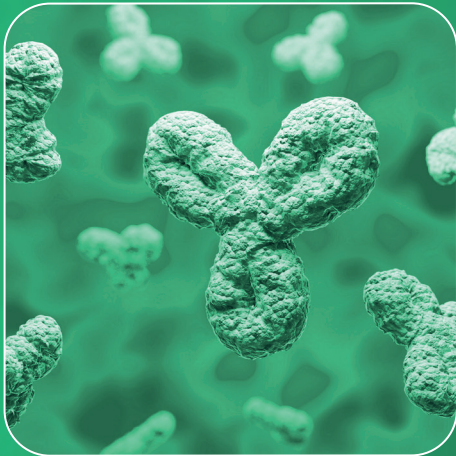
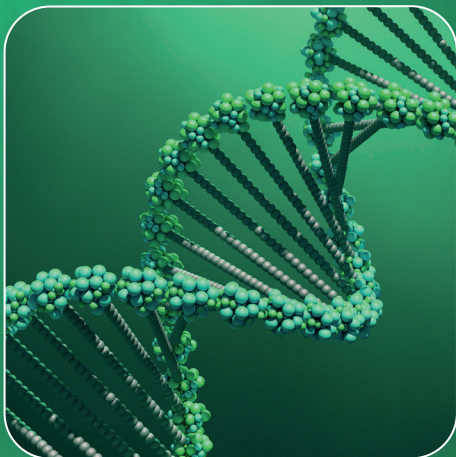


YMC

YMC Biochromatography Columns



RP
SEC
IEX
HIC
HILIC



HPLC Columns for Biochromatography

	Reversed Phase (RP)	Size Exclusion (SEC)	Ion Exchange (IEX)	Hydrophobic Interaction (HIC)	Hydrophilic Interaction (HILIC)
Separation principle	Hydrophobicity	Molecular size	Electric charge	Hydrophobicity	Hydrophilicity
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	Up to about 30,000 Da
Resolution	+++	++	+++	+++	+++
Speed	+++	+	++/+++	++/+++	++
Loading	++	+	+++	+++	++
Stability	+ / +++	+++	+++	+++	++
Usage (e.g.)	<ul style="list-style-type: none"> • Peptide mapping • LC/MS • Nucleic acids and oligonucleotides 	<ul style="list-style-type: none"> • Impurity analysis of antibody-drug conjugates • mAb separation 	<ul style="list-style-type: none"> • Proteins/mAb • Charge variant analysis • Isoform analysis • Nucleic acids and oligonucleotides 	<ul style="list-style-type: none"> • Drug-binding analysis of antibody-drug conjugates 	<ul style="list-style-type: none"> • Nucleic acids and oligonucleotides • Amino acids • Peptides

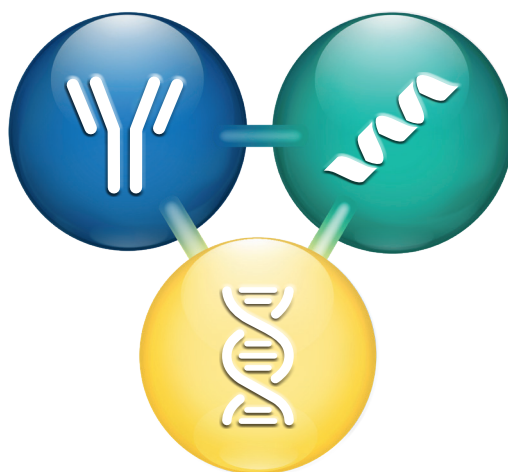
Application data mainly by courtesy of YMC Co., Ltd.

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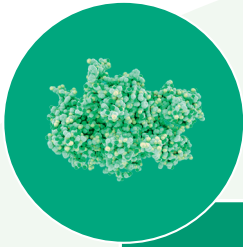
BEH, XBridge are trademarks of Waters Corp.
 Aeris is a trademark of Phenomenex Inc.
 MAbPac, ProPac are trademarks of Thermo Fisher Scientific Inc.
 AdvanceBio is a trademark of Agilent Technologies Inc.
 BioAssist, NPR, TSKgel are trademarks of Tosoh Corp.
 Mono Q, Mono S are trademarks of Cytiva.

Every effort has been taken to ensure this list is accurate at the time of printing this brochure.

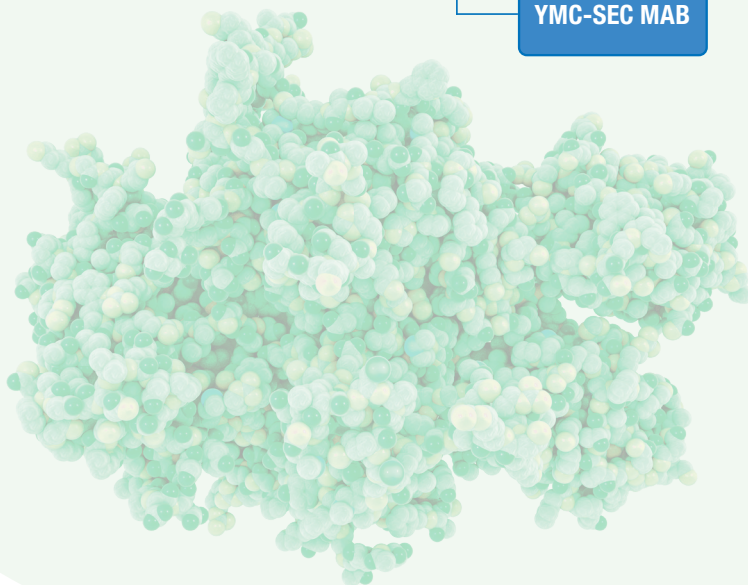
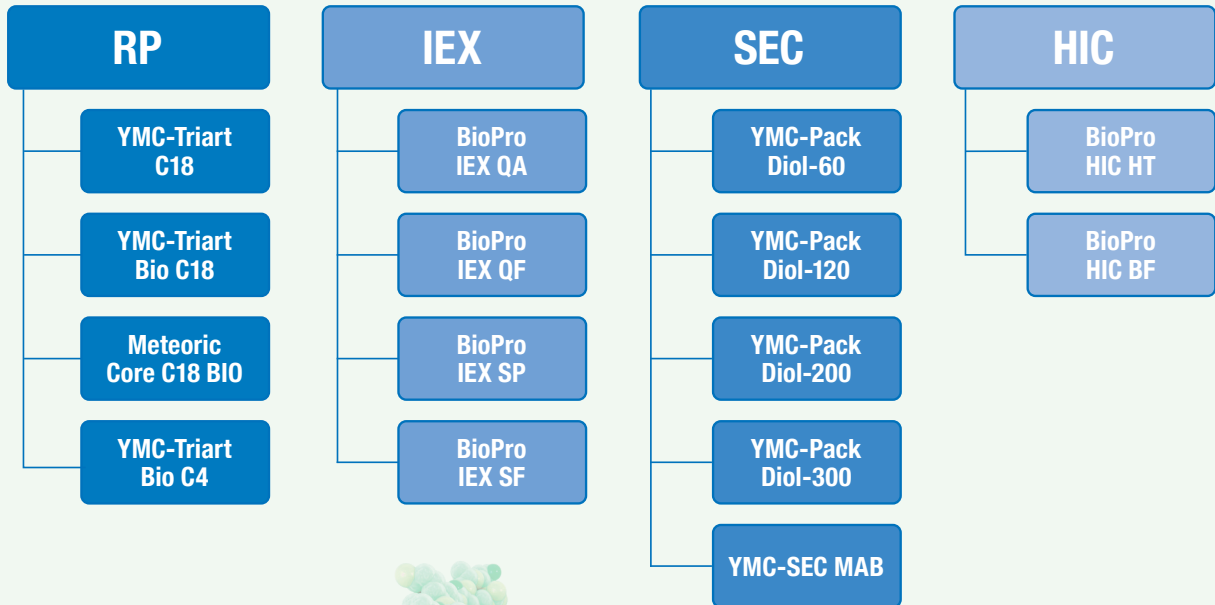
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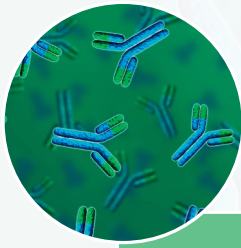


Phase selection guide

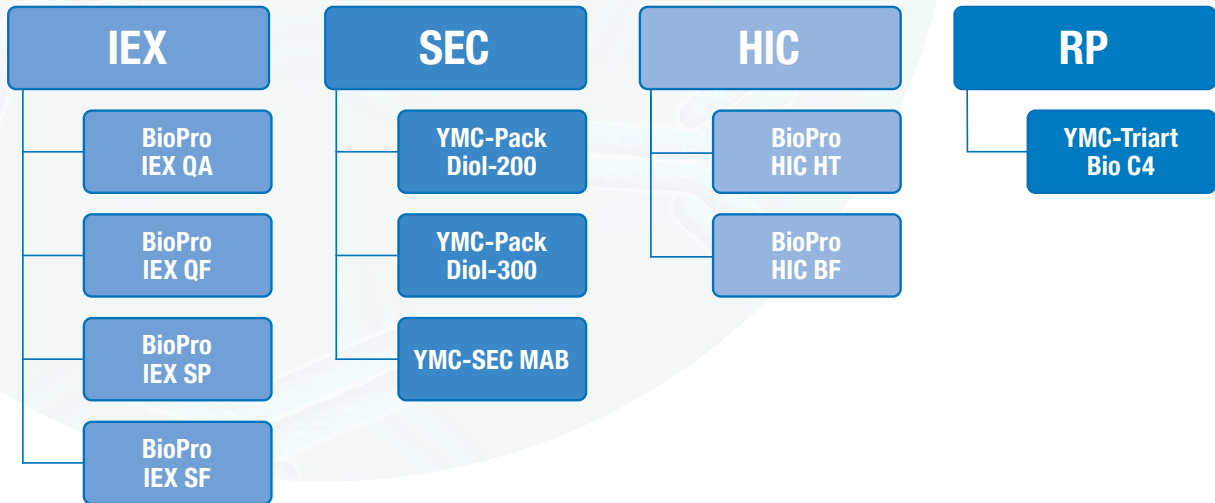


Proteins / Peptides

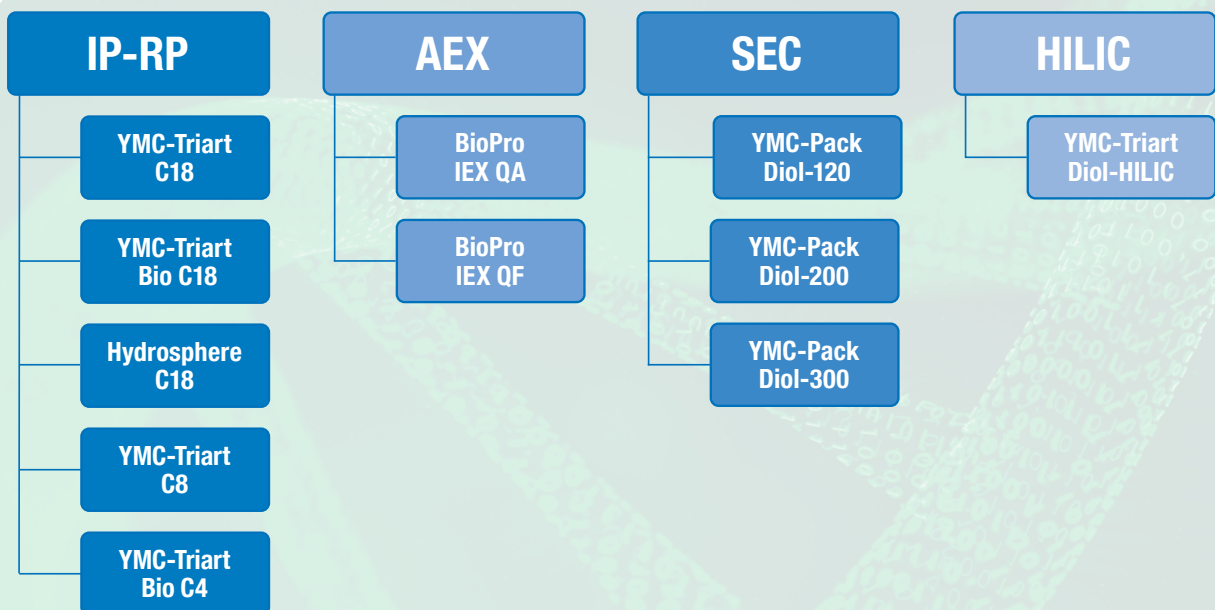


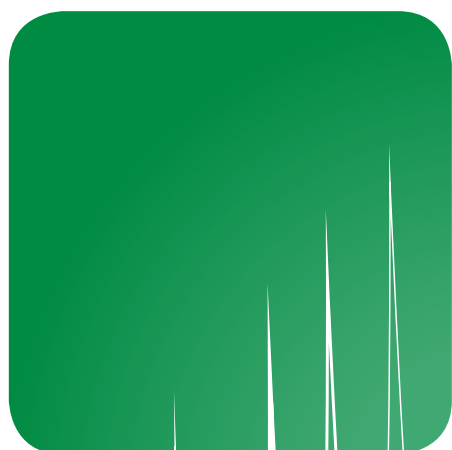


(Monoclonal) Antibodies

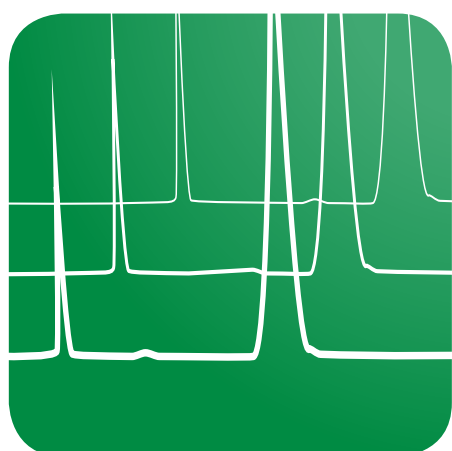


Oligonucleotides / Nucleic Acids





BioLC
Applications

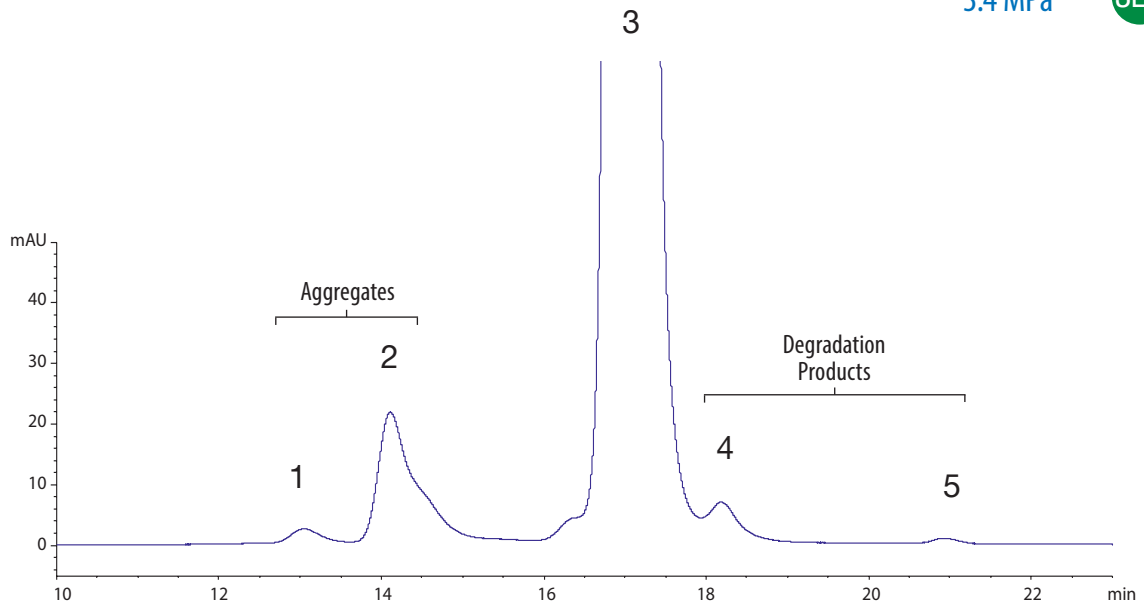


BioLC applications – Antibodies

Bevacizumab and its fragments and aggregates

3.4 MPa

SEC



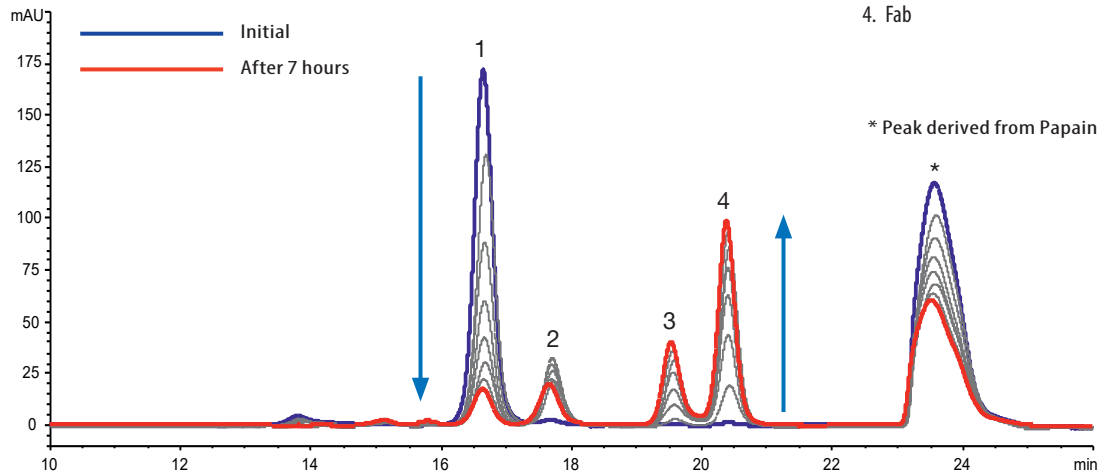
Column: YMC-SEC MAB (3 μ m, 25 nm) 300 x 4.6 mm ID
 Part No.: DLM25S03-3046WT
 Eluent: 0.1 M phosphate buffer (pH = 7) containing 0.2 M NaCl
 Flow rate: 0.165 mL/min
 Temperature: 25°C

Detection: UV at 280 nm
 Cell path: 10 mm
 Injection: 10 μ L (5 mg/mL)
 Sample: Bevacizumab (Avastin®)

Analysis of digested antibody

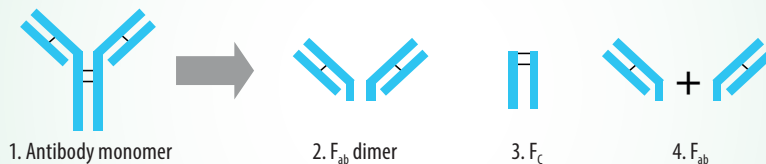
1. Antibody monomer
2. Fab dimer
3. F_C
4. Fab

SEC



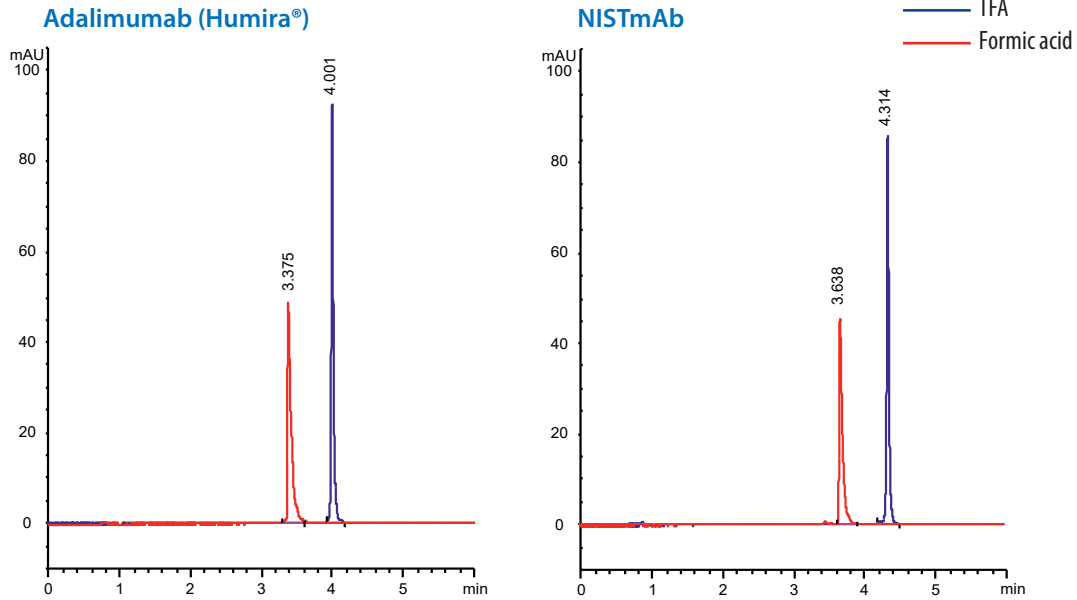
Column: YMC-SEC MAB (3 μ m, 25 nm) 300 x 4.6 mm ID
 Part No.: DLM25S03-3046WT
 Eluent: 0.1 M phosphate buffer (pH = 7) containing 0.2 M NaCl
 Flow rate: 0.165 mL/min

Temperature: 25°C
 Detection: UV at 280 nm
 Injection: 2 μ L (3 mg/mL)
 Sample: Humanised monoclonal IgG1 + Papain



Use of MS compatible conditions for antibody analysis by RP

RP

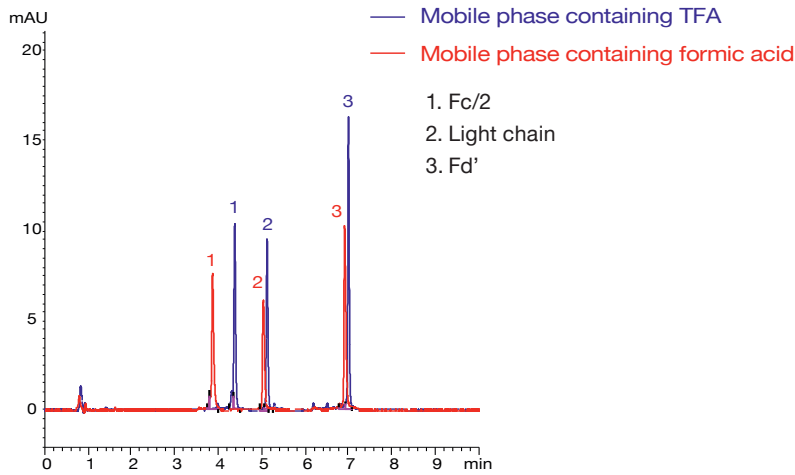


Column: YMC-Triart Bio C4 (1.9 μ m, 30 nm) 150 x 2.1 mm ID
 Part No.: TB30SP9-15Q1PT
 Eluent: A) water/TFA or formic acid (100/0.1)
 B) acetonitrile/TFA or formic acid (100/0.1)
 Gradient: 10–95%B (0–10 min)

Flow rate: 0.4 mL/min
 Temperature: 80°C
 Detection: UV at 280 nm (0.13 s, 40 Hz)
 Injection: 2 μ L (0.5 mg/mL)

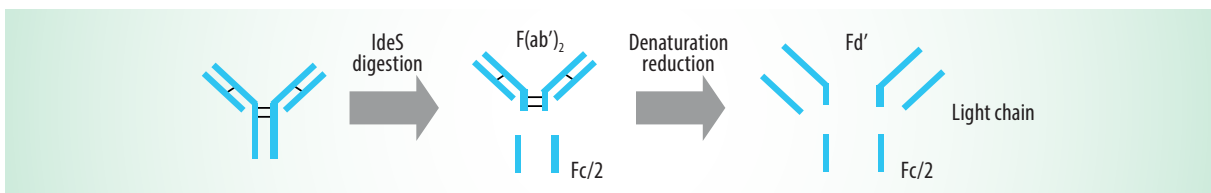
LC/MS compatible analysis of monoclonal antibody fragments

RP



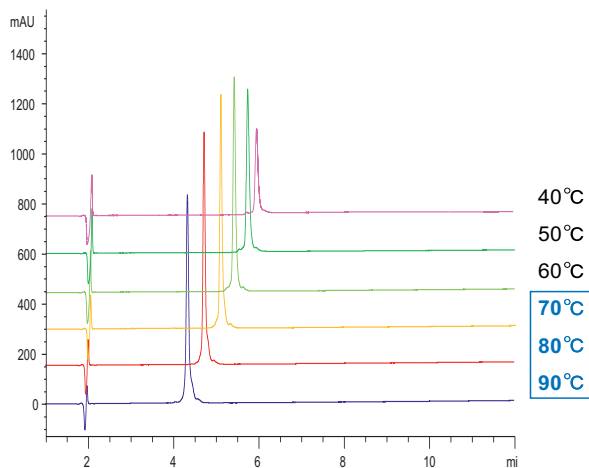
Column: YMC-Triart Bio C4 (1.9 μ m, 30 nm) 150 x 2.1 mm ID
 Part No.: TB30SP9-15Q1PT
 Eluent [TFA]: A) water/TFA (100/0.1)
 B) acetonitrile/TFA (100/0.1)
 Gradient [TFA]: 25–50%B (0–10 min), 90%B (10–12.5 min)
 Eluent [formic acid]: A) water/formic acid (100/0.1)
 B) acetonitrile/formic acid (100/0.1)

Gradient [formic acid]: 20–45%B (0–10 min), 90%B (10–12.5 min)
 Flow rate: 0.4 mL/min
 Temperature: 80°C
 Injection: 4 μ L (0.25 mg/mL)
 Detection: UV at 280 nm
 Sample: mAb Subunit Standard (Waters Corp.)



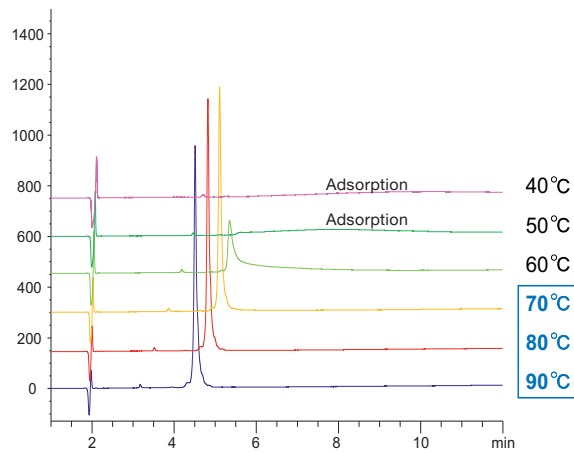
BioLC applications – Antibodies

Adalimumab (Humira®, MW: ca. 148 kDa)



Bevacizumab (Avastin®, MW: ca. 148 kDa)

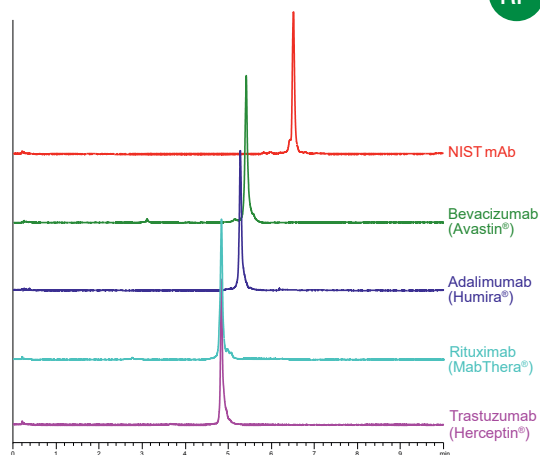
RP



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)
 Flow rate: 0.4 mL/min
 Detection: UV at 220 nm
 Injection: 4 µL
 Sample: Adalimumab (0.5 mg/mL) or Bevacizumab (0.5 mg/mL)

Analysis of different monoclonal antibodies

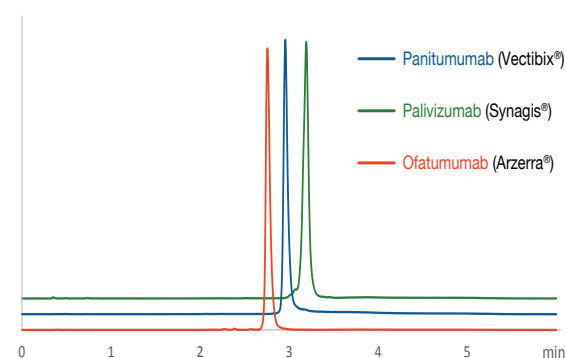
RP



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)
 Flow rate: 0.4 mL/min
 Temperature: 80 °C
 Detection: UV at 280 nm (0.13s, 40Hz)
 Injection: 2 µL (0.5 mg/mL)

Analysis of challenging monoclonal antibodies

RP



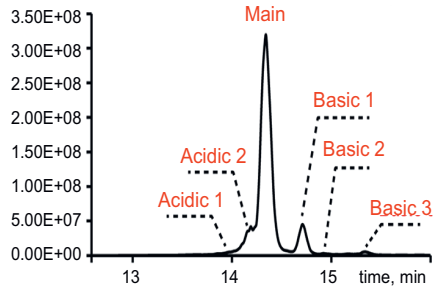
Column: YMC-Triart Bio C4 (1.9 µm, 30 nm) 50 x 2.1 mm ID
 Part No.: TB30SP9-05Q1PT
 Eluent: A) water/TFA (100/0.1)
 B) acetonitrile/TFA (100/0.1)
 Gradient: 25–50%B (0–4 min)
 Flow rate: 0.4 mL/min
 Temperature: 90 °C
 Detection: Fluorescence: ex 280nm, em 350nm
 Injection: 0.5 µL

By courtesy of University of Geneva, Institute of Pharmaceutical Sciences of Western Switzerland (ISPSO)

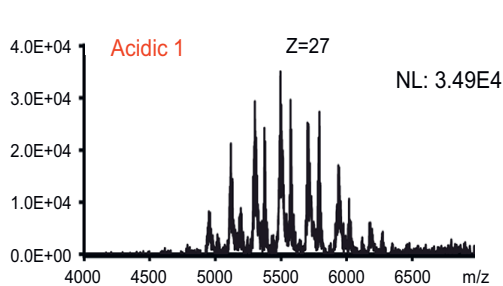
Native online CEX-MS analysis of monoclonal antibodies (IgG1 type)



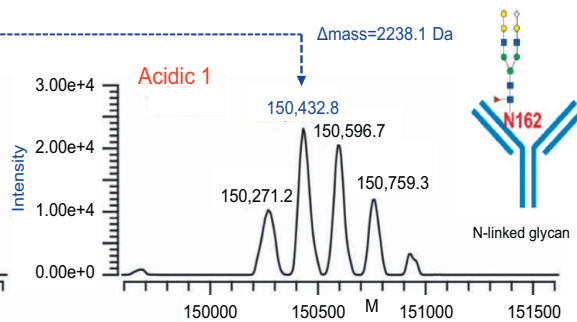
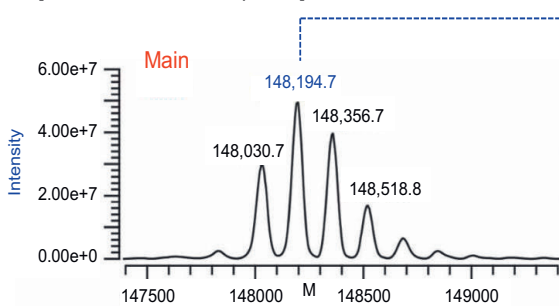
[TIC, native NISTmAb]



[Raw mass spectrum]



[Deconvoluted mass spectra]



Column: BioPro IEX SF (5 μm) 100 x 4.6 mm ID
 Part No.: SF00S05-1046WP
 Eluent: A) 20 mM CH₃COONH₄-CH₃COOH (pH 5.6)
 B) 140 mM CH₃COONH₄-10 mM NH₄HCO₃ (pH 7.4)
 Gradient: 0%B (0–2 min), 0–100%B (2–18 min), 100%B (18–22 min)
 Flow rate: 0.4 mL/min
 (To enable online simultaneous UV and MS detection, a post-column analytical splitter (~400:1 ratio) was connected)

Temperature: 45°C
 Detection: nanospray ionisation-mass spectrometry (NSI-MS)
 Load: 50 μg
 System: LC) ACQUITY UPLC I-Class system (Waters)
 MS) Exactive™ Plus EMR mass spectrometer (Thermo Fisher Scientific)

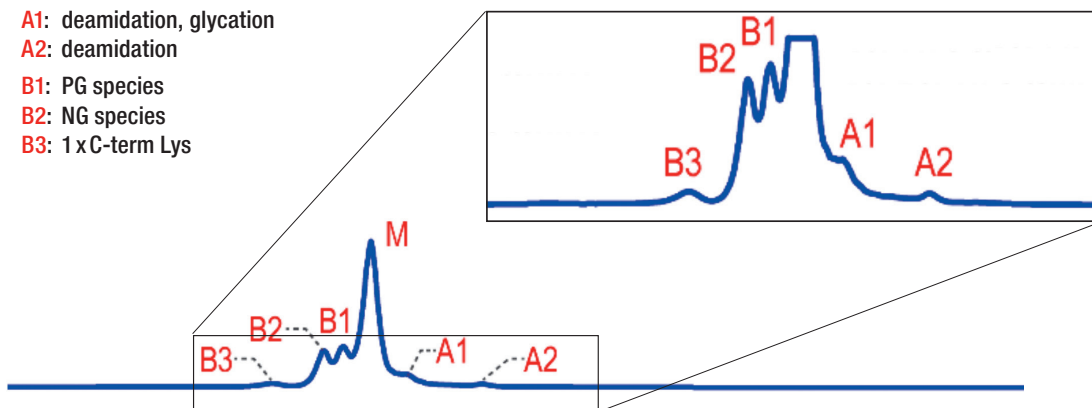
By courtesy of S. Wang, Regeneron Pharmaceuticals Inc.

Reference: Y. Yan, A. P. Liu, S. Wang, T. J. Daly und N. Li, Ultrasensitive Characterization of Charge Heterogeneity of Therapeutic Monoclonal Antibodies, Anal. Chem., 2018, 90, 13013-20.

Native online AEX-MS of IgG4 type mAbs



- A1: deamidation, glycation
- A2: deamidation
- B1: PG species
- B2: NG species
- B3: 1 x C-term Lys



Column: BioPro IEX QF (5 μm) 100 x 4.6 mm ID
 Part No.: QF00S05-1046WP
 Eluent: A) 10 mM ammonium acetate, pH 6.7
 B) 300 mM ammonium acetate, pH 6.8
 Gradient: 0%B (0–2 min), 0–100%B (2–18 min), 100%B (18–22 min)
 Flow rate: 0.4 mL/min
 Temperature: 45°C intact mAb
 25°C subunit analysis
 Injection: 5 or 10 μg mAb sample

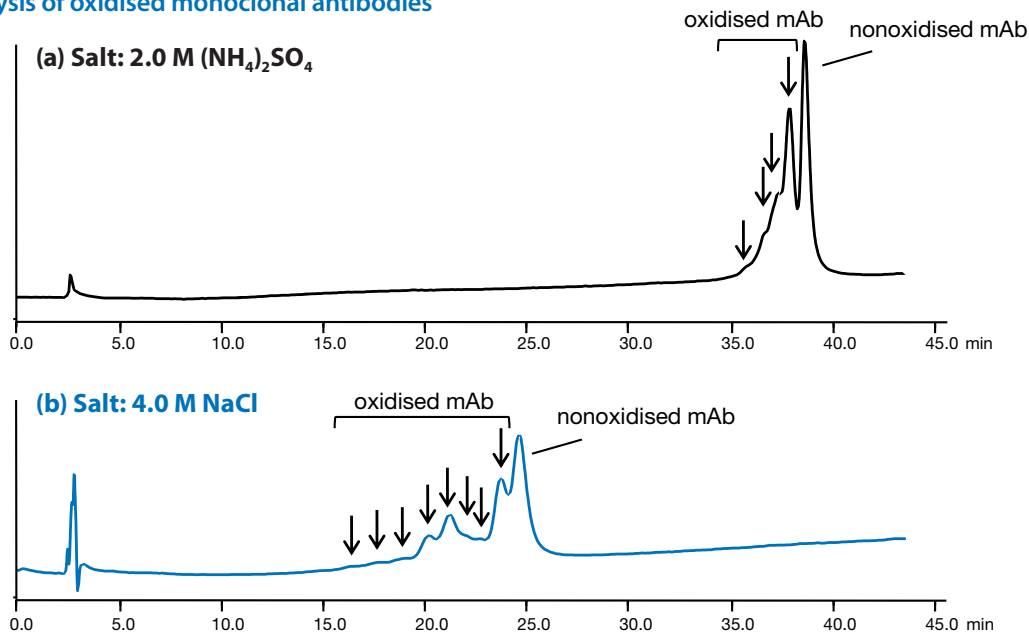
Detection: NSI-MS (nanoelectrospray ionisation)
 UV
 Sample: Inhouse IgG4-based mAb, pI=6.6 (Regeneron)
 Setup: Post column stainless-steel tee to direct the majority to the UV detector
 Remaining sub-microlitre per minute flow directed to the NSI-MS

By courtesy of S. Wang, Regeneron Pharmaceuticals Inc.

Reference: A. Liu, Y. Yan, S. Wang, N. Li, Coupling Anion Exchange Chromatography with Native Mass Spectrometry for Charge Heterogeneity Characterization of Monoclonal Antibodies, Anal. Chem. 2022, 94, 6355–6362.

BioLC applications – Antibodies

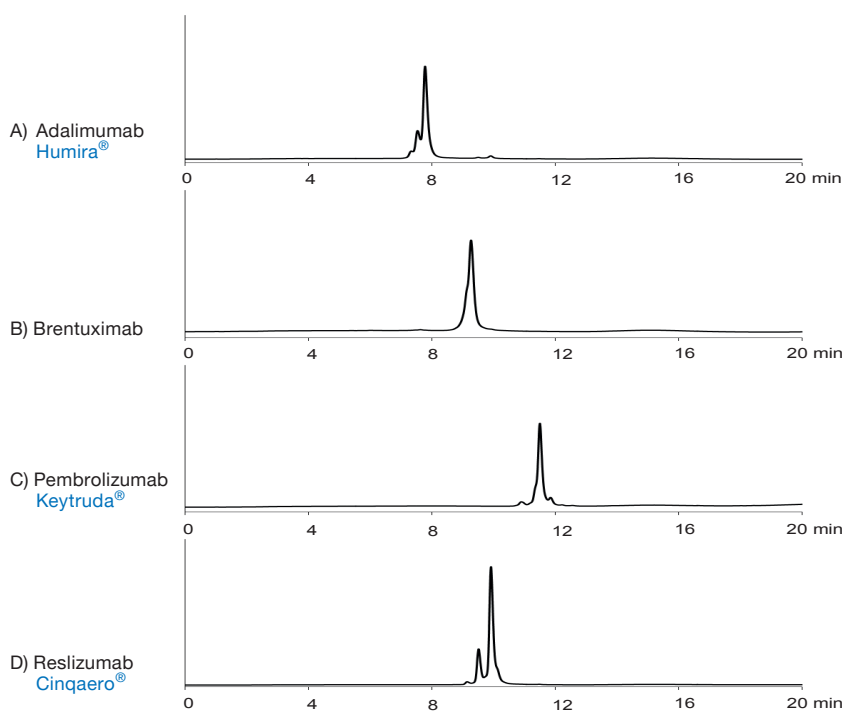
Analysis of oxidised monoclonal antibodies



HIC

Column:	BioPro HIC BF (4 μm) 100 x 4.6 mm ID	Flow rate:	0.3 mL/min
Part No.:	BHB00S04-1046WT	Temperature:	25 °C
Eluent:	A) 100 mM NaH ₂ PO ₄ -Na ₂ HPO ₄ (pH 7.0) containing salt	Detection:	UV at 280 nm
	B) 100mM NaH ₂ PO ₄ -Na ₂ HPO ₄ (pH 7.0)	Injection:	5 μL (1.0 mg/mL)
Gradient:	40–80%B (0–40 min), 80%B (40–45 min)	Sample:	oxidised NISTmAb

HIC analysis of different monoclonal antibodies using isopropanol as modifier



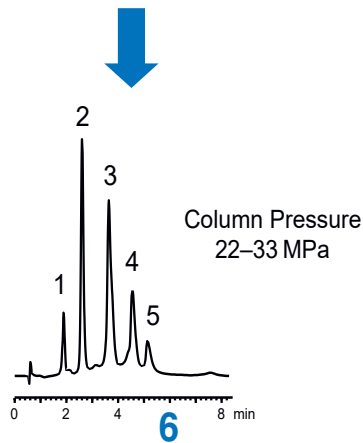
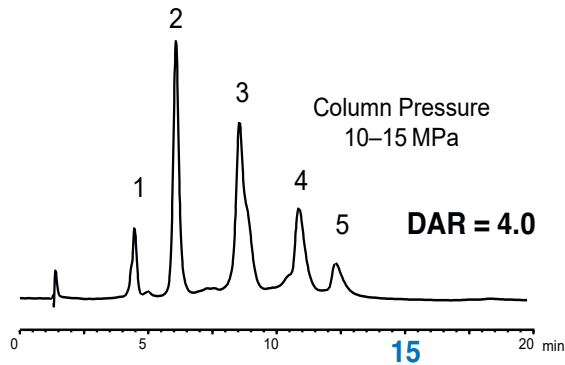
HIC

Column:	BioPro HIC BF (4 μm) 100 x 4.6 mm ID	Temperature:	20 °C
Part No.:	BHB00S04-1046WT	Detection:	Fluorescence: ex 280nm, em 360nm
Eluent:	A) 20 mM NaH ₂ PO ₄ -Na ₂ HPO ₄ (pH 7.4) containing 1.5 M (NH ₄) ₂ SO ₄	Injection:	3 μL (2 mg/mL)
	B) 20 mM NaH ₂ PO ₄ -Na ₂ HPO ₄ (pH 7.4) / 2-propanol (85/15)		
Gradient:	0–100%B (0–20 min)		
Flow rate:	1.0 mL/min		

By courtesy of University of Geneva, Institute of Pharmaceutical Sciences of Western Switzerland (ISPSO)

High throughput DAR determination by shortening analysis time

HIC



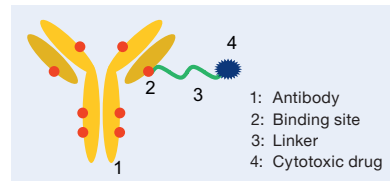
Flow rate
0.5 mL/min

2.5x faster

Flow rate
1.2 mL/min

1. DAR 0
2. DAR 2
3. DAR 4
4. DAR 6
5. DAR 8

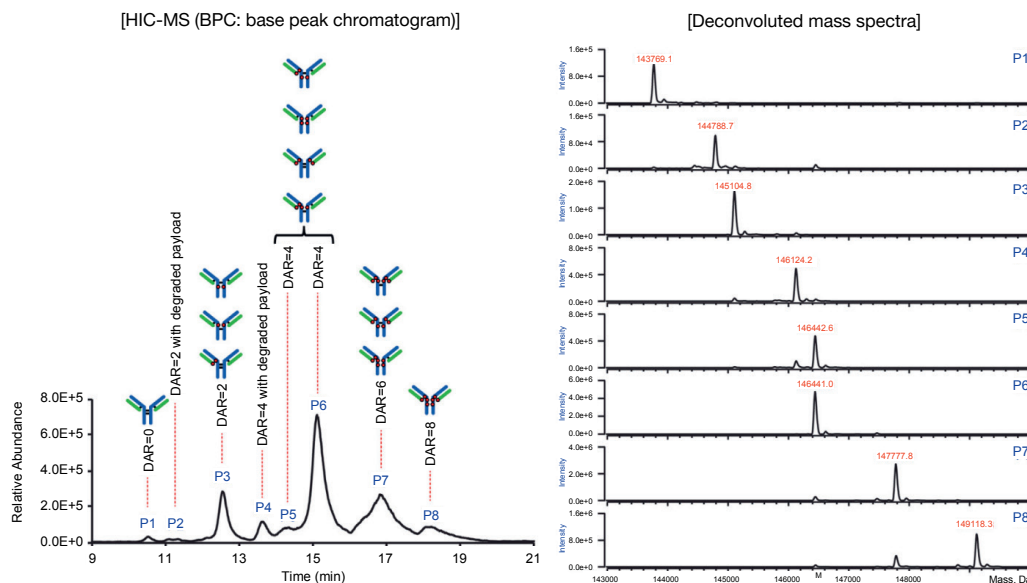
Column: BioPro HIC HT (2.3 μ m) 100 x 4.6 mm ID
 Part No.: BHH00SQ3-1046PTH
 Eluent: A) 20 mM NaH_2PO_4 - Na_2HPO_4 containing 1.0 M $(\text{NH}_4)_2\text{SO}_4$ (pH 7.0)
 B) 20 mM NaH_2PO_4 - Na_2HPO_4 (pH 7.0)/2-propanol (85/15)
 Gradient: 0–100%B (0–15 min), 100%B (15–20 min)
 0–100%B (0–6.25 min), 100%B (6.25–8.3 min)
 Temperature: 25°C
 Detection: UV at 280 nm
 Injection: 10 μ L
 Sample: Brentuximab vedotin (Adcetris[®]) (2.5 mg/mL)



BioLC applications – Antibody-Drug-Conjugates

Native online HIC-MS analysis of cys-linked ADCs

HIC



Column: BioPro HIC BF (4 μ m) 100 x 4.6 mm ID
 Part number: BHB00S04-1046WT
 Eluent: A) 3 M ammonium acetate in water
 B) 2-propanol/water (30/70)
 Gradient: 10%B (0–2 min), 10–97%B (2–18 min), 97%B (18–22 min)
 Flow rate: 0.3 mL/min
 Temperature: ambient
 Detection: UV at 280 nm, NSI-MS

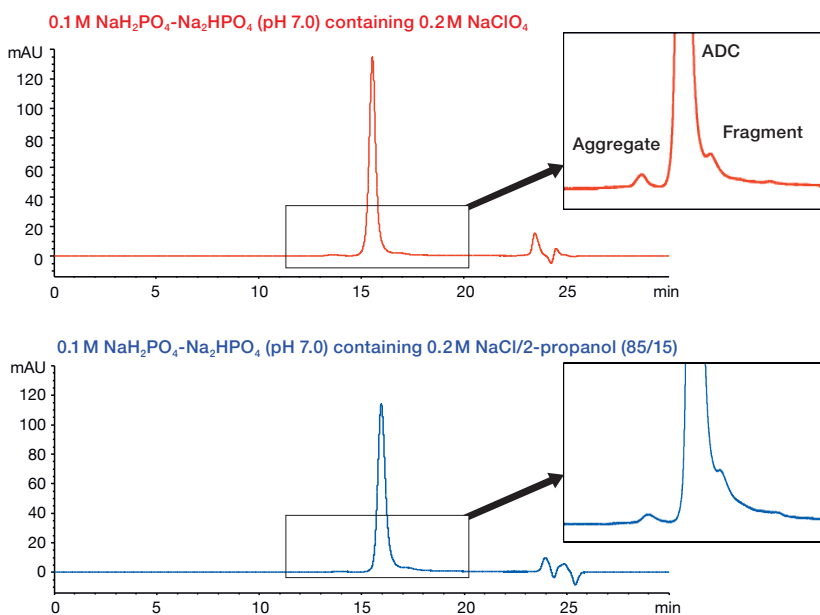
Injection: 10 μ g
 Sample: SigmaMAB ADC-mimic
 Setup: Post-column makeup flow:
 100% water at 1.5 mL/min (reducing salt conc. 6-fold)
 Splitter to reduce the flow rate to 1–5 μ L/min

By courtesy of S. Wang, Regeneron Pharmaceuticals Inc.

Reference: Y. Yan, T. Xing, S. Wang, T. J. Daly, N. Li, Online coupling of analytical hydrophobic interaction chromatography with native mass spectrometry for the characterization of monoclonal antibodies and related products, *J. Pharm. Biomed. Anal.* 186 (2020) 113313.

Separation of Brentuximab vedotin from its aggregates and fragments

SEC



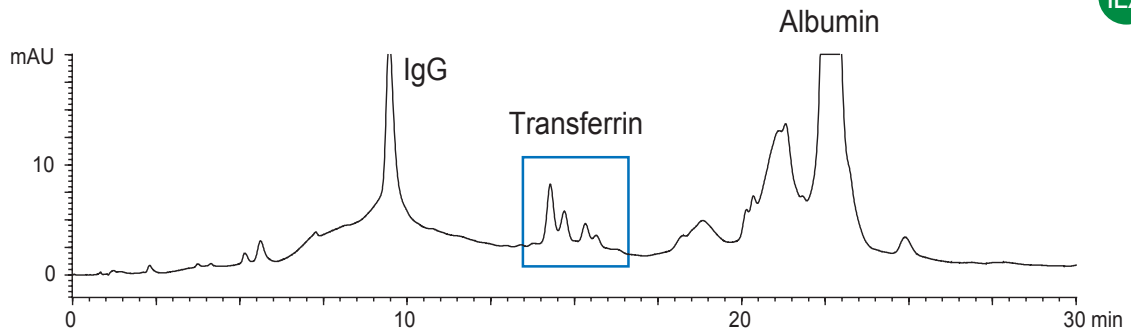
Column: YMC-SEC MAB (3 μ m, 25 nm) 300 x 4.6 mm ID
 Part No.: DLM25S03-3046WT
 Eluent: 0.1 M phosphate buffer (pH = 7) containing 0.2 M NaClO₄
 0.1 M phosphate buffer (pH = 7) containing 0.2 M NaCl/2-propanol (85/15)
 Flow rate: 0.165 mL/min

Temperature: 25 °C
 Detection: UV at 280 nm
 Injection: 4 μ L (2.5 mg/mL)
 Sample: Brentuximab vedotin (Adcetris®) for injection

By courtesy of Prof. S. Manabe, Hoshi University, Tokyo/Tohoku University, Sendai Japan.

Separation of proteins in human serum

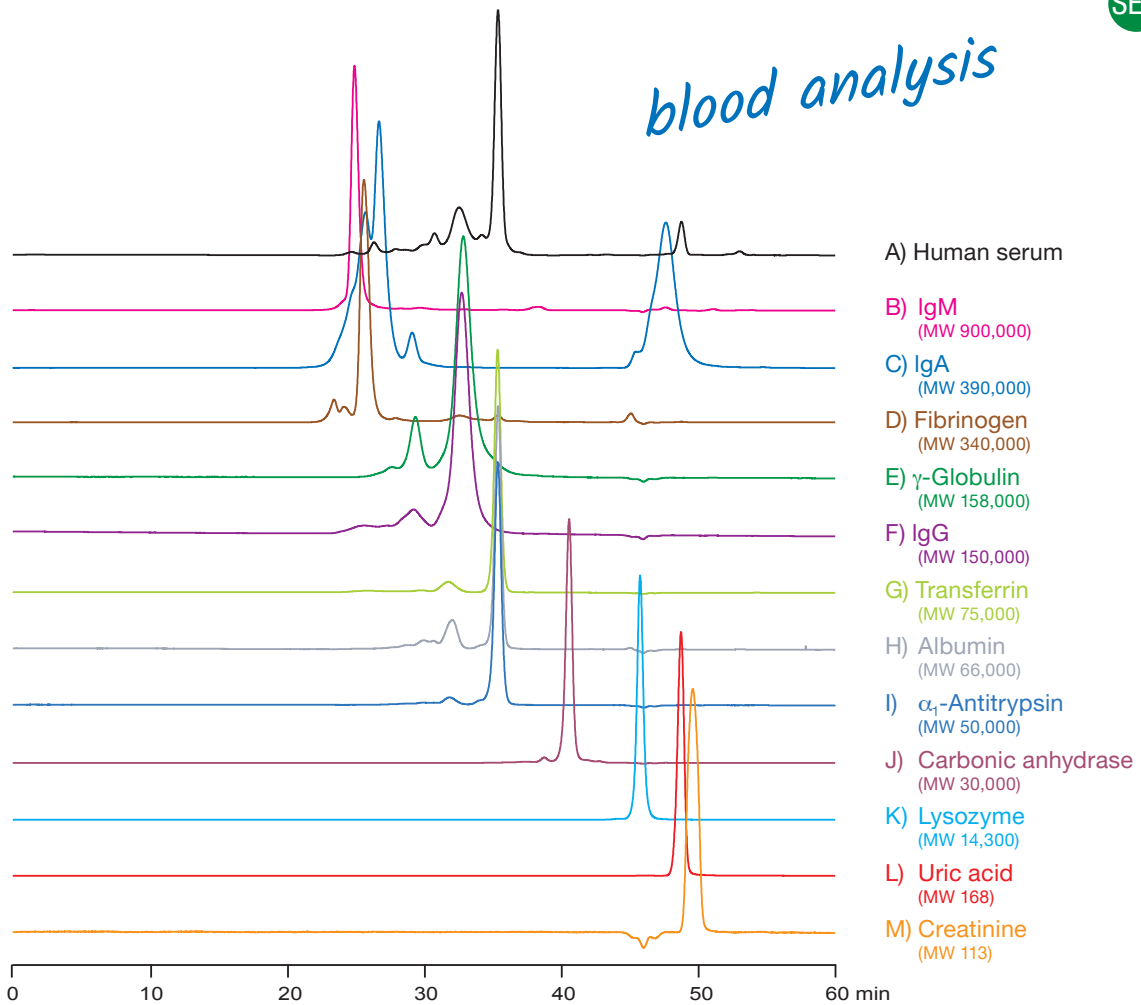
IEX



Column:	BioPro IEX QA (5 µm) 50 x 4.6 mm ID	Flow rate:	0.5 mL/min
Part No.:	QAA0S05-0546WP	Temperature:	25°C
Eluent:	A) 20 mM Tris-HCl (pH 8.6)	Detection:	UV at 280 nm
	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)

Plasma constituents

SEC



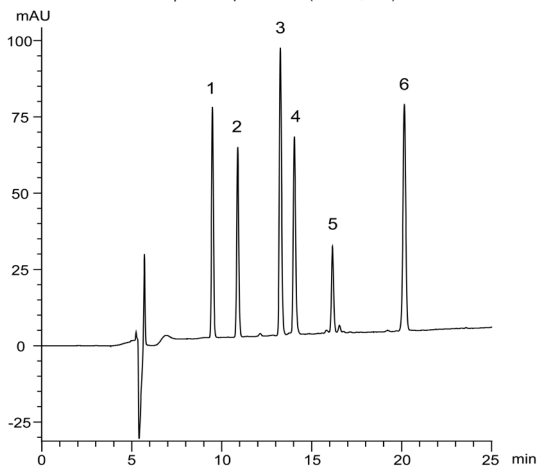
Columns:	YMC-Pack Diol-300 + Diol-200 (5 µm) 300 x 8.0 mm ID x 2	Temperature:	ambient (25°C)
Part Nos.:	DL30S05-3008WT + DL20S05-3008WT	Detection:	UV at 280 nm
Eluent:	0.1 M KH_2PO_4 - K_2HPO_4 (pH 7.0) containing 0.2 M NaCl	Injection:	20 µL (L: 1 µL)
Flow rate:	0.5 mL/min	Sample:	A) 100 µL/mL; B-M)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

BioLC applications – Peptides

Peptides covering different MW

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)

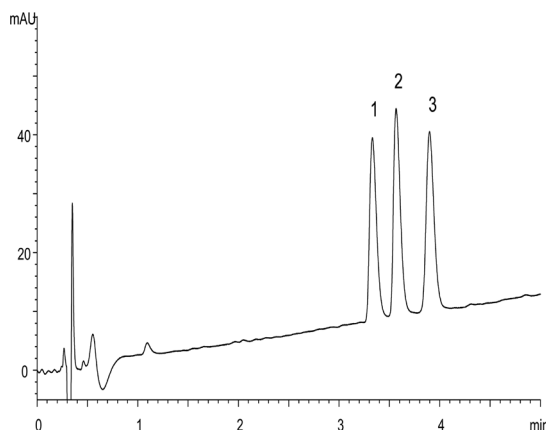


Column: YMC-Triart C18 (5 μ m, 12 nm) 150 x 2.0 mm ID
 Part No.: TA12S05-1502WT
 Eluent: A) water/TFA (100/0.1)
 B) acetonitrile/TFA (100/0.1)
 Gradient: 20%–45%B (0–25 min)
 Flow rate: 0.2 mL/min
 Temperature: 37 °C
 Detection: UV at 220 nm
 Injection: 2 μ L (0.075 \approx 0.25 mg/mL)

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)

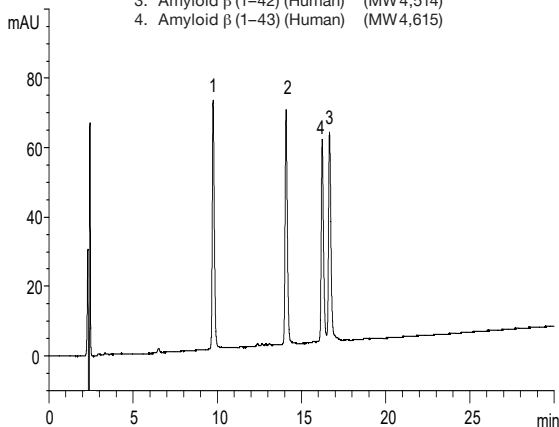


Column: YMC-Triart C18 (1.9 μ m, 12 nm) 50 x 2.0 mm ID
 Part No.: TA12SP9-0502PT
 Eluent: A) water/formic acid (100/0.1)
 B) 2-propanol/acetonitrile/formic acid (50/50/0.08)
 Gradient: 10%–25%B (0–10 min)
 Flow rate: 0.4 mL/min
 Temperature: 70 °C
 Detection: UV at 220 nm
 Injection: 1 μ L (50 μ g/mL)

Amyloid β -peptides

RP

1. Amyloid β (1–38) (Human) (MW 4,132)
2. Amyloid β (1–40) (Human) (MW 4,330)
3. Amyloid β (1–42) (Human) (MW 4,514)
4. Amyloid β (1–43) (Human) (MW 4,615)



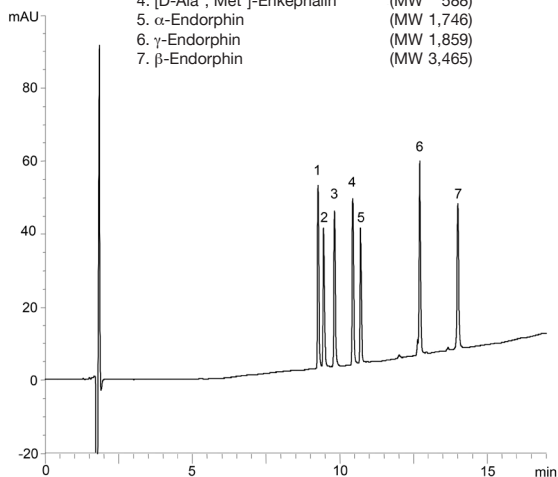
Amyloid β (1–43): Asp-Ala-Glu-Phe-Arg-His-Asp-Ser-Gly-Tyr-Glu-Val-His-His-Gln-Lys-Leu-Val-Phe-Phe-Ala-Glu-Asp-Val-Gly-Ser-Asn-Lys-Gly-Ala-Ile-Ile-Gly-Leu-Met-Val-Gly-Gly-Val-Val-Ile-Ala-Thr

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

Peptides

RP

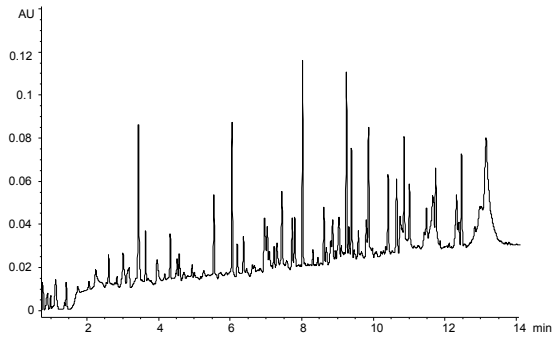
1. BAM-12P (MW 1,425)
2. [D-Ala², Met⁵]-Enkephalinamide (MW 587)
3. Met-Enkephalin (MW 574)
4. [D-Ala², Met⁵]-Enkephalin (MW 588)
5. α -Endorphin (MW 1,746)
6. γ -Endorphin (MW 1,859)
7. β -Endorphin (MW 3,465)



Column: Meteoric Core C18 BIO (2.7 μ m, 16 nm) 150 x 2.1 mm ID
 Part No.: CAW16SQ7-15Q1PT
 Eluent: A) water/TFA (100/0.1)
 B) acetonitrile/TFA (100/0.1)
 Gradient: 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 μ L (0.02–0.5 mg/mL)
 Pressure: 14.9–16.1 MPa (2,160–2,330 psi)

Peptide mapping of monoclonal antibody

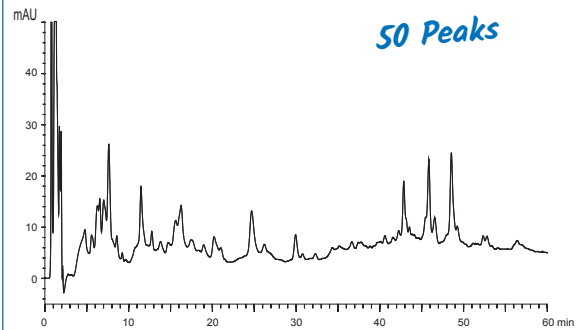
RP



Column: YMC-Triart C18 (1.9 μ m, 12 nm) 100 x 2.0 mm ID
 Part No.: TA12SP9-1002PT
 Eluent: A) water/TFA (100/0.1)
 B) acetonitrile/TFA (100/0.1)
 Gradient: 2%B (0–0.67 min), 2–45%B (0.67–14 min)
 Flow rate: 0.6 mL/min
 Temperature: 40 °C
 Detection: UV at 215 nm
 Injection: 10 μ L
 Sample: Tryptic digest of monoclonal antibody

Peptide mapping of tryptic digest of BSA with highest sensitivity

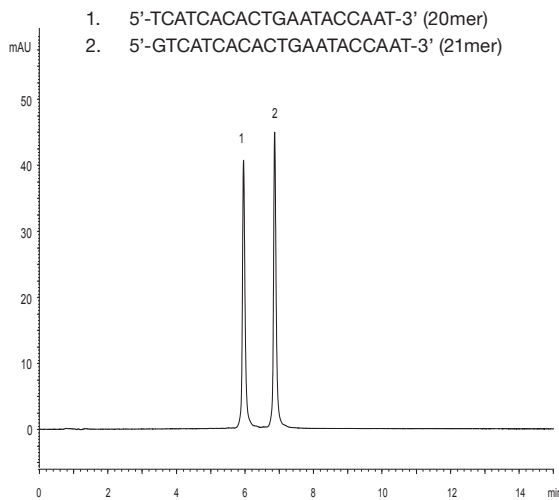
IEX



Column: BioPro IEX QA (5 μ m) 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 μ L
 Sample: Tryptic digest of BSA

Separation of synthetic oligonucleotides (single-strand DNA)

IEX

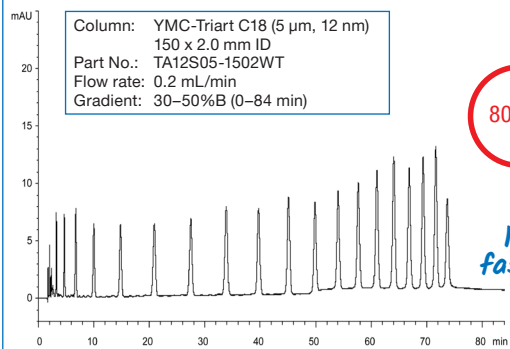


Column: BioPro IEX QF (5 μ m) 100 x 4.6 mm ID
 Part No.: QF00S05-1046WP
 Eluent: A) 10 mM NaOH
 B) 10 mM NaOH containing 1.0 M NaClO₄
 Gradient: 25–55%B (0–15 min), 100%B (15–20 min)
 Flow rate: 1.0 mL/min
 Temperature: 25 °C
 Detection: UV at 260 nm
 Injection: 4 μ L (5 nmol/L)

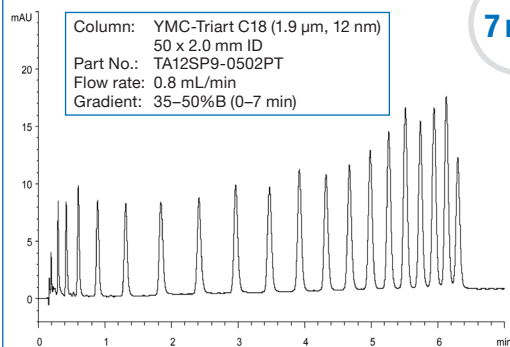
Oligonucleotides d(T)2-20 method transfer from HPLC to UHPLC

RP

Conventional LC method



UHPLC method



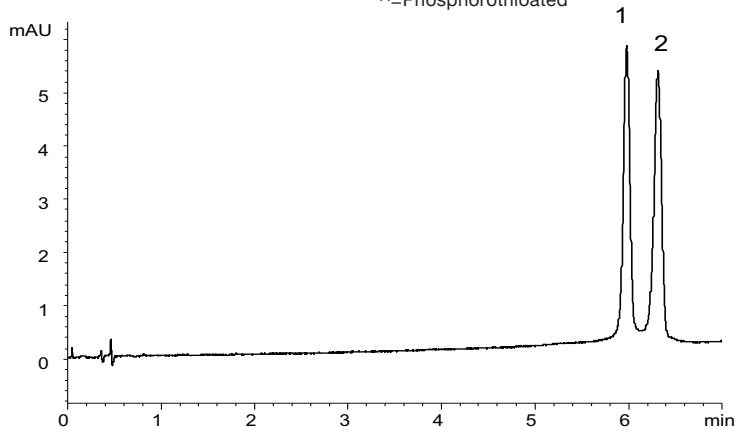
Eluent: A) 10 mM di-n-butylamine-acetic acid (pH 6.0)
 B) methanol
 Temperature: 37 °C
 Detection: UV at 269 nm
 Injection: 1 μ L (5 nmol/mL)

BioLC applications – Oligonucleotides

Challenging phosphorothioate oligonucleotides

RP

5'-U[^]C[^]A[^]U[^]C[^]A[^]C[^]A[^]C[^]A[^]U[^]G[^]A[^]A[^]U[^]A[^]C[^]A[^]A[^]U[^]-3' (RNA 20mer)
 5'-G[^]U[^]C[^]A[^]U[^]C[^]A[^]C[^]A[^]C[^]A[^]U[^]G[^]A[^]A[^]U[^]A[^]C[^]A[^]A[^]U[^]-3' (RNA 21mer)
 ^=Phosphorothioated

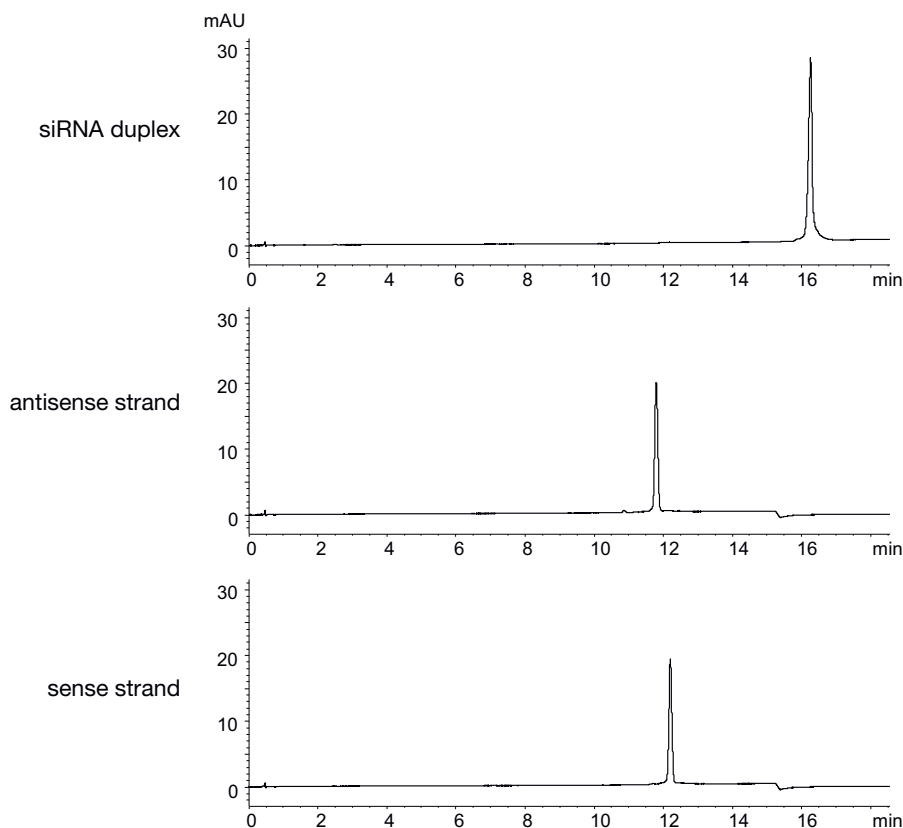


Column:	YMC Accura Triart Bio C18 (1.9 μm, 30 nm) 50 x 2.1 mm ID	Flow rate:	0.42 mL/min
Part No.:	TA30SP9-05Q1PTC	Temperature:	65 °C
Eluent:	A) 15 mM triethylamine - 400 mM HFIP* B) methanol	Detection:	UV at 260 nm
Gradient:	10–20%B (0–10 min)	Injection:	1 μL (each 1.0 nmol/mL)

*1,1,1,3,3,3-hexafluoro-2-propanol

siRNA under non-denaturing conditions

RP

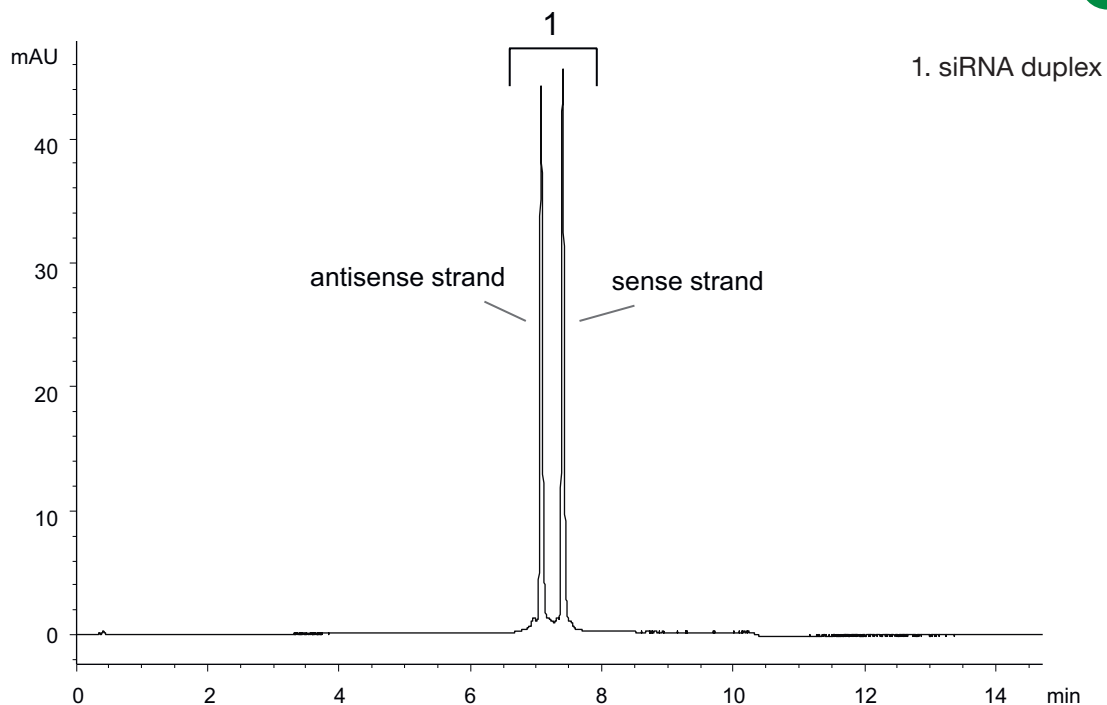


Column:	YMC Accura Triart Bio C18 (1.9 μm, 30 nm) 50 x 2.1 mm ID	Flow rate:	0.42 mL/min
Part No.:	TA30SP9-05Q1PTC	Temperature:	25 °C
Eluent:	A) 15 mM triethylamine - 400 mM HFIP* (pH 8) B) methanol	Detection:	UV at 260 nm
Gradient:	10%–28%B (0–18 min)	Injection:	1 μL (5 nmol/mL)
		Sample:	siRNA duplex & single strands

*1,1,1,3,3,3-hexafluoro-2-propanol

siRNA duplex under denaturing conditions

RP



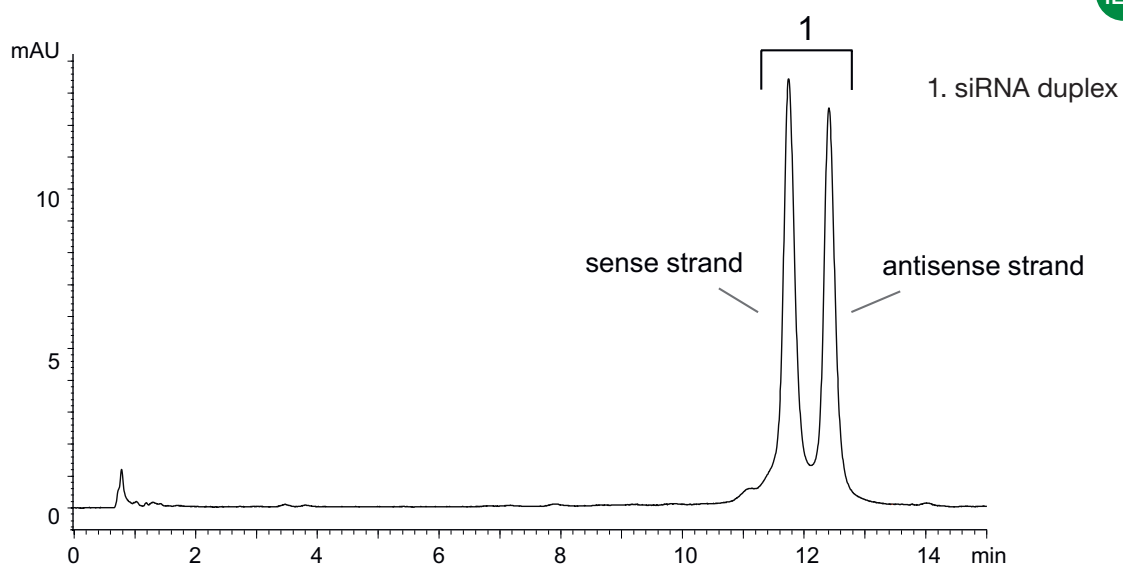
Column: YMC Accura Triart Bio C18 (1.9 μ m, 30 nm) 50 x 2.1 mm ID
 Part No.: TA30SP9-05Q1PTC
 Eluent: A) 15 mM TEAA* (pH 8)
 B) methanol
 Gradient: 5%–20%B (0–15 min)

Flow rate: 0.42 ml/min
 Temperature: 65°C
 Detection: UV at 260 nm
 Injection: 1 μ L (5 nmol/ml)
 Sample: siRNA duplex

*triethylammonium acetate

siRNA duplex under denaturing conditions

IEX



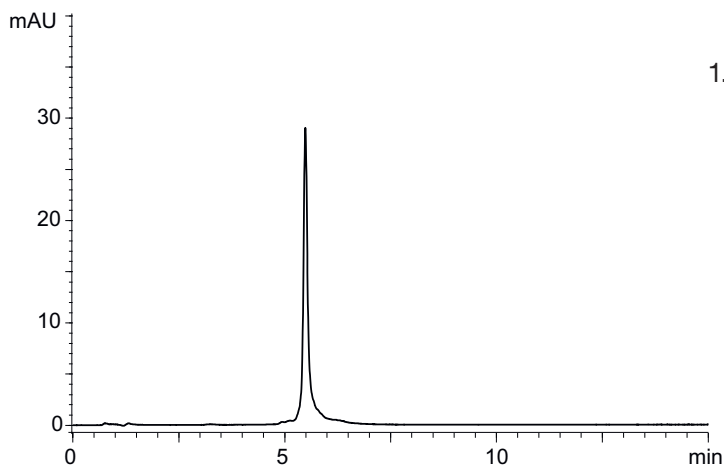
Column: BioPro IEX QF (5 μ m) 100 x 4.6 mm ID
 Part number: QF00S05-1046WP
 Eluent: A) 10 mM NaOH
 B) 10 mM NaOH containing 1 M NaClO₄
 Gradient: 30%–37%B (0–15 min)

Flow rate: 1.0 ml/min
 Temperature: 25°C
 Detection: UV at 260 nm
 Injection: 4 μ L (5 nmol/ml)
 Sample: siRNA duplex

BioLC applications – Oligonucleotides

AEX analysis of siRNA duplex under non-denaturing conditions

IEX



1. siRNA duplex

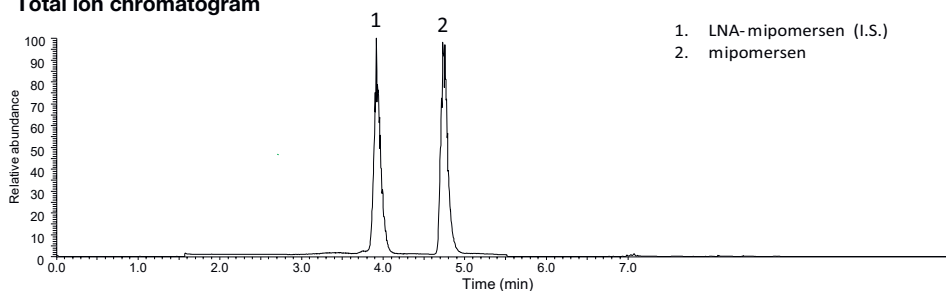
Column: BioPro IEX QF (5µm) 100 x 4.6 mm ID
 Part number: QF00S05-1046WP
 Eluent: A) 20 mM Tris-HCl (pH 8.1)
 B) 20 mM Tris-HCl (pH 8.1) containing 1 M NaClO₄
 Gradient: 25%–40%B (0–15 min)

Flow rate: 1.0 ml/min
 Temperature: 25 °C
 Detection: UV at 260 nm
 Injection: 4 µL (5 nmol/ml)
 Sample: siRNA duplex

LC-HRMS analysis of the antisense oligonucleotide mipomersen (Kynamro®)

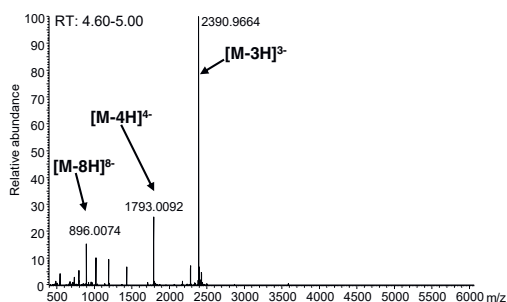
RP

Total ion chromatogram

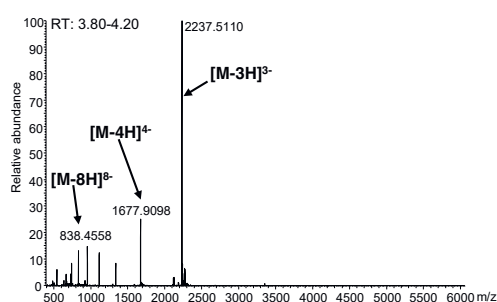


1. LNA-mipomersen (I.S.)
 2. mipomersen

Mass spectrum of mipomersen



Mass spectrum of LNA-mipomersen



Column: **YMC-Triart C8 metal-free PEEK-lined** (1.9µm, 12nm)* 100x2.1 mm ID
 Part No.: TO12SP9-10Q1PTP
 Eluent: A) water/triethylamine/HFIP** (100/0.4/2; triethylamine 28.0mM, HFIP 135.8mM)
 B) methanol/triethylamine/HFIP (100/0.4/2)
 Gradient: [Sample separation step]
 10–40%B (0–5.0 min)

[Column wash steps]
 40–70%B (5.0–5.1 min), 70%B (5.1–7.0 min), 70–10%B (7.0–7.1 min),
 10%B (7.1–8.0 min), 10–90%B (8.0–8.1 min), 90%B (8.1–9.0 min),
 90–10%B (9.0–9.1 min), 10%B (9.1–10.0 min),
 10–90%B (10.0–10.1 min), 90%B (10.1–11.0 min),
 90–10%B (11.0–11.1 min)

Flow rate: 0.3 mL/min
 Temperature: 50 °C
 Injection: 10 µL (1000 ng/mL)
 System: LC) Vanquish Binary Pump H system
 HRMS) Orbitrap HRMS Q Exactive Plus

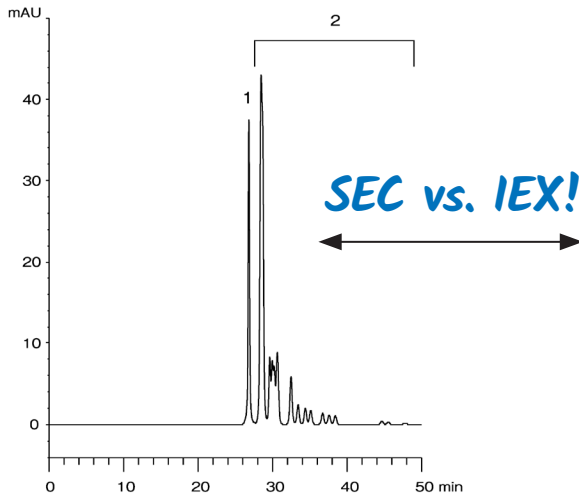
* Prewash the column prior to the first use with water/methanol/phosphoric acid (70/30/0.1) for 1 hour
 ** 1,1,1,3,3,3-hexafluoro-2-propanol

Reference: Y. Sun et al, Development of a bioanalytical method for an antisense therapeutic using high-resolution mass spectrometry, *Bioanalysis*, 2020 NOV 26, doi: 10.4155/bio-2020-0225.

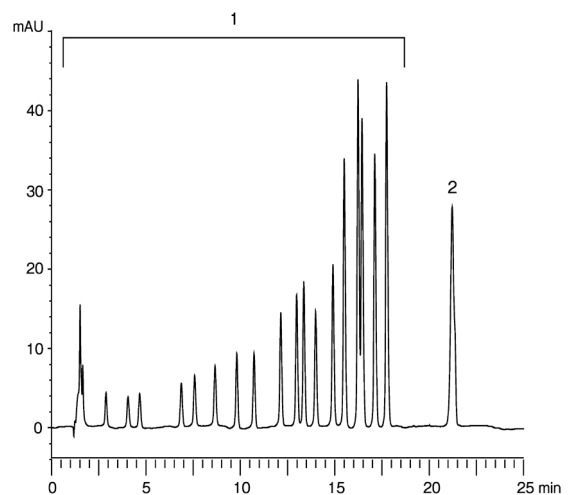
Plasmid pBR322 restriction and pBR322 Hae III restriction fragments

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)

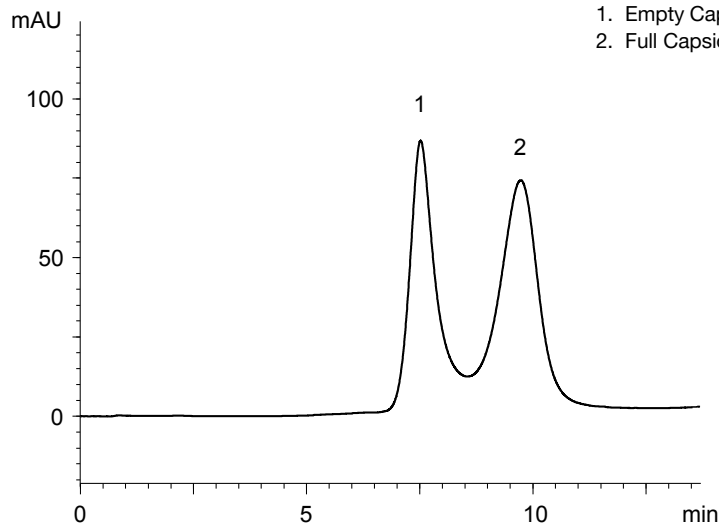


Columns: YMC-Pack Diol-300 + Diol-200 (5 μ m) 500 x 8.0 mm ID
 Part Nos.: DL30S05-5008WT + DL20S05-5008WT
 Eluent: 0.1 M KH_2PO_4 - K_2HPO_4 (pH 7.0) containing 0.2 M NaCl
 Flow rate: 0.7 mL/min
 Temperature: ambient (25 $^\circ\text{C}$)
 Detection: UV at 260 nm
 Injection: 10 μL

Column: BioPro IEX QF (5 μ m) 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 1.0 M NaCl
 Gradient: 70–85%B (0–20 min), 85%B (20–25 min)
 Flow rate: 0.5 mL/min
 Temperature: 35 $^\circ\text{C}$
 Detection: UV at 260 nm
 Injection: 10 μL

Intact adeno-associated virus

IEX



1. Empty Capsid
2. Full Capsid

Column: BioPro IEX QF (5 μ m) 30 x 4.6 mm ID
 Part number: QF00S05-0346WP
 Eluent: A) 20 mM Bis-trispropane-HCl (pH 9.0)
 B) 20 mM Bis-trispropane-HCl containing 0.5 M $(\text{CH}_3)_4\text{NCl}$ (pH 9.0)
 Gradient: 5%B (0–0.2 min), 20–45%B (0.2–10 min)
 Flow rate: 0.5 mL/min
 Temperature: 25 $^\circ\text{C}$
 Detection: FLS at Ex. 280 nm, Em. 348 nm
 Injection: 2 μL
 Sample: AAV2 (2.59×10^{12} vg/mL)

This research was supported by AMED under Grant Number JP18ae0201001.

BioLC applications – AAVs

RP

Denatured adeno-associated viruses

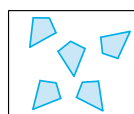
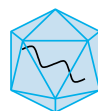
Sample preparation

AAV2
1.46 x 10¹² vg/mL
AAV5
3.95 x 10¹² vg/mL

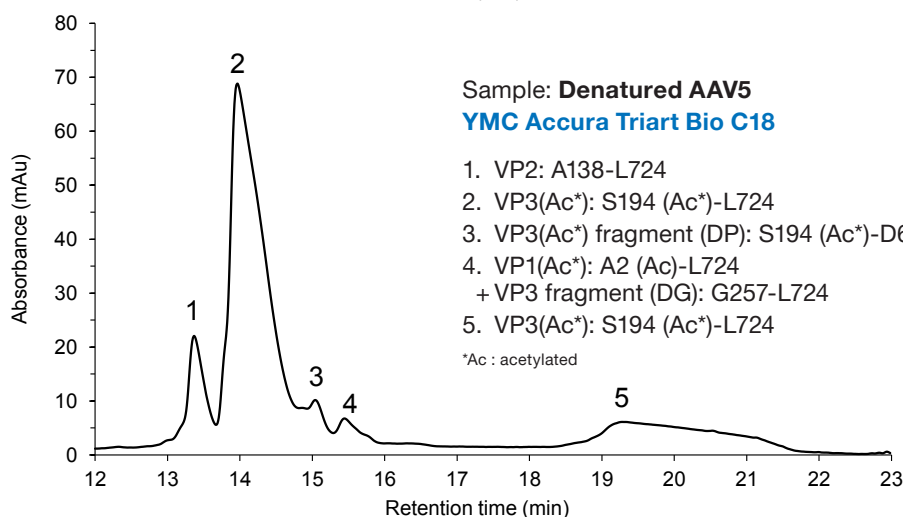
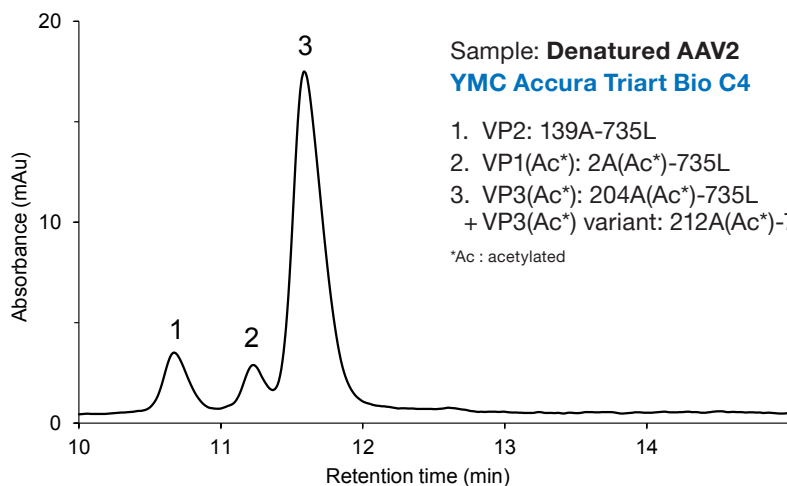
10% acetic acid treatment
RT, 15 min

Centrifuged at 12,000 rpm
5 min

analysis



VPs: 59~81 kDa



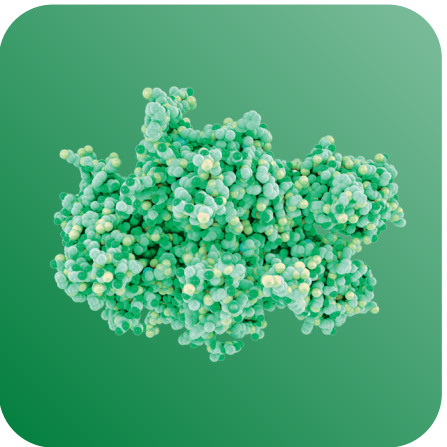
Columns: **YMC Accura Triart Bio C4** (1.9 μm, 30 nm) 150 x 2.1 mm ID
YMC Accura Triart Bio C18 (1.9 μm, 30 nm) 150 x 2.1 mm ID
Part Nos.: TB30SP9-15Q1PTC
TA30SP9-15Q1PTC
Eluent: A) water/difluoroacetic acid (100/0.1)
B) acetonitrile/difluoroacetic acid (100/0.1)
Gradient: 20–32%B (0–1 min), 32–36%B (1–16 min), 36–80%B (16–20 min)
Flow rate: 0.2 ml/min
Temperature: 80 °C
Detection: UV at 280 nm
ESI-MS (positive ion mode)
Injection: 50 μL

By courtesy of Prof. S. Uchiyama, Osaka University, Japan

This research was supported by AMED under Grant Number JP18ae0201001.



(Bioinert)
RP



RP – UHPLC / HPLC selectivities

- 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

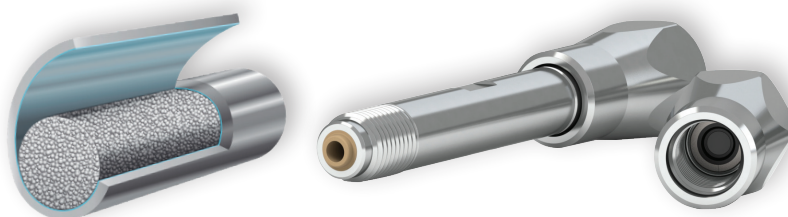
Selectivities for proteins / peptides and antibodies

	YMC-Triart Bio C4	YMC-Triart C18	YMC-Triart Bio C18	Meteoric Core C18 BIO
Base particle	organic/inorganic hybrid silica			core-shell type silica
Modification	C4 (USP L26)	C18 (USP L1)	C18 (USP L1)	C18 (USP L1)
Particle Size / μm	1.9, 3, 5	1.9, 3, 5	1.9, 3, 5	2.7
Pore Size / nm	30	12	30	16
pH range	1.0–10.0	1.0–12.0	1.0–12.0	1.5–10.0
Temperature range	pH < 7: 90 °C pH > 7: 50 °C	pH < 7: 90 °C pH > 7: 50 °C	pH < 9: 90 °C pH > 9: 50 °C	pH < 7: 70 °C pH > 7: 50 °C

Selectivities for oligonucleotides

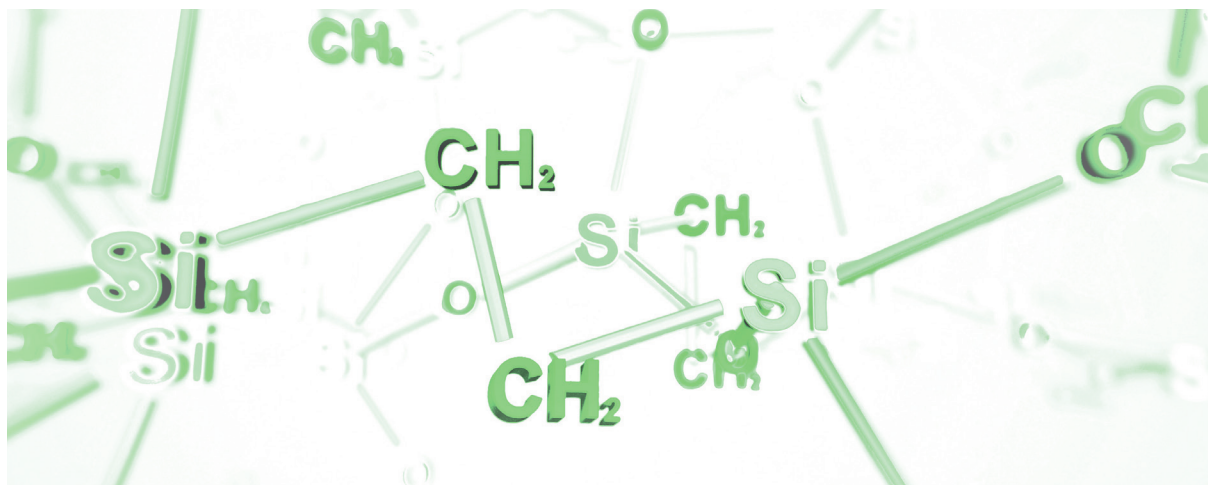
	YMC-Triart C18	YMC-Triart Bio C18	YMC-Triart C8	YMC-Triart Bio C4	Hydrosphere C18
Base particle	organic/inorganic hybrid silica				silica
Modification	C18 (USP L1)	C18 (USP L1)	C8 (USP L7)	C4 (USP L26)	C18 (USP L1)
Particle Size / μm	1.9, 3, 5	1.9, 3, 5	1.9, 3, 5	1.9, 3, 5	2, 3, 5
Pore Size / nm	12	30	12	30	12
pH range	1.0 – 12.0	1.0–12.0	1.0–12.0	1.0–10.0	2.0–8.0
Temperature range	pH < 7: 90 °C pH > 7: 50 °C	pH < 9: 90 °C pH > 9: 50 °C	pH < 7: 90 °C pH > 7: 50 °C	pH < 7: 90 °C pH > 7: 50 °C	50 °C

Bioinert hardware available!



Bioinert YMC-Triart columns are available for improved sensitivity, peak shape and recovery of coordinating compounds such as nucleotides, oligonucleotides or phosphorylated proteins/peptides.

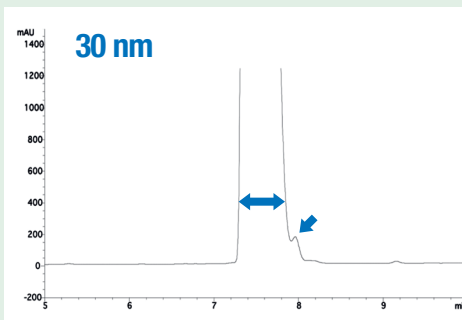
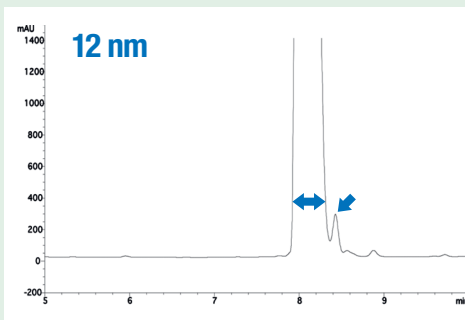
Organic / inorganic hybrid silica base particle



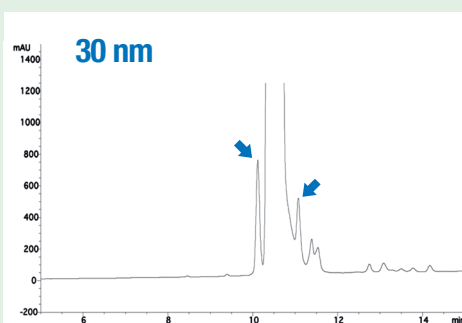
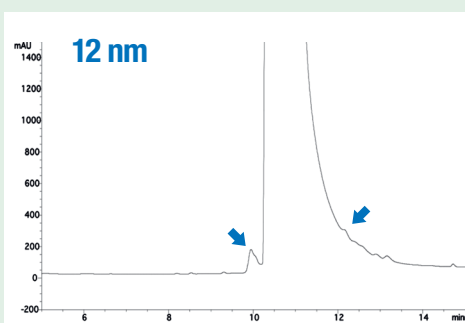
YMC-Triart is a versatile material prepared using tightly controlled particle formation technology. This production process developed by YMC results in exceptionally narrow particle and pore size distributions. With YMC-Triart, challenging pH and high temperature conditions are no longer a limitation to the day-to-day work in laboratories.

Influence of pore size

Angiotensin II
(MW 1,046)



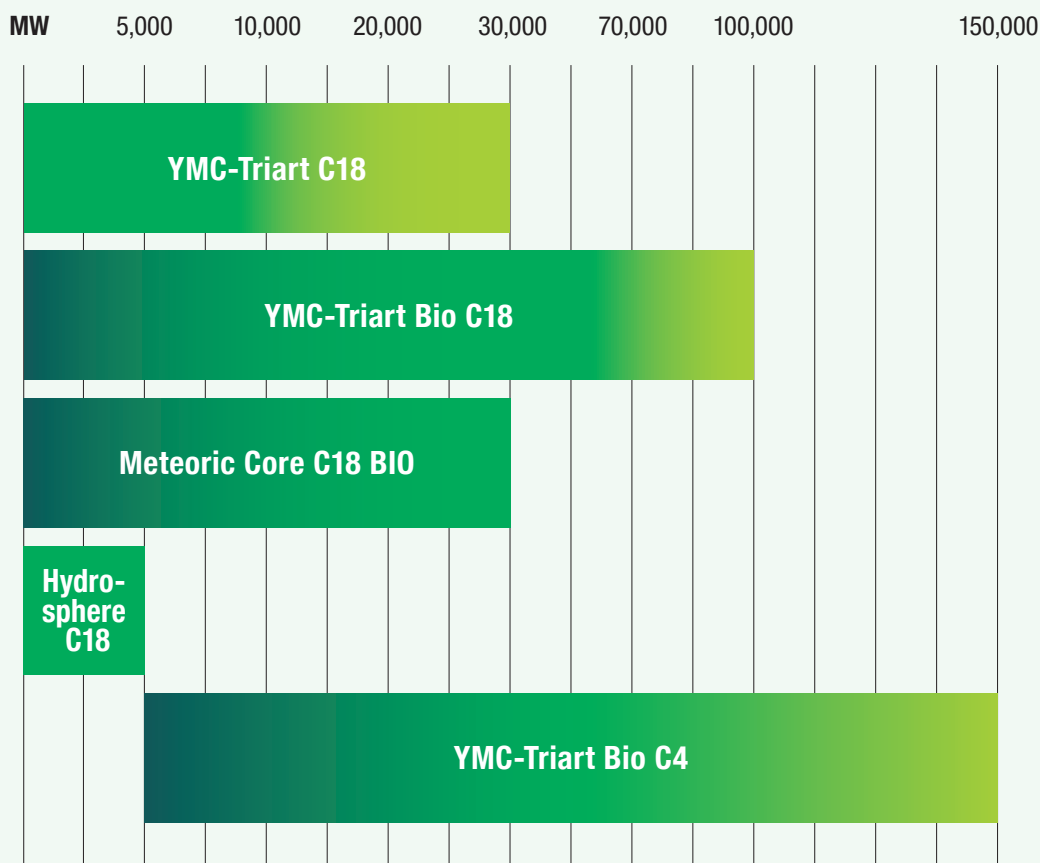
BSA
(MW 67,000)



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

RP – Columns for bioseparations

Column Selection Tool according to molecular weight



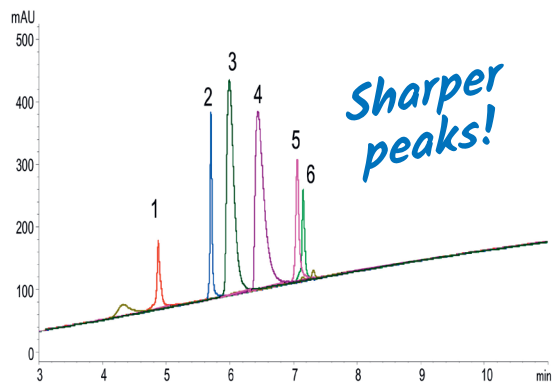
- most appropriate MW range
- extended MW range by elevated temperature
- appropriate MW range

For the separation of proteins, peptides or antibodies columns are selected on the basis of the molecular weight of the target compounds. YMC-Triart C18 with a pore size of 12 nm provides good separation at high temperatures of compounds with molecular weights up to 30,000Da. Widepore columns are effective for the separation of compounds with larger molecules. YMC-Triart Bio C4 with a pore size of 30nm can even separate compounds with molecular weights up to 150,000Da at high temperatures. Elevated temperature can improve efficiency and peak shape by reducing mobile phase viscosity and improving mass transfer. The appropriate molecular weight range for a given pore size of YMC-Triart can be extended compared to using the same pore size at a lower temperature.

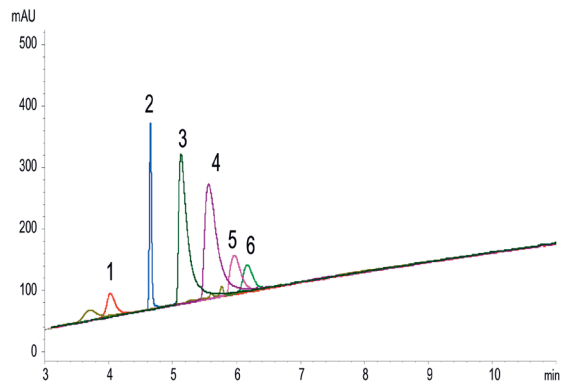
Better performance using YMC-Triart Bio C4

High sensitivity and sharp peaks under LC/MS compatible conditions

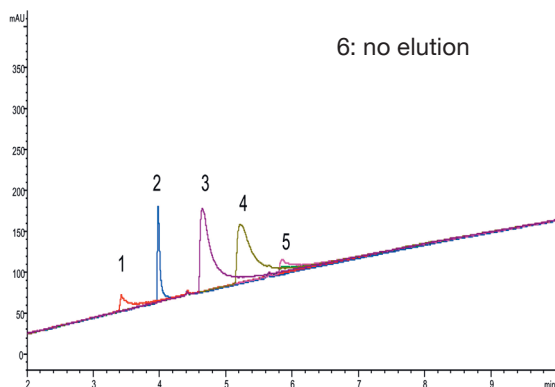
YMC-Triart Bio C4 (3 μ m, 30 nm)



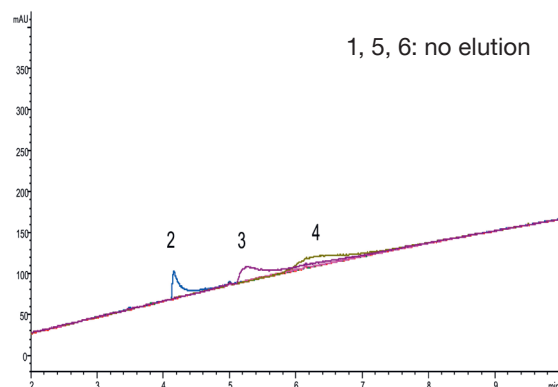
XBridge Protein BEH C4 (3.5 μ m, 30 nm)



AdvanceBio RP-mAb C4 (3.5 μ m, 45 nm)



Aeris widepore C4 (3.6 μ m, 20 nm)



Column: 150 x 3.0 mm ID
 Part No.: TB30S03-1503PTH
 Eluent: A) water/formic acid (100/0.1)
 B) acetonitrile/formic acid (100/0.1)
 Gradient: 10–95%B (0–15 min)
 Flow rate: 0.4 mL/min (for 3.0 mm ID)
 1.0 mL/min (for 4.6 mm ID)
 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)

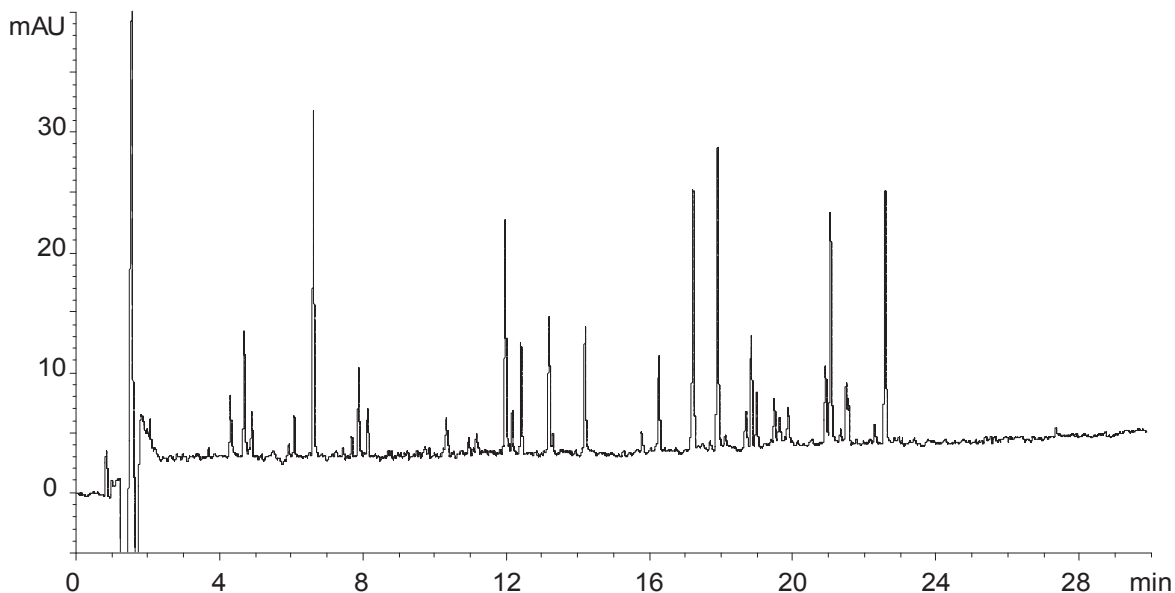
YMC-Triart Bio C4 shows better peak shape and recovery with a mobile phase containing formic acid, which is commonly used for LC/MS analysis. Therefore, YMC-Triart Bio C4 is ideal for high sensitivity analysis of proteins.

RP – YMC-Triart Bio C4: No column adsorption

Petide mapping with increased resolution

Coupling of 2 UHPLC columns

Peptide mapping



$$PC \text{ (peak capacity)} = 1 + (\text{gradient time} / \text{peak width}^*)$$

*peak width = $2W_{0.5h}$ average

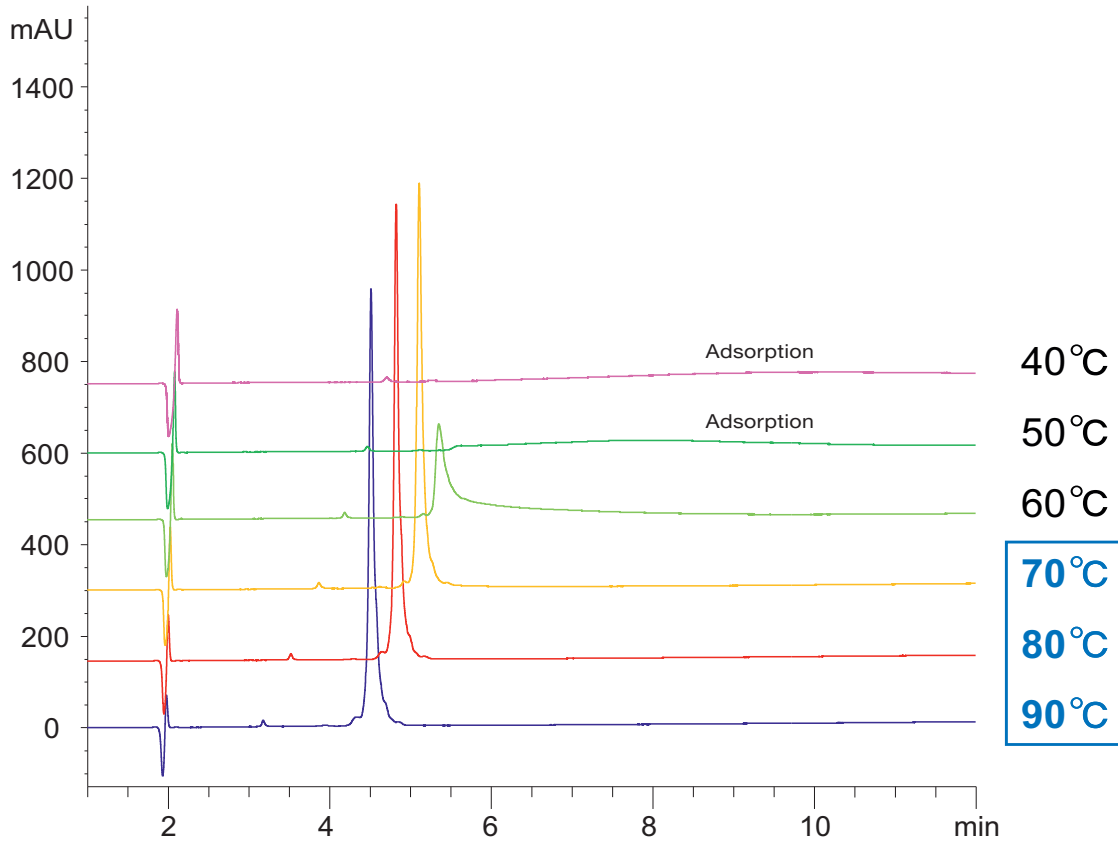
Column: YMC-Triart C18 (1.9 μ m, 12 nm) 200 x 2.0 mm ID (Two coupled 100 x 2.0 mm ID)
 Part No.: TA12SP9-1002PT (2x)
 Eluent: A) water/TFA (100/0.1)
 B) acetonitrile/TFA (100/0.08)
 Gradient: 5–40%B (0–30 min)
 Flow rate: 0.4 mL/min
 Temperature: 70 °C
 Detection: UV at 220 nm
 Injection: 20 μ L
 Sample: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



Coupling of two YMC-Triart UHPLC columns using the dead volume free MarvelIX™ connector.

High temperature tolerance allows antibody analysis

Bevacizumab (Avastin®, 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)
 Flow rate: 0.4 mL/min
 Detection: UV at 220 nm
 Injection: 4 µL
 Sample: Bevacizumab (0.5 mg/mL)

“

“The possibility to use temperatures up to 90 °C with YMC-Triart Bio C4 simplifies the development of analytical methods. Furthermore, a good peak shape can be obtained without the addition of TFA, which means that I have fewer problems when using it for MS.”

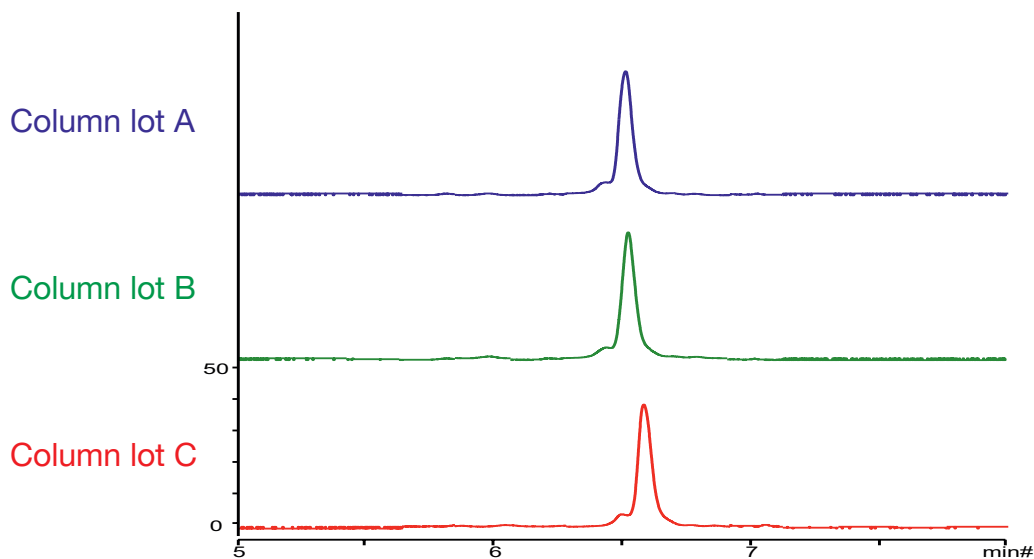
Lars M. H. Reinders, Institute for Energy and Environmental Technology e. V. (IUTA, DE)

”

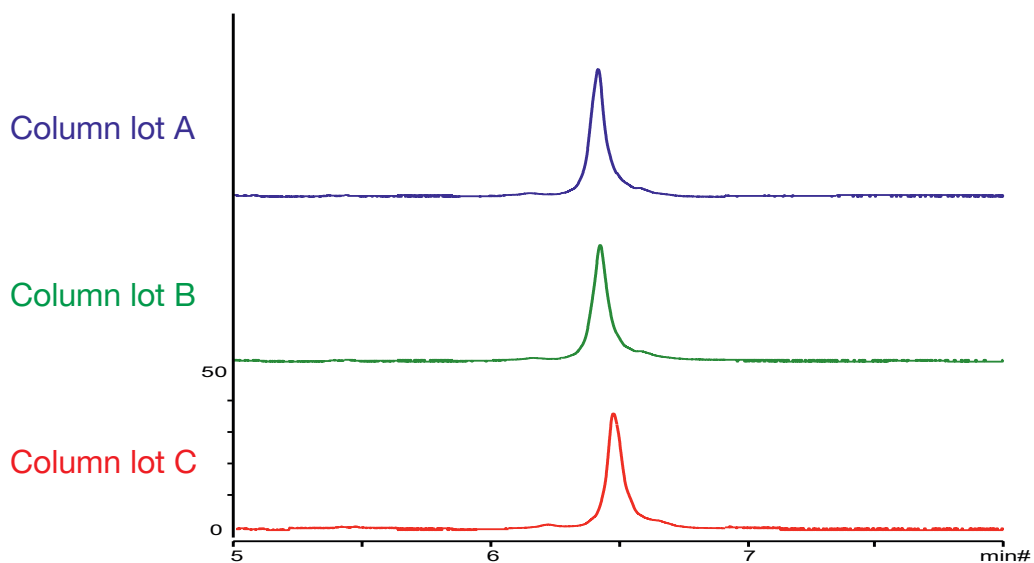
RP – YMC-Triart Bio C4: Reproducibility

Excellent Batch-to-batch reproducibility for antibody analysis

NISTmAb, 8671



Bevacizumab (Avastin®)



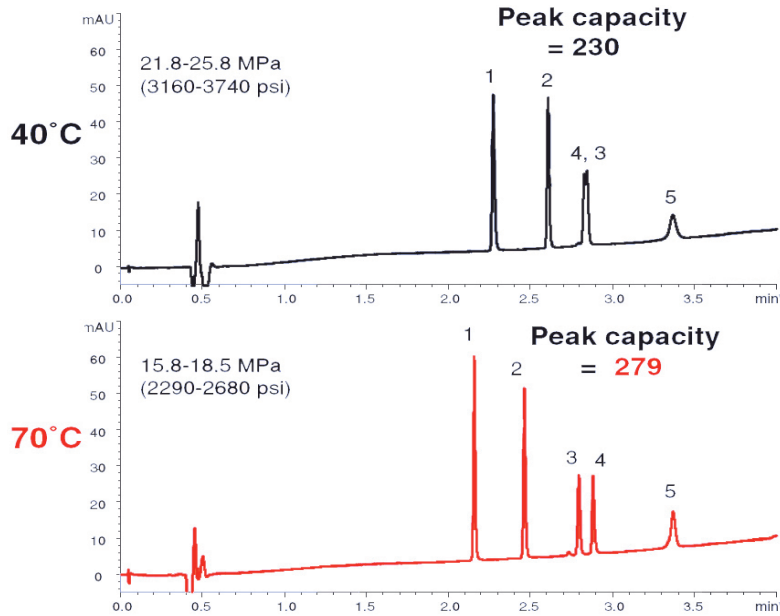
Column: YMC-Triart Bio C4 (1.9 μ m, 30 nm) 50 x 2.1 mm ID
 Part No.: TB30SP9-05Q1PT
 Eluent: A) water/TFA (100/0.1), B) acetonitrile/TFA (100/0.1)
 Gradient: 25–45%B (0–10 min)
 Flow rate: 0.4 mL/min
 Temperature: 80 °C
 Detection: UV at 280 nm
 Injection: 2 μ L (0.5 mg/mL)

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 flexibility using YMC-Triart

Highly efficient RP-HPLC separation of proteins

Mixture A (MW 500–18,400)



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

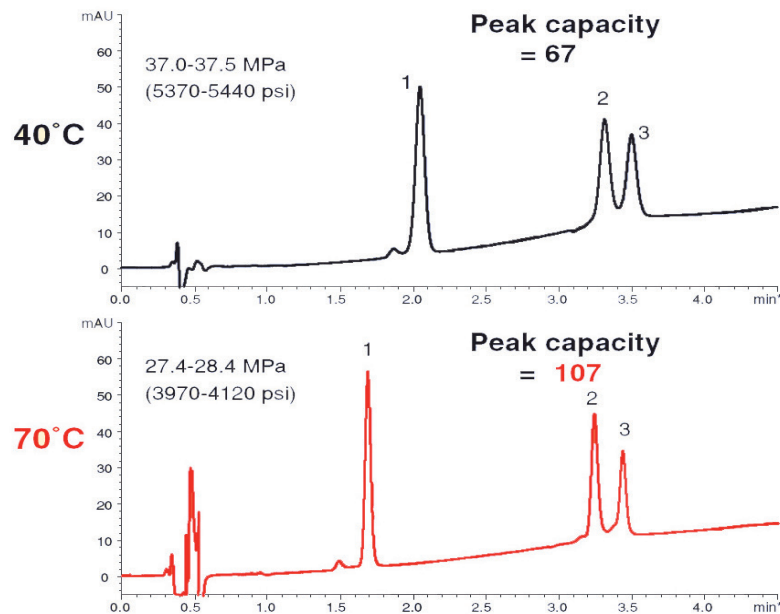
Mixture A

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

Mixture B (MW 14,300–25,700)



High temperatures only possible with YMC-Triart

Column: YMC-Triart C18 (1.9 μm, 12 nm) 50 x 2.0 mm ID
 Part-No.: TA12SP9-0502WT
 Eluent: A) water/TFA (100/0.1)
 B) acetonitrile/TFA (100/0.1) - mixture A
 B) acetonitrile/2-propanol/TFA (50/50/0.1) - mixture B
 Gradient: 10–80%B (0–5 min) - mixture A
 30–60%B (0–5 min) - mixture B

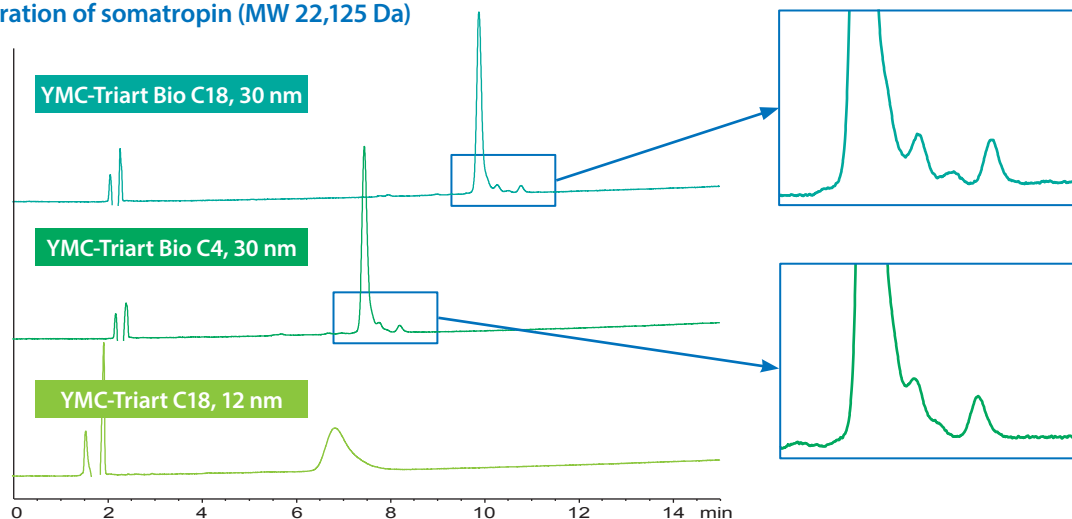
Flow rate: 0.4 mL/min
 Detection: UV at 220 nm
 Injection: 1 μL (50 μg/mL) - condition A
 1 μL (250 μg/mL) - condition B
 System: Agilent 1200SL

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

RP – YMC-Triart Bio C18: Great peak shapes

Ideal solutions for any kind of biomolecule

Separation of somatropin (MW 22,125 Da)



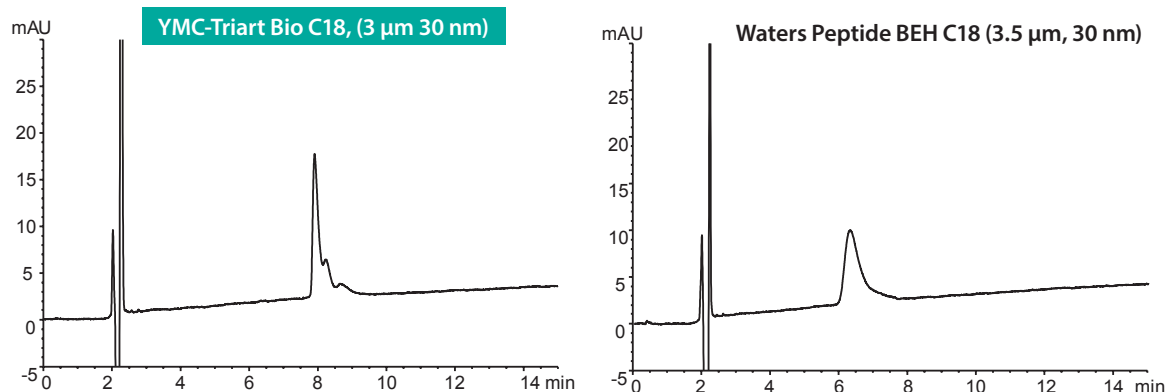
Columns: 150 x 3.0 mm ID (3 μ m)
 Part Nos.: TA30S03-1503PTH
 TB30S03-1503PTH
 TA12S03-1503PTH
 Eluent: A) water/TFA (100/0.1)
 B) acetonitrile/TFA (100/0.08)

Gradient: 50–70%B (0–15 min)
 Flow rate: 0.425 mL/min
 Temperature: 40 °C
 Detection: UV at 220 nm
 Injection: 4 μ L
 Sample: Somatropin (0.1 mg/mL)

In this example of somatropin, a peptide of 22,125 Da, good peak shape can be obtained with the widepore columns YMC-Triart Bio C18 and YMC-Triart Bio C4. Excellent separation was achieved using YMC-Triart Bio C18 with longer alkyl chains in its bonded phase.

Ideal for MS conditions

Good peak shape with mobile phase containing formic acid



Column: 150 x 3.0 mm ID; 150 x 4.6 mm ID
 Part No.: TA30S03-1503PTH
 Eluent: A) water/formic acid (100/0.1)
 B) acetonitrile/formic acid (100/0.08)
 Gradient: 45–65%B (0–15 min)

Flow rate: 0.425 mL/min for 3.0 mm ID; 1.0 mL/min for 4.6 mm ID
 Temperature: 40 °C
 Detection: UV at 220 nm
 Sample: Somatropin (0.1 mg/mL)

YMC-Triart Bio C18 is suitable for highly sensitive analysis and structural analysis of proteins using LC/MS since good peak shapes in mobile phase containing formic acid can be achieved.

RP – Hydrosphere C18: Oligonucleotide purification

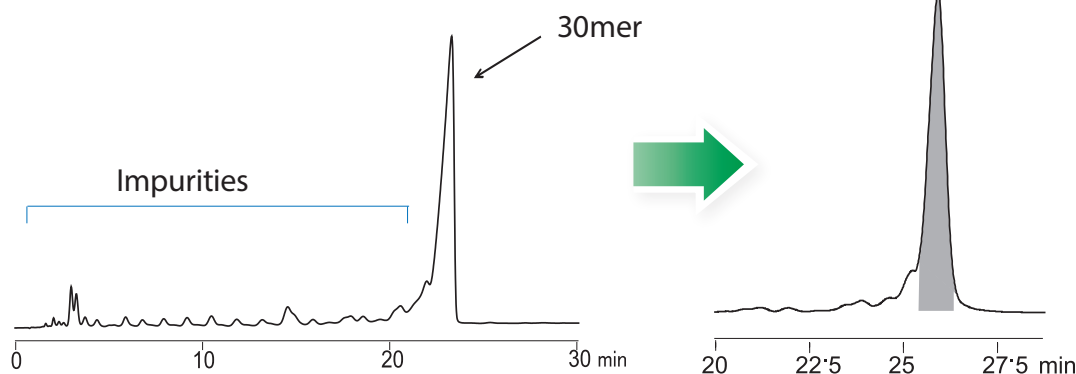
Easy purification of oligonucleotides with YMC-Actus semiprep columns

Purification of synthetic 30mer oligonucleotide

Analysis 1.0 mL/min, 5 μ L injection
Hydrosphere C18
 50 x 4.6 mm ID, 5 μ m

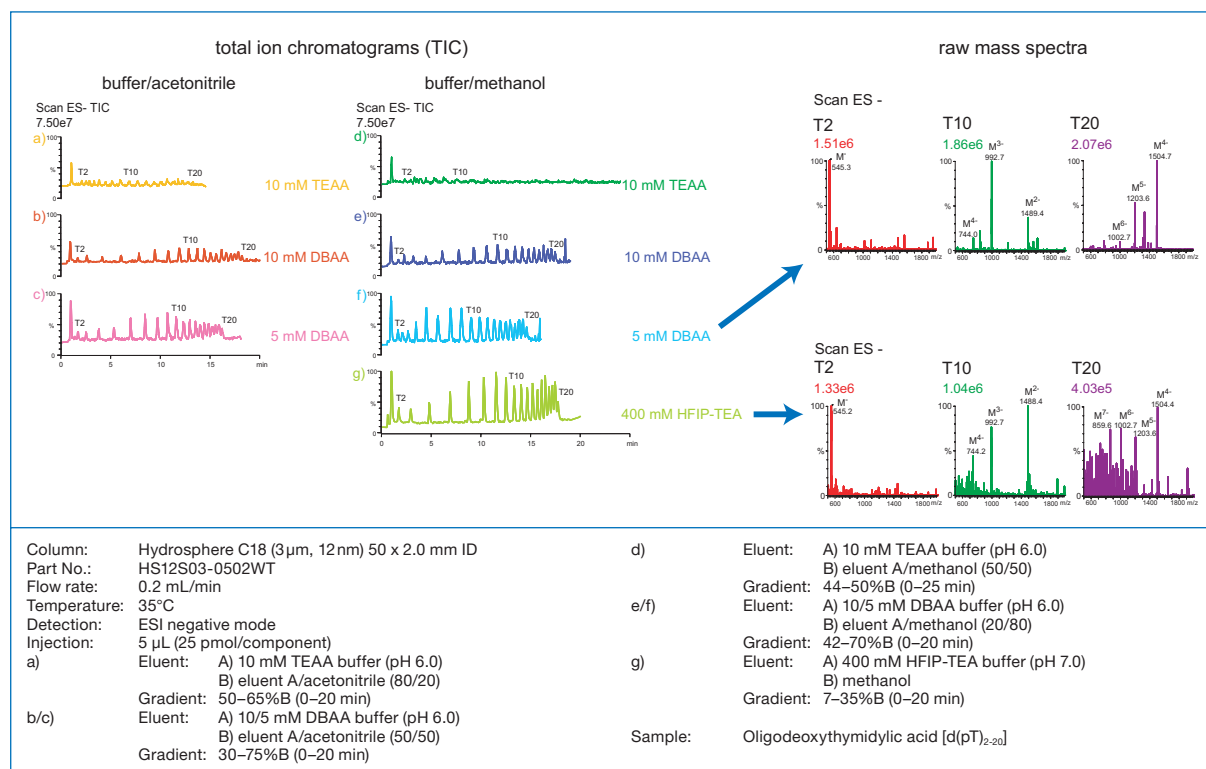
Purification 19 mL/min, 100 μ L injection
YMC-Actus Hydrosphere C18
 50 x 20 mm ID, 5 μ m

Recovery 56 %
Purity > 99 %



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 μ M)

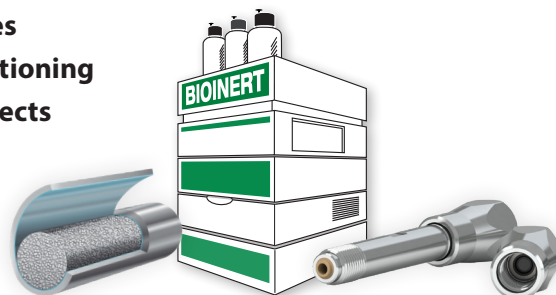
Influences of mobile phase conditions on intensity of ESI-MS



RP – YMC-Triart: Bioinert hardware

Bioinert columns for bioseparations and coordinating compounds

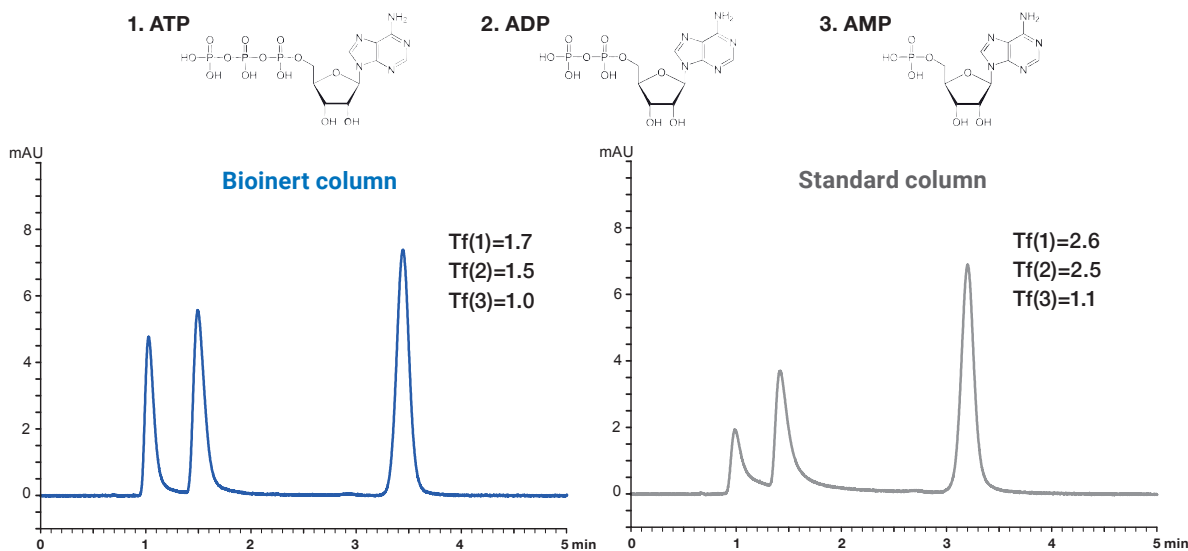
- Exceptional peak shapes with high sensitivities
- Excellent recoveries without column preconditioning
- Superior reproducibility and no carry-over effects
- Ideal for highly sensitive LC/MS analyses
- Different bioinert hardware options



Specification

	YMC Accura Triart	YMC-Triart metal-free PEEK-lined
YMC-Triart modifications	C18, C18 ExRS, Bio C18, C8, Bio C4, Phenyl, PFP, Diol-HILIC	
Particle Size	1.9, 3 and 5 μm	
Column hardware	Bioinert coated stainless steel	PEEK-lined stainless steel
Frit hardware	Bioinert coated stainless steel	PEEK
Hardware properties	Less hydrophobic	More hydrophobic
Pressure limit	1.9 μm : 100 MPa (15,000 psi) 3/5 μm : 45 MPa (6,525 psi)	
Column connection	No special connections required	Selected universal connectors such as MarvelXACT™

Improved sensitivity for coordination compounds



Column: YMC-Triart C18 (3 μm , 12nm) 50 x 2.1 mm ID
 Part Nos.: TA12S03-05Q1PTP (metal-free PEEK-lined) or
 TA12S03-05Q1PTH (standard hardware)
 Eluent: 5 mM HCOONH₄
 Flow rate: 0.21 mL/min

Temperature: 25 °C
 Detection: UV at 265 nm
 Injection: 1 μL (10 $\mu\text{g}/\text{mL}$)
 System: bioinert/"metal-free" 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 a bioinert column hardware, better peak shapes can be expected.

Nucleotides with phosphate groups also show better peak shapes when compared to the regular column hardware. The applied YMC-Triart metal-free PEEK-lined as well as the YMC Accura Triart column hardware are ideal for highly sensitive analyses using LC/MS.

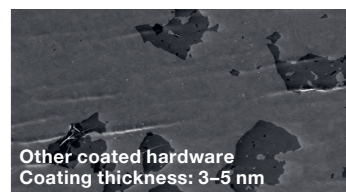
YMC Accura Triart: durable bioinert coating



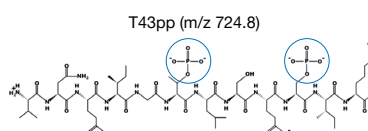
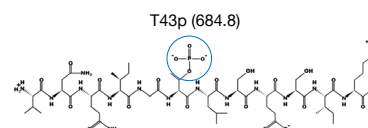
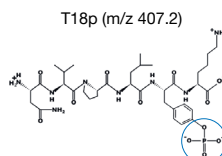
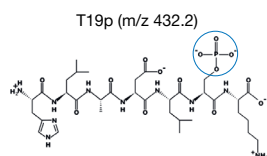
The robust bioinert coating used on YMC Accura hardware is 130 to 320-fold thicker making it more durable than other similar hardware concepts. A long-term inertness against sensitive substances is ensured. In order to demonstrate its robustness, a YMC Accura column was packed multiple times. Even though this is quite a challenge for the column surface, the coating remains unaffected (SEM* picture: top area is bare steel for comparison).

*Scanning Electron Microscope

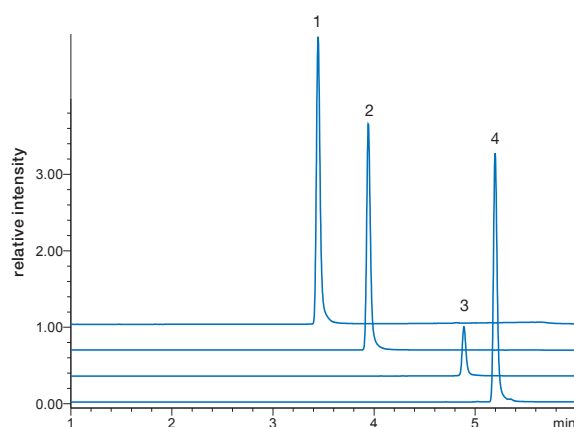
Other coated columns can lose their inertness over time. This will again lead to adsorption of sensitive compounds on the uncovered metallic surfaces. Peak tailing, loss of recovery and sample carry-over are typical results of the delamination of the coating. After only unpacking a coated competitor column most of the coating is already delaminated (dark spots: remaining coating).



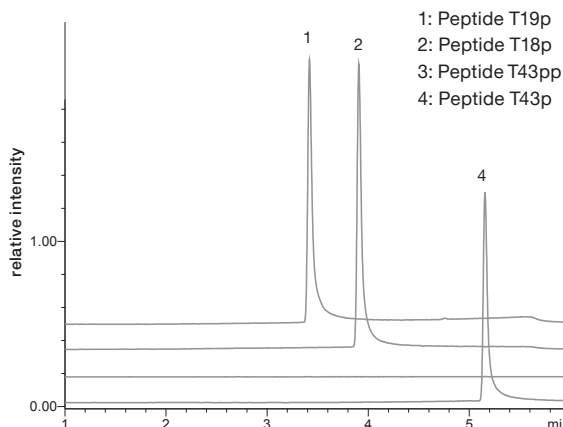
Full recovery of phosphorylated peptides



YMC Accura Triart C18



Standard column



- 1: Peptide T19p
- 2: Peptide T18p
- 3: Peptide T43pp
- 4: Peptide T43p

Columns: YMC Accura Triart C18 (1.9µm, 30 nm) 100 x 2.1 mm ID (bioinert hardware)
 YMC-Triart C18 (1.9µm, 30 nm) 100 x 2.1 mm ID (standard hardware)
 Part Nos.: TA12SP9-10Q1PTC
 TA12SP9-10Q1PT
 Eluent: A) water + 0.1% formic acid
 B) acetonitrile + 0.1% formic acid

Gradient: 0.7%–25%B (0–5 min), 25%B (5–6.6 min), 0.7%B (6.6–8 min)
 Flow rate: 0.6 ml/min
 Temperature: 60 °C
 Detection: ESI-MS
 Injection: 2 µL (10 pmol/µL)
 Sample: Massprep phosphopeptide enolase standard (Waters)
 System: Shimadzu Nexera XS inert
 Shimadzu LCMS-2020

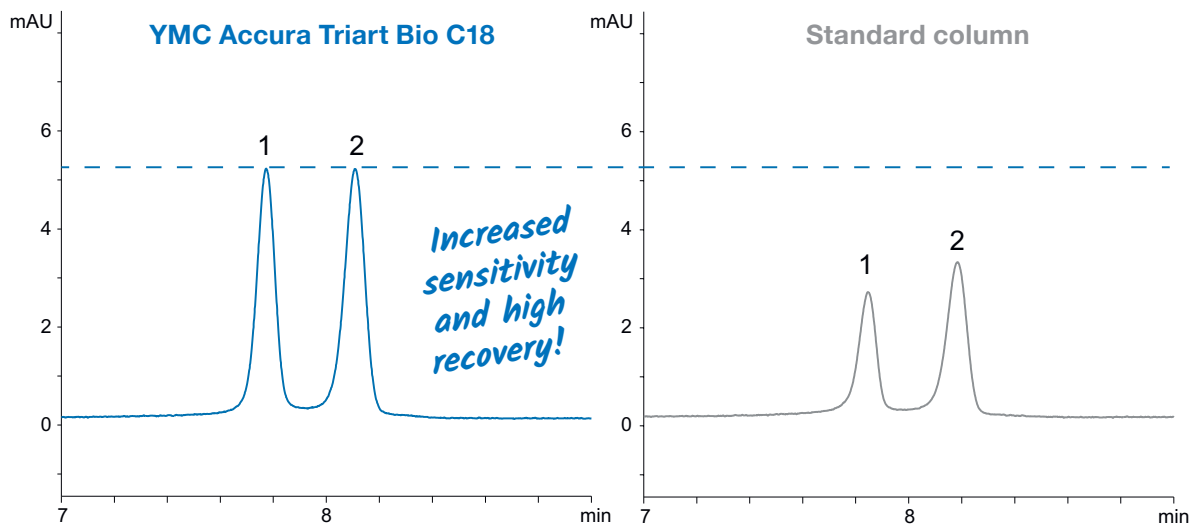
By courtesy of Shimadzu Europa.

The use of a bioinert coated **YMC Accura Triart C18** column led to higher intensities and peak areas of four phosphopeptides, compared to the stainless steel column. The high recovery rate of the **YMC Accura Triart C18** column also enabled the detection of the challenging phosphopeptide T43pp, which contains two phosphate residues. In contrast, detection of peptide T43pp was unsuccessful with the standard column, even after ten injections no signal was observed.

RP – YMC-Triart: Bioinert hardware

Significantly higher sensitivity and recovery

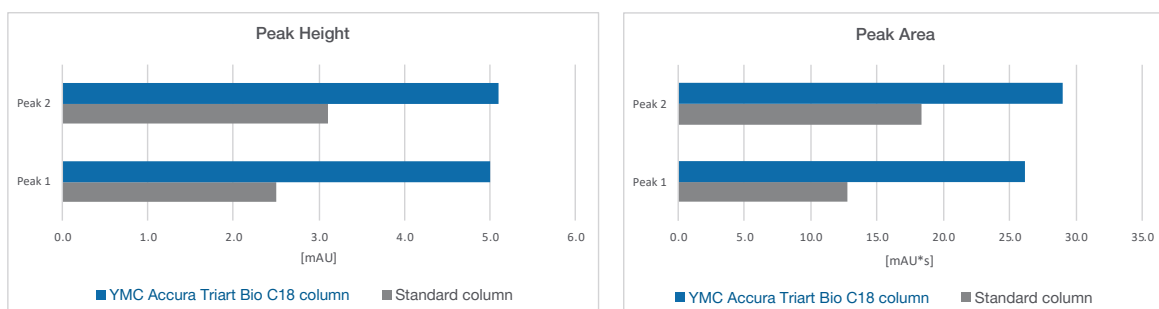
Ideal choice for challenging analytes such as phosphorothioate oligonucleotides



Column: YMC Accura Triart Bio C18 (1.9µm, 30 nm) 50 x 2.1 mm ID
 Part No.: TA30SP9-05Q1PTC
 Eluent: A) 15 mM triethylamine - 400 mM HFIP*
 B) methanol
 Gradient: 8–18%B (0–10 min)
 Flow rate: 0.42 mL/min
 Temperature: 65°C
 Detection: UV at 260 nm
 Injection: 1 µL
 Sample: All PS RNA 20mer (1) (5'-U[^]C[^]A[^]U[^]C[^]A[^]C[^]A[^]C[^]U[^]G[^]A[^]A[^]U[^]A[^]C[^]C[^]A[^]A[^]U[^]-3')
 All PS RNA 21mer (2) (5'-G[^]U[^]C[^]A[^]U[^]C[^]A[^]C[^]A[^]C[^]U[^]G[^]A[^]A[^]U[^]A[^]C[^]C[^]A[^]A[^]U[^]-3')
 ^=Phosphorothioate

*1,1,1,3,3,3-hexafluoro-2-propanol

High sensitivity and recovery

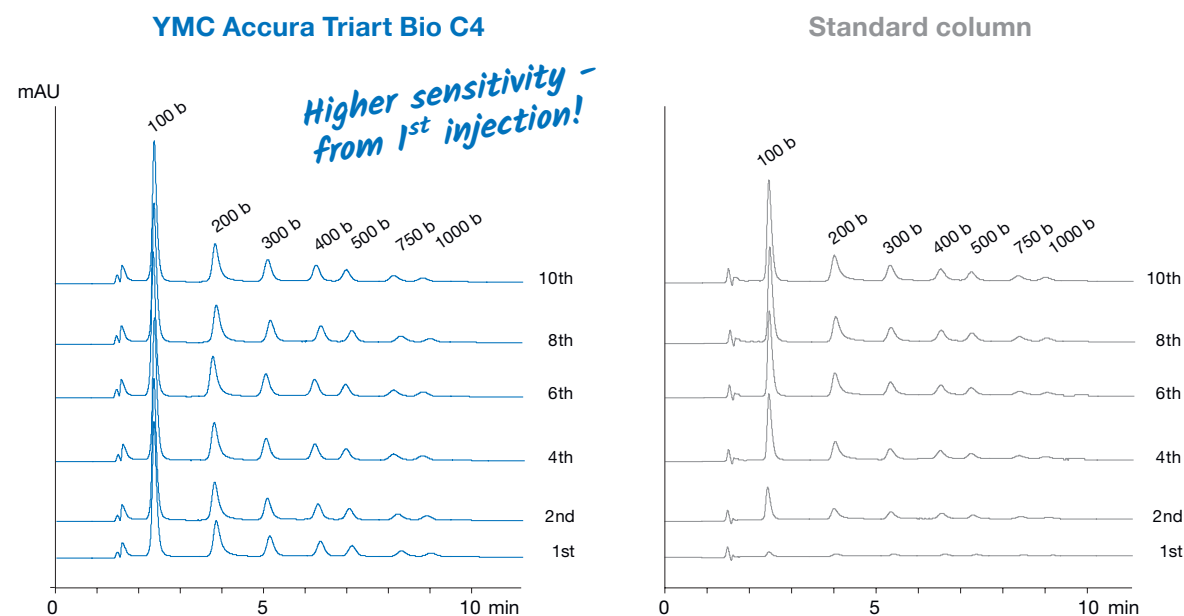


Doubled peak height and area!

The YMC Accura Triart Bio C18 column provides double peak heights and peak areas for the oligonucleotides compared to those for regular stainless-steel columns. YMC Accura Triart columns enhance the sensitivity significantly and help to save precious samples without any loss.

Reliable results from the first injection

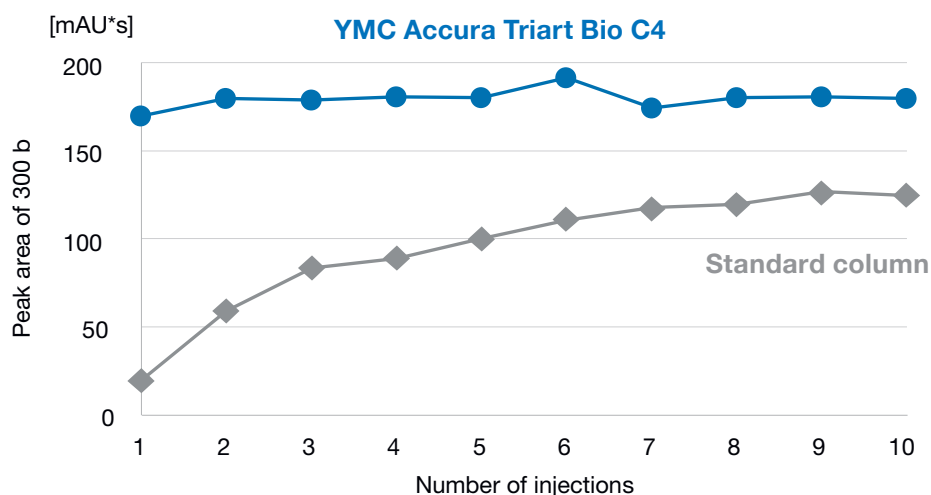
No preconditioning required for reliable results



Column:	YMC Accura Triart Bio C4 (3 μm, 30 nm) 100 x 2.1 mm ID	Flow rate:	0.2 mL/min
Part No.:	TA30S03-10Q1PTC	Temperature:	80°C
Eluent:	A) 50 mM TEAA* (pH 7.0)/acetonitrile (95/5)	Detection:	UV at 254 nm
	B) 50 mM TEAA (pH 7.0)/acetonitrile (50/50)	Injection:	1 μL (0.25 mg/mL)
Gradient:	9–14%B (0–10 min), 80%B (10–15 min)	Sample:	100–1,000 bases (Century™-Plus RNA Markers)

* triethylammonium acetate

Constantly higher peak areas and therefore recoveries

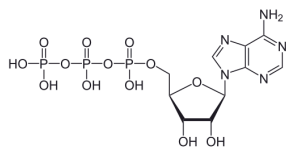


The YMC Accura Triart Bio C4 column shows stable peak areas from the first injection, while the standard stainless-steel column provides only 10% of the peak area (for the 300 base marker) with the first injection. Even after the tenth injection, the peak areas of the stainless-steel column are considerably less than those of the YMC Accura Triart column.

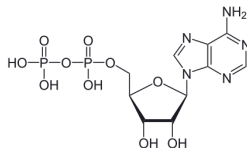
RP – Expert Tips: (Oligo)nucleotides

Influence of system and column hardware on the analysis of nucleotides

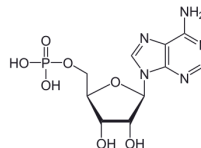
1 ATP



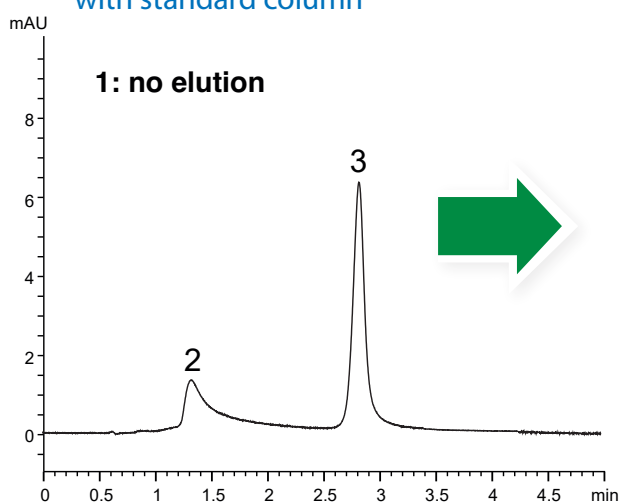
2 ADP



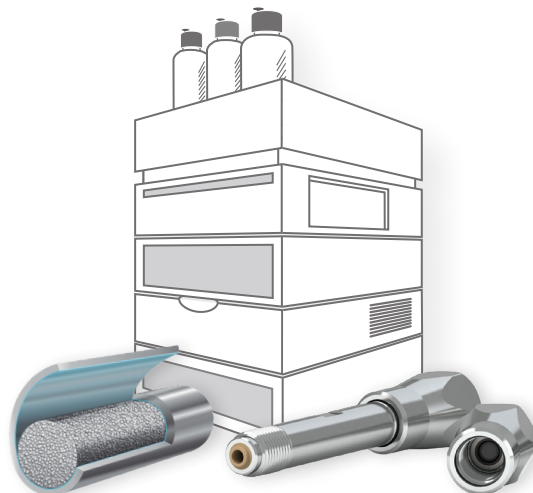
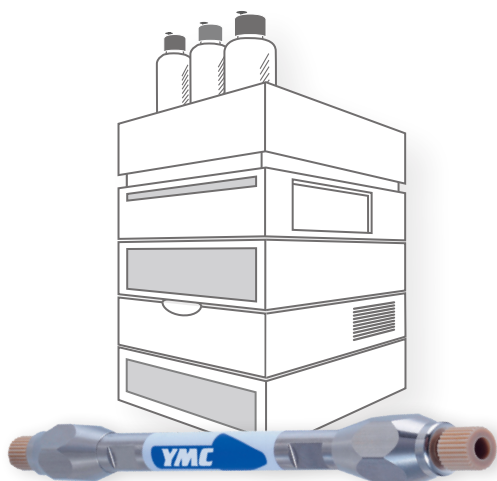
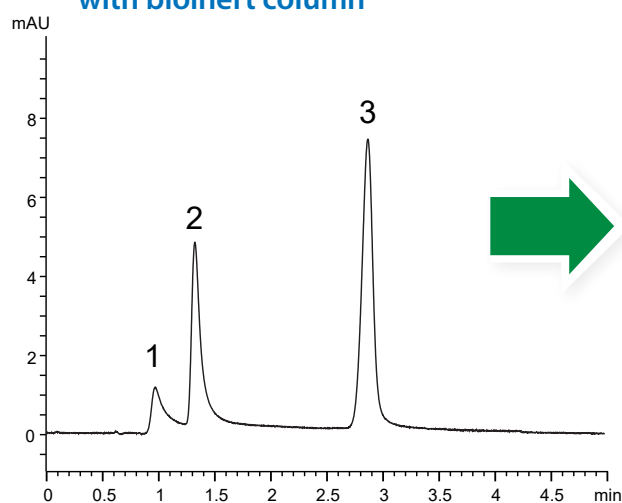
3 AMP



Ordinary HPLC system with standard column



Ordinary HPLC system with bioinert column

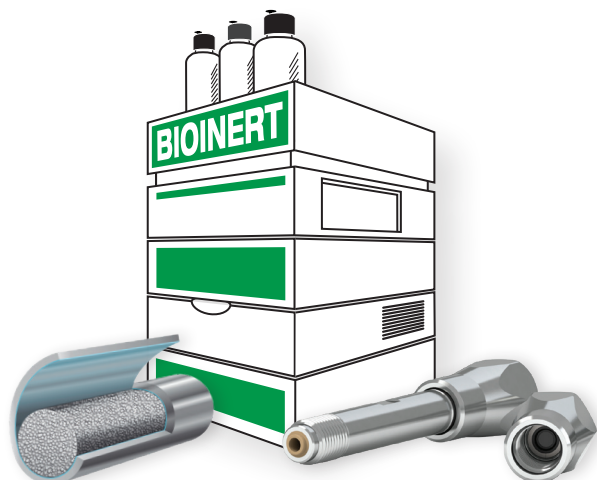
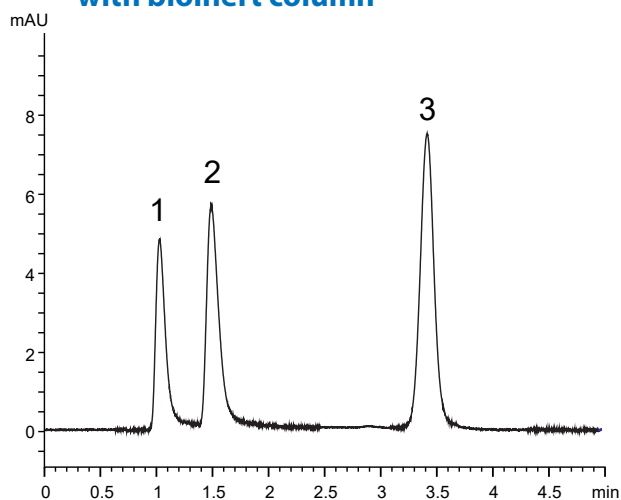


Column: YMC-Triart C18 (3 μ m, 12 nm) 50 x 2.1 mm ID
 Part Nos: TA12S03-05Q1PT (standard hardware)
 TA12S03-05Q1PTP (bioinert hardware)
 Eluent: 5 mM HCOONH₄
 Flow rate: 0.21 mL/min
 Temperature: 25°C
 Detection: UV at 265 nm
 Injection: 1 μ L (10 μ g/mL)

*Bioinert 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 a bioinert column.

Bioinert HPLC system* with bioinert column



“

“Metal-free YMC columns significantly reduce non-specific adsorption phenomena”

“YMC-Triart C18 metal-free columns significantly reduce non-specific adsorption phenomena during peptides analysis. We use these columns in our laboratory for a specific application. We obtain very good chromatographic resolution and excellent robustness, which is very appreciable during routine analysis.”

*Cynthia Mongongu, LADF,
Laboratoire AntiDopage Français,
Université Paris-Saclay (FR)*

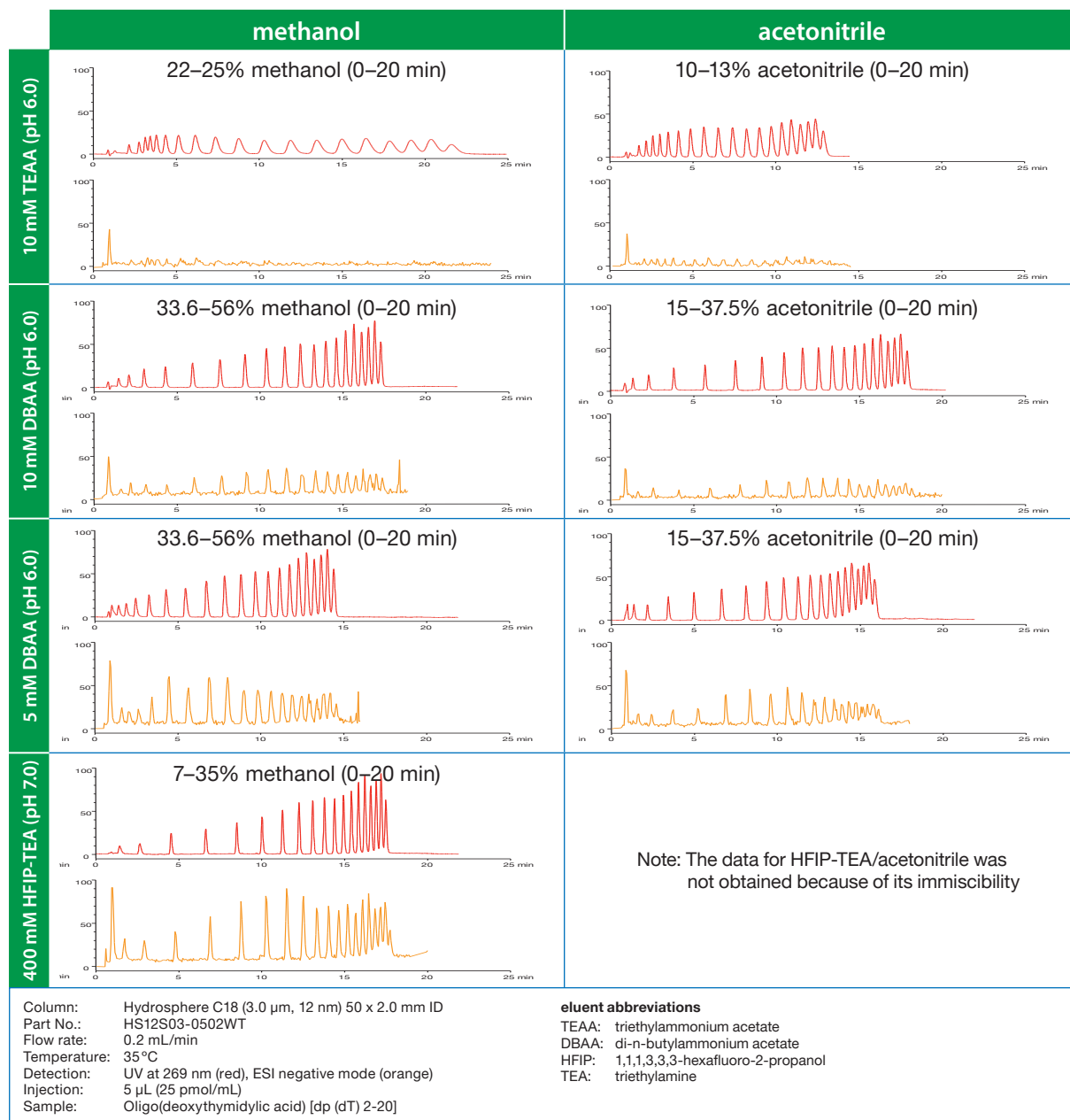
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Peak shape is greatly improved as a result of using a bioinert HPLC system.

RP – Expert Tips: Oligonucleotides

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



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.

1.9 µm UHPLC columns (max. pressure 100 MPa)

Phase	Column ID [mm]	Column length [mm]					Guard cartridges* with 5 mm length (pack of 3)
		30	50	75	100	150	
YMC-Triart C18	1.0	—	TA12SP9-0501WT	—	TA12SP9-1001WT	TA12SP9-1501WT	TA12SP9-E5Q1CC**
	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 Bio C18	2.0	TA30SP9-0302PT	TA30SP9-0502PT	TA30SP9-L502PT	TA30SP9-1002PT	TA30SP9-1502PT	TA30SP9-E5Q1CC**
	2.1	TA30SP9-03Q1PT	TA30SP9-05Q1PT	TA30SP9-L5Q1PT	TA30SP9-10Q1PT	TA30SP9-15Q1PT	TA30SP9-E5Q1CC**
	3.0	—	TA30SP9-0503PT	TA30SP9-L503PT	TA30SP9-1003PT	TA30SP9-1503PT	TA30SP9-E503CC
YMC-Triart C8	2.0	T012SP9-0302PT	T012SP9-0502PT	T012SP9-L502PT	T012SP9-1002PT	T012SP9-1502PT	T012SP9-E5Q1CC**
	2.1	T012SP9-03Q1PT	T012SP9-05Q1PT	T012SP9-L5Q1PT	T012SP9-10Q1PT	T012SP9-15Q1PT	T012SP9-E5Q1CC**
	3.0	—	T012SP9-0503PT	T012SP9-L503PT	T012SP9-1003PT	T012SP9-1503PT	T012SP9-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 bioinert coated UHPLC columns (max. pressure 100 MPa)

Phase	Column ID [mm]	Column length [mm]		
		50	100	150
YMC Accura Triart C18	2.1	TA12SP9-05Q1PTC	TA12SP9-100Q1PTC	TA12SP9-15Q1PTC
YMC Accura Triart Bio C18	2.1	TA30SP9-05Q1PTC	TA30SP9-10Q1PTC	TA30SP9-15Q1PTC
YMC Accura Triart C8	2.1	T012SP9-05Q1PTC	T012SP9-10Q1PTC	T012SP9-15Q1PTC
YMC Accura Triart Bio C4	2.1	TB30SP9-05Q1PTC	TB30SP9-10Q1PTC	TB30SP9-15Q1PTC

1.9 µm PEEK-lined UHPLC columns (max. pressure 100 MPa)

Phase	Column ID [mm]	Column length [mm]		
		50	100	150
YMC-Triart C18 metal-free	2.1	TA12SP9-05Q1PTP	TA12SP9-10Q1PTP	TA12SP9-15Q1PTP
YMC-Triart Bio C18 metal-free	2.1	TA30SP9-05Q1PTP	TA30SP9-10Q1PTP	TA30SP9-15Q1PTP
YMC-Triart C8 metal-free	2.1	T012SP9-05Q1PTP	T012SP9-10Q1PTP	T012SP9-15Q1PTP
YMC-Triart Bio C4 metal-free	2.1	TB30SP9-05Q1PTP	TB30SP9-10Q1PTP	TB30SP9-15Q1PTP

Special column connectors required.

RP – Ordering information

3 µm HPLC columns (max. pressure 25–45 MPa)

Phase	Column ID [mm]	Column length [mm]					Guard cartridges* with 10 mm length (pack of 5)
		50	75	100	150	250	
YMC-Triart C18	2.0	TA12S03-0502WT	TA12S03-L502WT	TA12S03-1002WT	TA12S03-1502WT	TA12S03-2502WT	TA12S03-01Q1GC
	2.1	TA12S03-05Q1PTH	TA12S03-L5Q1PTH	TA12S03-10Q1PTH	TA12S03-15Q1PTH	TA12S03-25Q1PTH	TA12S03-01Q1GC
	3.0	TA12S03-0503WT	TA12S03-L503WT	TA12S03-1003WT	TA12S03-1503WT	TA12S03-2503WT	TA12S03-0103GC
	4.6	TA12S03-0546WT	TA12S03-L546WT	TA12S03-1046WT	TA12S03-1546WT	TA12S03-2546WT	TA12S03-0104GC
YMC-Triart Bio C18	2.0	TA30S03-0502WT	TA30S03-L502WT	TA30S03-1002WT	TA30S03-1502WT	TA30S03-2502WT	TA30S03-01Q1GC
	2.1	TA30S03-05Q1PTH	TA30S03-L5Q1PTH	TA30S03-10Q1PTH	TA30S03-15Q1PTH	TA30S03-25Q1PTH	TA30S03-01Q1GC
	3.0	TA30S03-0503WT	TA30S03-L503WT	TA30S03-1003WT	TA30S03-1503WT	TA30S03-2503WT	TA30S03-0103GC
	4.6	TA30S03-0546WT	TA30S03-L546WT	TA30S03-1046WT	TA30S03-1546WT	TA30S03-2546WT	TA30S03-0104GC
YMC-Triart C8	2.0	T012S03-0502WT	T012S03-L502WT	T012S03-1002WT	T012S03-1502WT	T012S03-2502WT	T012S03-01Q1GC
	2.1	T012S03-05Q1PTH	T012S03-L5Q1PTH	T012S03-10Q1PTH	T012S03-15Q1PTH	T012S03-25Q1PTH	T012S03-01Q1GC
	3.0	T012S03-0503WT	T012S03-L503WT	T012S03-1003WT	T012S03-1503WT	T012S03-2503WT	T012S03-0103GC
	4.6	T012S03-0546WT	T012S03-L546WT	T012S03-1046WT	T012S03-1546WT	T012S03-2546WT	T012S03-0104GC
YMC-Triart Bio C4	2.0	TB30S03-0502WT	TB30S03-L502WT	TB30S03-1002WT	TB30S03-1502WT	TB30S03-2502WT	TB30S03-01Q1GC
	2.1	TB30S03-05Q1PTH	TB30S03-L5Q1PTH	TB30S03-10Q1PTH	TB30S03-15Q1PTH	TB30S03-25Q1PTH	TB30S03-01Q1GC
	3.0	TB30S03-0503WT	TB30S03-L503WT	TB30S03-1003WT	TB30S03-1503WT	TB30S03-2503WT	TB30S03-0103GC
	4.6	TB30S03-0546WT	TB30S03-L546WT	TB30S03-1046WT	TB30S03-1546WT	TB30S03-2546WT	TB30S03-0104GC
Hydrosphere C18	2.0	HS12S03-0502WT	HS12S03-L502WT	HS12S03-1002WT	HS12S03-1502WT	HS12S03-2502WT	HS12S03-01Q1GC
	2.1	HS12S03-05Q1WT	HS12S03-L5Q1WT	HS12S03-10Q1WT	HS12S03-15Q1WT	HS12S03-25Q1WT	HS12S03-01Q1GC
	3.0	HS12S03-0503WT	HS12S03-L503WT	HS12S03-1003WT	HS12S03-1503WT	HS12S03-2503WT	HS12S03-0103GC
	4.6	HS12S03-0546WT	HS12S03-L546WT	HS12S03-1046WT	HS12S03-1546WT	HS12S03-2546WT	HS12S03-0104GC

*Guard cartridge holder required, part no. XPGCH-Q1 (for EMEA) /XPGCHP1 (outside EMEA)

3 µm bioinert coated HPLC columns (max. pressure 45 MPa)

Phase	Column ID [mm]	Column length [mm]		
		50	100	150
YMC Accura Triart C18	2.1	TA12S03-05Q1PTC	TA12S03-10Q1PTC	TA12S03-15Q1PTC
	4.6	TA12S03-0546PTC	TA12S03-1046PTC	TA12S03-1546PTC
YMC Accura Triart Bio C18	2.1	TA30S03-05Q1PTC	TA30S03-10Q1PTC	TA30S03-15Q1PTC
	4.6	TA30S03-0546PTC	TA30S03-1046PTC	TA30S03-1546PTC
YMC Accura Triart C8	2.1	T012S03-05Q1PTC	T012S03-10Q1PTC	T012S03-15Q1PTC
	4.6	T012S03-0546PTC	T012S03-1046PTC	T012S03-1546PTC
YMC Accura Triart Bio C4	2.1	TB30S03-05Q1PTC	TB30S03-10Q1PTC	TB30S03-15Q1PTC
	4.6	TB30S03-0546PTC	TB30S03-1046PTC	TB30S03-1546PTC

3 µm PEEK-lined HPLC columns (max. pressure 45 MPa)

Phase	Column ID [mm]	Column length [mm]		
		50	100	150
YMC-Triart C18 metal-free	2.1	TA12S03-05Q1PTP	TA12S03-10Q1PTP	TA12S03-15Q1PTP
	4.6	TA12S03-0546PTP	TA12S03-1046PTP	TA12S03-1546PTP
YMC-Triart Bio C18 metal-free	2.1	TA30S03-05Q1PTP	TA30S03-10Q1PTP	TA30S03-15Q1PTP
	4.6	TA30S03-0546PTP	TA30S03-1046PTP	TA30S03-1546PTP
YMC-Triart C8 metal-free	2.1	T012S03-05Q1PTP	T012S03-10Q1PTP	T012S03-15Q1PTP
	4.6	T012S03-0546PTP	T012S03-1046PTP	T012S03-1546PTP
YMC-Triart Bio C4 metal-free	2.1	TB30S03-05Q1PTP	TB30S03-10Q1PTP	TB30S03-15Q1PTP
	4.6	TB30S03-0546PTP	TB30S03-1046PTP	TB30S03-1546PTP

Special column connectors required.

2.7 µm Core-Shell columns (max. pressure 60 MPa)

Phase	Column ID [mm]	Column length [mm]					Precolumn filter 0.5 µm*
		30	50	75	100	150	
Meteoritic Core C18 BIO	2.1	CAW16SQ7-03Q1PT	CAW16SQ7-05Q1PT	CAW16SQ7-L5Q1PT	CAW16SQ7-10Q1PT	CAW16SQ7-15Q1PT	XRPRCS35
	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 (max. pressure 25-45 MPa, 10 MPa (10 mm ID))

Phase	Column ID [mm]	Column length [mm]					Guard cartridges* with 10 mm length (pack of 5/2)
		50	75	100	150	250	
YMC-Triart C18	2.0	TA12S05-0502WT	TA12S05-L502WT	TA12S05-1002WT	TA12S05-1502WT	TA12S05-2502WT	TA12S05-01Q1GC
	2.1	TA12S05-05Q1PTH	TA12S05-L5Q1PTH	TA12S05-10Q1PTH	TA12S05-15Q1PTH	TA12S05-25Q1PTH	TA12S05-01Q1GC
	3.0	TA12S05-0503WT	TA12S05-L503WT	TA12S05-1003WT	TA12S05-1503WT	TA12S05-2503WT	TA12S05-0103GC
	4.6	TA12S05-0546WT	TA12S05-L546WT	TA12S05-1046WT	TA12S05-1546WT	TA12S05-2546WT	TA12S05-0104GC
	10	TA12S05-0510WT	-	TA12S05-1010WT	TA12S05-1510WT	TA12S05-2510WT	TA12S05-0110CC
YMC-Triart Bio C18	2.0	TA30S05-0502WT	TA30S05-L502WT	TA30S05-1002WT	TA30S05-1502WT	TA30S05-2502WT	TA30S05-01Q1GC
	2.1	TA30S05-05Q1PTH	TA30S05-L5Q1PTH	TA30S05-10Q1PTH	TA30S05-15Q1PTH	TA30S05-25Q1PTH	TA30S05-01Q1GC
	3.0	TA30S05-0503WT	TA30S05-L503WT	TA30S05-1003WT	TA30S05-1503WT	TA30S05-2503WT	TA30S05-0103GC
	4.6	TA30S05-0546WT	TA30S05-L546WT	TA30S05-1046WT	TA30S05-1546WT	TA30S05-2546WT	TA30S05-0104GC
	10	TA30S05-0510WT	-	-	TA30S05-1510WT	TA30S05-2510WT	TA30S05-0110CC
YMC-Triart C8	2.0	T012S05-0502WT	T012S05-L502WT	T012S05-1002WT	T012S05-1502WT	T012S05-2502WT	T012S05-01Q1GC
	2.1	T012S05-05Q1PTH	T012S05-L5Q1PTH	T012S05-10Q1PTH	T012S05-15Q1PTH	T012S05-25Q1PTH	T012S05-01Q1GC
	3.0	T012S05-0503WT	T012S05-L503WT	T012S05-1003WT	T012S05-1503WT	T012S05-2503WT	T012S05-0103GC
	4.6	T012S05-0546WT	T012S05-L546WT	T012S05-1046WT	T012S05-1546WT	T012S05-2546WT	T012S05-0104GC
	10	T012S05-0510WT	-	-	T012S05-1510WT	T012S05-2510WT	T012S05-0110CC
YMC-Triart Bio C4	2.0	TB30S05-0502WT	TB30S05-L502WT	TB30S05-1002WT	TB30S05-1502WT	TB30S05-2502WT	TB30S05-01Q1GC
	2.1	TB30S05-05Q1PTH	TB30S05-L5Q1PTH	TB30S05-10Q1PTH	TB30S05-15Q1PTH	TB30S05-25Q1PTH	TB30S05-01Q1GC
	3.0	TB30S05-0503WT	TB30S05-L503WT	TB30S05-1003WT	TB30S05-1503WT	TB30S05-2503WT	TB30S05-0103GC
	4.6	TB30S05-0546WT	TB30S05-L546WT	TB30S05-1046WT	TB30S05-1546WT	TB30S05-2546WT	TB30S05-0104GC
	10	TB30S05-0510WT	-	-	TB30S05-1510WT	TB30S05-2510WT	TB30S05-0110CC
Hydrosphere C18	2.0	HS12S05-0502WT	HS12S05-L502WT	HS12S05-1002WT	HS12S05-1502WT	HS12S05-2502WT	HS12S05-01Q1GC
	2.1	HS12S05-05Q1WT	HS12S05-L5Q1WT	HS12S05-10Q1WT	HS12S05-15Q1WT	HS12S05-25Q1WT	HS12S05-01Q1GC
	3.0	HS12S05-0503WT	HS12S05-L503WT	HS12S05-1003WT	HS12S05-1503WT	HS12S05-2503WT	HS12S05-0103GC
	4.6	HS12S05-0546WT	HS12S05-L546WT	HS12S05-1046WT	HS12S05-1546WT	HS12S05-2546WT	HS12S05-0104GC
	10	HS12S05-0510WT	-	-	HS12S05-1510WT	HS12S05-2510WT	HS12S05-0110CC

*Guard cartridge holder required, part no. XPGCH-Q1 (for EMEA) /XPGCHP1 (outside EMEA) XPCCHSPW1 (10 mm ID)

5 µm bioinert coated HPLC columns (max. pressure 45 MPa)

Phase	Column ID [mm]	Column length [mm]		
		50	100	150
YMC Accura Triart C18	2.1	TA12S05-05Q1PTC	TA12S05-10Q1PTC	TA12S05-15Q1PTC
	4.6	TA12S05-0546PTC	TA12S05-1046PTC	TA12S05-1546PTC
YMC Accura Triart Bio C18	2.1	TA30S05-05Q1PTC	TA30S05-10Q1PTC	TA30S05-15Q1PTC
	4.6	TA30S05-0546PTC	TA30S05-1046PTC	TA30S05-1546PTC
YMC Accura Triart C8	2.1	T012S05-05Q1PTC	T012S05-10Q1PTC	T012S05-15Q1PTC
	4.6	T012S05-0546PTC	T012S05-1046PTC	T012S05-1546PTC
YMC Accura Triart Bio C4	2.1	TB30S05-05Q1PTC	TB30S05-10Q1PTC	TB30S05-15Q1PTC
	4.6	TB30S05-0546PTC	TB30S05-1046PTC	TB30S05-1546PTC

RP – Ordering information

5 µm PEEK-lined HPLC columns (max. pressure 45 MPa)

Phase	Column ID [mm]	Column length [mm]		
		50	100	150
YMC-Triart C18 metal-free	2.1	TA12S05-05Q1PTP	TA12S05-10Q1PTP	TA12S05-15Q1PTP
	4.6	TA12S05-0546PTP	TA12S05-1046PTP	TA12S05-1546PTP
YMC-Triart Bio C18 metal-free	2.1	TA30S05-05Q1PTP	TA30S05-10Q1PTP	TA30S05-15Q1PTP
	4.6	TA30S05-0546PTP	TA30S05-1046PTP	TA30S05-1546PTP
YMC-Triart C8 metal-free	2.1	T012S05-05Q1PTP	T012S05-10Q1PTP	T012S05-15Q1PTP
	4.6	T012S05-0546PTP	T012S05-1046PTP	T012S05-1546PTP
YMC-Triart Bio C4 metal-free	2.1	TB30S05-05Q1PTP	TB30S05-10Q1PTP	TB30S05-15Q1PTP
	4.6	TB30S05-0546PTP	TB30S05-1046PTP	TB30S05-1546PTP

Special column connectors required.

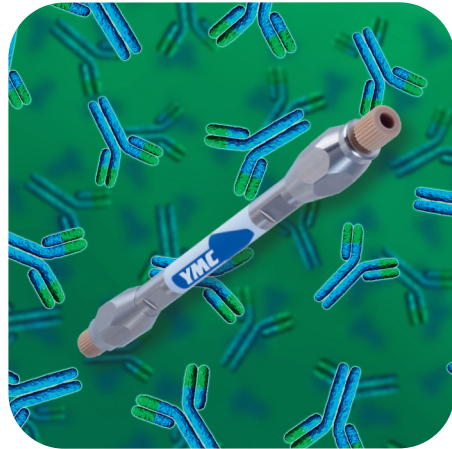
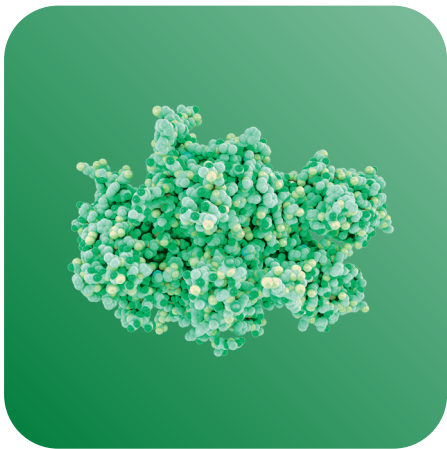
5 µm YMC-Actus high-throughput (semi)preparative columns (max. pressure 20–30 MPa)

Phase	Column ID [mm]	Column length [mm]					Guard cartridges* with 10 mm length (pack of 2)
		50	75	100	150	250	
YMC-Triart C18	20	TA12S05-0520WX	TA12S05-L520WX	TA12S05-1020WX	TA12S05-1520WX	TA12S05-2520WX	TA12S05-0120CCN
	30	TA12S05-0530WX	TA12S05-L530WX	TA12S05-1030WX	TA12S05-1530WX	TA12S05-2530WX	TA12S05-0130CCN
	50	TA12S05-0553DX	–	TA12S05-1053DX	TA12S05-1553DX	TA12S05-2553DX	TA12S05-0553DXG**
YMC-Triart Bio C18	20	TA30S05-0520WX	TA30S05-L520WX	TA30S05-1020WX	TA30S05-1520WX	TA30S05-2520WX	TA30S05-0120CCN
	30	TA30S05-0530WX	TA30S05-L530WX	TA30S05-1030WX	TA30S05-1530WX	TA30S05-2530WX	TA30S05-0130CCN
	50	TA30S05-0553DX	–	TA30S05-1053DX	TA30S05-1553DX	TA30S05-2553DX	TA30S05-0553DXG**
YMC-Triart C8	20	T012S05-0520WX	T012S05-L520WX	T012S05-1020WX	T012S05-1520WX	T012S05-2520WX	T012S05-0120CCN
	30	T012S05-0530WX	T012S05-L530WX	T012S05-1030WX	T012S05-1530WX	T012S05-2530WX	T012S05-0130CCN
	50	T012S05-0553DX	–	T012S05-1053DX	T012S05-1553DX	T012S05-2553DX	T012S05-0553DXG**
YMC-Triart Bio C4	20	TB30S05-0520WX	TB30S05-L520WX	TB30S05-1020WX	TB30S05-1520WX	TB30S05-2520WX	TB30S05-0120CCN
	30	TB30S05-0530WX	TB30S05-L530WX	TB30S05-1030WX	TB30S05-1530WX	TB30S05-2530WX	TB30S05-0130CCN
	50	TB30S05-0553DX	–	TB30S05-1053DX	TB30S05-1553DX	TB30S05-2553DX	TB30S05-0553DXG**
Hydrosphere C18	20	HS12S05-0520WX	HS12S05-L520WX	HS12S05-1020WX	HS12S05-1520WX	HS12S05-2520WX	HS12S05-0120CCN
	30	HS12S05-0530WX	HS12S05-L530WX	HS12S05-1030WX	HS12S05-1530WX	HS12S05-2530WX	HS12S05-0130CCN

*Guard cartridge holder required, part no. XPGHF2P20ID (20 mm ID)
XPGHF2P30ID (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 oligonucleotides and carbohydrates
- 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
Dedicated for	peptides, small proteins	intermediate proteins, short oligonucleotides	large proteins, intermediate oligonucleotides	very large proteins, longer oligonucleotides	antibodies, fragments and aggregates
Base particle	Silica				
Particle Size / µm	3, 5	3, 5	2, 3, 5	2, 3, 5	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)				14 MPa (2,030 psi)

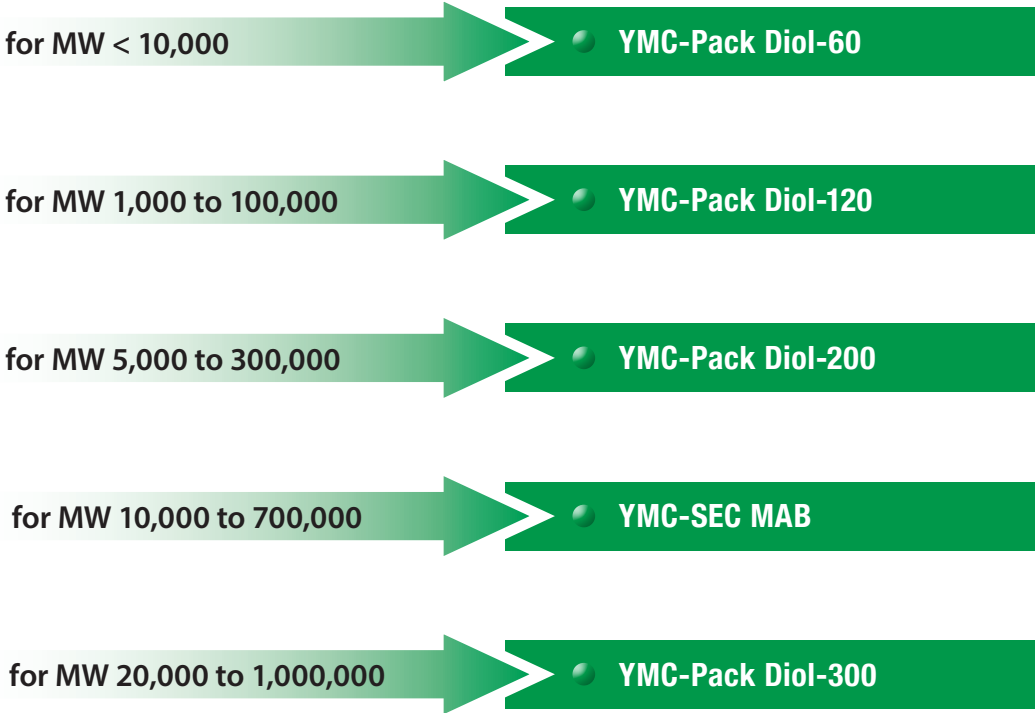
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“The YMC-Pack Diol SEC column has been successfully used for subsequent method validation.”

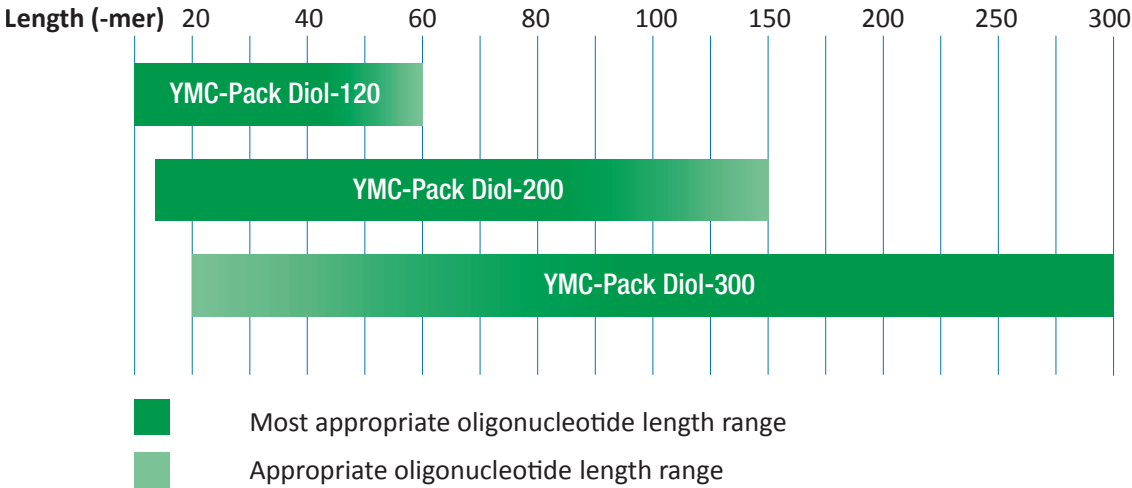
Rubén Pedrosa Segon, Head of Quality Control Pharmaceutical Department, OFICE S.L. (ES)

”

Column Selection Tool according to MW



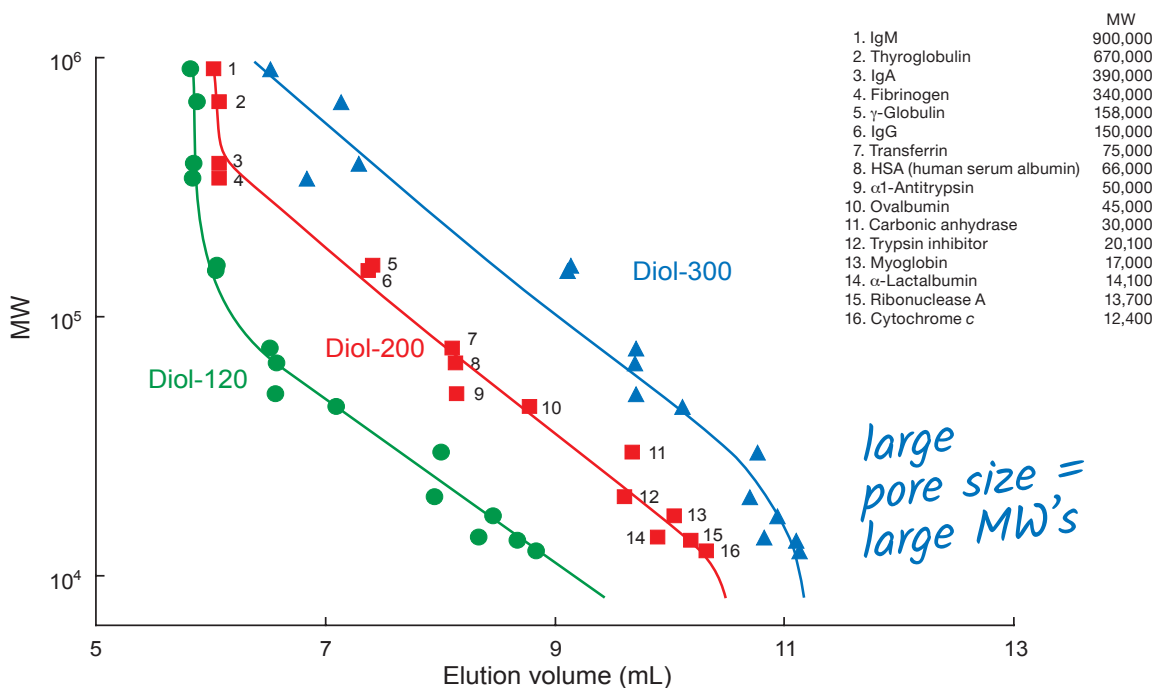
Column Selection Tool according to DNA length



SEC – YMC-Pack Diol: Phase selection for proteins & saccharides

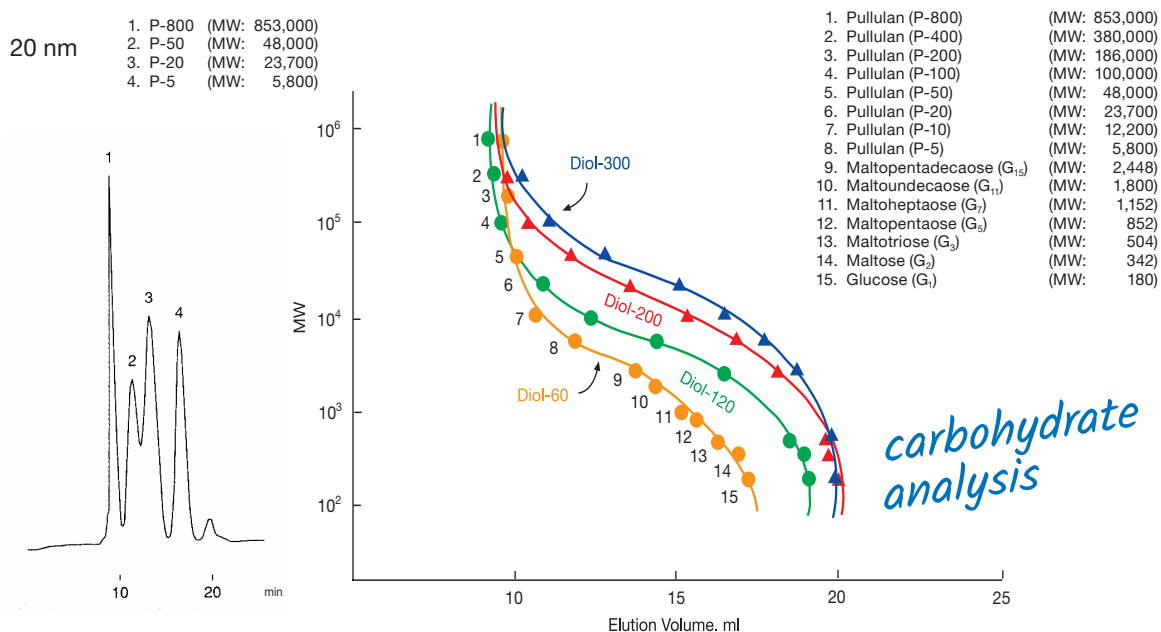
Phases for different MW ranges

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



Column: YMC-Pack Diol, 300 x 8.0 mm ID
 Part Nos.: DL12S05-3008WT, DL20S05-3008WT, DL30S05-3008WT
 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

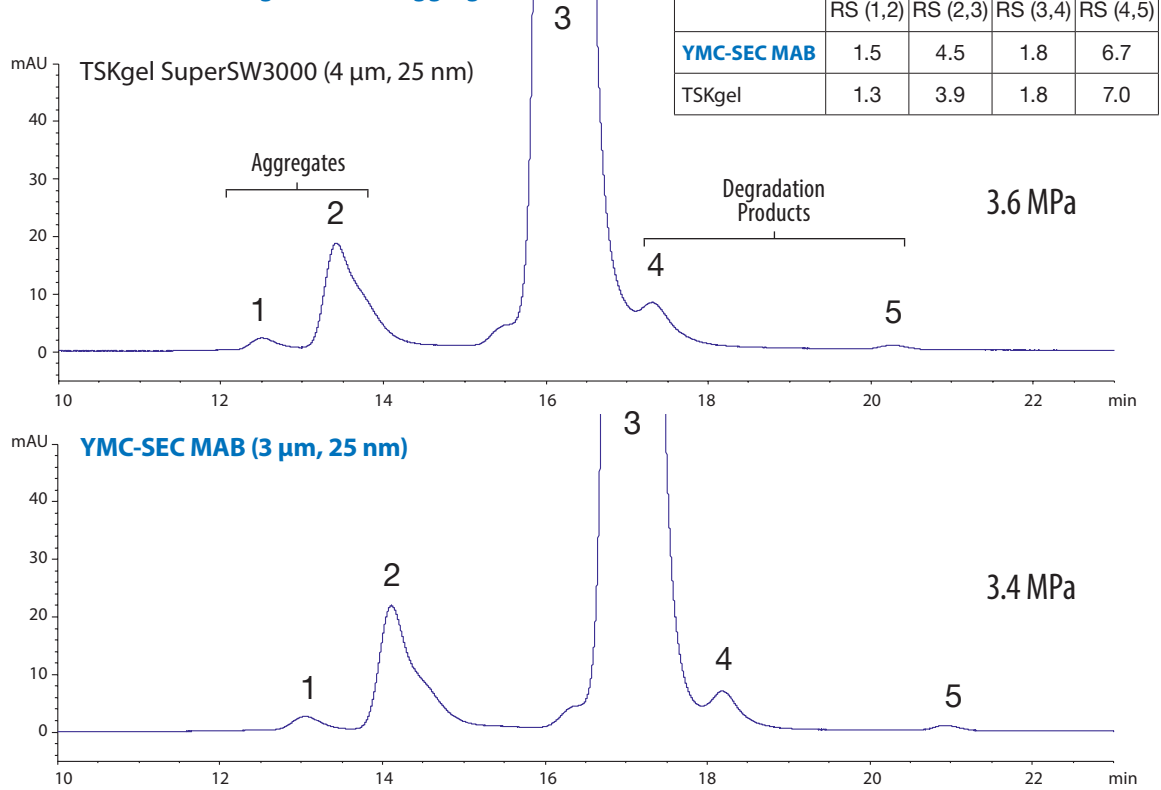
For molecular weight determination of oligosaccharides and polysaccharides



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

Ideal choice for monoclonal antibodies

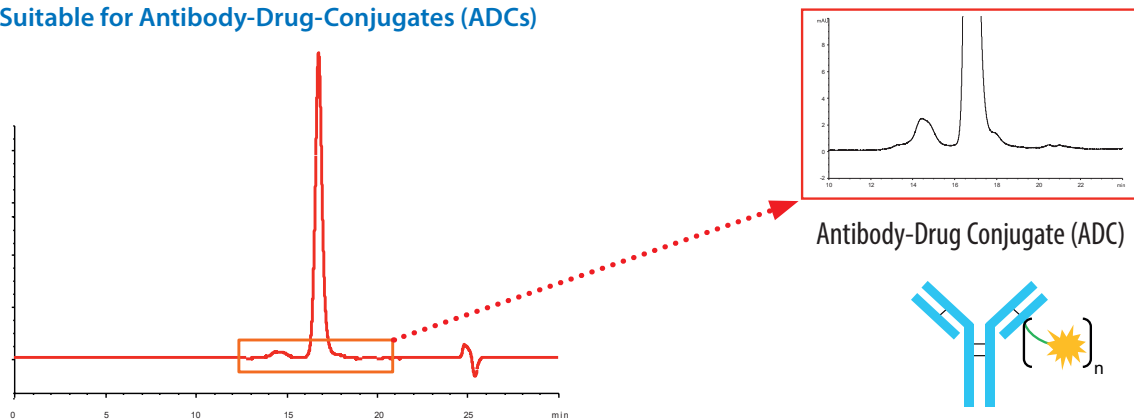
Bevacizumab and its fragments and aggregates



Column: 300 x 4.6 mm ID
 Part No.: DLM25S03-3046WT
 Eluent: 0.1 M phosphate buffer (pH = 7) containing 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: Bevacizumab (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) containing 0.2 M NaCl / 2-propanol (85 / 15)
 Flow rate: 0.165 mL/min

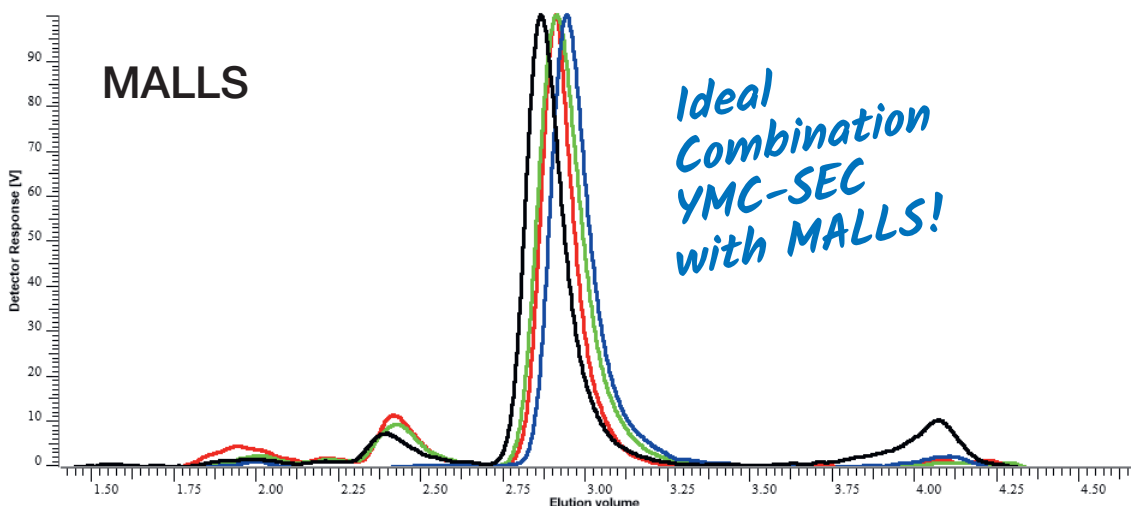
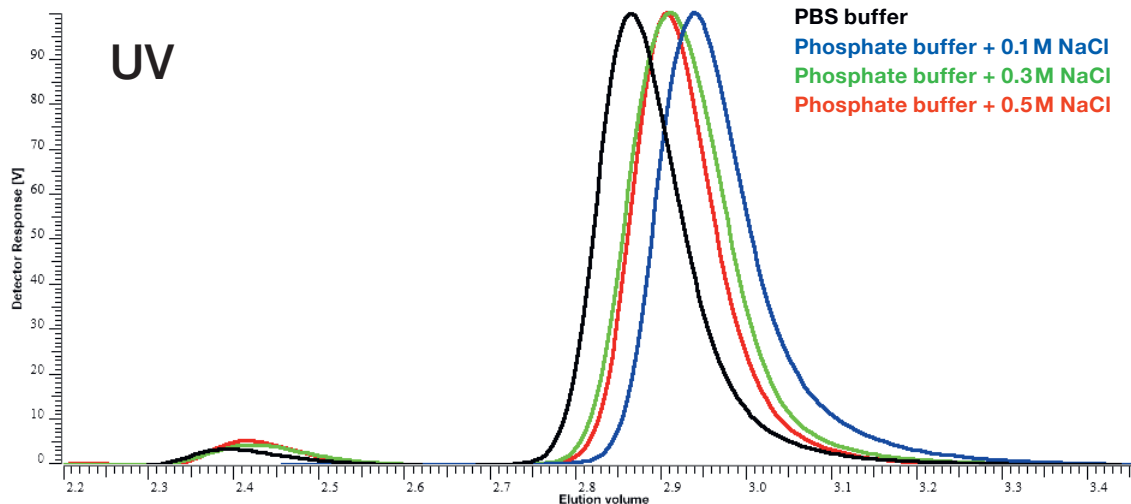
Temperature: 25 °C
 Detection: UV at 280 nm
 Injection: 4 µL (2.5 mg/mL)
 Sample: SigmaMAb Antibody Drug Conjugate Mimic

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

SEC – YMC-SEC MAB: MALLS

YMC-SEC columns ideally combined with light scattering detection

Detection of higher molar mass species by MALLS



Column: YMC-SEC MAB (3 μ m, 25 nm) 300 x 4.6 mm ID
 Part No.: DLM25S03-3046WT
 Eluent: Phosphate buffer pH 6.6 containing 0.3 M NaCl
 Flow rate: 0.33 mL/min
 Temperature: 25 °C
 Detection: MALLS at 90° angle (PSS SLD7100), UV at 280 nm
 Injection volume: 10 μ L
 Sample: Bevacizumab (Avastin®) dosage form (10 mg/mL, diluted to 1 mg/mL)
 System: PSS-SECcurity GPC systems, 1260 Infinity II
 Software: WinGPC Unichrom

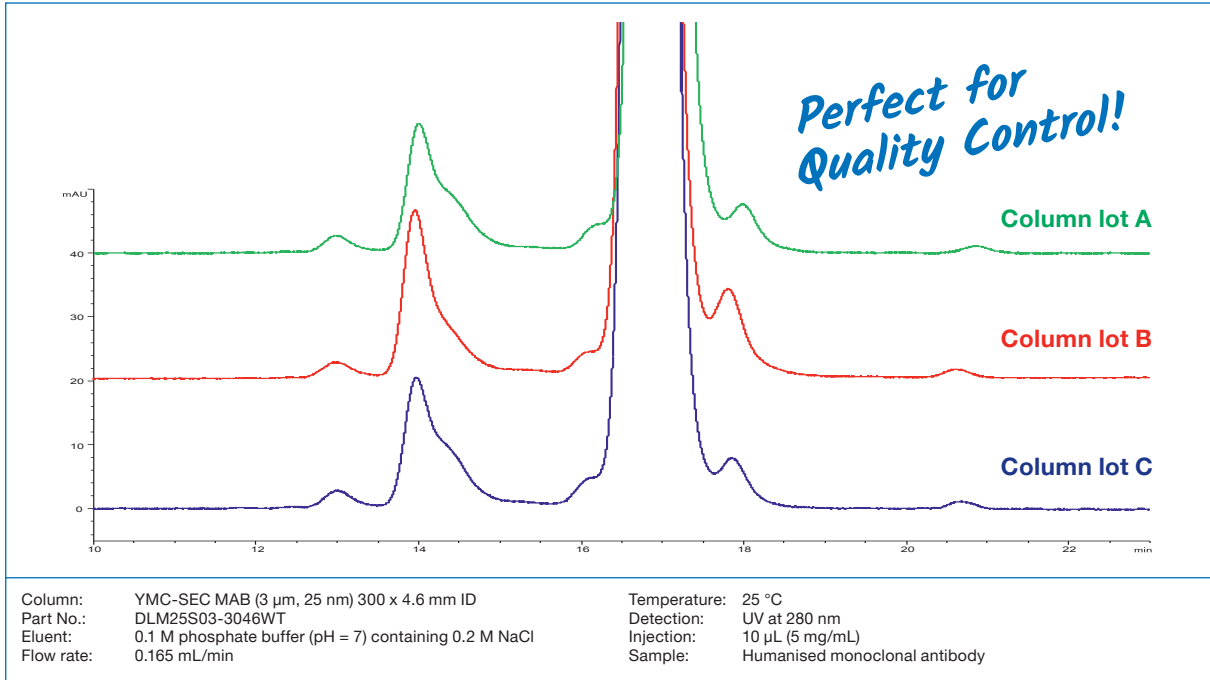
Courtesy of Thorsten Hofe, PSS Polymer Standards Service GmbH, Mainz, Germany.

Four different buffers, a phosphate buffered saline (PBS) pH 7.4 and phosphate buffers pH 6.6 with varying concentrations of NaCl, were used to develop a suitable MALLS detection method for mAbs.

A defined minimum ionic strength is necessary to achieve a robust method with good resolution. The phosphate buffer with 0.3 M NaCl appeared to be the most suitable eluent.

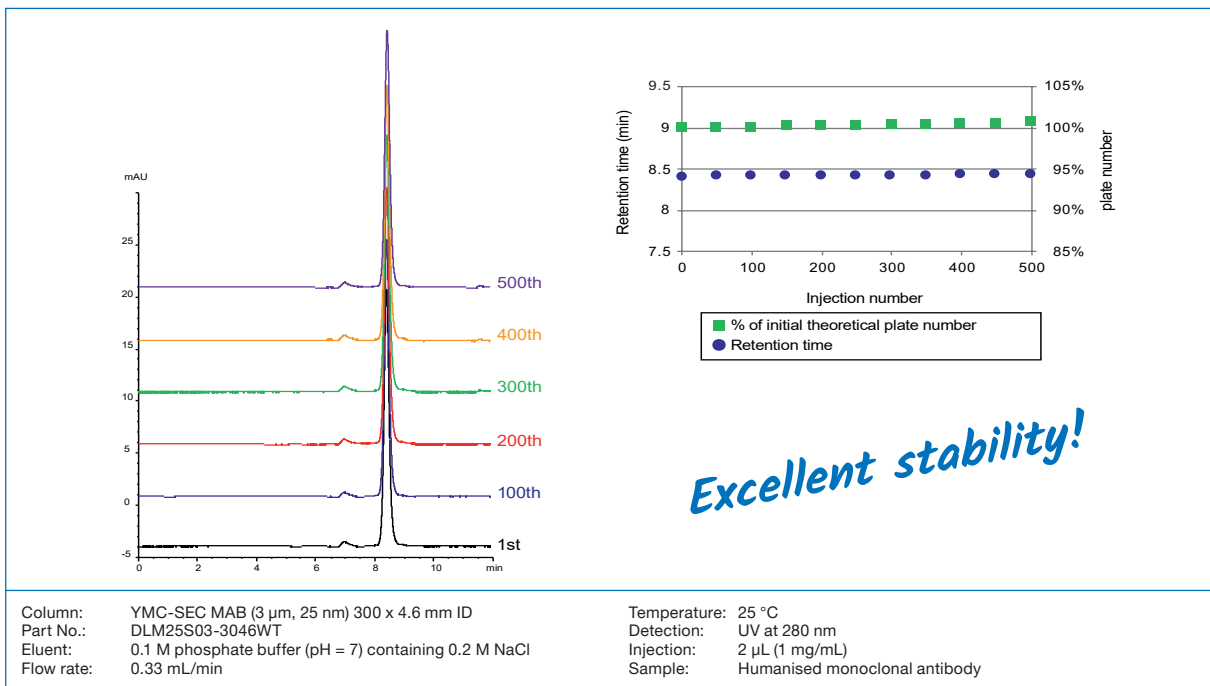
Compared to UV detection, the MALLS signal shows 2 higher molar mass species, aggregates of Bevacizumab, at about 2.0 mL and 2.3 mL elution volume.

Excellent lot-to-lot reproducibility



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

High column stability



Excellent stability is provided for monoclonal antibody analysis without any changes in theoretical plate number or elution time even after more than 500 injections.

SEC – YMC-Pack Diol: Resolution & throughput

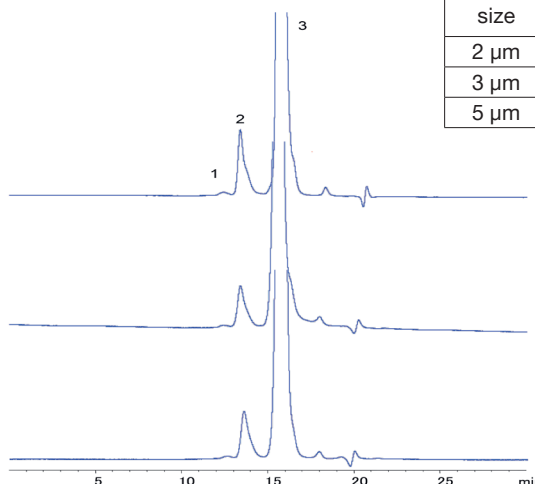
Benefits of using smaller particles

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)

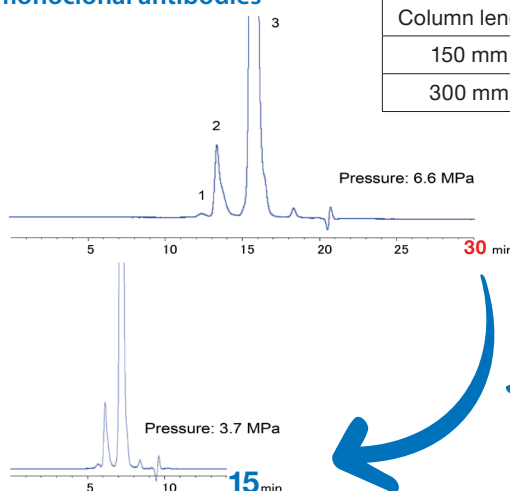
All three particle sizes show identical separation patterns for monoclonal antibody analysis. This allows easy method transfer between HPLC and UHPLC. A method developed using conventional HPLC can be directly transferred to UHPLC using a 2 μm YMC-Pack Diol

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

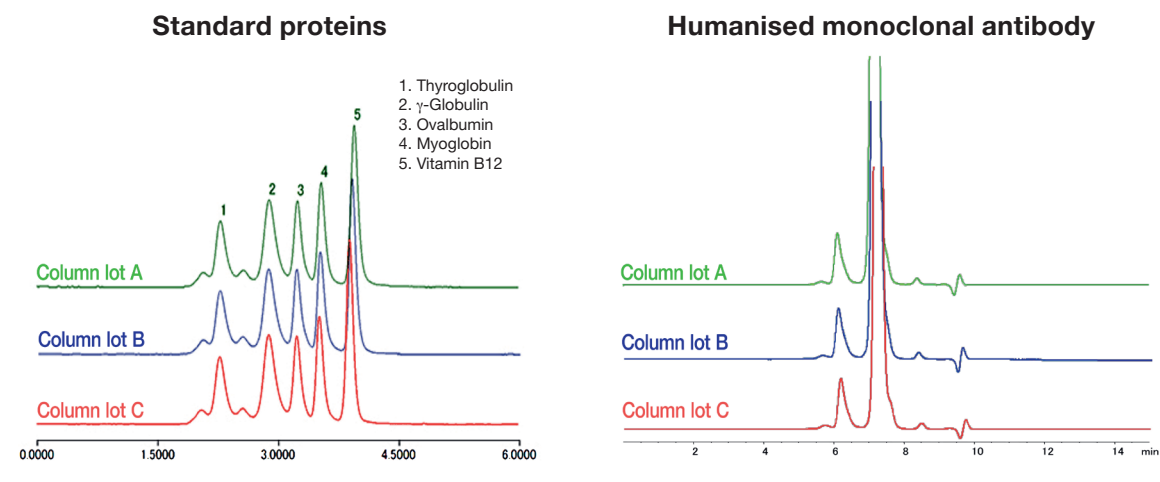
Columns: YMC-Pack Diol-300, 150 or 300 x 4.6 mm ID
Part Nos.: DL30S02-3046PTH / DL30S02-1546PTH
Eluent: 0.1 M KH_2PO_4 - K_2HPO_4 (pH 7.0) containing 0.2 M NaCl
Flow rate: 0.2 mL/min

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 good resolution as for a 300 mm length column (compare upper and lower chromatograms). This allows an increase in throughput to be achieved.

Reproducibility and stability data

Excellent batch-to-batch reproducibility

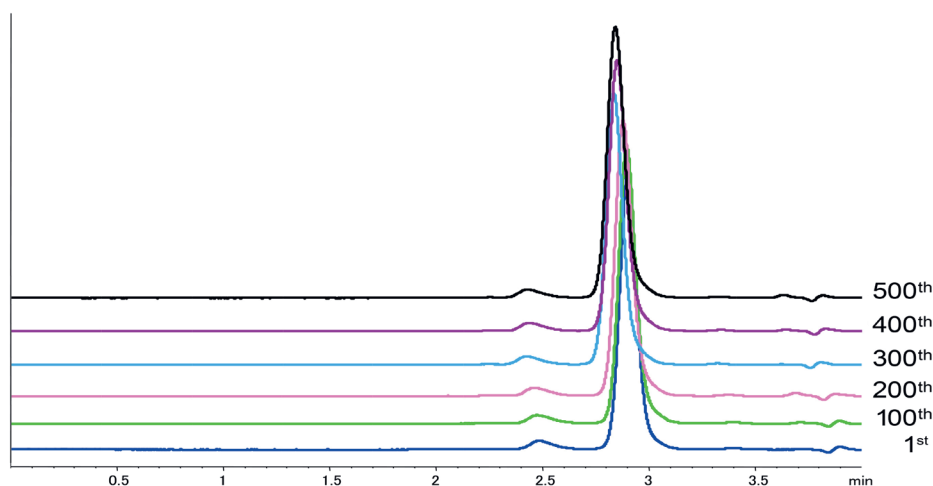


Column: YMC-Pack Diol-300 (2 μ m) 150 x 4.6 mm ID
 Part No.: DL30S02-1546PTH
 Eluent: 0.1 M NaH_2PO_4 - Na_2HPO_4 (pH 7.0) containing 0.2 M NaCl
 Flow rate: 0.5 mL/min
 Temperature: ambient
 Detection: UV at 280 nm
 Sample: Standard proteins

Column: YMC-Pack Diol-300 (2 μ m) 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 $^\circ\text{C}$
 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 μ m columns the ideal choice for the quality control of bio-based drugs including monoclonal antibodies.

Long-term stability



Column: YMC-Pack Diol-300 (2 μ m) 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 $^\circ\text{C}$
 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.

SEC – Ordering information

2 µm UHPLC columns (max. pressure 45 MPa)

Phase	Column ID [mm]	Column length [mm]		Guard cartridges* with 10 mm length (pack of 5)
		150	300	
YMC-Pack Diol-200	4.6	DL20S02-1546PTH	DL20S02-3046PTH	DL20S02-0104GC
YMC-Pack Diol-300	4.6	DL30S02-1546PTH	DL30S02-3046PTH	DL30S02-0104GC

*Guard cartridge holder required, part no. XPGCH-Q1 (for EMEA) /XPGCHP1 (outside EMEA)

3 µm HPLC columns (max. pressure 14–20 MPa)

Phase	Column ID [mm]	Column length [mm]			Guard cartridges/columns* with 10/30 mm length (pack of 5)
		150	250	300	
YMC-SEC MAB	4.6	–	–	DLM25S03-3046WT	DLM25S03-0104GC
	6.0	–	–	–	–
	8.0	–	–	DLM25S03-3008WT	–
YMC-Pack Diol-60	4.6	DL06S03-1546WT	DL06S03-2546WT	DL06S03-3046WT	DL06S03-0104GC
	6.0	–	–	DL06S03-3006WT	–
	8.0	DL06S03-1508WT	–	DL06S03-3008WT	DL06S03-0308WTG**
YMC-Pack Diol-120	4.6	DL12S03-1546WT	DL12S03-2546WT	DL12S03-3046WT	DL12S03-0104GC
	6.0	–	–	DL12S03-3006WT	–
	8.0	DL12S03-1508WT	–	DL12S03-3008WT	DL12S03-0308WTG**
YMC-Pack Diol-200	4.6	DL20S03-1546WT	DL20S03-2546WT	DL20S03-3046WT	DL20S03-0104GC
	6.0	–	–	DL20S03-3006WT	–
	8.0	DL20S03-1508WT	–	DL20S03-3008WT	DL20S03-0308WTG**
YMC-Pack Diol-300	4.6	DL30S03-1546WT	DL30S03-2546WT	DL30S03-3046WT	DL30S03-0104GC
	6.0	–	–	DL30S03-3006WT	–
	8.0	DL30S03-1508WT	–	DL30S03-3008WT	DL30S03-0308WTG**

*Guard cartridge holder required, part no. XPGCH-Q1 (for EMEA) /XPGCHP1 (outside EMEA)

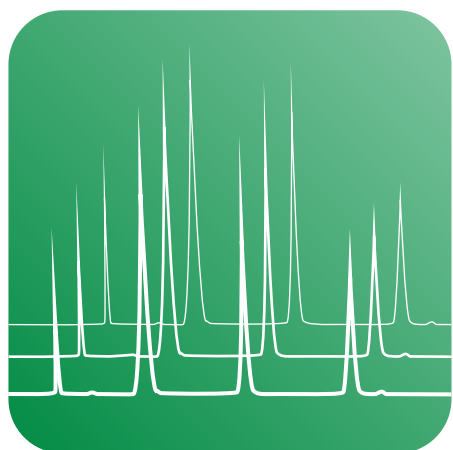
**no holder required for 30 x 8 mm ID guard columns (1 piece)
recommended column coupler part no. XRCP1602

5 µm HPLC columns (max. pressure 20 MPa)

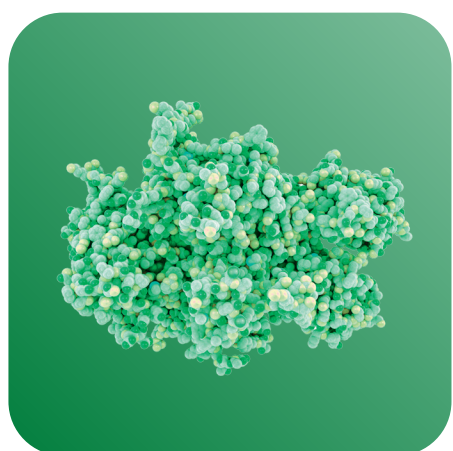
Phase	Column ID [mm]	Column length [mm]			Guard cartridges/columns* with 10/30 mm length (pack of 5)
		250	300	500	
YMC-Pack Diol-60	4.6	DL06S05-2546WT	DL06S05-3046WT	–	DL06S05-0104GC
	6.0	DL06S05-2506WT	DL06S05-3006WT	DL06S05-5006WT	–
	8.0	–	DL06S05-3008WT	DL06S05-5008WT	DL06S05-0308WTG**
	10.0	DL06S05-2510WT	DL06S05-3010WT	DL06S05-5010WT	DL06S05-0310WTG**
YMC-Pack Diol-120	4.6	DL12S05-2546WT	DL12S05-3046WT	–	DL12S05-0104GC
	6.0	DL12S05-2506WT	DL12S05-3006WT	DL12S05-5006WT	–
	8.0	–	DL12S05-3008WT	DL12S05-5008WT	DL12S05-0308WTG**
	10.0	DL12S05-2510WT	DL12S05-3010WT	DL12S05-5010WT	DL12S05-0310WTG**
YMC-Pack Diol-200	4.6	DL20S05-2546WT	DL20S05-3046WT	–	DL20S05-0104GC
	6.0	DL20S05-2506WT	DL20S05-3006WT	DL20S05-5006WT	–
	8.0	–	DL20S05-3008WT	DL20S05-5008WT	DL20S05-0308WTG**
	10.0	DL20S05-2510WT	DL20S05-3010WT	DL20S05-5010WT	DL20S05-0310WTG**
YMC-Pack Diol-300	4.6	DL30S05-2546WT	DL30S05-3046WT	–	DL30S05-0104GC
	6.0	DL30S05-2506WT	DL30S05-3006WT	DL30S05-5006WT	–
	8.0	–	DL30S05-3008WT	DL30S05-5008WT	DL30S05-0308WTG**
	10.0	DL30S05-2510WT	DL30S05-3010WT	DL30S05-5010WT	DL30S05-0310WTG**

*Guard cartridge holder required, part no. XPGCH-Q1 (for EMEA) /XPGCHP1 (outside EMEA)

**no holder required for 30 x 8 mm ID guard columns (1 piece)
recommended column coupler part no. XRCP1602 (for 8 mm ID) and XRCP1605 (for 10 mm ID)

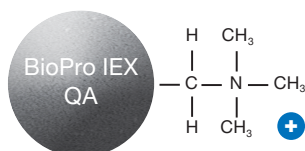


IEX

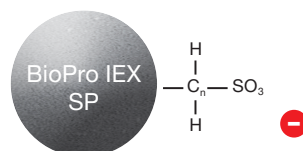


IEX – HPLC selectivities

- Porous or non-porous hydrophilic polymers
- High binding capacity and recovery of biomolecules
- Very high resolution
- Low nonspecific adsorption
- Excellent reproducibility

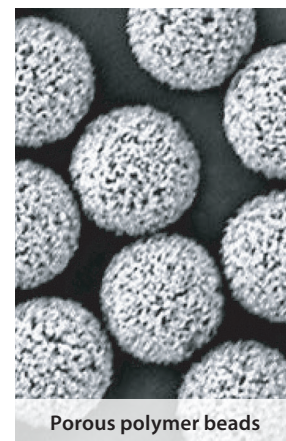


strong anion exchanger



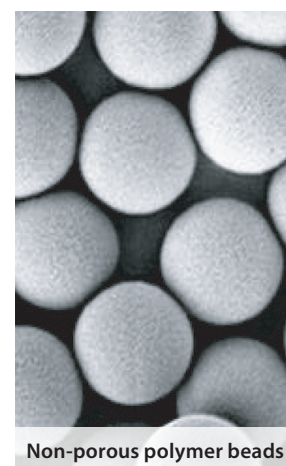
strong cation exchanger

	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	$-\text{CH}_2\text{N}^+(\text{CH}_3)_3$	$-(\text{CH}_2)_3\text{SO}_3^-$
Counter ion	Cl^-	Na^+
Available pH range	2.0–12.0	2.0–12.0
Temperature range	4–60 °C	
Pressure limit	2.5–3.5 MPa (360–510 psi)	
Column hardware	PEEK	



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

	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	$-\text{CH}_2\text{N}^+(\text{CH}_3)_3$	$-(\text{CH}_2)_3\text{SO}_3^-$
Counter ion	Cl^-	Na^+
Available pH range	2.0–12.0	2.0–12.0
Temperature range	4–60 °C	
Pressure limit	3 μm : 18–25 MPa (2,610–3,625 psi) 5 μm : 6–12 MPa (870–1,740 psi)	
Column hardware	PEEK	



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

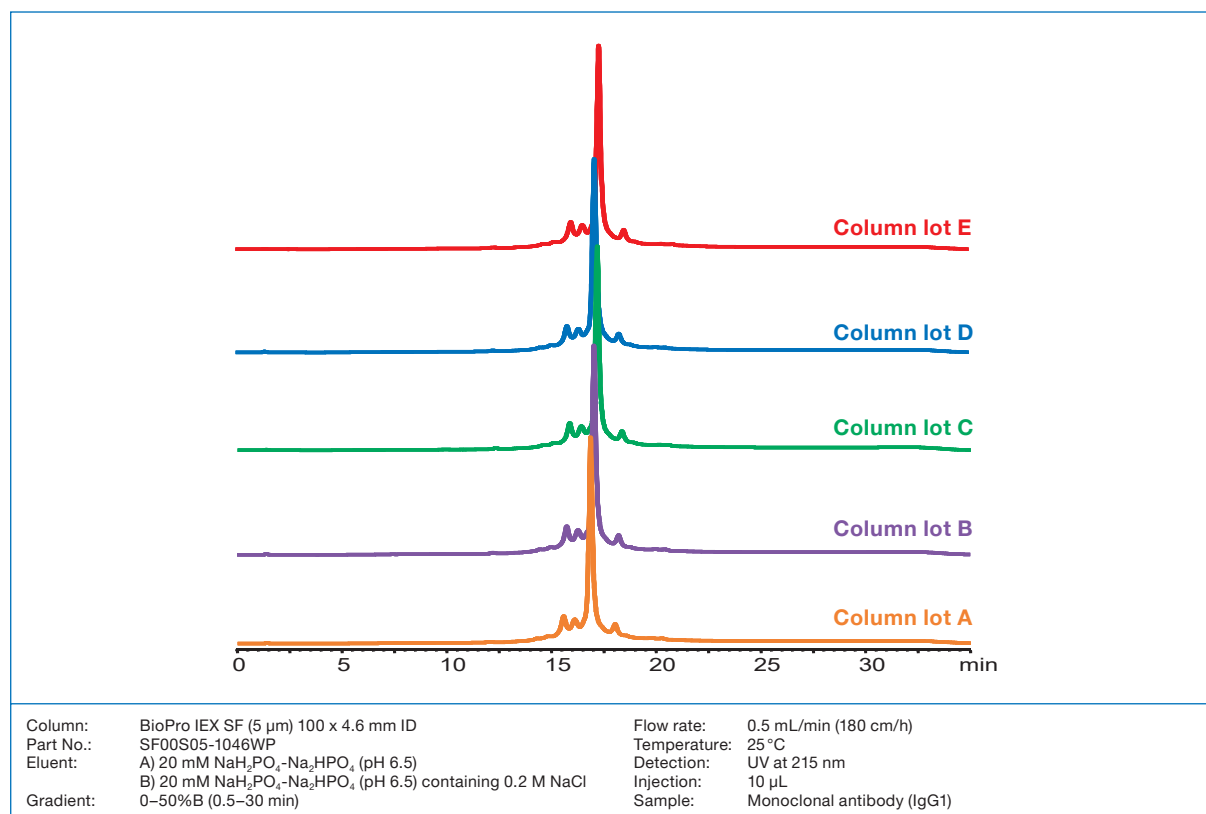
	Dynamic binding capacity (mg/mL-gel, 10% breakthrough)	Eluted amount (mg/mL-gel)	Recovery* (%)
BioPro IEX QA	126	120	95
Mono Q	100	35	35
TSKgel BioAssist Q	73	58	79

High recovery rates for BioPro IEX

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

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



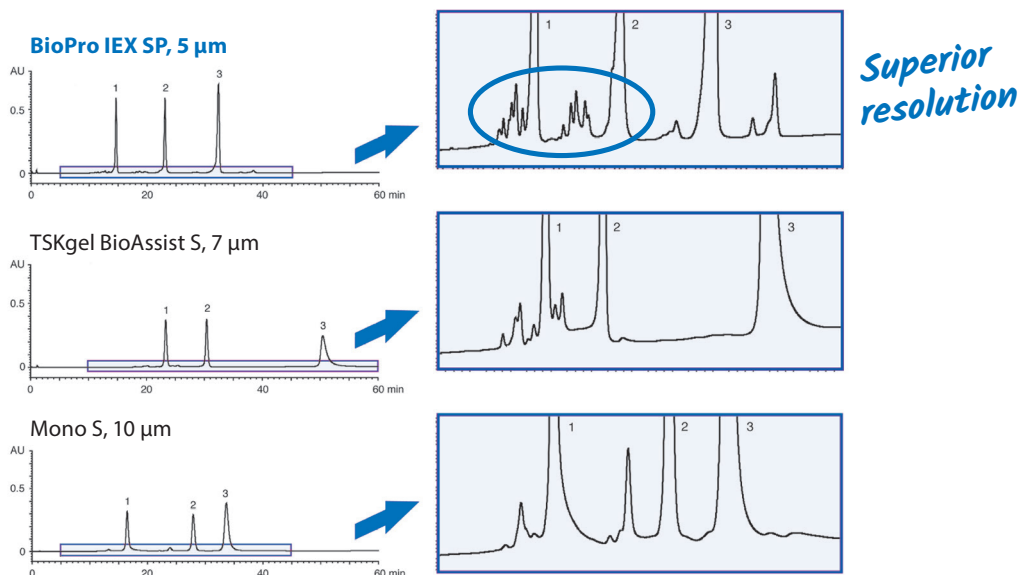
BioPro IEX SF columns exhibit excellent batch-to-batch reproducibility for mAb analysis with resolution of peaks for small charge variants. All gel batches are inspected by rigorous quality control tests, including HPLC analysis of mAbs, and must meet the required criteria before release.

BioPro IEX columns are the best choice for the quality control of mAbs, proteins, oligonucleotides and other biopharmaceuticals.

IEX – BioPro IEX: Resolution & throughput

Superior resolution

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

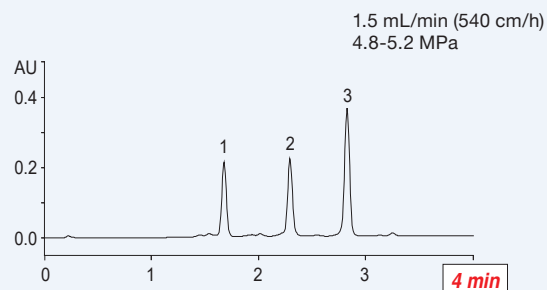


Eluent:	A) 20 mM KH_2PO_4 - K_2HPO_4 (pH 6.8) B) 20 mM KH_2PO_4 - K_2HPO_4 (pH 6.8) containing 0.5 M NaCl	Detection:	UV at 220 nm	
Gradient:	0–100%B (0–60 min)	Injection:	BioPro IEX SP, TSKgel BioAssist S Mono S	20 μL 23.6 μL
Flow rate:	BioPro IEX SP, TSKgel BioAssist S 0.5 mL/min Mono S 0.59 mL/min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

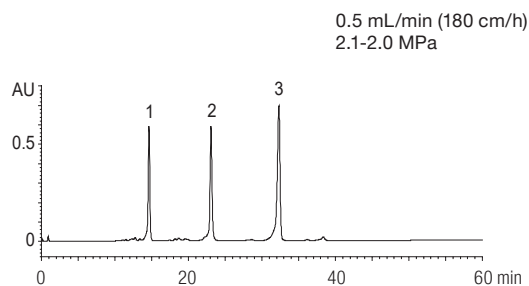
Only BioPro IEX is available in the smaller particle size and is therefore able to provide superior resolution.

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

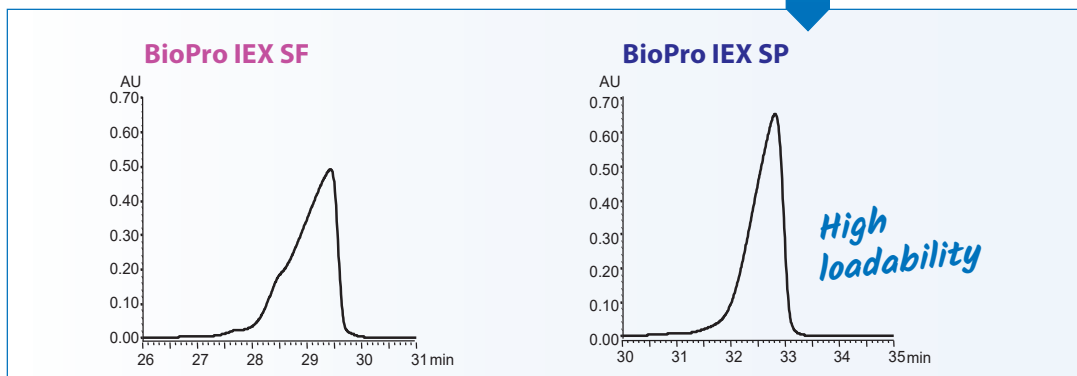
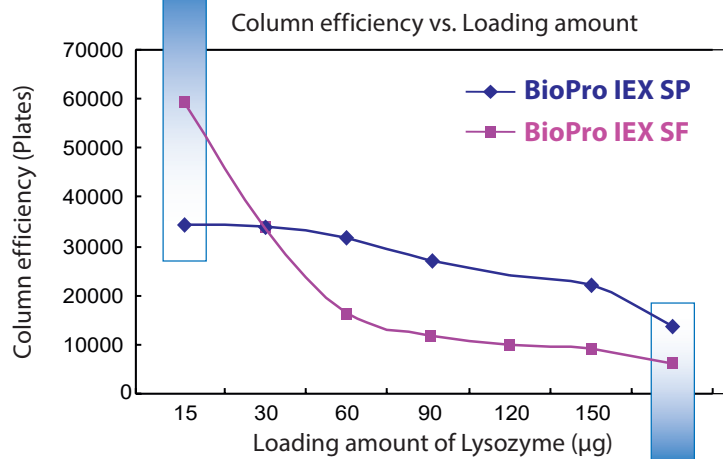
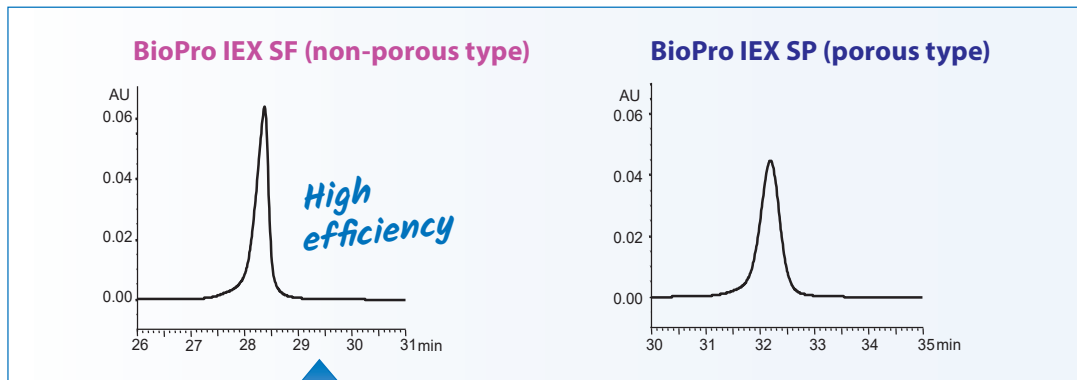


Part Nos.:	SF00S05-0346WP (non-porous) SPA0S05-0546WP (porous)	Temperature:	25 °C
Eluent:	A) 20 mM KH_2PO_4 - K_2HPO_4 (pH 6.8) B) 20 mM KH_2PO_4 - K_2HPO_4 (pH 6.8) containing 0.5 M NaCl	Detection:	UV at 220 nm
Gradient:	0–100%B (0–4 min) for BioPro IEX SF 0–100%B (0–60 min) for BioPro IEX SP	Injection: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

Column efficiency and loadability

When to use porous and non-porous BioPro IEX



Columns: (5 µm) 50 x 4.6 mm ID
 Part Nos.: SF00S05-0546WP
 SPA0S05-0546WP
 Eluent: A) 20 mM NaH₂PO₄-Na₂HPO₄ (pH 6.8)
 B) 20 mM NaH₂PO₄-Na₂HPO₄ (pH 6.8) containing 0.5 M NaCl
 Gradient: 0–100%B (0–60 min)

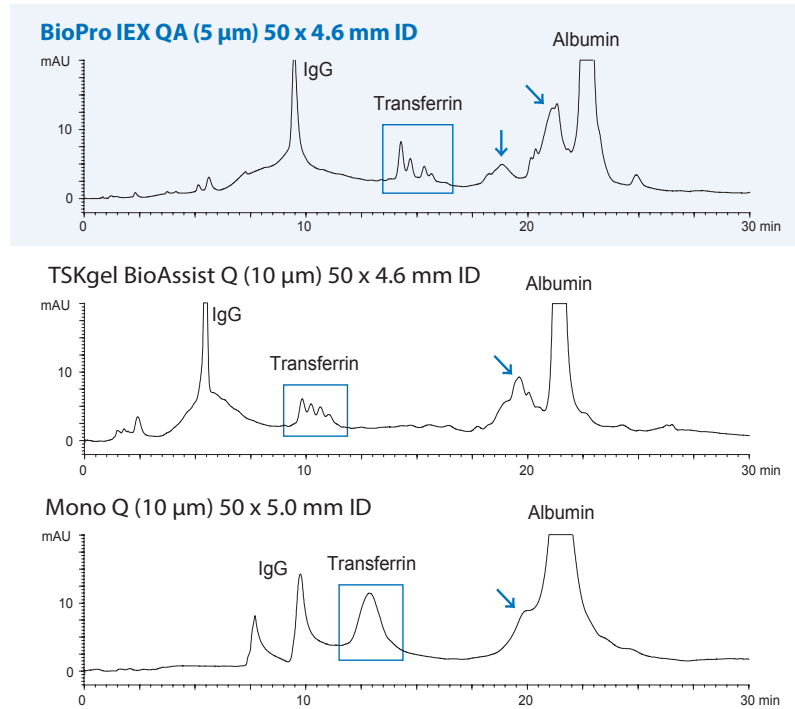
Flow rate: 0.5 mL/min
 Temperature: 25°C
 Detection: UV at 280 nm
 Injection: 100 µL
 Sample: Lysozyme

Non-porous BioPro IEX columns offer outstanding column efficiency for small sample loading amounts. These columns are especially suitable for microscale analysis, which requires higher resolution. Porous BioPro IEX columns maintain good peak shape even when the loading amount increases. These high-capacity columns are useful for high-load analytical separations and laboratory-scale purification.

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

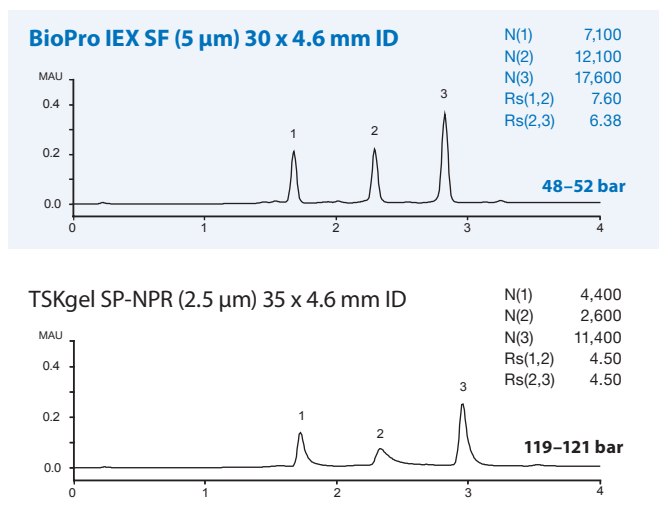


For high resolution porous BioPro IEX QA/SP is recommended!

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



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.

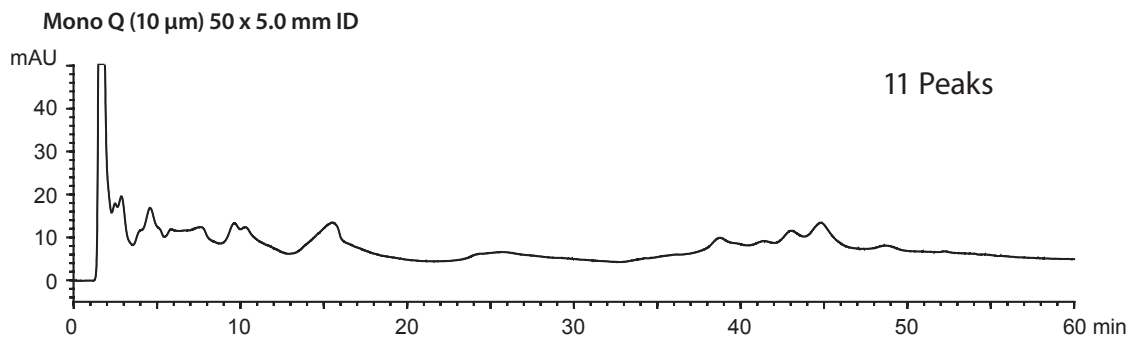
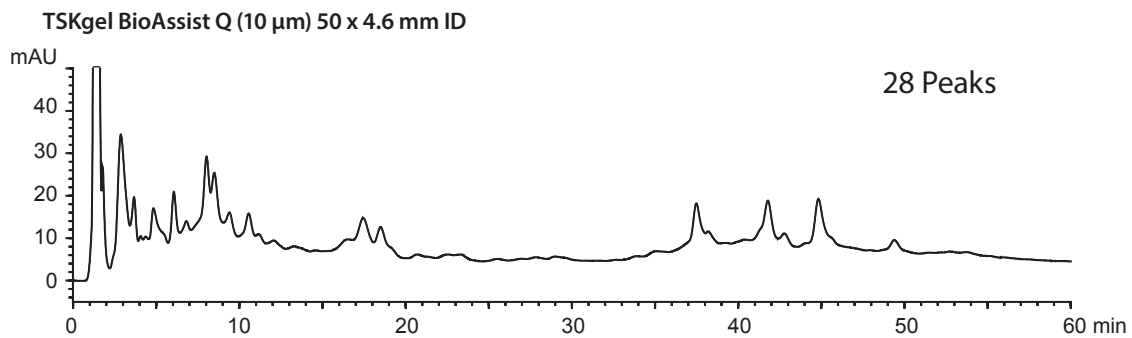
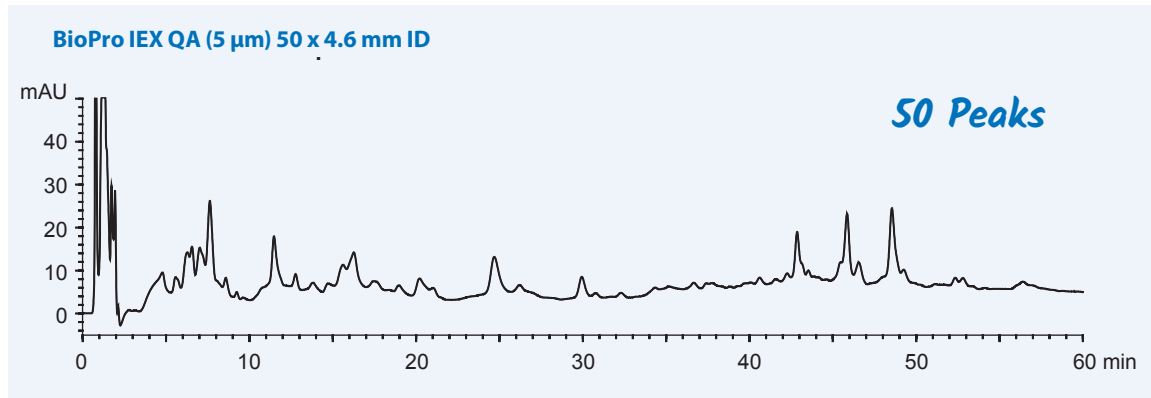
higher plate count

Part No.: SF00S05-0346WP
 Eluent: A) 20 mM KH₂PO₄-K₂HPO₄ (pH 6.8)
 B) 20 mM KH₂PO₄-K₂HPO₄ (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 (0.1 mg/mL)
 2. Cytochrome c (0.1 mg/mL)
 3. Lysozyme (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

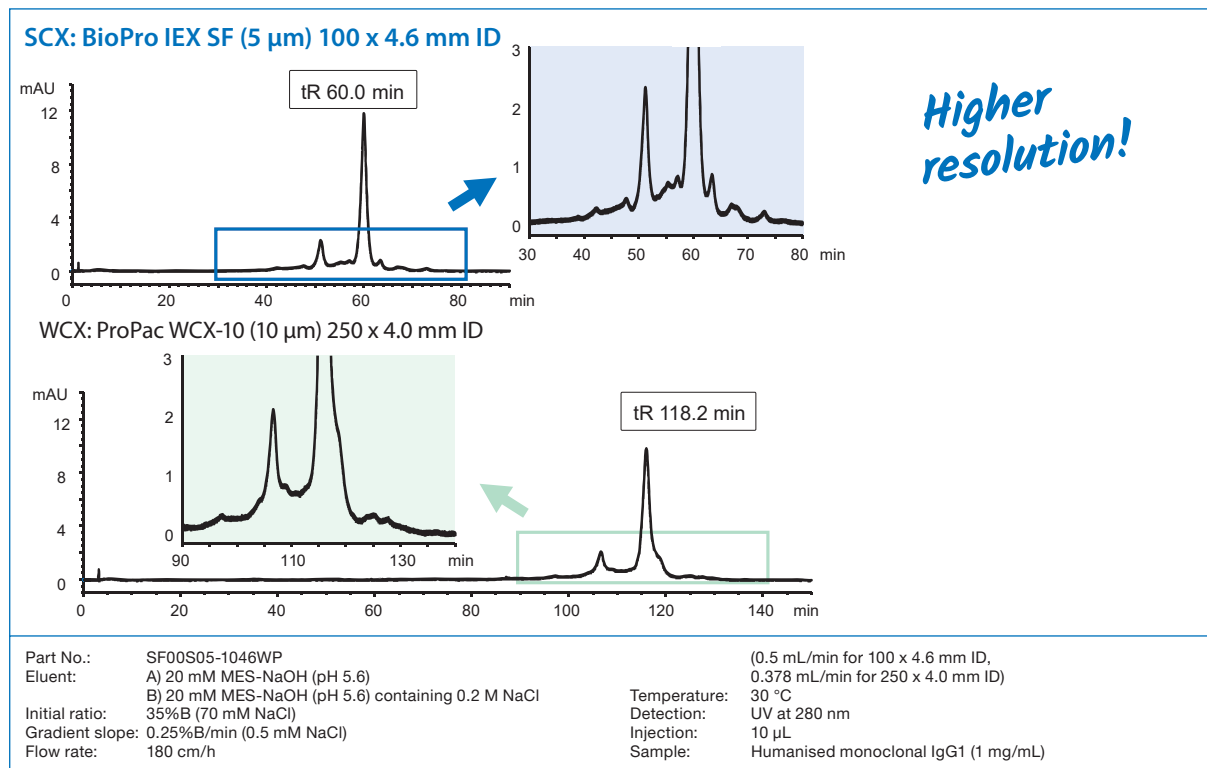
Peptide mapping of tryptic digests of BSA with enhanced sensitivity



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 μ L
 Sample: Tryptic digest of BSA

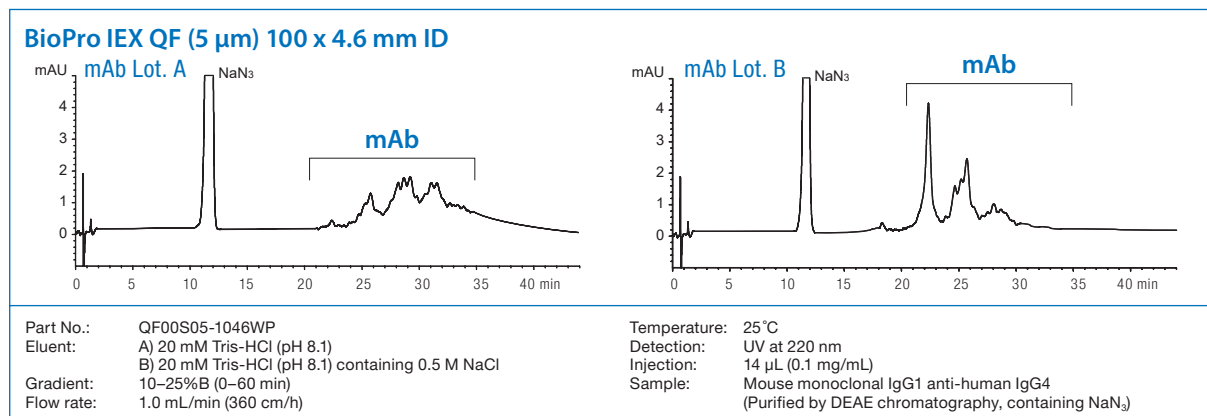
IEX – BioPro IEX: Antibody analysis

Monoclonal antibody analysis with non-porous cation exchange columns



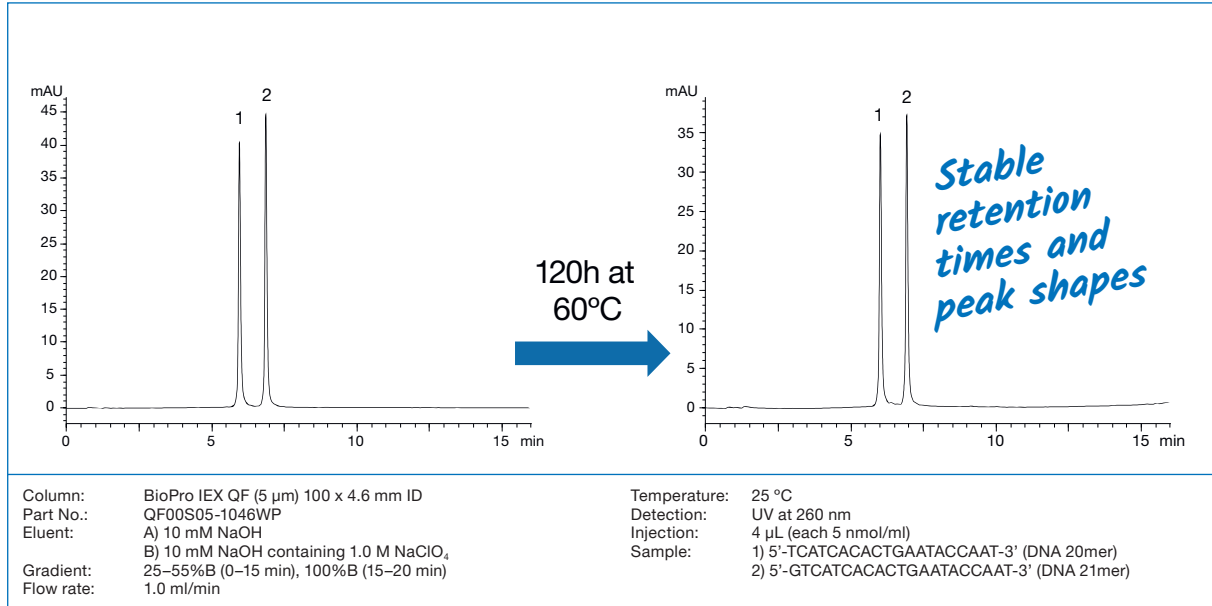
The separation of a mAb is compared using a strong cation (BioPro IEX SF) and a weak cation exchange column (ProPac WCX-10) under the same gradient conditions at pH 5.6. BioPro IEX SF can achieve a higher resolution of the mAb than the competitor's column in a shorter analysis time.

QC of monoclonal antibodies with non-porous BioPro IEX QF

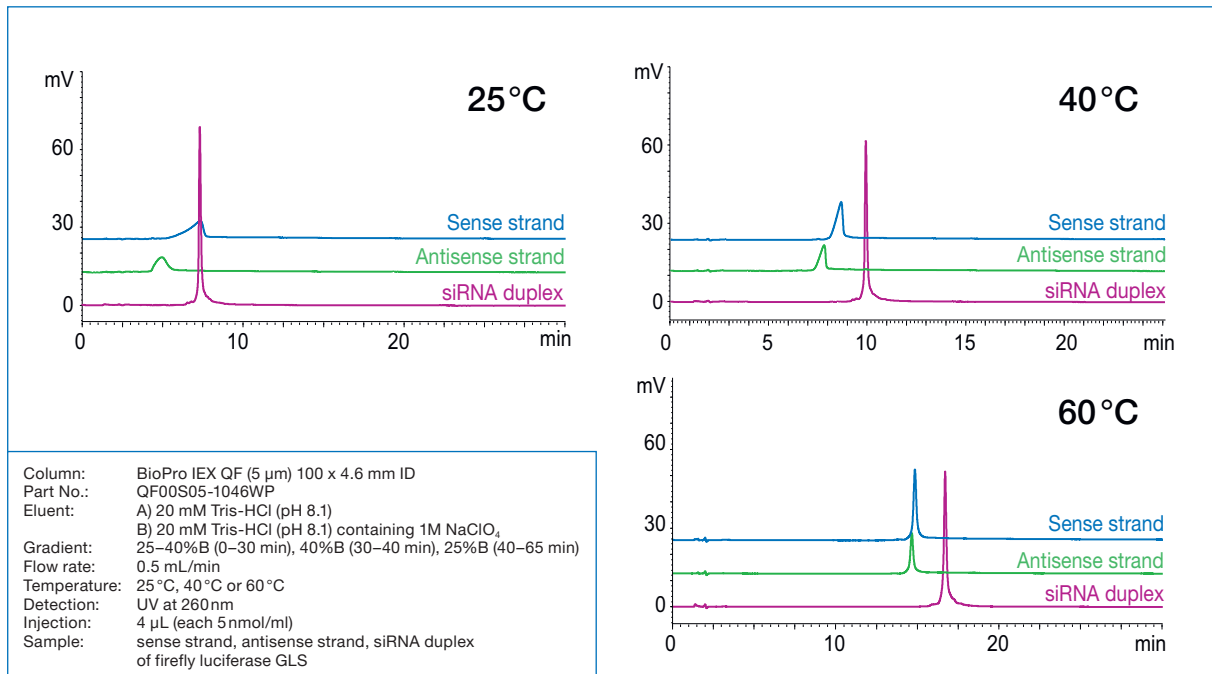


Two different batches of a commercially available mAb purified by DEAE chromatography were analysed on a BioPro IEX QF column (100 mm length). The mAb was separated into several peaks, and the batch-to-batch variability is observed. The BioPro IEX QF/SF 100mm length columns, which have high efficiency,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

High temperature stability of BioPro IEX columns



Influence of temperature on the analysis of a non-denaturated siRNA



A higher temperature tends to show improved peak shape. Slightly better peak shapes of the ssRNAs are observed at 40 °C, while the dsRNA shows comparable and relatively good peak shape regardless of the temperature. An even higher temperature of 60 °C provides better peak shape of the sense and antisense strands. However, peak height of the siRNA duplex decreases due to partial denaturation. It is considered that the higher order structure of ssRNAs is denatured when increasing temperature. The ssRNAs as well as dsRNA retain longer on the stationary phase, as the ion exchange group can access the phosphate groups more easily.

IEX – Ordering information

3 µm non-porous analytical columns, PEEK hardware (max. pressure 18–25 MPa)

Phase	Column ID [mm]	Column length [mm]				Precolumn filter 2 µm* (pack of 5)
		30 (25 MPa)	50 (25 MPa)	100 (25 MPa)	150 (18 MPa)	
BioPro IEX QF	4.6	QF00S03-0346WP	QF00S03-0546WP	QF00S03-1046WP	QF00S03-1546WP	XRPRCP25
BioPro IEX SF	4.6	SF00S03-0346WP	SF00S03-0546WP	SF00S03-1046WP	SF00S03-1546WP	

5 µm non-porous analytical columns, PEEK hardware (max. pressure 6–12 MPa)

Phase	Column ID [mm]	Column length [mm]				Precolumn filter 2 µm* (pack of 5)
		30 (6 MPa)	50 (10 MPa)	100 (12 MPa)	150 (12 MPa)	
BioPro IEX QF	4.6	QF00S05-0346WP	QF00S05-0546WP	QF00S05-1046WP	QF00S05-1546WP	XRPRCP25
BioPro IEX SF	4.6	SF00S05-0346WP	SF00S05-0546WP	SF00S05-1046WP	SF00S05-1546WP	

5 µm porous analytical columns, PEEK hardware (max. pressure 2.5–3.5 MPa)

Phase	Column ID [mm]	Column length [mm]			Precolumn filter 2 µm* (pack of 5)
		30 (2.5 MPa)	50 (3.0 MPa)	100 (3.5 MPa)	
BioPro IEX QA	4.6	QAA0S05-0346WP	QAA0S05-0546WP	QAA0S05-1046WP	XRPRCP25
BioPro IEX SP	4.6	SPA0S05-0346WP	SPA0S05-0546WP	SPA0S05-1046WP	

* Holder required, part no. XRPRCP02

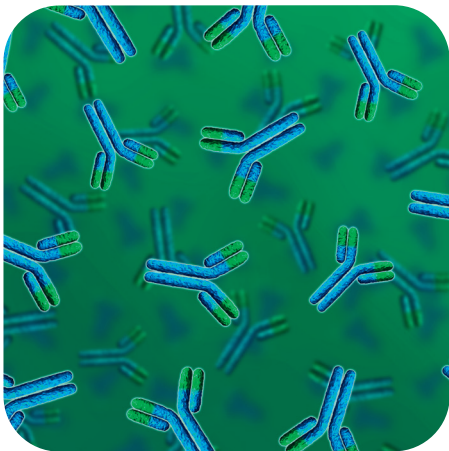
6 µm non-porous semiprep. columns, stainless steel hardware (max. pressure 3–9 MPa)**

Phase	Column ID [mm]	Column length [mm]	
		100	
BioPro IEX QF	10	QF00S06-1010WT	
	20	QF00S06-1020WT	
	30	QF00S06-1030WT	
BioPro IEX SF	10	SF00S06-1010WT	
	20	SF00S06-1020WT	
	30	SF00S06-1030WT	

** optionally bioinert coated stainless steel hardware available



HIC



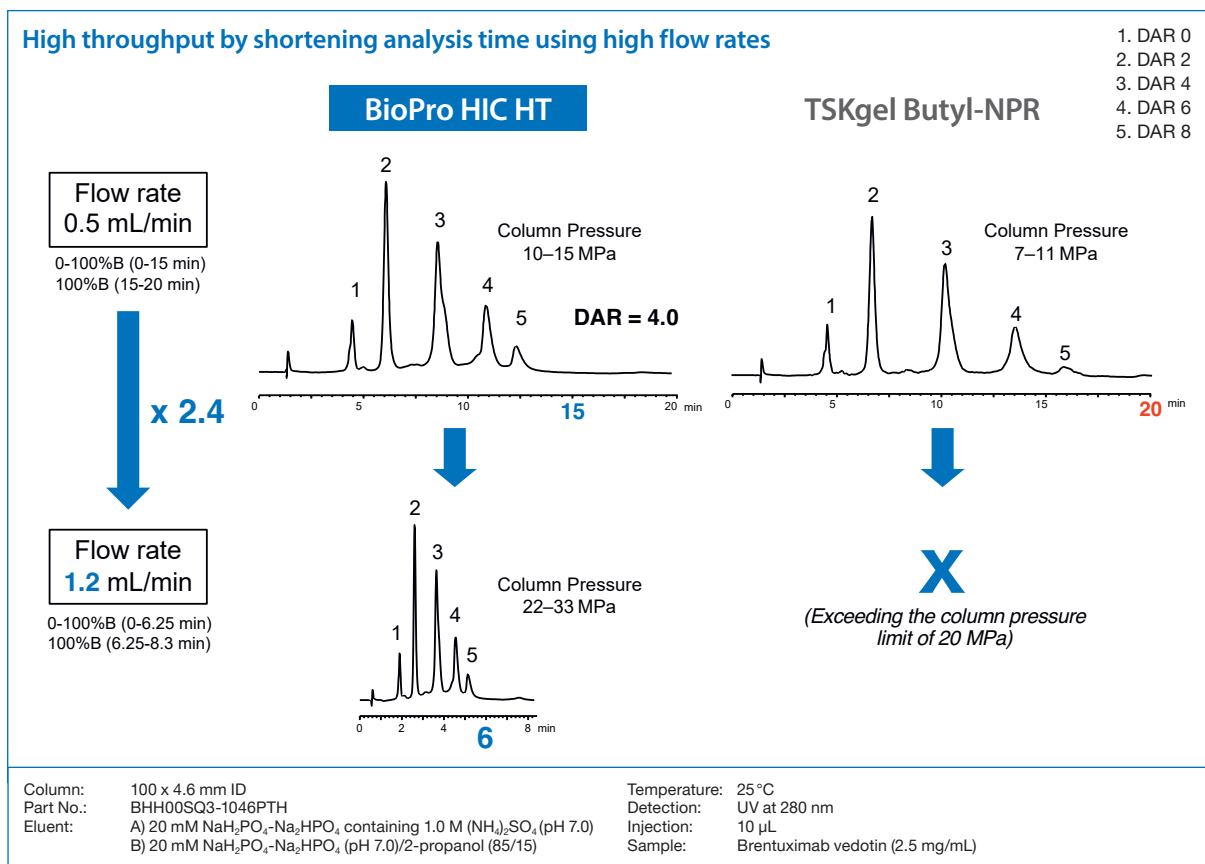
HIC – HPLC selectivities

- Specifically designed for drug-to-antibody conjugates (ADCs) and antibodies
- Ideal drug-to-antibody ratio (DAR) analysis
- High throughput by reducing analysis time
- Excellent batch-to-batch reproducibility
- Long term stability

	BioPro HIC HT	BioPro HIC BF
Base particle	hydrophilic polymer (polymethacrylate)	hydrophilic polymer (polymethacrylate)
Particle size / μm	2.3	4
Pore	non-porous	non-porous
Functional group	butyl	butyl
pH range	2–12	2–12
Pressure limit (for 100 mm)	40 MPa (5,800 psi)	20 MPa (2,900 psi)
Temperature range	10–60°C	10–60°C

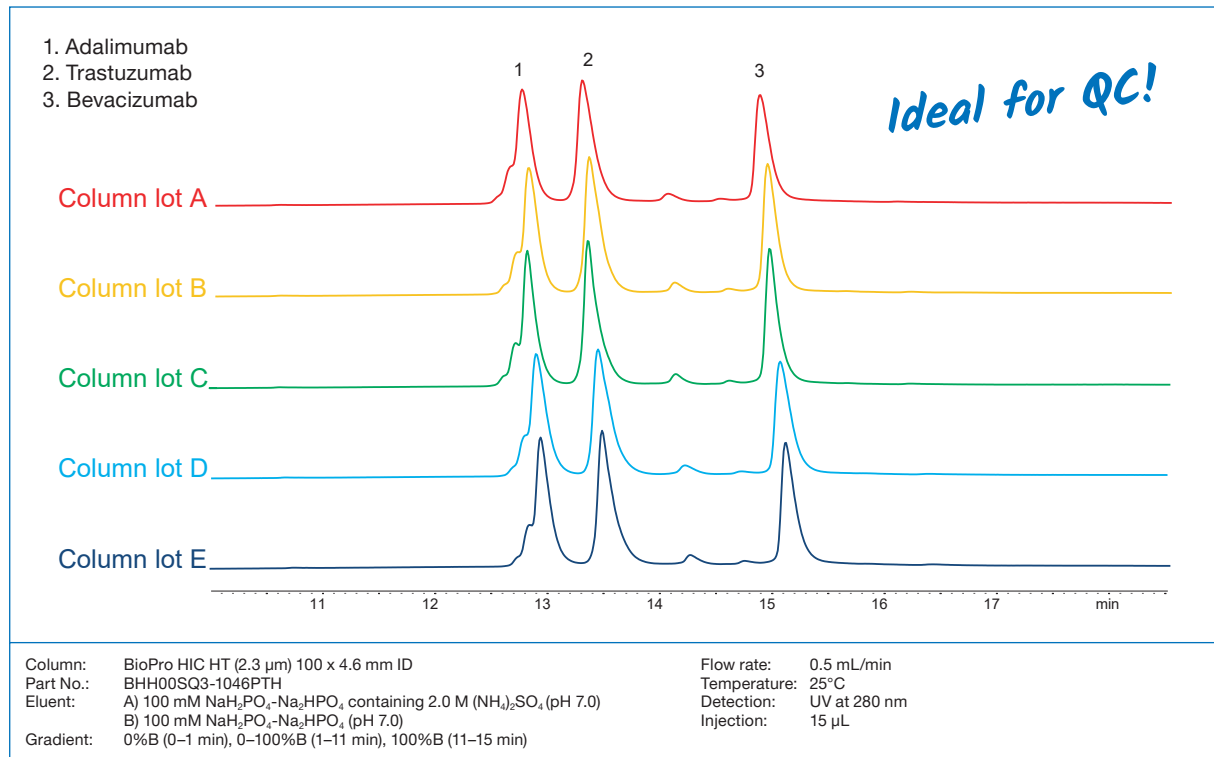
High column stability

High throughput by shortening analysis time using high flow rates



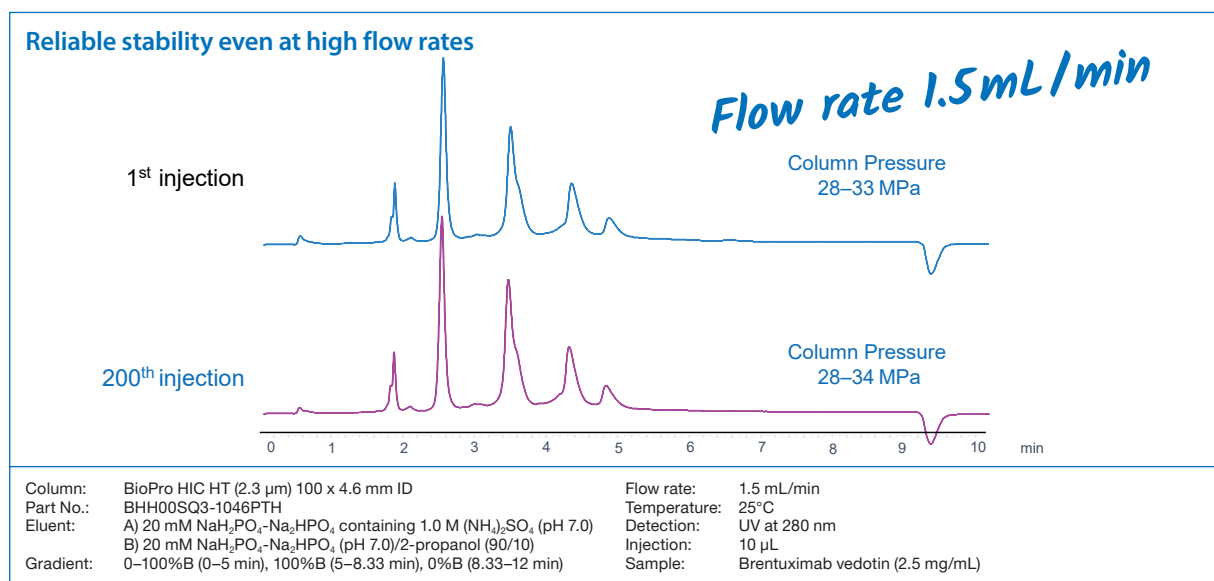
BioPro HIC HT improves analysis throughput of ADCs by 2–3 times with an excellent Drug-to-Antibody Ratio (DAR). The rapid analysis is possible without loss of resolution. Competitor HIC columns fail under these conditions.

Excellent batch-to-batch reproducibility



BioPro HIC HT exhibits an excellent batch-to-batch reproducibility making it the ideal choice for quality control analysis of biopharmaceuticals such as mAbs.

Exceptional stability



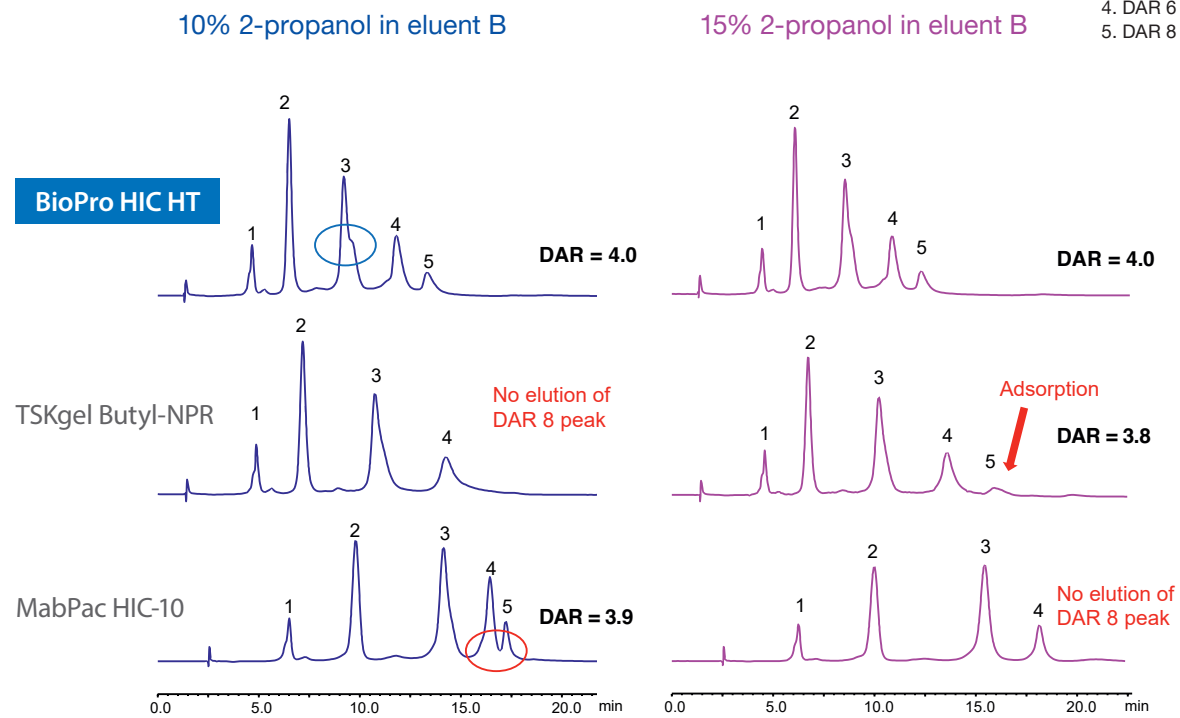
BioPro HIC HT offers excellent stability under high flow rates/high pressure conditions due to its unique rigid particle and optimised column packing technology.

HIC – BioPro HIC: ADC analysis

Designed for analysis of ADCs

Novel surface chemistry for drug-to-antibody ratio (DAR) analysis

- 1. DAR 0
- 2. DAR 2
- 3. DAR 4
- 4. DAR 6
- 5. DAR 8



Column: 100 x 4.6 mm ID
 Part No.: BHH00SQ3-1046PTH
 Eluent: A) 20 mM NaH₂PO₄-Na₂HPO₄ containing 1.0 M (NH₄)₂SO₄ (pH 7.0)
 B) 20 mM NaH₂PO₄-Na₂HPO₄ (pH 7.0)/2-propanol (90/10) or (85/15)
 Gradient: 0–100%B (0–15 min), 100%B (15–20 min), 0%B (20–35 min)
 Flow rate: 0.5 mL/min
 Temperature: 25°C
 Detection: UV at 280 nm
 Injection: 10 µL
 Sample: Brentuximab vedotin (2.5 mg/mL)

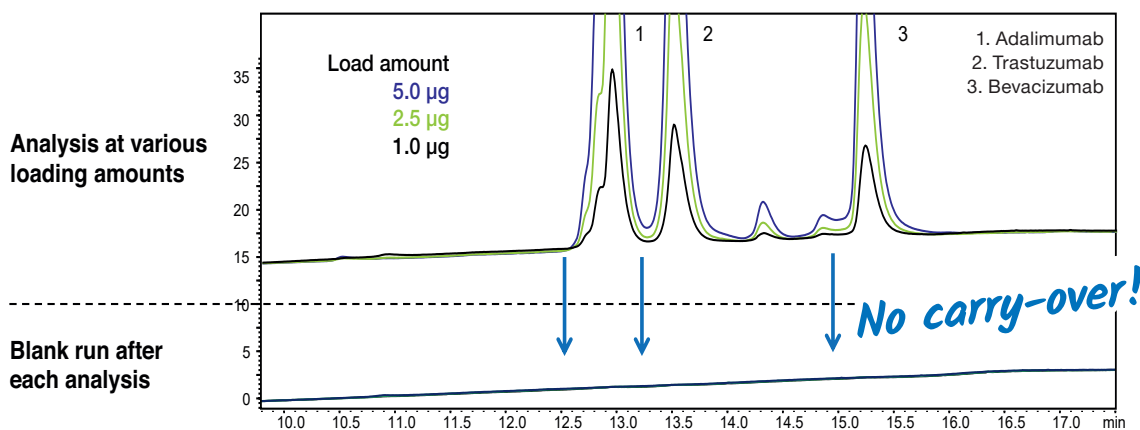
BioPro HIC HT offers higher resolution than conventional HIC columns. Its surface modification suppresses excessive or too strong adsorption of ADCs and results in highly reliable quantification. With varying 2-propanol content, all peaks are completely eluted from the BioPro HIC HT column with high resolution. Another peak is partially separated from peak 3. Additionally, the same DAR values are observed at any content of 2-propanol.

BioPro HIC HT offers:

- Higher resolution than conventional HIC columns
- Highly reliable quantification
- Flexible method development

Excellent recovery and virtually no carry-over

Highly accurate quantification of ADCs and antibodies



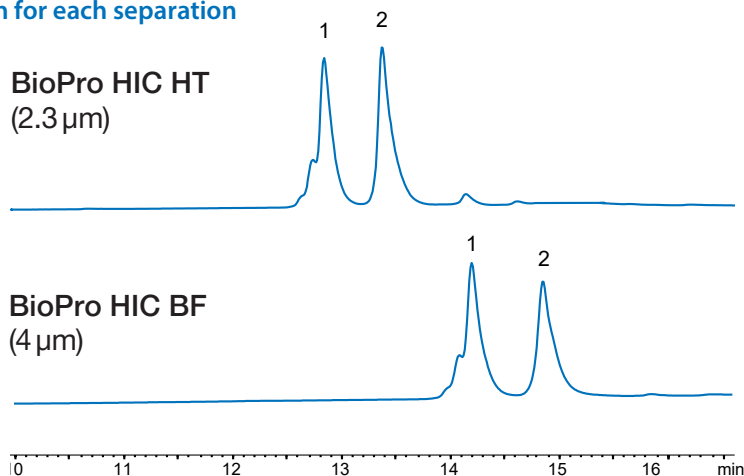
Column: BioPro HIC HT (2.3 µm) 100 x 4.6 mm ID
 Part No.: BHH00SQ3-1046PTH
 Eluent: A) 100 mM NaH₂PO₄-Na₂HPO₄ containing 2.0 M (NH₄)₂SO₄ (pH 7.0)
 B) 100 mM NaH₂PO₄-Na₂HPO₄ (pH 7.0)

Gradient: 0%B (0–1 min), 0–100%B (1–11 min), 100%B (11–15 min)
 Flow rate: 0.5 mL/min
 Temperature: 25 °C
 Detection: UV at 280 nm

BioPro HIC HT offers higher linearity over wide loading and virtually no carry-over. This contributes to highly accurate quantitation of ADCs and antibodies.

Different hydrophobicity

The right column for each separation



Column: 100 x 4.6 mm ID
 Part Nos.: BHH00SQ3-1046PTH
 BHB00S04-1046WT
 Eluent: A) 100 mM NaH₂PO₄-Na₂HPO₄ containing 2.0 M (NH₄)₂SO₄ (pH 7.0)
 B) 100 mM NaH₂PO₄-Na₂HPO₄ (pH 7.0)
 Gradient: 0%B (0–1 min), 0–100%B (1–11 min), 100%B (11–15 min)

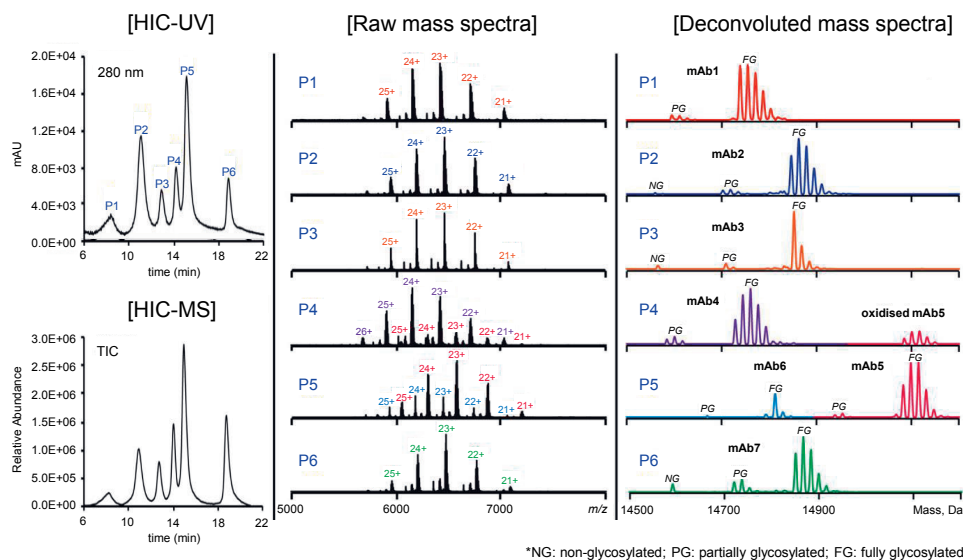
Flow rate: 0.5 mL/min
 Temperature: 25 °C
 Detection: UV at 280 nm
 Injection: 15 µL
 Sample: 1. Adalimumab (Humira®; 0.5 mg/mL)
 2. Trastuzumab (Herceptin®; 0.5 mg/mL)

BioPro HIC HT is the first choice for ADCs or mAbs. BioPro HIC BF columns show a stronger retention and can therefore be used for the separation of low hydrophobic proteins or especially for the analysis of oxidised mAbs.

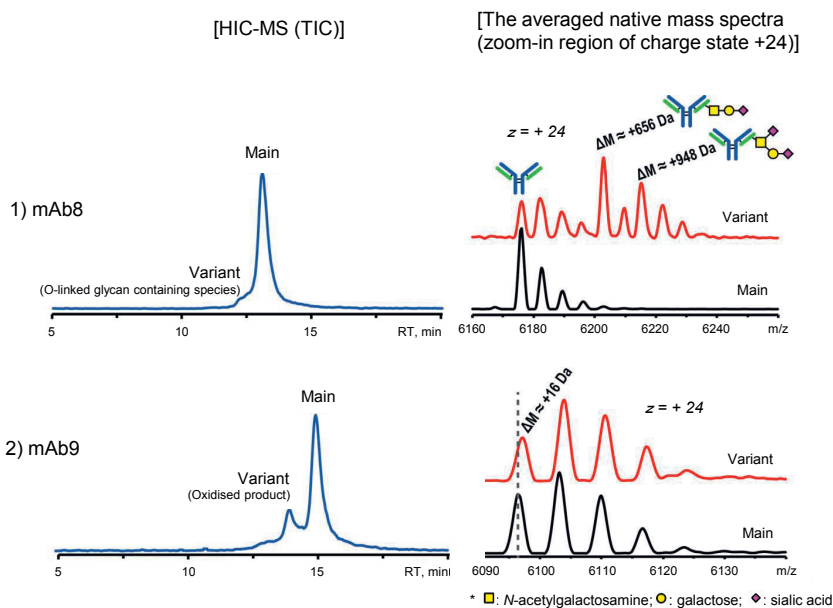
HIC – BioPro HIC: Direct HIC-MS coupling

Online native HIC-MS analysis of mAbs and their molecular variants

Separation of an antibody mixture of seven different mAbs



Separation of two mAbs from their molecular variants



Column: BioPro HIC BF (4 μ m) 100 x 4.6 mm ID
 Part No.: BHB00S04-1046WT
 Eluent: A) 3 M ammonium acetate in water
 B) 100% water
 Gradient: 0%B (0–2min), 0–90%B (2–18min), 90%B (18–22 min)
 Flow rate: 0.3 mL/min
 Temperature: ambient
 Detection: UV at 280 nm, NSI-MS

Injection: mAb mixture: 3 μ L (3–6 μ g)
 mAb 8 and mAb 9: 10 μ g each
 Sample: Mixture of 7 in-house mAbs at 1–2 mg/mL each
 2 in-house mAbs with molecular variants
 Setup: Post-column makeup flow:
 100% water at 1.5 mL/min (reducing salt conc. 6-fold)
 Splitter to reduce the flow rate to 1–5 μ L/min

Courtesy by S. Wang, Regeneron Pharmaceuticals Inc.

To enable simultaneous UV and MS detection a post-column makeup flow and a splitter were used. The makeup flow decreases the salt concentration while the splitter reduces the flow rate to enable the coupling to MS. A nanospray ionisation (NSI) was chosen because of its high sensitivity and salt tolerance.

Reference: Y. Yan, T. Xing, S. Wang, T. J. Daly, N. Li, Online coupling of analytical hydrophobic interaction chromatography with native mass spectrometry for the characterization of monoclonal antibodies and related products, J. Pharm. Biomed. Anal. 186 (2020) 113313.

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 or decrease in the charge on the protein due to the ionisation of acidic or basic groups.

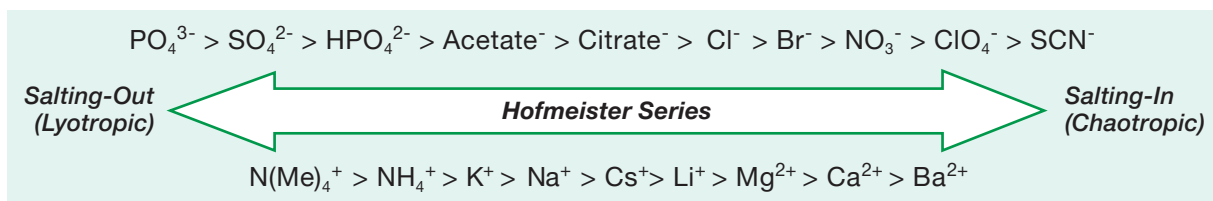


Fig. 1: The Hofmeister Series of lyotropic and chaotropic ions.

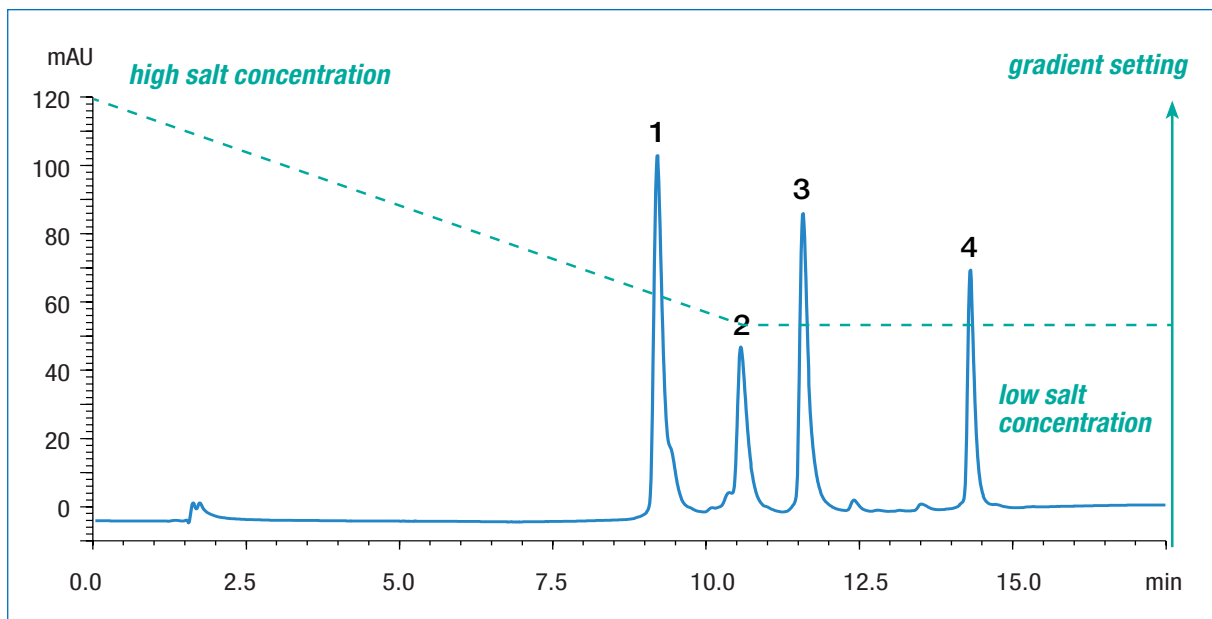


Fig. 2: Method with decreasing salt gradient.

Column:	BioPro HIC BF (100 x 4.6 mm ID)	Samples:	1. Myoglobin (0.73 mg/mL)
Part No.:	BHB00S04-1046WT		2. Ribonuclease A (0.75 mg/mL)
Eluent:	A) 100 mM NaH_2PO_4 - Na_2HPO_4 containing 2.0 M $(\text{NH}_4)_2\text{SO}_4$ (pH 7.0)		3. Lysozyme (0.25 mg/mL)
	B) 100 mM NaH_2PO_4 - Na_2HPO_4 (pH 7.0)		4. α -Chymotrypsinogen A (0.25 gm/mL)
Flow rate:	0.5 mL/min		
Gradient:	0–100%B (0–11 min), 100%B (11–15 min)		
Temperature:	25°C		
Detection:	UV at 280 nm		
Injection:	15 μL		

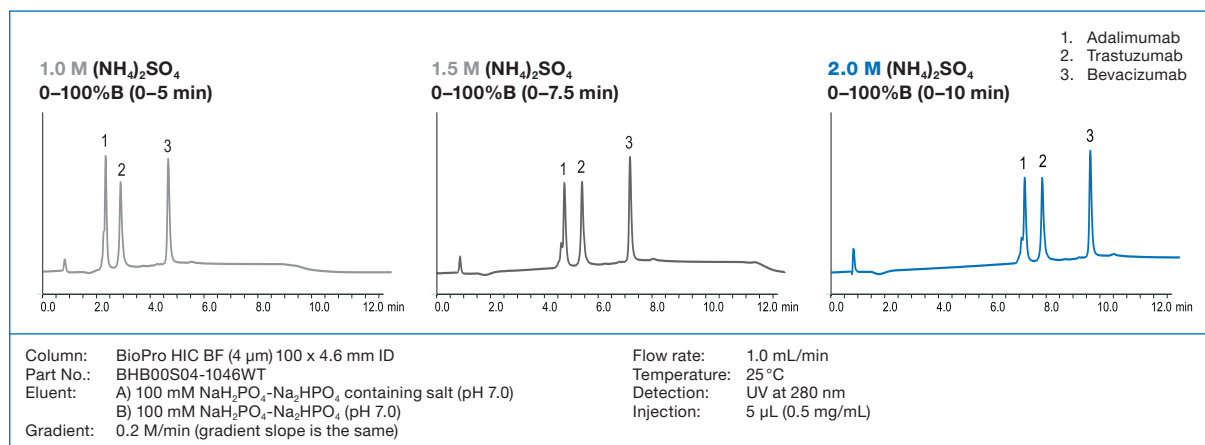
HIC is particularly effective when used to separate proteins and monoclonal antibodies. The separation of monoclonal antibodies, mAb aggregates and glycosylated mAbs can be achieved due to their specific hydrophobic properties. It also provides an excellent method for determination of drug-to-antibody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

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

HIC – Expert Tips: Separation factors

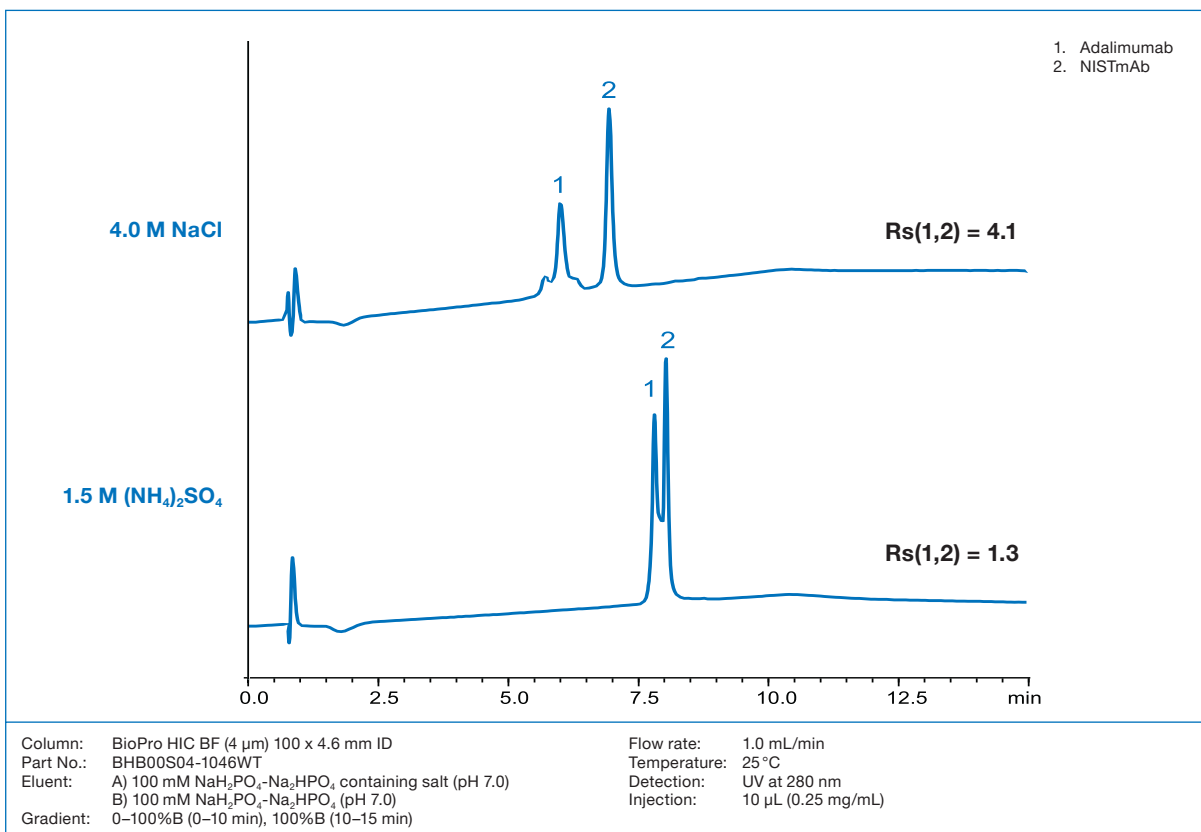
Effect of initial salt concentration

Buffers containing $(\text{NH}_4)_2\text{SO}_4$ are often used as a mobile phase in HIC mode because $(\text{NH}_4)_2\text{SO}_4$ has a strong salting-out effect. The higher the initial concentration of $(\text{NH}_4)_2\text{SO}_4$, the stronger will be the retention of proteins. Therefore, a buffer with a high salt concentration is more suitable for the separation of low hydrophobic proteins with weak retention.



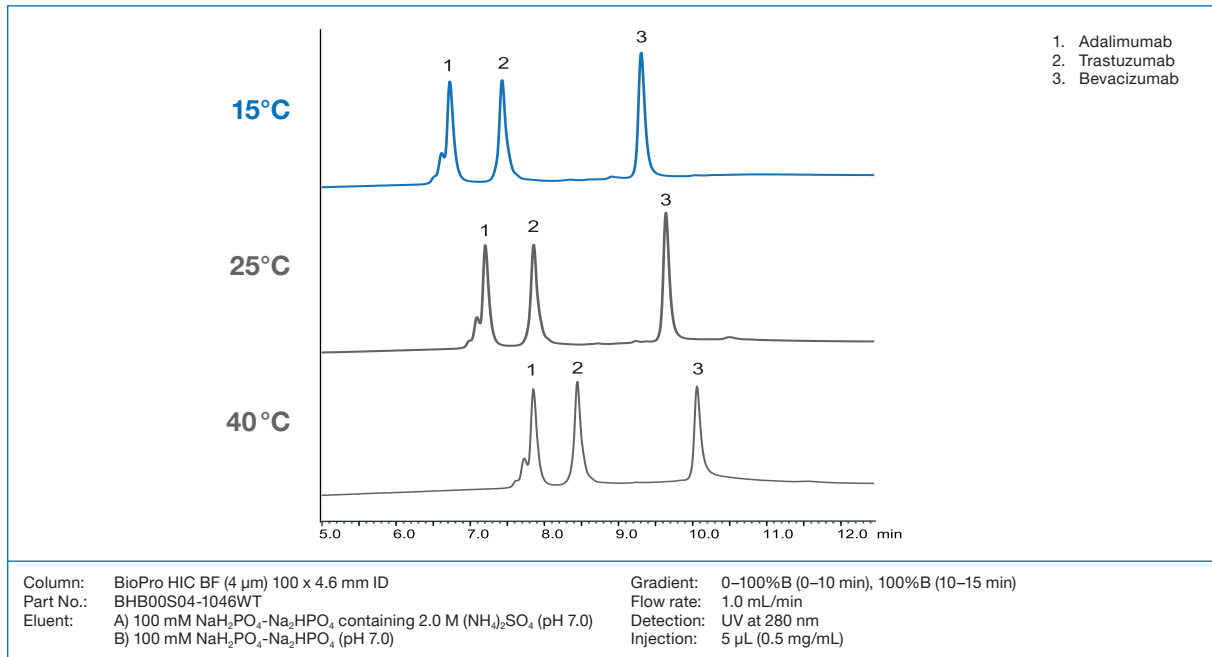
Influence of the type of salt

NaCl and $\text{CH}_3\text{COONH}_4$ are also used as buffer salts. The separation selectivity varies with the type of salt used in some cases, so changing the type of salt can also be effective when the separation is not sufficient. However, these salts have to be used at very high concentrations to gain retentions comparable to $(\text{NH}_4)_2\text{SO}_4$. Attention needs to be paid to the prevention of precipitation of salts in the buffer and damage of the LC system.



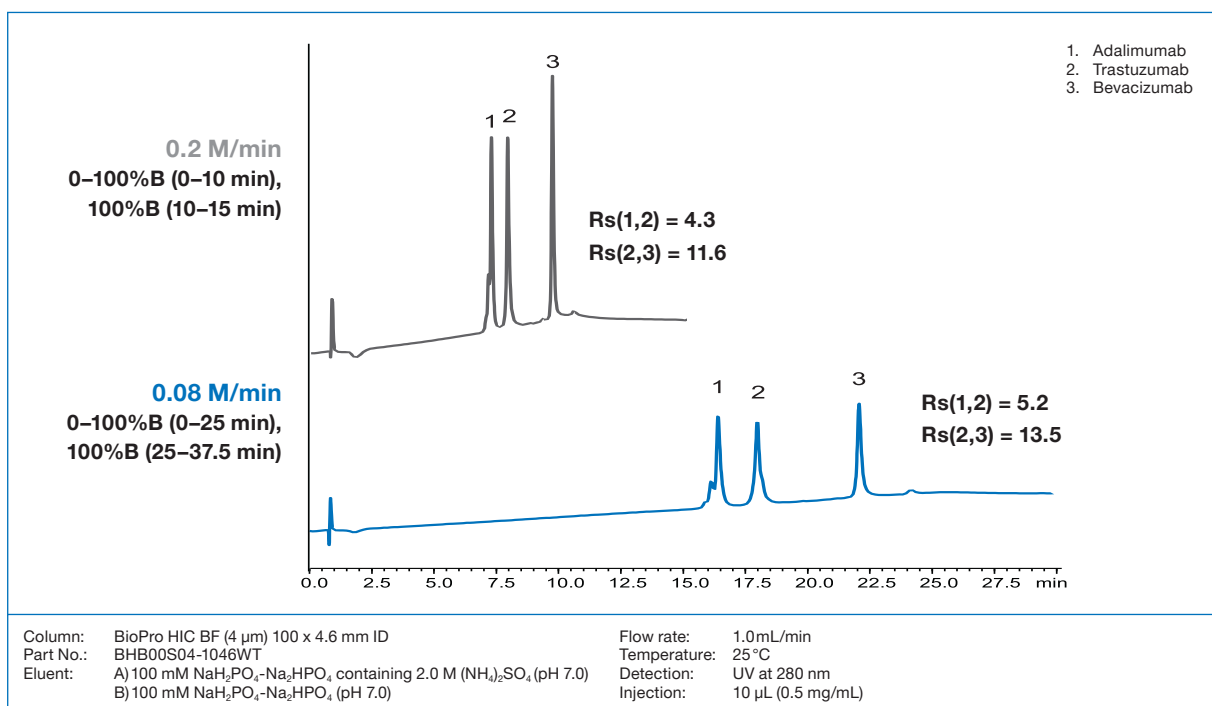
Temperature influence

In HIC mode, higher temperatures result in longer retention times of proteins. This assumes that the hydrophobic area interacting with the stationary phase becomes larger due to a change in the structure of proteins with increasing temperature so that the hydrophobic interactions become stronger.



Variation of gradient slope

In general, shallower gradients improve the separation and the resulting resolution.



HIC – Ordering information

2.3 and 4 µm non-porous analytical columns (max. pressure 20–40 MPa)

Phase	Particle size [µm]	Column ID [mm]	Column length [mm]	Part number	Precolumn filter 2 µm* (pack of 5)
BioPro HIC HT	2.3	4.6	100	BHH00SQ3-1046PTH	XRPRCS35
		4.6	33	BHH00SQ3-H346PTH	XRPRCS35
BioPro HIC BF	4	4.6	100	BHB00S04-1046WT	XRPRCS35

*Holder required, part no XRPRCS03
Other dimensions on demand



HILIC



HILIC – UHPLC/HPLC selectivity

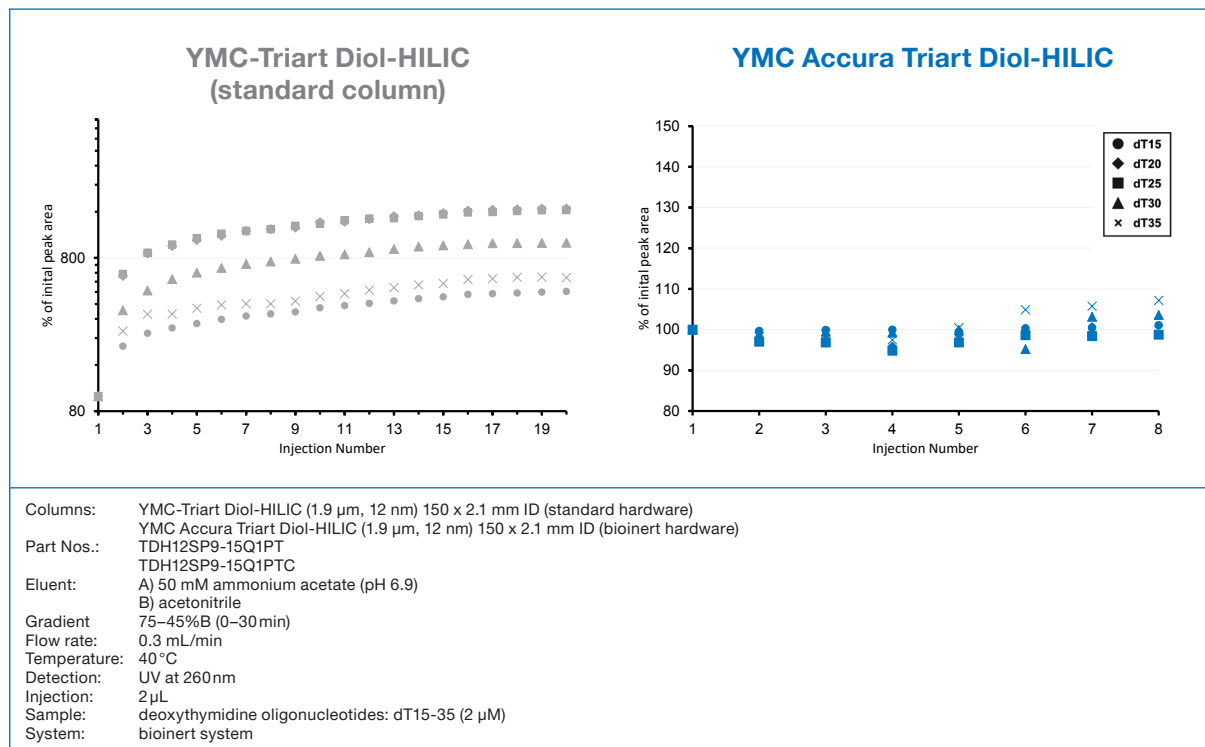
- Applicable to oligonucleotides, peptides, amino acids
- pH- and temperature stable
- Superior reproducibility
- Bioinert hardware available

	Base particle	Modification	Particle Size / μm	Pore Size / nm	pH range	Temperature range
YMC-Triart Diol-HILIC	organic/inorganic hybrid silica	Diol (USP L20)	1.9, 3, 5	12	2–10	50 °C



Bioinert YMC-Triart columns are available for improved sensitivity, peak shape and recovery of coordinating compounds such as nucleotides, oligonucleotides or phosphorylated proteins/peptides.

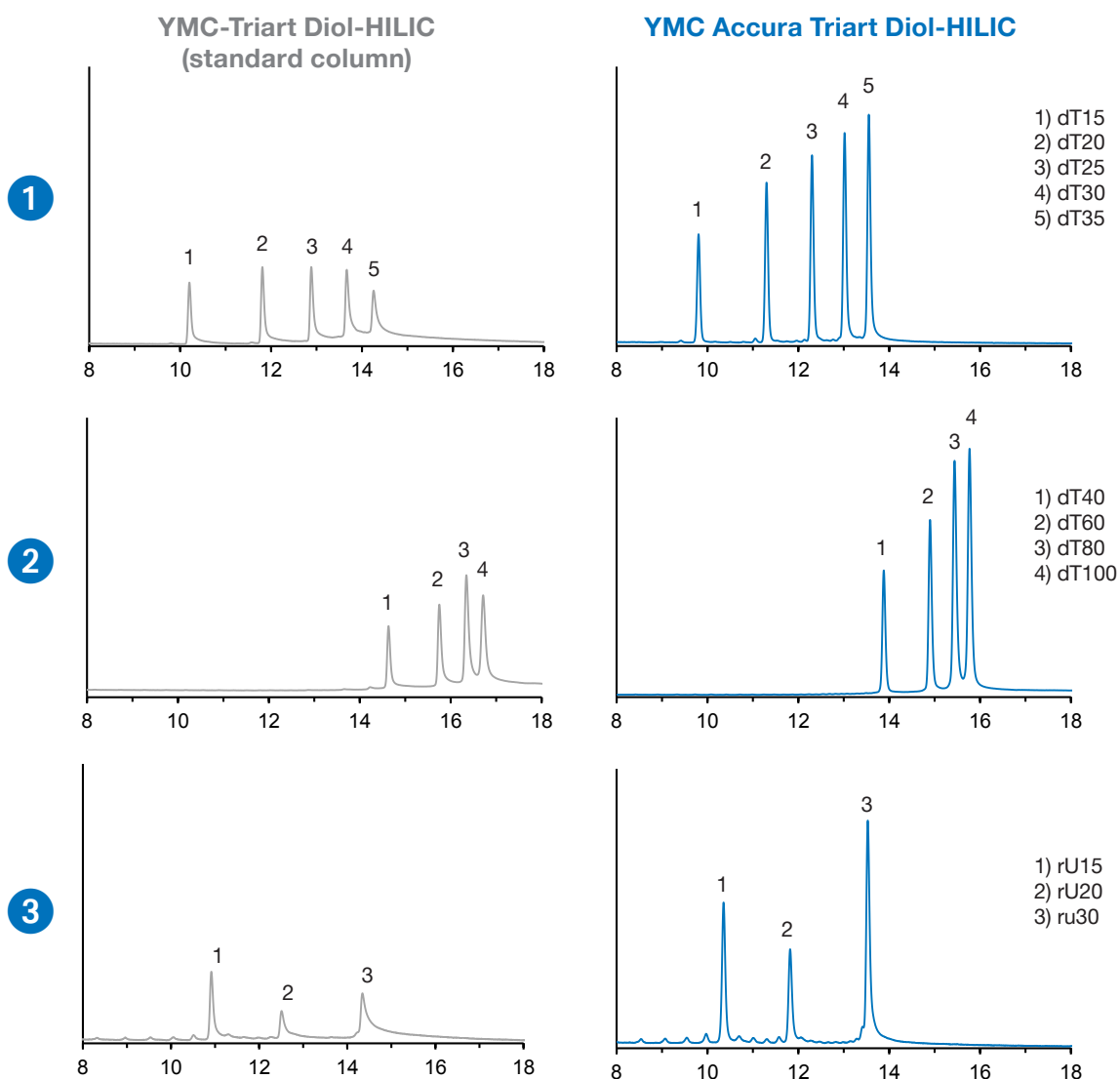
Pre-conditioning of a stainless-steel and a bioinert coated column with short DNA mixture



Pre-conditioning is a typical procedure when working with stainless-steel columns. Using a bioinert column such as YMC Accura Triart usually achieves great performance from the first injection when working with an IP-RP phase. HILIC phases still need some pre-conditioning when a bioinert column is used; however, the number of injections is remarkably reduced. While 20 injections are necessary for the stainless-steel column, the YMC Accura column is already conditioned after 8 injections, with very little difference (less than 10%) between initial and final peak areas.

Significantly better oligonucleotide separation

Improved chromatographic results using bioinert coated YMC Accura Triart column



Columns: YMC-Triart Diol-HILIC (1.9 μ m, 12 nm) 150 x 2.1 mm ID (standard hardware)
 YMC Accura Triart Diol-HILIC (1.9 μ m, 12 nm) 150 x 2.1 mm ID (bioinert hardware)

Part Nos.: TDH12SP9-15Q1PT
 TDH12SP9-15Q1PTC

Eluent: A) 50 mM ammonium acetate (pH 6.9)
 B) acetonitrile

Gradient: 75–45%B (0–30 min)

Flow rate: 0.3 mL/min

Temperature: 40 °C

Detection: UV at 260nm

Injection: 2 μ L

Sample: deoxythymidine oligonucleotides: dT15-35 (2 μ M) and dT40-100 (2 μ M)
 RNA oligonucleotides: rU15-30 (2 μ M)

System: bioinert system

dT15-35 **1**
 dT40-100 **2**
 rU15-30 **3**

By courtesy of University of Geneva,
 Institute of Pharmaceutical Sciences of Western Switzerland (ISPSO)

After conditioning and analysing the short DNA oligonucleotide mixture of dT15-35, longer DNA oligonucleotides dT40-100 and short RNA oligonucleotides rU15-30 are analysed. Higher sensitivities, peak areas and less tailing are achieved using the bioinert YMC Accura Triart Diol-HILIC column. Non-specific adsorption does not vary according to length, even though the adsorption is usually higher for longer oligonucleotides in IP-RP.

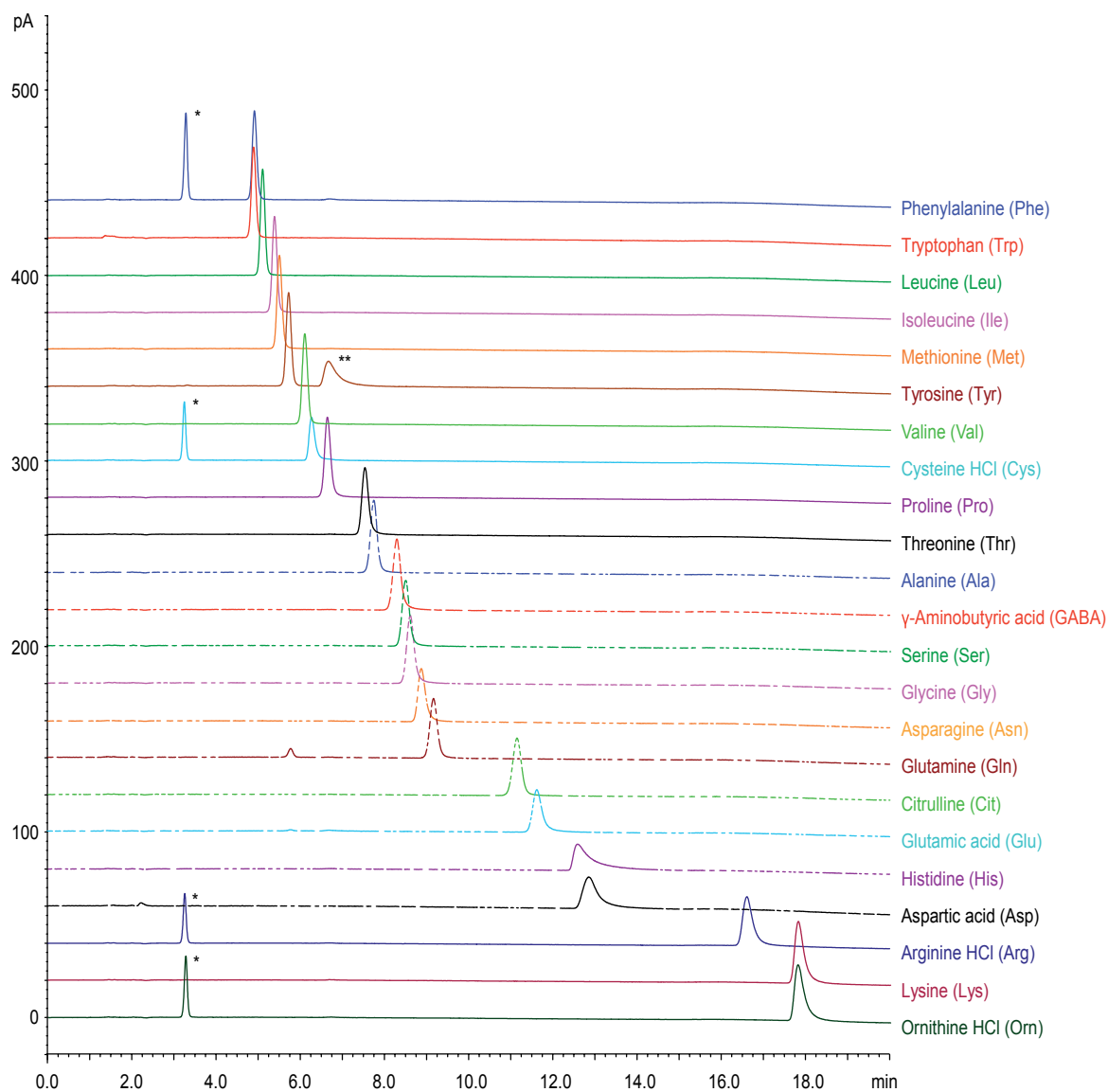
Reference: H. Lardeux, A. Goyon, K. Zhang, J.M. Nguyen, M.A. Lauber, D. Guillaume, V. D'Atri, The impact of low adsorption surfaces for the analysis of DNA and RNA oligonucleotides, J. Chromatogr. A 1677 (2022) 463324.

HILIC – Amino acids

Ideal choice for reliable analysis of hydrophilic compounds

Free amino acids in HILIC mode

* Chloride ion contained in the sample solution
 ** Sodium ion contained in the sample solution



Column: YMC-Triart Diol-HILIC (5 μm, 12 nm) 150 x 4.6 mm ID
 Part No.: TDH12S05-1546PTH
 Eluent: A) 100 mM HCOOH-HCOONH₄ (pH 3.6)
 B) acetonitrile
 Gradient: 83–80%B (0–12 min), 80–68%B (12–20 min)
 Flow rate: 1.0 mL/min
 Temperature: 40 °C
 Detection: Corona® CAD® (Charged Aerosol Detector)
 Injection: 10 μL (0.1 mg/mL)

Corona and CAD are trademarks of Thermo Fisher Scientific.

1.9 µm UHPLC columns (max. pressure 100 MPa)

Phase	Column ID [mm]	Column length [mm]				
		30	50	75	100	150
YMC-Triart Diol-HILIC	2.0	TDH12SP9-0302PT	TDH12SP9-0502PT	TDH12SP9-L502PT	TDH12SP9-1002PT	TDH12SP9-1502PT
	2.1	TDH12SP9-03Q1PT	TDH12SP9-05Q1PT	TDH12SP9-L5Q1PT	TDH12SP9-10Q1PT	TDH12SP9-15Q1PT
	3.0	–	TDH12SP9-0503PT	TDH12SP9-L503PT	TDH12SP9-1003PT	TDH12SP9-1503PT

1.9 µm bioinert coated UHPLC columns (max. pressure 100 MPa)

Phase	Column ID [mm]	Column length [mm]		
		50	100	150
YMC Accura Triart Diol-HILIC	2.1	TDH12SP9-05Q1PTC	TDH12SP9-10Q1PTC	TDH12SP9-15Q1PTC

1.9 µm PEEK-lined UHPLC columns (max. pressure 100 MPa)

Phase	Column ID [mm]	Column length [mm]		
		50	100	150
YMC-Triart Diol-HILIC metal-free	2.1	TDH12SP9-05Q1PTP	TDH12SP9-10Q1PTP	TDH12SP9-15Q1PTP

Special column connectors required.

3 µm HPLC columns (max. pressure 20–45 MPa)

Phase	Column ID [mm]	Column length [mm]					
		50	75	100	150	250	(pack of 5)
YMC-Triart Diol-HILIC	2.0	TDH12S03-0502WT	TDH12S03-L502WT	TDH12S03-1002WT	TDH12S03-1502WT	TDH12S03-2502WT	TDH12S03-01Q1GC
	2.1	TDH12S03-05Q1PTH	TDH12S03-L5Q1PTH	TDH12S03-10Q1PTH	TDH12S03-15Q1PTH	TDH12S03-25Q1PTH	TDH12S03-01Q1GC
	3.0	TDH12S03-0503WT	TDH12S03-L503WT	TDH12S03-1003WT	TDH12S03-1503WT	TDH12S03-2503WT	TDH12S03-0103GC
	4.6	TDH12S03-0546WT	TDH12S03-L546WT	TDH12S03-1046WT	TDH12S03-1546WT	TDH12S03-2546WT	TDH12S03-0104GC

*Guard cartridge holder required, part no. XPGCH-Q1 (for EMEA) /XPGCHP1 (outside EMEA)

3 µm bioinert coated HPLC columns (max. pressure 45 MPa)

Phase	Column ID [mm]	Column length [mm]		
		50	100	150
YMC Accura Triart Diol-HILIC	2.1	TDH12S03-05Q1PTC	TDH12S03-10Q1PTC	TDH12S03-15Q1PTC
	4.6	TDH12S03-0546PTC	TDH12S03-1046PTC	TDH12S03-1546PTC

3 µm PEEK-lined HPLC columns (max. pressure 45 MPa)

Phase	Column ID [mm]	Column length [mm]		
		50	100	150
YMC-Triart Diol-HILIC metal-free	2.1	TDH12S03-05Q1PTP	TDH12S03-10Q1PTP	TDH12S03-15Q1PTP
	4.6	TDH12S03-0546PTP	TDH12S03-1046PTP	TDH12S03-1546PTP

Special column connectors required.

HILIC – Ordering Information

5 µm HPLC columns (max. pressure 20–45 MPa)

Phase	Column ID [mm]	Column length [mm]					
		50	75	100	150	250	(pack of 5)
YMC-Triart Diol-HILIC	2.0	TDH12S05-0502WT	TDH12S05-L502WT	TDH12S05-1002WT	TDH12S05-1502WT	TDH12S05-2502WT	TDH12S05-01Q1GC
	2.1	TDH12S05-05Q1PTH	TDH12S05-L5Q1PTH	TDH12S05-10Q1PTH	TDH12S05-15Q1PTH	TDH12S05-25Q1PTH	TDH12S05-01Q1GC
	3.0	TDH12S05-0503WT	TDH12S05-L503WT	TDH12S05-1003WT	TDH12S05-1503WT	TDH12S05-2503WT	TDH12S05-0103GC
	4.6	TDH12S05-0546WT	TDH12S05-L546WT	TDH12S05-1046WT	TDH12S05-1546WT	TDH12S05-2546WT	TDH12S05-0104GC

*Guard cartridge holder required, part no. XPGCH-Q1 (for EMEA) /XPGCHP1 (outside EMEA)

5 µm bioinert coated HPLC columns (max. pressure 45 MPa)

Phase	Column ID [mm]	Column length [mm]		
		50	100	150
YMC Accura Triart Diol-HILIC	2.1	TDH12S05-05Q1PTC	TDH12S05-10Q1PTC	TDH12S05-15Q1PTC
	4.6	TDH12S05-0546PTC	TDH12S05-1046PTC	TDH12S05-1546PTC

5 µm PEEK-lined HPLC columns (max. pressure 45 MPa)

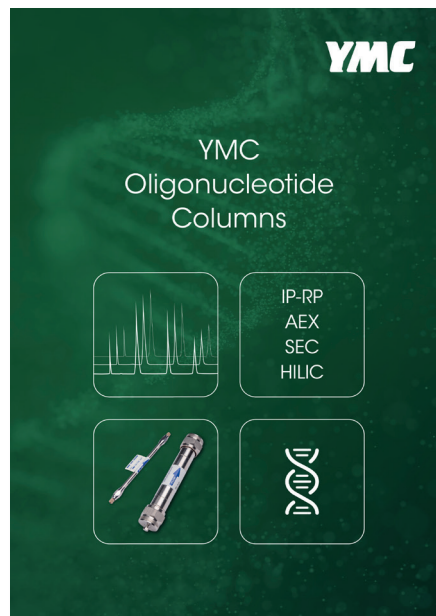
Phase	Column ID [mm]	Column length [mm]		
		50	100	150
YMC-Triart Diol-HILIC metal-free	2.1	TDH12S05-05Q1PTP	TDH12S05-10Q1PTP	TDH12S05-15Q1PTP
	4.6	TDH12S05-0546PTP	TDH12S05-1046PTP	TDH12S05-1546PTP

Special column connectors required.

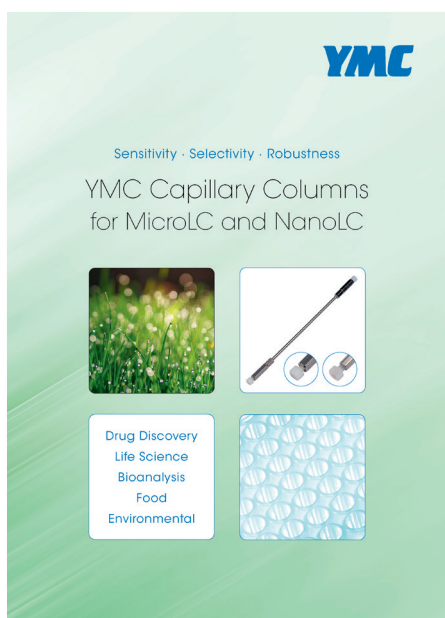
Further dimensions and guard cartridges available in regular stainless-steel hardware.



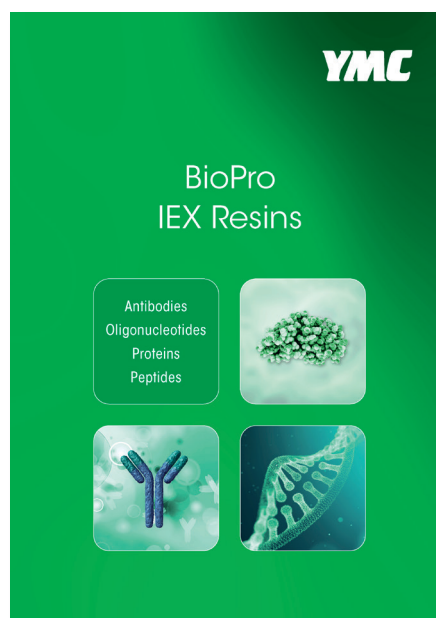
YMC-Triart



YMC Oligonucleotide Columns



YMC Capillary Columns



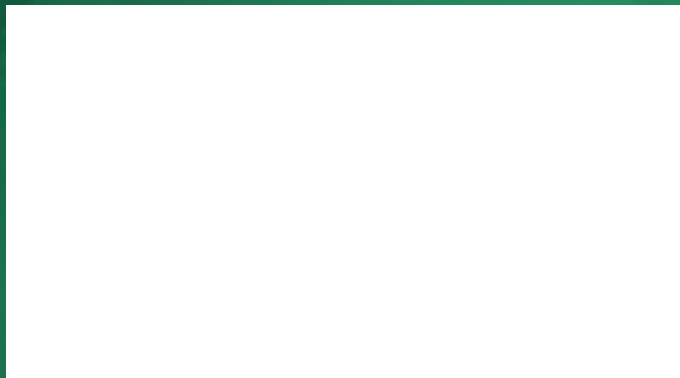
BioPro IEX Resins

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