

APPLICATION DATA

Amino acid·Peptide·Protein

HPLC/IEX/SEC

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Technical Data

- Tips for optimization of peptides and proteins separation

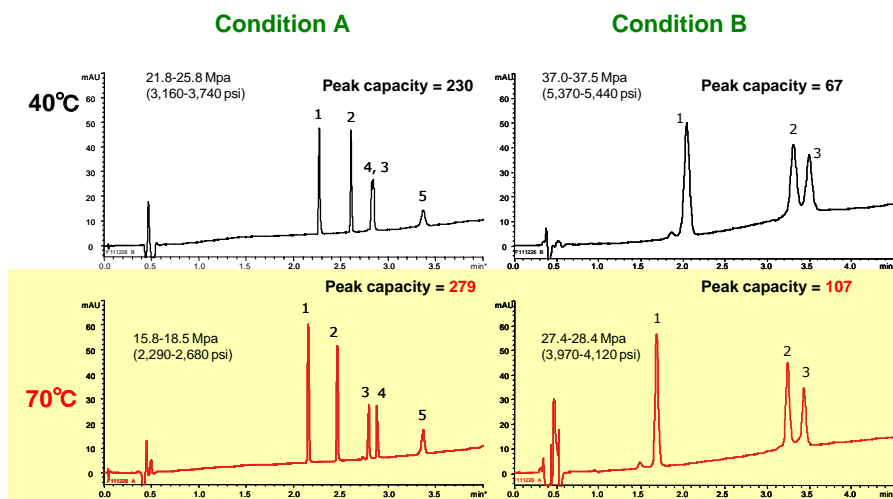
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Tips for optimization of peptides and proteins separation

R150610AE

Effect of column temperature on separation of peptides and proteins

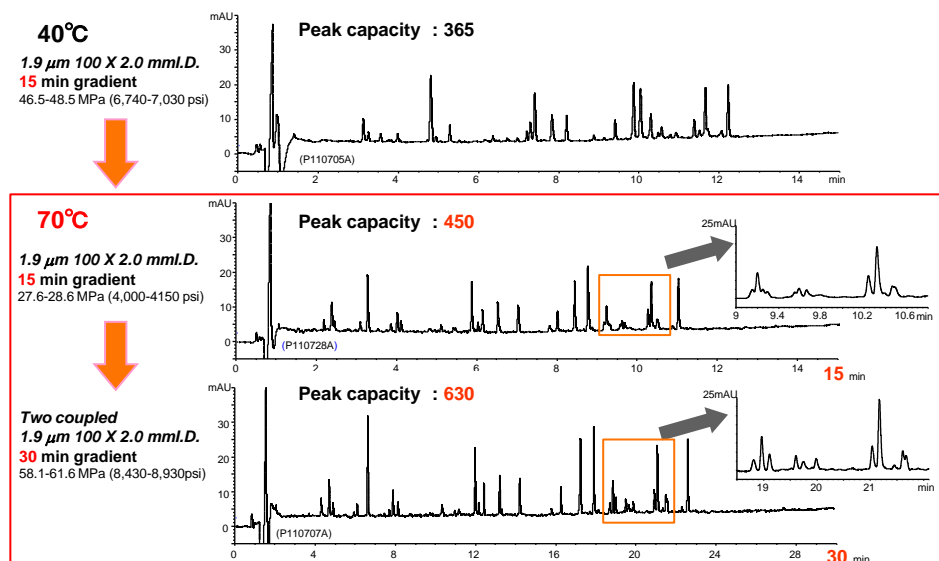


Analytes	MW	Peak width 1/2(min)	
		40°C	70°C
Condition A			
1. Oxytocin	1,007	0.017	0.014
2. Leu-Enkephalin	556	0.015	0.015
3. β -Endorphin	3,465	-	0.016
4. Insulin	5,733	-	0.015
5. β -Lactoglobulin A	18,400	0.043	0.030
Condition B			
1. Lysozyme	14,300	0.069	0.044
2. α -Chymotrypsinogen	25,700	0.080	0.049
3. β -Lactoglobulin A	18,400	0.080	0.048

Column : YMC-Triart C18 (1.9 μ m, 120 \AA), 50 X 2.0 mmI.D.
 Eluent : A) water/TFA (100/0.1)
 B) acetonitrile/TFA (100/0.1) – condition A
 C) acetonitrile/2-propanol/TFA (50/50/0.1) – condition B
 Gradient : 10-80%B (0-5 min) – condition A
 30-60%B (0-5 min) – condition B
 Flow rate : 0.4 mL/min
 Detection : UV at 220 nm

- The effect of temperature on separation of peptides and proteins with a variety of molecular weight (MW) is estimated. The separations at 40°C and 70°C are compared.
- By increasing column temperature to 70°C, selectivity change is observed, and peaks become sharper. Thus, improved resolution especially for larger molecules is obtained. Generally, larger molecules diffuse very slowly compared to small molecules. An elevated temperature can improve efficiency and peak shape by lowering mobile phase viscosity and improving mass transfer.
- Temperature is a simple and effective tool to increase resolution in separation of proteins and peptides.

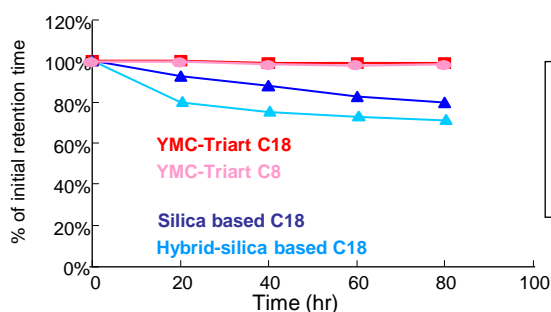
Improvement of resolution by increasing column temperature and coupling of 1.9 μ m columns



- 23% more peaks can be resolved by increasing the column temperature to 70°C in the separation of tryptic digest of Hemoglobin.
- The outstanding efficiency obtained by a coupling of two 100 mm length of Triart 1.9 μ m columns reduces co-elution peaks and allows the precise separation in an analysis of complicated samples, such as peptide mapping.

Column : YMC-Triart C18 (1.9 μ m, 120 \AA)
 Eluent : A) water/TFA (100/0.1)
 B) acetonitrile/TFA (100/0.08)
 5-40%B (0-15 min) for a single column
 5-40%B (0-30 min) for two coupled columns
 Flow rate : 0.4 mL/min
 Detection : UV at 220 nm
 Sample : Tryptic digest of Bovine Hemoglobin

Durability in pH 1 (1% TFA), 70°C



Test conditions
 Column : 5 μ m, 50 X 2.0 mmI.D.
 Eluent : acetonitrile/water (60/40)
 Flow rate : 0.2 mL/min
 Temperature : 37°C
 Sample : butyl benzoate

The columns are kept in acetonitrile/water/TFA (10/90/1, pH 1) at 70°C, and tested for performance every 20 hours

- YMC-Triart C18, which offers excellent durability even under elevated temperature and low-pH conditions, is effective for the separation of peptides and proteins.

Analytical Data

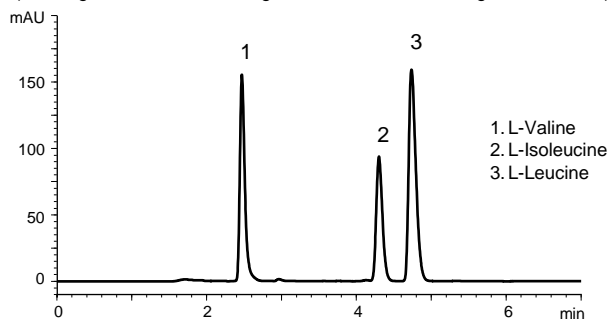
イソロイシン、バリン、ロイシン顆粒

L-Isoleucine, L-Leucine and L-Valine granules

U120210A

Standard solution

(1.10 mg/mL L-Valine, 0.92 mg/mL L-Isoleucine, 1.84 mg/mL L-Leucine)



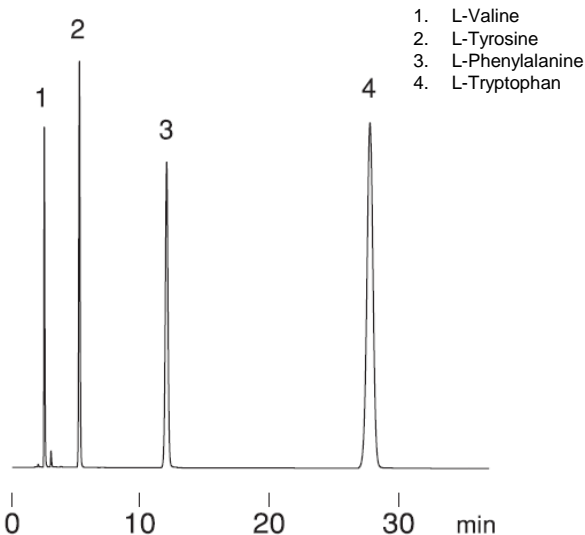
	System suitability requirement	result	
Resolution (2, 3)	≥ 1.5	2.68	
Relative standard deviation of the retention time (each of the peaks)	≤ 1.0%	1	0.02%
		2	0.02%
		3	0.02%

Column : YMC-Triart C18 (3 μm, 12 nm)
150 X 4.6 mmI.D.
Eluent : phosphate buffer (pH 2.8)*1/acetonitrile (97/3)
*1 Dissolve 31.2 g of NaH₂PO₄·2H₂O in 1000 mL of water and adjust pH 2.8 with H₃PO₄
Flow rate : 0.9 mL/min (adjust the flow rate so that the retention time of L-Valine is about 2.5 min)
Temperature : 40°C
Detection : UV at 210 nm
Injection : 20 μL
(The Japanese Pharmacopoeia 16th; Identification)

アミノ酸

Amino acids

A000419B

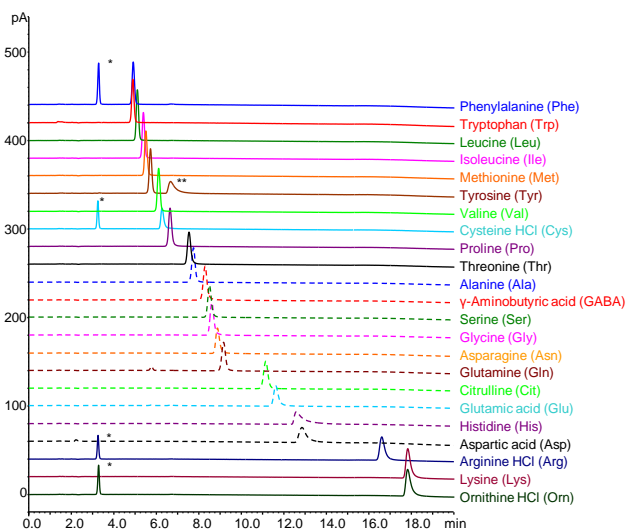


Column : Hydrosphere C18 (5 μm, 12 nm)
150 X 4.6 mmI.D.
Eluent : 20 mM KH₂PO₄
Flow rate : 1.0 mL/min
Temperature : 37°C
Detection : UV at 210 nm
Injection : 5 μL (0.05-1.6 mg/mL)

アミノ酸

Amino acids

F130618A



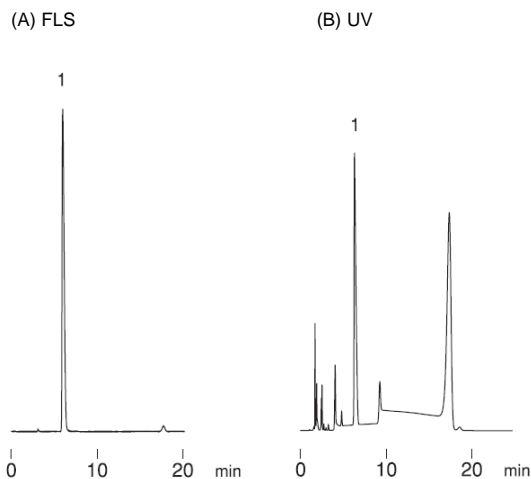
Column : YMC-Triart Diol-HILIC (5 μm, 12 nm)
150 X 4.6 mmI.D.
Eluent : A) 100 mM HCOOH-HCOONH₄ (pH 3.6)
B) acetonitrile
83-80%B (0-12 min), 80-68%B (12-20 min)
Flow rate : 1.0 mL/min
Temperature : 40°C
Detection : Corona® CAD® (Charged Aerosol Detector)
Injection : 10 μL (0.1 mg/mL)
* Corona and CAD are trademarks of Thermo Fisher Scientific.

市販ドリンク剤中のタウリン

Taurine in nutritious drink

F001115C

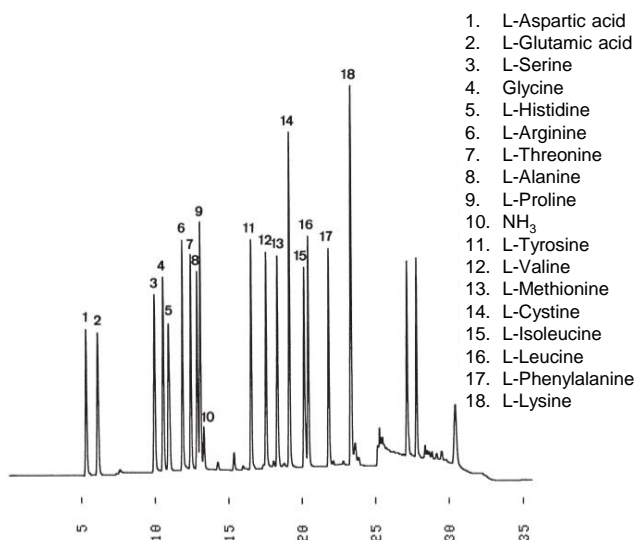
1. Taurine



Column : Hydrosphere C18 (5 μm, 12 nm)
150 X 4.6 mmI.D.
Eluent : 10 mM KH₂PO₄-K₂HPO₄ (pH 6.8)/acetonitrile (85/15)
Flow rate : 1.0 mL/min
Temperature : 30°C
Detection : (A) FLS at Ex 345 nm, Em 450 nm
(B) UV at 254 nm
Injection : 15 μL
Sample : OPA derivative

PTC-アミノ酸
PTC-amino acids

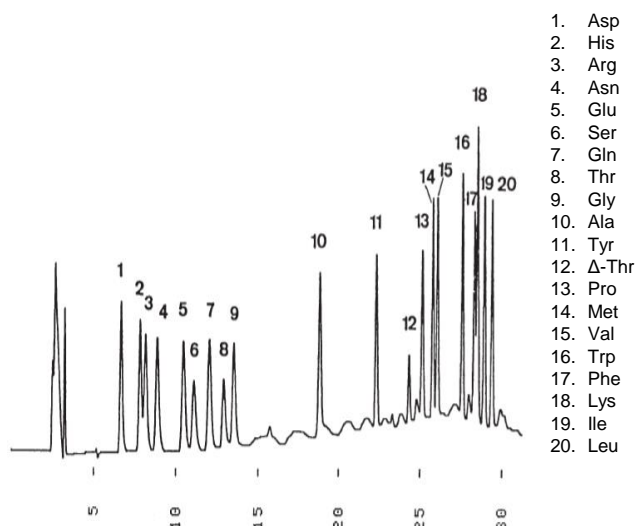
G910523A



Column : YMC-Pack ODS-AM (5 μ m, 12 nm), 250 X 4.6 mmI.D.
 Eluent : A) 60 mM CH₃COONa-CH₃COOH (pH 6.6)/acetonitrile (94/6)
 B) 60 mM CH₃COONa-CH₃COOH (pH 6.6)/acetonitrile (40/60)
 0-55%B (0-20 min), 100%B (20-25 min), 0%B (25-35 min)
 Flow rate : 1.0 mL/min
 Temperature : 37°C
 Detection : UV at 250 nm
 Injection : 5 μ L (62.5 pmol/ μ L)
 Sample : PTC derivatives

PTH-アミノ酸
PTH-amino acids

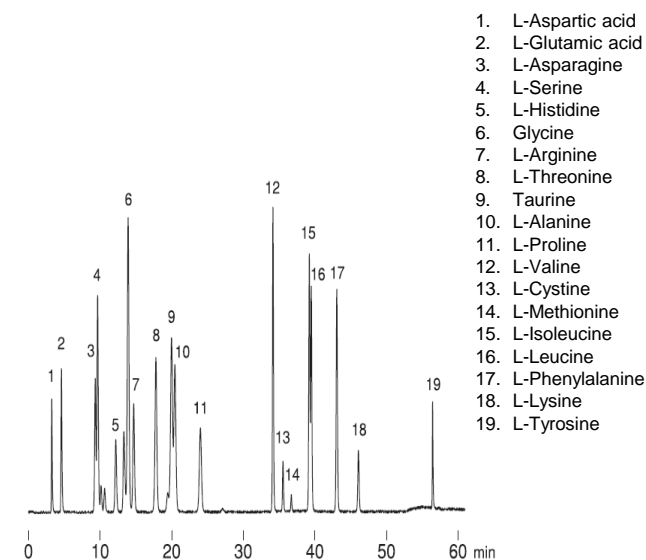
K940331A



Column : J'sphere ODS-H80 (4 μ m, 8 nm)
 250 X 2.0 mmI.D.
 Eluent : A) acetonitrile/THF/300 mM TEAA* (pH 5.1) (10/4/90)
 B) acetonitrile/THF/300 mM TEAA* (pH 5.1) (75/4/25)
 0-30%B (0-15 min), 30-80%B (15-30 min)
 Flow rate : 0.2 mL/min
 Temperature : 50°C
 Detection : UV at 270 nm
 Injection : 30 μ L (25 pmol/10 μ L)
 *TEAA: triethylamine-acetic acid

NBD-アミノ酸
NBD-amino acids

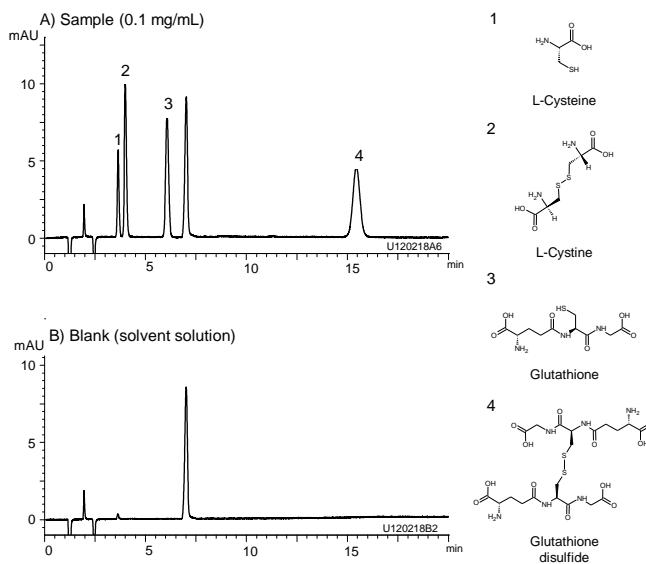
F001129E



Column : Hydrosphere C18 (5 μ m, 12 nm), 150 X 4.6 mmI.D.
 Eluent : A) 20 mM KH₂PO₄-K₂HPO₄ (pH 6.5)
 B) 20 mM KH₂PO₄-K₂HPO₄ (pH 6.5)/acetonitrile (50/50)
 15-25%B (0-25 min), 25-40%B (25-30 min),
 40-65%B (30-50 min), 65-100%B (50-55 min),
 100%B (55-60 min)
 Flow rate : 1.0 mL/min (0-25 min), 1.5 mL/min (25-60 min)
 Temperature : 30°C
 Detection : FLS at Ex 470 nm, Em 530 nm
 Injection : 20 μ L
 Sample : NBD derivatives

グルタチオンおよび関連化合物
Glutathione and related compounds

U120218A



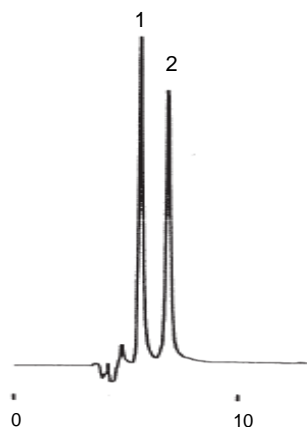
Column : YMC-Triart C18 (5 μ m, 12 nm)
 150 X 3.0 mmI.D.
 Eluent : acetonitrile/water/heptafluorobutyric acid (8/92/0.1)
 Flow rate : 0.425 mL/min
 Temperature : 30°C
 Detection : UV at 220 nm
 Injection : 2 μ L

酸化型および還元型グルタチオン

Glutathion, oxidized and reduced form

H900710D

1. Glutathione (GSH)
2. Glutathione disulfide (GSSG)



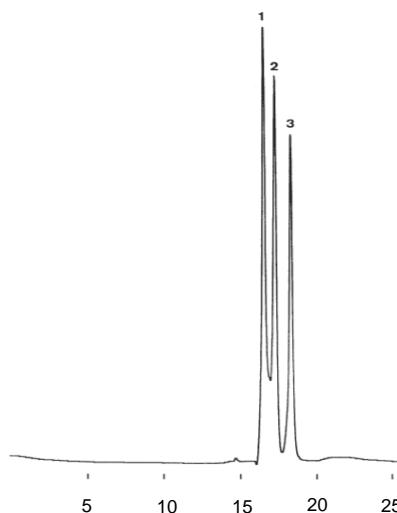
Column	: YMC-Pack ODS-A (5 μ m, 12 nm) 150 X 4.6 mm.I.D.
Eluent	: 0.1 M sodium perchlorate in A A) acetonitrile/water/TFA (5/95/0.05)
Flow rate	: 0.5 mL/min
Temperature	: 30°C
Detection	: UV at 220 nm
Injection	: 10 μ L

グリシンオリゴマー

Glycine oligomers

G911227B

1. Pentaglycine
2. Triglycine
3. Glycine



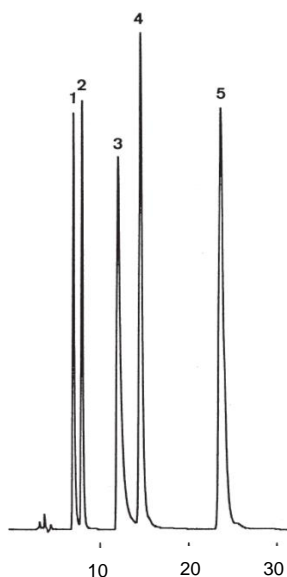
Column	: YMC-Pack Diol-60 (5 μ m) 500 X 8.0 mm.I.D.
Eluent	: 0.1 M KH_2PO_4 - K_2HPO_4 (pH 7.0)/acetonitrile (70/30)
Flow rate	: 1.0 mL/min
Temperature	: ambient (24°C)
Detection	: UV at 215 nm
Injection	: 20 μ L (0.25-2.5 mg/mL)

アンジオテンシン

Angiotensins

G910520A

1. Angiotensin III
2. Angiotensin II
3. [Asn¹, Val⁵]-Angiotensin I
4. [Val⁵]-Angiotensin I
5. Angiotensin I



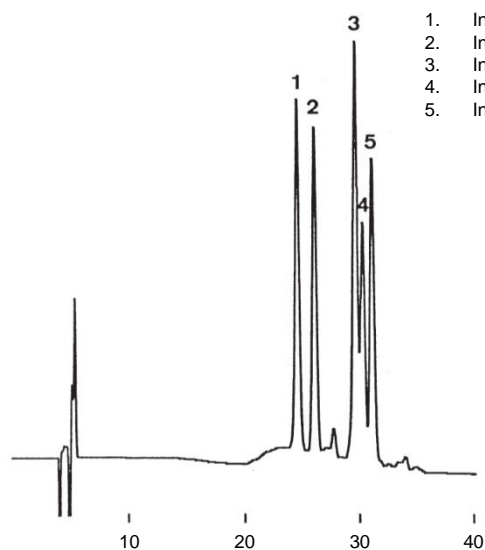
Column	: YMC-Pack ODS-AM (5 μ m, 12 nm) 150 X 4.6 mm.I.D.
Eluent	: acetonitrile/20 mM KH_2PO_4 (20/80)
Flow rate	: 0.5 mL/min
Temperature	: 30°C
Detection	: UV at 220 nm
Injection	: 8 μ L (0.05-0.15 mg/mL)

インスリン

Insulins

G910522C

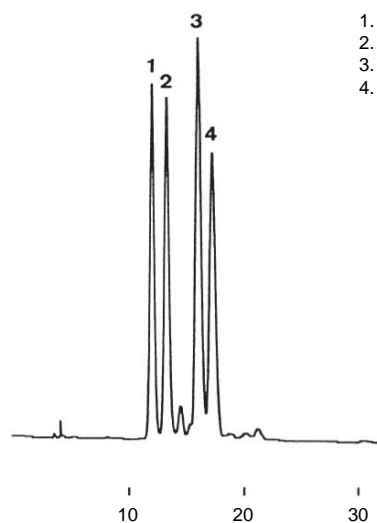
1. Insulin (Bovine)
2. Insulin (Sheep)
3. Insulin (Human)
4. Insulin (Equine)
5. Insulin (Porcine)



Column	: YMC-Pack ODS-AM (5 μ m, 12 nm) 250 X 4.6 mm.I.D.
Eluent	: A) acetonitrile/water/TFA (30/70/0.1) B) acetonitrile/water/TFA (32/68/0.1) 0%B (0-3 min), 0-100%B (3-25 min), 100%B (25-40 min)
Flow rate	: 0.6 mL/min
Temperature	: 30°C
Detection	: UV at 220 nm
Injection	: 15 μ L (0.2 mg/mL)

インスリン Insulins

G910522D



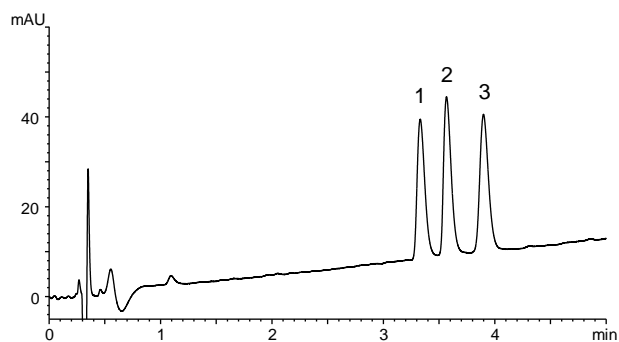
1. Insulin (Bovine)
2. Insulin (Sheep)
3. Insulin (Human)
4. Insulin (Porcine)

Column : YMC-Pack ODS-AM (5 μ m, 12 nm)
150 X 6.0 mmI.D.
Eluent : A) acetonitrile/0.01 N HCl (28/72)
B) acetonitrile/0.01 N HCl (35/65)
0-100%B (0-60 min)
Flow rate : 1.0 mL/min
Temperature : 30°C
Detection : UV at 220 nm
Injection : 15 μ L (0.25 mg/mL)

抗菌ペプチド Antimicrobial peptides

F120501D

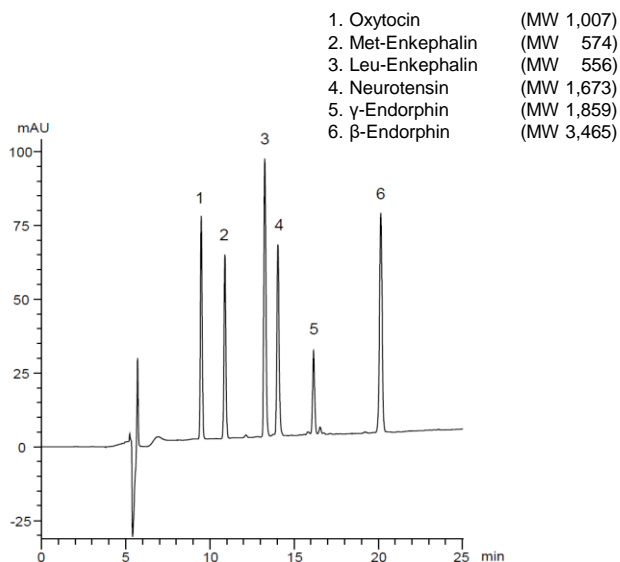
Analytes	Sequence	Molecular weight
1. α -Defensin-1 (human)	ACYCRIPACIAGERRYGTCIYQGRLWAFCC	3,442
2. α -Defensin-2 (human)	CYCRIPACIAGERRYGTCIYQGRLWAFCC	3,371
3. α -Defensin-3 (human)	DCYCRIPACIAGERRYGTCIYQGRLWAFCC	3,486



Column : YMC-Triart C18 (1.9 μ m, 12 nm)
50 X 2.0 mmI.D.
Eluent : A) water/formic acid (100/0.1)
B) 2-propanol/acetonitrile/formic acid (50/50/0.08)
10-25%B (0-10 min)
Flow rate : 0.4 mL/min
Temperature : 70°C
Detection : UV at 220 nm
Injection : 1 μ L (50 μ g/mL)

ペプチド(MW 556-3,465) Peptides (MW 556-3,465)

R091009C

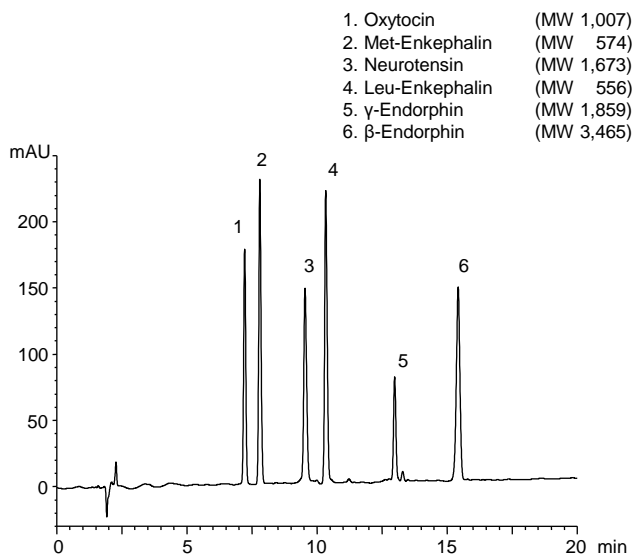


1. Oxytocin (MW 1,007)
2. Met-Enkephalin (MW 574)
3. Leu-Enkephalin (MW 556)
4. Neurotensin (MW 1,673)
5. γ -Endorphin (MW 1,859)
6. β -Endorphin (MW 3,465)

Column : YMC-Triart C18 (5 μ m, 12 nm)
150 X 2.0 mmI.D.
Eluent : A) water/TFA (100/0.1)
B) acetonitrile/TFA (100/0.1)
20-45%B (0-25 min)
Flow rate : 0.2 mL/min
Temperature : 37°C
Detection : UV at 220 nm
Injection : 2 μ L (0.075-0.25 mg/mL)

ペプチド(MW 556-3,465) Peptides (MW 556-3,465)

N041021C



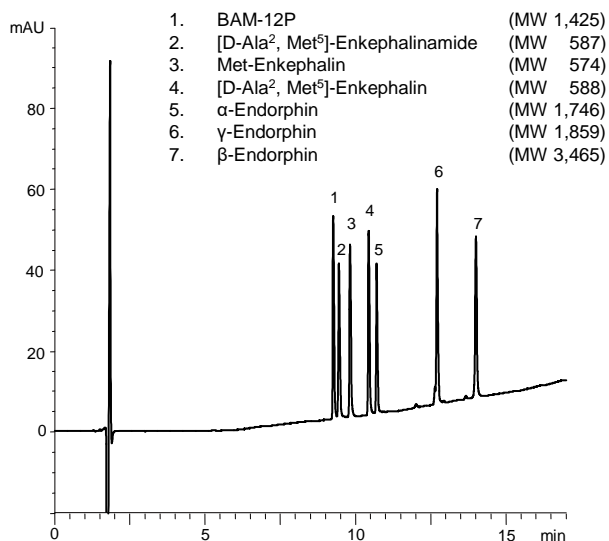
1. Oxytocin (MW 1,007)
2. Met-Enkephalin (MW 574)
3. Neurotensin (MW 1,673)
4. Leu-Enkephalin (MW 556)
5. γ -Endorphin (MW 1,859)
6. β -Endorphin (MW 3,465)

Column : YMC-Pack Pro C18 (5 μ m, 12 nm)
150 X 4.6 mmI.D.
Eluent : A) water/TFA (100/0.05)
B) acetonitrile/TFA (100/0.05)
20-40%B (0-20 min)
Flow rate : 1.0 mL/min
Temperature : 37°C
Detection : UV at 220 nm
Injection : 10 μ L (0.075-0.25 mg/mL)

ペプチド (MW 574-3,465)

Peptides (MW 574-3,465)

H140303A

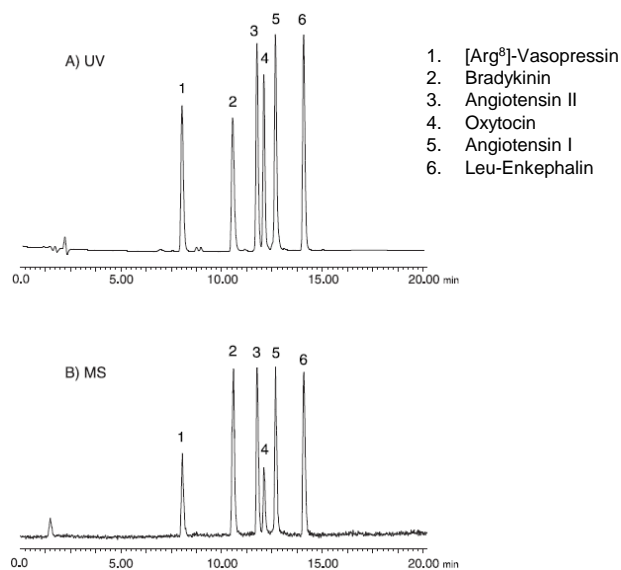


Column : Meteoric Core C18 BIO (2.7 μm, 16 nm)
 150 X 2.1 mmI.D.
 Eluent : A) water/TFA (100/0.1)
 B) acetonitrile/TFA (100/0.1)
 15-55%B (0-15 min), 55%B (15-17 min)
 Flow rate : 0.2 mL/min
 Temperature : 40°C
 Detection : UV at 220 nm
 Injection : 2 μL (0.02-0.05 mg/mL)
 Pressure : 14.9-16.1 MPa (2160-2330 psi)

ペプチド-LC/MS

Peptides-LC/MS

A020808A

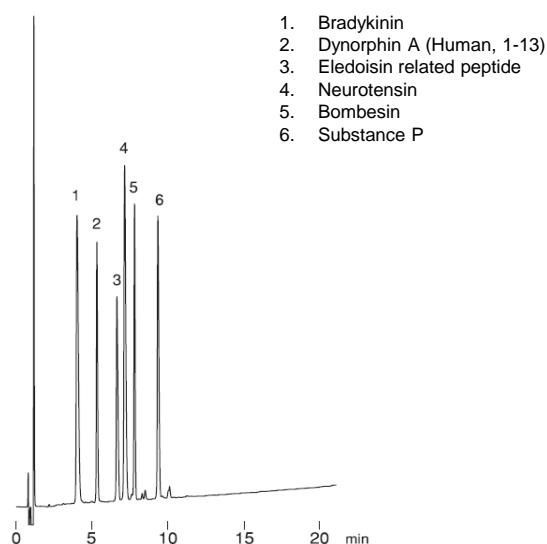


Column : YMC-Pack Pro C18 RS (5 μm, 8 nm)
 150 X 2.0 mmI.D.
 Eluent : A) water/TFA (100/0.01)
 B) acetonitrile/TFA (100/0.01)
 10-35%B (0-15 min), 35%B (15-20 min)
 Flow rate : 0.2 mL/min
 Temperature : 37°C
 Detection : A) UV at 220 nm, B) ESI positive-mode
 Injection : 4 μL (0.05 mg/mL)

ペプチド

Peptides

A980827D

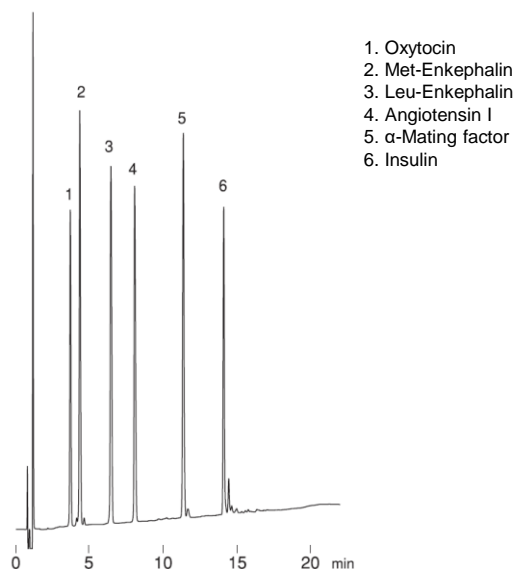


Column : YMC-Pack Pro C18 (3 μm, 12 nm)
 75 X 4.6 mmI.D.
 Eluent : A) water/TFA (100/0.1)
 B) acetonitrile/TFA (100/0.1)
 20-40%B (0-20 min)
 Flow rate : 1.0 mL/min
 Temperature : 37°C
 Detection : UV at 220 nm
 Injection : 15 μL (0.05-0.15 mg/mL)

ペプチド

Peptides

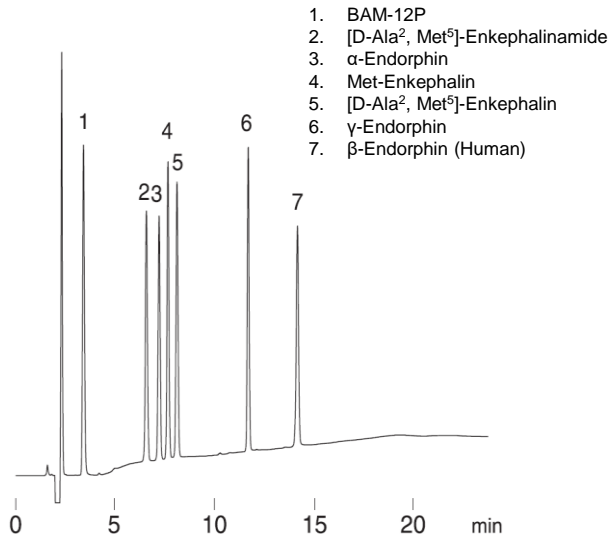
A980827C



Column : YMC-Pack Pro C18 (3 μm, 12 nm)
 75 X 4.6 mmI.D.
 Eluent : A) water/TFA (100/0.1)
 B) acetonitrile/TFA (100/0.1)
 20-40%B (0-20 min)
 Flow rate : 1.0 mL/min
 Temperature : 37°C
 Detection : UV at 220 nm
 Injection : 15 μL (0.04-0.15 mg/mL)

ペプチド
Peptides

A000308B

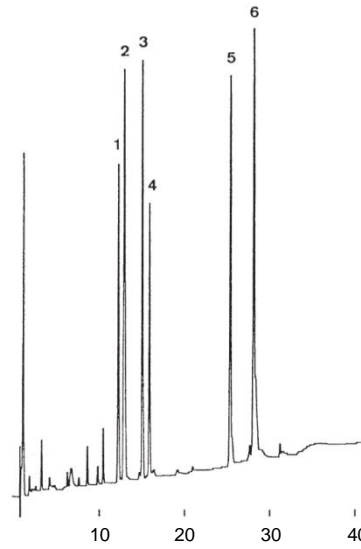


1. BAM-12P
2. [D-Ala², Met⁵]-Enkephalinamide
3. α-Endorphin
4. Met-Enkephalin
5. [D-Ala², Met⁵]-Enkephalin
6. γ-Endorphin
7. β-Endorphin (Human)

Column	: Hydrosphere C18 (5 μm, 12 nm) 150 X 4.6 mmI.D.
Eluent	: A) water/TFA (100/0.1) B) acetonitrile/TFA (100/0.1) 20-40%B (0-15 min), 40%B (15-20 min)
Flow rate	: 1.0 mL/min
Temperature	: 37°C
Detection	: UV at 220 nm
Injection	: 10 μL (0.05-0.1 mg/mL)

ペプチド
Peptides

S930909D

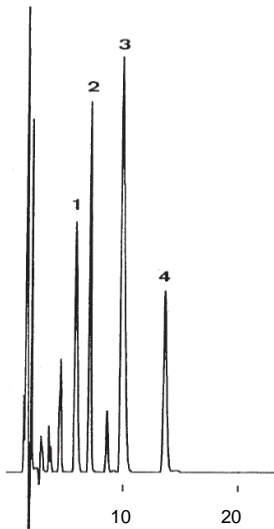


1. Met-Enkephalin
2. Bradykinin
3. Angiotensin II
4. Leu-Enkephalin
5. CCK-Octapeptide
6. Insulin Chain B

Column	: J'sphere ODS-H80 (4 μm, 8 nm) 150 X 4.6 mmI.D.
Eluent	: A) acetonitrile/water/TFA (5/95/0.1) B) acetonitrile/water/TFA (40/60/0.1) 20-100%B (0-40 min)
Flow rate	: 1.5 mL/min
Temperature	: 37°C
Detection	: UV at 220 nm
Injection	: 40 μL (0.067-0.13 mg/mL)

ペプチド
Peptides

O940804A

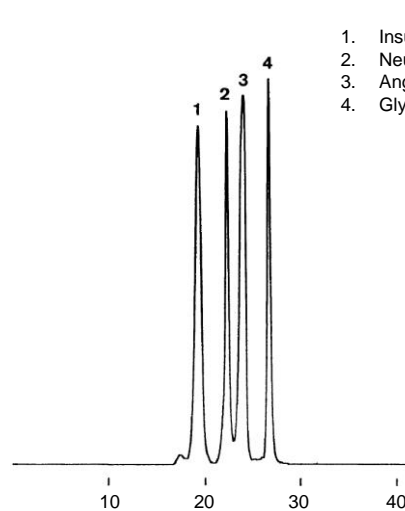


1. Bradykinin
2. Met-Enkephalin
3. Angiotensin III
4. Leu-Enkephalin

Column	: J'sphere ODS-H80 (4 μm, 8 nm) 150 X 2.0 mmI.D.
Eluent	: acetonitrile/water/TFA (20/80/0.1)
Flow rate	: 0.2 mL/min
Temperature	: 37°C
Detection	: UV at 220 nm
Injection	: 4 μL (34-65 μg/mL)

ペプチド (MW 75-5,700)
Peptides (MW 75-5,700)

G911217A



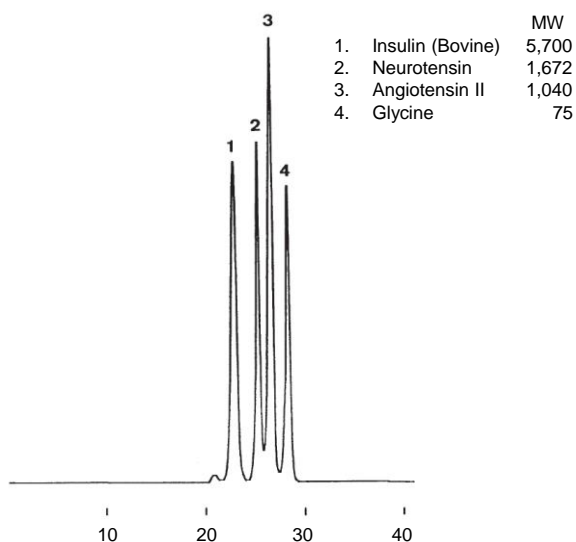
- | | MW |
|---------------------|-------|
| 1. Insulin (Bovine) | 5,700 |
| 2. Neurotensin | 1,672 |
| 3. Angiotensin II | 1,040 |
| 4. Glycine | 75 |

Column	: YMC-Pack Diol-60 (5 μm) 500 X 8.0 mmI.D.
Eluent	: 0.1 M KH ₂ PO ₄ -K ₂ HPO ₄ (pH 7.0) containing 0.2 M NaCl/acetonitrile (70/30)
Flow rate	: 0.7 mL/min
Temperature	: ambient (25°C)
Detection	: UV at 215 nm
Injection	: 25 μL (0.07-5.3 mg/mL)

ペプチド(MW 75-5,700)

Peptides (MW 75-5,700)

G911217B

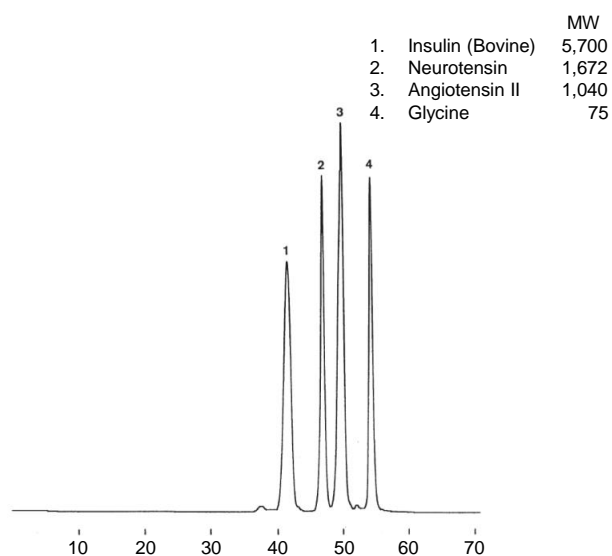


Column	: YMC-Pack Diol-120 (5 μm) 500 X 8.0 mmI.D.
Eluent	: 0.1 M KH ₂ PO ₄ -K ₂ HPO ₄ (pH 7.0) containing 0.2 M NaCl/acetonitrile (70/30)
Flow rate	: 0.7 mL/min
Temperature	: ambient (25°C)
Detection	: UV at 215 nm
Injection	: 25 μL (0.07-5.3 mg/mL)

ペプチド(MW 75-5,700)

Peptides (MW 75-5,700)

G911217C



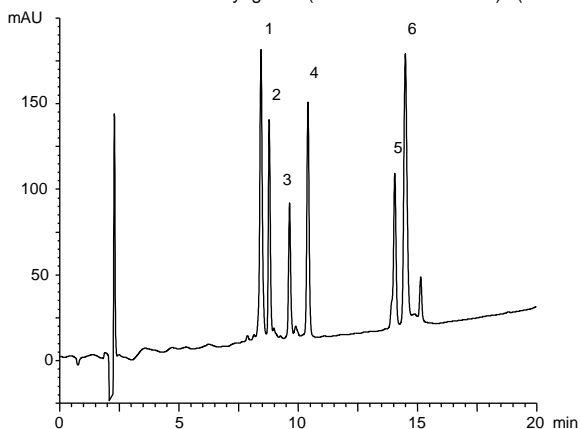
Column	: YMC-Pack Diol-120 + Diol-60 (5 μm) 500 X 8.0 mmI.D. X 2
Eluent	: 0.1 M KH ₂ PO ₄ -K ₂ HPO ₄ (pH 7.0) containing 0.2 M NaCl/acetonitrile (70/30)
Flow rate	: 0.7 mL/min
Temperature	: ambient (25°C)
Detection	: UV at 215 nm
Injection	: 25 μL (0.07-5.3 mg/mL)

ペプチドおよびタンパク質(MW 4,330-17,000)

Peptides and proteins (MW 4,330-17,000)

N040820A

- | | |
|--------------------------------------|-------------|
| 1. Cytochrome c (Horse heart) | (MW 12,400) |
| 2. Insulin (Bovine pancreas) | (MW 5,733) |
| 3. Amyloid β-protein (1-40) | (MW 4,330) |
| 4. Lysozyme (Chicken egg white) | (MW 14,300) |
| 5. α-Lactalbumin (Human milk) | (MW 14,100) |
| 6. Myoglobin (Horse skeletal muscle) | (MW 17,000) |

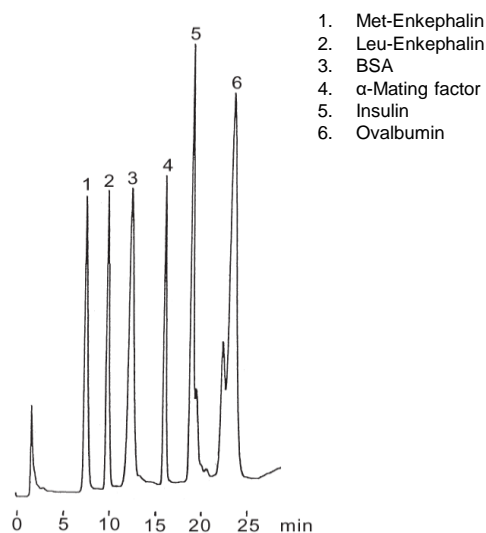


Column	: YMC-Pack C ₈ (5 μm, 20 nm) 150 X 4.6 mmI.D.
Eluent	: A) water/TFA (100/0.1) B) acetonitrile/TFA (100/0.1) 25-60%B (0-20 min)
Flow rate	: 1.0 mL/min
Temperature	: 37°C
Detection	: UV at 220 nm
Injection	: 10 μL (0.1-0.2 mg/mL)

ペプチドおよびタンパク質

Peptides and proteins

H900606A

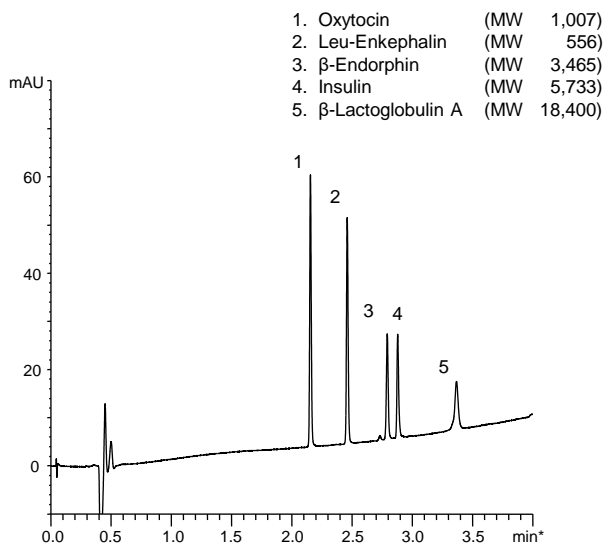


Column	: YMC-Pack PolymerC18 150 X 4.6 mmI.D.
Eluent	: A) acetonitrile/water/TFA (20/80/0.05) B) acetonitrile/water/TFA (45/55/0.05) 0-100%B (0-30 min)
Flow rate	: 1.0 mL/min
Temperature	: 30°C
Detection	: UV at 220 nm
Injection	: 30 μL

ペプチドおよびタンパク質 (MW 556-18,400)

Peptides and proteins (MW 556-18,400)

F111228A

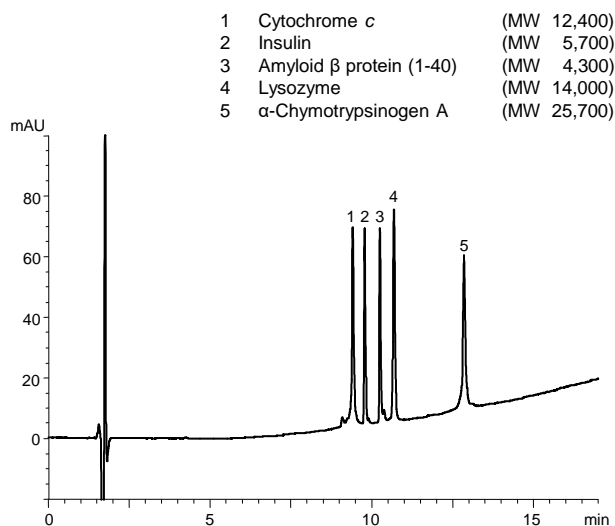


Column : YMC-Triart C18 (1.9 μ m, 12 nm)
50 X 2.0 mmI.D.
Eluent : A) water/TFA (100/0.1)
B) acetonitrile/TFA (100/0.1)
10-80%B (0-5 min)
Flow rate : 0.4 mL/min
Temperature : 70°C
Detection : UV at 220 nm
Injection : 1 μ L (50 μ g/mL)
Pressure : 15.8-18.5 MPa (2290-2680 psi)

ペプチドおよびタンパク質 (MW 4,300-25,700)

Peptides and proteins (MW 4,300-25,700)

H140219D

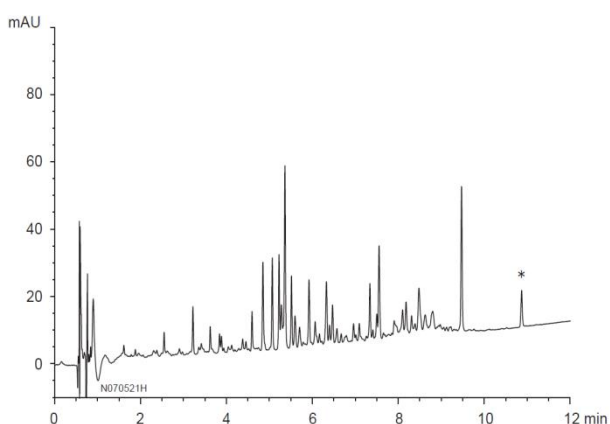


Column : Meteoric Core C18 BIO (2.7 μ m, 16 nm)
150 X 2.1 mmI.D.
Eluent : A) water/TFA (100/0.1)
B) acetonitrile/TFA (100/0.1)
25-70%B (0-15 min), 70%B (15-17 min)
Flow rate : 0.2 mL/min
Temperature : 40°C
Detection : UV at 220 nm
Injection : 2 μ L (0.05-0.2 mg/mL)
Pressure : 12.8-16.1 MPa (1860-2330 psi)

ペプチドマッピング-2 μ mカラムによる高分解能分析

High resolution peptide mapping

N070521H



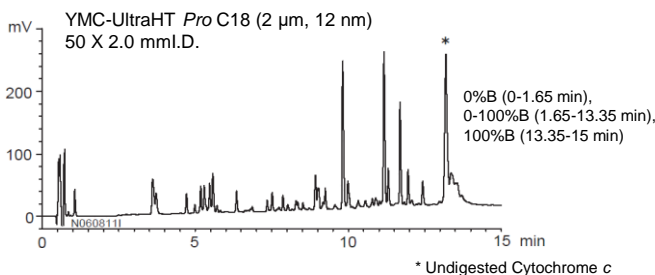
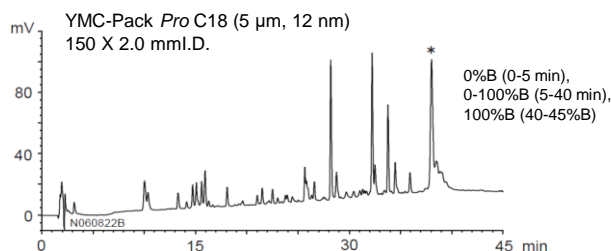
* Undigested β -Lactoglobulin B

Column : YMC-UltraHT Pro C18 (2 μ m, 12 nm)
100 X 2.0 mmI.D.
Eluent : A) water/TFA (100/0.1)
B) acetonitrile/TFA (100/0.1)
5-50%B (0-12 min)
Flow rate : 0.5 mL/min
Temperature : 37°C
Detection : UV at 220 nm
Injection : 1 μ L
Sample : Tryptic digest of β -Lactoglobulin B

ペプチドマッピング-粒子間の優れた再現性

Peptide mapping – excellent reproducibility between 5 and 2 μ m

N070629E



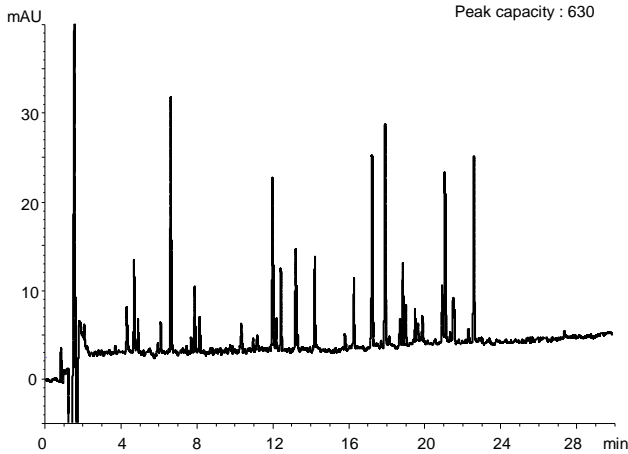
* Undigested Cytochrome c

Eluent : A) acetonitrile/water/TFA (10/90/0.1)
B) acetonitrile/water/TFA (35/65/0.1)
Flow rate : 0.2 mL/min
Temperature : 37°C
Detection : UV at 220 nm
Injection : 1 μ L (5 nmol/mL)
Sample : Tryptic digest of Cytochrome c

ペプチドマッピング

Peptide mapping

P110707A



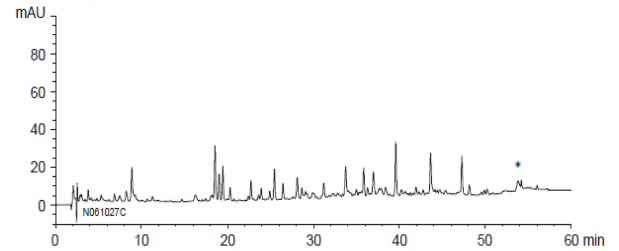
Column	: YMC-Triart C18 (1.9 μ m, 12 nm) 200 X 2.0 mmI.D. (Two coupled 100 X 2.0 mmI.D.)
Eluent	: A) water/TFA (100/0.1) B) acetonitrile/TFA (100/0.08) 5-40%B (0-30 min)
Flow rate	: 0.4 mL/min
Temperature	: 70°C
Detection	: UV at 220 nm
Injection	: 20 μ L
Sample	: Tryptic digest of Bovine Hemoglobin (2.5 nmol/mL)
Pressure	: 58.1-61.6 MPa (8430-8930 psi)

ペプチドマッピング

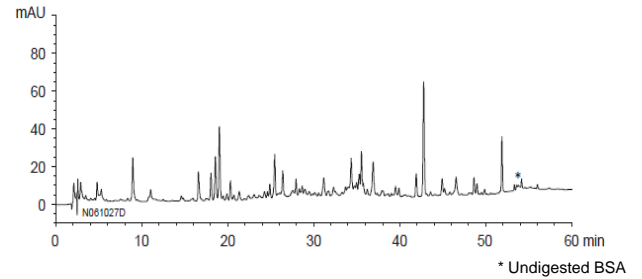
Peptide mapping

N070629D

Tryptic digest of BSA



Lysyl endopeptidase digest of BSA

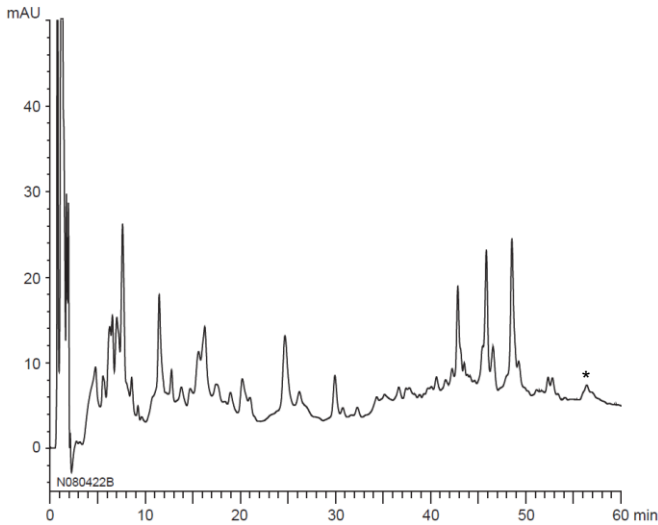


Column	: YMCbasic (5 μ m) 150 X 2.0 mmI.D.
Eluent	: A) water/TFA (100/0.1) B) acetonitrile/TFA (100/0.1) 5-35%B (0-50 min), 35-45%B (50-55 min), 45%B (55-60 min)
Flow rate	: 0.2 mL/min
Temperature	: 37°C
Detection	: UV at 220 nm
Injection	: 1 μ L

ペプチドマッピング

Peptide mapping

N080422B



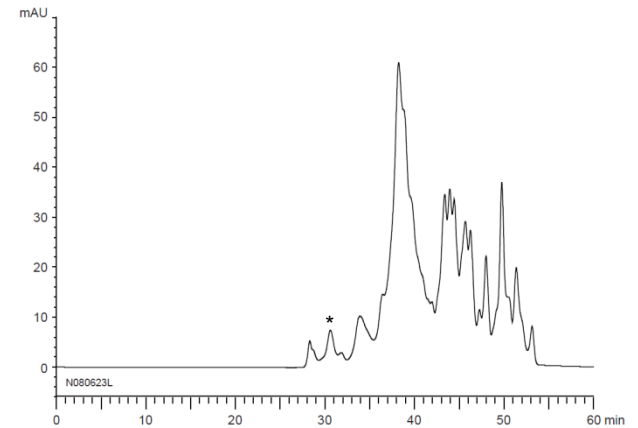
* Undigested BSA

Column	: BioPro IEX QA (5 μ m) 50 X 4.6 mmI.D.
Eluent	: A) 20 mM Tris-HCl (pH 8.6) B) 20 mM Tris-HCl (pH 8.6) containing 0.5 M NaCl 0-15%B (0-30 min), 15-60%B (30-60 min)
Flow rate	: 0.5 mL/min
Temperature	: 25°C
Detection	: UV at 220 nm
Injection	: 20 μ L
Sample	: Tryptic digest of BSA

ペプチドマッピング

Peptide mapping

N080623L



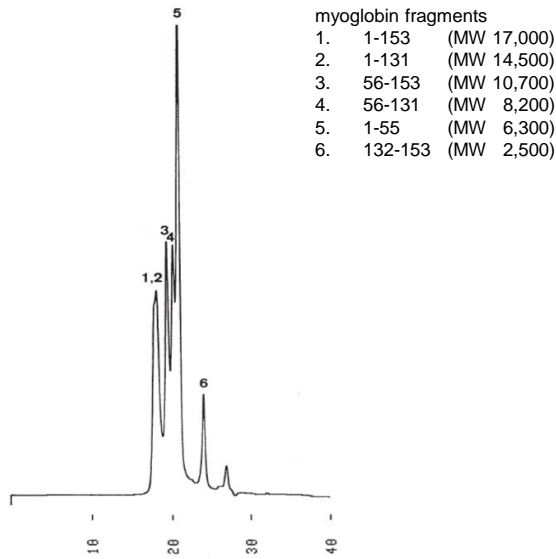
* Undigested BSA

Column	: YMC-Pack Diol-120 + Diol-60 (5 μ m) 500 X 8.0 mmI.D. X 2
Eluent	: 0.1 M KH_2PO_4 - K_2HPO_4 (pH 7.0) containing 0.2 M NaCl/acetonitrile (70/30)
Flow rate	: 0.7 mL/min
Temperature	: ambient (25°C)
Detection	: UV at 220 nm
Injection	: 5 μ L
Sample	: Tryptic digest of BSA

ミオグロビン分解物

Peptide fragments from myoglobin

G911218A



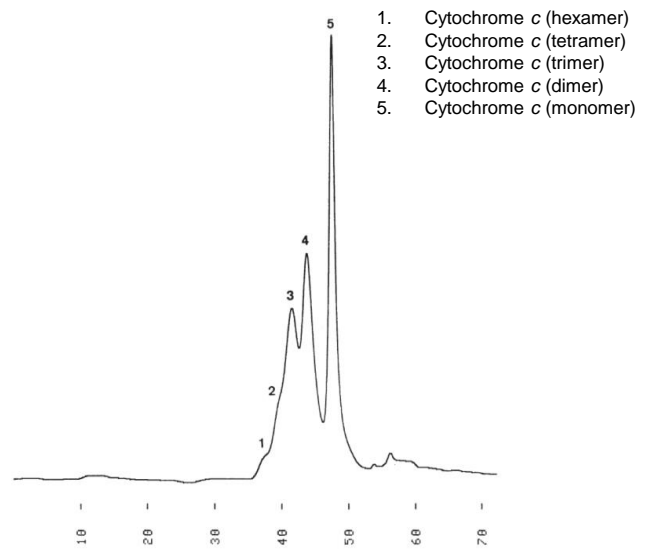
Column : YMC-Pack Diol-120 (5 μ m)
500 X 8.0 mm.I.D.
Eluent : 0.1 M KH_2PO_4 - K_2HPO_4 (pH 7.0)
containing 0.2 M NaCl/acetonitrile (70/30)
Flow rate : 0.7 mL/min
Temperature : ambient (25°C)
Detection : UV at 215 nm
Injection : 20 μ L (2.0 mg/mL)

*Cyanogen bromide cleavages of horse heart myoglobin. Molecular Weight Marker for proteins, manufactured by Fulka Chemie AG.

チトクロームc

Cytochrome c

G911217D



Column : YMC-Pack Diol-200 + Diol-120 (5 μ m)
500 X 8.0 mm.I.D. X 2
Eluent : 0.1 M KH_2PO_4 - K_2HPO_4 (pH 7.0) containing
0.2 M NaCl
Flow rate : 1.0 mL/min
Temperature : ambient (24°C)
Detection : UV at 215 nm
Injection : 30 μ L (0.2 mg/mL)

アミロイドβタンパク質

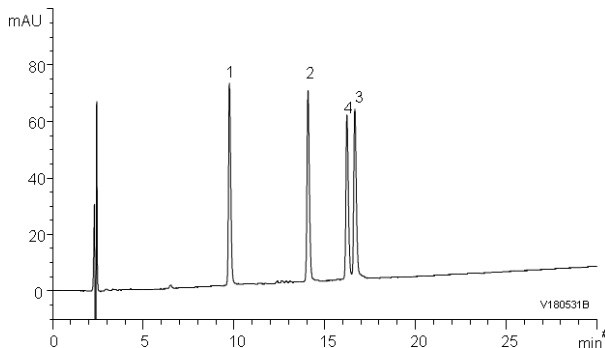
Amyloid β proteins

V180531B

1. Amyloid β (1-38) (Human) (MW 41,32)
2. Amyloid β (1-40) (Human) (MW 4,330)
3. Amyloid β (1-42) (Human) (MW 4,514)
4. Amyloid β (1-43) (Human) (MW 4,615)

Amyloid β (1-43) :

Asp-Ala-Glu-Phe-Arg-His-Asp-Ser-Gly-Tyr-Glu-Val-His-His-Gln-Lys-
Leu-Val-Phe-Phe-Ala-Glu-Asp-Val-Gly-Ser-Asn-Lys-Gly-Ala-Ile-Ile-
Gly-Leu-Met-Val-Gly-Gly-Val-Val-Ile-Ala-Thr



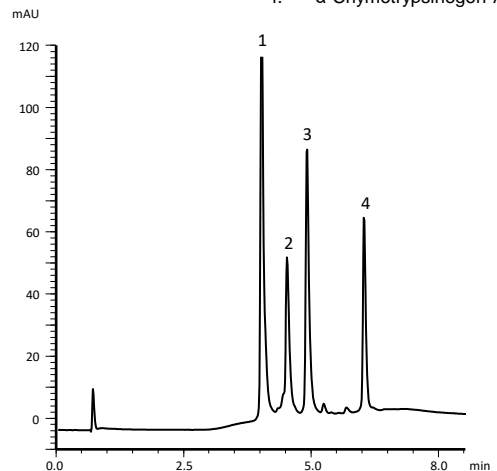
Column : YMC-Triart Bio C4 (3 μ m, 30 nm)
150 X 3.0 mm.I.D.
Eluent : A) water/TFA (100/0.1)
B) acetonitrile/TFA (100/0.1)
25-40%B (0-30 min), 90%B (30-40 min)
Flow rate : 0.4 mL/min
Temperature : 70°C
Detection : UV at 220 nm
Injection : 4 μ L (each 0.1 mg/mL)

タンパク質

Proteins

L171208F02

1. Myoglobin (0.73 mg/mL)
2. Ribonuclease A (0.75 mg/mL)
3. Lysozyme (0.25 mg/mL)
4. α -Chymotrypsinogen A (0.25 mg/mL)

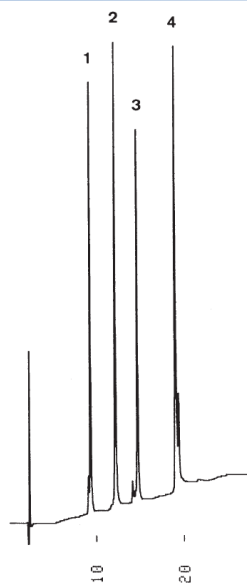


Column : BioPro HIC BF (4 μ m)
100 X 4.6 mm.I.D.
Eluent : A) 100 mM NaH_2PO_4 - Na_2HPO_4 (pH 7.0)
containing 2.0 M $(\text{NH}_4)_2\text{SO}_4$
B) 100 mM NaH_2PO_4 - Na_2HPO_4 (pH 7.0)
0-100%B (0-4.58 min), 100%B (4.58-6.58 min)
Flow rate : 1.2 mL/min
Temperature : 25°C
Detection : UV at 280 nm
Injection : 15 μ L

タンパク質

Proteins

T931029A



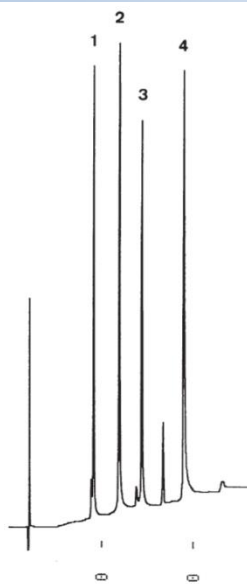
1. Ribonuclease A
2. Cytochrome c
3. Lysozyme
4. Myoglobin

Column	: YMC-Pack ODS-A (5 μm, 30 nm) 150 X 4.6 mmI.D.
Eluent	: A) acetonitrile/water/TFA (5/95/0.1) B) acetonitrile/water/TFA (60/40/0.1) 30-90%B (0-20 min), 90%B (20-25 min)
Flow rate	: 1.0 mL/min
Temperature	: 37°C
Detection	: UV at 220 nm
Injection	: 16 μL (0.16-0.33 mg/mL)

タンパク質

Proteins

B931023A



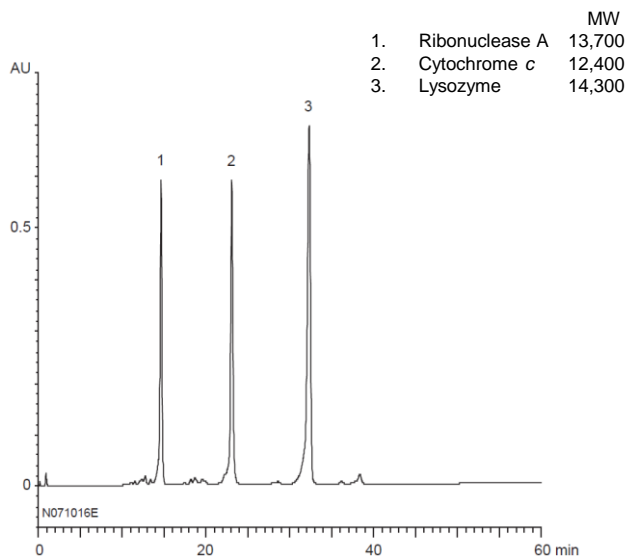
1. Ribonuclease A
2. Cytochrome c
3. Lysozyme
4. Myoglobin

Column	: YMC-Pack C ₄ (5 μm, 30 nm) 150 X 4.6 mmI.D.
Eluent	: A) acetonitrile/water/TFA (5/95/0.1) B) acetonitrile/water/TFA (60/40/0.1) 30-90%B (0-20 min), 90%B (20-25 min)
Flow rate	: 1.0 mL/min
Temperature	: 37°C
Detection	: UV at 220 nm
Injection	: 16 μL (0.16-0.33 mg/mL)

タンパク質 (MW 12,400-13,700)

Proteins (MW 12,400-13,700)

N071016E

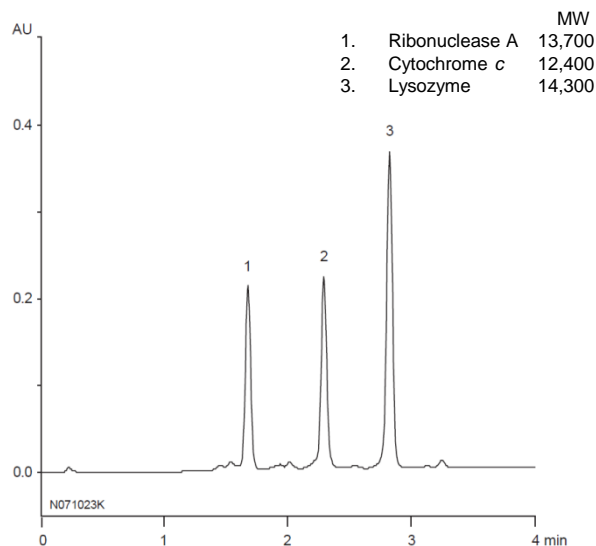


Column	: BioPro IEX SP (5 μm) 50 X 4.6 mmI.D.
Eluent	: A) 20 mM KH ₂ PO ₄ -K ₂ HPO ₄ (pH 6.8) B) 20 mM KH ₂ PO ₄ -K ₂ HPO ₄ (pH 6.8) containing 0.5 M NaCl 0-100%B (0-60 min)
Flow rate	: 0.5 mL/min
Temperature	: 25°C
Detection	: UV at 220 nm
Injection	: 20 μL (0.5 mg/mL)

タンパク質 (MW 12,400-13,700)

Proteins (MW 12,400-13,700)

N071023K

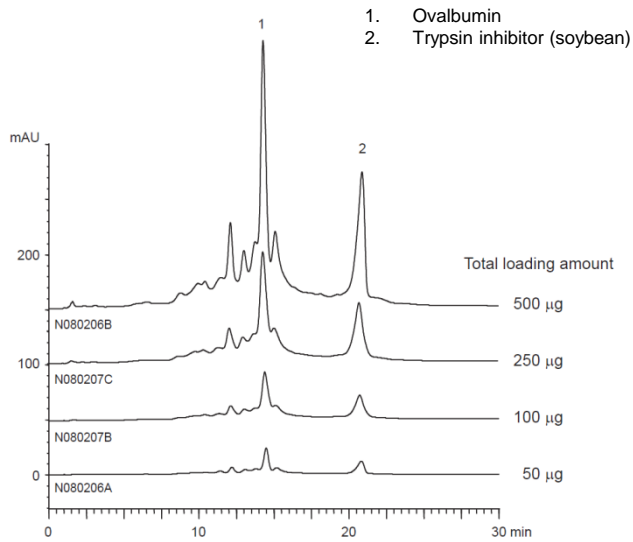


Column	: BioPro IEX SF (5 μm) 30 X 4.6 mmI.D.
Eluent	: A) 20 mM KH ₂ PO ₄ -K ₂ HPO ₄ (pH 6.8) B) 20 mM KH ₂ PO ₄ -K ₂ HPO ₄ (pH 6.8) containing 0.5 M NaCl 0-100%B (0-4 min)
Flow rate	: 1.5 mL/min
Temperature	: 25°C
Detection	: UV at 220 nm
Injection	: 20 μL (0.1 mg/mL)

タンパク質

Proteins

N080417W

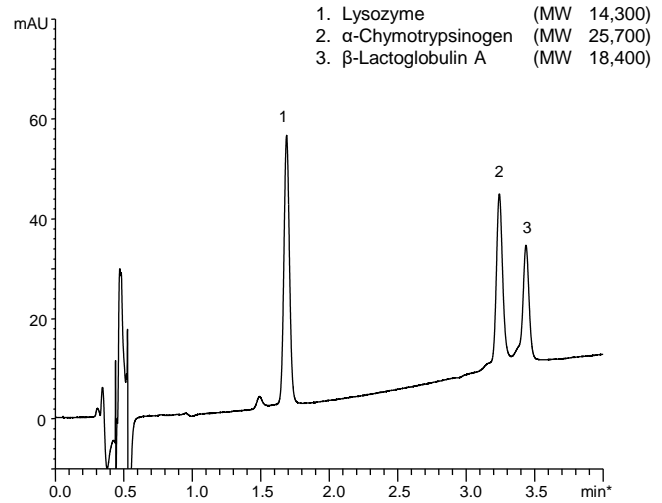


Column	: BioPro IEX QA (5 µm) 50 X 4.6 mmI.D.
Eluent	: A) 20 mM Tris-HCl (pH 8.1) B) 20 mM Tris-HCl (pH 8.1) containing 0.5 M NaCl 10-80%B (0-30 min)
Flow rate	: 0.5 mL/min
Temperature	: 25°C
Detection	: UV at 280 nm
Injection	: 100 µL

タンパク質 (MW 14,300-25,700)

Proteins (MW 14,300-25,700)

F111226A



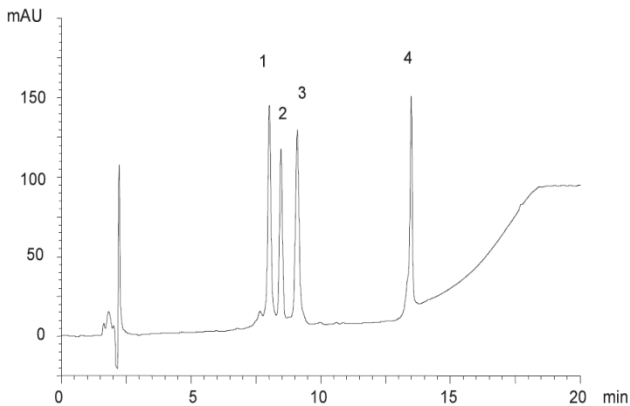
Column	: YMC-Triart C18 (1.9 µm, 12 nm) 50 X 2.0 mmI.D.
Eluent	: A) water/TFA (100/0.1) B) acetonitrile/2-propanol/TFA (50/50/0.1) 30-60%B (0-5 min)
Flow rate	: 0.4 mL/min
Temperature	: 70°C
Detection	: UV at 220 nm
Injection	: 1 µL (250 µg/mL)
Pressure	: 27.4-28.4 MPa (3790-4120 psi)

タンパク質 (MW 18,300-45,000)

Proteins (MW 18,300-45,000)

N041126A

1. β-Lactoglobulin B (Bovine milk) (MW 18,300)
2. β-Lactoglobulin A (Bovine milk) (MW 18,400)
3. α-Chymotrypsinogen A (Bovine pancreas) (MW 25,700)
4. Ovalbumin (MW 45,000)



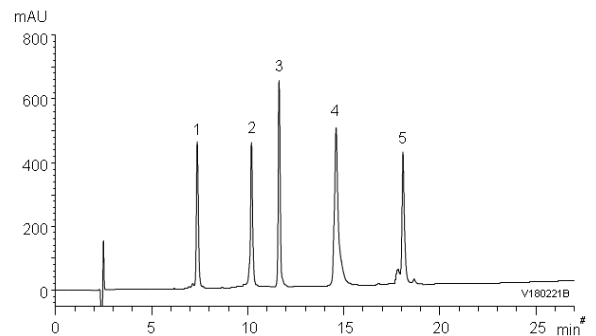
Column	: YMC-Pack C ₄ (5 µm, 30 nm) 150 X 4.6 mmI.D.
Eluent	: A) water/TFA (100/0.1) B) acetonitrile/TFA (100/0.1) 40-50%B (0-10 min), 50-90%B (10-15 min), 90%B (15-20 min)
Flow rate	: 1.0 mL/min
Temperature	: 37°C
Detection	: UV at 220 nm
Injection	: 10 µL (0.2-0.3 mg/mL)

タンパク質 (MW 5,700-66,000)

Proteins (MW 5,700-66,000)

V180221B

1. Ribonuclease A (Bovine pancreas) (MW 13,700)
2. Cytochrome c (Horse heart) (MW 12,400)
3. Insulin (Bovine pancreas) (MW 5,700)
4. BSA (MW 66,000)
5. α-Chymotrypsinogen A (Bovine pancreas) (MW 25,700)



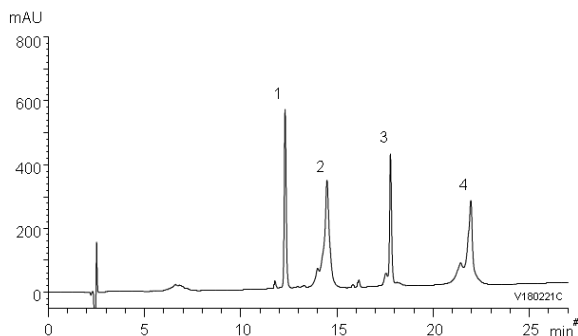
Column	: YMC-Triart Bio C4 (5 µm, 30 nm) 150 X 3.0 mmI.D.
Eluent	: A) water/TFA (100/0.1) B) acetonitrile/TFA (100/0.1) 20-60%B (0-27 min), 90%B (27-35 min)
Flow rate	: 0.4 mL/min
Temperature	: 70°C
Detection	: UV at 220 nm
Injection	: 10 µL (0.25-0.50 mg/mL)

タンパク質 (MW 14,300-77,000)

Proteins (MW 14,300-77,000)

V180221C

- | | |
|---|-------------|
| 1. Lysozyme (Chicken egg white) | (MW 14,300) |
| 2. Conalbumin (Chicken egg white) | (MW 77,000) |
| 3. β -Lactoglobulin A (Bovine milk) | (MW 18,400) |
| 4. Ovalbumin | (MW 45,000) |



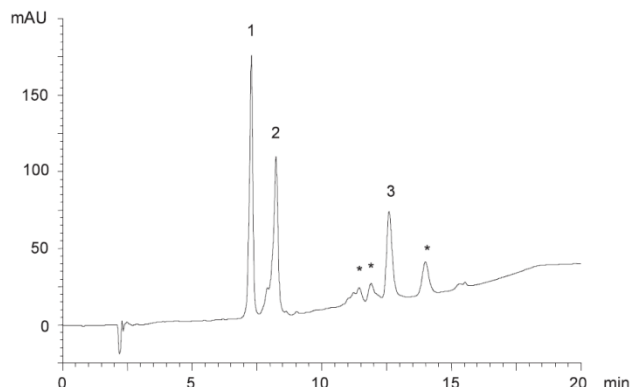
Column	: YMC-Triart Bio C4 (5 μ m, 30 nm) 150 X 3.0 mmI.D.
Eluent	: A) water/TFA (100/0.1) B) acetonitrile/TFA (100/0.1) 20-60%B (0-27 min), 90%B (27-35 min)
Flow rate	: 0.4 mL/min
Temperature	: 70°C
Detection	: UV at 220 nm
Injection	: 10 μ L (0.15-0.60 mg/mL)

タンパク質 (MW 66,000-96,000)

Proteins (MW 66,000-96,000)

N040930G

- | | |
|-----------------------------------|-------------|
| 1. BSA | (MW 66,000) |
| 2. Conalbumin (Chicken egg white) | (MW 77,000) |
| 3. Lipoxidase (Soybean) | (MW 96,000) |



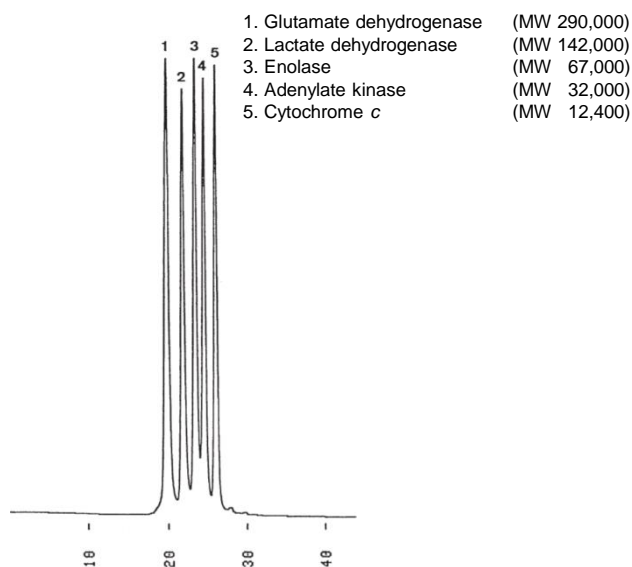
* Impurities in commercial lipoxidase

Column	: YMC-Pack C ₄ (5 μ m, 30 nm) 150 X 4.6 mmI.D.
Eluent	: A) water/TFA (100/0.1) B) acetonitrile/2-propanol/TFA (50/50/0.1) 30-75%B (0-15 min), 75%B (15-20 min)
Flow rate	: 1.0 mL/min
Temperature	: 37°C
Detection	: UV at 220 nm
Injection	: 10 μ L (0.25-1.0 mg/mL)

タンパク質分子量マーカー

Proteins for molecular weight marker

G911213A

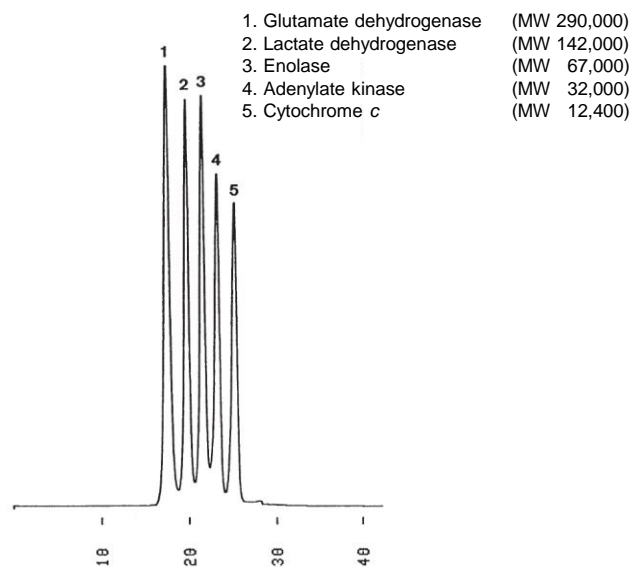


Column	: YMC-Pack Diol-300 (5 μ m) 500 X 8.0 mmI.D.
Eluent	: 0.1 M KH ₂ PO ₄ -K ₂ HPO ₄ (pH 7.0) containing 0.2 M NaCl
Flow rate	: 0.7 mL/min
Temperature	: ambient (26°C)
Detection	: UV at 280 nm
Injection	: 15 μ L (100 μ L/1 vial)
Sample	: MW-Marker (HPLC), manufactured by ORIENTAL YEAST CO., LTD.

タンパク質分子量マーカー

Proteins for molecular weight marker

G911214A

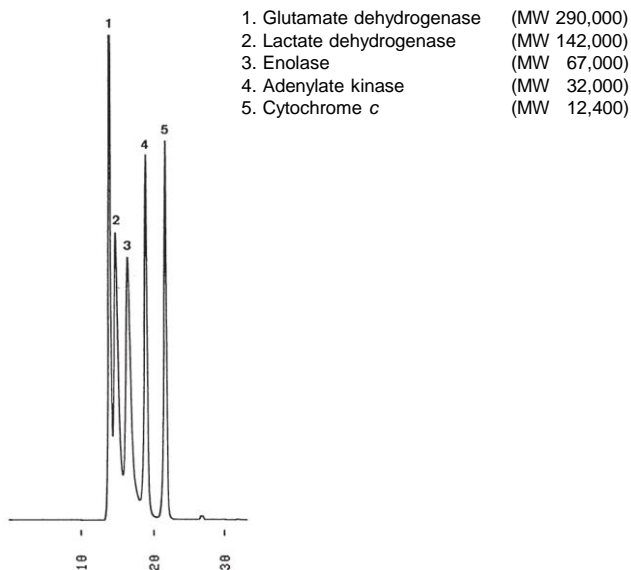


Column	: YMC-Pack Diol-200 (5 μ m) 500 X 8.0 mmI.D.
Eluent	: 0.1 M KH ₂ PO ₄ -K ₂ HPO ₄ (pH 7.0) containing 0.2 M NaCl
Flow rate	: 0.7 mL/min
Temperature	: ambient (26°C)
Detection	: UV at 280 nm
Injection	: 15 μ L (100 μ L/1 vial)
Sample	: MW-Marker (HPLC), manufactured by ORIENTAL YEAST CO., LTD.

タンパク質分子量マーカー

Proteins for molecular weight marker

G911214B

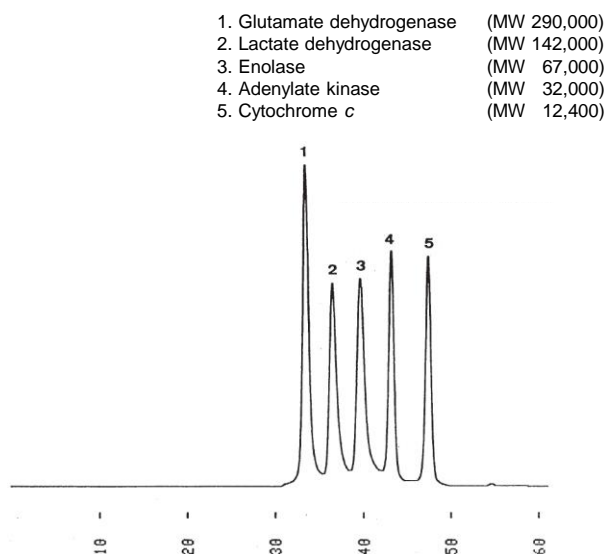


Column	: YMC-Pack Diol-120 (5 μm) 500 X 8.0 mmI.D.
Eluent	: 0.1 M KH ₂ PO ₄ -K ₂ HPO ₄ (pH 7.0) containing 0.2 M NaCl
Flow rate	: 0.7 mL/min
Temperature	: ambient (26°C)
Detection	: UV at 280 nm
Injection	: 15 μL (100 μL/1 vial)
Sample	: MW-Marker (HPLC), manufactured by ORIENTAL YEAST CO., LTD.

タンパク質分子量マーカー

Proteins for molecular weight marker

G911214C



Column	: YMC-Pack Diol-120 + Diol-300 (5 μm) 500 X 8.0 mmI.D. X 2
Eluent	: 0.1 M KH ₂ PO ₄ -K ₂ HPO ₄ (pH 7.0) containing 0.2 M NaCl
Flow rate	: 0.7 mL/min
Temperature	: ambient (26°C)
Detection	: UV at 280 nm
Injection	: 15 μL (100 μL/1 vial)
Sample	: MW-Marker (HPLC), manufactured by ORIENTAL YEAST CO., LTD.

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