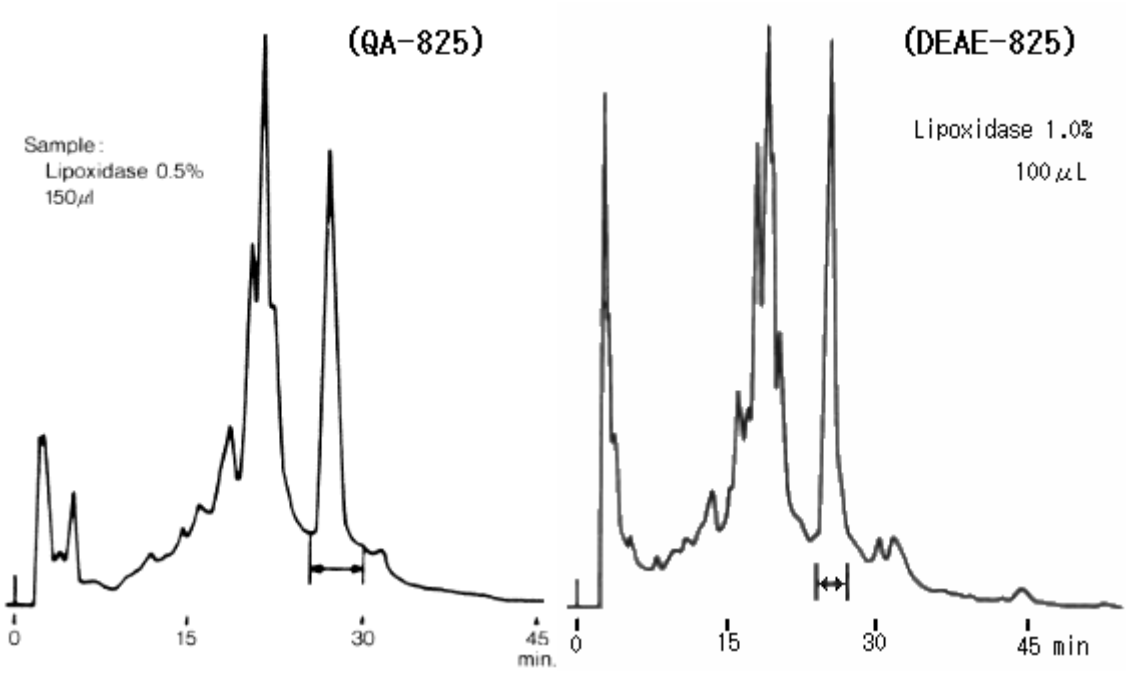
1. Ovalbumin 0.8mg/mL
2. Trypsinogen 1.6mg/mL
3. Ribonuclease A 1.6mg/mL
4. Cytochrome c 0.8mg/mL
5. Lysozyme 0.4mg/mL



Column : Shodex IEC SP-FT 4A (4.6mm I.D. x 10mm)
Eluent : (A); 20mM *MES buffer (pH6.0)
(B); (A) + 0.5M Na_2SO_4
Linear gradient: (A) to (B) (2min) □□□□
Flow rate : 1.5, 3.0mL/min
Detector : UV(280nm)
Column temp. : Room temp.
*MES : 2-(N-Morpholino) ethanesulfonic acid

Lipoxidase from Soybean (1) (QA-825, DEAE-825)

Lipoxidase from soybean was analyzed using IEC QA-825 (a column for strong anion exchange chromatography) and IEC DEAE-825 (a column for weak anion exchange chromatography). The enzyme activity of this sample was measured by using hydrogen peroxide as a substrate. Enzyme activity was found at the arrowhead position.



Sample : Lipoxidase from soybean

QA-825

Column：Shodex IEC QA-825 (8.0 mm I.D. x 75 mm)
Eluent：(A); 20 mM Ethanolamine-HCl buffer (pH9.0)
(B); (A) + 0.5 M NaCl
Linear gradient; 0 min to 60 min, (A) to (B)
Flow rate：0.6 mL/min
Detector：UV (280 nm)
Column temp.：Room temp.

DEAE-825

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

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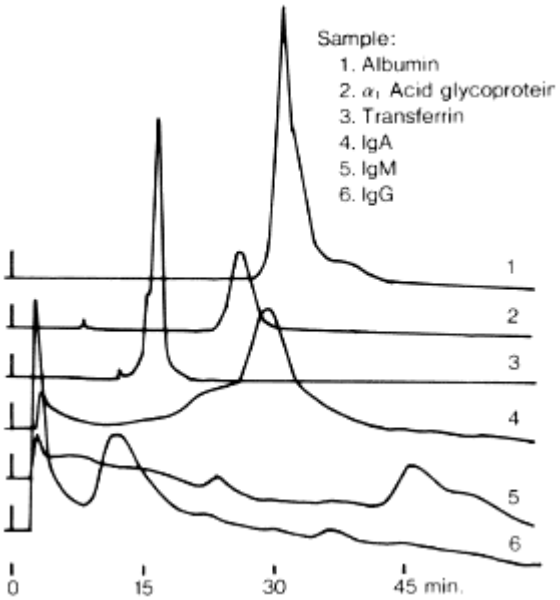
Proteins in Human Serum (2) (DEAE-825)

Proteins in human serum were analyzed using IEC DEAE-825 (a column for weak anion exchange chromatography).

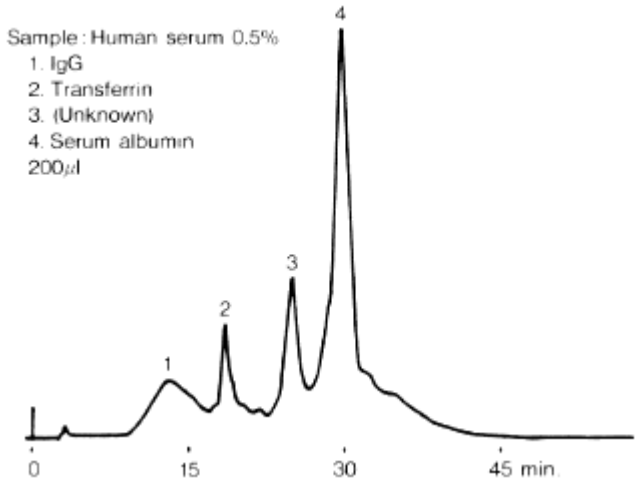


Sample : Human Serum
1. Albumin
2. alpha1 Acid glycoprotein
3. Transferrin
4. IgA
5. IgM
6. IgG

Column : Shodex IEC DEAE-825 (8.0mmID*75mm)
Eluent : (A); 20mM Tris-CH_3COOH buffer(pH8.0)
(B); (A) + 0.5M CH_3COONa
Linear gradient: 0min to 60min, (A) to (B)
Flow rate : 1.0mL/min
Detector : UV(280nm)
Column temp. : Room temp.

Proteins in Human Serum (1) (QA-825)

Proteins in Human serum were analyzed using IEC QA-825 (a column for strong anion exchange chromatography).
In this analysis, the sample was separated into four peaks including the transferrin peak; the largest one is serum albumin.

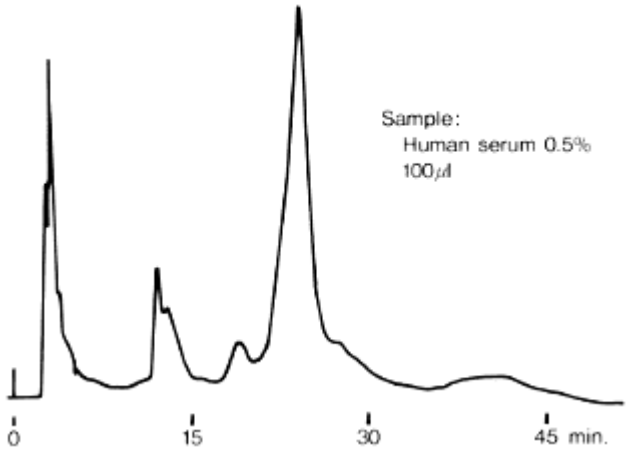


Sample : Human serum
1. IgG
2. Transferrin
3. (Unknown)
4. Serum albumin

Column : Shodex IEC QA-825 (8.0mmID*75mm)
Eluent : (A); 20mM Tris-HCl buffer(pH8.6)
(B); (A) + 0.5M NaCl
Linear gradient: 0min to 60min, 100% (A) to 50% (B)
Flow rate : 1.0mL/min
Detector : UV(280nm)
Column temp. : Room temp.

Human Serum (4) (DEAE-825)

Human serum was analyzed using IEC DEAE-825 (a column for waek anion exchange chromatography).



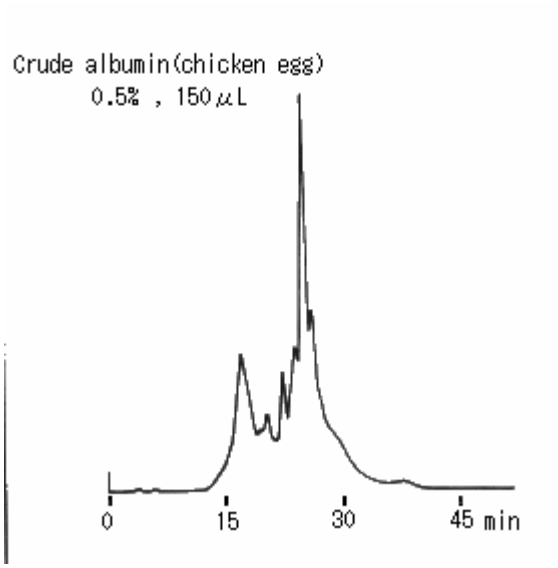
Sample : Human serum

Column : Shodex IEC DEAE-825 (8.0mmID*75mm)
Eluent : (A); 50mM 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.

>

Crude Albumin (Chicken Egg)

Chicken egg albumin was analyzed using IEC QA-825 (a column for strong cation exchange chromatography).

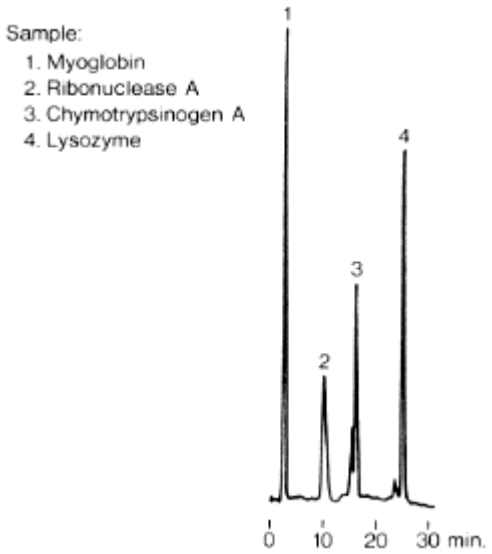


Sample: Crude albumin (chicken egg)

Column : Shodex IEC QA-825 (8.0mmID*75mm)
Eluent : (A); 20mM Tris-HCl buffer(pH8.5)
(B); (A) + 0.5M NaCl
Linear gradient: 0min to 60min, (A) to (B)
Flow rate : 1.0mL/min
Detector : UV(280nm)
Column temp. : 25°C

Standard Proteins (8) (ES-502C 7C)

Four kinds of protien standard were analyzed using Asahipak ES-502C 7C (a column for weak cation exchange chromatography).

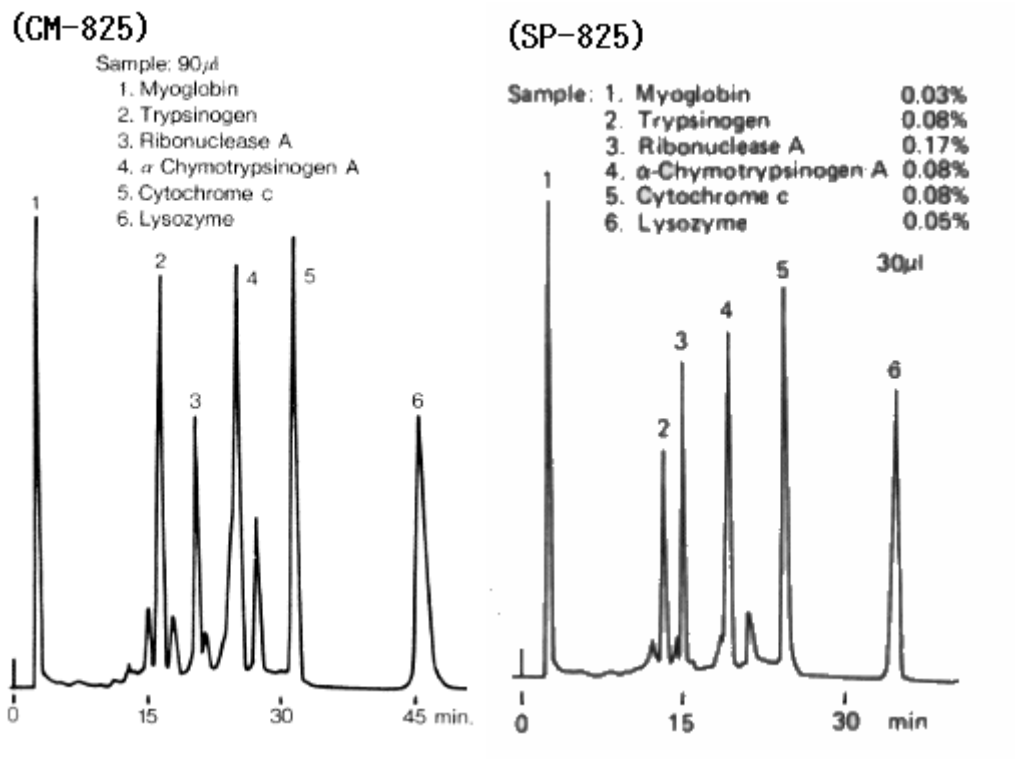


Sample :
1. Myoglobin
2. Ribonuclease A
3. Chymotrypsinogen A
4. Lysozyme

Column : Shodex Asahipak ES-502C 7C (7.6mmID*100mm)
Eluent : (A); 50mM Sodium phosphate buffer(pH7.0)
(B); (A) + 0.5M NaCl
Linear gradient: 0min to 20min, (A) to (B)
Flow rate : 1.0mL/min
Detector : UV(280nm)

Standard Proteins (7) (CM-825, SP-825)

Six kinds of protien standard were analyzed using IEC CM-825 (a column for weak cation exchange chromatography) and IEC SP-825 (a column for strong cation exchange chromatography).

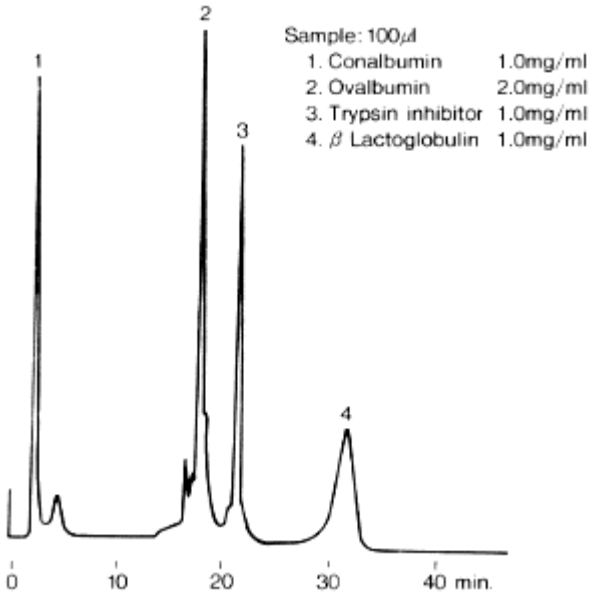


Sample :
1. Myoglobin, 2. Trypsinogen, 3. Ribonuclease A, 4. alpha Chymotrypsinogen A, 5. Cytochrome c, 6. Lysozyme

Columns : (Left) Shodex IEC CM-825 (8.0mmID*75mm)
(Right) Shodex IEC SP-825 (8.0mmID*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.

Standard Proteins (6) (ES-502N 7C)

The base packing material of the IEC DEAE-825 (a column for weak anion exchange chromatography) is poly(hydroxy methacrylate) and that of the Asahipak ES-502N 7C (a column for weak anion exchange chromatography) is poly(vinyl alcohol). In case of poly(vinyl alcohol) material, not only the ion exchange mode affect the separation, but also the reversed phase mode contributes to some degree to the separation of high molecular weight compounds such as proteins. There are some cases in which the difference in the packing material base may affect the separation.

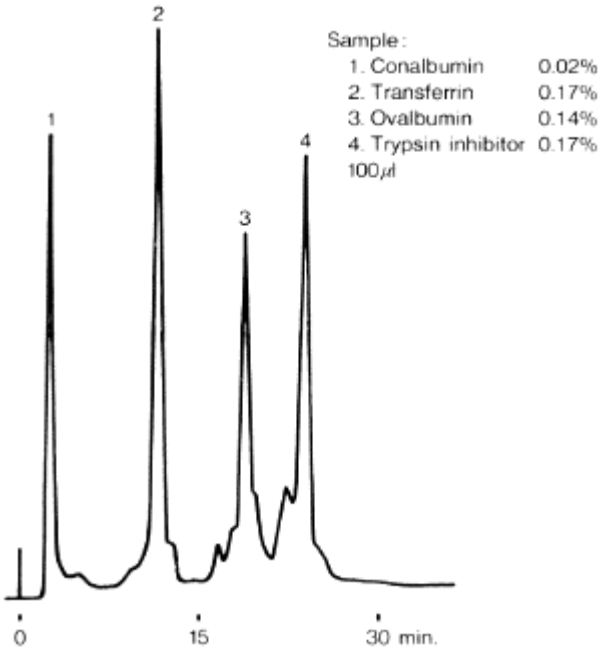


Sample :
1. Conalbumin
2. Ovalbumin
3. Trypsin inhibitor
4. beta Lactoglobulin

Column : Shodex Asahipak ES-502N 7C (7.6mmID*100mm)
Eluent : (A); 50mM Bis-Tris propane HCl(pH7.0)
(B); 50mM Bis-Tris propane HCl(pH7.0) + 500mM NaCl
Linear gradient: 0min to 20min, 100% (A) to 100% (B)
Flow rate : 1.0mL/min
Detector : UV(280nm)
Column temp. : 30°C

Standard Proteins (5) (QA-825)

Four kinds of protein standards were analyzed using IEC QA-825 (a column for strong anion exchange chromatography). Since the pH value of the eluent is 6.0 and the pI value of conalbumin is in the range of 6.0 to 6.8, the conalbumin cannot be retained by the packing material and therefore it elutes rapidly. The other proteins like transferrin, ovalbumin and trypsin are negatively charged because their pIs are lower than 6.0 and therefore they are retained by the packing material. These proteins are released from the surface of the packing material and elute from the column by gradient elution with increasing salt concentration.

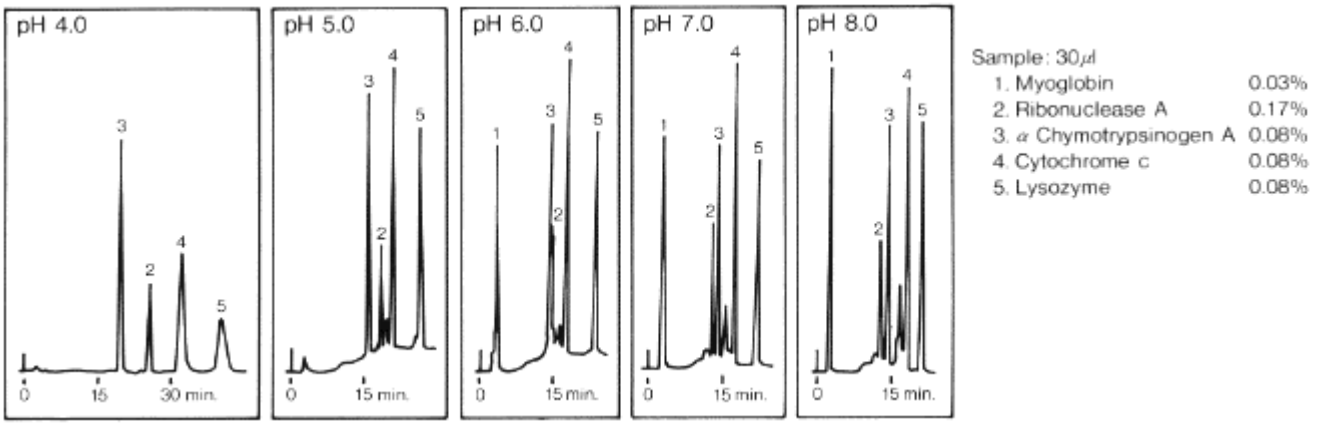


Sample :
1. Conalbumin
2. Transferrin
3. Ovalbumin
4. Trypsin inhibitor

Column : Shodex IEC QA-825 (8.0mmID*75mm)
Eluent : (A); 20mM Piperazine-HCl buffer(pH6.0)
(B); (A) + 0.5M NaCl
Linear gradient: 0min to 30min, 100% (A) to 50% (B)
Flow rate : 1.0mL/min
Detector : UV(280nm)
Column temp. : Room temp.

Effect of pH on Elution Pattern (SP-825)

Using an eluent at pH of 7.0 or 8.0, five types of proteins are well separated using IEC SP-825 (a column for strong cation exchange chromatography). The retention time of ribonuclease A increases with decreasing pH value and, at pH of less than 6.0, ribonuclease A elutes after alpha-chymotrypsinogen A. At pH 6.0, the separation of ribonuclease A from alpha-chymotrypsinogen A is insufficient. At pH 4.0 or 5.0, myoglobin does not elute sharply.

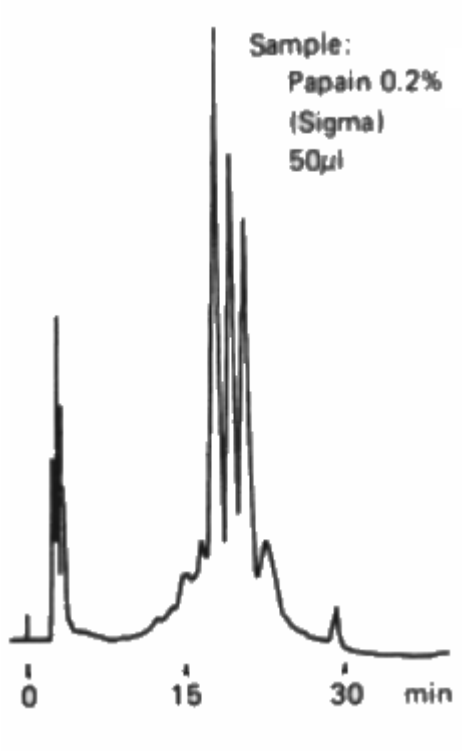


Sample :
1. Myoglobin, 2. Ribonuclease A, 3. alpha Chymotrypsinogen A, 4. Cytochrome c, 5. Lysozyme

Column : Shodex IEC SP-825 (8.0mmID*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

Papain (SP-825)

Papain was analyzed using IEC SP-825 (a column for strong cation exchange chromatography). Papain is a polypeptide chain of MW 23,406 and pI 8.75. It is very stable protelytic enzyme. It is used as the reagent for the study of structure of proteins and also for industrial use.

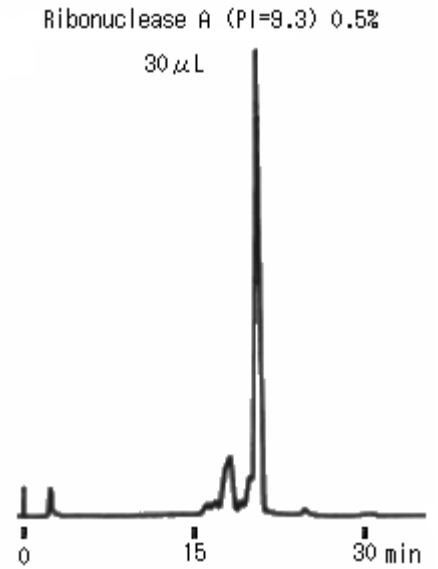


Sample : Papain

Column : Shodex IEC SP-825 (8.0mmID*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. : 25°C

Ribonuclease A from Equine Pancreas (CM-825)

Ribonuclease A from equine pancreas was analyzed using IEC CM-825 (a column for weak cation exchange chromatography). This enzyme contains rather small impurities.

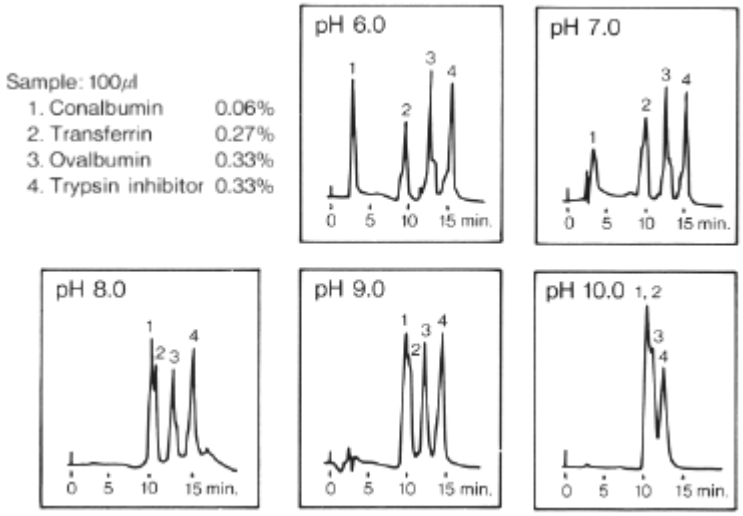


Sample: 0.5% Ribonuclease A (pI=9.3), 30micro-L

Column : Shodex IEC CM-825 (8.0mmID*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. : 25°C

Effect of pH on Elution Pattern (DEAE-825)

When the buffer eluent is acidic, four types of proteins are separated using IEC DEAE-825 (a column for weak anion exchange chromatography) well. In opposition to this, when the eluent is alkaline, the separation of the proteins is poor. By increasing the pH value, the four peaks tend to converge into one peak. The reason for that is the ion exchange groups of the packing material cannot dissociate adequately when the pH value is high.



Sample :

1. Conalbumin
0.1%
2. Transferrin
0.2%
3. Ovalbumin
0.2%
4. Trypsin inhibitor 0.2%

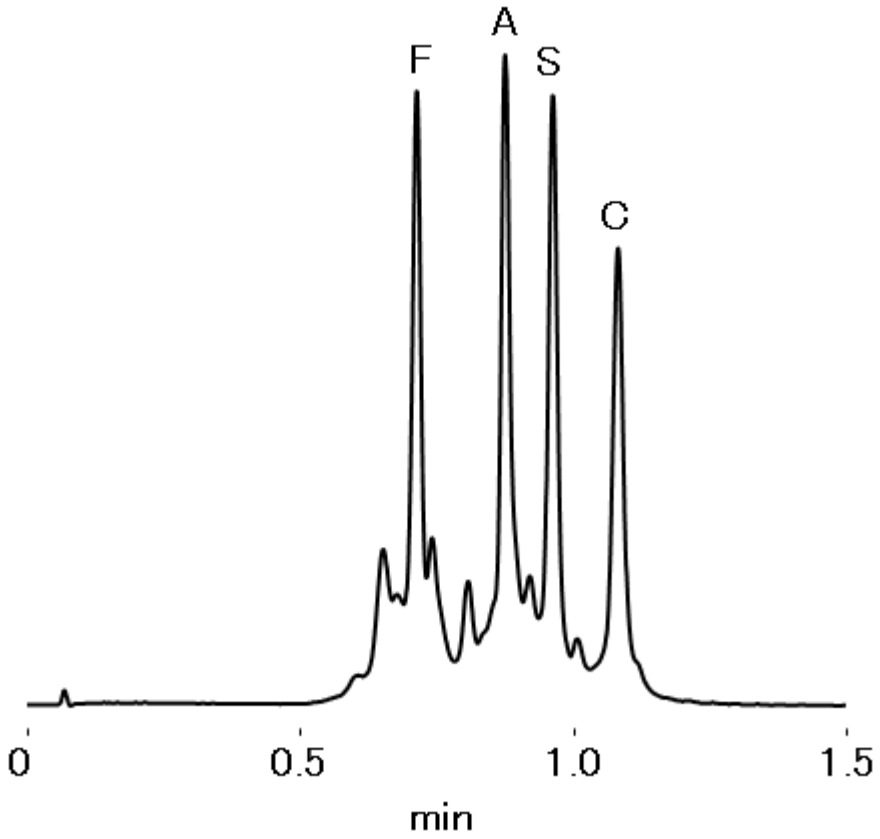
Column : Shodex IEC DEAE-825 (8.0mmID*75mm)
Eluent : (A); 20mM Piperazine-HCl buffer(pH6.0)
20mM Bis-Tris-HCl buffer(pH7.0)
20mM Tris-HCl buffer(pH8.0)
20mM Ethanolamine-HCl buffer(pH9.0)
20mM 1,3-Diaminopropane-HCl buffer(pH10.0)
(B); (A) + 0.5M NaCl

Linear gradient: 0min to 20min, (A) to (B)
Flow rate : 1.0mL/min
Detector : UV(280nm)
Column temp. : 25°C

Ultra-rapid Analysis of Hemoglobin A, F, S and C (SP-FT 4A)

Hemoglobin A, F, S and C were analyzed using IEC SP-FT 4A, a non-porous type column for cation exchange chromatography. SP-FT 4A can provide ultra-rapid and high-resolution analysis using conventional devices as same as when using UHPLC.

(Caution) Backpressure will differ depends on the LC system used and the analytical conditions such as eluent compositions and column temperature. Please set the system pressure (sum of column and system pressures) less than 20 MPa.



Sample : 5µL
AFSC Hemoglobin Control
(51-fold diluted)

Column : Shodex IEC SP-FT 4A (4.6mmI.D. x 10mm)
Eluent : (A); 20mM *MES buffer(pH5.6)
(B); (A) + 0.5M Na_2SO_4
Linear gradient: 5% (B) to 100% (B) (2min)

Flow rate : 1.7mL/min
Detector : VIS(415nm)
Column temp. : 30°C
*MES : 2-(N-Morpholino) ethanesulfonic acid

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