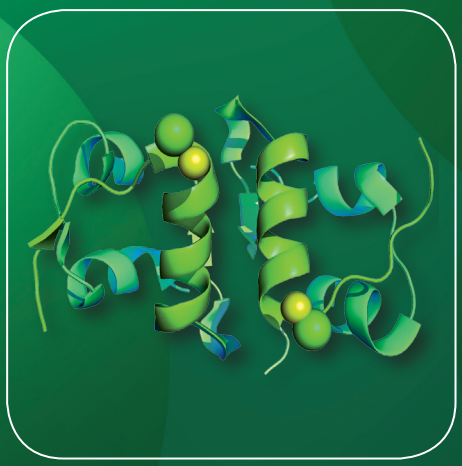
YMC
EUROPE GMBH
The Selectivity Company

YMC Biochromatography Columns

RP
SEC
IEX
HIC



www.ymc.de

HPLC Columns for Biochromatography

	Reversed Phase (RP)	Size Exclusion (SEC)	Ion Exchange (IEX)	Hydrophobic Interaction (HIC)
Separation principle	Hydrophobicity	Molecular weight	Electric charge	Hydrophobicity
Max. MW	Up to about 150,000 Da	Up to about 1,000,000 Da	Up to several millions Da	Up to about 1,000,000 Da
Resolution	+++	++	+++	+++
Speed	+++	+	++/+++	+++
Loading	++	++	+++	+++
Stability	+/++	+++	+++	+++
Usage (e.g.)	• Peptide mapping • LC/MS • Nucleic acids and oligonucleotides	• Impurity analysis of antibody-drug conjugates • MAb separation	• Proteins/MAb • Charge variant analysis • Isoform analysis • Nucleic acids and oligonucleotides	• Drug-binding analysis of antibody-drug conjugates

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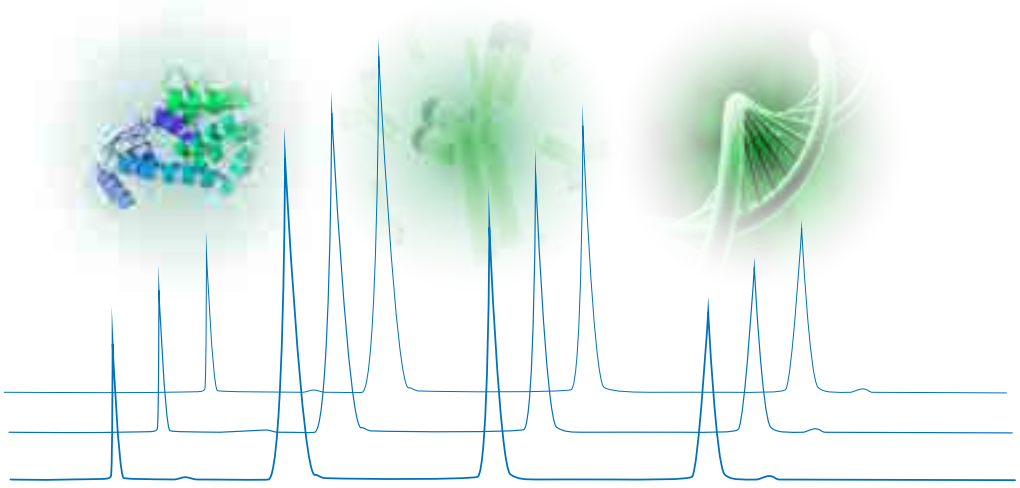
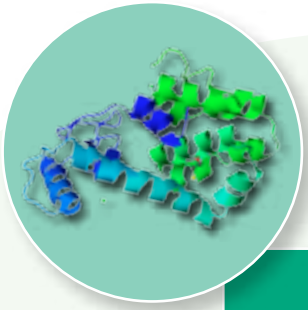


Phase selection guide



Proteins / Peptides

HIC
- BioPro HIC BF

IEX
- BioPro IEX QA
- BioPro IEX QF
- BioPro IEX SP
- BioPro IEX SF

SEC
- YMC-Pack Diol-60
- YMC-Pack Diol-120
- YMC-Pack Diol-200
- YMC-Pack Diol-300
- YMC-SEC MAB

RP

<5 kDa
- YMC-Triart C18
- YMC-Pack Pro C18
- Hydrosphere C18
- Meteoric Core C18

5–20 kDa
- YMC-Pack C8, 200 Å
- YMCbasic
- Meteoric Core C18 BIO

20–100 kDa
- YMC-Triart Bio C4
- YMC-Pack C4, 300 Å

(Monoclonal) Antibodies

- **HIC**
  - BioPro HIC BF
- **IEX**
  - BioPro IEX QA
  - BioPro IEX QF
  - BioPro IEX SP
  - BioPro IEX SF
- **SEC**
  - YMC-Pack Diol-200
  - YMC-Pack Diol-300
  - YMC-SEC MAB

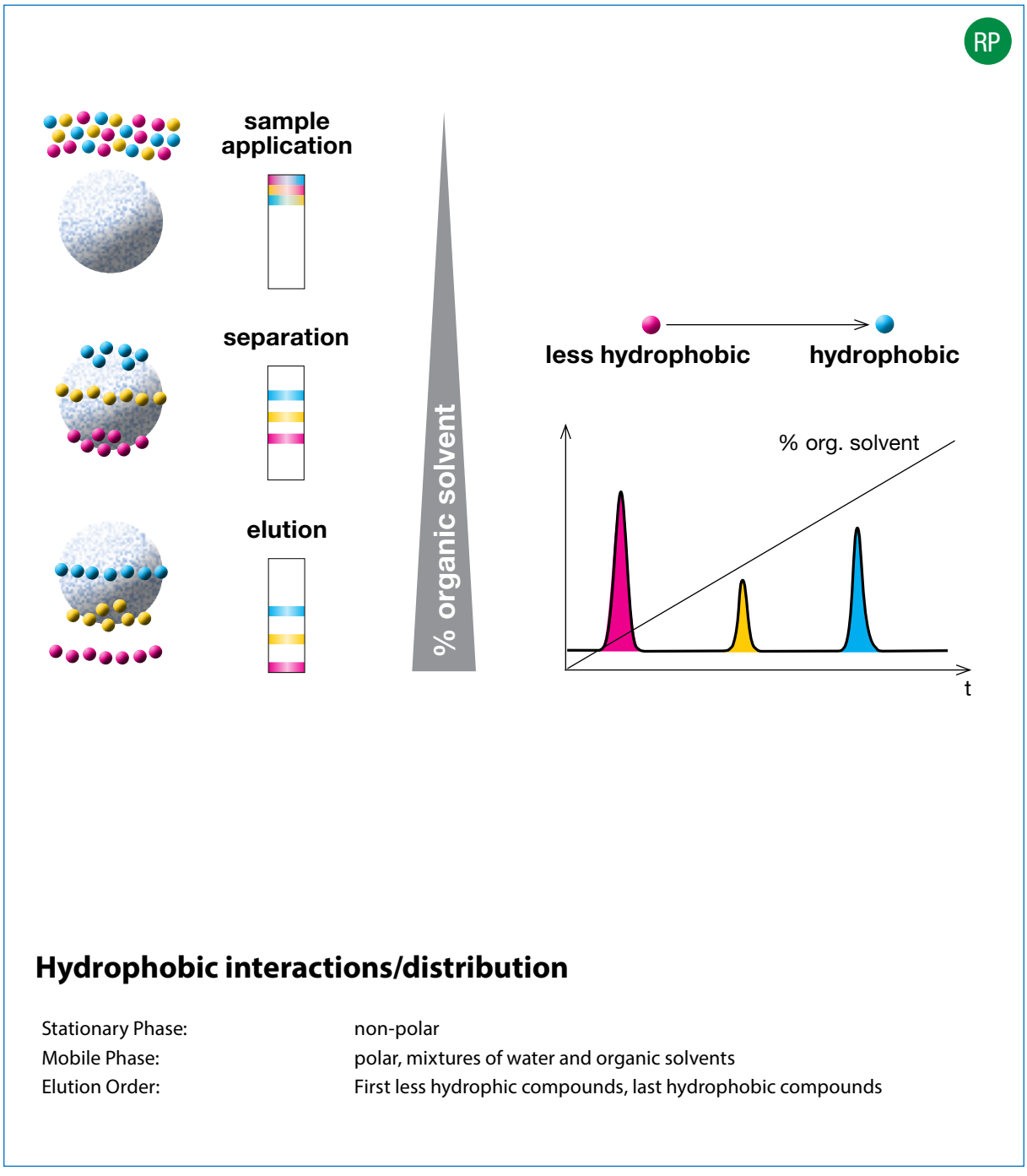
Oligonucleotides / Nucleic Acids

- **RP**
  - YMC-Triart C18
  - YMC-Triart C8
  - Hydrosphere C18
- **IEX**
  - BioPro IEX QA
  - BioPro IEX QF

Separation mechanisms

Reversed phase chromatography

Reversed phase chromatography is a technique used to separate molecules using the hydrophobic interactions between compounds and a stationary phase and between compounds and a mobile phase. Stationary phases include silica gels and hybrid silica to which hydrophobic functional groups such as C18, C8, and C4 are bonded. They are used with mobile phases consisting of mixtures of organic solvents and aqueous acid solutions or buffers.
As the concentration of organic solvent in the mobile phase is increased, the molecules with lower hydrophobicity are eluted first.

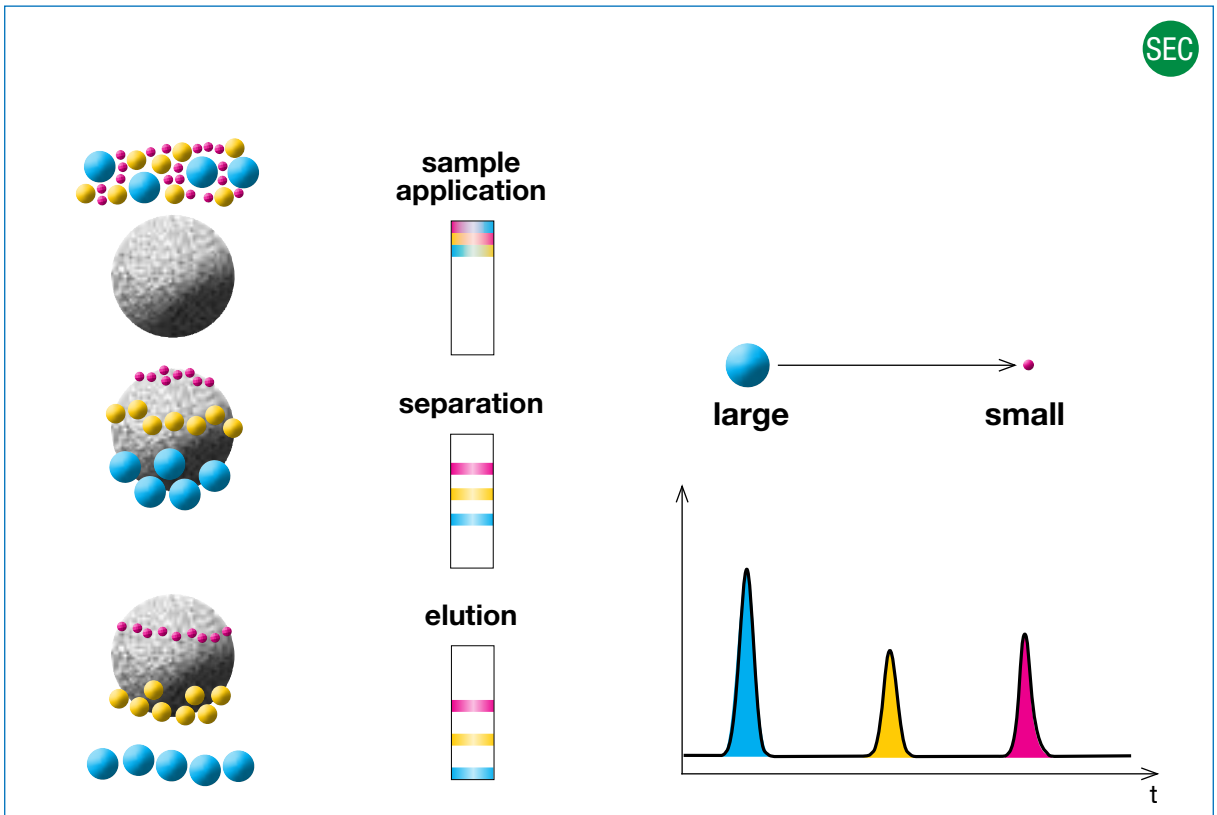


Hydrophobic interactions/distribution

Stationary Phase:	non-polar
Mobile Phase:	polar, mixtures of water and organic solvents
Elution Order:	First less hydrophic compounds, last hydrophobic compounds

Size exclusion chromatography

Size exclusion chromatography is a technique used to separate molecules by their size. Stationary phases used consist of silica gels or polymers which have closely defined pores with a network structure.
Mobile phases such as buffers which have high solubility and pH stability towards the compounds are used.
Smaller molecules which can penetrate deeper into the pores are eluted later from the column while larger molecules which cannot enter the pores are eluted earlier from the column.



Size exclusion by hydrodynamic volume

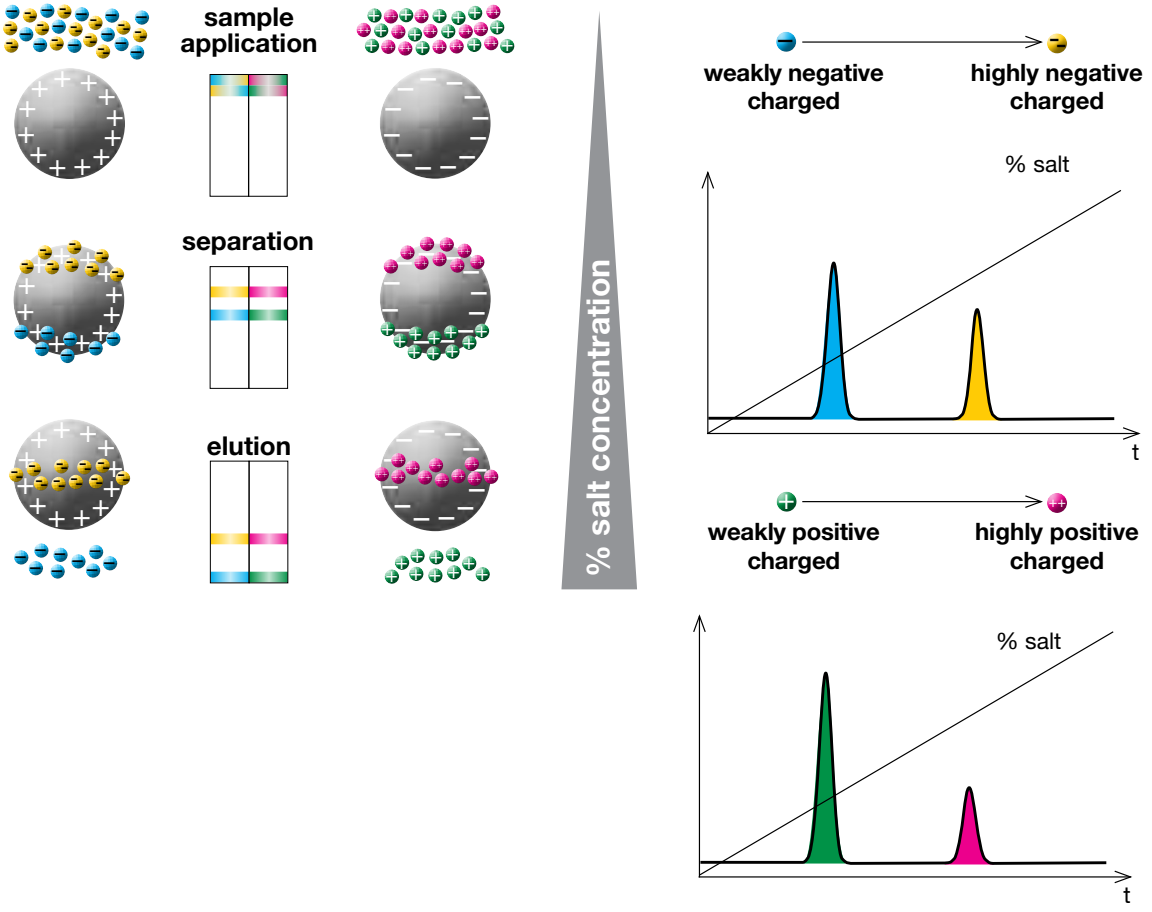
Stationary Phase:	Porous packing material
Mobile Phase:	Aqueous solutions
Elution order:	First large molecules, last small molecules

Separation mechanisms

Ion exchange chromatography

Ion exchange chromatography is a technique used to separate ionic molecules by differences in their net charge. Generally, resins (media), which have cationic or anionic groups, are used as stationary phases and buffers containing counter-ions are used as mobile phases.
In the sample application step, molecules with opposite charge to the media bind to the media by ionic interaction. Elution is achieved by increasing the concentration of the counter-ions in the mobile phase. Molecules with the lowest net charge are eluted first and those with higher charge are eluted later.

IEX



Ion exchange

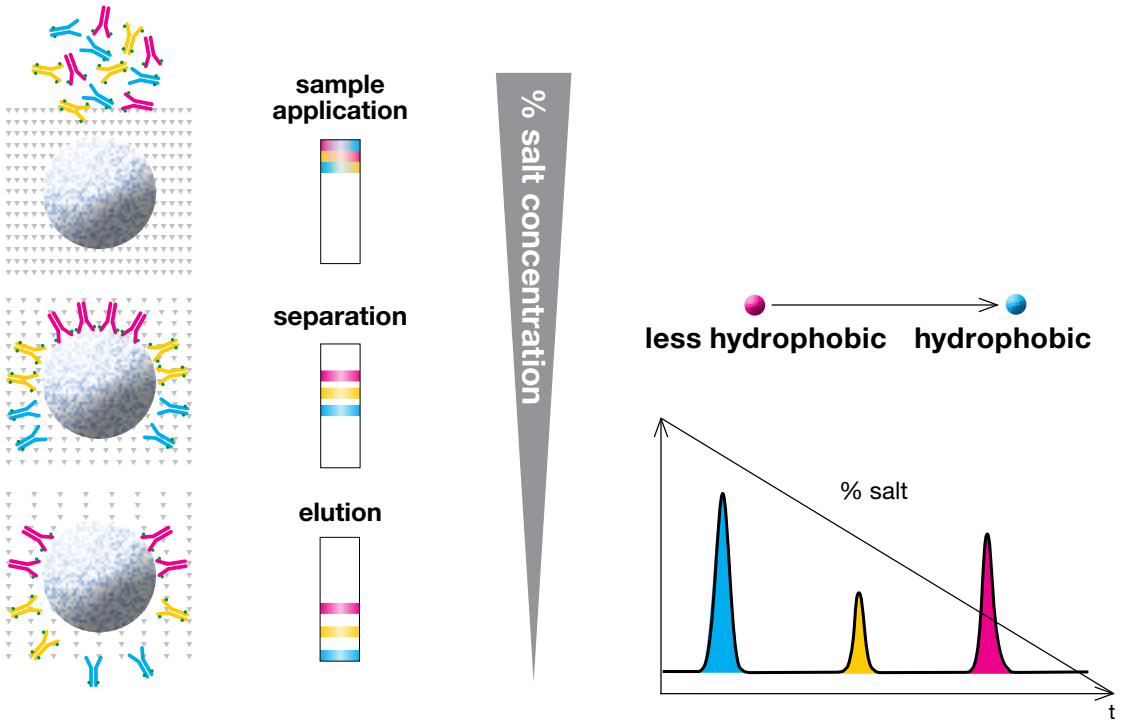
Stationary Phase:	Charged surface (positive: anion exchange, negative: cation exchange)
Mobile Phase:	Buffers for elution with increasing salt concentration. Alternatively, the pH of the mobile phase can be changed for elution.
Elution order:	First compounds which have the same charge as the surface, last compounds which are oppositely charged to the surface.

Hydrophobic interaction chromatography

Hydrophobic interaction chromatography separates molecules using hydrophobic interaction between the stationary phase and compounds such as proteins. Unlike reversed phase, proteins can be separated without any denaturation, thereby maintaining its activity, with the use of ammonium sulphate as a mobile phase.

By lowering the concentration of ammonium sulphate in the mobile phase, bonding power between proteins and the mobile phase is weakened and the proteins are eluted.

HIC



Hydrophobic interaction

Stationary Phase:	Non-polar
Mobile Phase:	Buffer for elution with decreasing salt concentration
Elution Order:	First least hydrophic compounds, last greatest hydrophobic compounds

Bio QC – Validation kit

Method Validation Kits for BioLC

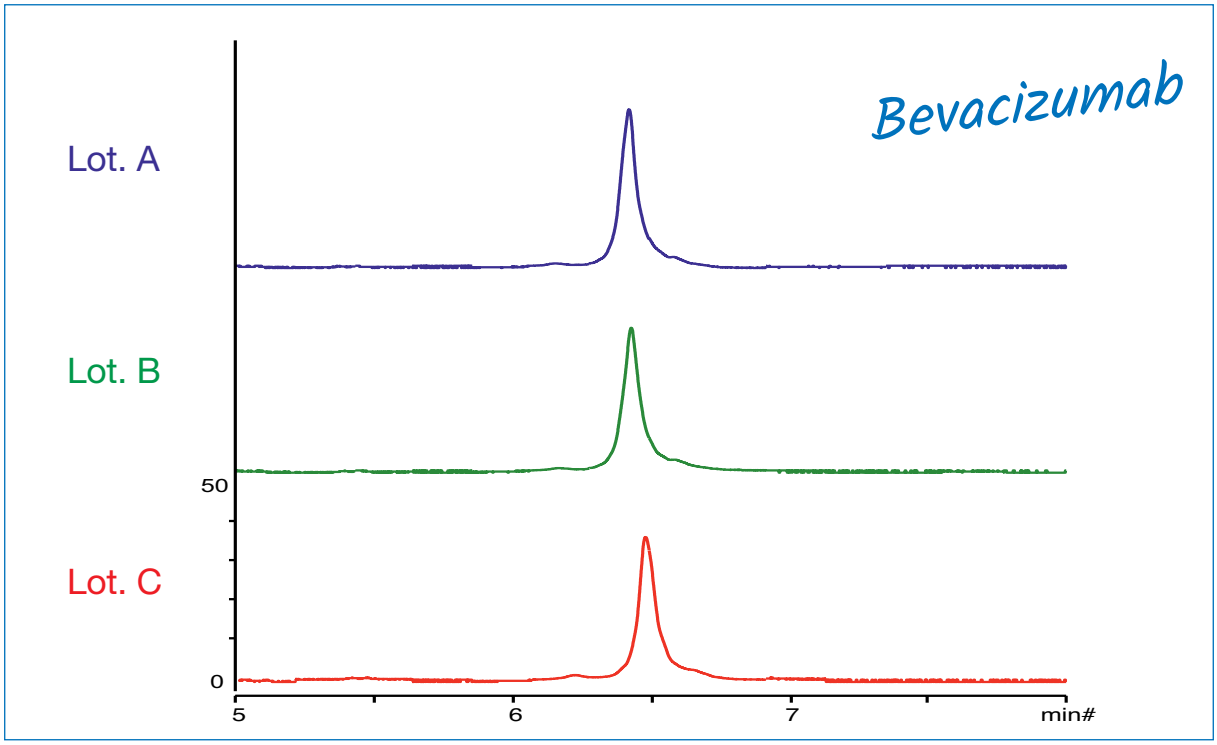
- **for documentation of robustness and reproducibility**
- **three analytical columns from specified lots**

Validation kit:

contains three analytical columns packed with stationary phases from three different batches, in order to solely test the robustness of the particular method.

Available dimensions:

Length: 30 or 33, 50, 75, 100, 150, 250, 300 mm
ID: 2.0 or 2.1, 3.0, 4.0, 4.6, 8.0 mm



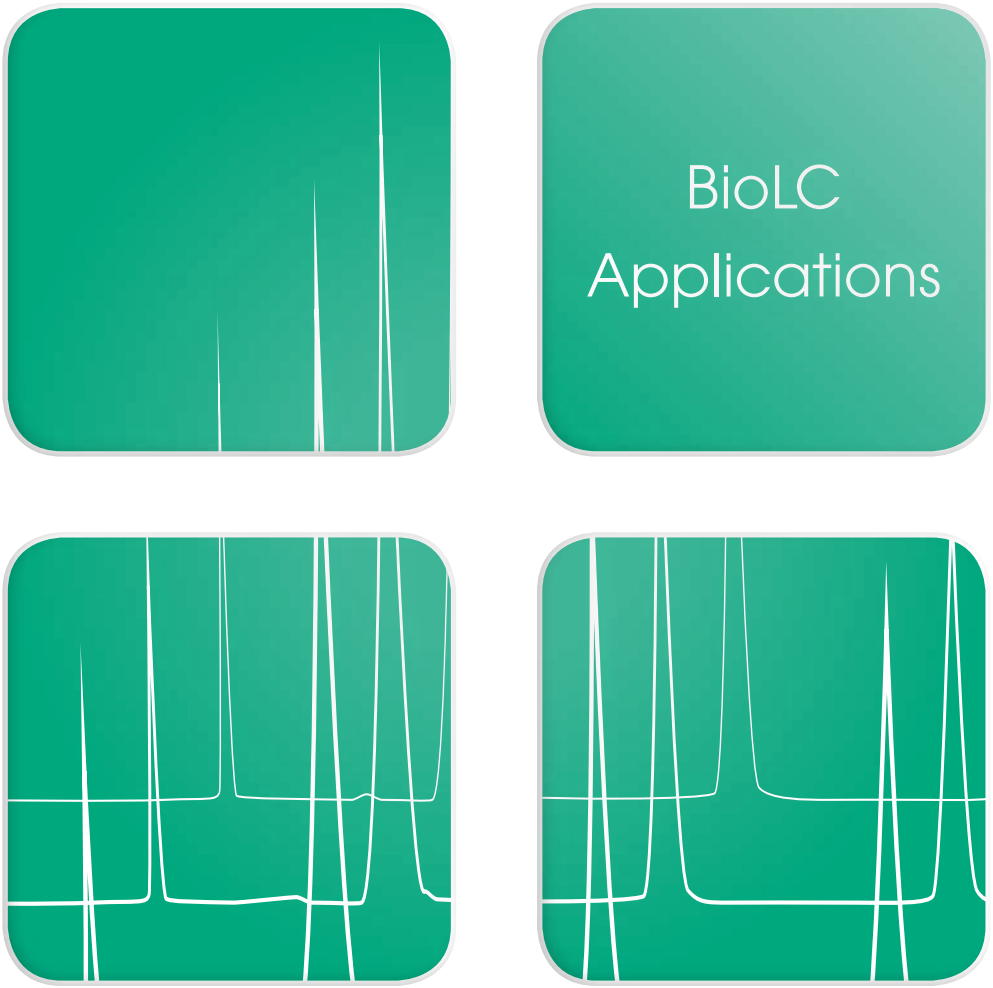
To order a validation kit simply use the ordering number for the column of interest,
e.g. **TB30SP9-05Q1PT** and add V1: **TB30SP9-05Q1PTV1.**

For details on YMC selectivities and the International Product Code please refer to the specific product sections in this catalogue.

Batch Reservation Service

Occasionally, a critical analytical method may not prove as robust as you would prefer. Columns from a particular media batch may be the only way that you can, for example, isolate a critical process impurity. In such cases, YMC will reserve a specific batch of material for the use of an individual customer. YMC will also reserve prepacked columns for release according to a pre-arranged schedule.

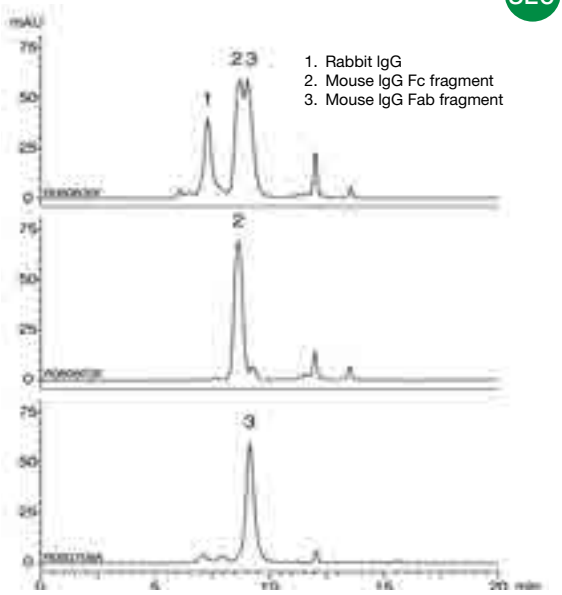
Please call YMC or contact your YMC representative for details.


BioLC
Applications

BioLC applications – Antibodies

IgG, Fab and Fc fragments

SEC

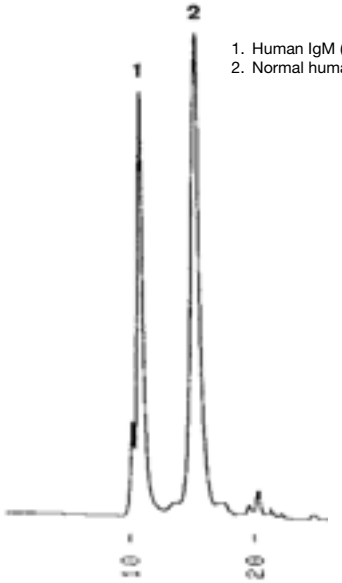


1. Rabbit IgG
2. Mouse IgG Fc fragment
3. Mouse IgG Fab fragment

Column:	YMC-Pack Diol-200 (5 µm) 300 x 8.0 mm ID
Part No.:	DL20S05-3008WT
Eluent:	0.1 M KH_2PO_4-K_2HPO_4 (pH 6.9) containing 0.2 M NaCl
Flow rate:	1.0 mL/min
Temperature:	ambient (27 °C)
Detection:	UV at 220 nm
Injection:	5 µL (0.4, 0.5 mg/mL)

Human Immunglobulin

SEC



1. Human IgM (*k*),myeloma protein
2. Normal human IgG

Column:	YMC-Pack Diol-300 (5 µm) 500 x 8.0 mm ID
Part No.:	DL30S05-5008WT
Eluent:	0.1 M NaH_2PO_4-Na_2HPO_4 (pH 6.8) containing 0.1 M Na_2SO_4
Flow rate:	1.0 mL/min
Temperature:	ambient (24 °C)
Detection:	UV at 280 nm, 0.04 AUFS
Injection:	40 µL (0.5 mg/mL)

Human IgG (λ), myeloma protein

SEC

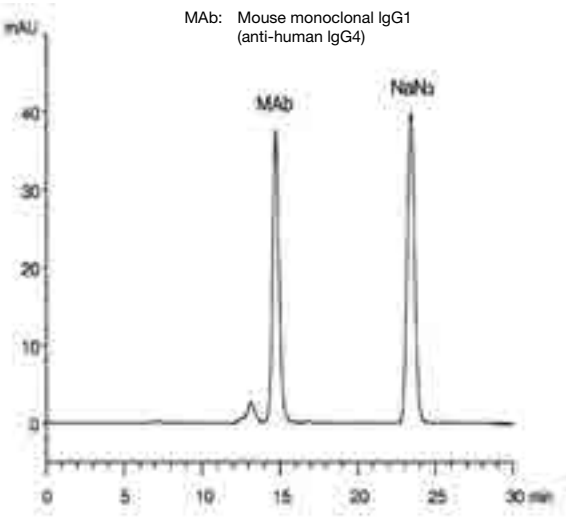
1. Human IgG (λ), myeloma protein

Column:	YMC-Pack Diol-300 (5 µm) 500 × 8.0 mm ID
Part No.:	DL30S05-5008WT
Eluent:	0.1 M NaH_2PO_4-Na_2HPO_4 (pH 6.8) containing 0.1 M Na_2SO_4
Flow rate:	1.0 mL/min
Temperature:	ambient (24 °C)
Detection:	UV at 280 nm, 0.04 AUFS
Injection:	20 µL (1.0 mg/mL)

Monoclonal antibody (MAb)

SEC

MAb: Mouse monoclonal IgG1 (anti-human IgG4)

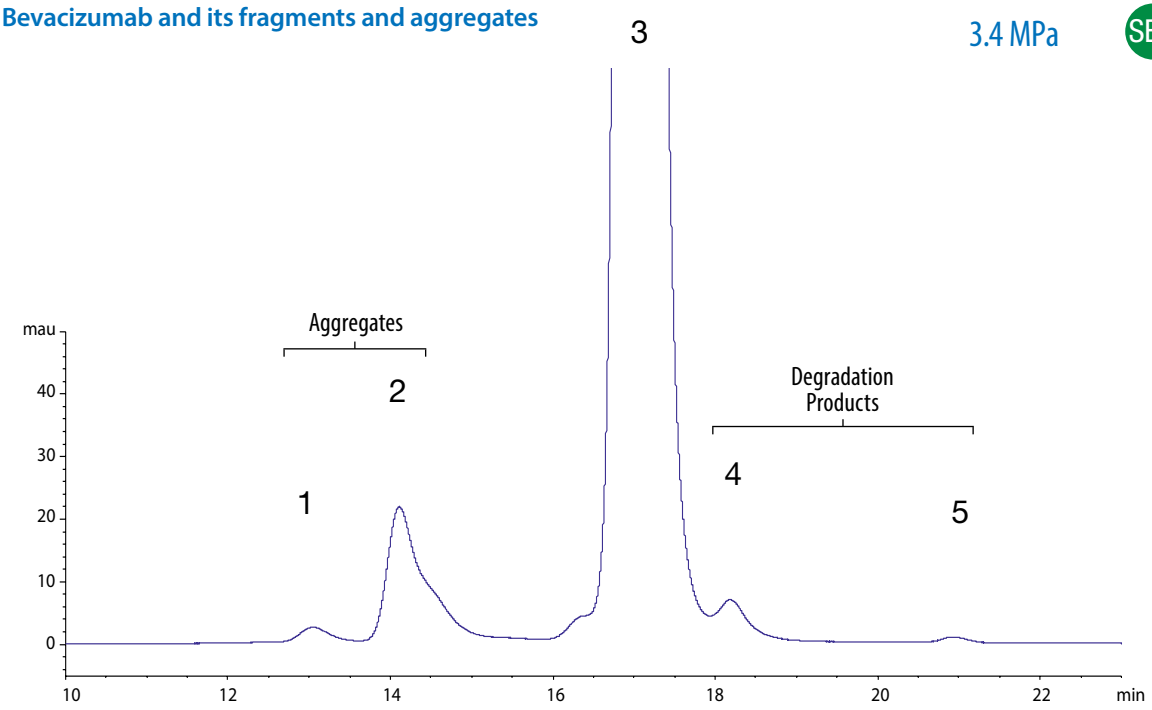


Column:	YMC-Pack Diol-200 (5 µm) 300 x 4.6 mm ID
Part No.:	DL20S05-3046WT
Eluent:	0.1 M KH_2PO_4-K_2HPO_4 (pH 7.0)
Flow rate:	0.17 mL/min
Temperature:	ambient (25 °C)
Detection:	UV at 220 nm
Injection:	10 µL
Sample:	a commercially available mouse monoclonal IgG1 (0.05 mg/mL) (purified by DEAE chromatography, containing NaN_3)

Bevacizumab and its fragments and aggregates

SEC

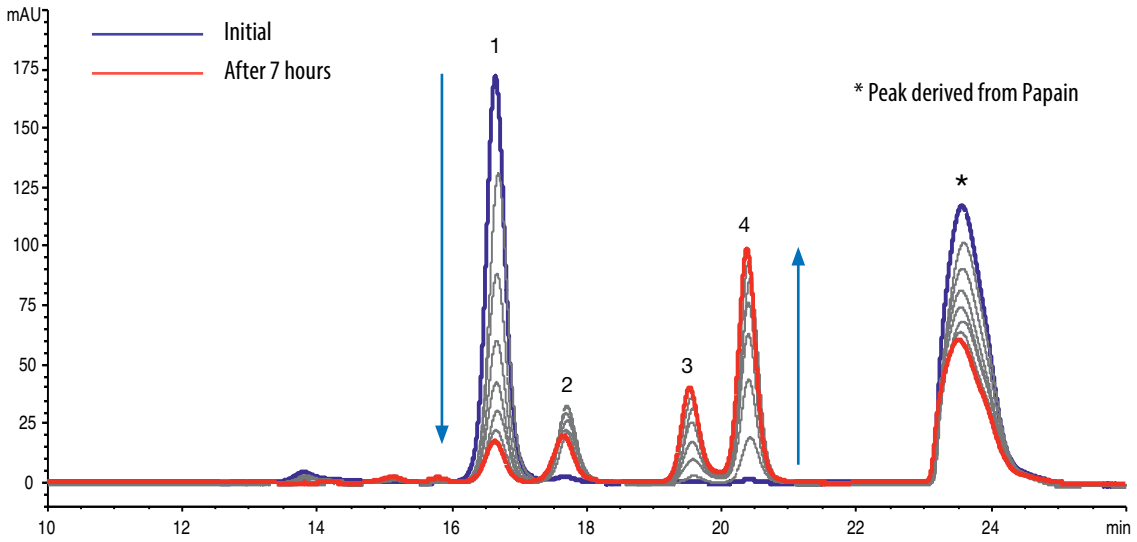
3.4 MPa



Column:	YMC-SEC MAB (3 µm, 25 nm) 300 x 4.6 mm ID
Part No.:	DLM25S03-3046WT
Eluent:	0.1 M phosphate buffer (pH = 7) cont. 0.2 M NaCl
Flow rate:	0.165 mL/min
Temperature:	25 °C
Detection:	UV at 280 nm
Cell path:	10 mm
Injection:	10 µL (5 mg/mL)
Sample:	Bavacizumab

Immunglobulin digest

SEC

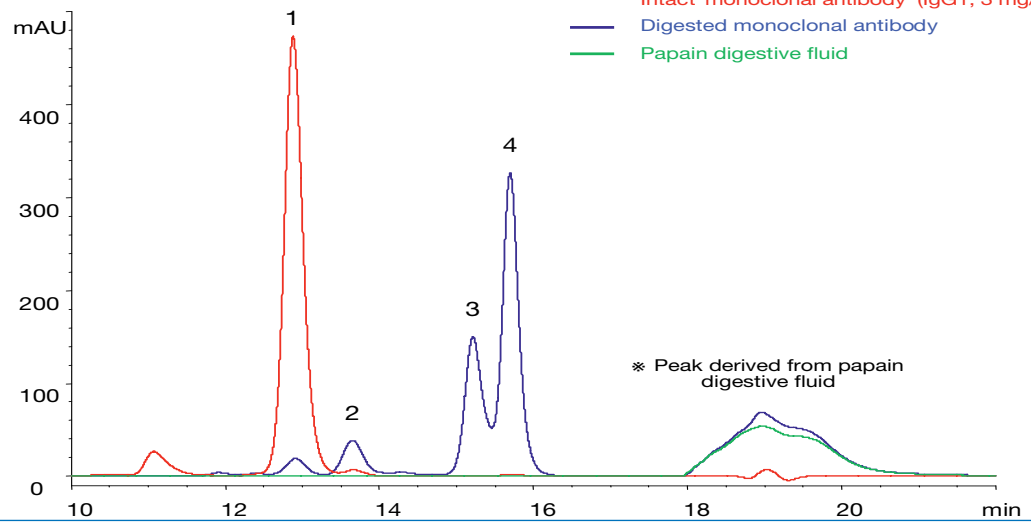


Column:	YMC-SEC MAB (3 µm, 25 nm) 300 x 4.6 mm ID	Temperature:	25 °C
Part No.:	DLM25S03-3046WT	Detection:	UV at 280 nm
Eluent:	0.1 M phosphate buffer (pH = 7) cont. 0.2 M NaCl	Injection:	2 µL (3 mg/mL)
Flow rate:	0.165 mL/min	Sample:	Humanised monoclonal IgG1 + Papain

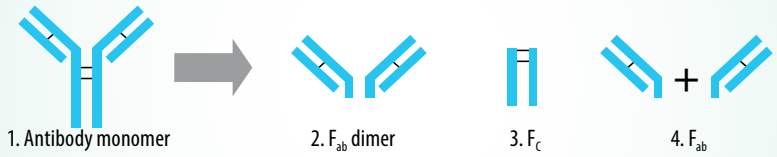
BioLC applications – Antibodies

Immunglobulin digest

SEC

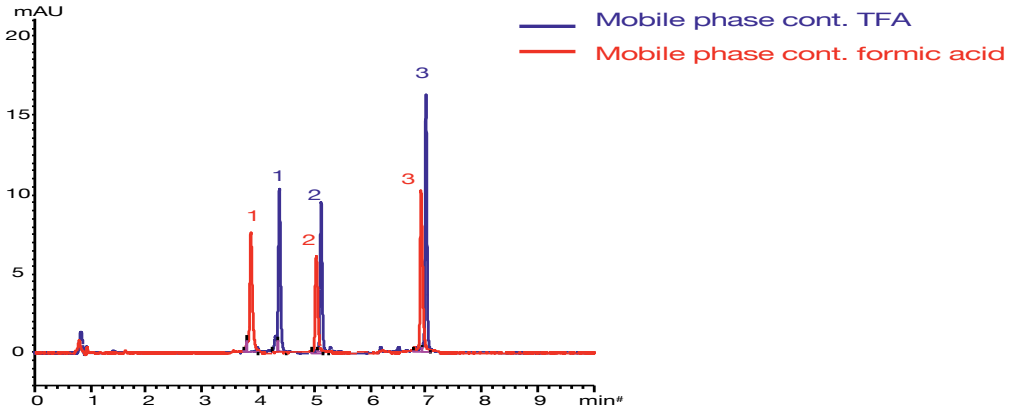


Column:	YMC-Pack Diol-200 (2 µm, 20 nm) 300 x 4.6 mm ID	Temperature:	ambient
Part no:	DL20S02-3046PTH	Detection:	UV at 280 nm
Eluent:	0.1 M KH_2PO_4-K_2HPO_4 (pH 7.0) containing 0.2 M NaCl	Sample:	IgG1 (3 mg/ml)
Flow rate:	0.2 mL/min		

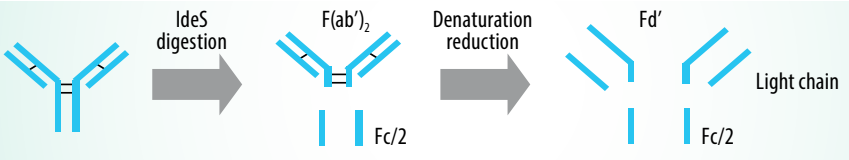


LC/MS compatible analysis of monoclonal antibody fragments

RP

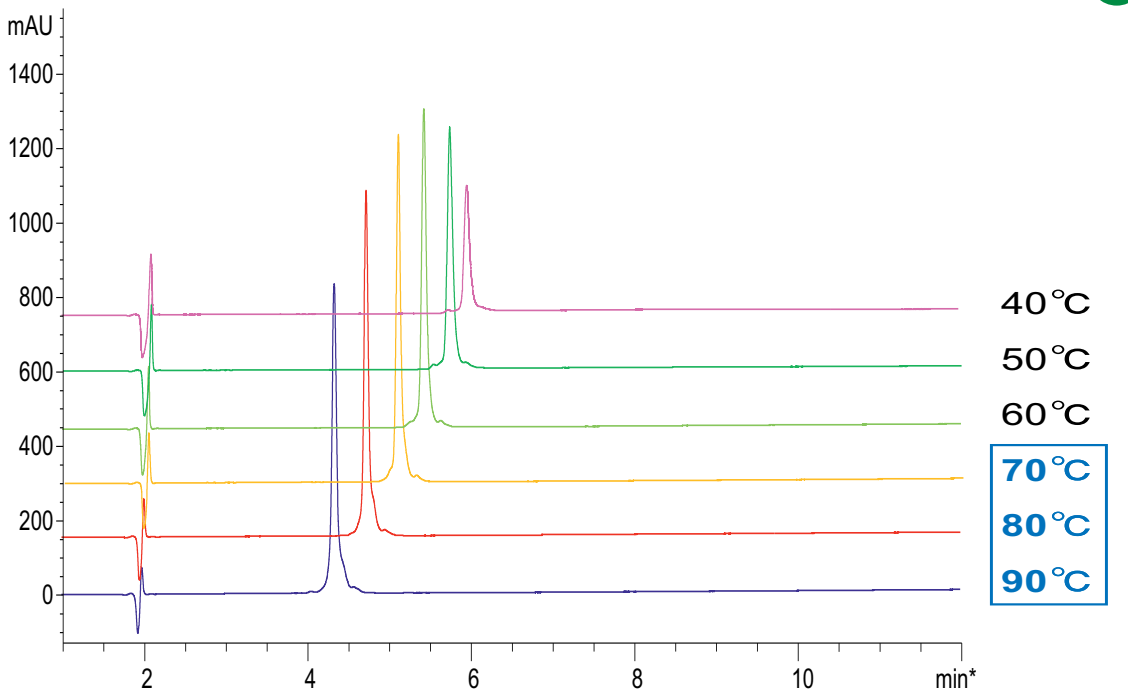


Column size:	YMC-Triart BioC4 (1.9 µm, 30 nm) 150 x 2.1 mm ID	Detection:	UV at 280 nm
Part no:	TB30SP9-15Q1PT	Flow rate:	0.4 mL/min
Eluent <TFA>:	A) water/TFA (100/0.1)	Temperature:	80 °C
	B) acetonitrile/TFA (100/0.1)	Injection:	4 µL (0.25 mg/mL)
Gradient <TFA>:	25-50%B (0-10 min), 90%B (10-12.5 min)	Sample:	mAb Subunit Standard (Waters Corp.)
Eluent <Formic acid>:	A) water/formic acid (100/0.1)		
	B) acetonitrile/formic acid (100/0.1)		
Gradient <Formic acid>:	20-45%B (0-10 min), 90%B (10-12.5 min)		

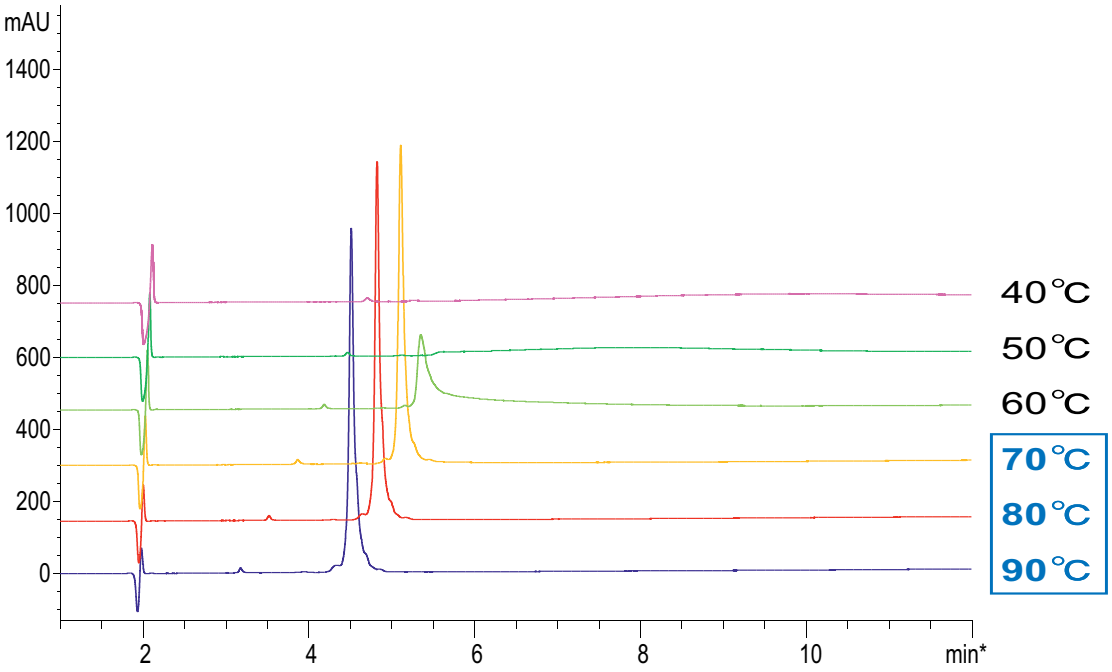


Adalimumab (MW: ca. 148 kDa)

RP



Bevacizumab (MW: ca. 148 kDa)

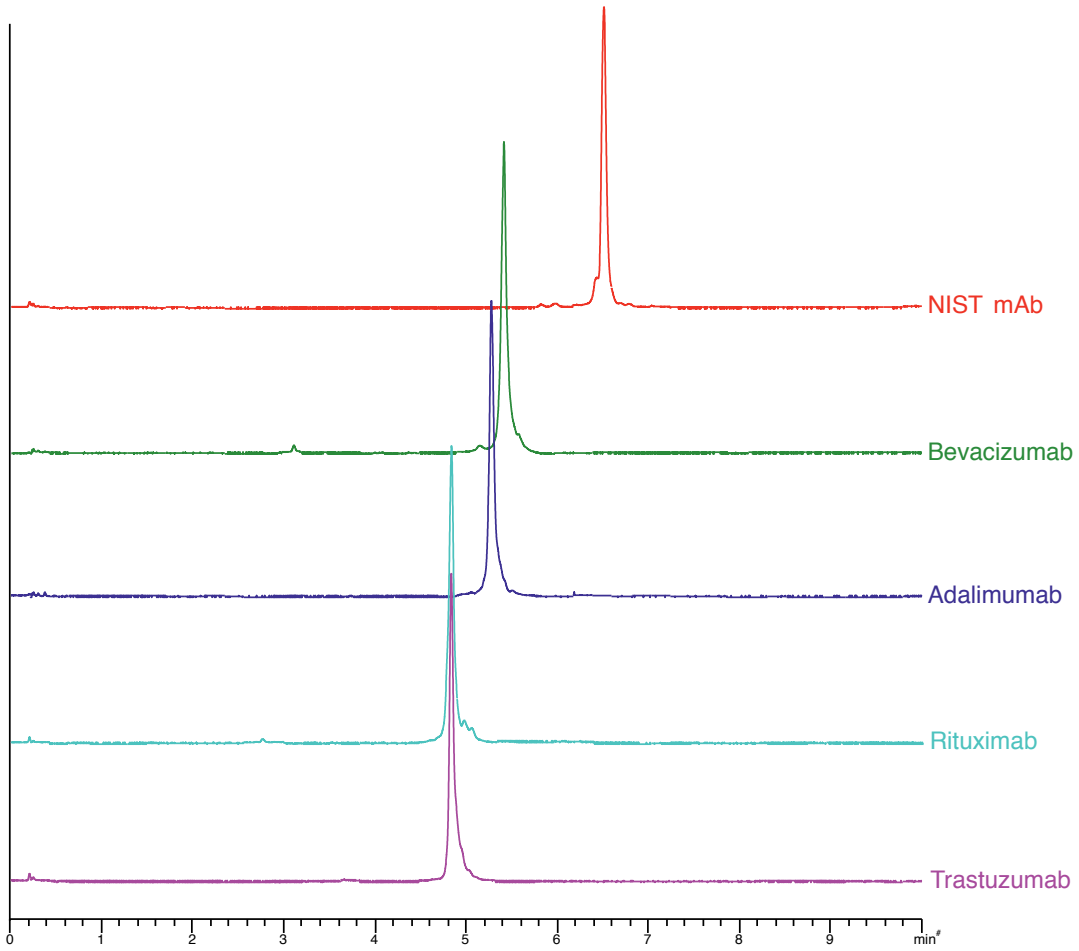


Column:	YMC-Triart Bio C4 (3 µm, 30 nm) 150 x 3.0 mm ID
Eluent:	A) water/TFA (100/0.1) B) acetonitrile/TFA (100/0.1)
Gradient:	30-60%B (0-15 min), 90%B (15-30min)
Detection:	UV at 220 nm
Flow rate:	0.4 mL/min
Sample:	Adalimumab (0.5 mg/mL) or Bevacizumab (0.5 mg/mL)
Injection:	4 µL

BioLC applications – Antibodies

Analysis of different monoclonal antibodies

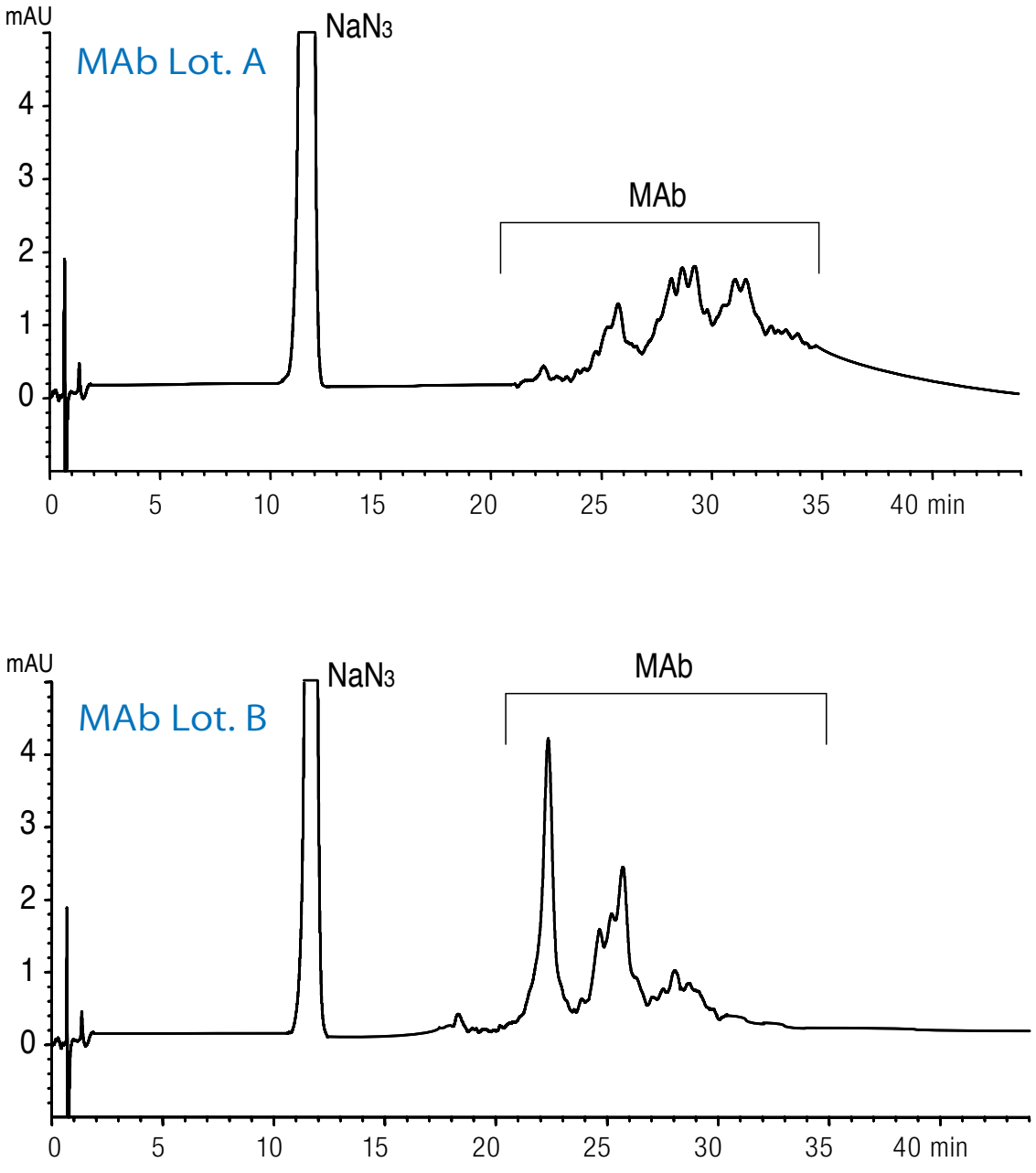
RP



Column:	YMC-Triart Bio C4 (1.9 µm, 30 nm) 50 x 2.1 mm ID
Part No.:	TB30SP9-05Q1PT
Eluent:	A) water/TFA (100/0.1)
	B) acetonitrile/TFA (100/0.1)
Gradient:	25-45%B (0-10 min)
Detection:	UV at 280 nm (0.13s, 40Hz)
Flow rate:	0.4 mL/min
Temperature:	80 °C
Sample conc.:	0.5 mg/mL
Injection:	2 µL

Different production batches of IgG1

IEX

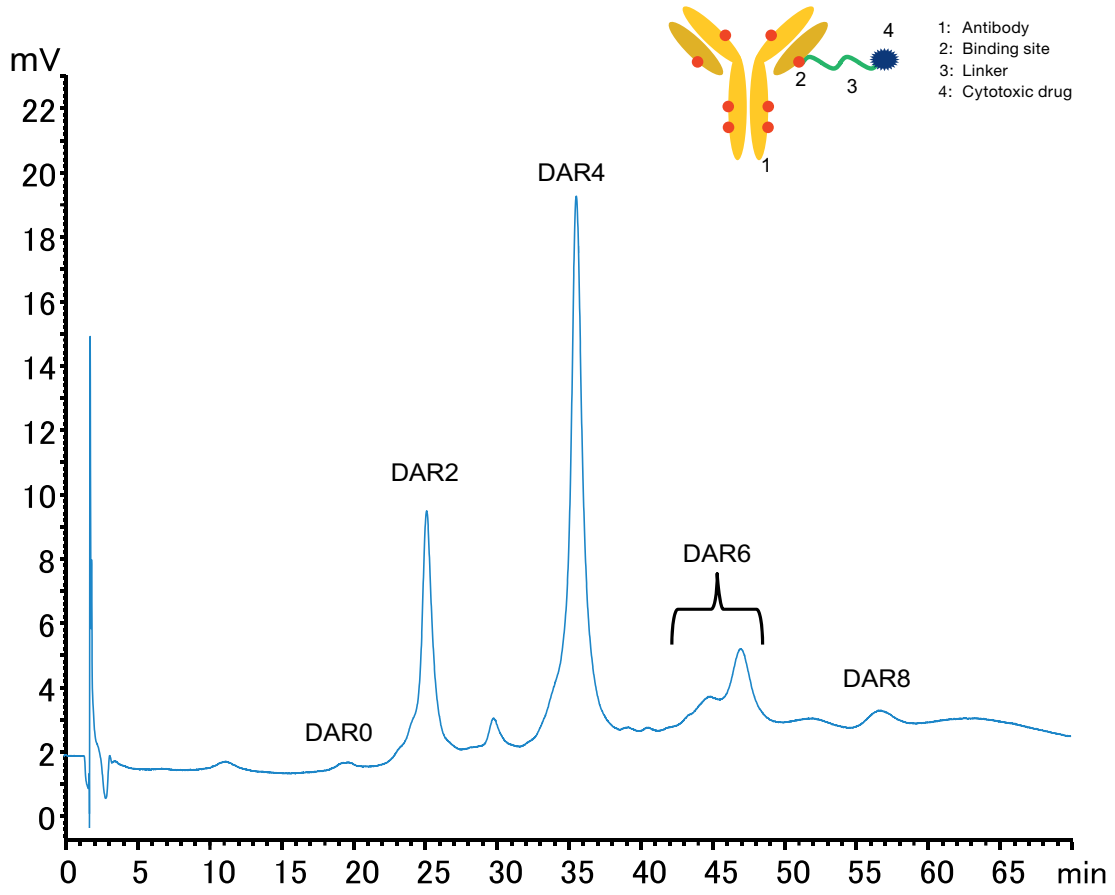


Column:	BioPro IEX QF (5 µm) 100 x 4.6 mm ID
Part No.:	QF00S05-1046WP
Eluent:	A) 20 mM Tris-HCl (pH 8.1) B) 20 mM Tris-HCl (pH 8.1) containing 0.5 M NaCl
Gradient:	10-25%B (0-60 min)
Flow rate:	1.0 mL/min (360 cm/hr)
Temperature:	25 ˚C
Detection:	UV at 220 nm
Injection:	14 µL (0.1 mg/mL)
Sample:	Mouse monoclonal IgG1 anti-human IgG4 (Purified by DEAE chromatography, containing NaN_3)

BioLC applications – Antibody-Drug-Conjugates

Drug to Antibody Ratio

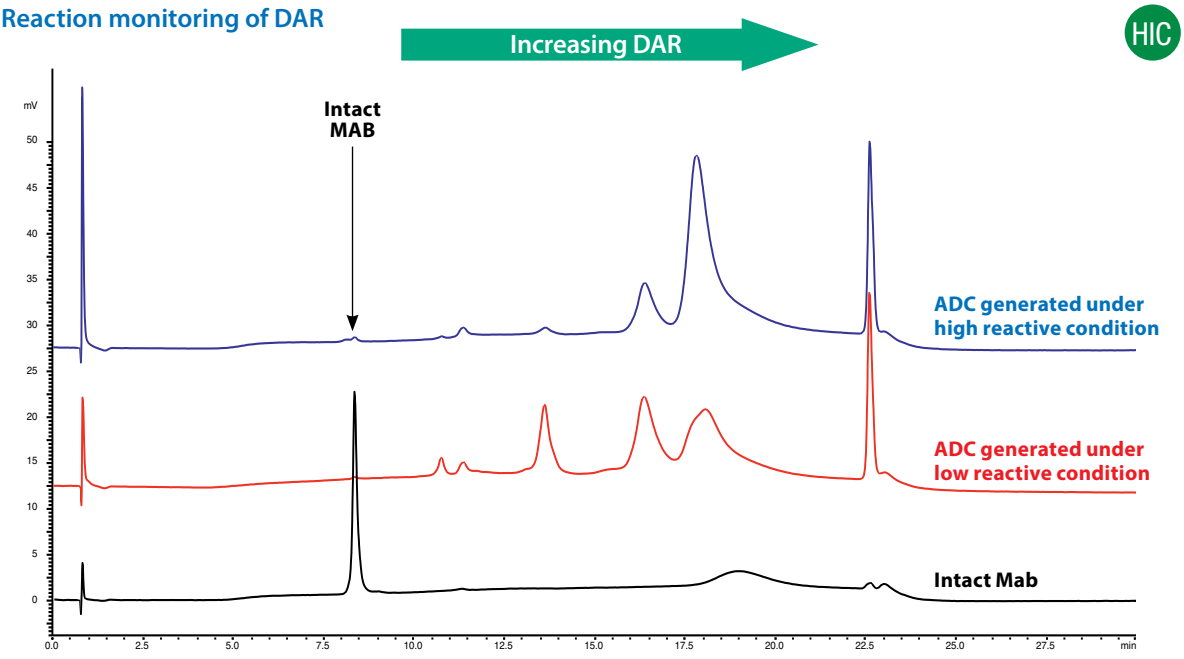
HIC



Column:	BioPro HIC BF (4 µm) 100 x 4.6 mm ID
Part No.:	BHB00S04-1046WT
Eluent:	A) 50 mM NaH_2PO_4-Na_2HPO_4 (pH 6.8) containing 1.5 M $(NH_4)_2SO_4$/IPA (95/5)
	B) 50 mM NaH_2PO_4-Na_2HPO_4 (pH 6.8)/IPA (80/20)
Gradient:	30%B (0-5 min)
	30-80%B (5-45 min)
	80%B (45-70 min)
Flow rate:	0.4 mL/min
Temperature:	25 °C
Detection:	UV at 280 nm
Injection:	20 µL
Sample:	Cystein-conjugated ADC mimic (1.25 mg/mL)

Reaction monitoring of DAR

HIC

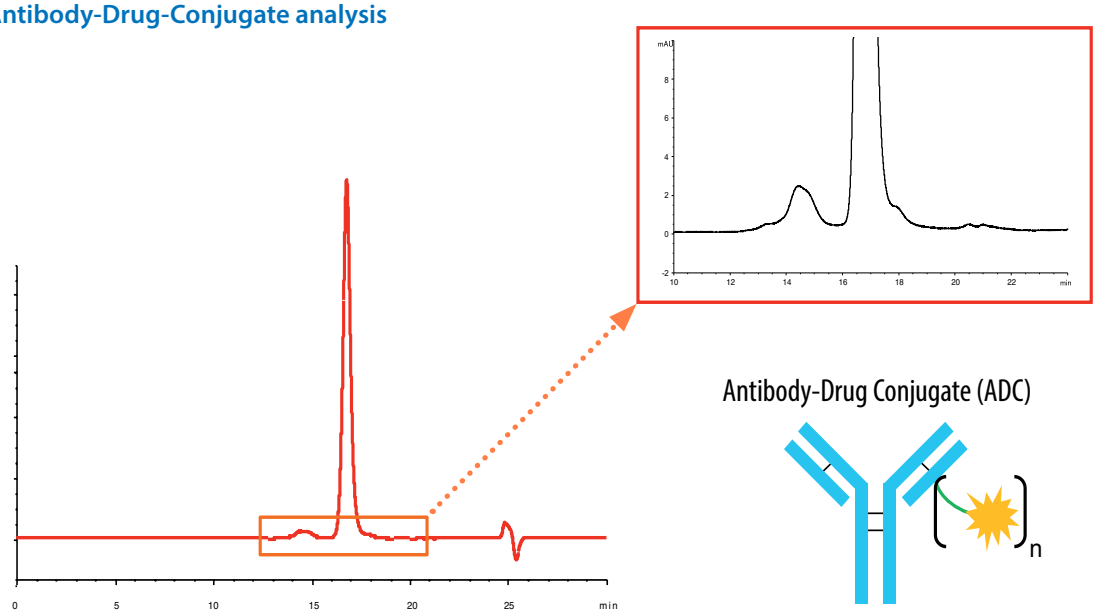


Column: BioPro HIC BF (4 µm) 100 x 4.6 mm ID
Part No.: BHB00S04-1046WT
Eluent: A) 50 mM NaH_2PO_4-Na_2HPO_4 (pH 7.0) containing 1.5 M $(NH_4)_2SO_4$/2-propanol (95/5)
B) 50 mM NaH_2PO_4-Na_2HPO_4 (pH 7.0)/2-propanol (80/20)
Gradient: 0%B (0-1 min), 0-100%B (1-15 min), 100%B (15-20 min), 0%B (20-30 min)
Flow rate: 1.0 mL/min
Temperature: 25 °C
Detection: UV at 280 nm
Sample: Antibody Drug Conjugate*

*Courtesy of RIKEN

Antibody-Drug-Conjugate analysis

SEC

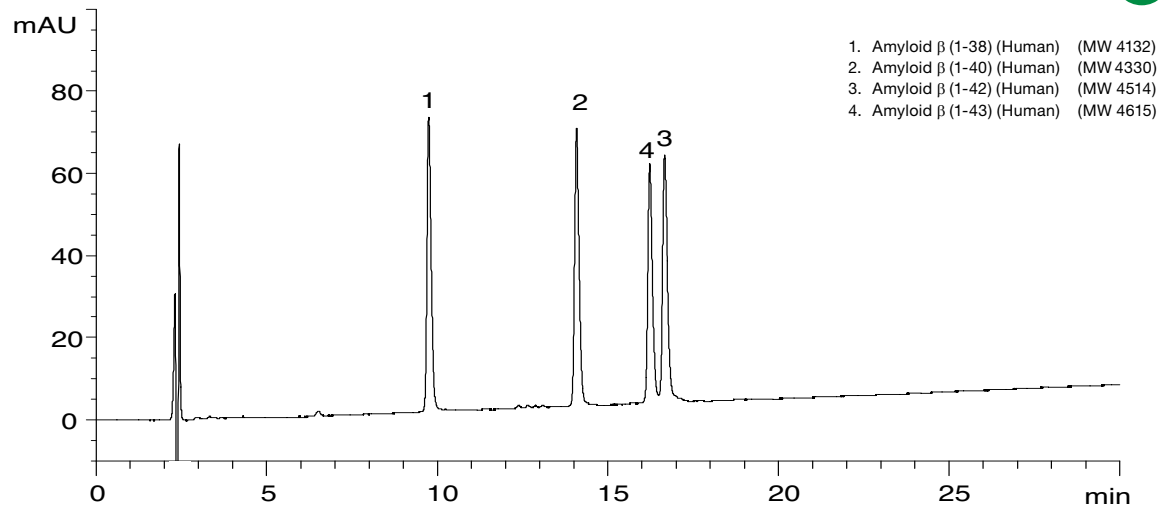


Column: YMC-SEC MAB (3 µm, 25 nm) 300 x 4.6 mm ID
Part No.: DLM25S03-3046WT
Eluent: 0.1 M phosphate buffer (pH = 7) cont. 0.2 M NaCl / 2-propanol (85 / 15)
Flow rate: 0.165 mL/min
Temperature: 25 °C
Detection: UV at 280 nm
Injection: 4 µL (2.5 mg/mL)
Sample: SigmaMAb Antibody Drug Conjugate Mimic

BioLC applications – Proteins

Amyloid β-proteins

RP

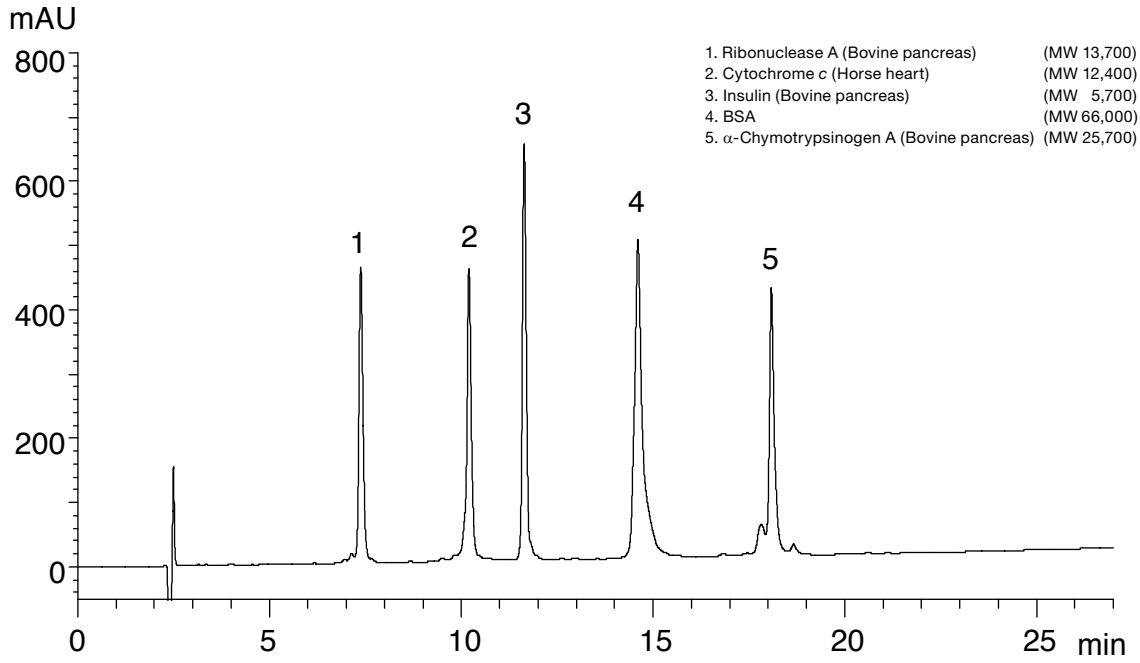


Amyloid (1-43) : Asp-Ala-Glu-Phe-Arg-His-Asp-Ser-Gly-Tyr-Glu-Val-His-His-Gln-Lys-Leu-Val-Phe-Phe-Ala-Glu-Asp-Val-Gly-Ser-Asn-Lys-Gly-Ala-Ile-Ile-Gly-Leu-Met-Val-Gly-Gly-Val-Val-Ile-Ala-Thr

Column:	YMC-Triart Bio C4 (3 µm, 30 nm) 150 x 3.0 mm ID	Flow rate:	0.4 mL/min
Part No.:	TB30S03-1503PTH	Temperature:	70 °C
Eluent:	A) water/TFA (100/0.1)	Detection:	UV at 220 nm
	B) acetonitrile/TFA (100/0.1)	Injection:	4 µL (each 0.1 mg/mL)
Gradient:	25-40%B (0-30 min), 90%B (30-40 min)		

Proteins (MW 5,700 ~ 66,000)

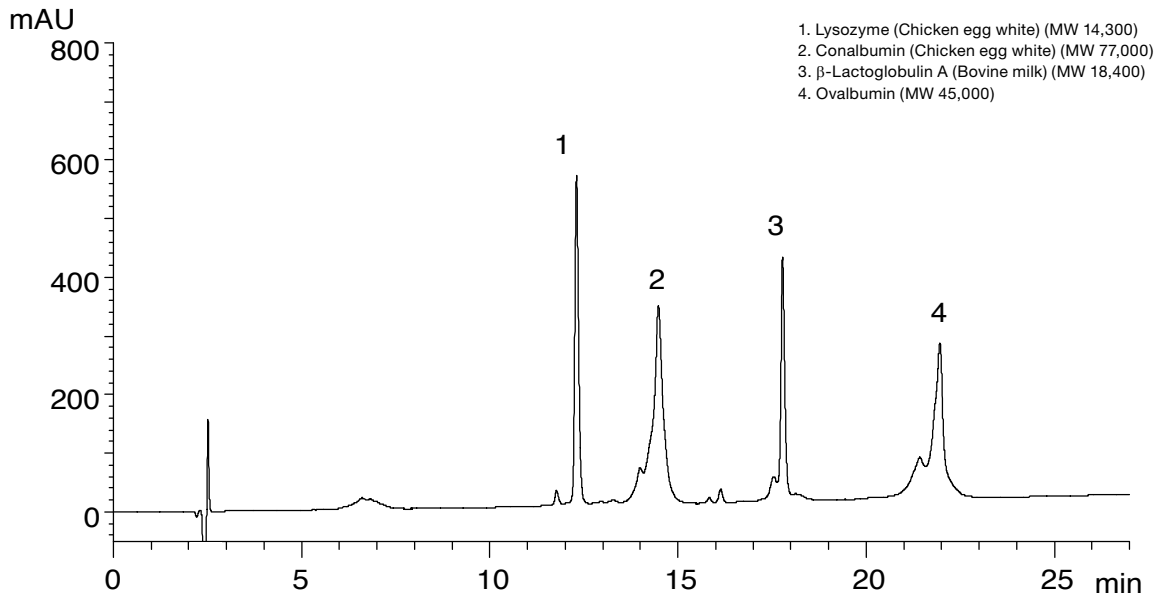
RP



Column:	YMC-Triart Bio C4 (5 µm, 30 nm) 150 x 3.0 mm ID	Flow rate:	0.4 mL/min
Part No.:	TB30S03-1503PTH	Temperature:	70 °C
Eluent:	A) water/TFA (100/0.1)	Detection:	UV at 220 nm
	B) acetonitrile/TFA (100/0.1)	Injection:	10 µL (0.25 ~ 0.50 mg/mL)
Gradient:	20-60%B (0-27 min), 90%B (27-35 min)		

Proteins (MW 14,300 ~ 77,000)

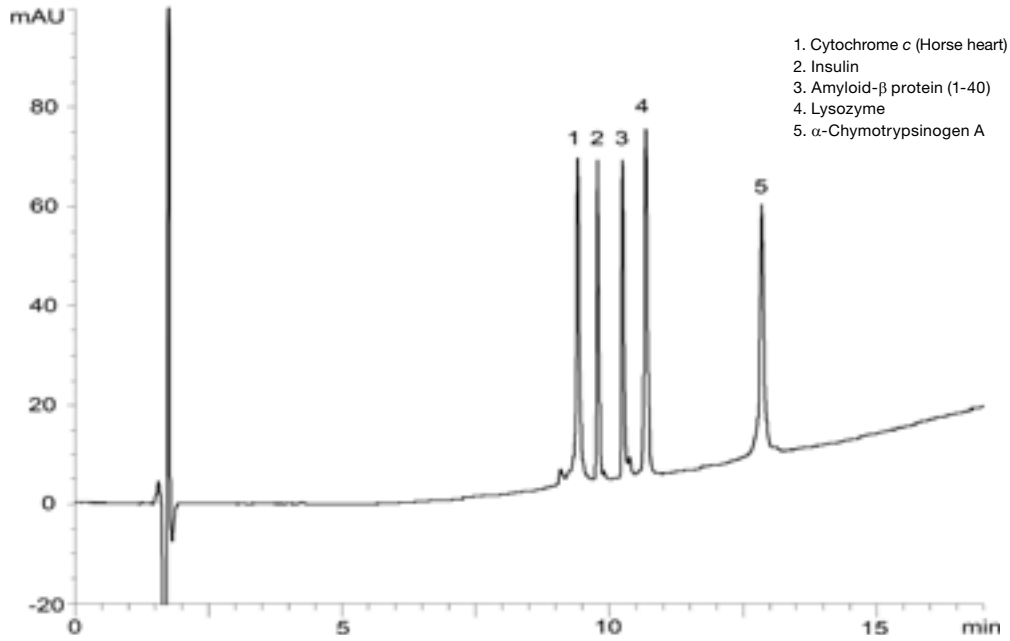
RP



Column:	YMC-Triart Bio C4 (5 µm, 30 nm) 150 x 3.0 mm ID	Flow rate:	0.4 mL/min
Part No.:	TB30S03-1503PTH	Temperature:	70 °C
Eluent:	A) water/TFA (100/0.1)	Detection:	UV at 220 nm
	B) acetonitrile/TFA (100/0.1)	Injection:	10 µL (0.25 ~ 0.50 mg/mL)
Gradient:	20-60%B (0-27 min), 90%B (27-35 min)		

Peptides and proteins

RP

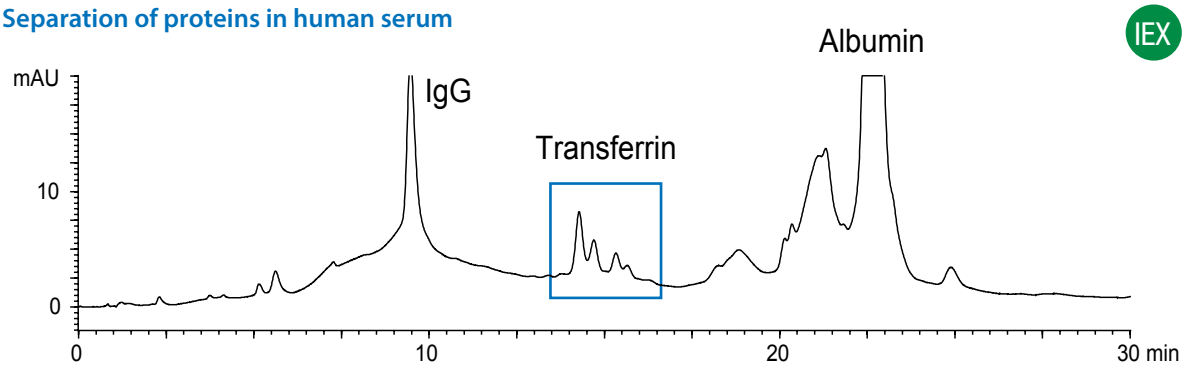


Column:	Meteoric Core C18 BIO (2.7 µm, 16 nm) 150 × 2.1 mm ID	Flow rate:	0.2 mL/min
Part No.:	CAW16SQ7-15Q1PT	Temperature:	40 °C
Eluent:	A) water/TFA (100/0.1)	Detection:	UV at 220 nm
	B) acetonitrile/TFA (100/0.1)	Injection:	2 µL (0.05-0.2 mg/mL)
	20-70% B (0-15 min), 70% B (15-17 min)	Pressure:	12.8-16.1 MPa (1860-2330 psi)

BioLC applications – Proteins

Separation of proteins in human serum

IEX

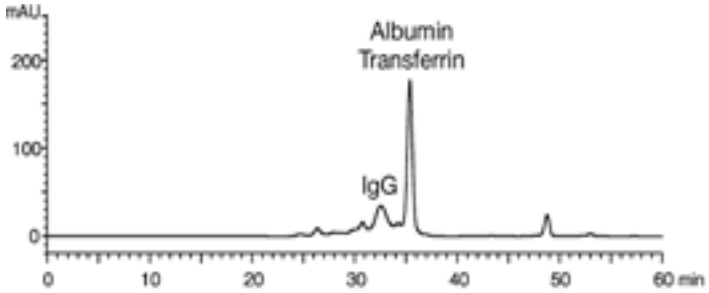


Column:	BioPro IEX QA (5 µm) 50 x 4.6 mm ID	Temperature:	25 °C
Part No.:	QAA0S05-0546WP	Detection:	UV at 280 nm
Eluent:	A) 20 mM Tris-HCl (pH 8.6) B) 20 mM Tris-HCl (pH 8.6) containing 0.5 M NaCl	Injection:	20 µL
Gradient:	0-30%B (0-15 min), 30-100%B (15-30 min)	Sample:	Human serum (100 µL/mL)
Flow rate:	0.5 mL/min		

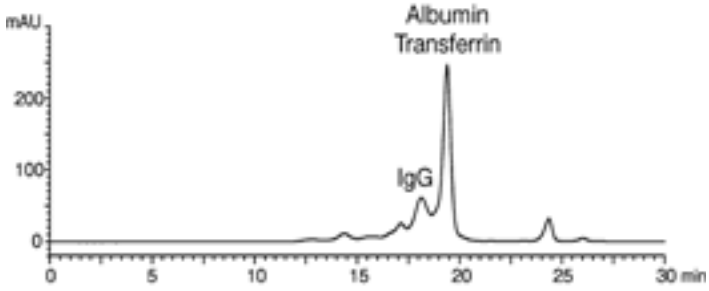
Proteins in human serum

SEC

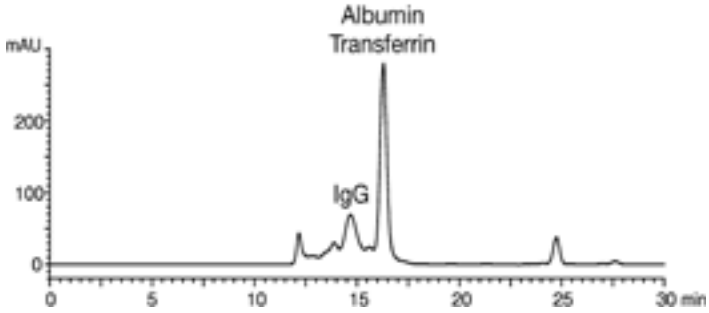
YMC-Pack Diol-300 + Diol-200
5 µm, 300 x 8.0 mm ID x 2



YMC-Pack Diol-300
5 µm, 300 x 8.0 mm ID



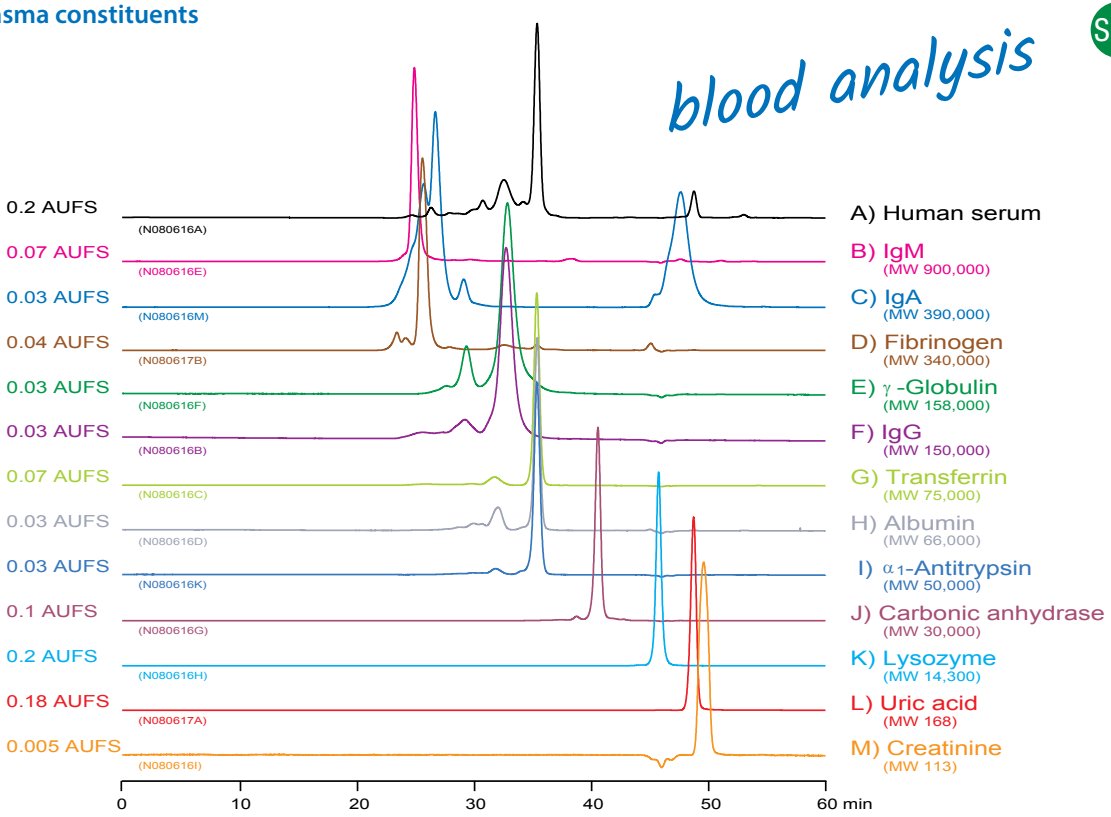
YMC-Pack Diol-200
5 µm, 300 x 8.0 mm ID



Eluent:	0.1 M KH_2PO_4-K_2HPO_4 (pH 7.0) containing 0.2 M NaCl
Part Nos.:	DL30S05-3008WT + DL30S05-3008WT
Flow rate:	0.5 mL/min
Temperature:	ambient (25 °C)
Detection:	UV at 280 nm
Injection:	20 µL
Sample:	Human serum (100 µL/mL)

Plasma constituents

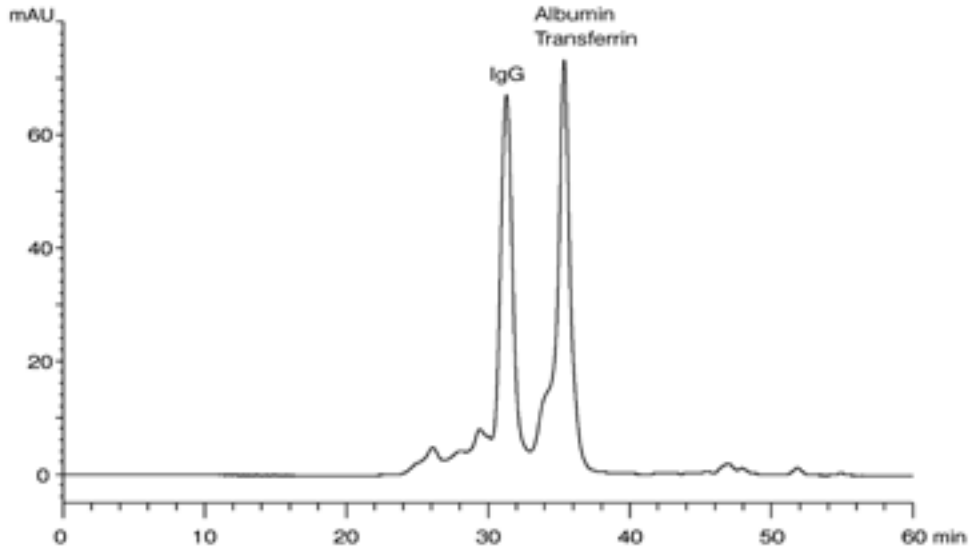
SEC



Columns:	YMC-Pack Diol-300 + Diol-200 (5 µm) 300 x 8.0 mm ID x 2	Temperature:	ambient (25 °C)
Part Nos.:	DL30S05-3008WT + DL20S05-3008WT	Detection:	UV at 280 nm
Eluent:	0.1 M KH_2PO_4-K_2HPO_4 (pH 7.0) containing 0.2 M NaCl	Injection:	20 µL (L: 1 µL)
Flow rate:	0.5 mL/min	Sample:	A) 100 µL/mL; B-M) 1.0 mg/mL

Proteins in mouse ascites fluid

SEC



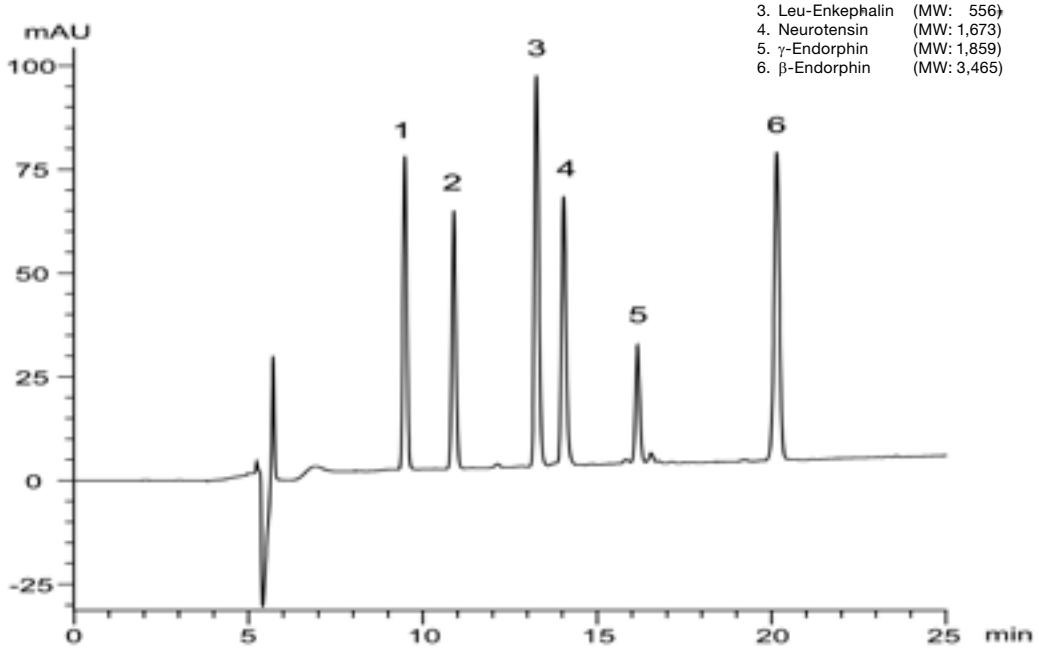
Columns:	YMC-Pack Diol-300 + Diol-200 (5 µm) 300 x 4.6 mm ID	Temperature:	ambient (25 °C)
Part Nos.:	DL30S05-3046WT + DL20S05-3046WT	Detection:	UV at 220 nm
Eluent:	0.1 M KH_2PO_4-K_2HPO_4 (pH 7.0)	Injection:	10 µL (60 times dilution with water)
Flow rate:	0.17 mL/min		

BioLC applications – Peptides

Peptides

RP

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)

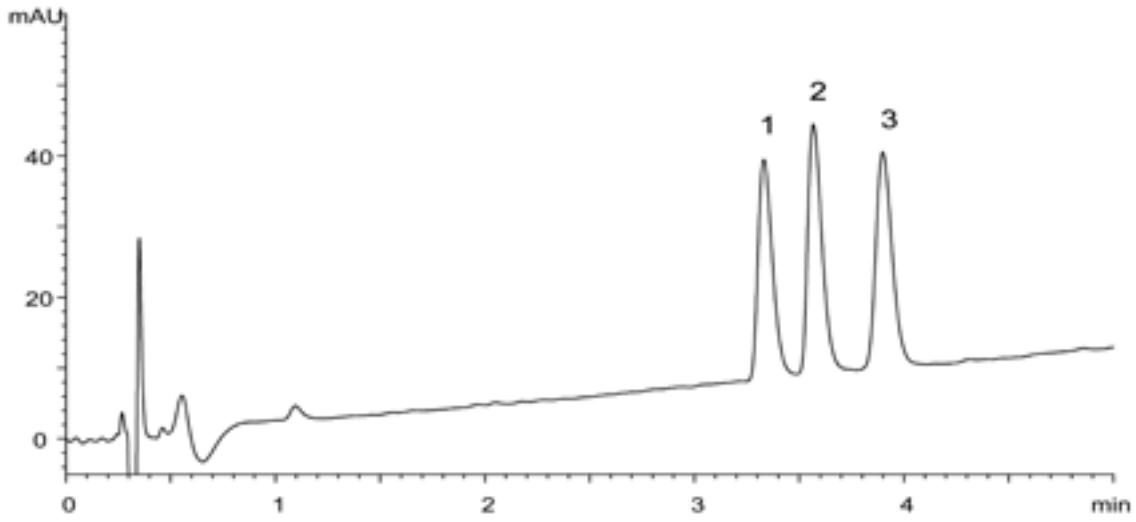


Column:	YMC-Triart C18 (5 µm, 12 nm) 150 x 2.0 mm ID	Flow rate:	0.2 mL/min
Part No.:	TA12S05-1502WT	Temperature:	37 °C
Eluent:	A) water / TFA (100/0.1)	Detection:	UV at 220 nm
	B) acetonitrile / TFA (100/0.1)	Injection:	2 µL (0.075 ≈ 0.25 mg/mL)
Gradient:	20%-45% B (0-25 min)		

Antimicrobial peptides

RP

1. α-Defensin-1 (human) (MW: 3,442)
2. α-Defensin-2 (human) (MW: 3,371)
3. α-Defensin-3 (human) (MW: 3,486)

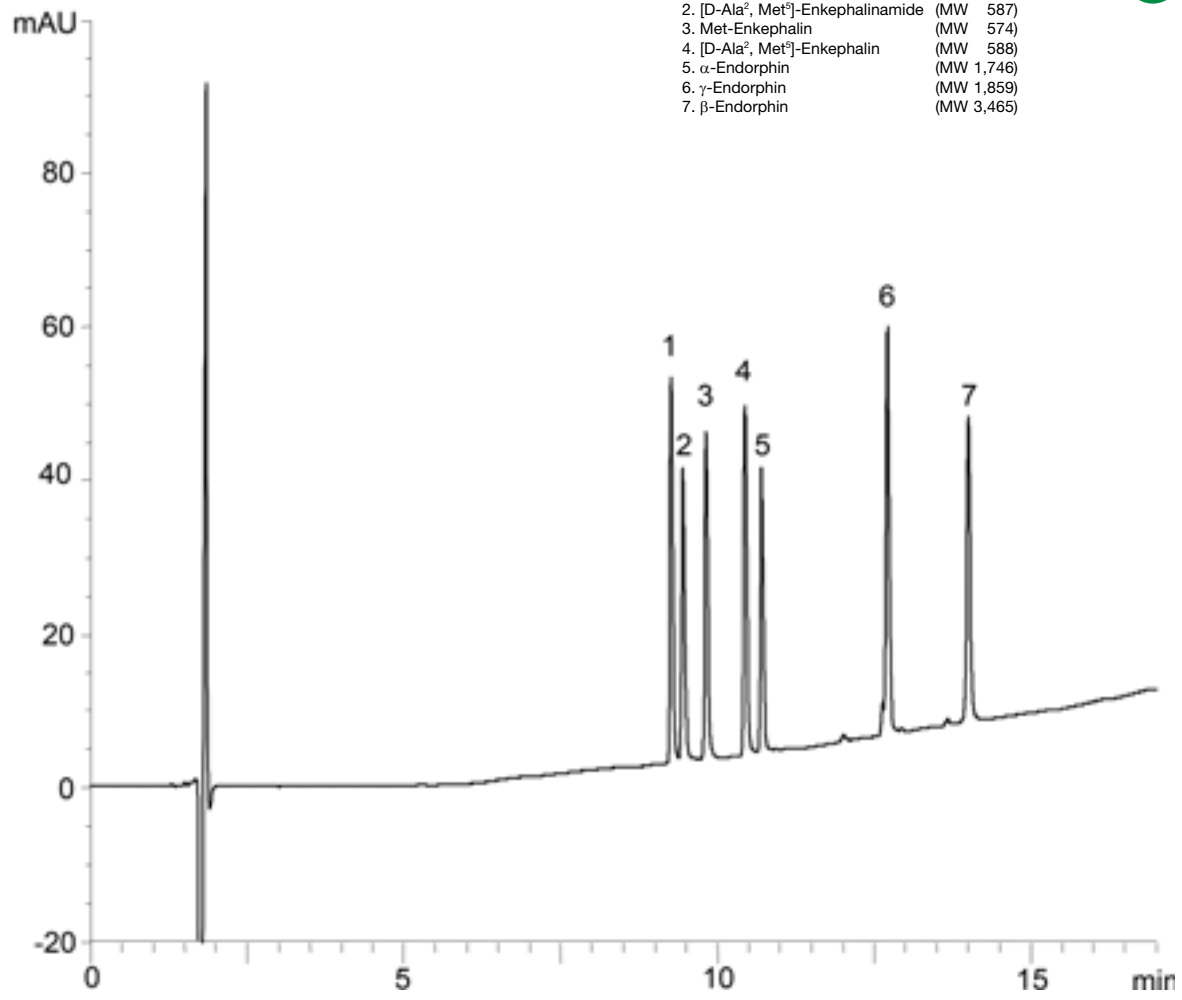


Column:	YMC-Triart C18 (1.9 µm, 12 nm) 50 x 2.0 mm ID	Flow rate:	0.4 mL/min
Part No.:	TA12SP9-0502PT	Temperature:	70 °C
Eluent:	A) water / formic acid (100/0.1)	Detection:	UV at 220 nm
	B) 2-propanol / acetonitrile / formic acid (50/50/0.08)	Injection:	1 µL (50 µg/mL)
Gradient:	10%-25% B (0-10 min)		

Peptides

RP

1. BAM-12P (MW 1,425)
2. [D-Ala^2, Met^5]-Enkephalinamide (MW 587)
3. Met-Enkephalin (MW 574)
4. [D-Ala^2, Met^5]-Enkephalin (MW 588)
5. α-Endorphin (MW 1,746)
6. γ-Endorphin (MW 1,859)
7. β-Endorphin (MW 3,465)

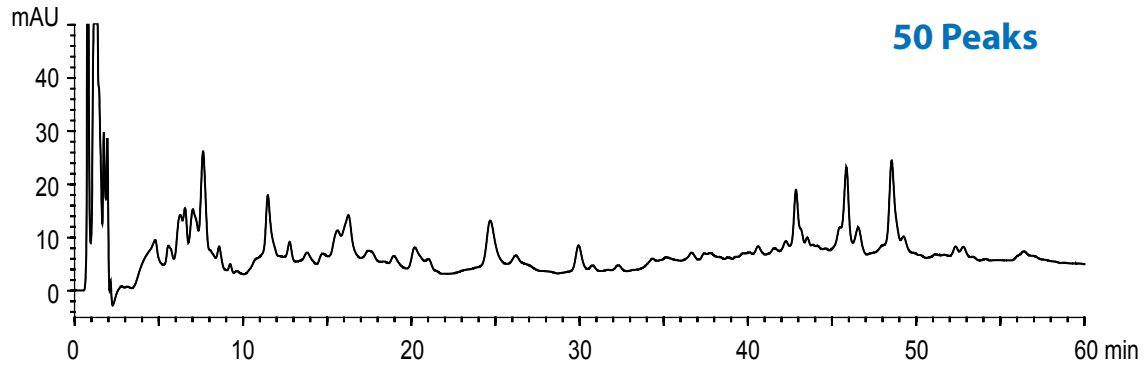


Column:	Meteoric Core C18 BIO (2.7 µm, 16 nm) 150 × 2.1 mm ID
Part No.:	CAW16SQ7-15Q1PT
Eluent:	A) water/TFA (100/0.1) B) acetonitrile/TFA (100/0.1) 15-55% B (0-15 min), 55% B (15-17 min)
Flow rate:	0.2 mL/min
Temperature:	40 °C
Detection:	UV at 220 nm
Injection:	2 µL (0.02-0.5 mg/mL)
Pressure:	14.9-16.1 MPa (2160-2330 psi)

BioLC applications – Peptide Mapping

Peptide mapping of tryptic digests of BSA with highest sensitivity

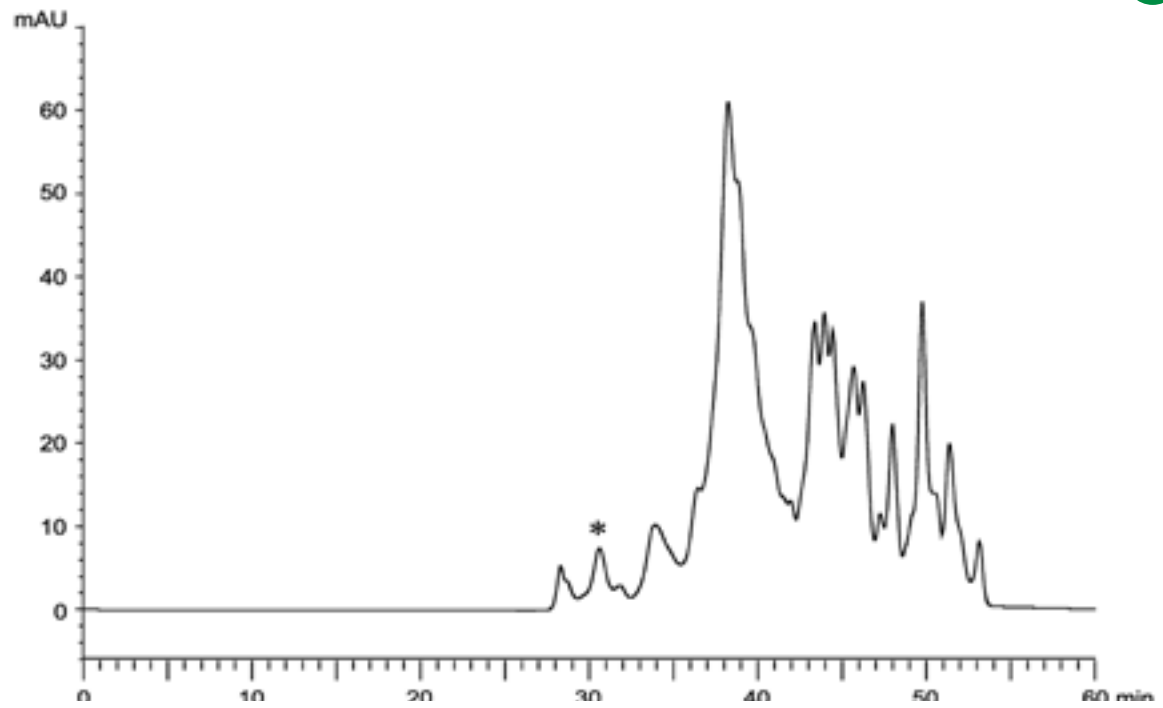
IEX



Column:	BioPro IEX QA (5 µm) 50 x 4.6 mm ID
Part No.:	QAA0S05-0546WP
Eluent:	A) 20 mM Tris-HCl (pH 8.6)
	B) 20 mM Tris-HCl (pH 8.6) containing 0.5 M NaCl
Gradient:	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 µL
Sample:	Tryptic digest of BSA

Tryptic digest of BSA

SEC

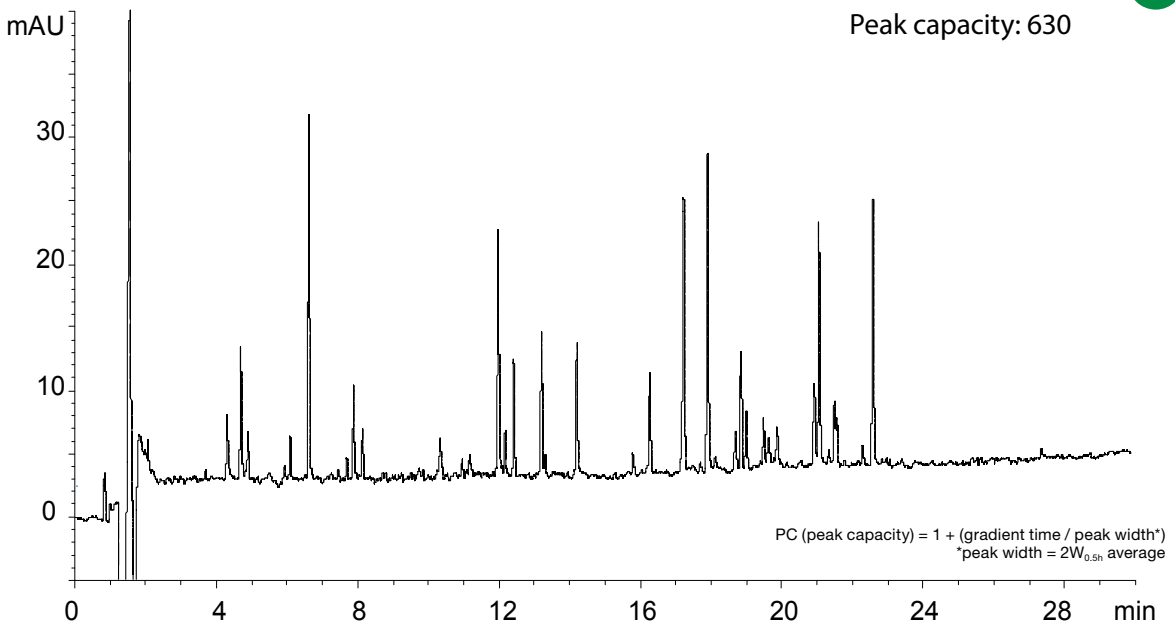


Columns:	YMC-Pack Diol-120 + Diol-60 (5 µm) 500 x 8.0 mm ID
Part Nos.:	DL12S05-5008WT + DL06S05-5008WT
Eluent:	0.1 M KH_2PO_4-K_2HPO_4 (pH 7.0)
	containing 0.2 M NaCl/acetonitrile (70/30)
Flow rate:	0.7 mL/min
Temperature:	ambient (25 °C)
Detection:	UV at 220 nm
Injection:	5 µL
Sample:	Tryptic digest of BSA

*undigested BSA

Tryptic digest of Hemoglobin

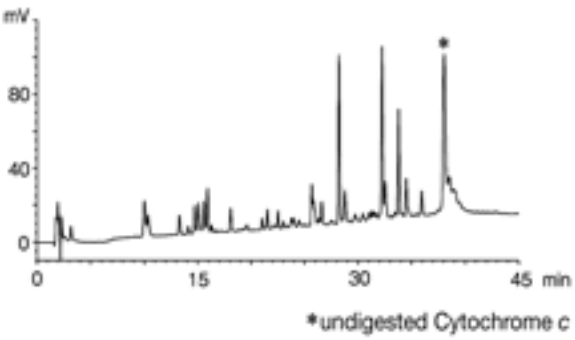
RP

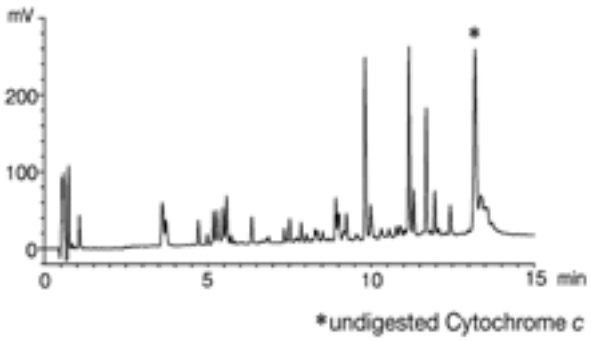


Column:	YMC-Triart C18 (1.9 µm, 12 nm) 200 x 2.0 mm ID (Two coupled 100 x 2.0 mm ID)
Part No.:	TA12SP9-1002PT (2x)
Eluent:	A) water/TFA (100/0.1) B) acetonitrile/TFA (100/0.08)
Gradient:	5-40%B (0-30 min)
Flow rate:	0.4 mL/min
Temperature:	70 °C
Detection:	UV at 220 nm
Injection:	20 µL
Sample:	Triptic digest of Bovine Hemoglobin (2.5 nmol/mL)
Pressure:	58.1-61.6 MPa (8,430-8,930 psi)

Peptide mapping – excellent reproducibility between 5 µm and 2 µm

RP





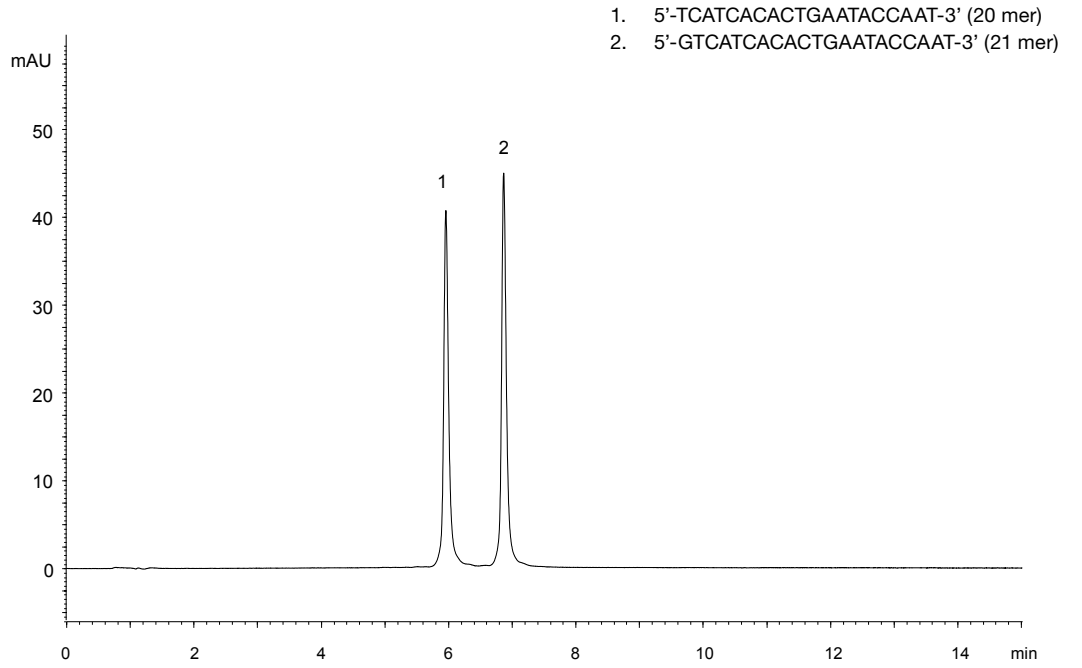
Column:	YMC-Pack *Pro* C18 (5 µm, 12 nm) 150 × 2.0 mm ID
Part No.:	AS12S05-1502WT
Eluent:	A) acetonitrile/water/trifluoroacetic acid (10/90/0.1) B) acetonitrile/water/trifluoroacetic acid (35/65/0.1)
Gradient:	Time A (in %) B (in %) 0 100 0 5 100 0 40 0 100 45 0 100
Flow rate:	0.2 mL/min
Temperature:	37 °C
Detection:	UV at 220 nm
Injection:	1 µL
Sample:	Tryptic digest of Cytochrome *c*

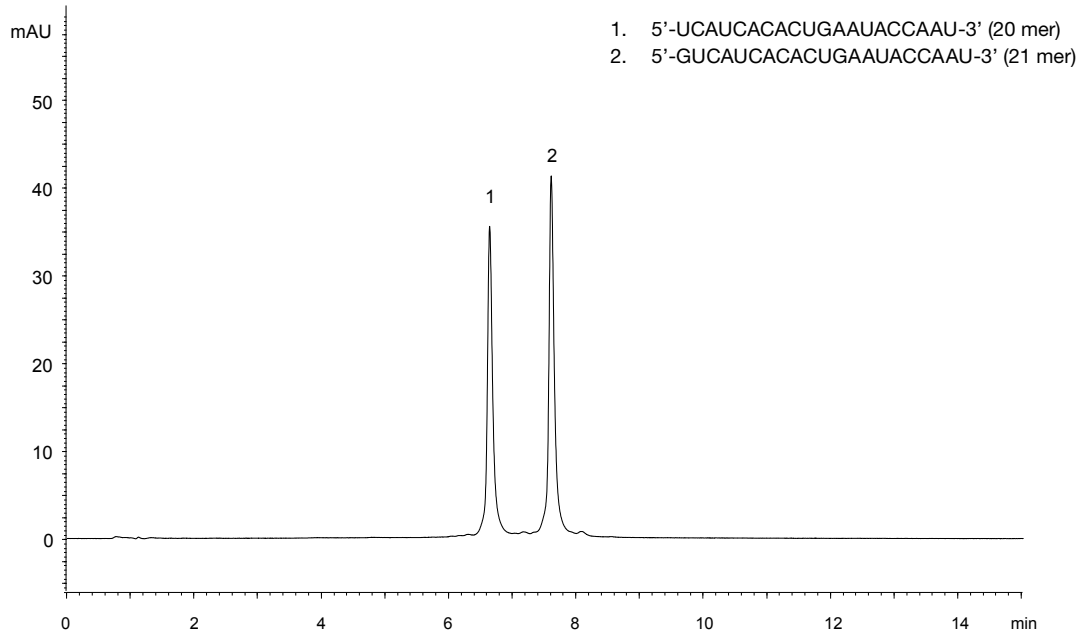
Column:	YMC-UltraHT *Pro* C18 (2 µm, 12 nm) 50 × 2.0 mm ID
Part No.:	AS12S02-0502WT
Eluent:	A) acetonitrile/water/trifluoroacetic acid (10/90/0.1) B) acetonitrile/water/trifluoroacetic acid (35/65/0.1)
Gradient:	Time A (in %) B (in %) 0 100 0 1.65 100 0 13.35 0 100 15.00 0 100
Flow rate:	0.2 mL/min
Temperature:	37 °C
Detection:	UV at 220 nm
Injection:	1 µL
Sample:	Tryptic digest of Cytochrome c

BioLC applications – Oligonucleotides

Separation of synthetic oligonucleotides (single-strand DNA)

IEX





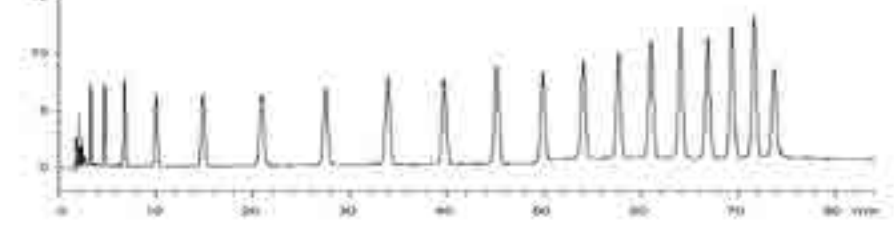
Column:	BioPro IEX QF (5 µm) 100 x 4.6 mm ID
Part no.:	QF00S05-1046WP
Eluent:	A) 10 mM NaOH
	B) 10 mM NaOH containing 1.0 M $NaClO_4$
Gradient:	25-55%B (0-15 min), 100%B (15-20 min)
Flow rate:	1.0 mL/min
Temperature:	25 °C
Detection:	UV at 260 nm
Injection:	4 µL (5 nmol/L)

Oligonucleotides d(T)2-20 method transfer from HPLC to UHPLC

RP

Conventional LC method

Column: YMC-Triart C18 (5 µm, 12 nm)
150 x 2.0 mm ID
Part No. TA12S05-1502WT
Flow rate: 0.2 mL/min
Gradient: 30-50% B (0-84 min)

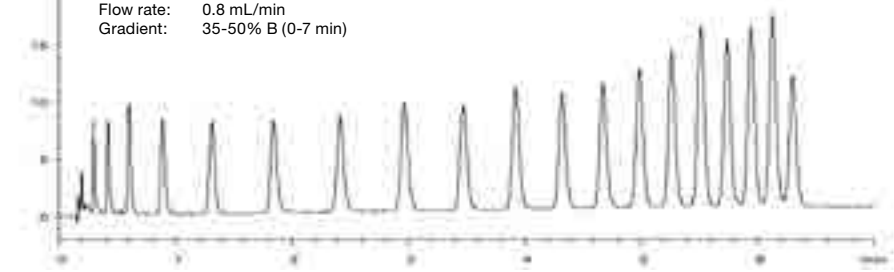


80 min

11x faster

UHPLC method

Column: YMC-Triart C18 (1.9 µm, 12 nm)
50 x 2.0 mm ID
Part No. TA12SP9-0502PT
Flow rate: 0.8 mL/min
Gradient: 35-50% B (0-7 min)

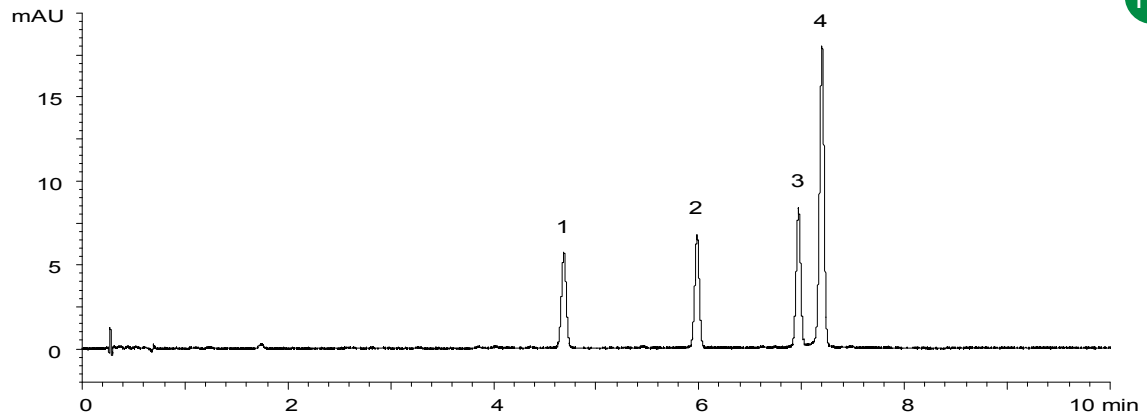


7 min

Eluent: A) 10 mM di-n-butylamine-acetic acid (pH 6.0)
B) methanol
Detection: UV at 269 nm
Injection: 1 µL (5 nmol/mL)
Temperature: 37 °C

Synthetic oligonucleotides

RP



1. 5′-CAC UGA AUA CCA AU-3′ (14mer)
2. 5′-UCA CAC UGA AUA CCA AU-3′ (17mer)
3. 5′-UCA UCA CAC UGA AUA CCA AU-3′ (20mer)
4. 5′-GUC AUC ACA CUG AAU ACC AAU-3′ (21mer)

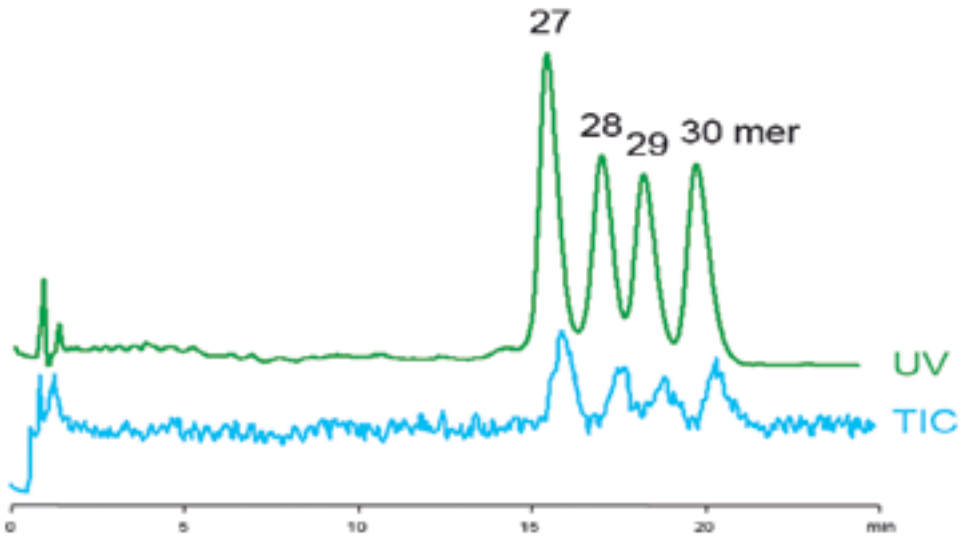
Column: YMC-Triart C18 (1.9 µm, 12 nm) 50 x 2.1 mm ID
Part No.: TA12SP9-05Q1PT
Eluent: A) 200 mM HFIP*-8 mM triethylamine
B) methanol
Gradient: 10-20%B (0-10 min)

*hexafluoroisopropanol
Flow rate: 0.42 mL/min
Temperature: 65 °C
Detection: UV at 260 nm
Injection: 1 µL (2-4 nmol/mL)

BioLC applications – Oligonucleotides

LC-MS analysis of synthetic 27-30 mer oligonucleotides

RP

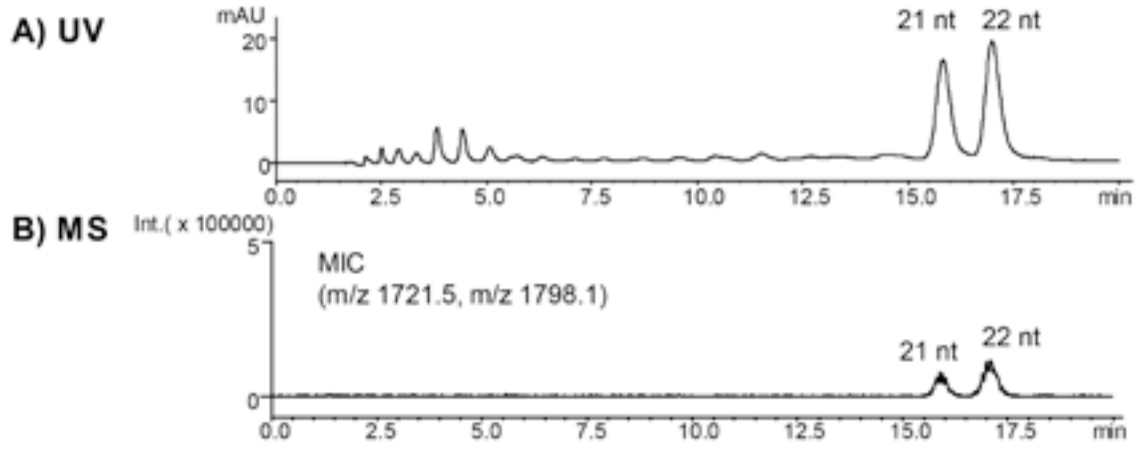


Sample: Primer of DNA sequencing
5'-CCGCTCGAGCTAAAAAAAGCCTGTGTTACC-3' (30 mer)

Column:	Hydrosphere C18 (3 µm) 50 x 2.0 mm ID
Part No.:	HS12S03-0502WT
Eluent:	A) 10 mM DBAA (pH 6.0)
	B) Mobile phase A / acetonitrile (50/50)
Gradient:	58%-62% B (0-20 min), 62% B (20-25 min)
Flow rate:	0.2 mL/min
Temperature:	35 °C
Detection:	UV at 269 nm and ESI negative-mode
Injection:	1 µL (10 pmol/component)

LC/MS analysis of miRNA

RP



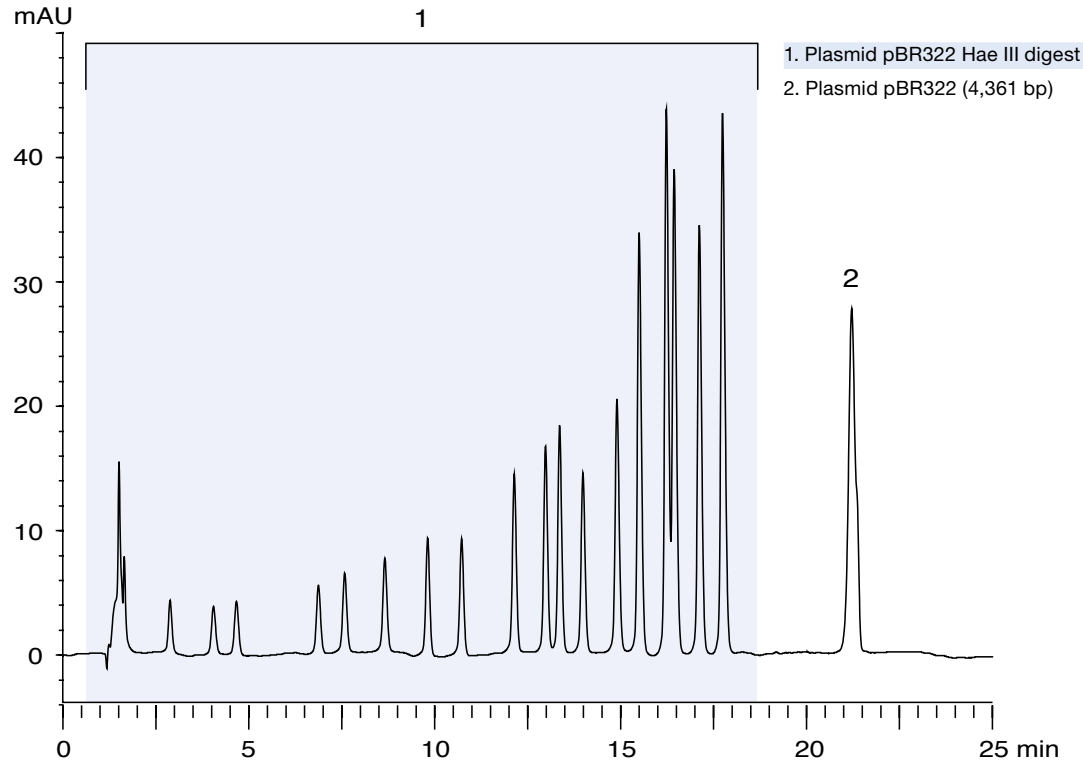
Courtesy of M. Yamada, SHIMADZU CORPORATION

5'-pUGG AGU GUG ACA AUG GUG UUG-3'	(21 nt, MW 6890.1)
5'-pUGG AGU GUG ACA AUG GUG UUG U-3'	(22 nt, MW 7196.3)

Column:	YMC-Triart C18 (3 µm, 12 nm) 150 x 2.0 mm ID
Part No.:	TA12S03-1502WT
Eluent:	A) 10 mM di-n-butylamine-acetic acid (pH 7.5)
	B) 10 mM di-n-butylamine-acetic acid (pH 7.5)/acetonitrile (50/50)
Gradient:	62-72%B (0-20 min)
Flow rate:	0.2 mL/min
Temperature:	30 °C
Detection:	A) UV at 260 nm
	B) ESI-negative mode
Injection:	4 µL (5 nmol/mL)
System:	LC) Shimadzu Prominence
	MS) Shimadzu LCMS2020

High resolution analysis using non-porous BioPro IEX QF for fragment identification

IEX



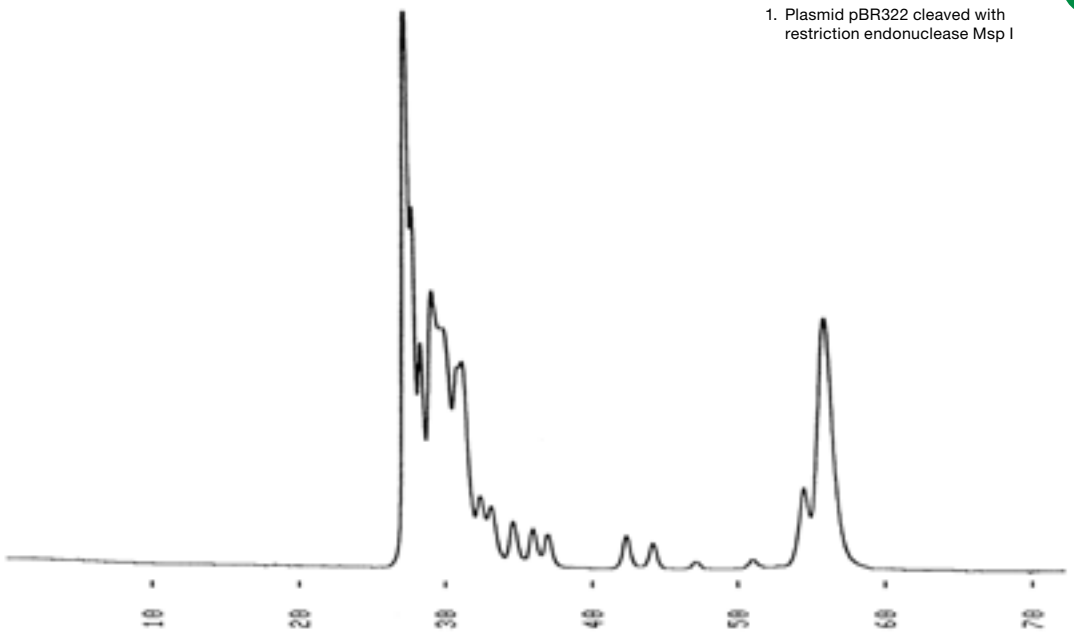
Column:	BioPro IEX QF (5 µm) 100 x 4.6 mm ID	Temperature:	35 °C
Part No.:	QF00S05-1046WP	Detection:	UV at 260 nm
Eluent:	A) 20 mM Tris-HCl (pH 8.1)	Injection:	10 µL
	B) 20 mM Tris-HCl (pH 8.1) containing 1.0 M NaCl	Sample:	Plasmid pBR322 Hae III digest (0.13 mg/mL)
Gradient:	70-85%B (0-20 min), 85%B (20-25 min)		Plasmid pBR322 (0.03 mg/mL)
Flow rate:	0.5 mL/min		

BioLC applications – Plasmids

Plasmid pBR322 restriction fragment

SEC

1. Plasmid pBR322 cleaved with restriction endonuclease Msp I



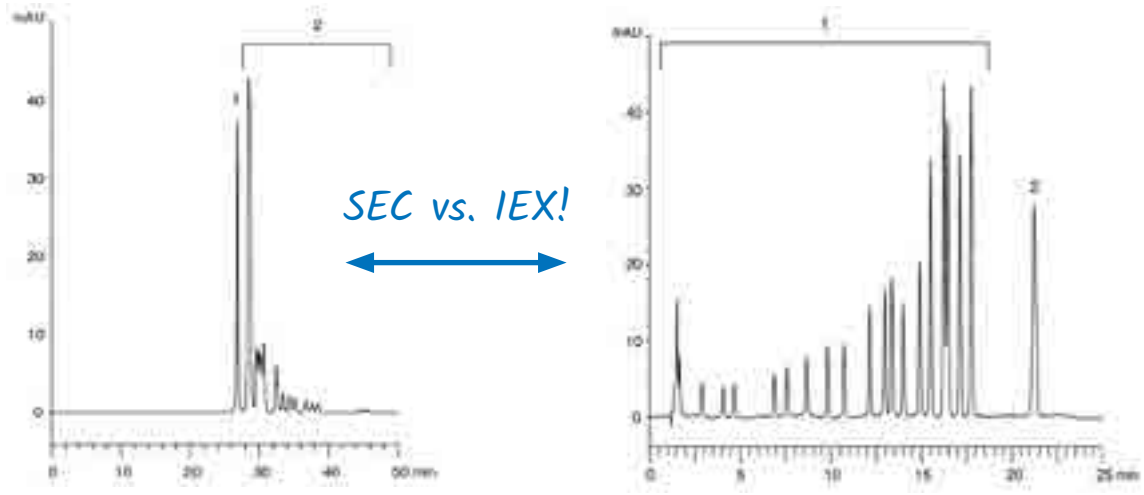
Columns:	YMC-Pack Diol-300 + Diol-200 (5 µm) 500 x 8.0 mm ID	Temperature:	ambient (26 °C)
Part Nos.:	DL30S05-5008WT + DL20S05-5008WT	Detection:	UV at 260 nm, 0.01 AUFS
Eluent:	0.1M KH_2PO_4-K_2HPO_4 (pH 7.0) containing 0.2M NaCl	Injection:	3 µL (0.49 mg/mL)
Flow rate:	0.7 mL/min	Sample:	Plasmid pBR322 cleaved with restriction endonuclease Msp I

Plasmid pBR322 restriction and pBR322 Hae III restriction fragment

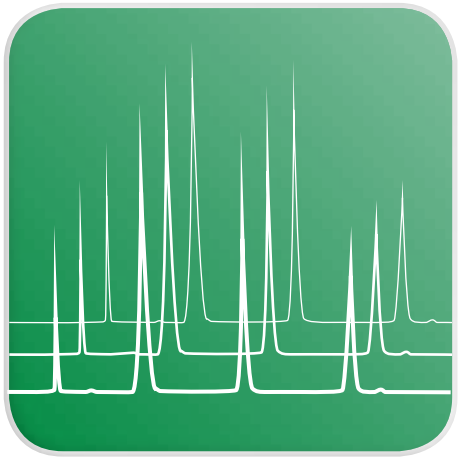
SEC IEX

1. Plasmid pBR322 (4,361 bp)
2. Plasmid pBR322 Hae III digest (8-587 bp)

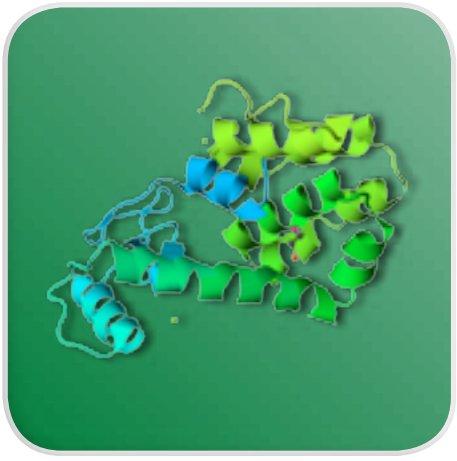
1. Plasmid pBR322 Hae III digest (8-587 bp)
2. Plasmid pBR322 (4,361 bp)



Columns:	YMC-Pack Diol-300 + Diol-200 (5 µm) 500 x 8.0 mm ID	Column:	BioPro IEX QF (5 µm) 100 x 4.6 mm ID
Part Nos.:	DL30S05-5008WT + DL20S05-5008WT	Part No.:	QF00S05-1046WP
Eluent:	0.1 M KH_2PO_4-K_2HPO_4 (pH 7.0) containing 0.2 M NaCl	Eluent:	A) 20 mM Tris-HCl (pH 8.1) B) 20 mM Tris-HCl (pH 8.1) containing 0.1 M NaCl
Flow rate:	0.7 mL/min	Gradient:	70-85% B (0-20 min), 85% B (20-25 min)
Temperature:	ambient (25 °C)	Flow rate:	0.5 mL/min
Detection:	UV at 260 nm	Temperature:	35 °C
Injection:	10 µL	Detection:	UV at 260 nm
		Injection:	10 µL



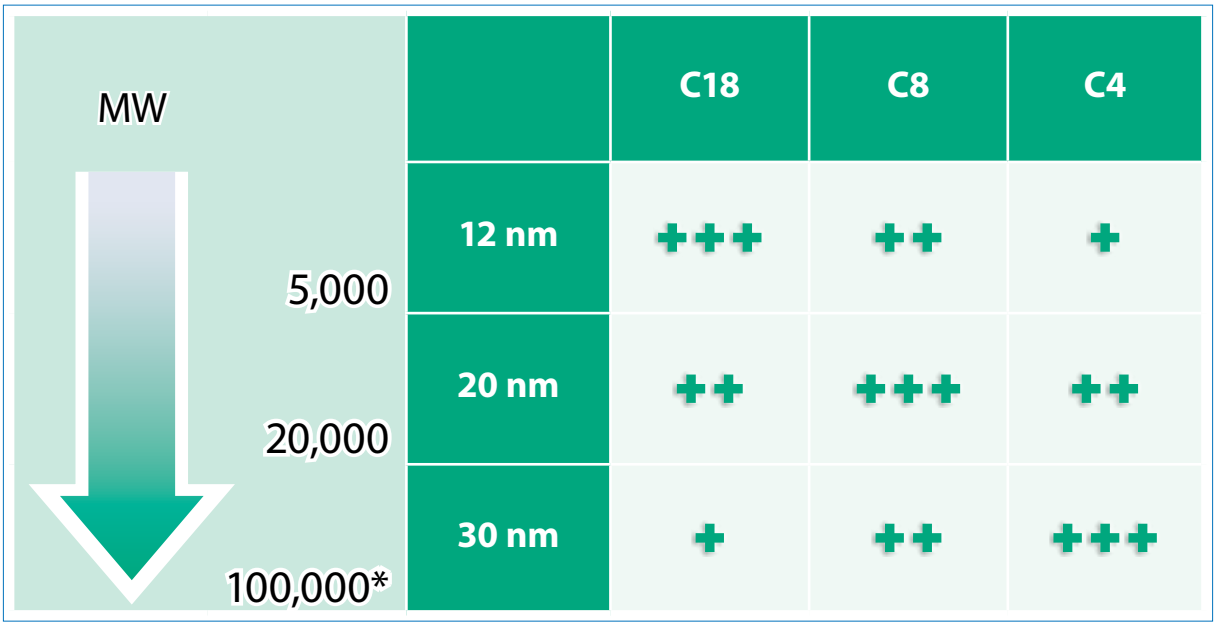

RP



RP – Bioseparation Columns

- **Applicable to proteins, antibodies, peptides and oligonucleotides**
- **Selection of C18, C8 and C4 columns**
- **For UHPLC and HPLC**
- **pH- and temperature stable phases**
- **Superior reproducibility**

Column Selection Tool according to molecular weight



	C18	C8	C4
12 nm	+++	++	+
20 nm	++	+++	++
30 nm	+	++	+++

* up to 150,000 Da possible using e.g. higher temperature

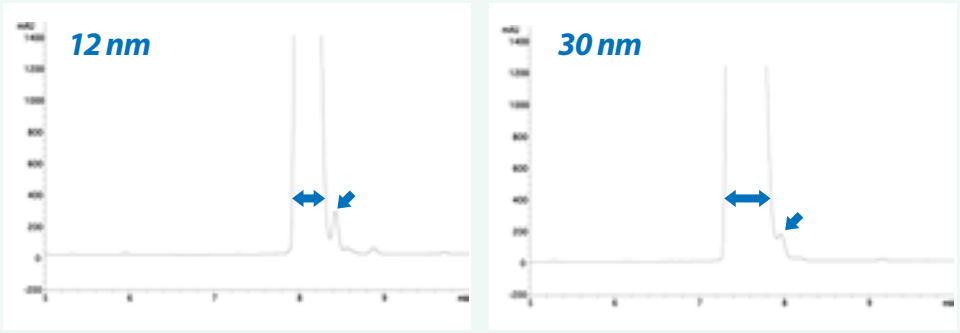
Influence of pore size

As shown in the table (below), the C18 column with 12 nm pore size is suitable for small peptides up to a MW of 5000 Da. The highest efficiency for large peptides or small proteins can be obtained by using a wide pore C8 phase with 20 nm porosity. Most proteins can be eluted efficiently with a wide pore C4 column with 30 nm porosity.

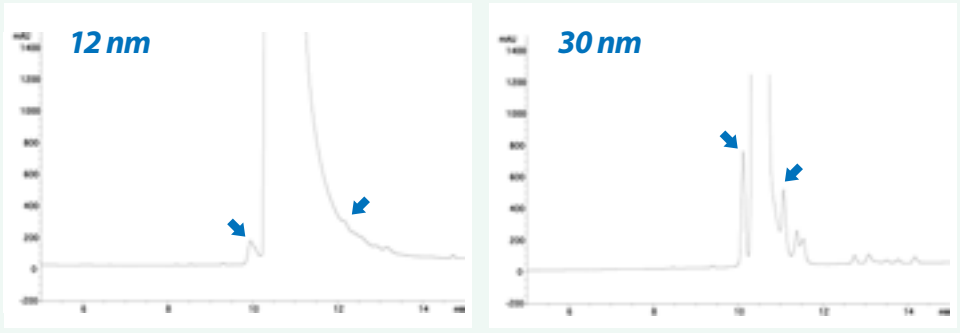
However, the separation may also be influenced by the hydrophobicity of the peptide/protein and the nature of the column's bonded phase. Therefore, for initial method development, it can be useful, in the first instance, to follow the arrow shown in the ***Column Selection Tool*** for method optimisation.

Comparison of peaks on C4 with 12 nm and 30 nm

Angiotensin II
(MW 1,046)



BSA
(MW 67,000)



For smaller peptides a small pore size is more successful. Larger molecules are separated much better with larger pore sizes!

RP – UHPLC / HPLC Selectivities

C4 and C8 selectivities for proteins and peptides and antibodies

	YMC-Triart Bio C4	YMC-Pack C4	YMC-Pack C8	YMC basic
Modification	C4 (USP L26)	C4 (USP L26)	C8 (USP L7)	C8 (USP L7)
Particle Size / µm	1.9, 3, 5	3, 5	3, 5	3, 5
Pore Size / nm	30	30	20	20
pH range	1.0 – 10.0	2.0 – 7.5	2.0 – 7.5	2.0 – 7.5
Temperature range	pH < 7: 90 °C pH > 7: 50 °C	50 °C	50 °C	50 °C

C18 selectivities for peptides

	YMC-Triart C18	YMC-Pack Pro C18	Hydrosphere C18	Meteoric Core C18 (BIO)
Modification	C18 (USP L1)	C18 (USP L1)	C18 (USP L1)	C18 (USP L1)
Particle Size / µm	1.9, 3, 5	2, 3, 5	2, 3, 5	2.7
Pore Size / nm	12	12	12	8 (16)
pH range	1.0 – 12.0	2.0 – 8.0	2.0 – 8.0	1.5 – 10.0
Temperature range	pH < 7: 90 °C pH > 7: 50 °C	50 °C	50 °C	pH < 7: 70 °C pH > 7: 50 °C

C18 and C8 selectivities for oligonucleotides

	YMC-Triart C18	Hydrosphere C18	YMC-Triart C8
Modification	C18 (USP L1)	C18 (USP L1)	C8 (USP L7)
Particle Size / µm	1.9, 3, 5	2, 3, 5	1.9, 3, 5
Pore Size / nm	12	12	12
pH range	1.0 – 12.0	2.0 – 8.0	1.0 – 12.0
Temperature range	pH < 7: 90 °C pH > 7: 50 °C	50 °C	pH < 7: 90 °C pH > 7: 50 °C

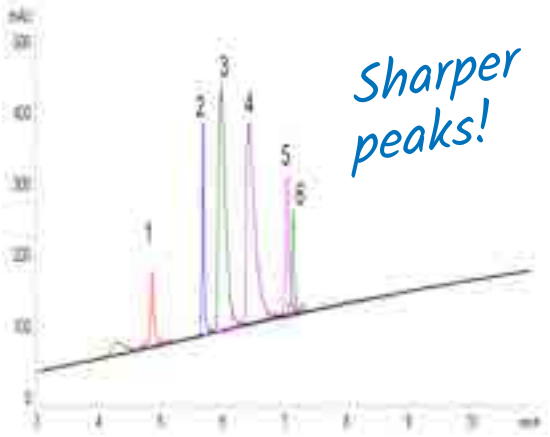
Biocompatible hardware available!

YMC-Triart metal-free columns are available for improved sensitivity and peak shape of coordinating compounds such as nucleotides or oligonucleotides, see page 43.

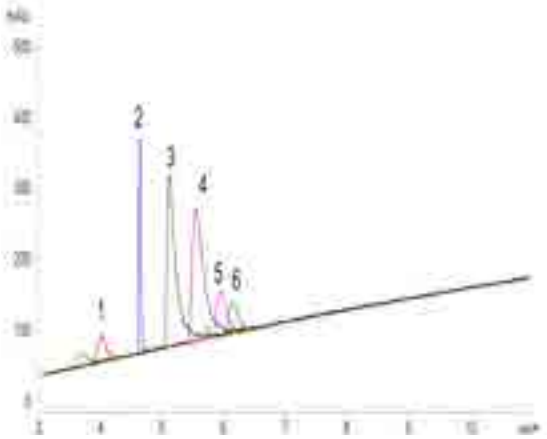
Better performance using YMC-Triart Bio C4

High sensitivity and sharp peaks under LC/MS compatible conditions

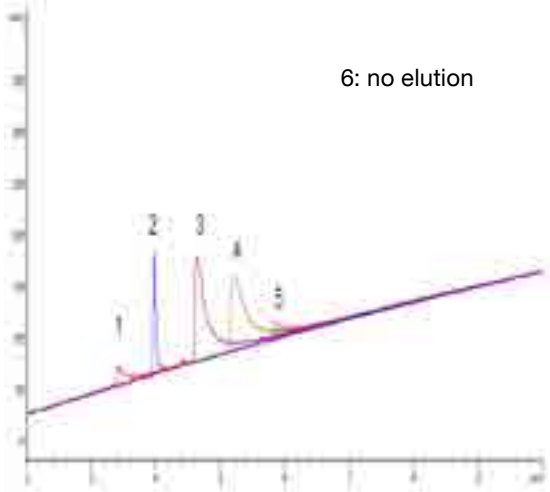
YMC-Triart Bio C4 (3 µm, 30 nm)



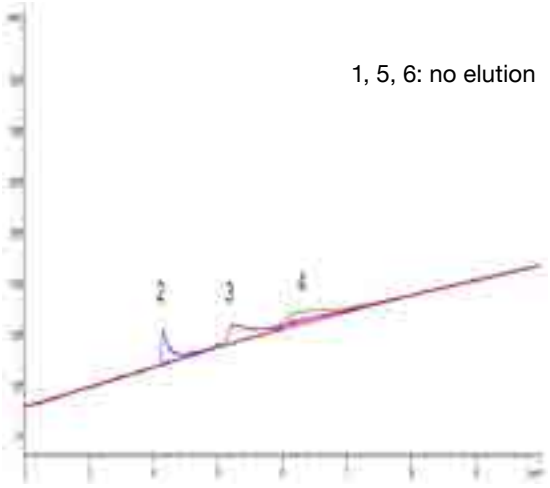
XBridge Protein BEH C4 (3.5 µm, 30 nm)



AdvanceBio RP-mAb C4 (3.5 µm, 45 nm)



Aeris widepore C4 (3.6 µm, 20 nm)



Column: 150 x 3.0 mm ID
Eluent: A) water/formic acid (100/0.1)
B) acetonitrile/formic acid (100/0.1)
Gradient: 10-95%B (0-15 min)
Temperature: 40 °C
Detection: UV at 220 nm

Sample:
1. Cytochrome c (Horse heart)
2. Insulin (Bovine pancreas)
3. Transferrin (Human)
4. BSA
5. β-Lactoglobulin (Bovine)
6. α-Chymotrypsinogen A (Bovine pancreas)

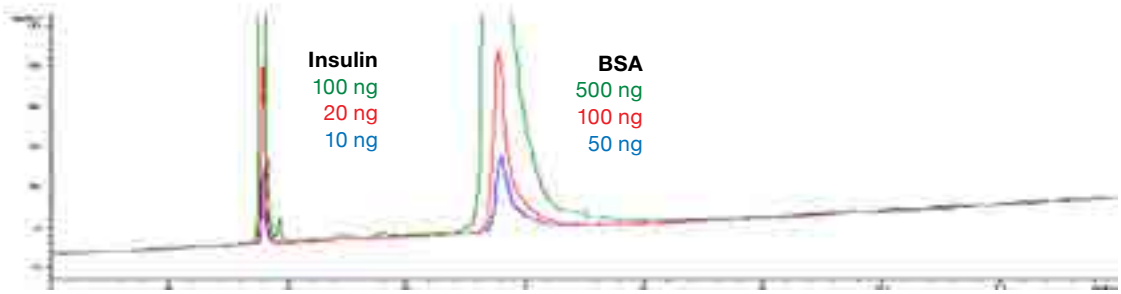
YMC-Triart Bio C4 shows better peak shape and recovery with a mobile phase containing formic acid, which is commonly used for LC/MS analysis. Therefore, YMC-Triart Bio C4 is ideal for high sensitivity analysis of proteins.

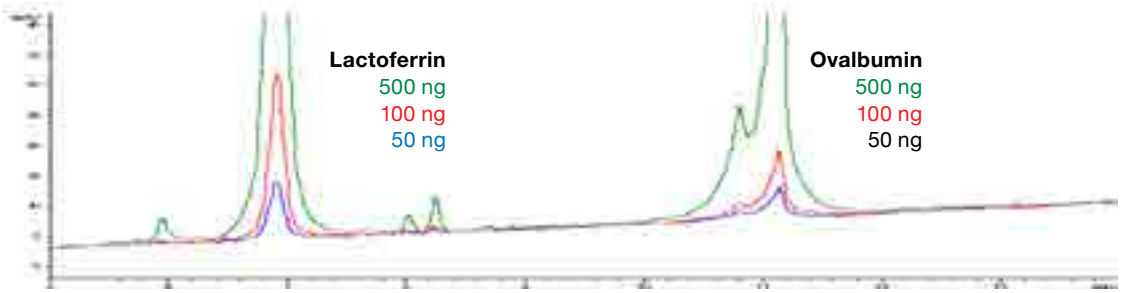
RP – YMC-Triart Bio C4: No column adsorption

No sample adsorption by YMC-Triart Bio C4 columns

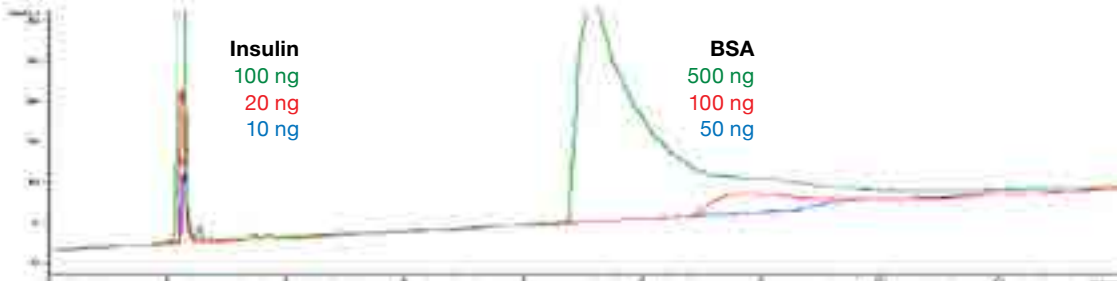
Ideal for Microanalysis

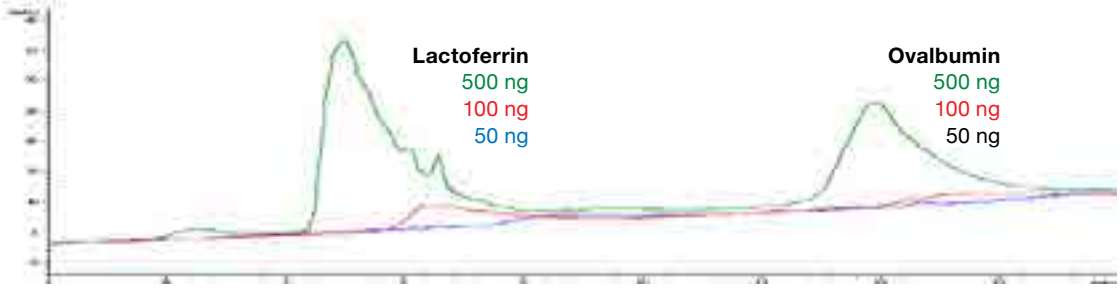
YMC-Triart Bio C4 (1.9 µm, 30 nm)





Aeris widepore C4 (3.6 µm, 20 nm)



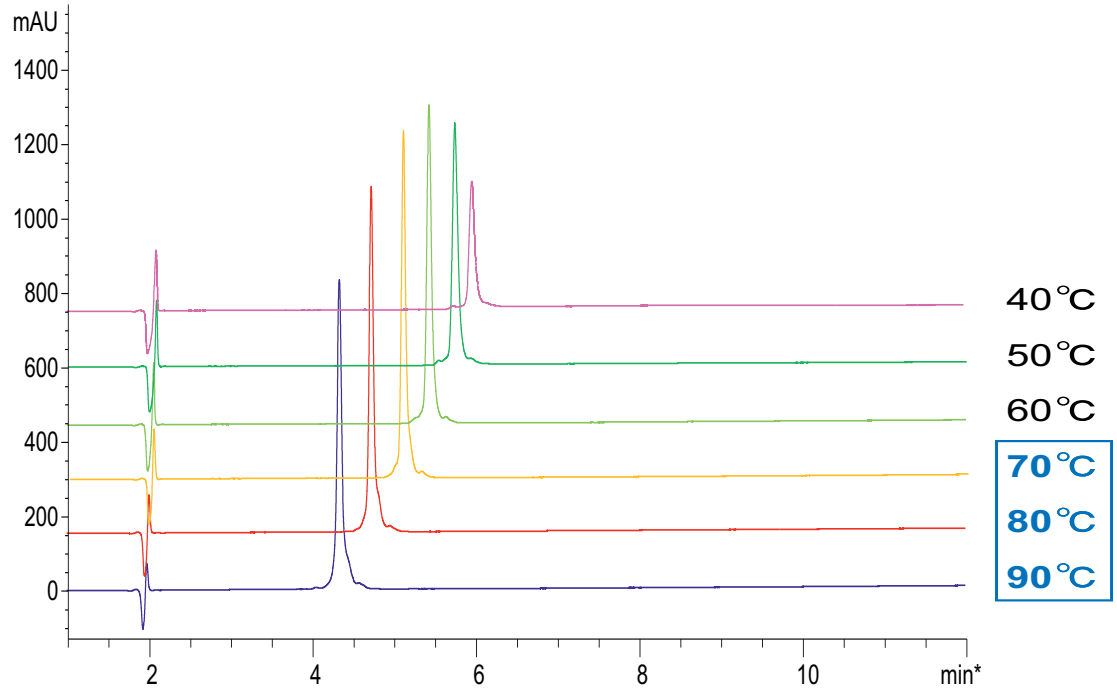


Column:	150 x 2.1 mm ID
Eluent:	A) water/TFA (100/0.05) B) acetonitrile/TFA (100/0.05)
Gradient:	25-60%B (0-15 min), 90%B (15-20 min), 25%B (20-35 min)
Detection:	UV at 220 nm
Temperature:	40 °C

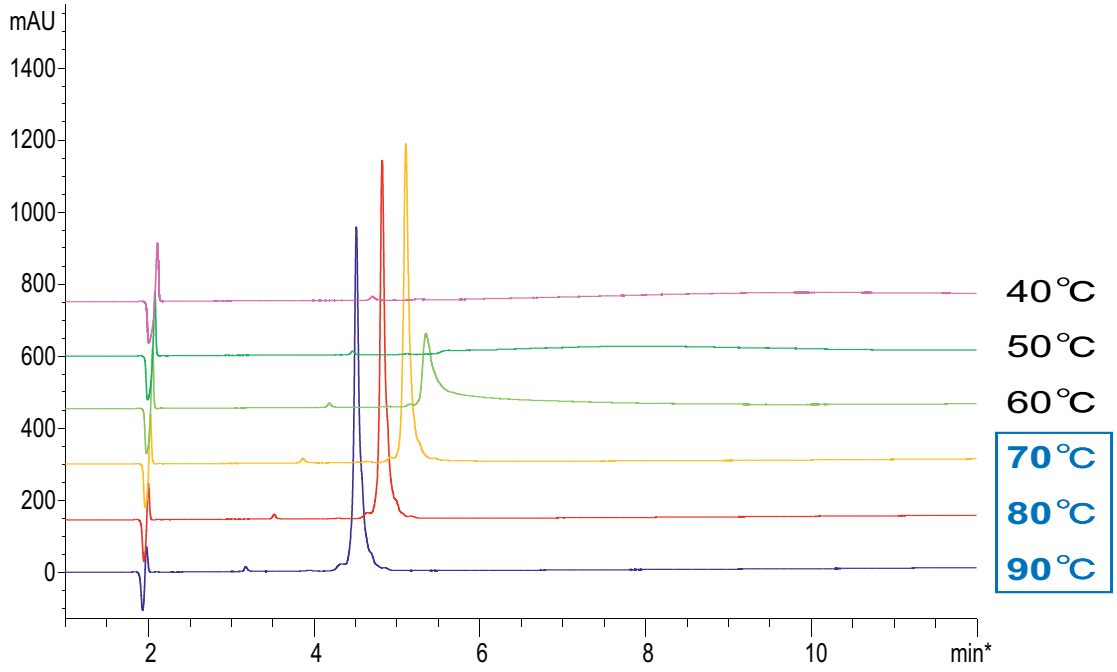
No sample adsorption was observed on YMC-Triart Bio C4 even at a low sample loading. This makes YMC-Triart Bio C4 ideal for microanalysis of proteins.

High temperature tolerance allows antibody analyses

Adalimumab (MW: ca. 148 kDa)



Bevacizumab (MW: ca. 148 kDa)

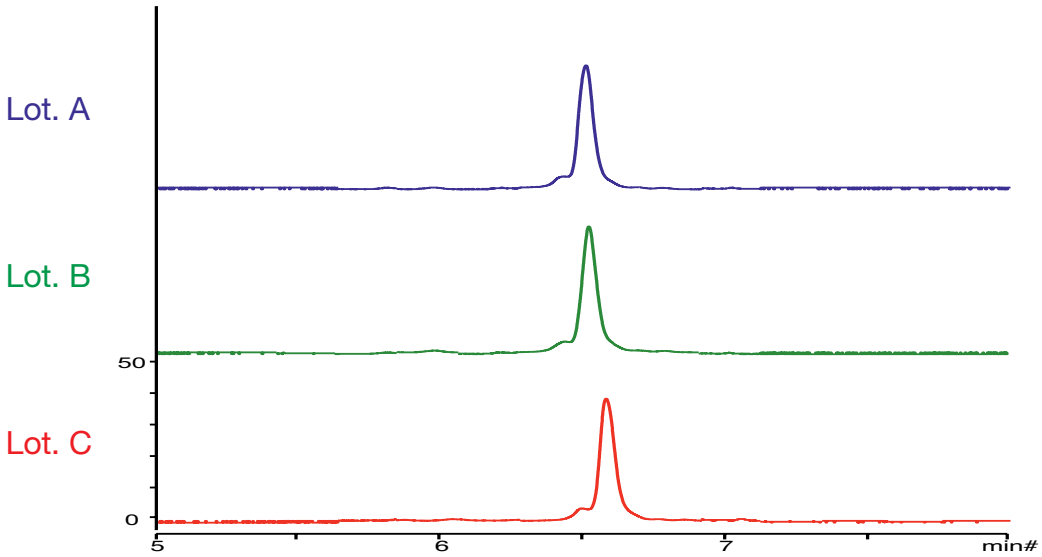


Column:	YMC-Triart Bio C4 (3 µm, 30 nm) 150 x 3.0 mm ID
Part No:	TB30S03-1503PTH
Eluent:	A) water/TFA (100/0.1).
	B) acetonitrile/TFA (100/0.1)
Gradient:	30-60%B (0-15 min), 90%B (15-30min)
Detection:	UV at 220 nm
Flow rate:	0.4 mL/min
Sample:	Adalimumab (0.5 mg/mL) or Bevacizumab (0.5 mg/mL)
Injection:	4 µL

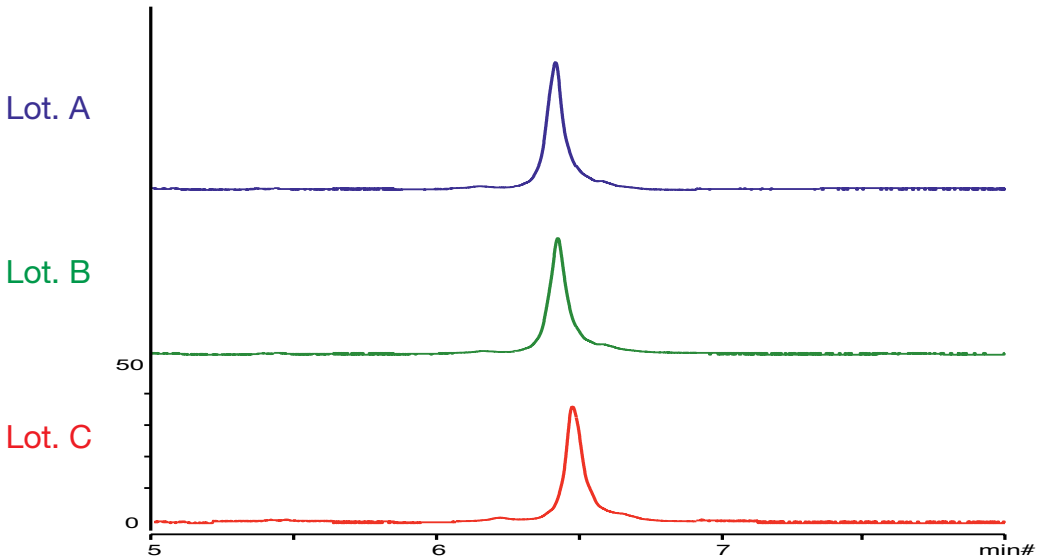
RP – YMC-Triart Bio C4: Reproducibility

Excellent Batch-to-batch reproducibility for antibody analysis

NIST mAb, 8671



Bevacizumab



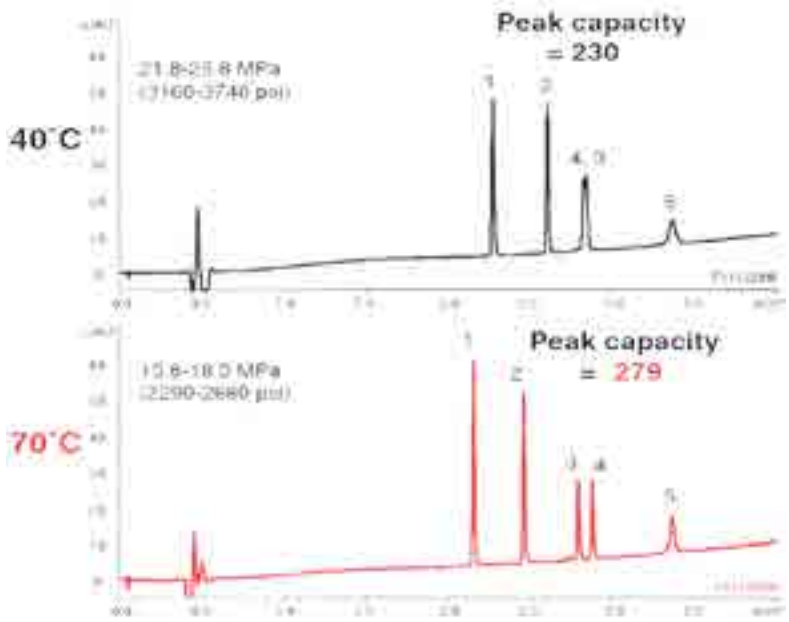
Column:	YMC-Triart Bio C4 (1.9 µm, 30 nm) 50 x 2.1 mm ID
Part No.:	TB30SP9-05Q1PT
Eluent:	A) water/TFA (100/0.1), B) acetonitrile/TFA (100/0.1)
Gradient:	25-45%B (0-10 min)
Detection:	UV at 280 nm
Flow rate:	0.4 mL/min
Temperature:	80 °C
Injection:	2 µL (0.5 mg/mL)

YMC-Triart Bio C4 shows excellent lot-to-lot reproducibility for antibodies. Not only is retention time highly reproducible, but also the resolution of minor impurity peaks. This makes YMC-Triart Bio C4 ideal for quality control of biopharmaceuticals.

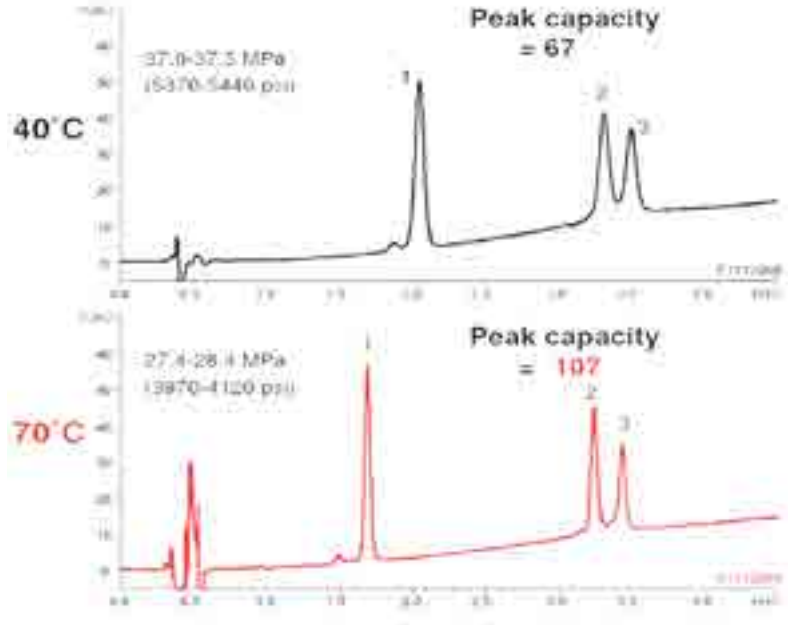
More temperature flexiblity using YMC-Triart

Highly efficient RP-HPLC separation of proteins

Mixture A (MW 500–18,400)



Mixture B (MW 14,300–25,700)



Analytes	MW	Peak width ½h (min)	
		40 °C	70 °C
Mixture 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
Mixture 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

High temperatures only possible with YMC-Triart

Column:	YMC-Triart C18 (1.9 µm, 12 nm) 50 x 2.0 mm ID	Flow rate:	0.4 mL/min
Part-No.:	TA12SP9-0502WT	Detection:	UV at 220 nm
Eluent:	A) water / TFA (100/0.1)	Injection:	1 µL (50 µg/mL) - condition A
	B) acetonitrile / TFA (100/0.1) - mixture A		1 µl (250 µg/mL) - condition B
	B) acetonitrile / 2-propanol / TFA (50/50/0.1) - mixture B	System:	Agilent 1200SL
Gradient:	10-80% B (0-5 min) - mixture A		
	30-60% B (0-5 min) - mixture B		

PC (peak capacity) = 1 + (gradient time / peak width*)
*peak width = $2W_{0.5h}$ average

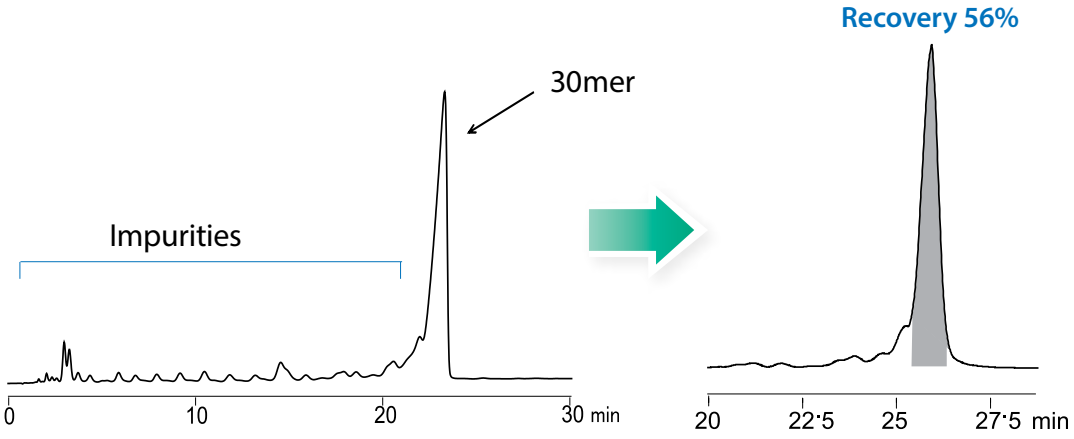
RP – Hydrosphere C18: Oligonucleotide purification

Easy purification of Oligonucleotides with YMC-Actus semi prep columns

Purification of synthetic 30mer oligonucleotide

Analysis 1.0 mL/min, 5 µL injection
Hydrosphere C18
50 x 4.6 mm ID, 5 µm

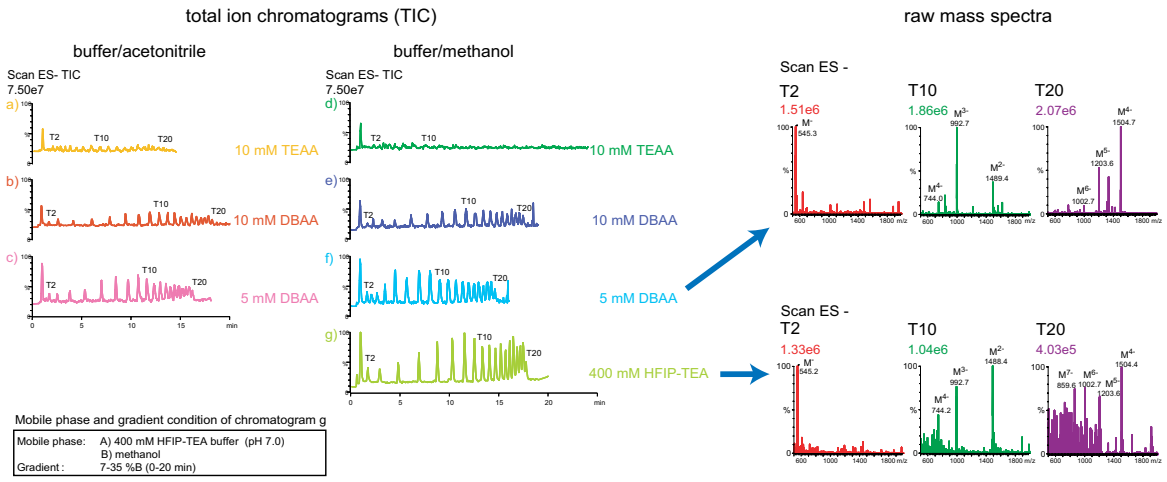
Purification 19 mL/min, 100 µL injection
YMC-Actus Hydrosphere C18
50 x 20 mm ID, 5 µm



Part Nos.:	HS12S05-0546WT HS12S05-0520WX
Eluent:	A) 10 mM DBA-acetic acid (pH 6.0) / methanol (60/40) B) 10 mM DBA-acetic acid (pH 6.0) / methanol (20/80)
Gradient:	10%-35% B (0-30 min.)
Temperature:	ambient
Detection:	UV at 269 nm
Sample:	synthetic oligonucleotide (100 µM)

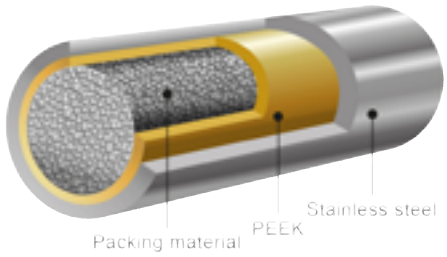
purity>99%

Influences of mobile phase conditions on intensity of ESI-MS



Column:	Hydrosphere C18 (3 µm) 50 x 2.0 mm	
Part No.:	HS12S03-0502WT	
Flow rate:	0.2 mL/min	
Temperature:	35 °C	
Detection:	ESI negative mode	
Injection:	5 µL (25 pmol/component)	
a)	Eluent:	A) 10 mM TEAA buffer (pH 6.0) B) eluent A/acetonitrile (80/20)
	Gradient:	50-65% B (0-20 min)
b/c)	Eluent:	A) 10/5 mM DBAA buffer (pH 6.0) B) eluent A/acetonitrile (50/50)
	Gradient:	30-75% B (0-20 min)
d)	Eluent:	A) 10 mM TEAA buffer (pH 6.0) B) eluent A/methanol (50/50)
	Gradient:	44-50% B (0-25 min)
e/f)	Eluent:	A) 10/5 mM DBAA buffer (pH 6.0) B) eluent A/methanol (20/80)
	Gradient:	42-70% B (0-20 min)
g)	Eluent:	A) 400 mM HFIP-TEA buffer (pH 7.0) B) methanol
	Gradient:	7-35% B (0-20 min)
Sample:	Oligodeoxythymidylic acid $[d(pT)_{2\text{-}20}]$	

Metal-free column hardware ideal for oligonucleotide analysis

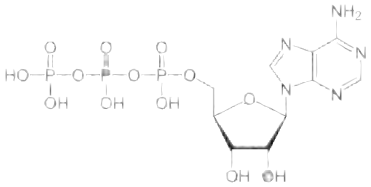


Specifications	
YMC-Triart Phases	C18, C8, Bio C4
Particle Size	1.9, 3, 5 µm
Inner layer	PEEK
Outer layer	Stainless steel
Frit	PEEK
Pressure limit	1.9 µm: 100 MPa (15,000 psi) 3/5 µm: 45 MPa (6,525 psi)

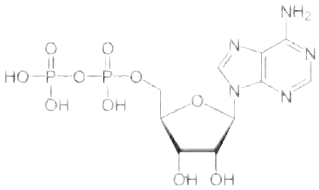
Special column connectors required.
See ordering information recommendations.

Improved sensitivity for coordination compounds

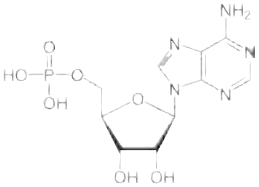
1. ATP

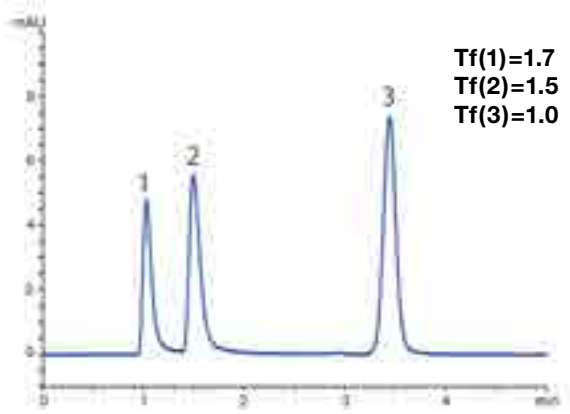


2. ADP

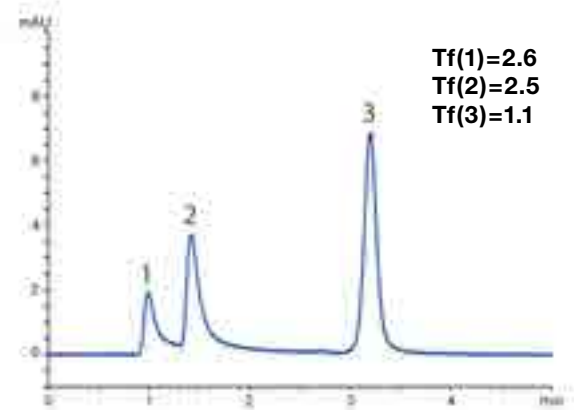


3. AMP





Tf(1)=1.7
Tf(2)=1.5
Tf(3)=1.0



Tf(1)=2.6
Tf(2)=2.5
Tf(3)=1.1

Column:	YMC-Triart C18 (3 µm) 50 x 2.1 mm ID
Part Nos.:	TA12S03-05Q1PTP (metal-free) or TA12S03-05Q1PTH (regular hardware)
Eluent:	5 mM $HCOONH_4$
Flow rate:	0.21 mL/min
Temperature:	25 °C
Detection:	UV at 265 nm
Injection:	1 mL (10 mg/mL)
System:	bioinert/"non-metal" HPLC system

Metal coordinating compounds, which have a phosphate group in their structure, tend to show poor peak shape due to interactions with metals, such as the stainless steel in column bodies and frits. By using the metal-free column hardware, better peak shapes can be expected. Nucleotides with phosphate groups show better peak shapes when compared to the regular column hardware. The metal-free column hardware is very suitable for highly sensitive analyses using LC/MS.

RP – Expert Tips: Proteins/Peptides

Optimisation of peptides and proteins separation by reversed-phase

Factors to be considered for method optimisation of peptides and proteins

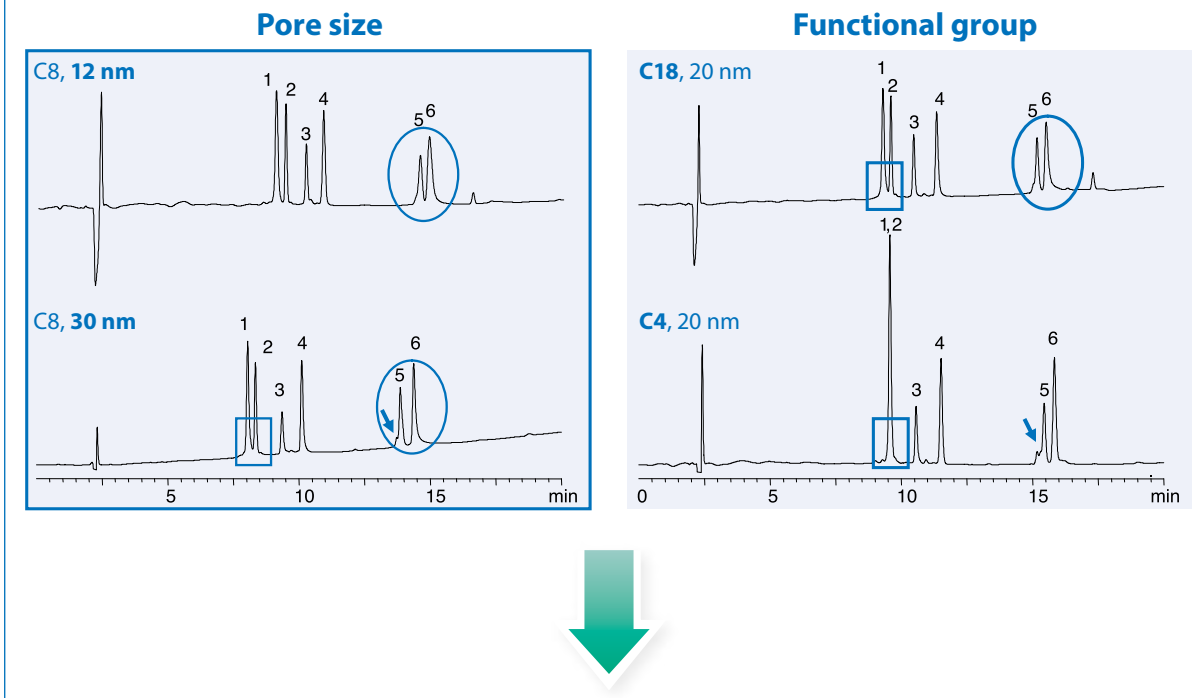
Column	**Combination of functional group and pore diameter** ▶ Choose an optimal combination for the molecular weight and hydrophobicity of peptides and proteins. Generally, a column with large pore size and low surface hydrophobicity is suitable for large molecules
Mobile phase	**Gradient elution with 0.1% TFA/acetonitrile system as first choice** ▶ Change 1) concentration of TFA, 2) acid species and/or pH if a sample is mixture of compounds with various ionic characteristics. ▶ Adjust the gradient conditions, 2-propanol might be effective for separation of large proteins
Temperature	**Effective for changing separation selectivity or improving peak shape.** However, usable temperature range is limited by column stability. (strongly acidic conditions and heating will accelerate the loss of functional groups resulting in shorter retention times and/or increase in unfavourable secondary interactions between the packing material and sample)

Comparison of separation on columns with different pore sizes and functional groups

The separation characteristics of proteins and peptides with molecular weights of 4,300–17,000 are compared using columns with different pore sizes and functional groups. The figure below shows that the most suitable column for compounds with a molecular weight within this range is C8, 20 nm.

If either the pore size or functional group of the packing material is not optimised, peak broadening and poor resolution are observed.

By using the most suitable column (C8, 20 nm) for the target compounds, sharp peak shapes and excellent separation are achieved.



Columns: all 5 µm, 150 x 4.6 mm ID
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

1. Cytochrome c (MW 12,400)
2. Insulin (Bovine) (MW 5,700)
3. Amyloid β-protein (MW 4,300)
4. Lysozyme (MW 14,300)
5. α-Lactalbumin (MW 14,100)
6. Myoglobin (MW 17,000)

RP – Expert Tips: Proteins/Peptides

Effect of column temperature and mobile phase composition (Optimisation of antimicrobial peptides separation)

Structure (antimicrobial peptides)

1. α-Defensin-1: MW 3,442	**A**	**CYCRIPACIAGERRYGTCIYQGRLWAFCC**
2. α-Defensin-2: MW 3,371		**CYCRIPACIAGERRYGTCIYQGRLWAFCC**
3. α-Defensin-3: MW 3,486	**D**	**CYCRIPACIAGERRYGTCIYQGRLWAFCC**

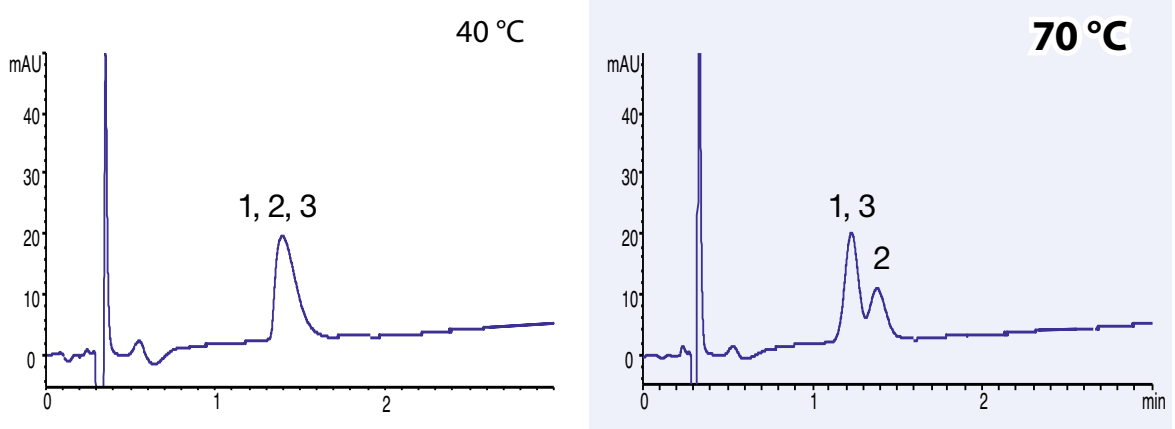
* Difference in amino acid residue on N-terminal

Common HPLC conditions

Column: **YMC-Triart C18** (1.9 µm, 12 nm) 50 x 2.0 mm ID
Part No.: TA12SP9-0502PT
Flow rate: 0.4 mL/min
Detection: 220 nm

1 Effect of column temperature

Changing the column temperature will provide selectivity changes as well as peak shape improvement. The high stability column, YMC-Triart, can offer a wider usable temperature range. Temperature can be used as a tool for method optimisation.

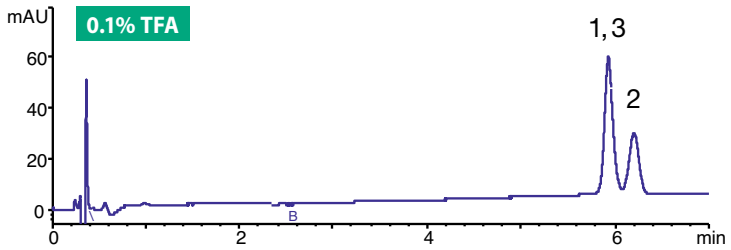


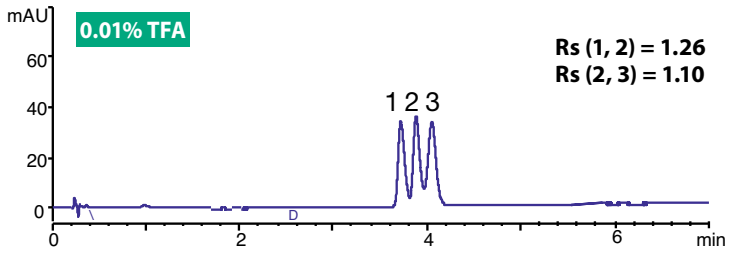
Eluent: A) water/TFA (100/0.1)
B) acetonitrile/TFA (100/0.1)
25-45%B (0-5 min)

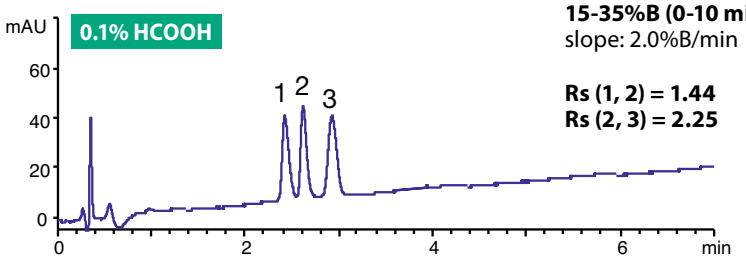
2 Effect of acid type, acid concentration and gradient conditions (at 70°C)

Selectivity changes can be expected when changing acid type and/or concentration. It is useful when there is a large difference in ionic characteristics of compounds.

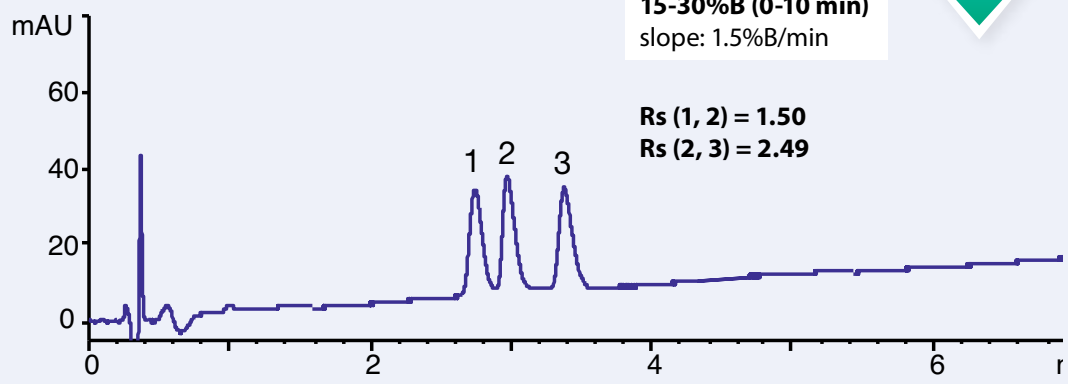
Acid type/Acid concentration







Gradient slope



Eluent: A) acid-containing aqueous solution
B) acid-containing acetonitrile solution
(0.1% HCOOH in B solution is 0.08%)

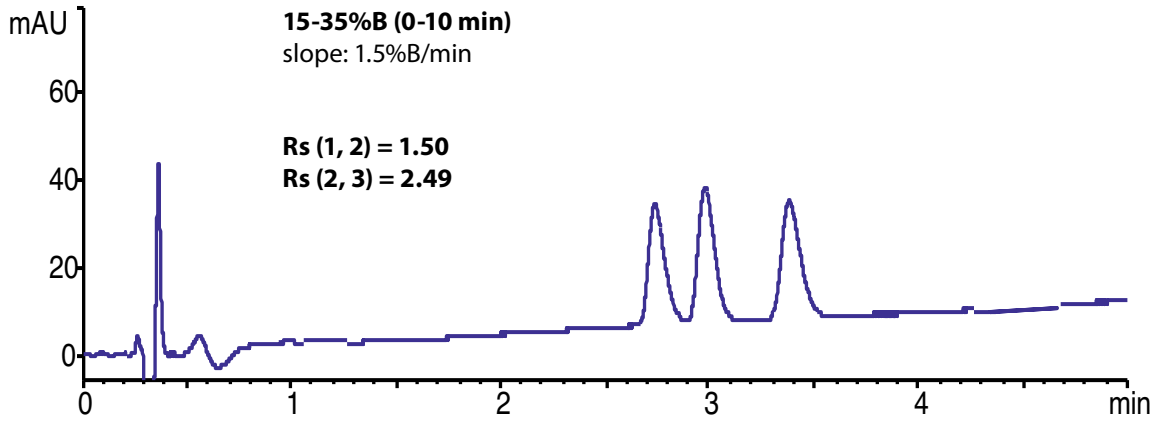
Temperature: 70 °C

RP – Expert Tips: Proteins/Peptides

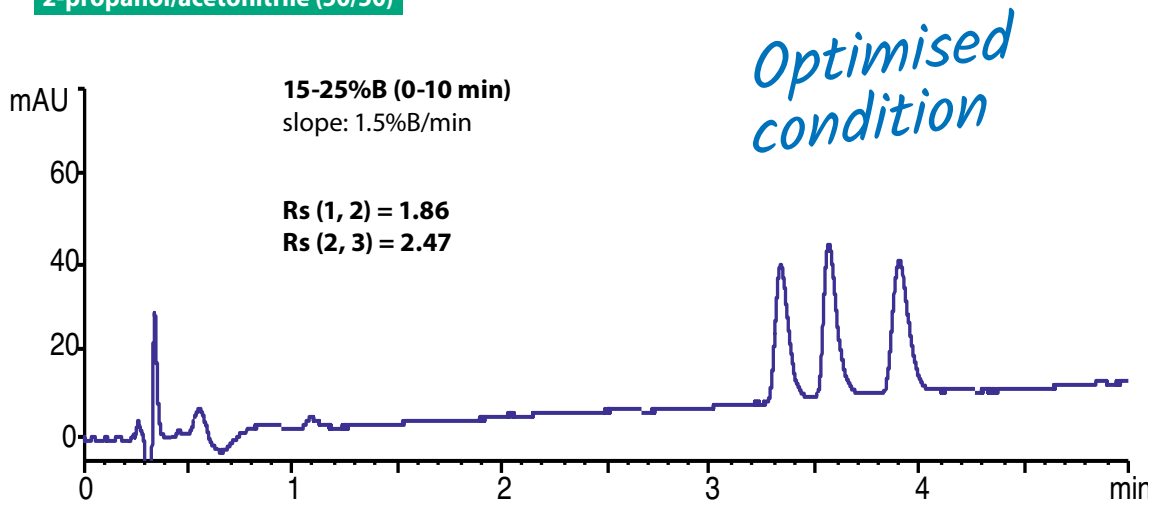
3 Addition of 2-propanol in a mobile phase

When 2-propanol is added in the mobile phase and the gradient slope is optimised resolution between peaks 1 and 2 is improved while the analysis time remains the same.

acetonitrile



2-propanol/acetonitrile (50/50)

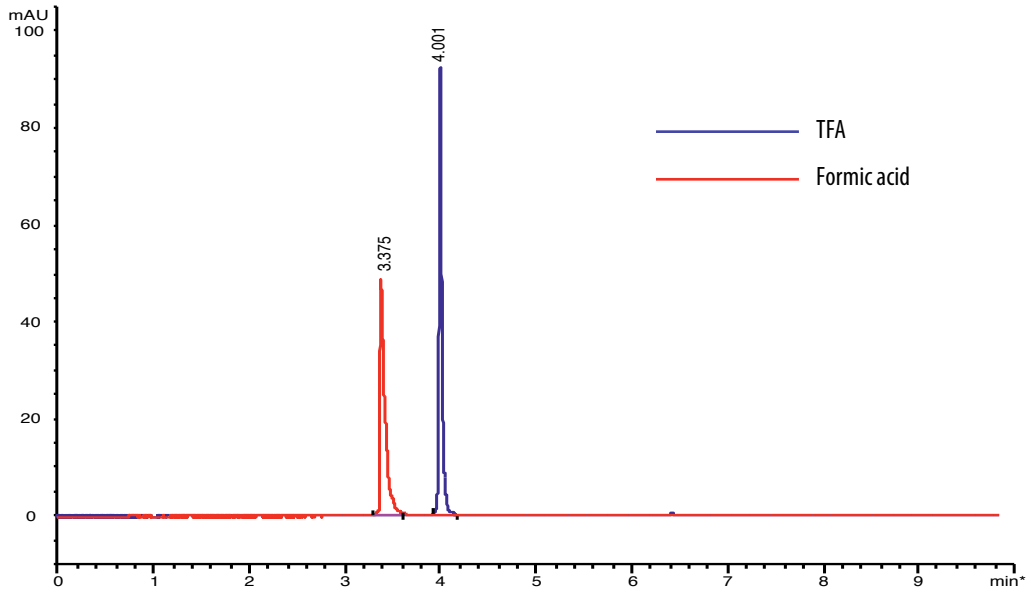


Eluent:	A) 0.1% formic acid in water
	B) 0.08% formic acid in organic solvent
Temperature:	70 °C

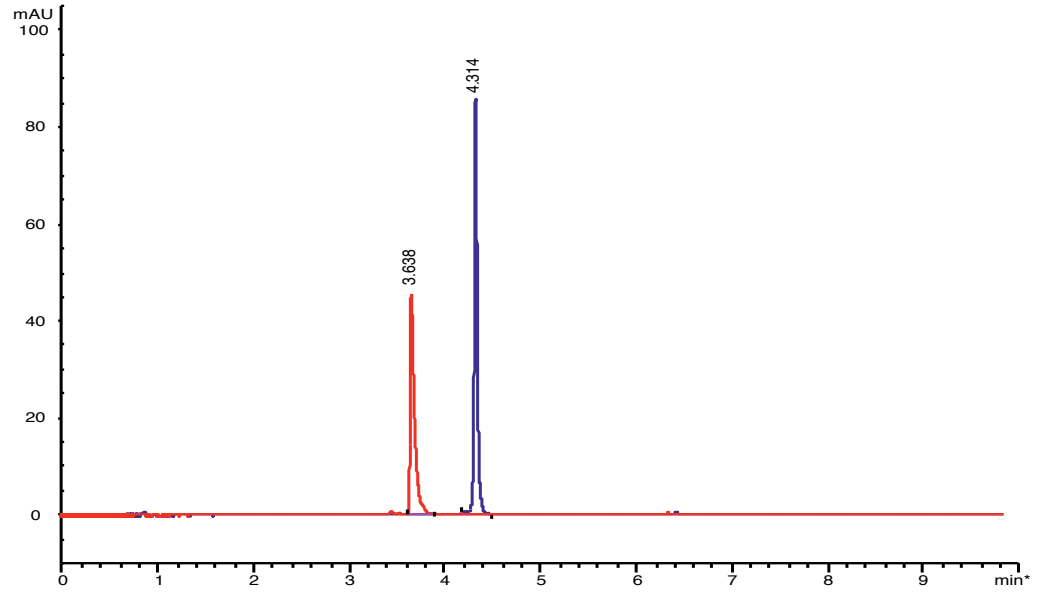
Use of MS compatible conditions for antibody analysis by RP

Although the best peak shapes and greater sensitivity can be provided by mobile phases containing TFA, suitable peaks can also be obtained with mobile phases containing formic acid – especially in combination with YMC-Triart Bio C4.

Adalimumab



NIST Mab

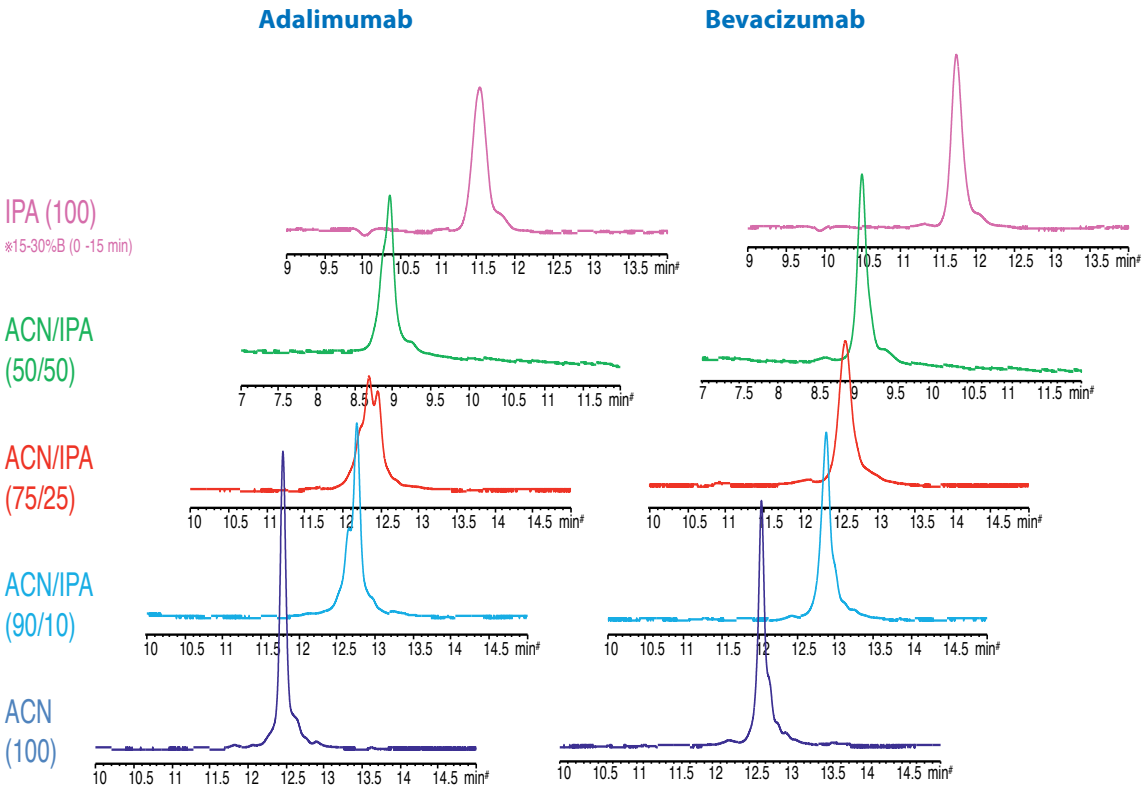


Column:	YMC-Triart Bio C4 (1.9 µm, 30 nm) 150 x 2.1 mm ID
Part No:	TB30SP9-15Q1PT
Eluent:	A) water/TFA or Formic acid (100/0.1)
	B) acetonitrile/TFA or Formic acid (100/0.1)
Gradient:	10-95%B (0-10 min)
Detection:	UV at 280 nm (0.13s, 40Hz)
Flow rate:	0.4 mL/min
Temperature:	80 °C
Sample conc.:	0.5 mg/mL
Injection:	2 µL

RP – Expert Tips: Antibodies

Influence of organic solvents on antibody analysis

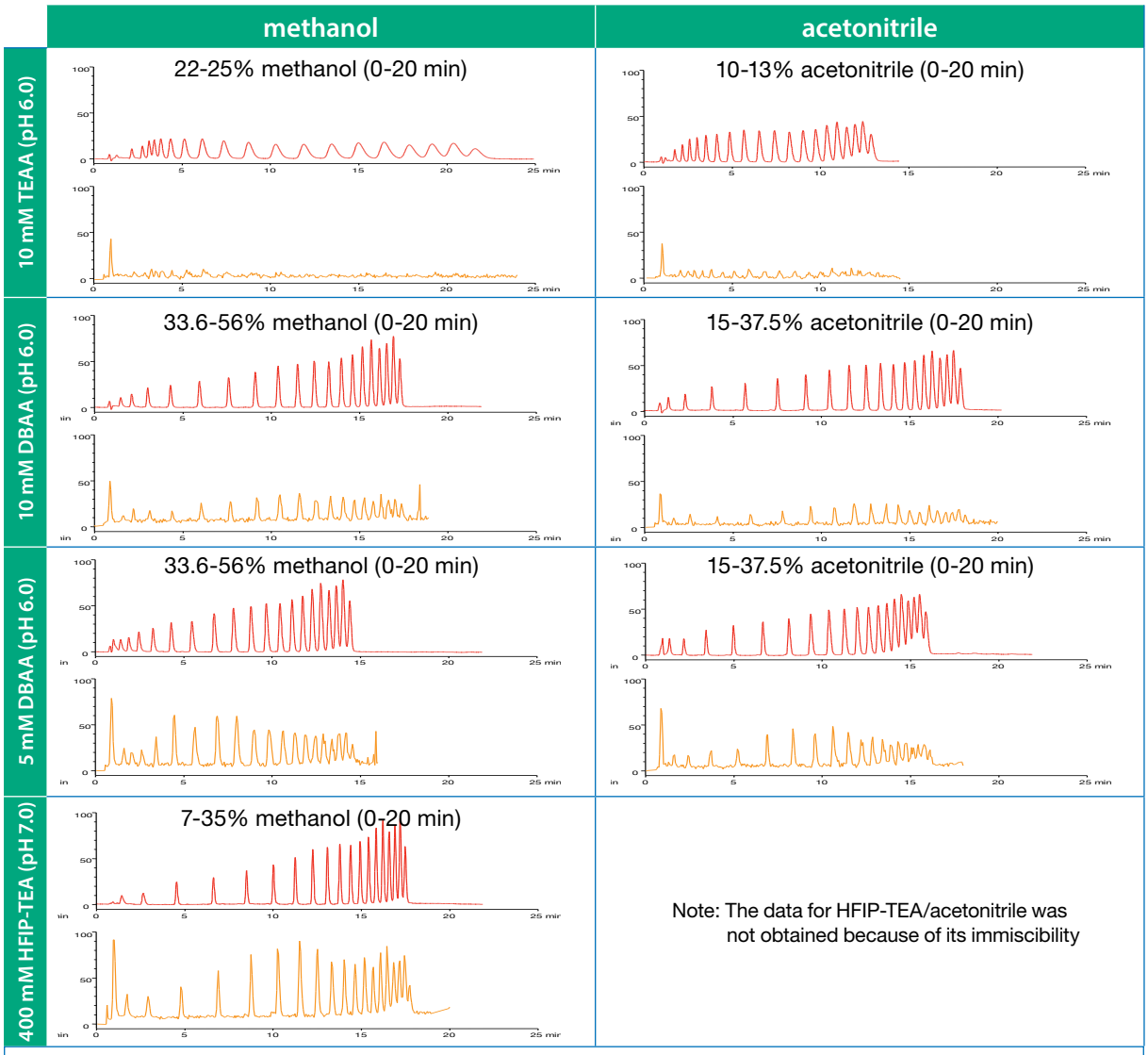
The separation behaviour can be influenced by a large amount by an organic solvent in the mobile phase.Using the optimum organic solvent is beneficial for Mab characterisation.



Column:	YMC-Triart Bio C4 (3 µm, 30 nm) 150 x 3.0 mm ID
Part No:	TB30S03-1503PTH
Eluent:	A) water/TFA (100/0.1).
	B) organic solvent/TFA (100/0.1)
Gradient:	25-40%B (0-15 min), 90%B (15-30 min)
Detection:	UV at 220 nm
Flow rate:	0.4 mL/min
Temperature:	80 °C
Sample:	Adalimumab (0.5 mg/mL) or Bevacizumab (0.5 mg/mL)
Injection:	4 µL

Effect of composition and salt concentration of ion-pairing mobile phase on the separation and signal intensity

Comparison of separation and ESI-MS signal intensity using different ion-pairing buffers and organic solvents



Column:	Hydrosphere C18 (3.0 µm, 12 nm) 50 x 2.0 mm ID
Part No:	HS12S03-0502WT
Flow rate:	0.2 ml/min
Detection:	UV at 269 nm (red), ESI negative mode (orange)
Temperature:	35 °C
Sample:	Oligo(deoxythymidylic acid) [dp (dT) 2-20]
Injection:	5 µl (25 pmol/ml)

eluent abbreviations

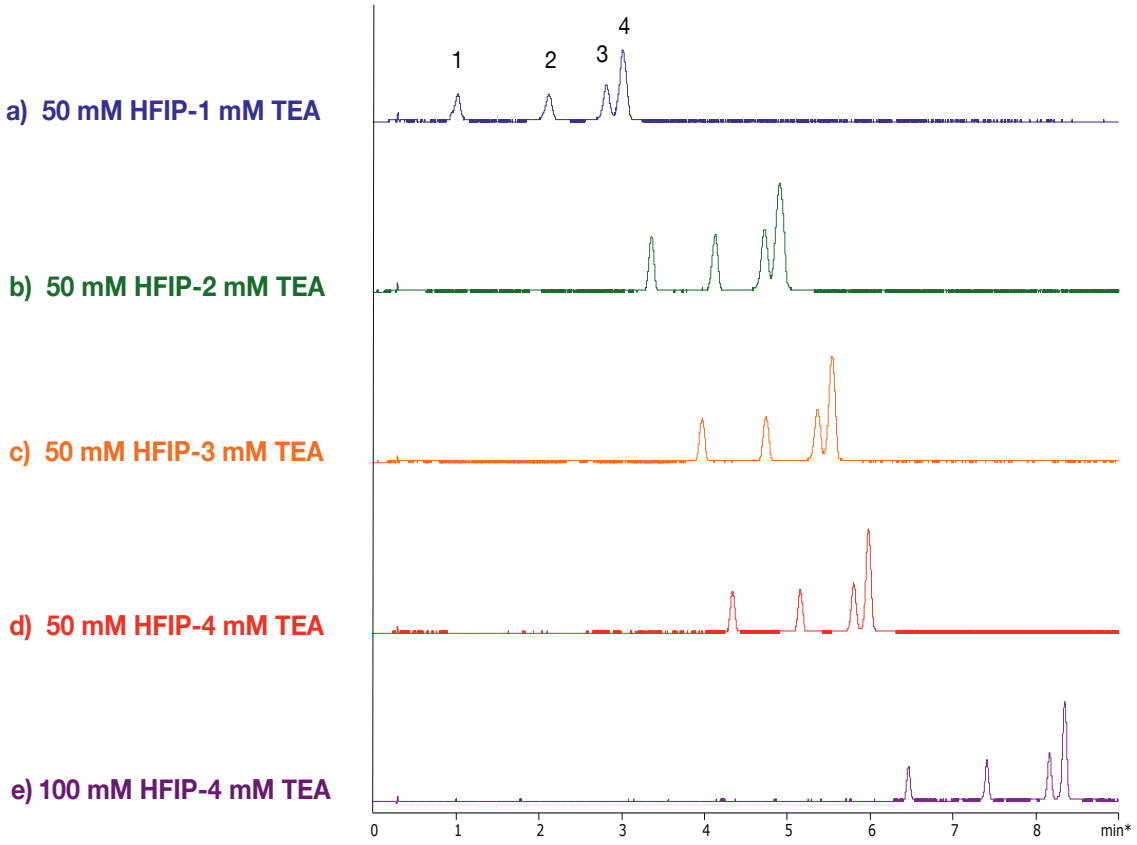
TEAA:	triethylammonium acetate
DBAA:	di-n-butylammonium acetate
HFIP:	1,1,1,3,3,3-hexafluoro-2-propanol
TEA:	triethylamine

The mobile phase composition has different effects on the separation and signal intensity in electrospray ionisation mass spectrometry (ESI-MS) of oligonucleotides. Using different gradient conditions, acceptable retention and resolution can be achieved (upper UV chromatograms; red trace) for each separation by optimising the gradient slope of the organic solvent regardless of the type of mobile phase. The ESI-MS intensity is significantly influenced by the type and concentration of ion-pairing buffer as shown in the lower MS chromatograms (orange trace). HFIP-TEA buffer/methanol systems provide the maximum MS intensity. Enhanced retention and MS intensity are obtained using 10 mM DBAA buffer compared to 10 mM TEAA buffer, and the lower DBAA concentration results in approximately 1.5–3 times increase in the intensity without any change in the concentration of organic solvent.

RP – Expert Tips: Oligonucleotides

Salt concentration in HFIP-TEA buffer

RNA Sample
rCrArCrUrGrArArUrArCrCrArArU (14mer)
rUrCrArCrArCrUrGrArArUrArCrCrArArU (17mer)
rUrCrArUrCrArCrArCrUrGrArArUrArCrCrArArU (20mer)
rGrUrCrArUrCrArCrArCrUrGrArArUrArCrCrArArU (21mer)

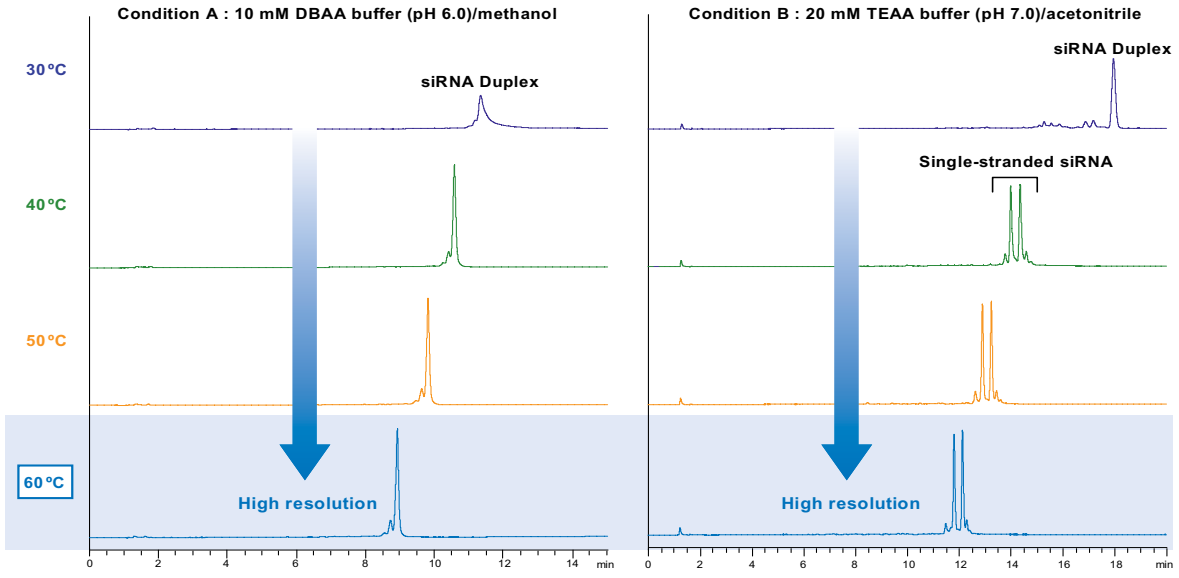


Column:	YMC-Triart C18 (1.9 µm, 12 nm) 50 x 2.1 mm ID
Part No:	TA12SP9-05Q1PT
Eluent:	A) shown in figure
	B) methanol
Gradient:	5-15%B (0-10 min)
Flow rate:	0.42 ml/min
Detection:	UV at 260 nm
Temperature:	65 °C
Injection:	1.0 µl (2-4 nmol/ml)

Oligonucleotide separations are influenced by the use of mobile phases consisting of different ratios of HFIP and TEA. The retention time is approximately doubled when the TEA concentration is increased from 1 to 4 mM at a constant 50 mM HFIP concentration (chromatograms a – d), and increased approximately 1.5 times when the HFIP concentration is increased from 50 mM to 100 mM at a constant 4 mM TEA concentration (chromatograms d and e).

These results indicate that small changes in concentration of basic ion-pairing reagents such as TEA and DBA provide a great impact on oligonucleotide separations.

Effect of mobile phase and column temperature on the separation of siRNA duplex



Crude synthetic siRNA duplex (19 bp): 5′-CGU ACG CGG AAU ACU UCG AdTdT-3′
3′-dTdTGCA UGC GCC UUA UGA AGC U-5′

Column:	YMC-Triart C18 (1.9 µm, 12 nm) 100 x 2.0 mm ID	**Condition A** Eluent:	A) 10 mM di-n-butylamine-acetic acid (pH 6.0)
Part No.:	TA12SP9-1002PT		B) methanol
Flow rate:	0.2 mL/min	Gradient:	35-60%B (0-15 min)
Detection:	UV at 269 nm	**Condition B** Eluent:	A) 20 mM triethylamine-acetic acid (pH 7.0)
Injection:	1 µL (5 nmol/mL)		B) acetonitrile
System:	Agilent 1290	Gradient:	5-12%B (0-20 min)

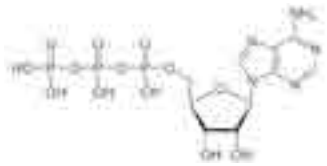
Using different mobile phase conditions, peak shape and resolution between intermediate peaks is improved by increasing the column temperature. As a result of enhanced dispersion and distribution velocity when increasing column temperature, biomolecules such as RNA and DNA generally exhibit sharper peak shapes and improved resolution.
Under condition B at 40°C or above, two peaks of single-stranded RNA, generated by the denaturation of the siRNA duplex, are observed. This HPLC technique using high temperature to generate single-stranded RNA is called "Denaturing HPLC", and is widely used in the field of gene mutation analysis.

Denaturation of duplex DNA or RNA is also influenced by ionic strength (type and concentration), pH and polarity. It is recommended that these analysis conditions (temperature and mobile phase) should be optimised depending on characteristics of the target analyte and the purpose of the analysis.

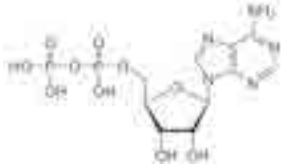
RP – Expert Tips: (Oligo)nucleotides

Influence of system and column hardware on the analysis of nucleotides

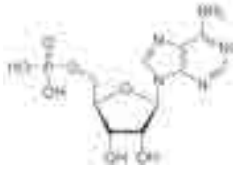
1 ATP



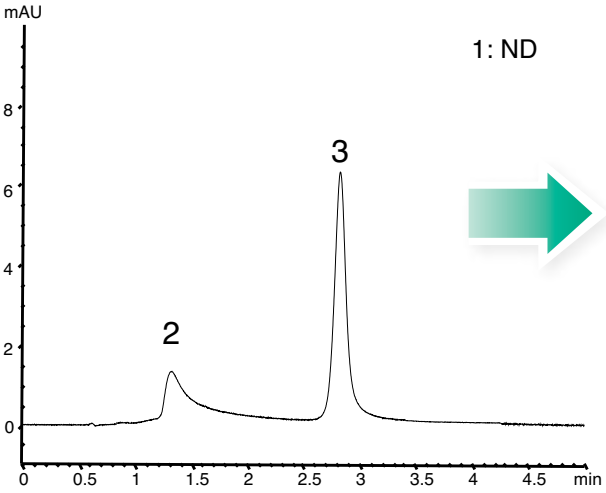
2 ADP



3 AMP

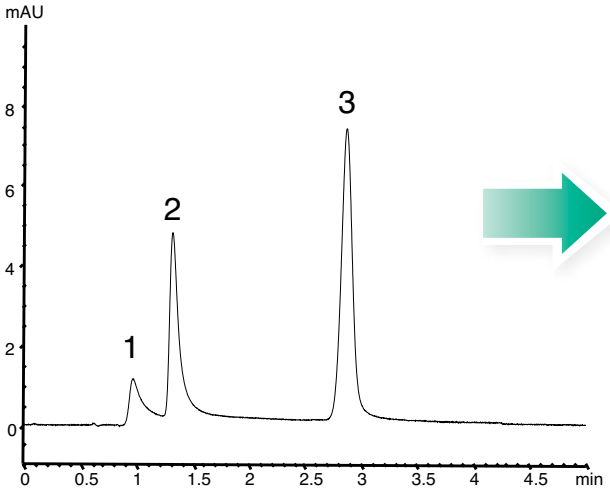


Ordinary HPLC system with standard column



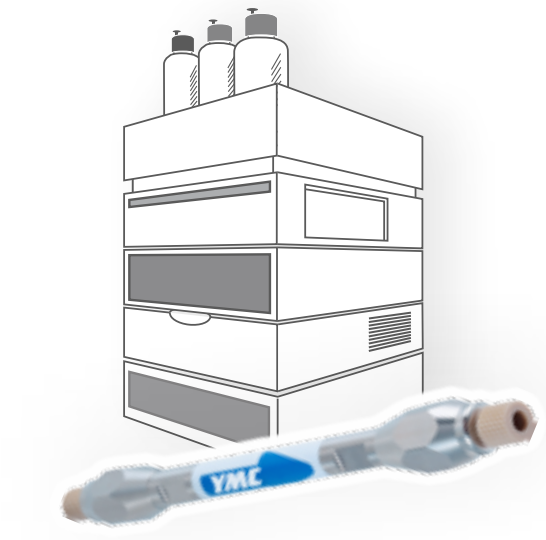
1: ND

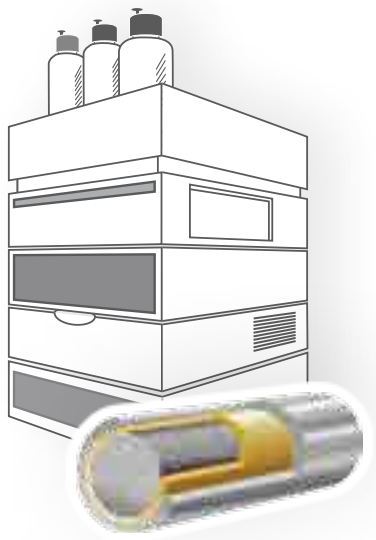
Ordinary HPLC system with metal-free column



Column:	YMC-Triart C18 (3 µm, 12 nm) 50 x 2.1 mm ID
Part Nos:	TA12S03-05Q1PT (standard hardware)
	TA12S03-05Q1PTP (metal-free hardware)
Eluent:	5 mM $HCOONH_4$
Flow rate:	0.21 mL/min
Temperature:	25 °C
Detection:	UV at 265 nm
Injection:	1 µL (10 µg/mL)

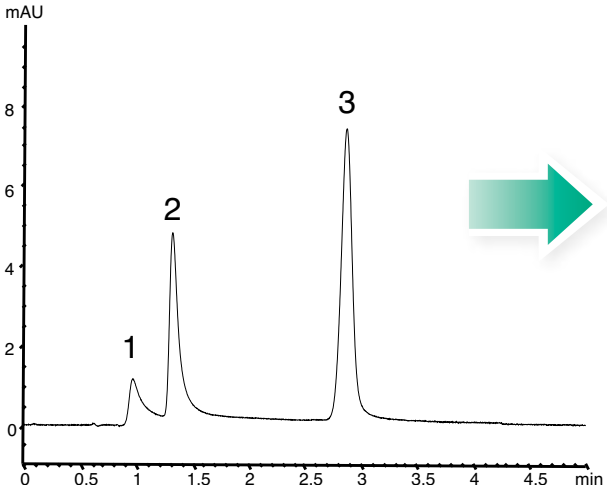
*"Non-metal" HPLC system: PEEK sample loop, PEEK injector port, and PEEK tubing are used.



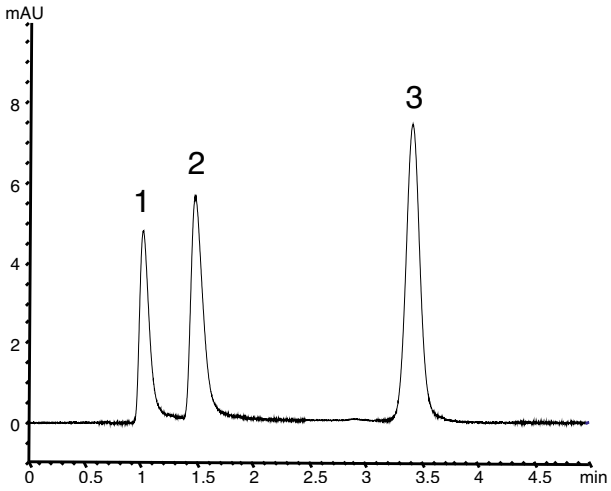


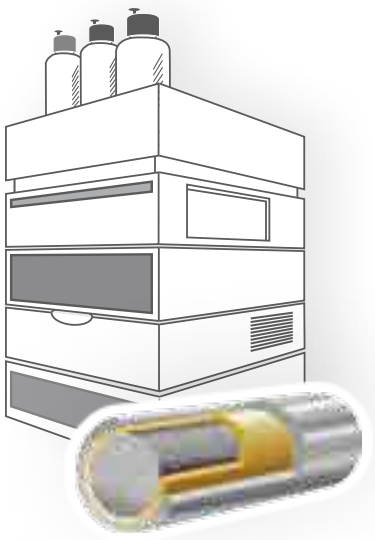
ATP peak is detected, and peak shape of ADP is improved as a result of using the metal-free column.

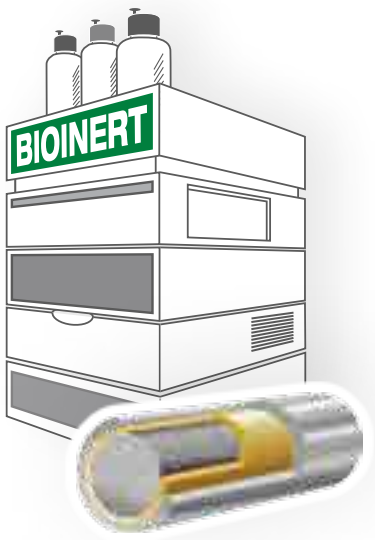
Ordinary HPLC system with metal-free column



"Non-metal" HPLC system* with metal-free column







Peak shape is greatly improved as a result of using "non-metal" HPLC system

RP – Ordering information

1.9 µm UHPLC columns

Phase	Column ID [mm]	Column length [mm]					Guard cartridges* with 5 mm length
		30	50	75	100	150	(pack of 3)
YMC-Triart C18	2.0	TA12SP9-0302PT	TA12SP9-0502PT	TA12SP9-L502PT	TA12SP9-1002PT	TA12SP9-1502PT	TA12SP9-E5Q1CC**
	2.1	TA12SP9-03Q1PT	TA12SP9-05Q1PT	TA12SP9-L5Q1PT	TA12SP9-10Q1PT	TA12SP9-15Q1PT	TA12SP9-E5Q1CC**
	3.0	—	TA12SP9-0503PT	TA12SP9-L503PT	TA12SP9-1003PT	TA12SP9-1503PT	TA12SP9-E503CC
YMC-Triart C8	2.0	TO12SP9-0302PT	TO12SP9-0502PT	TO12SP9-L502PT	TO12SP9-1002PT	TO12SP9-1502PT	TO12SP9-E5Q1CC**
	2.1	TO12SP9-03Q1PT	TO12SP9-05Q1PT	TO12SP9-L5Q1PT	TO12SP9-10Q1PT	TO12SP9-15Q1PT	TO12SP9-E5Q1CC**
	3.0	—	TO12SP9-0503PT	TO12SP9-L503PT	TO12SP9-1003PT	TO12SP9-1503PT	TO12SP9-E503CC
YMC-Triart Bio C4	2.0	TB30SP9-0302PT	TB30SP9-0502PT	TB30SP9-L502PT	TB30SP9-1002PT	TB30SP9-1502PT	TB30SP9-E5Q1CC**
	2.1	TB30SP9-03Q1PT	TB30SP9-05Q1PT	TB30SP9-L5Q1PT	TB30SP9-10Q1PT	TB30SP9-15Q1PT	TB30SP9-E5Q1CC**
	3.0	—	TB30SP9-0503PT	TB30SP9-L503PT	TB30SP9-1003PT	TB30SP9-1503PT	TB30SP9-E503CC

*Guard cartridge holder required, part no. XPCHUHP
**Guard cartridge : 2.1 mm ID

1.9 µm metal-free UHPLC columns

Phase	Column ID [mm]	Column length [mm]		
		50	100	150
YMC-Triart C18	2.1	TA12SP9-05Q1PTP	TA12SP9-10Q1PTP	TA12SP9-15Q1PTP
YMC-Triart C8	2.1	TO12SP9-05Q1PTP	TO12SP9-10Q1PTP	TO12SP9-15Q1PTP
YMC-Triart BioC4	2.1	TB30SP9-05Q1PTP	TB30SP9-10Q1PTP	TB30SP9-15Q1PTP

Special column connectors required.

Column connectors

Recommendation	✓ ✓		✓	
Ferrule	no		replaceable	
Product	MarvelX™	MarvelXACT™	Handy connector 2	Hand-tight EXP® fitting
Manufacturer	IDEX Health & Science LLC	IDEX Health & Science LLC	YMC Co., Ltd.	Optimize Technologies, Inc.
				
Pressure rating	131 MPa / 1,310 bar	131 MPa / 1,310 bar	42 MPa / 420 bar	137 MPa / 1,370 bar
Product code	e.g. UPFP-6050250	e.g. UPFP-YM7050250	XRP0204	XRHTF-01

MarvelX (ACT) is a registered trademark of IDEX Health & Science LLC · EXP® is a registered trademark of Optimize Technologies, Inc.

3 µm HPLC columns

Phase	Column ID [mm]	Column length [mm]						Guard cartridges* with 10 mm length
		30/33	50	75	100	150	250	(pack of 5)
YMC-Triart C18	2.1	TA12S03-H3Q1PTH	TA12S03-05Q1PTH	TA12S03-L5Q1PTH	TA12S03-10Q1PTH	TA12S03-15Q1PTH	–	TA12S03-01Q1GC
	3.0	–	TA12S03-0503PTH	TA12S03-L503PTH	TA12S03-1003PTH	TA12S03-1503PTH	–	TA12S03-0103GC
	4.6	TA12S03-H346PTH	TA12S03-0546PTH	TA12S03-L546PTH	TA12S03-1046PTH	TA12S03-1546PTH	TA12S03-2546PTH	TA12S03-0104GC
YMC-Triart C8	2.1	TO12S03-H3Q1PTH	TO12S03-05Q1PTH	TO12S03-L5Q1PTH	TO12S03-10Q1PTH	TO12S03-15Q1PTH	–	TO12S03-01Q1GC
	3.0	–	TO12S03-0503PTH	TO12S03-L503PTH	TO12S03-1003PTH	TO12S03-1503PTH	–	TO12S03-0103GC
	4.6	TO12S03-H346PTH	TO12S03-0546PTH	TO12S03-L546PTH	TO12S03-1046PTH	TO12S03-1546PTH	TO12S03-2546PTH	TO12S03-0104GC
YMC-Triart Bio C4	2.1	TB30S03-H3Q1PTH	TB30S03-05Q1PTH	TB30S03-L5Q1PTH	TB30S03-10Q1PTH	TB30S03-15Q1PTH	–	TB30S03-01Q1GC
	3.0	–	TB30S03-0503PTH	TB30S03-L503PTH	TB30S03-1003PTH	TB30S03-1503PTH	–	TB30S03-0103GC
	4.6	TB30S03-H346PTH	TB30S03-0546PTH	TB30S03-L546PTH	TB30S03-1046PTH	TB30S03-1546PTH	TB30S03-2546PTH	TB30S03-0104GC
YMC-Pack Pro C18	2.1	AS12S03-H3Q1QT	AS12S03-05Q1QT	AS12S03-L5Q1QT	AS12S03-10Q1QT	AS12S03-15Q1QT	AS12S03-25Q1QT	AS12S03-01Q1GC
	3.0	AS12S03-H303QT	AS12S03-0503QT	AS12S03-L503QT	AS12S03-1003QT	AS12S03-1503QT	AS12S03-2503QT	AS12S03-0103GC
	4.6	AS12S03-0346WT	AS12S03-0546WT	AS12S03-L546WT	AS12S03-1046WT	AS12S03-1546WT	AS12S03-2546WT	AS12S03-0104GC
Hydrosphere C18	2.1	HS12S03-H3Q1QT	HS12S03-05Q1QT	HS12S03-L5Q1QT	HS12S03-10Q1QT	HS12S03-15Q1QT	HS12S03-25Q1QT	HS12S03-01Q1GC
	3.0	HS12S03-H303QT	HS12S03-0503QT	HS12S03-L503QT	HS12S03-1003QT	HS12S03-1503QT	HS12S03-2503QT	HS12S03-0103GC
	4.6	HS12S03-0346WT	HS12S03-0546WT	HS12S03-L546WT	HS12S03-1046WT	HS12S03-1546WT	HS12S03-2546WT	HS12S03-0104GC
YMC-Pack C8, 20 nm	2.1	OC20S03-H3Q1QT	OC20S03-05Q1QT	OC20S03-L5Q1QT	OC20S03-10Q1QT	OC20S03-15Q1QT	OC20S03-25Q1QT	OC20S03-01Q1GC
	3.0	OC20S03-H303QT	OC20S03-0503QT	OC20S03-L503QT	OC20S03-1003QT	OC20S03-1503QT	OC20S03-2503QT	OC20S03-0103GC
	4.6	OC20S03-0346WT	OC20S03-0546WT	OC20S03-L546WT	OC20S03-1046WT	OC20S03-1546WT	OC20S03-2546WT	OC20S03-0104GC
YMCbasic (eq. C8)	2.1	BA99S03-H3Q1QT	BA99S03-05Q1QT	BA99S03-L5Q1QT	BA99S03-10Q1QT	BA99S03-15Q1QT	BA99S03-25Q1QT	BA99S03-01Q1GC
	3.0	BA99S03-H303QT	BA99S03-0503QT	BA99S03-L503QT	BA99S03-1003QT	BA99S03-1503QT	BA99S03-2503QT	BA99S03-0103GC
	4.6	BA99S03-0346WT	BA99S03-0546WT	BA99S03-L546WT	BA99S03-1046WT	BA99S03-1546WT	BA99S03-2546WT	BA99S03-0104GC
YMC-Pack C4, 30 nm	2.1	BU30S03-H3Q1QT	BU30S03-05Q1QT	BU30S03-L5Q1QT	BU30S03-10Q1QT	BU30S03-15Q1QT	BU30S03-25Q1QT	BU30S03-01Q1GC
	3.0	BU30S03-H303QT	BU30S03-0503QT	BU30S03-L503QT	BU30S03-1003QT	BU30S03-1503QT	BU30S03-2503QT	BU30S03-0103GC
	4.6	BU30S03-0346WT	BU30S03-0546WT	BU30S03-L546WT	BU30S03-1046WT	BU30S03-1546WT	BU30S03-2546WT	BU30S03-0104GC

*Guard cartridge holder required, part no. XPGCH-Q1

3 µm metal-free UHPLC columns

Phase	Column ID [mm]	Column length [mm]		
		50	100	150
YMC-Triart C18	2.1	TA12S03-05Q1PTP	TA12S03-10Q1PTP	TA12S03-15Q1PTP
	4.6	TA12S03-0546PTP	TA12S03-1046PTP	TA12S03-1546PTP
YMC-Triart C8	2.1	TO12S03-05Q1PTP	TO12S03-10Q1PTP	TO12S03-15Q1PTP
	4.6	TO12S03-0546PTP	TO12S03-1046PTP	TO12S03-1546PTP
YMC-Triart BioC4	2.1	TB30S03-05Q1PTP	TB30S03-10Q1PTP	TB30S03-15Q1PTP
	4.6	TB30S03-0546PTP	TB30S03-1046PTP	TB30S03-1546PTP

Special column connectors required.

RP – Ordering information

2.7 µm Core-Shell columns

Phase	Column ID [mm]	Column length [mm]					Precolumn filter 0.5 µm
		30	50	75	100	150	(pack of 3)
Meteoric Core C18, 8 nm	2.1	CAS08SQ7-03Q1PT	CAS08SQ7-05Q1PT	CAS08SQ7-L5Q1PT	CAS08SQ7-10Q1PT	CAS08SQ7-15Q1PT	XRPRCS35
	3.0	CAS08SQ7-0303PT	CAS08SQ7-0503PT	CAS08SQ7-L503PT	CAS08SQ7-1003PT	CAS08SQ7-1503PT	
	4.6	CAS08SQ7-0346PT	CAS08SQ7-0546PT	CAS08SQ7-L546PT	CAS08SQ7-1046PT	CAS08SQ7-1546PT	
Meteoric Core C18 BIO, 16 nm	2.1	CAW16SQ7-03Q1PT	CAW16SQ7-05Q1PT	CAW16SQ7-L5Q1PT	CAW16SQ7-10Q1PT	CAW16SQ7-15Q1PT	
	3.0	CAW16SQ7-0303PT	CAW16SQ7-0503PT	CAW16SQ7-L503PT	CAW16SQ7-1003PT	CAW16SQ7-1503PT	
	4.6	CAW16SQ7-0346PT	CAW16SQ7-0546PT	CAW16SQ7-L546PT	CAW16SQ7-1046PT	CAW16SQ7-1546PT	

*Holder required, part no. XRPRCS03

5 µm HPLC columns

Phase	Column ID [mm]	Column length [mm]						Guard cartridges* with 10 mm length
		30/33	50	75	100	150	250	(pack of 5)
YMC-Triart C18	2.1	TA12S05-H3Q1PTH	TA12S05-05Q1PTH	TA12S05-L5Q1PTH	TA12S05-10Q1PTH	TA12S05-15Q1PTH	–	TA12S05-01Q1GC
	3.0	–	TA12S05-0503PTH	TA12S05-L503PTH	TA12S05-1003PTH	TA12S05-1503PTH	–	TA12S05-0103GC
	4.6	TA12S05-H346PTH	TA12S05-0546PTH	TA12S05-L546PTH	TA12S05-1046PTH	TA12S05-1546PTH	TA12S05-2546PTH	TA12S05-0104GC
YMC-Triart C8	2.1	TO12S05-H3Q1PTH	TO12S05-05Q1PTH	TO12S05-L5Q1PTH	TO12S05-10Q1PTH	TO12S05-15Q1PTH	–	TO12S05-01Q1GC
	3.0	–	TO12S05-0503PTH	TO12S05-L503PTH	TO12S05-1003PTH	TO12S05-1503PTH	–	TO12S05-0103GC
	4.6	TO12S05-H346PTH	TO12S05-0546PTH	TO12S05-L546PTH	TO12S05-1046PTH	TO12S05-1546PTH	TO12S05-2546PTH	TO12S05-0104GC
YMC-Triart Bio C4	2.1	TB30S05-H3Q1PTH	TB30S05-05Q1PTH	TB30S05-L5Q1PTH	TB30S05-10Q1PTH	TB30S05-15Q1PTH	–	TB30S05-01Q1GC
	3.0	–	TB30S05-0503PTH	TB30S05-L503PTH	TB30S05-1003PTH	TB30S05-1503PTH	–	TB30S05-0103GC
	4.6	TB30S05-H346PTH	TB30S05-0546PTH	TB30S05-L546PTH	TB30S05-1046PTH	TB30S05-1546PTH	TB30S05-2546PTH	TB30S05-0104GC
YMC-Pack Pro C18	2.1	AS12S05-H3Q1QT	AS12S05-05Q1QT	AS12S05-L5Q1QT	AS12S05-10Q1QT	AS12S05-15Q1QT	AS12S05-25Q1QT	AS12S05-01Q1GC
	3.0	AS12S05-H303QT	AS12S05-0503QT	AS12S05-L503QT	AS12S05-1003QT	AS12S05-1503QT	AS12S05-2503QT	AS12S05-0103GC
	4.6	AS12S05-0346WT	AS12S05-0546WT	AS12S05-L546WT	AS12S05-1046WT	AS12S05-1546WT	AS12S05-2546WT	AS12S05-0104GC
Hydrosphere C18	2.1	HS12S05-H3Q1QT	HS12S05-05Q1QT	HS12S05-L5Q1QT	HS12S05-10Q1QT	HS12S05-15Q1QT	HS12S05-25Q1QT	HS12S05-01Q1GC
	3.0	HS12S05-H303QT	HS12S05-0503QT	HS12S05-L503QT	HS12S05-1003QT	HS12S05-1503QT	HS12S05-2503QT	HS12S05-0103GC
	4.6	HS12S05-0346WT	HS12S05-0546WT	HS12S05-L546WT	HS12S05-1046WT	HS12S05-1546WT	HS12S05-2546WT	HS12S05-0104GC
YMC-Pack C8, 20 nm	2.1	OC20S05-H3Q1QT	OC20S05-05Q1QT	OC20S05-L5Q1QT	OC20S05-10Q1QT	OC20S05-15Q1QT	OC20S05-25Q1QT	OC20S05-01Q1GC
	3.0	OC20S05-H303QT	OC20S05-0503QT	OC20S05-L503QT	OC20S05-1003QT	OC20S05-1503QT	OC20S05-2503QT	OC20S05-0103GC
	4.6	OC20S05-0346WT	OC20S05-0546WT	OC20S05-L546WT	OC20S05-1046WT	OC20S05-1546WT	OC20S05-2546WT	OC20S05-0104GC
YMCbasic (eq. C8)	2.1	BA99S05-H3Q1QT	BA99S05-05Q1QT	BA99S05-L5Q1QT	BA99S05-10Q1QT	BA99S05-15Q1QT	BA99S05-25Q1QT	BA99S05-01Q1GC
	3.0	BA99S05-H303QT	BA99S05-0503QT	BA99S05-L503QT	BA99S05-1003QT	BA99S05-1503QT	BA99S05-2503QT	BA99S05-0103GC
	4.6	BA99S05-0346WT	BA99S05-0546WT	BA99S05-L546WT	BA99S05-1046WT	BA99S05-1546WT	BA99S05-2546WT	BA99S05-0104GC
YMC-Pack C4, 30 nm	2.1	BU30S05-H3Q1QT	BU30S05-05Q1QT	BU30S05-L5Q1QT	BU30S05-10Q1QT	BU30S05-15Q1QT	BU30S05-25Q1QT	BU30S05-01Q1GC
	3.0	BU30S05-H303QT	BU30S05-0503QT	BU30S05-L503QT	BU30S05-1003QT	BU30S05-1503QT	BU30S05-2503QT	BU30S05-0103GC
	4.6	BU30S05-0346WT	BU30S05-0546WT	BU30S05-L546WT	BU30S05-1046WT	BU30S05-1546WT	BU30S05-2546WT	BU30S05-0104GC

*Guard cartridge holder required, part no. XPGCH-Q1

5 µm metal-free UHPLC columns

Phase	Column ID [mm]	Column length [mm]		
		50	100	150
YMC-Triart C18	2.1	TA12S05-05Q1PTP	TA12S05-10Q1PTP	TA12S05-15Q1PTP
	4.6	TA12S05-0546PTP	TA12S05-1046PTP	TA12S05-1546PTP
YMC-Triart C8	2.1	TO12S05-05Q1PTP	TO12S05-10Q1PTP	TO12S05-15Q1PTP
	4.6	TO12S05-0546PTP	TO12S05-1046PTP	TO12S05-1546PTP
YMC-Triart BioC4	2.1	TB30S05-05Q1PTP	TB30S05-10Q1PTP	TB30S05-15Q1PTP
	4.6	TB30S05-0546PTP	TB30S05-1046PTP	TB30S05-1546PTP

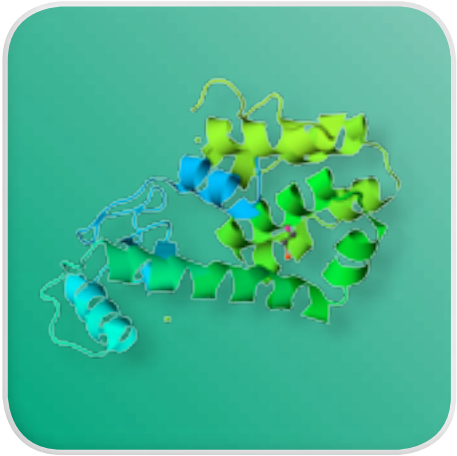
Special column connectors required.

5 µm YMC-Actus high-throughput semipreparative columns

Phase	Column ID [mm]	Column length [mm]					Guard cartridges* with 10 mm length
		50	75	100	150	250	(pack of 5)
YMC-Triart C18	20	TA12S05-0520WX	TA12S05-L520WX	TA12S05-1020WX	TA12S05-1520WX	TA12S05-2520WX	TA12S05-0120CC
	30	TA12S03-0530WX	TA12S03-L530WX	TA12S03-1030WX	TA12S03-1530WX	TA12S03-2530WX	TA12C05-0130CC
	50	TA12S05-0553DX	–	TA12S05-1053DX	TA12S05-1553DX	TA12S05-2553DX	TA12S05-0553DXG**
YMC-Triart C8	20	TO12S05-0520WX	TO12S05-L520WX	TO12S05-1020WX	TO12S05-1520WX	TO12S05-2520WX	TO12S05-0120CC
	30	TO12S03-0530WX	TO12S03-L530WX	TO12S03-1030WX	TO12S03-1530WX	TO12S03-2530WX	TO12C05-0130CC
	50	TO12S05-0553DX	–	TO12S05-1053DX	TO12S05-1553DX	TO12S05-2553DX	TO12S05-0553DXG**
YMC-Triart Bio C4	20	TB30S05-0520WX	TB30S05-L520WX	TB30S05-1020WX	TB30S05-1520WX	TB30S05-2520WX	TB30S05-0120CC
	30	TB30S03-0530WX	TB30S03-L530WX	TB30S03-1030WX	TB30S03-1530WX	TB30S03-2530WX	TB30C05-0130CC
	50	TB30S05-0553DX	–	TB30S05-1053DX	TB30S05-1553DX	TB30S05-2553DX	TB30S05-0553DXG**
YMC-Pack Pro C18	20	AS12S05-0520WX	AS12S05-L520WX	AS12S05-1020WX	AS12S05-1520WX	AS12S05-2520WX	AS12S05-0120CC
	30	AS12S05-0530WX	AS12S05-L530WX	AS12S05-1030WX	AS12S05-1530WX	AS12S05-2530WX	AS12S05-0130CC
	50	AS12S05-0553DX	–	AS12S05-1053DX	AS12S05-1553DX	AS12S05-2553DX	–
Hydrosphere C18	20	HS12S05-0520WX	HS12S05-L520WX	HS12S05-1020WX	HS12S05-1520WX	HS12S05-2520WX	HS12S05-0120CC
	30	HS12S05-0530WX	HS12S05-L530WX	HS12S05-1030WX	HS12S05-1530WX	HS12S05-2530WX	HS12S05-0130CC
	50	HS12S05-0553DX	–	HS12S05-1053DX	HS12S05-1553DX	HS12S05-2553DX	–
YMCbasic (eq. C8)	20	BA99S05-0520WX	BA99S05-L520WX	BA99S05-1020WX	BA99S05-1520WX	BA99S05-2520WX	BA99S05-0120CC
	30	BA99S05-0530WX	BA99S05-L530WX	BA99S05-1030WX	BA99S05-1530WX	BA99S05-2530WX	BA99S05-0130CC
	50	BA99S05-0553DX	–	BA99S05-1053DX	BA99S05-1553DX	BA99S05-2553DX	–

*Guard cartridge holder required, part no. XPCHSPW2 (20 mm ID)
XPCHSPW3 (30 mm ID)
no holder required for 50 mm

SEC

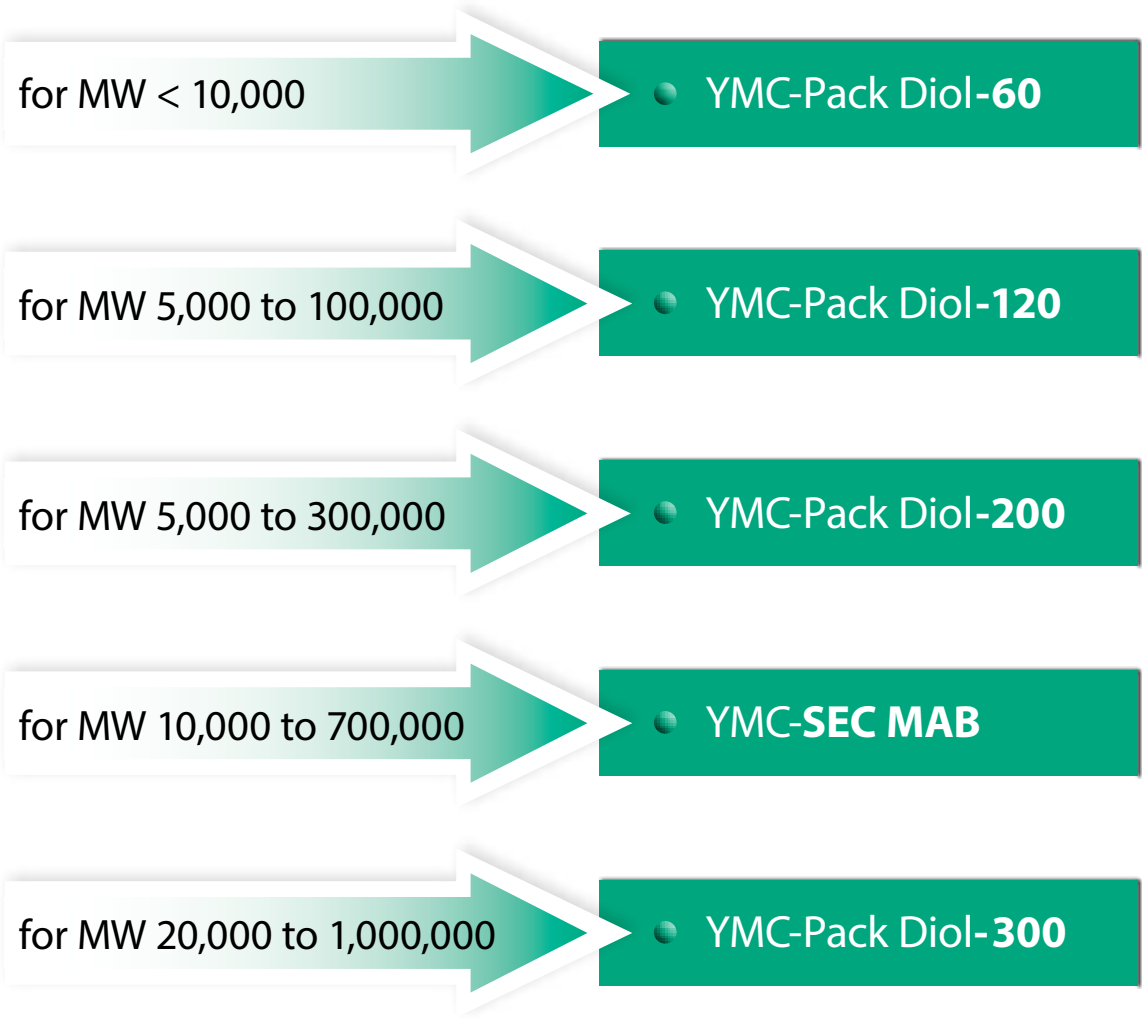


SEC – UHPLC / HPLC Selectivities

- **Applicable to proteins, antibodies, their fragments and peptides**
- **Also applicable to carbohydrates and nucleic acid components**
- **Excellent reproducibility with minimal secondary interactions**
- **2 µm for UHPLC**
- **Cost effective**

	YMC-Pack Diol-60	YMC-Pack Diol-120	YMC-Pack Diol-200	YMC-Pack Diol-300	YMC-SEC MAB
	For peptides and small proteins	For intermediate proteins	For large proteins	For very large proteins	For antibodies, fragments and aggregates
Base particle	Silica				
Pore Size / µm	3, 5	3,5	2, 3, 5	2, 3, 5	(<2), 3
Pore Size / nm	6	12	20	30	25
Modification	Dihydroxypropyl				
Temperature range	40 °C				
Pressure limit	2 µm: 45 MPa (6,525 psi); 3/5 µm: 20 MPa (3,000 psi)				3 µm: 14 MPa (2,030 psi)

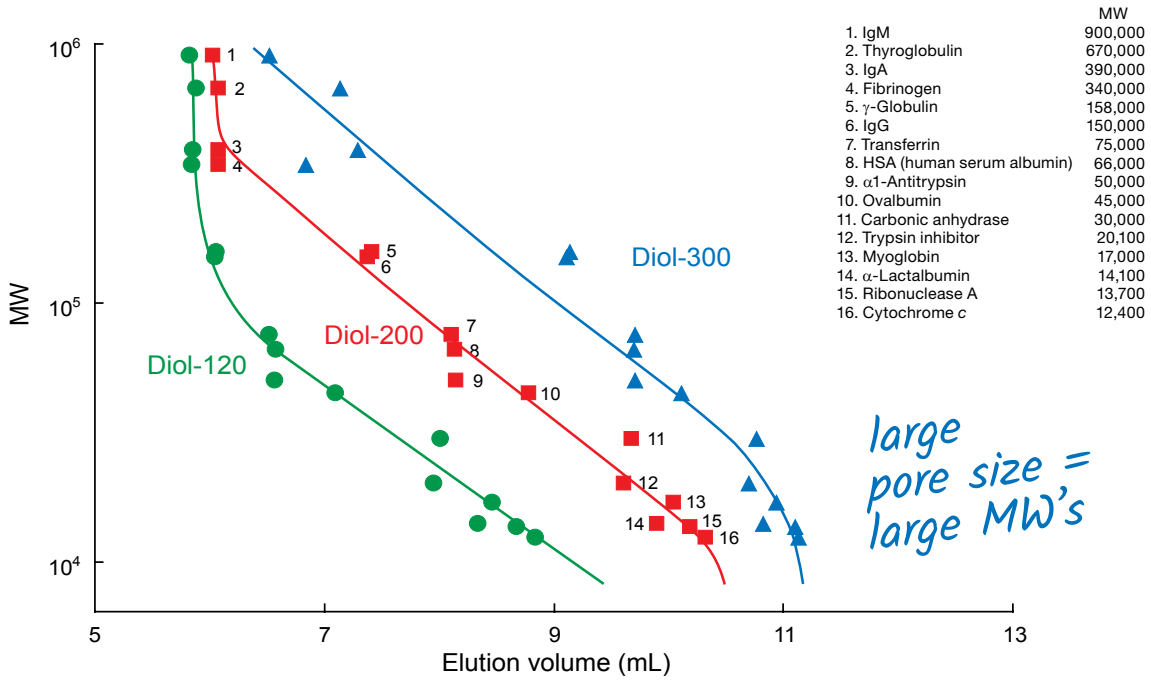
Column Selection Tool



SEC – YMC-Pack Diol: Phase selection for proteins

Phases for different MW ranges

For separation of proteins with molecular weights from 10,000 to several 100,000 Da

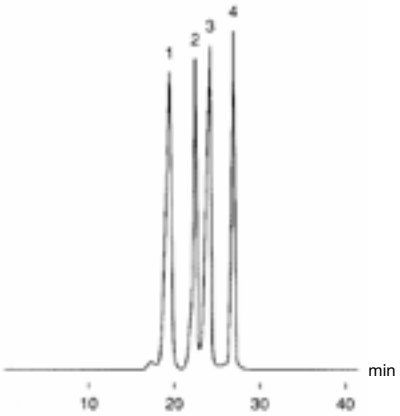


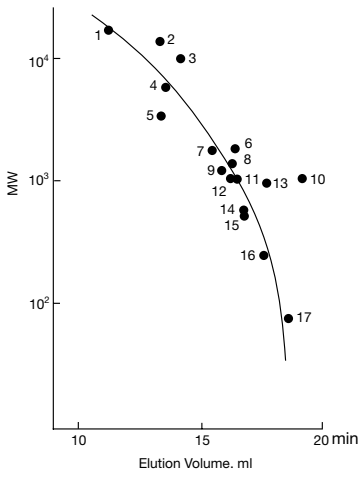
	MW
1. IgM	900,000
2. Thyroglobulin	670,000
3. IgA	390,000
4. Fibrinogen	340,000
5. γ-Globulin	158,000
6. IgG	150,000
7. Transferrin	75,000
8. HSA (human serum albumin)	66,000
9. α1-Antitrypsin	50,000
10. Ovalbumin	45,000
11. Carbonic anhydrase	30,000
12. Trypsin inhibitor	20,100
13. Myoglobin	17,000
14. α-Lactalbumin	14,100
15. Ribonuclease A	13,700
16. Cytochrome *c*	12,400

Column:	YMC-Pack Diol, 300 x 8.0 mm ID
Part Nos.:	DL12S05-3008WT, DL20S05-3008WT, DL30S05-5008WT
Eluent:	0.1 M KH_2PO_4-K_2HPO_4 (pH 7.0) containing 0.2 M NaCl
Flow rate:	0.5 mL/min
Temperature:	25 °C
Detection:	UV at 280 nm

Separation of proteins with molecular weights below 10,000 Da

1. Insulin (bovine)
2. Neurotensin
3. Angiotensin-II
4. Glycine





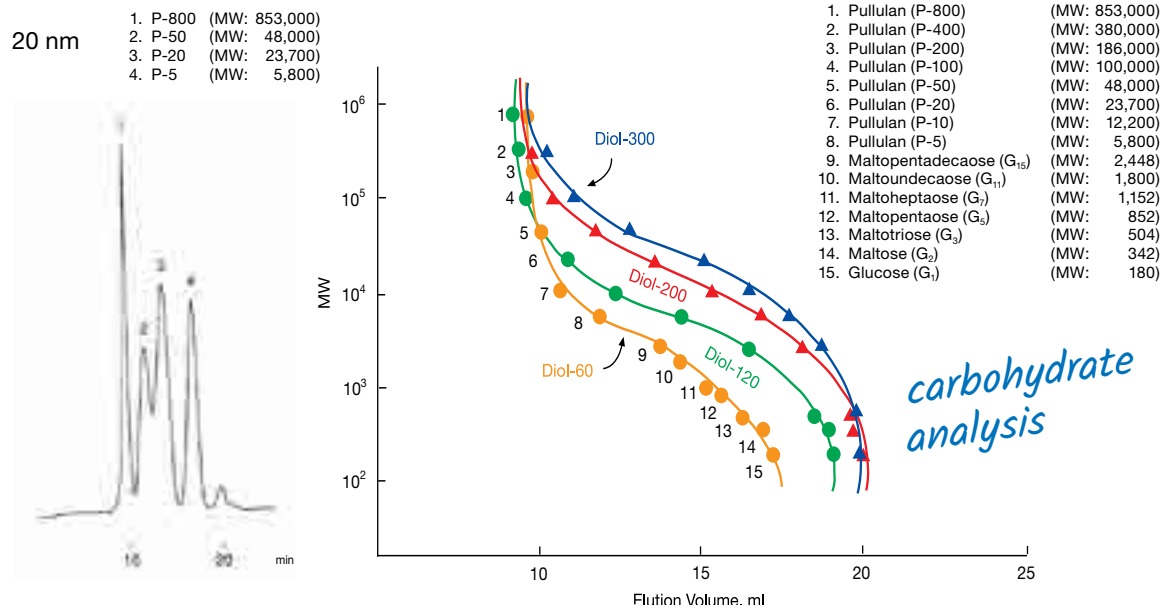
1. Myoglobin	(MW:	17,000)
2. Ribonuclease A	(MW:	13,700)
3. Cytochrome *c*	(MW:	12,400)
4. Insulin (bovine)	(MW:	5,700)
5. Insulin B chain	(MW:	3,496)
6. α-Mating factor	(MW:	1,684)
7. Neurotensin	(MW:	1,672)
8. Angiotensin-I	(MW:	1,296)
9. CCK-octapeptide	(MW:	1,143)
10. Bradykinin	(MW:	1,060)
11. Angiotensin-II	(MW:	1,040)
12. Oxytocin	(MW:	1,007)
13. Angiotensin-III	(MW:	931)
14. Met-enkephalin	(MW:	573)
15. Leu-enkephalin	(MW:	555)
16. Tetraglycine	(MW:	246)
17. Glycine	(MW:	75)

small pore size = small MW's

Column:	YMC-Pack Diol (6 nm) 500 x 8.0 mm ID
Part No.:	DL06S05-5008WT
Eluent:	0.1 M KH_2PO_4-K_2HPO_4 (pH 7) containing 0.2 M NaCl / acetonitrile (70/30)
Flow rate:	0.7 mL/min
Temperature:	ambient
Detection:	UV at 215 nm

Phases for different MW ranges

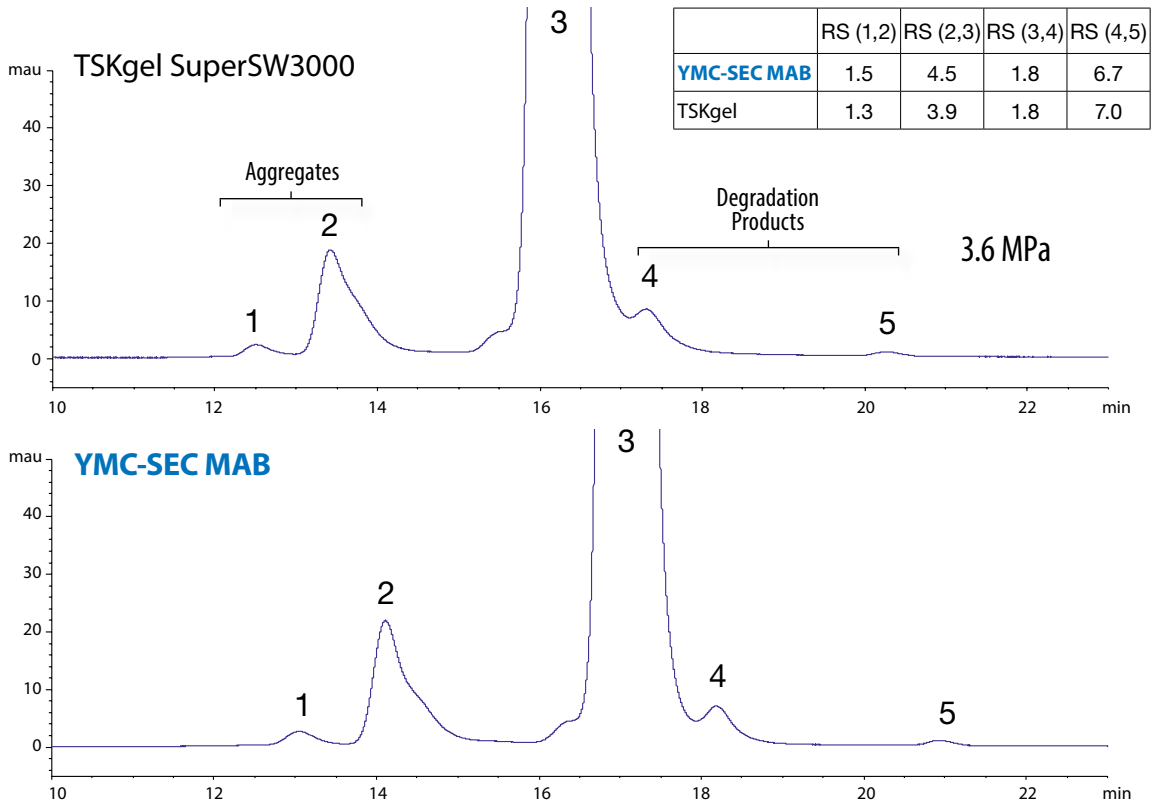
For molecular weight determination of oligosaccharides and polysaccharides



Column: YMC-Pack Diol (20 nm) 500 x 8.0 mm ID
Part No.: DL20S05-5008WT
Eluent: water
Flow rate: 1.0 mL/min
Temperature: ambient
Detection: RI

SEC – YMC-SEC MAB: MAb & ADC analysis

Ideal choice for monoclonal antibodies

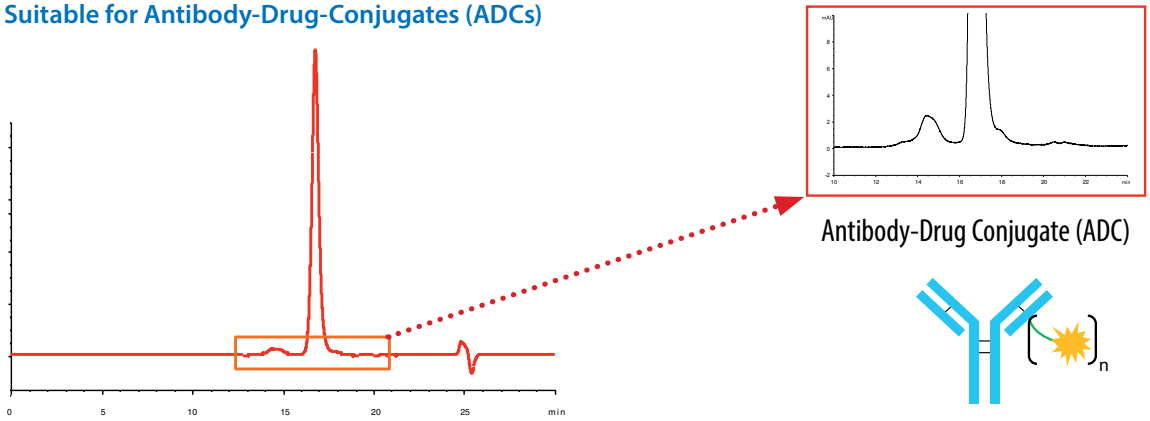


	RS (1,2)	RS (2,3)	RS (3,4)	RS (4,5)
YMC-SEC MAB	1.5	4.5	1.8	6.7
TSKgel	1.3	3.9	1.8	7.0

Column: YMC-SEC MAB (3 µm, 25 nm) 300 x 4.6 mm ID
Part No.: DLM25S03-3046WT
Eluent: 0.1 M phosphate buffer (pH = 7) cont. 0.2 M NaCl
Flow rate: 0.165 mL/min
Temperature: 25 °C

Detection: UV at 280 nm
Cell path: 10 mm
Injection: 10 µL (5 mg/mL)
Sample: Avastin

Suitable for Antibody-Drug-Conjugates (ADCs)

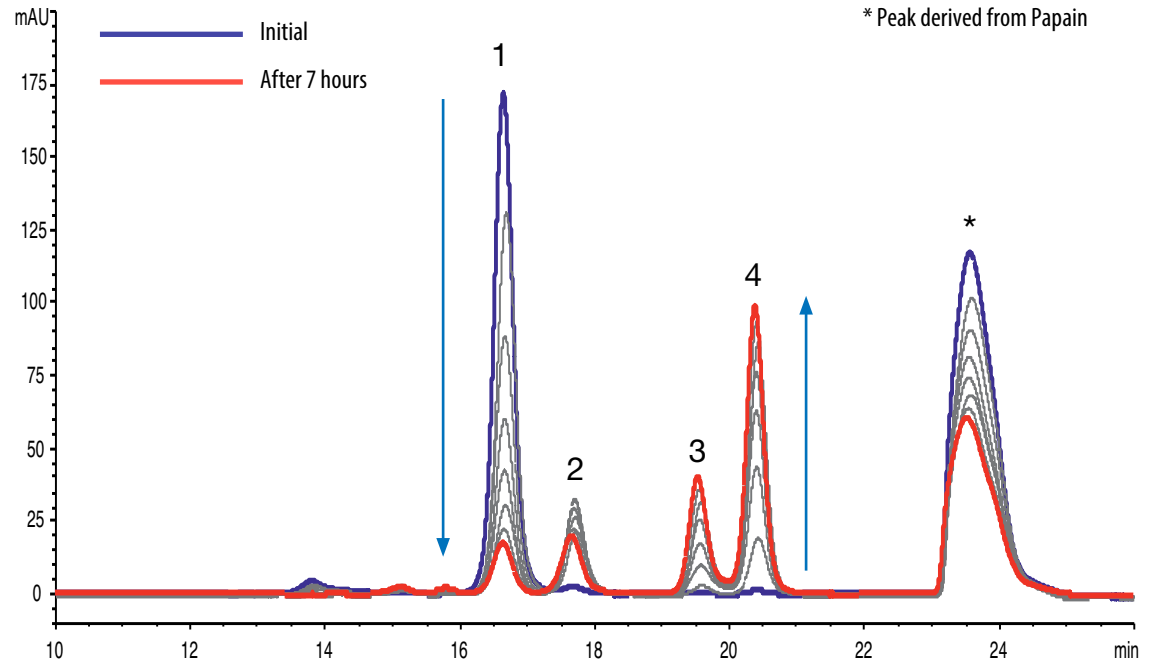


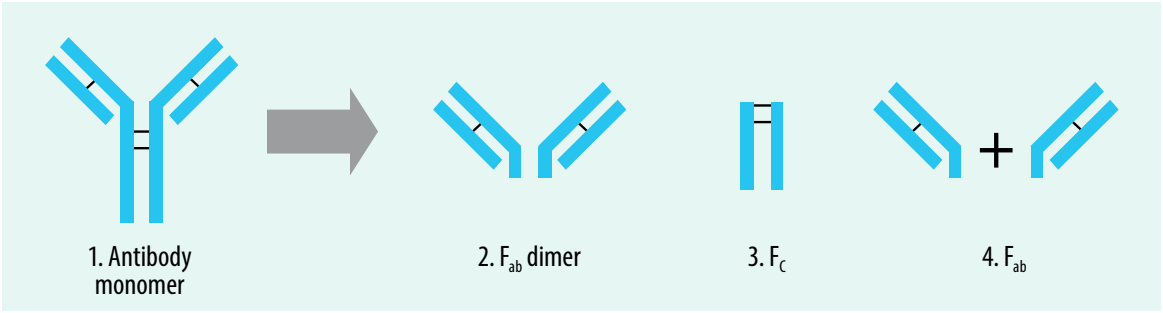
Column: YMC-SEC MAB (3 µm, 25 nm) 300 x 4.6 mm ID
Part No.: DLM25S03-3046WT
Eluent: 0.1 M phosphate buffer (pH = 7) cont. 0.2 M NaCl / 2-propanol (85 / 15)
Flow rate: 0.165 mL/min

Temperature: 25 °C
Detection: UV at 280 nm
Injection: 4 µL (2.5 mg/mL)
Sample: SigmaMAb Antibody Drug Conjugate Mimic

YMC-SEC MAB is also suitable for the analysis of Antibody-Drug Conjugates (ADC). The addition of an organic solvent to the mobile phase can improve the results obtained for ADC analysis.

Ideal for analysis of digested antibodies



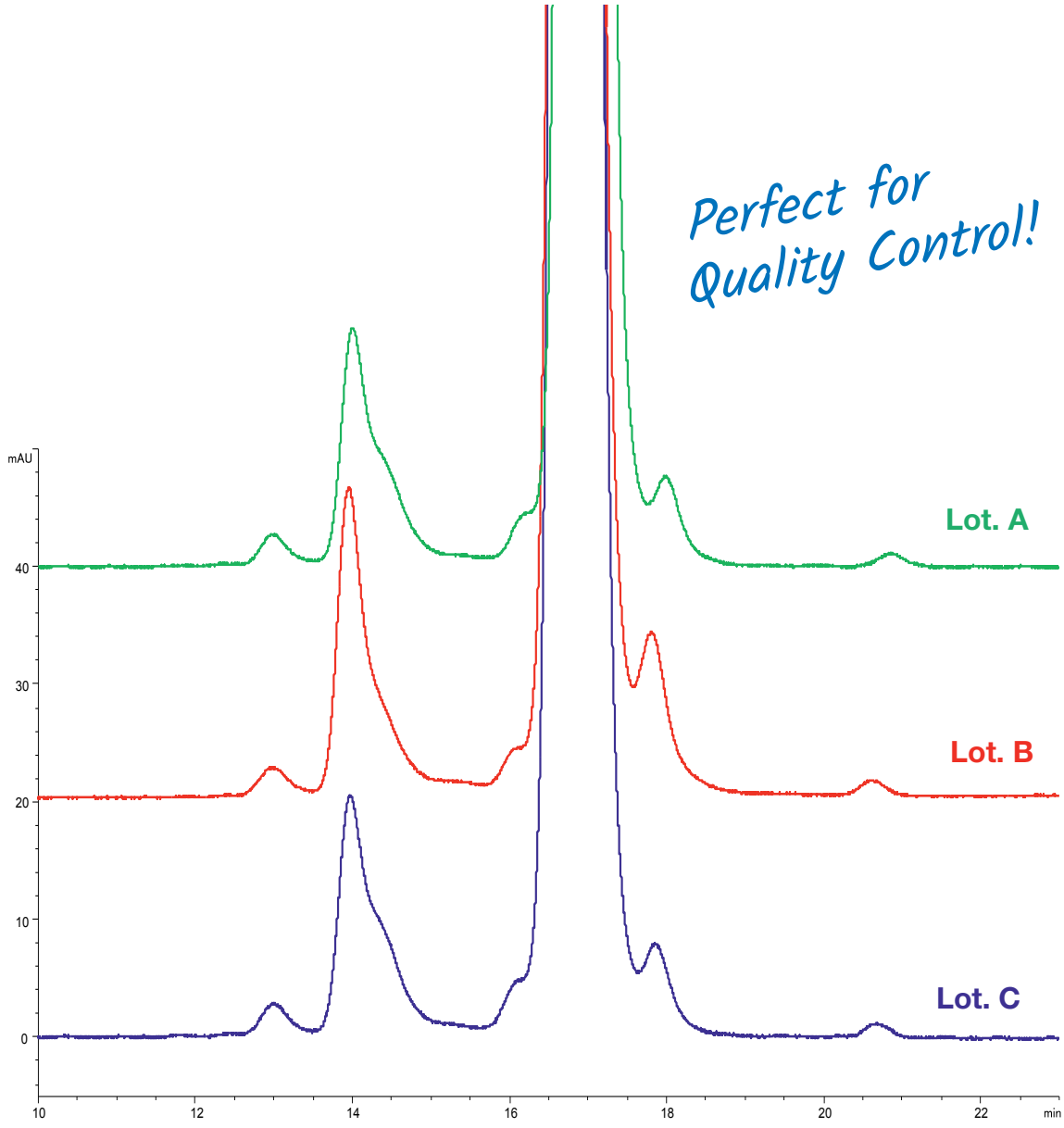


Column:	YMC-SEC MAB (3 µm, 25 nm) 300 x 4.6 mm ID
Part No.:	DLM25S03-3046WT
Eluent:	0.1 M phosphate buffer (pH = 7) cont. 0.2 M NaCl
Flow rate:	0.165 mL/min
Temperature:	25 °C
Detection:	UV at 280 nm
Injection:	2 µL (3 mg/mL)
Sample:	Humanised monoclonal IgG1 + Papain

Ideal for the analysis of fragments/degradation products of antibodies: Digestion of a monoclonal antibody with papain was monitored for 7 hours. The peak of the monomer decreased as digestion proceeded, while peaks for degradation products increased.

SEC – YMC-SEC MAB: Reproducibility

Excellent lot-to-lot reproducibility



Column:	YMC-SEC MAB (3 µm, 25 nm) 300 x 4.6 mm ID
Part No.:	DLM25S03-3046WT
Eluent:	0.1 M phosphate buffer (pH = 7) cont. 0.2 M NaCl
Flow rate:	0.165 mL/min
Temperature:	25 °C
Detection:	UV at 280 nm
Injection:	10 µL (5 mg/mL)
Sample:	humanised monoclonal antibody

YMC-SEC MAB provides excellent reproducibility for the separation of monomer and aggregates as well as for monomer and their fragments, making it very effective for quality control of antibody drugs.

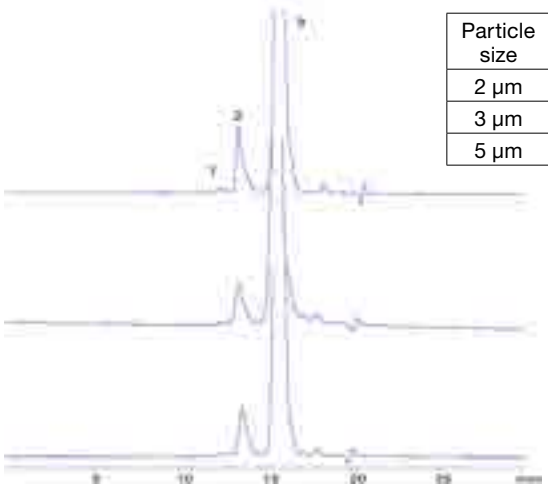
Benefits of using smaller particles

Higher resolution for analysis of monoclonal antibodies

(A) YMC-Pack Diol-300 (2 µm)
300 x 4.6 mm ID

(B) YMC-Pack Diol-300 (3 µm)
300 x 4.6 mm ID

(C) YMC-Pack Diol-300 (5 µm)
300 x 4.6 mm ID



Particle size	Rs (1,2)	Rs (2,3)	N (3)
2 µm	1.17	4.15	16,200
3 µm	1.03	3.18	10,400
5 µm	0.88	2.67	**8,500**

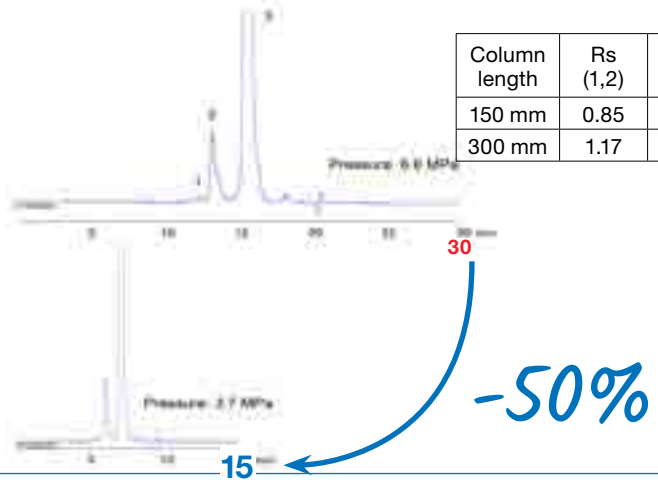
Columns: YMC-Pack Diol-300, 300 x 4.6 mm ID
Part Nos.: (A) DL30S02-3046PTH
(B) DL30S03-3046WT
(C) DL30S05-3046WT
Eluent: 0.1 M KH_2PO_4-K_2HPO_4 (pH 7.0) containing 0.2 M NaCl
Flow rate: 0.2 mL/min
Temperature: ambient
Detection: UV at 280 nm
Sample: Humanised monoclonal antibody (IgG1)

All three particle sizes show identical separation patterns for monoclonal antibody analysis. This allows easy method transfer between HPLC and UHPLC.
A method developed using conventional HPLC can be directly transferred to UHPLC using a 2 µm YMC-Pack Diol column. YMC-Pack Diol UHPLC columns greatly improve the resolution between aggregates and the monomer peak. In addition, a shoulder peak which can be observed after the monomer peak can be partially separated using the 2 µm column.

High throughput analysis of monoclonal antibodies

YMC-Pack Diol-300 (2 µm)
300 x 4.6 mm ID

YMC-Pack Diol-300 (2 µm)
150 x 4.6 mm ID



Column length	Rs (1,2)	Rs (2,3)	N (3)
150 mm	0.85	2.75	8,700
300 mm	1.17	4.15	16,200

Columns: YMC-Pack Diol-300, 150 or 300 x 4.6 mm ID
Part Nos.: DL30S02-3046PTH / DL30S02-1546PTH
Eluent: 0.1 M KH_2PO_4-K_2HPO_4 (pH 7.0) containing 0.2 M NaCl
Flow rate: 0.2 mL/min
Temperature: ambient
Detection: UV at 280 nm
Sample: Humanised monoclonal antibody (IgG1)

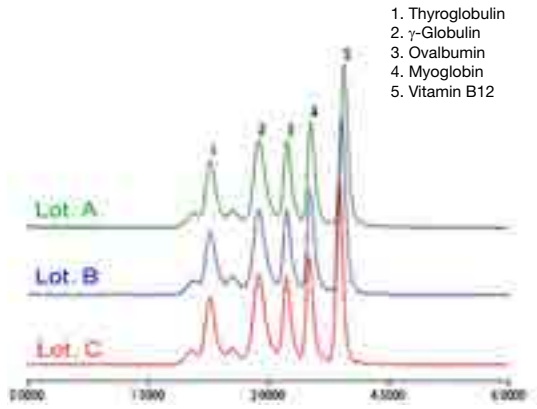
By using a 150 mm length column, 50% shorter run times can be achieved with the same resolution as for a 5 µm 300 mm length column (compare upper and lower chromatograms). This allows an increase in throughput to be achieved. The backpressure is only 6.6 MPa, even for the 300 mm column. Therefore, YMC-Pack Diol 2 µm columns can be used with both UHPLC and HPLC systems.

SEC – YMC-Pack Diol: Reproducibility & stability

Reproducibility and stability data

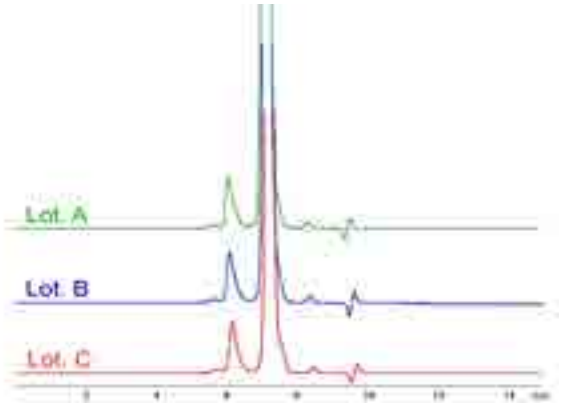
Excellent batch to batch reproducibility

Standard proteins



Column:	YMC-Pack Diol-300 (2 µm) 150 x 4.6 mm ID
Part No.:	DL30S02-1546PTH
Eluent:	0.1 M NaH_2PO_4-Na_2HPO_4 (pH 7.0) containing 0.2 M NaCl
Flow rate:	0.5 mL/min
Temperature:	ambient
Detection:	UV at 280 nm
Sample:	Standard proteins

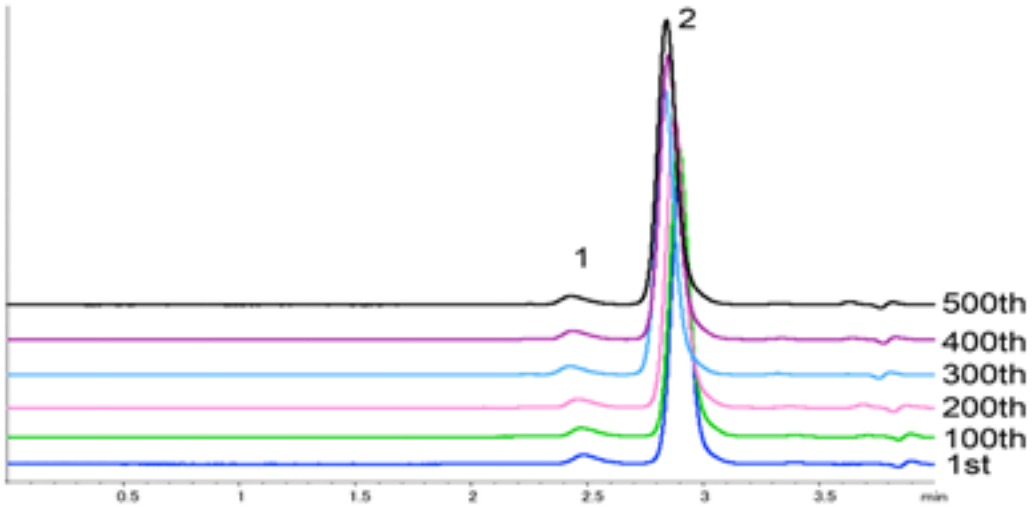
Humanised monoclonal antibody



Column:	YMC-Pack Diol-300 (2 µm) 150 x 4.6 mm ID
Part No.:	DL30S02-1546PTH
Eluent:	0.1 M KH_2PO_4-K_2HPO_4 (pH 7.0) containing 0.2 M NaCl
Flow rate:	0.2 mL/min
Temperature:	25 °C
Detection:	UV at 280 nm
Sample:	Humanised monoclonal antibody

YMC-Pack Diol UHPLC columns have excellent batch-to-batch reproducibility. This makes YMC-Pack Diol 2 µm columns the ideal choice for the quality control of bio-based drugs including monoclonal antibodies.

Long-term stability



Column:	YMC-Pack Diol-300, 2 µm, 150 x 4.6 mm ID
Part No.:	DL30S02-1546PTH
Eluent:	0.1 M KH_2PO_4-K_2HPO_4 (pH 7.0) containing 0.2 M NaCl
Flow rate:	0.5 mL/min
Temperature:	25 °C
Detection:	UV at 280 nm
Sample:	Humanised monoclonal antibody

YMC-Pack Diol UHPLC columns maintain their performance for more than 500 injections of sample during monoclonal antibody analysis. This ensures reproducible and reliable quality control of bio-based drugs including monoclonal antibodies.

2 µm UHPLC columns

Phase	Column ID [mm]	Column length [mm]		Precolumn filter 0.5 µm
		150	300	(pack of 5)
YMC-Pack Diol-200	4.6	DL20S02-1546PTH	DL20S02-3046PTH	XRPRCS35
YMC-Pack Diol-300	4.6	DL30S02-1546PTH	DL30S02-3046PTH	

Holder required, part no. XRPRCS03

3 µm HPLC columns

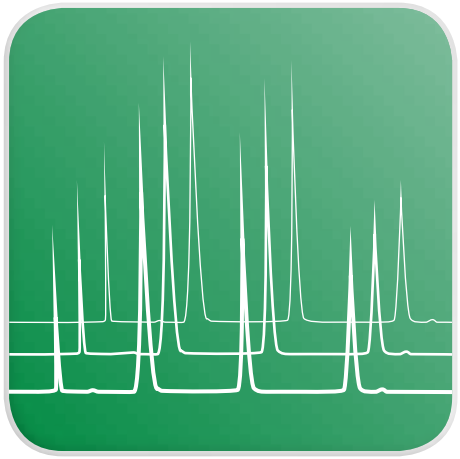
Phase	Column ID [mm]	Column length [mm]			Guard cartridges* with 10/30 mm length
		150	250	300	(pack of 5)
YMC-SEC MAB	4.6 6.0 8.0	DLM25S03-1546WT – –	– – –	DLM25S03-3046WT – DLM25S03-3008WT	DLM25S03-0104GC – –
YMC-Pack Diol-60	4.6 6.0 8.0	DL06S03-1546WT – DL06S03-1508WT	DL06S03-2546WT – –	DL06S03-3046WT DL06S03-3006WT DL06S03-3008WT	DL06S03-0104GC – DL06S03-0310WTG**
YMC-Pack Diol-120	4.6 6.0 8.0	DL12S03-1546WT – DL12S03-1508WT	DL12S03-2546WT – –	DL12S03-3046WT DL12S03-3006WT DL12S03-3008WT	DL12S03-0104GC – DL12S03-0310WTG**
YMC-Pack Diol-200	4.6 6.0 8.0	DL20S03-1546WT – DL20S03-1508WT	DL20S03-2546WT – –	DL20S03-3046WT DL20S03-3006WT DL20S03-3008WT	DL20S03-0104GC – DL20S03-0310WTG**
YMC-Pack Diol-300	4.6 6.0 8.0	DL30S03-1546WT – DL30S03-1508WT	DL30S03-2546WT – –	DL30S03-3046WT DL30S03-3006WT DL30S03-3008WT	DL30S03-0104GC – DL30S03-0310WTG**

*Guard cartridge holder required, part no. XPGCH-Q1
**no holder required for 30 x 8 mm ID guards

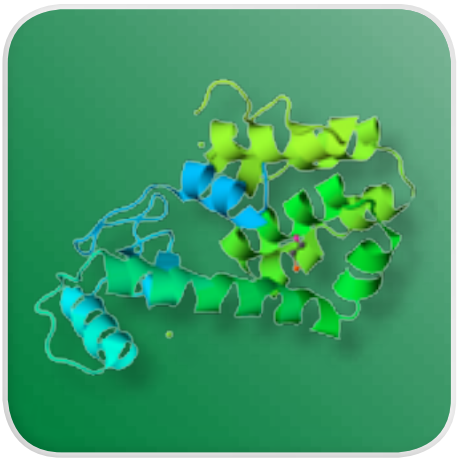
5 µm HPLC columns

Phase	Column ID [mm]	Column length [mm]			Guard cartridges* with 10/30 mm length
		250	300	500	(pack of 5)
YMC-Pack Diol-60	4.6 6.0 8.0 10.0	DL06S05-2546WT DL06S05-2506WT – DL06S05-2510WT	DL06S05-3046WT DL06S05-3006WT DL06S05-3008WT DL06S05-3010WT	– DL06S05-5006WT DL06S05-5008WT DL06S05-5010WT	DL06S05-0104GC – DL06S05-0308WTG** DL06S05-3010WTG**
YMC-Pack Diol-120	4.6 6.0 8.0 10.0	DL12S05-2546WT DL12S05-2506WT – DL12S05-2510WT	DL12S05-3046WT DL12S05-3006WT DL12S05-3008WT DL12S05-3010WT	– DL12S05-5006WT DL12S05-5008WT DL12S05-5010WT	DL12S05-0104GC – DL12S05-0308WTG** DL12S05-3010WTG**
YMC-Pack Diol-200	4.6 6.0 8.0 10.0	DL20S05-2546WT DL20S05-2506WT – DL20S05-2510WT	DL20S05-3046WT DL20S05-3006WT DL20S05-3008WT DL20S05-3010WT	– DL20S05-5006WT DL20S05-5008WT DL20S05-5010WT	DL12S05-0104GC – DL12S05-0308WTG** DL12S05-3010WTG**
YMC-Pack Diol-300	4.6 6.0 8.0 10.0	DL30S05-2546WT DL30S05-2506WT – DL30S05-2510WT	DL30S05-3046WT DL30S05-3006WT DL30S05-3008WT DL30S05-3010WT	– DL30S05-5006WT DL30S05-5008WT DL30S05-5010WT	DL20S05-0104GC – DL20S05-0308WTG** DL20S05-3010WTG**

*Guard cartridge holder required, part no. XPGCH-Q1
**no holder required for 30 x 8 mm ID guards

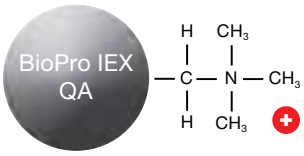


IEX

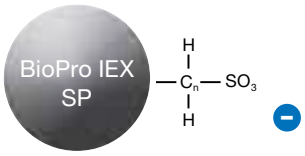


IEX – Bio Pro Series

- **porous or non-porous hydrophilic polymers**
- **high binding capacity and recovery of biomolecules**
- **very high resolution**
- **low nonspecific adsorption**
- **excellent reproducibility**



strong anion exchanger



strong cation exchanger

	BioPro IEX QA	BioPro IEX SP
Matrix	hydrophilic polymer (polymethacrylate)	hydrophilic polymer (polymethacrylate)
Particle size / µm	5	5
Pore size / nm	100	100
Charged group	$-CH_2N^+(CH_3)_3$	$-(CH_2)_3SO_3^-$
Counter ion	Cl^-	Na^+
Available pH range	2.0 – 12.0	2.0 – 12.0
Temperature range	4 – 60 °C	
Presssure limit	2.5 – 3.5 MPa (360 – 510 psi)	
Column hardware	PEEK	

Also available in 10, 20, 30 or 75 µm for preparative scale

Porous polymer beads

	BioPro IEX QF	BioPro IEX SF
Matrix	hydrophilic polymer (polymethacrylate)	hydrophilic polymer (polymethacrylate)
Particle size / µm	3; 5	3; 5
Pore size / nm	non-porous	non-porous
Charged group	$-CH_2N^+(CH_3)_3$	$-(CH_2)_3SO_3^-$
Counter ion	Cl^-	Na^+
Available pH range	2.0 - 12.0	2.0 - 12.0
Temperature range	4 – 60 °C	
Presssure limit	3 µm: 25 MPa (3,625 psi) 5 µm: 6 – 12 MPa (870 – 1,740 psi)	
Column hardware	PEEK	

Non-porous polymer beads

General

YMC´s BioPro IEX series of ion exchange columns are available in QA and SP chemistries, based on 5 µm porous (QA or SP columns) or on 3 or 5 µm non-porous (QF and SF columns) hydrophilic polymer beads.

The porous materials offer excellent binding capacity with exceptionally high efficiency and low operating pressure, whilst the non-porous particles offer high efficiency, very high resolution and low operating pressures.

High binding capacity and high recovery for porous type

The porous versions of YMC´s BioPro IEX show high dynamic binding capacity and excellent recovery, making them useful for semi-preparative separations of proteins and antibodies.

Comparison of dynamic binding capacity (DBC) for BSA

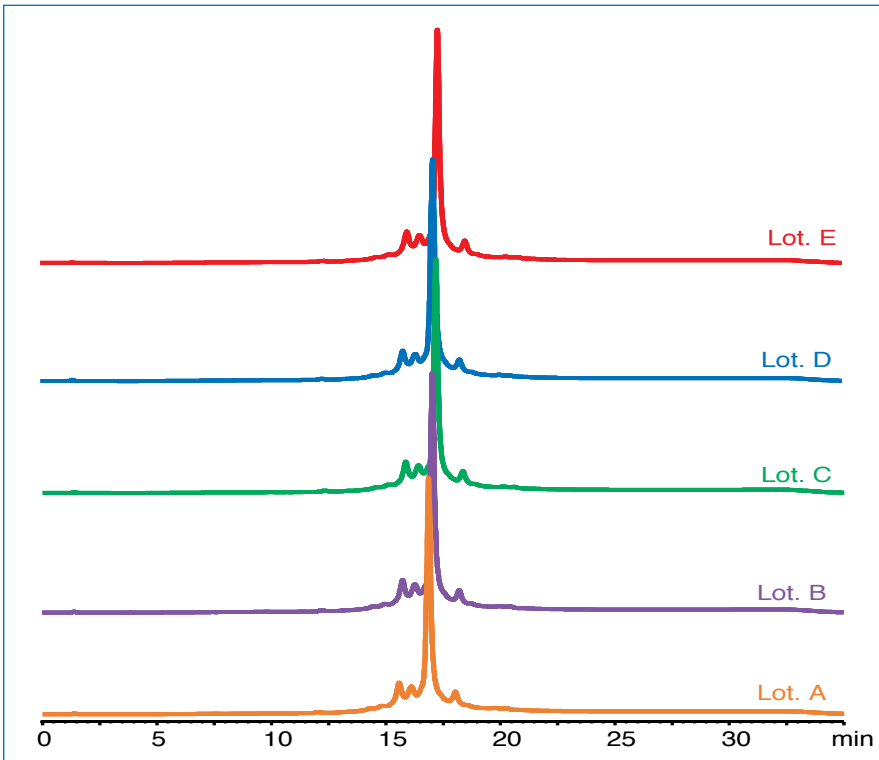
	Dynamic binding capacity (mg/mL-gel, 10% breakthrough)	Eluted amount (mg/mL-gel)	Recovery* (%)
BioPro IEX QA	126	120	95
Mono Q (GE Healthcare)	100	35	35
BioAssist Q (Tosoh Bioscience)	73	58	79

* Recovery: (Eluted amount/Dynamic binding capacity) x 100

High recovery rates for BioPro IEX

Compared with conventional porous polymer anion exchange columns, BioPro IEX QA provides higher DBC and recovery rates. This indicates that BioPro IEX has a much lower nonspecific adsorption compared to conventional columns.

Excellent batch-to-batch reproducibility



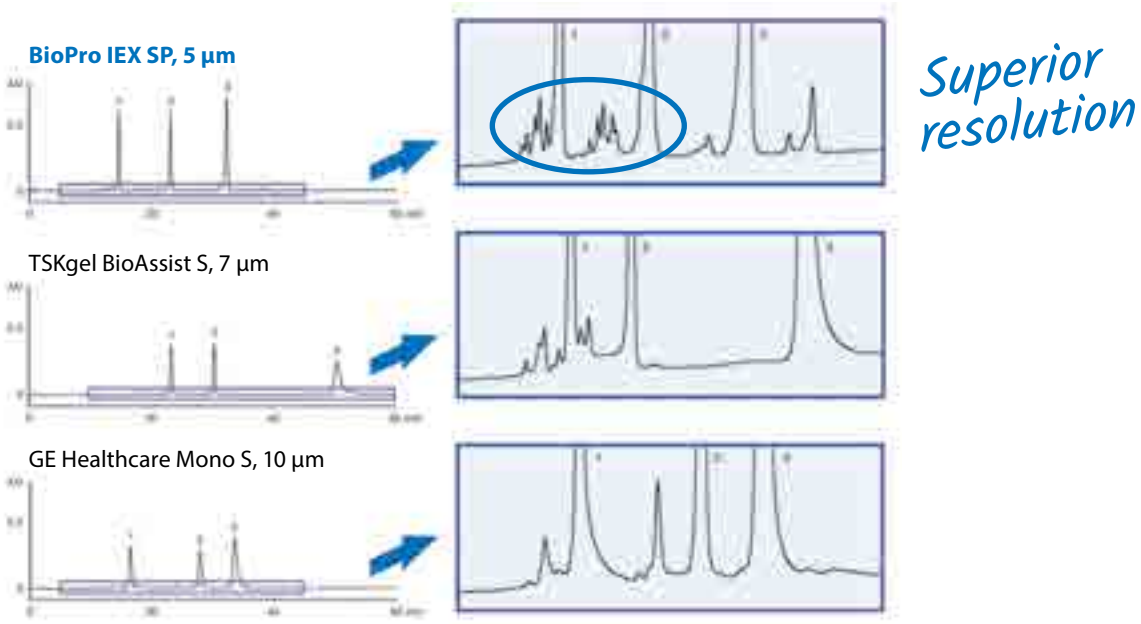
Column: BioPro IEX SF (5 µm) 100 x 4.6 mm ID
Part No.: SF00S05-1046WP
Eluent: A) 20 mM NaH_2PO_4-Na_2HPO_4 (pH 6.5)
B) 20 mM NaH_2PO_4-Na_2HPO_4 (pH 6.5) containing 0.2 M NaCl
Gradient: 0-50%B (0.5-30 min)
Flow rate: 0.5 mL/min (180 cm/hr)
Temperature: 25 °C
Detection: UV at 215 nm
Injection: 10 µL
Sample: monoclonal antibody (IgG1)

BioPro IEX SF columns exhibit excellent batch-to-batch reproducibility for MAb analysis with resolution of peaks for small charge variants.
All gel batches are inspected by rigorous quality control tests, including HPLC analysis of MAb, and must meet the required criteria before release.
BioPro IEX columns are the best choice for the quality control of MAb and other biopharmaceuticals.

IEX – BioPro IEX: Resolution & throughput

Superior resolution

Comparison of standard protein separation on BioPro IEX SP and commercial S type products

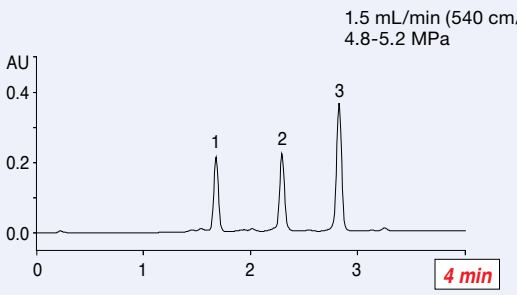


Eluent:	A) 20 mM KH_2PO_4-K_2HPO_4 (pH 6.8)		Detection:	UV at 220 nm	
	B) 20 mM KH_2PO_4-K_2HPO_4 (pH 6.8) containing 0.5 M NaCl		Injection:	BioPro IEX SP, TSKgel BioAssist S	20 µL
Gradient:	0-100%B (0-60 min)			GE Healthcare Mono S	23.6 µL
Flow rate:	BioPro IEX SP, TSKgel BioAssist S	0.5 mL/min	Sample:	1. Ribonuclease A (0.5 mg/mL)	
	GE Healthcare Mono S	0.59 mL/min		2. Cytochrome *c* (0.5 mg/mL)	
Temperature:	25 °C			3. Lysozyme (0.5 mg/mL)	

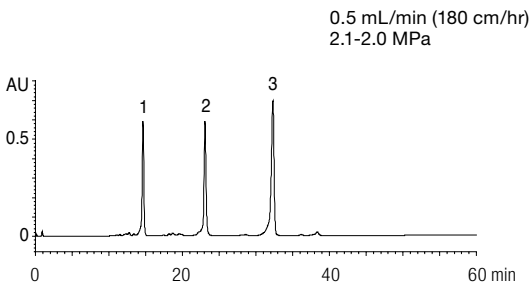
Only BioPro IEX is available in the smaller particle size and is therefore able to provide superior resolution.

Ultra-high-throughput analysis with non-porous BioPro IEX

Non-porous type
BioPro IEX SF (5 µm) 30 x 4.6 mm ID



Porous type
BioPro IEX SP (5 µm) 50 x 4.6 mm ID



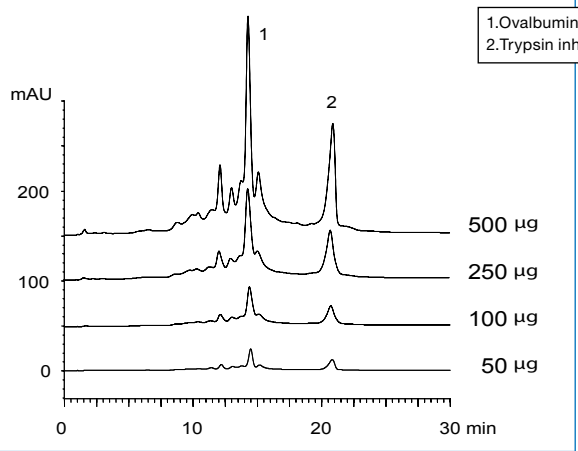
Eluent:	A) 20 mM KH_2PO_4-K_2HPO_4 (pH 6.8)	Temperature:	25 °C	
	B) 20 mM KH_2PO_4-K_2HPO_4 (pH 6.8) containing 0.5 M NaCl	Detection:	UV at 220 nm	
Part Nos.:	SF00S05-0346WP (non-porous)	Injection:	20 µL	
	SPA0S05-0546WP (porous)	Sample:	1. Ribonuclease A (0.5 mg/mL)	
Gradient:	0-100%B (0-4 min) for BioPro IEX SF		2. Cytochrome *c* (0.5 mg/mL)	
	0-100%B (0-60 min) for BioProIEX SP		3. Lysozyme (0.5 mg/mL)	

The high mechanical stability of non-porous polymer beads and the short column length allow faster elution of proteins at a higher flow rate without any loss of resolution.

High loadability

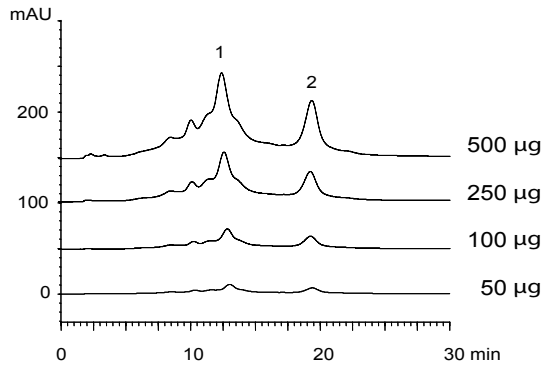
Loading study for BioPro IEX QA (porous)

BioPro IEX QA (5 µm) 50 x 4.6 mm ID



Part No.: QAA0S05-0546WP
Eluent: A) 20 mM Tris-HCl (pH 8.1)
B) 20 mM Tris-HCl (pH 8.1) containing 0.5 M NaCl
Gradient: 10-80%B (0-30 min)
Flow rate: 0.5 mL/min
Temperature: 25 °C
Detection: UV at 280 nm
Injection: 100 µL

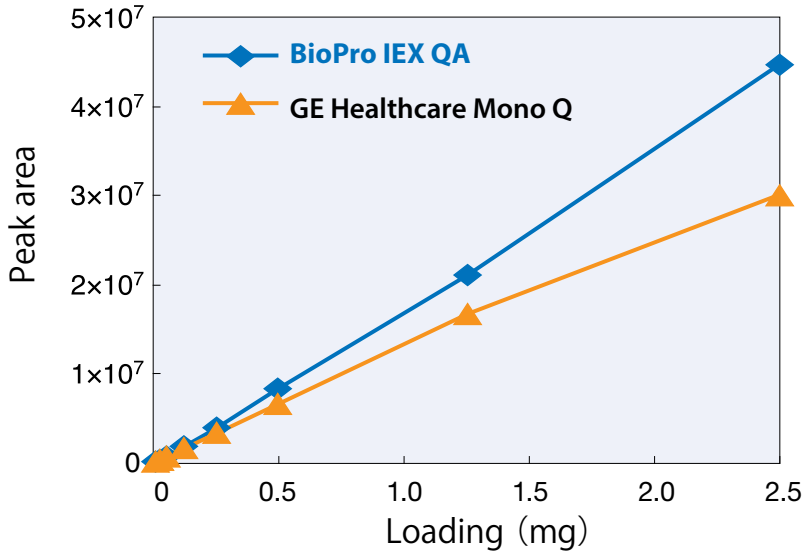
GE Healthcare Mono Q (10 µm) 50 x 5.0 mm ID



Eluent: A) 20 mM Tris-HCl (pH 8.1)
B) 20 mM Tris-HCl (pH 8.1) containing 0.5 M NaCl
Gradient: 10-80%B (0-30 min)
Flow rate: 0.5 mL/min
Temperature: 25 °C
Detection: UV at 280 nm
Injection: 100 µL

High recovery

Recovery of trypsin inhibitor



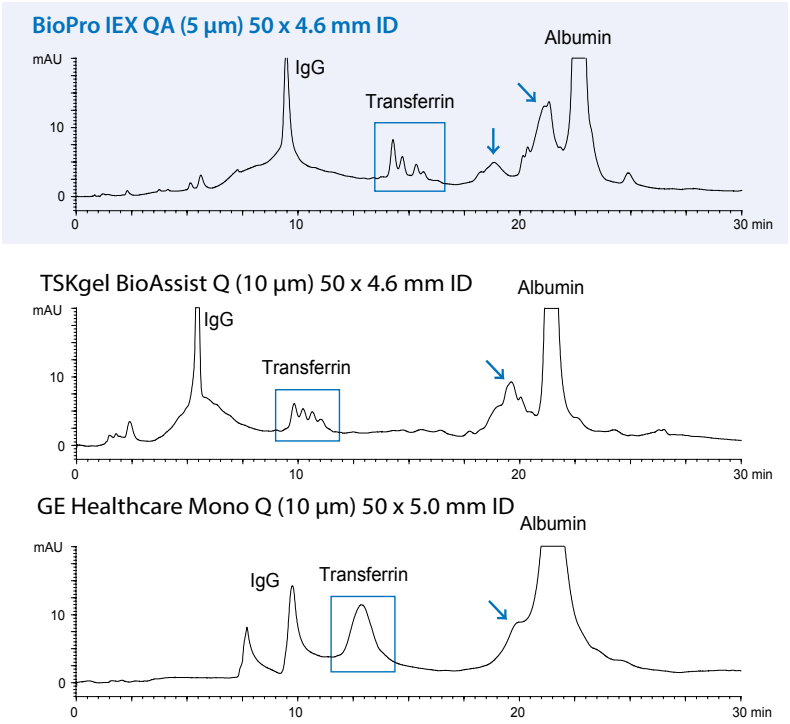
Eluent: A) 20 mM Tris-HCl (pH 8.1)
B) 20 mM Tris-HCl (pH 8.1) containing 0.5 M NaCl
Gradient: 10-80%B (0-30 min)
Flow rate: 0.5 mL/min (180 cm/hr for 4.6 mm ID,
150 cm/hr for 5.0 mm ID)
Temperature: 25 °C
Detection: UV at 280 nm
Injection: 100 µL

BioPro IEX QA shows excellent resolution and peak shapes even when the loading amount is increased. The porous type BioPro IEX columns are suitable for laboratory-scale purification of proteins.

IEX – BioPro IEX: Challenging separations

Protein separation in challenging matrices

Separation of proteins in human serum on BioPro IEX QA and commercial Q-type products



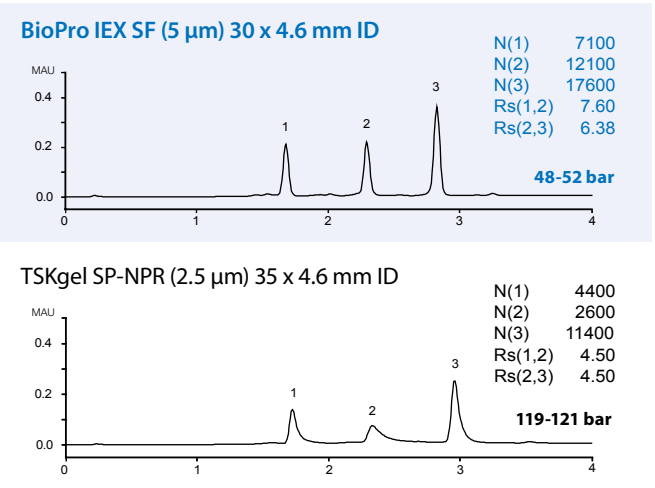
For high resolution porous BioPro IEX QA/SP is recommended!

Part No.:	QAA0S05-0546WP	Flow rate:	0.5 mL/min
Eluent:	A) 20 mM Tris-HCl (pH 8.6) B) 20 mM Tris-HCl (pH 8.6) containing 0.5 M NaCl	Temperature: Detection: Injection:	25 °C UV at 280 nm 20 µL
Gradient:	0-30%B (0-15 min), 30-100%B (15-30 min)	Sample:	Human serum (100 µL/mL)

Better performance at lower backpressure

Comparison of standard protein separation on BioPro IEX SF and a commercial SP-type product

higher plate count



BioPro IEX SF elutes the proteins in sharper peaks without peak-tailing compared to TSKgel SP-NPR.
Despite the larger particle size, the theoretical plate count for BioPro IEX SF is higher than that for TSKgel SP-NPR.

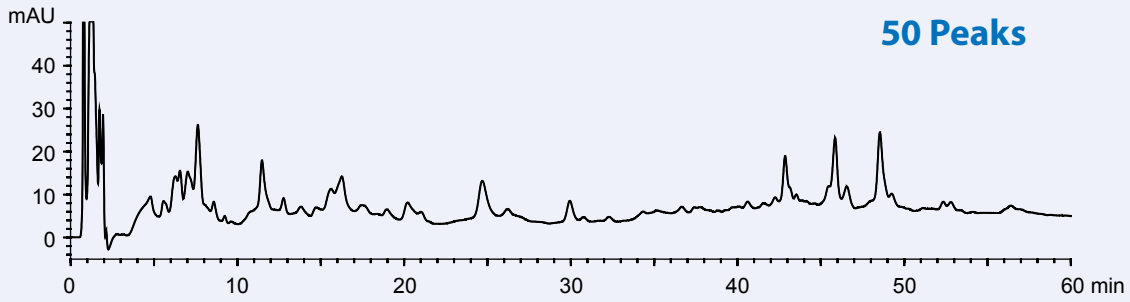
Part No.:	SF00S05-0346WP	
Eluent:	A) 20 mM KH_2PO_4-K_2HPO_4 (pH 6.8) B) 20 mM KH_2PO_4-K_2HPO_4 (pH 6.8) containing 0.5 M NaCl	
Gradient:	BioPro IEX SF 0-100%B (0-4 min) TSKgel SP-NPR 0-100%B (0-4.67 min)	
Flow rate:	1.5 mL/min	
Temperature:	25 °C	
Detection:	UV at 220 nm	
Injection:	20 µL	
Sample:	1. Ribonuclease A 2. Cytochrome *c* 3. Lysozyme	(0.1 mg/mL) (0.1 mg/mL) (0.1 mg/mL)

Compared to the competitor's column, BioPro IEX SF gives higher theoretical plate counts, excellent peak shapes, and lower backpressures. This makes BioPro IEX SF most suitable for high-throughput analysis.

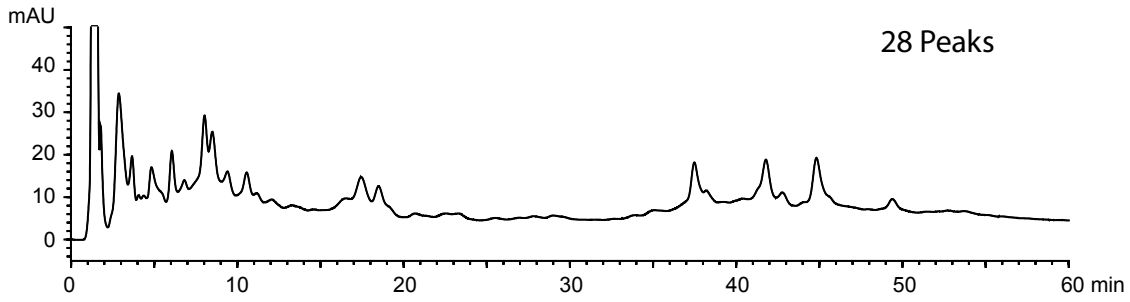
Peptide mapping

Peptide mapping of tryptic digests of BSA with enhanced sensitivity

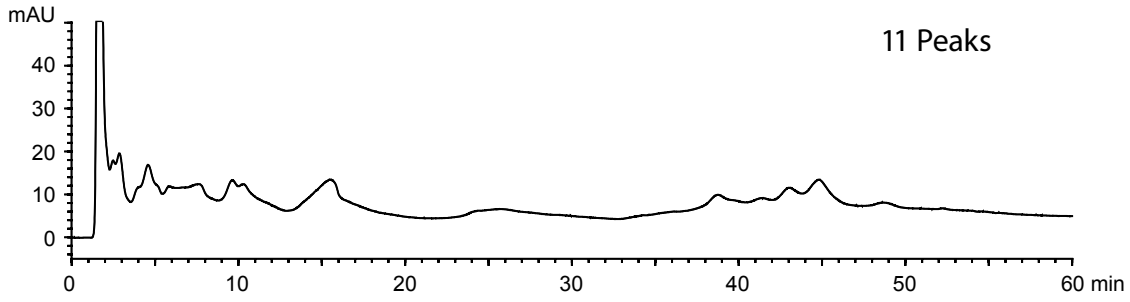
BioPro IEX QA (5 µm) 50 x 4.6 mm ID



TSKgel BioAssist Q (10 µm) 50 x 4.6 mm ID



GE Healthcare Mono Q (10 µm) 50 x 5.0 mm ID

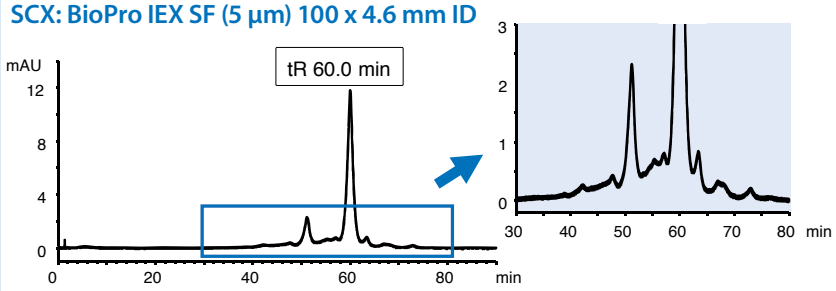


Part No.:	QAA0S05-0546WP
Eluent:	A) 20 mM Tris-HCl (pH 8.6) B) 20 mM Tris-HCl (pH 8.6) containing 0.5 M NaCl
Gradient:	0-15%B (0-30 min), 15-60%B (30-60 min)
Flow rate:	0.5 mL/min
Temperature:	25 °C
Detection:	UV at 220 nm
Injection:	20 µL
Sample:	Tryptic digest of BSA

IEX – BioPro IEX: Antibody analysis

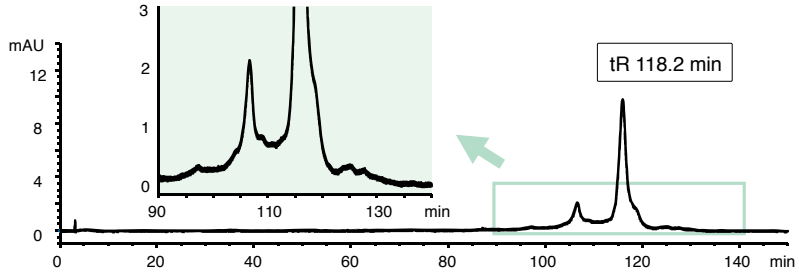
Monoclonal antibody analysis with non-porous cation exchange columns

SCX: BioPro IEX SF (5 µm) 100 x 4.6 mm ID



Higher Resolution!

WCX: Pro Pac WCX-10 (10 µm) 250 x 4.0 mm ID

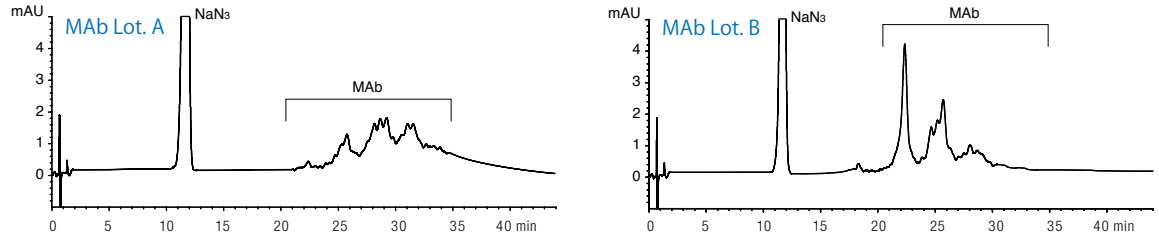


Part No.:	SF00S05-1046WP	Temperature:	30 °C
Eluent:	A) 20 mM MES-NaOH (pH 5.6) B) 20 mM MES-NaOH (pH 5.6) containing 0.2 M NaCl	Detection:	UV at 280 nm
		Injection:	10 µL
Gradient:	35%B (70 mM NaCl	Sample:	Humanized monoclonal IgG1 (1 mg/mL)
Gradient slope:	0.25%B/min (0.5 mM NaCl)		
Flow rate:	180 cm/hr (0.5 mL/min for 100 x 4.6 mm ID, 0.378 mL/min for 250 x 4.0 mm ID)		

The separation of MAb is compared using a strong cation (BioPro IEX SF) and a weak cation exchange column (ProPac WCX-10) under the same gradient conditions at pH 5.6. BioPro IEX SF can achieve a higher resolution of MAb than the competitor's column in a shorter analysis time.

QC of monoclonal antibodies with non-porous BioPro IEX QF

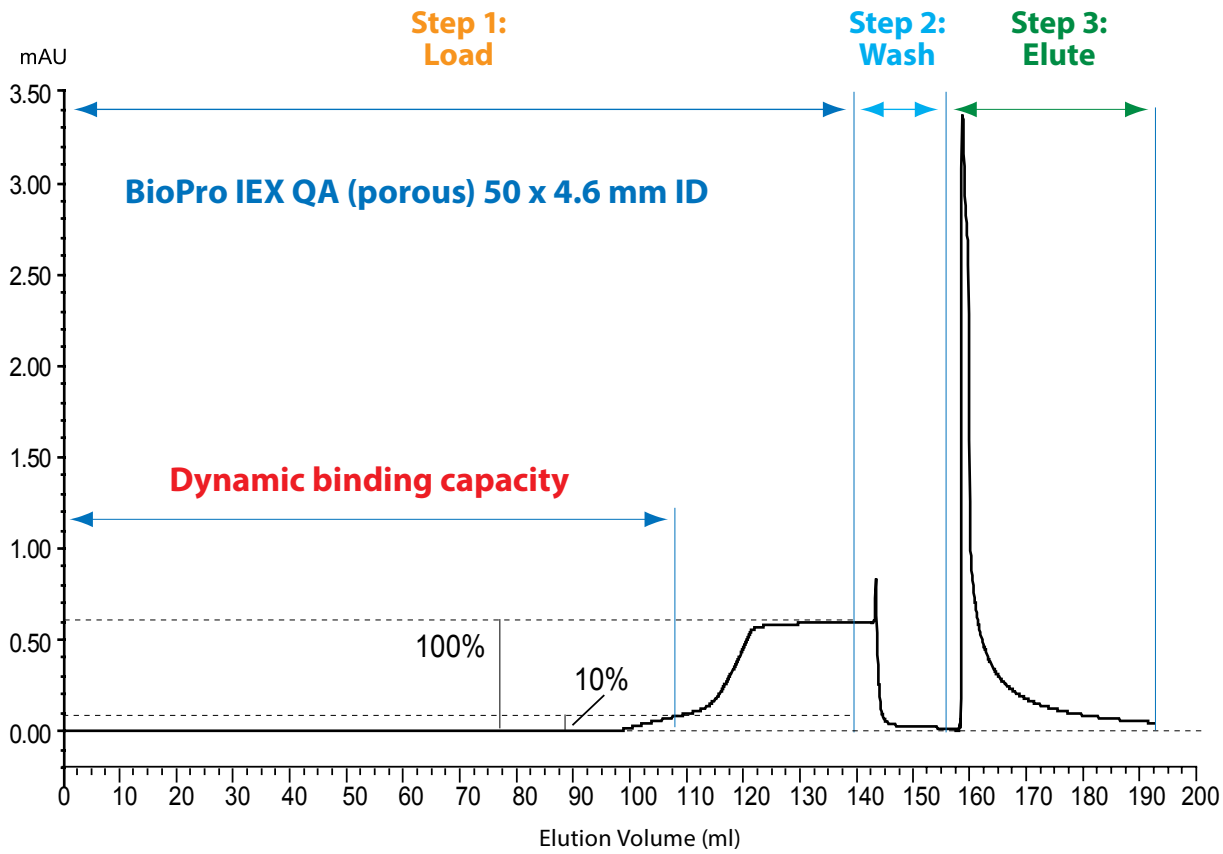
BioPro IEX QF (5 µm) 100 x 4.6 mm ID



Part No.:	QF00S05-1046WP	Temperature:	25 °C
Eluent:	A) 20 mM Tris-HCl (pH 8.1) B) 20 mM Tris-HCl (pH 8.1) containing 0.5 M NaCl	Detection:	UV at 220 nm
		Injection:	14 µL (0.1 mg/mL)
Gradient:	10-25%B (0-60 min)	Sample:	Mouse monoclonal IgG1 anti-human IgG4 (Purified by DEAE chromatography, containing NaN_3)
Flow rate:	1.0 mL/min (360 cm/hr)		

Two different batches of commercially available MAb purified by DEAE chromatography were analyzed on a BioPro IEX QF column (100 mm length).
The MAb was separated into several peaks, and the batch-to-batch variability is observed. The BioPro IEX QF/SF 100 mm length columns, which have high efficiency, are ideal for characterization of glycoproteins, such as monoclonal antibodies, and for quality control assessment of biopharmaceuticals.

Determination of Dynamic Binding Capacity*



Before determination, equilibrate the column with equilibration buffer.

Step 1: Load

A protein solution of known concentration is continuously loaded onto the column at the desired flow rate and the absorbance of the eluate is monitored until full saturation is achieved (100% UV absorbance of the pure sample solution).

Step 2: Wash

Wash the column with equilibration buffer until no more protein elutes (0% UV absorbance).

Step 3: Elute

The DBC of the medium is a measure of the volume of protein solution that has been applied up to a specific breakthrough point (usually 5 or 10%).

* Application data by courtesy YMC Co., Ltd.

IEX – Ordering information

3 µm non-porous analytical columns in PEEK hardware (max. pressure 250 bar)

Phase	Column ID [mm]	Column length [mm]			Precolumn filter 2 µm*
		30 (250 bar)	50 (250 bar)	100 (250 bar)	(pack of 5)
BioPro IEX QF	4.6	QF00S03-0346WP	QF00S03-0546WP	QF00S03-1046WP	XRPRCP25
BioPro IEX SF	4.6	SF00S03-0346WP	SF00S03-0546WP	SF00S03-1046WP	

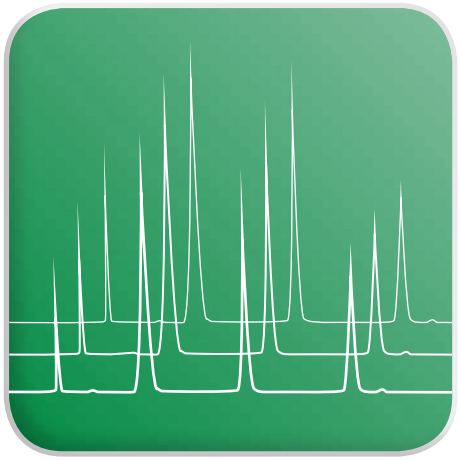
5 µm non-porous analytical columns in PEEK hardware (max. pressure 60–120 bar)

Phase	Column ID [mm]	Column length [mm]			Precolumn filter 2 µm*
		30 (60 bar)	50 (100 bar)	100 (120 bar)	(pack of 5)
BioPro IEX QF	4.6	QF00S05-0346WP	QF00S05-0546WP	QF00S05-1046WP	XRPRCP25
BioPro IEX SF	4.6	SF00S05-0346WP	SF00S05-0546WP	SF00S05-1046WP	

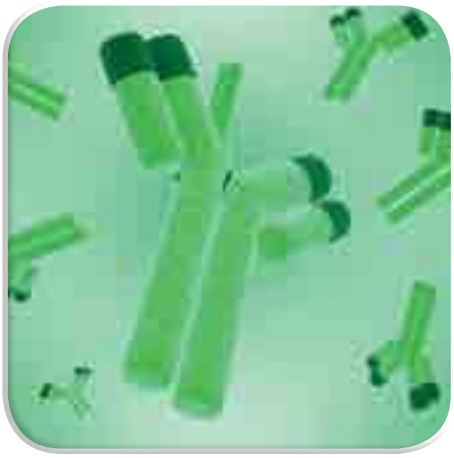
5 µm porous analytical columns in PEEK hardware (max. pressure 25–35 bar)

Phase	Column ID [mm]	Column length [mm]			Precolumn filter 2 µm*
		30 (25 bar)	50 (30 bar)	100 (35 bar)	(pack of 5)
BioPro IEX QA	4.6	QAA0S05-0346WP	QAA0S05-0546WP	QAA0S05-1046WP	XRPRCP25
BioPro IEX SP	4.6	SPA0S05-0346WP	SPA0S05-0546WP	SPA0S05-1046WP	

Other dimensions on demand
* Holder required, part no. XRPRCP02




HIC

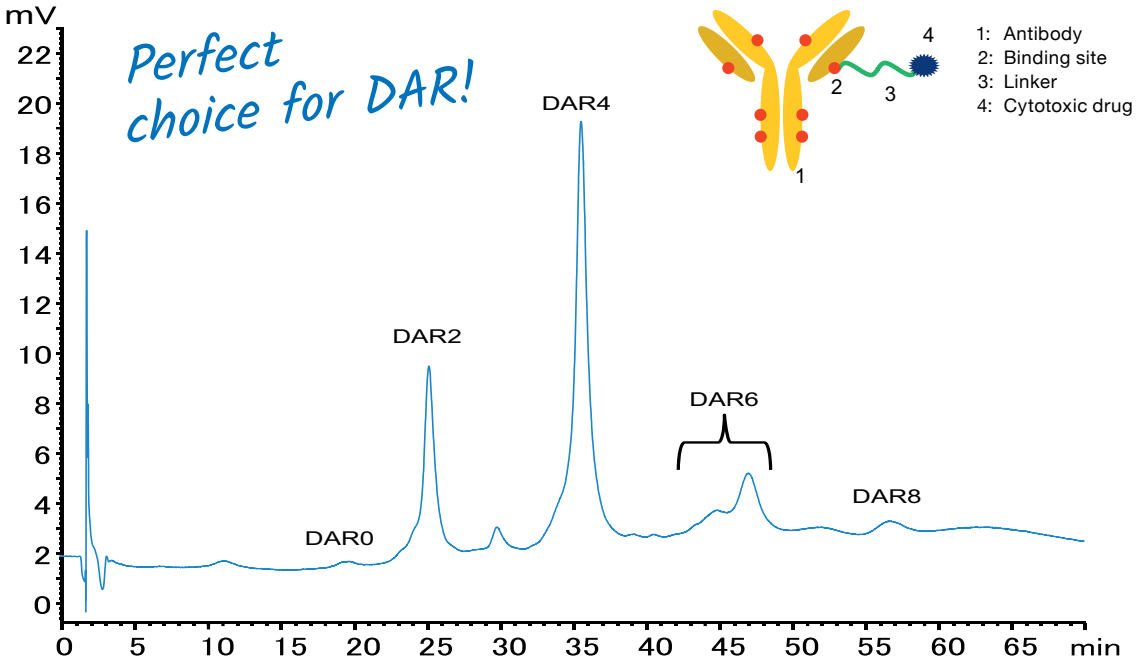


HIC – BioPro Series

- **Specifically designed for antibodies, antibody-drug conjugates (ADCs), proteins**
- **High performance separation**
- **High throughput analyses**
- **Excellent lot-to-lot reproducibility**
- **Long term stability**
- **Virtually no carryover effects**

	BioPro HIC BF
Base particle	hydrophilic polymer (polymethacrylate)
Particle size	4 µm
Pore	non-porous
Functional group	butyl
pH range	2 – 12
Temperature range	10 – 60 °C

Designed for Drug-to-Antibody Ratio (DAR) analysis of ADCs

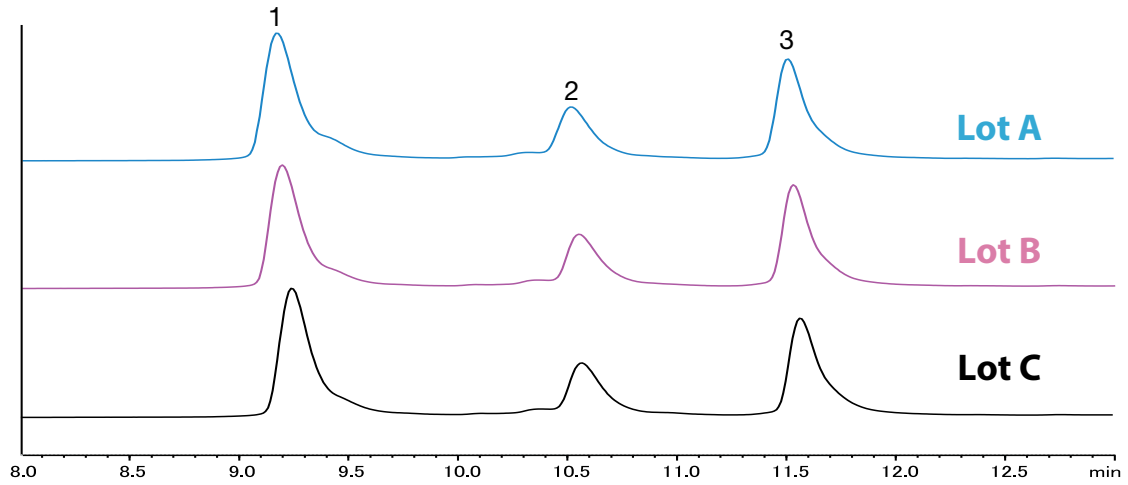


Column: BioPro HIC BF (4 µm) 100 x 4.6 mm ID
Part No.: BHB00S04-1046WT
Eluent: A) 50 mM NaH_2PO_4-Na_2HPO_4 (pH 6.8) containing 1.5 M $(NH_4)_2SO_4$/IPA (95/5)
B) 50 mM NaH_2PO_4-Na_2HPO_4 (pH 6.8)/IPA (80/20)
Gradient: 30%B (0-5 min)
30-80%B (5-45 min)
80%B (45-70 min)
Flow rate: 0.4 mL/min
Temperature: 25 °C
Detection: UV at 280 nm
Injection: 20 µL

Sample:
Cystein-conjugated ADC mimic (1.25 mg/mL)

BioPro HIC BF is suitable for ADC (antibody-drug conjugate) analysis, and is especially effective for monitoring of DAR (drug-to-antibody ratio).

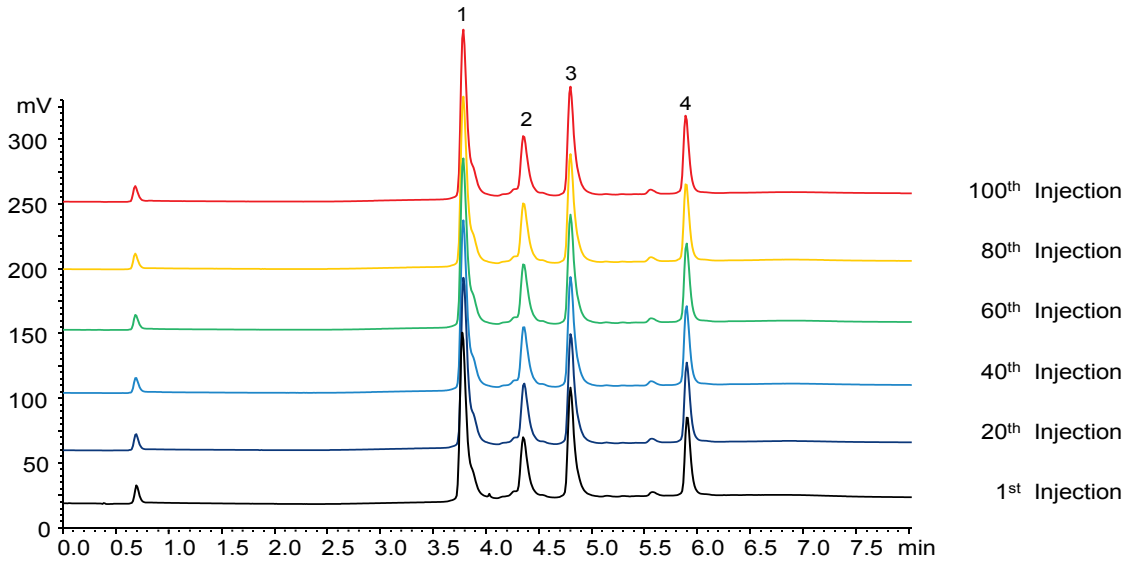
Excellent lot-to-lot reproducibility



Column: BioPro HIC BF (4 µm) 100 x 4.6 mm ID
Part No.: BHB00S04-1046WT
Eluent: A) 100 mM NaH_2PO_4-Na_2HPO_4 (pH 7.0) containing 2.0 M $(NH_4)_2SO_4$
B) 100 mM NaH_2PO_4-Na_2HPO_4 (pH 7.0)
Gradient: 0-100%B (0-11 min), 100%B (11-15 min)
Flow rate: 0.5 mL/min
Temperature: 25 °C
Detection: UV at 280 nm
Injection: 15 µL

Samples:
1. Myoglobin (0.73 mg/mL)
2. Ribonuclease A (0.75 mg/mL)
3. Lysozyme (0.25 mg/mL)

High column stability

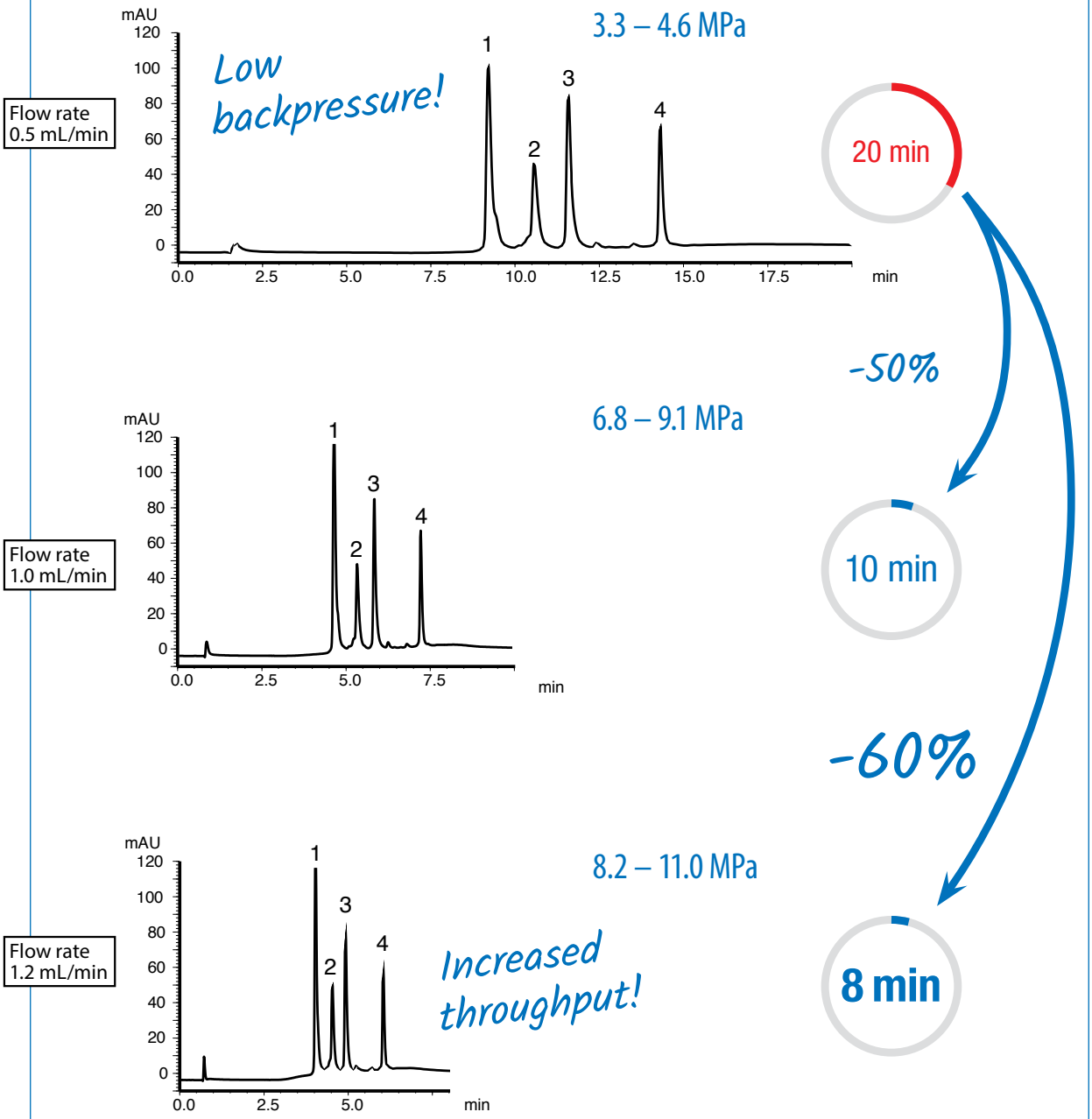


Column: BioPro HIC BF (4 µm) 100 x 4.6 mm ID
Part No.: BHB00S04-1046WT
Eluent: A) 100 mM NaH_2PO_4-Na_2HPO_4 (pH 7.0) containing 2.0 M $(NH4)_2SO_4$
B) 100 mM NaH_2PO_4-Na_2HPO_4 (pH 7.0)
0-100%B (0-4.58 min), 100%B (4.58-6.25 min)
Flow rate: 1.2 mL/min
Temperature: 25 °C
Detection: UV at 280 nm
Injection: 15 µL

Optimised column packing method results in highly stable performance over a long time.

HIC – BioPro HIC: Throughput & resolution

High throughput at high resolution



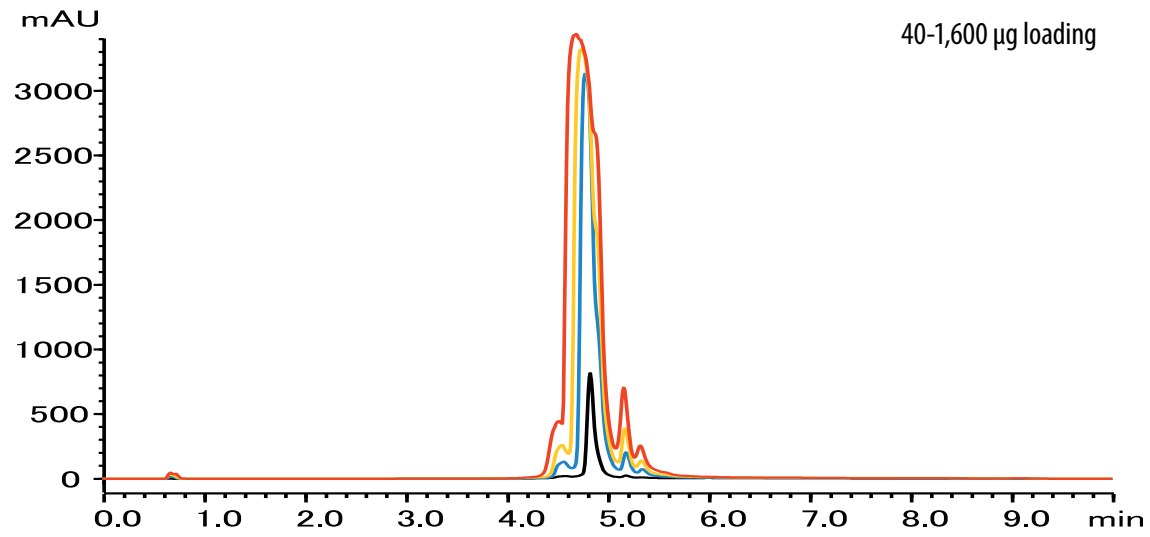
Column:	BioPro HIC BF (4 µm) 100 × 4.6 mm ID		
Part No.:	BHB00S04-1046WT		
Eluent:	A) 100 mM NaH_2PO_4-Na_2HPO_4 (pH 7.0) containing 2.0 M $(NH_4)_2SO_4$		
	B) 100 mM NaH_2PO_4-Na_2HPO_4 (pH 7.0)		
Gradient 0.5 mL/min:	0-100% B (0-11 min) 100% B (11-15 min)		
1.0 mL/min:	0-100% B (0-5.5 min) 100% B (5.5-7.5 min)		
1.2 mL/min:	0-100% B (0-4.58 min) 100% B (4.58-6.58 min)		
Temperature:	25 °C		
Detection:	UV at 280 nm	Injection:	15 µL

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

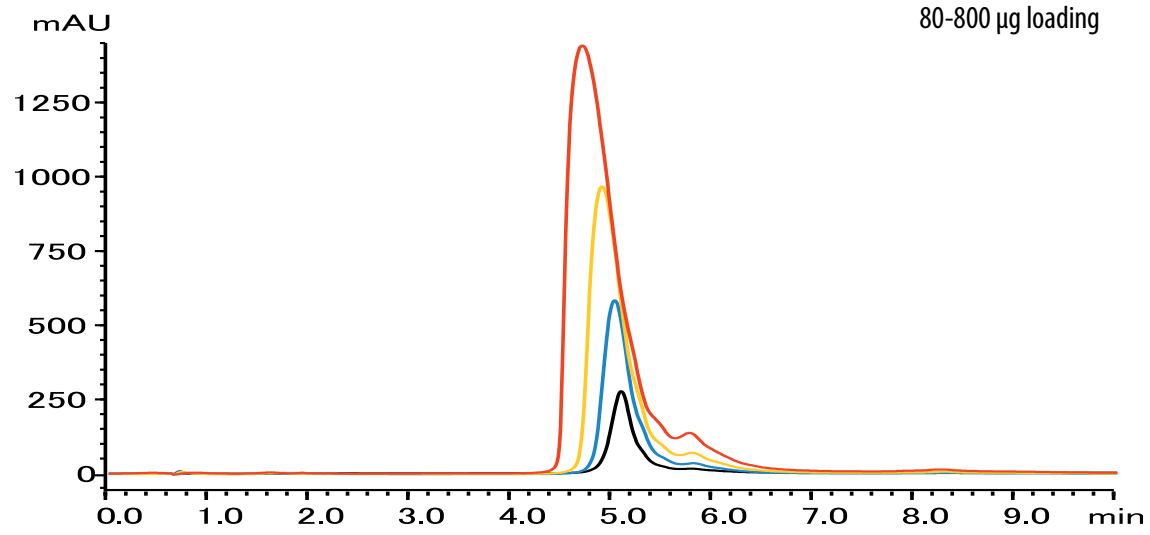
Due to its intermediate particle size, BioPro HIC BF can improve analysis throughput.

High loadability

Lysozyme



Humanized monoclonal IgG

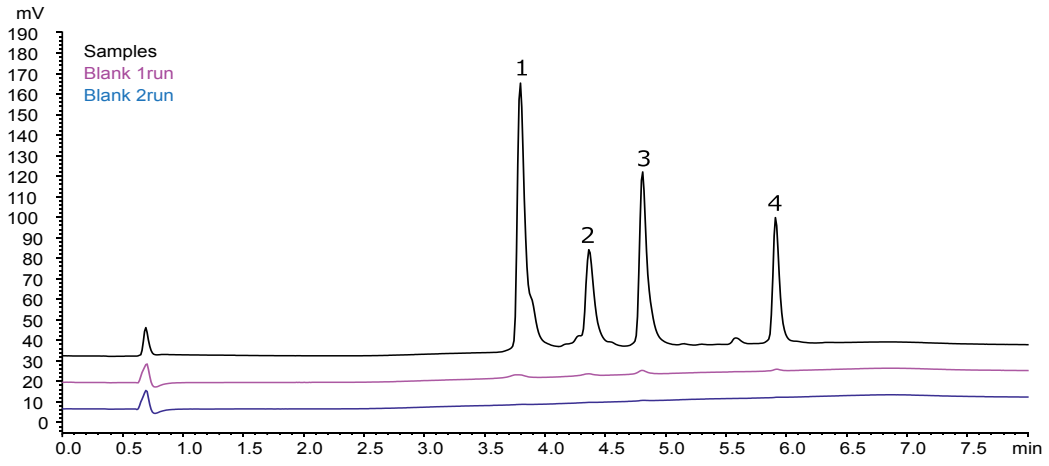


Column:	BioPro HIC BF (4 µm) 100 x 4.6 mm ID
Part No.:	BHB00S04-1046WT
Eluent:	A) 100 mM NaH_2PO_4-Na_2HPO_4 (pH 7.0) containing 2.0 M $(NH_4)_2SO_4$ B) 100 mM NaH_2PO_4-Na_2HPO_4 (pH 7.0)
Gradient:	0-100%B (0-4.58 min), 100%B (4.58-6.58 min) for lysozyme 60%B(0-0.5 min), 60-100%B (0.5-7.5 min), 100%B (7.5-10 min) for IgG
Flow rate:	1.2 mL/min
Temperature:	25 °C for lysozyme 30 °C for IgG
Detection:	UV at 280 nm
Injection:	4, 40, 80, 160 µL for lysozyme 32, 80, 160, 320 µL for IgG
Sample:	Lysozyme (10 mg/mL) Humanized monoclonal IgG (2.5 mg/mL)

BioPro HIC BF provides superior peak shapes, even under high loading conditions. This allows detection of very tiny amounts of impurities in the sample.
In addition, it can be used for lab-scale purifications e.g. for isolation of variants for various research requirements (e. g. structural analysis).

HIC – BioPro HIC: No carry over & ADC monitoring

Suppressed carryover

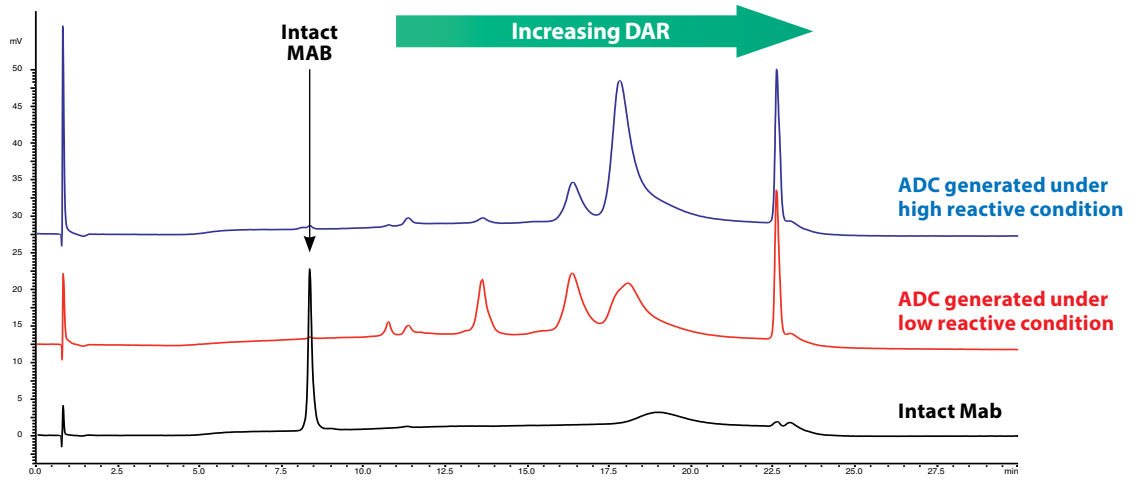


Column:	BioPro HIC BF (4 µm) 100 x 4.6 mm ID
Part No.:	BHB00S04-1046WT
Eluent:	A 100 mM NaH_2PO_4-Na_2HPO4 (pH 7.0) containing 2.0 M $(NH_4)_2SO_4$
	B)100 mM NaH_2PO_4-Na_2HPO_4 (pH 7.0)
Gradient:	0-100%B (0-4.58 min), 100%B (4.58-6.25 min)
Flow rate:	1.2 mL/min
Temperature:	25 °C
Detection:	UV at 280 nm
Injection:	15 µL

Samples:
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)

Carryover between analyses is minimized on BioPro HIC BF. This contributes significantly to reproducibility of analyses.

Characterisation and reaction monitoring of ADCs



Column:	BioPro HIC BF (4 µm) 100 x 4.6 mm ID
Par No.:	BHB00S04-1046WT
Eluent:	A) 50 mM NaH_2PO_4-Na_2HPO_4 (pH 7.0) containing 1.5 M $(NH_4)_2SO_4$/2-propanol (95/5)
	B) 50 mM NaH_2PO_4-Na_2HPO_4 (pH 7.0)/2-propanol (80/20)
Gradient:	0%B (0-1 min), 0-100%B (1-15 min), 100%B (15-20 min), 0%B (20-30 min)
Flow rate:	1.0 mL/min
Temperature:	25 °C
Detection:	UV at 280 nm
Sample:	Antibody Drug Conjugate*

ADCs generated under various reaction conditions are shown. BioPro HIC BF is suitable for characterisation as well as reaction monitoring of ADC production.

The influence of salts in HIC separations

The technique known as hydrophobic interaction chromatography is a mode of chromatography that separates proteins by differences in surface hydrophobicity.[1] This method utilises reversible interactions that occur between protein molecules and hydrophobic stationary phase ligands attached to the particle surface.

Certain non-denaturing salts are used to improve the hydrophobic interactions between proteins and the stationary phase. The mobile phase is typically an aqueous solution of salts such as ammonium sulfate or sodium chloride and a buffer to control pH (usually phosphate buffer between pH 6 and 7). The Hofmeister series of lyotropic and chaotropic ions shown below in Fig. 1 provides a template for salt selection. High concentrations of salt, particularly ammonium sulfate, may precipitate proteins; therefore, solubility should be checked under the initial gradient (binding) conditions. The strength of the interaction between the protein and stationary phase decreases with decreasing salt gradient (see Fig. 2). Another option is a change of pH which results in an increase in the charge on the protein due to the ionisation of acidic groups.

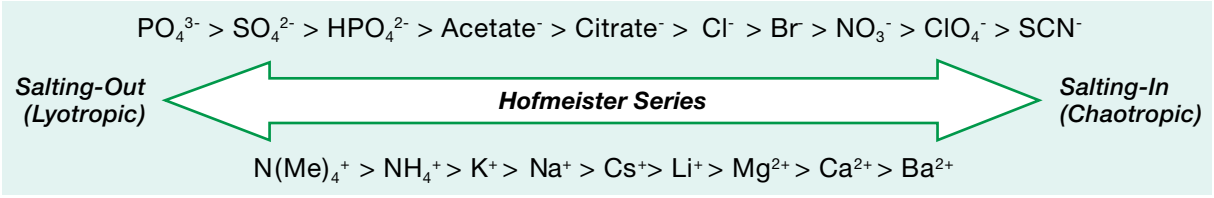


Fig. 1: The Hofmeister Series of lyotropic and chaotropic ions.

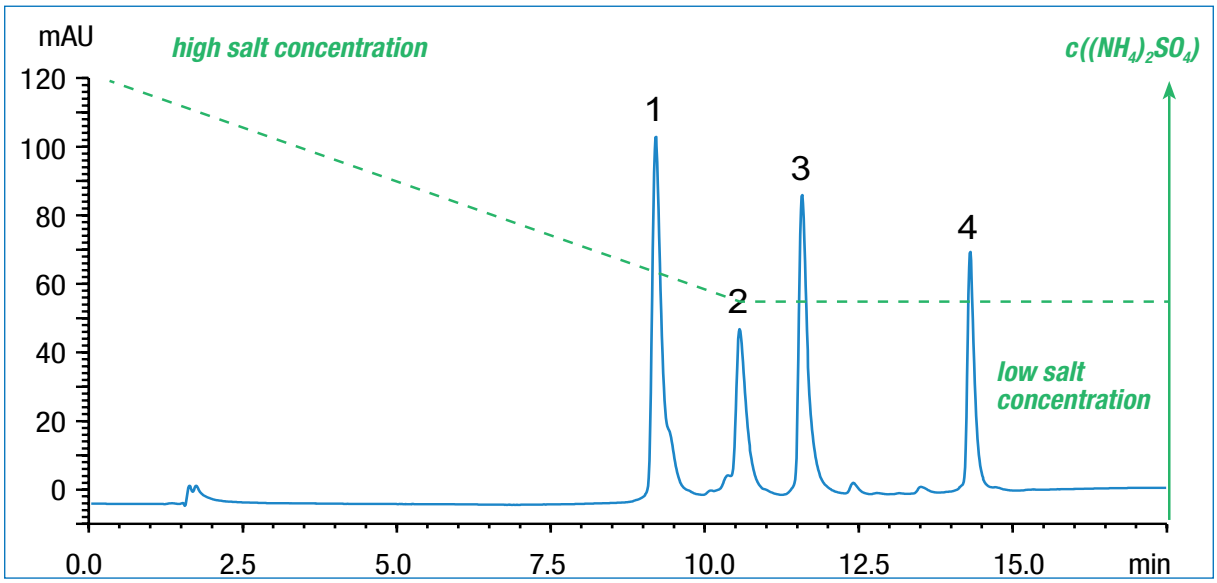


Fig. 2: Method with decreasing salt gradient.

Column:	BioPro HIC BF (100 x 4.6 mm ID)
Part No.:	BHB00S04-1046WT
Eluent:	A) 100 mM NaH2PO4-Na2HPO4 (pH 7.0) containing 2.0 M (NH4)2SO4 B) 100 mM NaH2PO4-Na2HPO4 (pH 7.0)
Flow:	0.5 mL/min
Gradient:	0-100% B (0-11 min) 100% B (11-15 min)
Temp.:	25 °C Detection: UV at 280 nm
Injection:	15 µL
Samples:	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 gm/mL).

HIC is particularly effective when used to separate proteins and monoconal antibodies. The separation of monoclonal antibodies (MAb), MAb aggregates and glycosylated MAbs can be achieved due to their specific hydrophobic properties. It also provides an excellent method for determination of drug-to-antibody ratios (DAR) in antibody-drug conjugates (ADCs).

[1] Queiroza, J.A.; Tomaza, C.T.; Cabral, J.M.: Hydrophobic interaction chromatography of proteins, J Biotechnol. 2001, 87, 143-159.

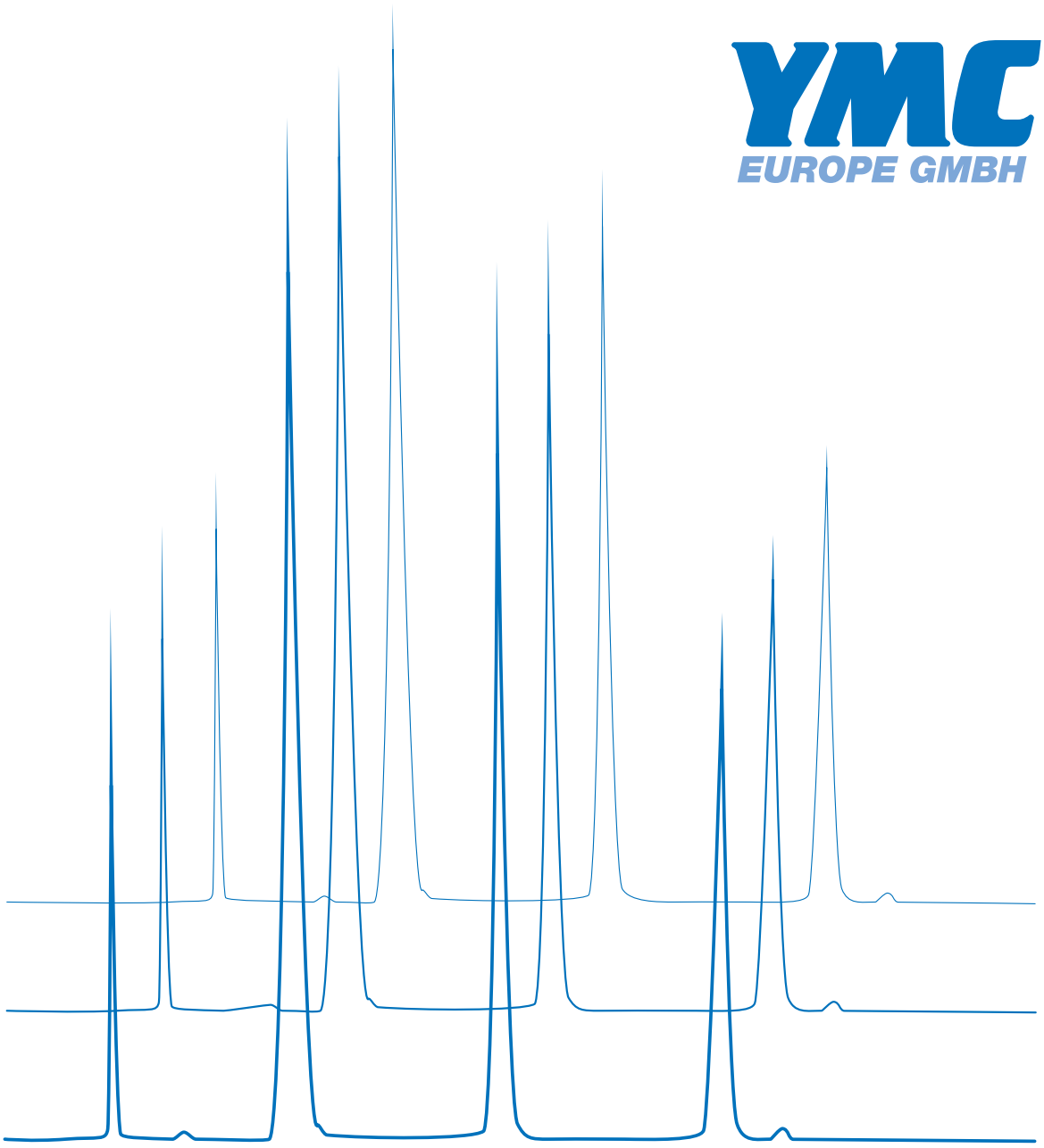
HIC – Ordering information

4 µm HPLC column

Phase	Column ID [mm]	Column length [mm]	Precolumn filter 2 µm
		100	(pack of 5)
BioPro HIC BF	4.6	BHB00S04-1046WT	XRPRCP25

Holder required, part no. XRPRCP02

YMC
EUROPE GMBH



Reproducibility...

...YMC

Application data mainly by courtesy of YMC Co., Ltd.

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(U)HPLC columns YMC-Triart

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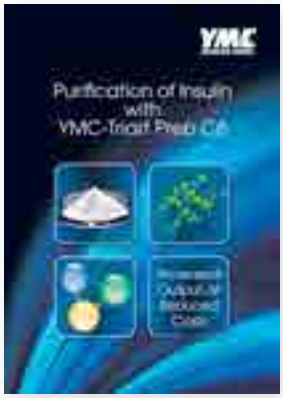
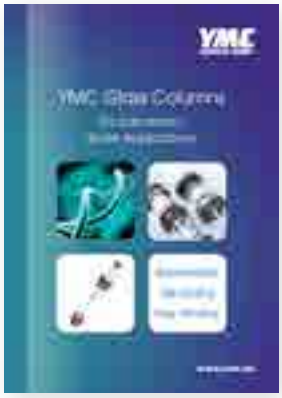


Purification of Insulin with YMC-Triart Prep C8



YMC Glass Columns

BioPro IEX Resins

YMC Pilot^{Plus}

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Substance index

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