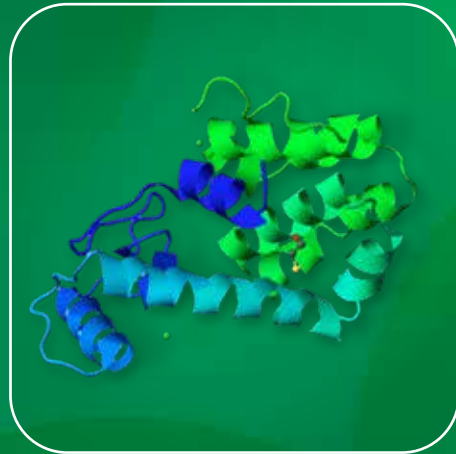


# YMC

## Biochromatography Columns

RP  
SEC  
IEX  
HIC



## HPLC Columns for Biochromatography

	Reversed Phase (RP)	Size Exclusion (SEC)	Ion Exchange (IEX)	Hydrophobic Interaction (HIC)
Separation principle	Hydrophobicity	Molecular weight	Electric charge	Hydrophobicity
Max. MW	Up to about 150,000 Da	Up to about 1,000,000 Da	Up to several millions Da	Up to about 1,000,000 Da
Resolution	+++	++	+++	+++
Speed	+++	+	++ / +++	+++
Loading	++	++	+++	+++
Stability	+ / ++	+++	+++	+++
Usage (e.g.)	<ul style="list-style-type: none"> <li>• Peptide mapping</li> <li>• LC/MS</li> <li>• Nucleic acids and oligonucleotides</li> </ul>	<ul style="list-style-type: none"> <li>• Impurity analysis of antibody-drug conjugates</li> <li>• MAb separation</li> </ul>	<ul style="list-style-type: none"> <li>• Proteins/MAB</li> <li>• Charge variant analysis</li> <li>• Isoform analysis</li> <li>• Nucleic acids and oligonucleotides</li> </ul>	<ul style="list-style-type: none"> <li>• Drug-binding analysis of antibody-drug conjugates</li> </ul>

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

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

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Pro Pac WCX-10, MabPac HIC-10 are trademarks of Thermo Fisher Scientific.

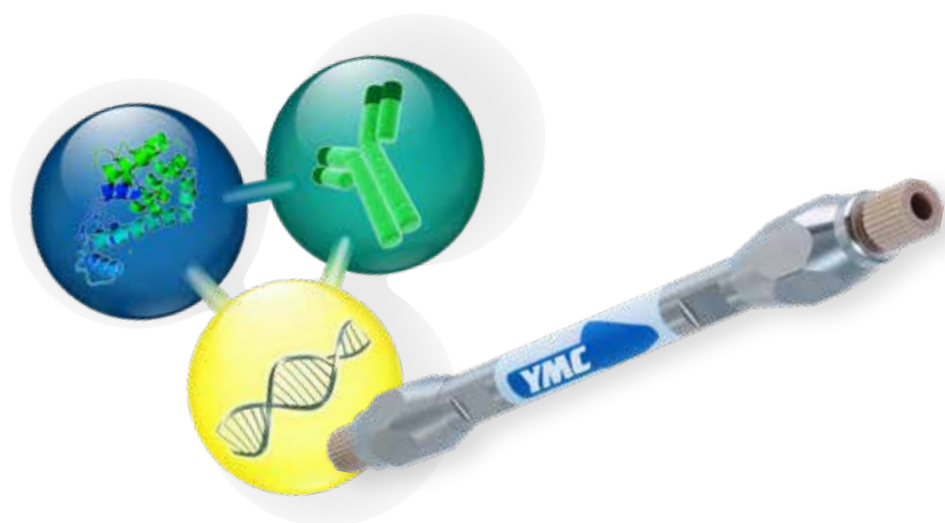
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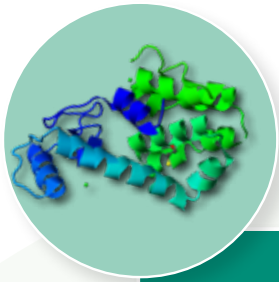
GE Healthcare Mono Q/S is a trademark of GE Corp.

New BioLC phase:  
**BioPro HIC HT**

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# Phase selection guide



## Proteins / Peptides

### HIC

BioPro  
HIC HT

BioPro  
HIC BF

### IEX

BioPro  
IEX QA

BioPro  
IEX QF

BioPro  
IEX SP

BioPro  
IEX SF

### SEC

YMC-Pack  
Diol-60

YMC-Pack  
Diol-120

YMC-Pack  
Diol-200

YMC-Pack  
Diol-300

YMC-SEC MAB

### RP

YMC-Triart  
C18

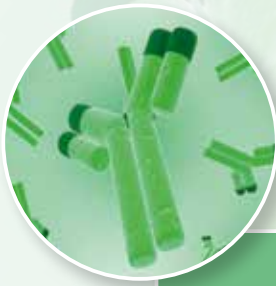
YMC-Triart  
Bio C18

Meteoric  
Core C18 BIO

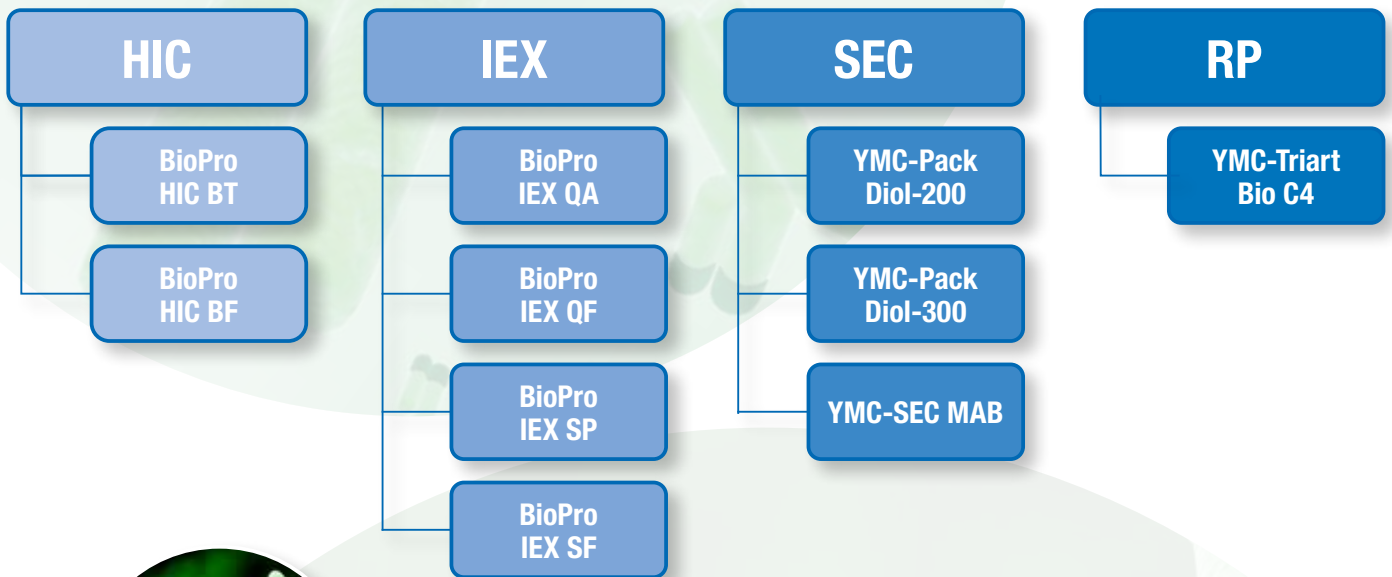
YMCbasic

YMC-Triart  
Bio C4

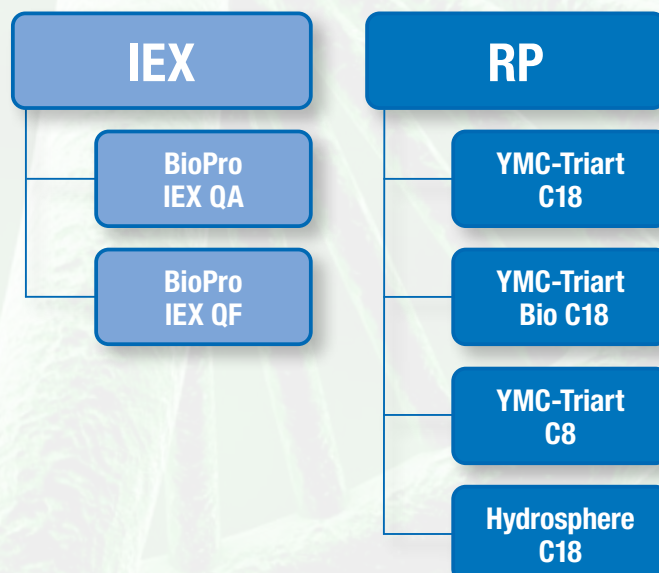




## (Monoclonal) Antibodies



## Oligonucleotides / Nucleic Acids



## Bio QC – Validation kit

### Method Validation Kits for BioLC

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

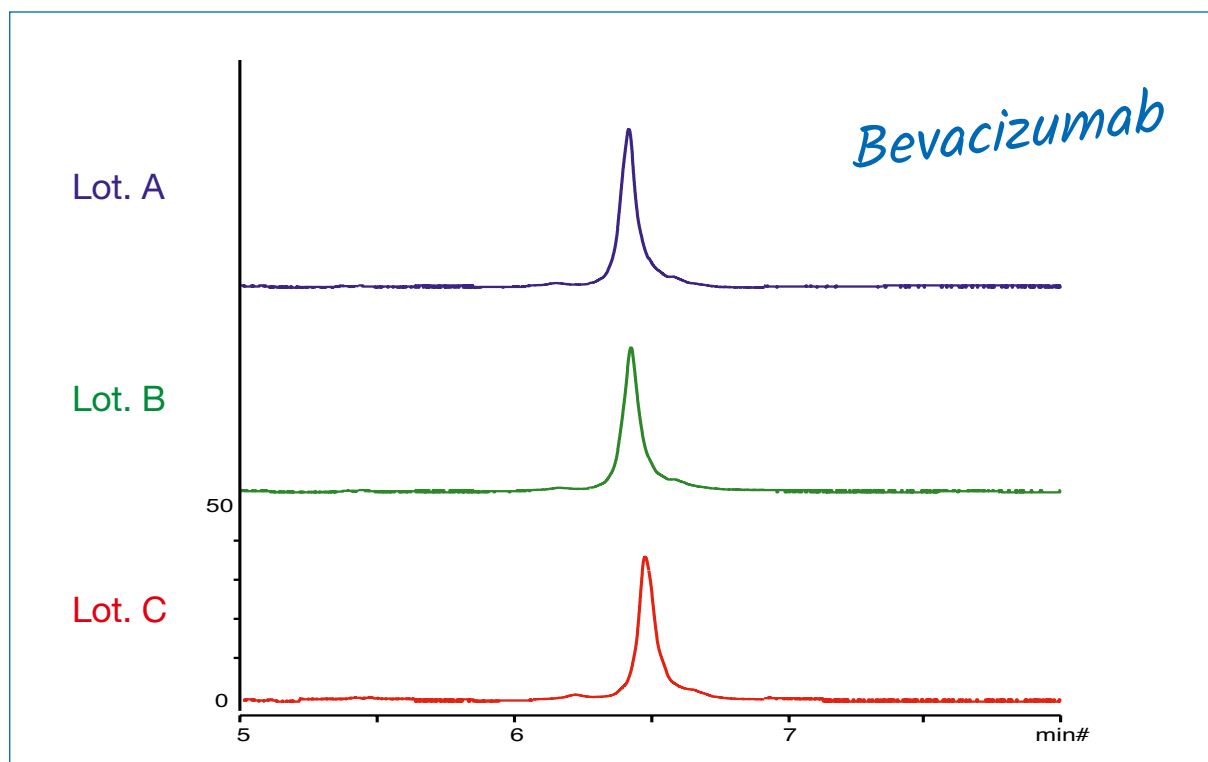
#### Validation kit:

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

#### Available dimensions:

Length: 30 or 33, 50, 75, 100, 150, 250, 300 mm

ID: 2.0 or 2.1, 3.0, 4.0, 4.6, 8.0 mm



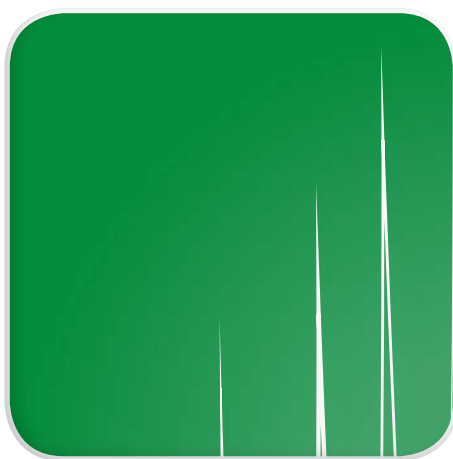
To order a validation kit simply use the ordering number for the column of interest, e.g. **TB30SP9-05Q1PT** and add V1: **TB30SP9-05Q1PTV1**.

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

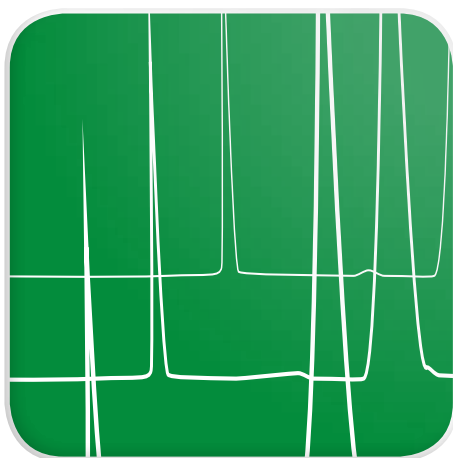
### Batch Reservation Service

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

**Please call YMC or contact your YMC representative for details.**



BioLC  
Applications

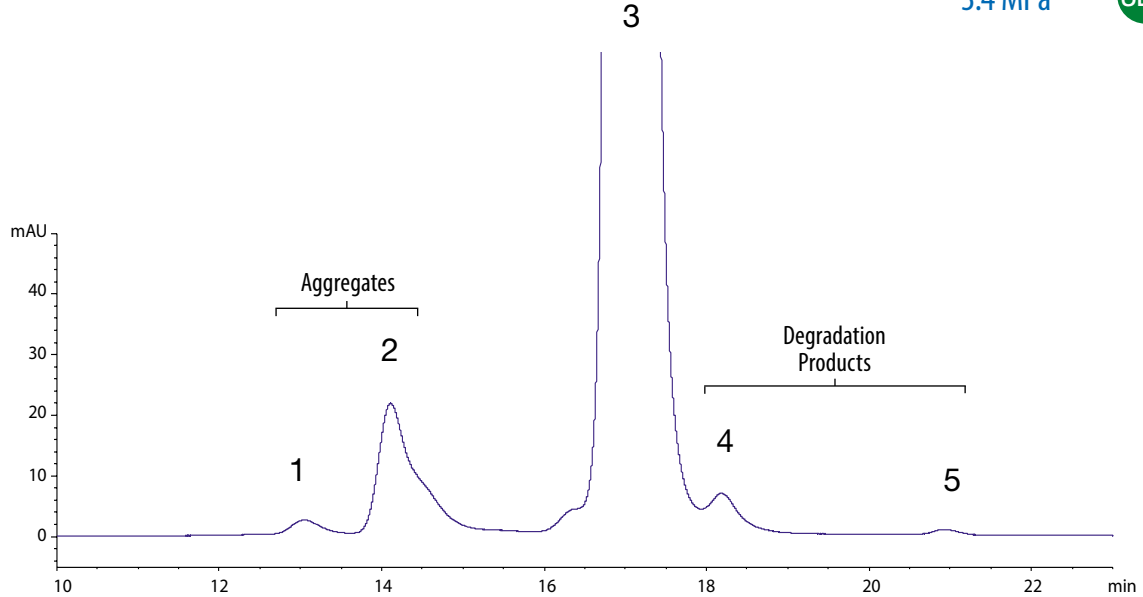


# BioLC applications – Antibodies

## Bevacizumab and its fragments and aggregates

3.4 MPa

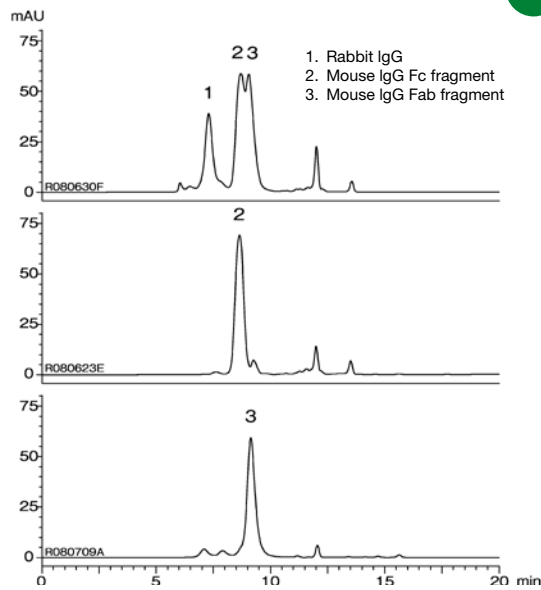
SEC



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

## IgG, Fab and Fc fragments

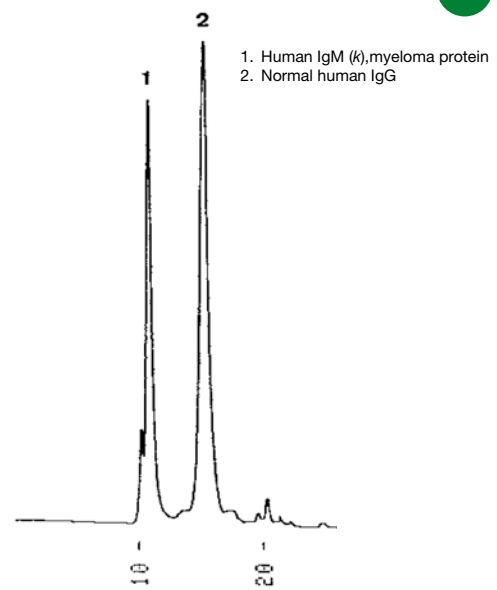
SEC



Column: YMC-Pack Diol-200 (5  $\mu$ m) 300 x 8.0 mm ID  
 Part No.: DL20S05-3008WT  
 Eluent: 0.1 M  $\text{KH}_2\text{PO}_4$ - $\text{K}_2\text{HPO}_4$  (pH 6.9) containing 0.2 M NaCl  
 Flow rate: 1.0 mL/min  
 Temperature: ambient (27 °C)  
 Detection: UV at 220 nm  
 Injection: 5  $\mu$ L (0.4, 0.5 mg/mL)

## Human Immunglobulin

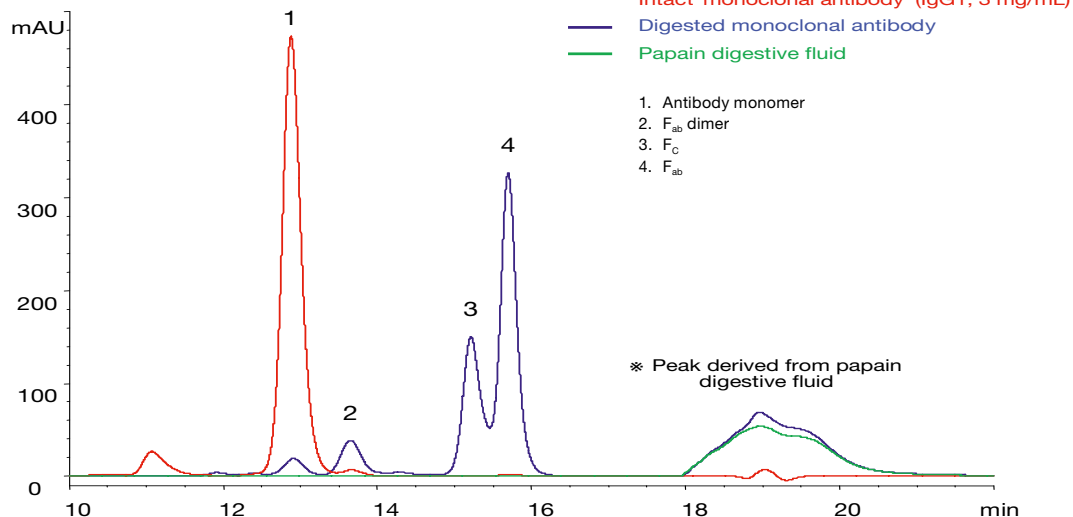
SEC



Column: YMC-Pack Diol-300 (5  $\mu$ m) 500 x 8.0 mm ID  
 Part No.: DL30S05-5008WT  
 Eluent: 0.1 M  $\text{NaH}_2\text{PO}_4$ - $\text{Na}_2\text{HPO}_4$  (pH 6.8) containing 0.1 M  $\text{Na}_2\text{SO}_4$   
 Flow rate: 1.0 mL/min  
 Temperature: ambient (24 °C)  
 Detection: UV at 280 nm, 0.04 AUFS  
 Injection: 40  $\mu$ L (0.5 mg/mL)

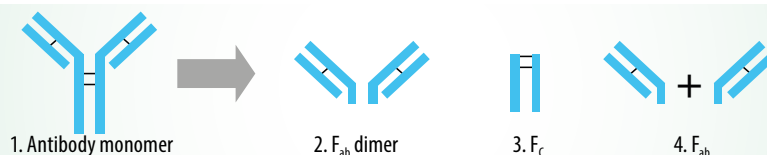


## Immunglobulin digest

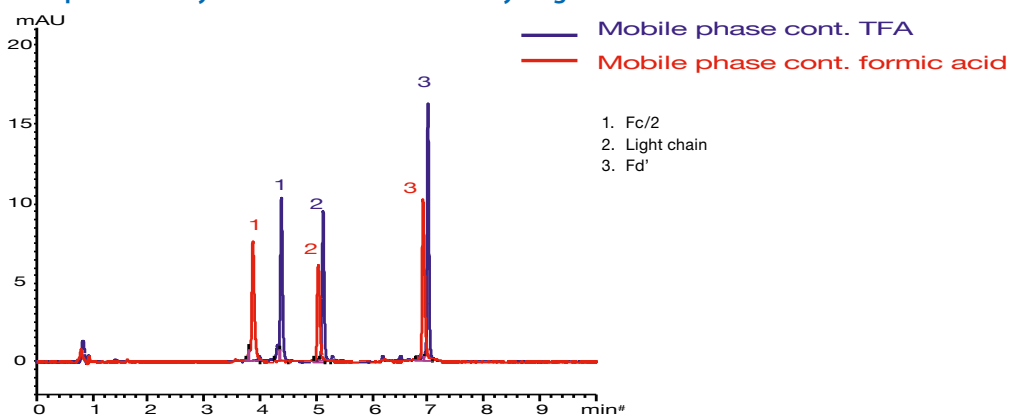


SEC

Column: YMC-Pack Diol-200 (2  $\mu$ m, 20 nm) 300 x 4.6 mm ID  
 Part no: DL20S02-3046PTH  
 Eluent: 0.1 M  $\text{KH}_2\text{PO}_4$ - $\text{K}_2\text{HPO}_4$  (pH 7.0) containing 0.2 M NaCl  
 Flow rate: 0.2 mL/min  
 Temperature: ambient  
 Detection: UV at 280 nm  
 Sample: IgG1 (3 mg/ml)

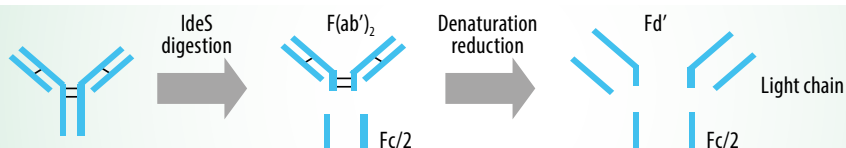


## LC/MS compatible analysis of monoclonal antibody fragments



RP

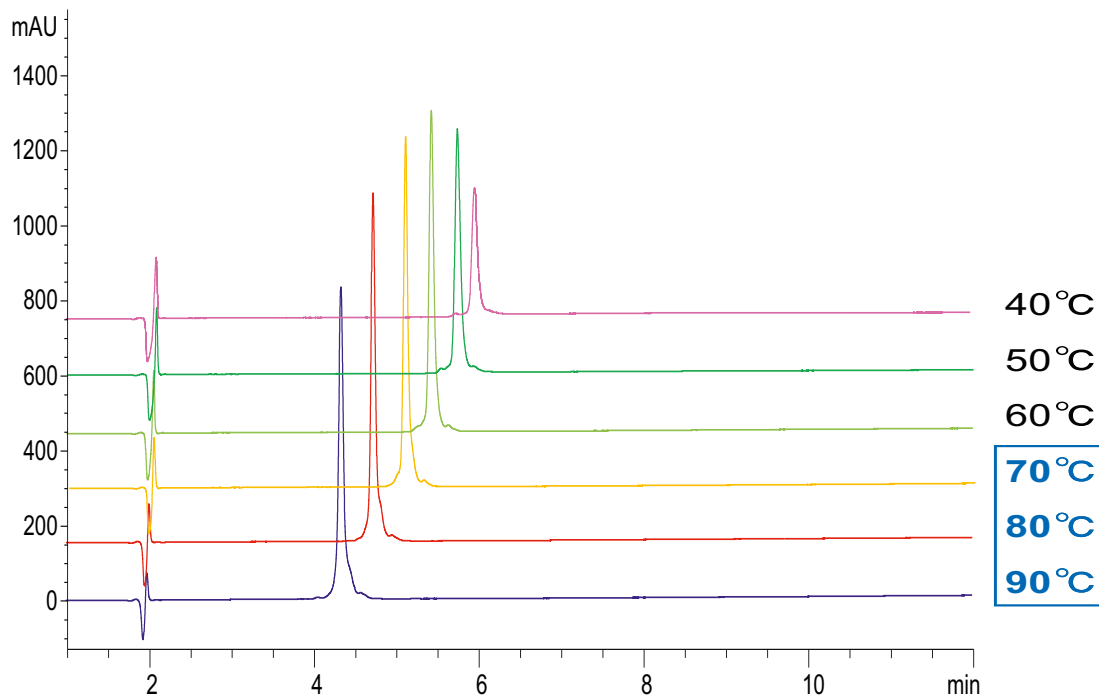
Column size: YMC-Triart Bio C4 (1.9  $\mu$ m, 30 nm) 150 x 2.1 mm ID  
 Part no: TB30SP9-15Q1PT  
 Eluent <TFA>: A) water/TFA (100/0.1)  
 B) acetonitrile/TFA (100/0.1)  
 Gradient <TFA>: 25-50%B (0-10 min), 90%B (10-12.5 min)  
 Eluent <Formic acid>: A) water/formic acid (100/0.1)  
 B) acetonitrile/formic acid (100/0.1)  
 Gradient <Formic acid>: 20-45%B (0-10 min), 90%B (10-12.5 min)  
 Detection: UV at 280 nm  
 Flow rate: 0.4 mL/min  
 Temperature: 80 °C  
 Injection: 4  $\mu$ L (0.25 mg/mL)  
 Sample: mAb Subunit Standard (Waters Corp.)



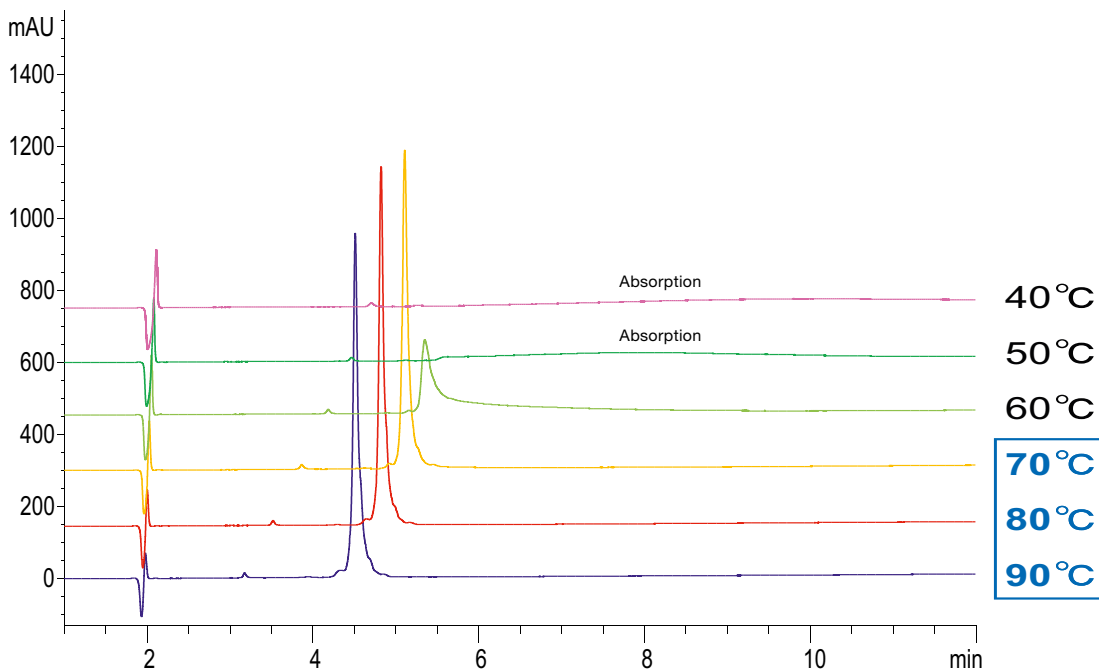
# BioLC applications – Antibodies

Adalimumab (Humira®, MW: ca. 148 kDa)

RP



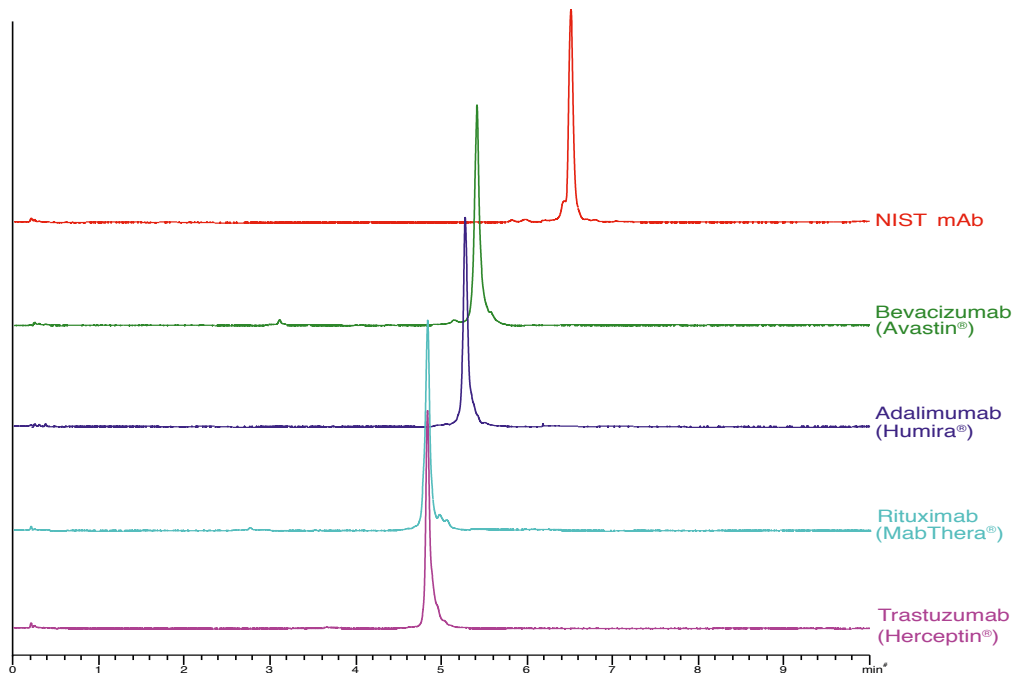
Bevacizumab (Avastin®, MW: ca. 148 kDa)



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

## Analysis of different monoclonal antibodies

RP

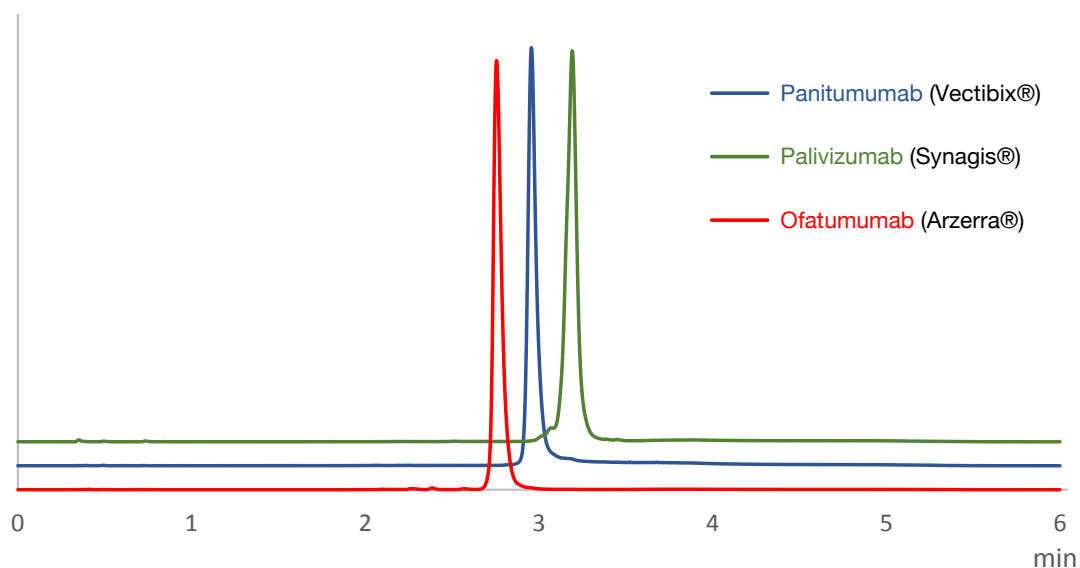


Column: YMC-Triart Bio C4 (1.9  $\mu$ m, 30 nm) 50 x 2.1 mm ID  
 Part No.: TB30SP9-05Q1PT  
 Eluent: A) water/TFA (100/0.1)  
 Gradient: 25-45%B (0-10 min)

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

## Analysis of challenging monoclonal antibodies

RP

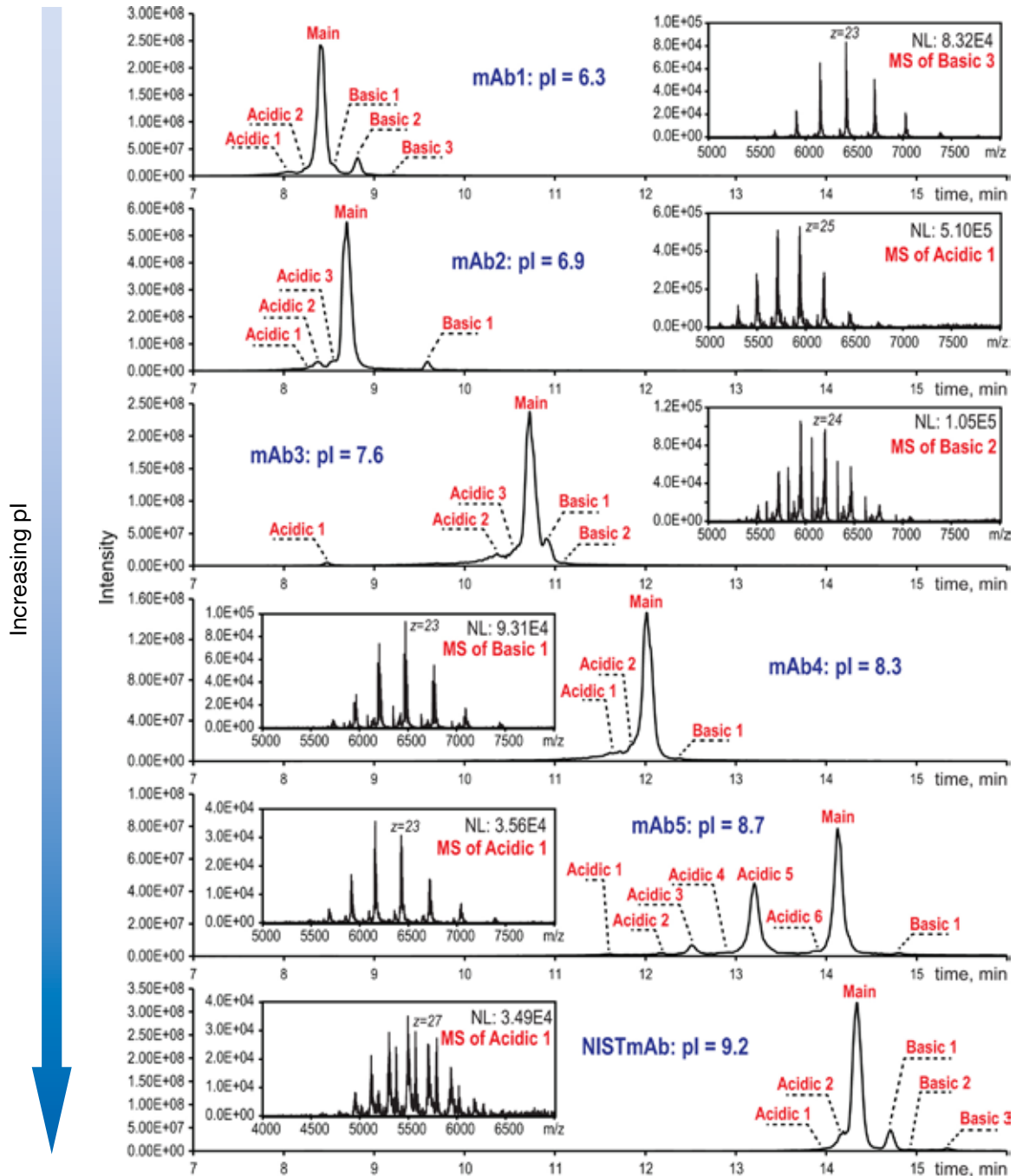


Column: YMC-Triart Bio C4 (1.9  $\mu$ m, 30 nm) 50 x 2.1 mm ID  
 Productcode: TB30SP9-05Q1PT  
 Eluents: A: 0.1 % TFA in water  
 Gradient: 25-50 % B (0-4 min)  
 Flow rate: 0.4 mL/min  
 Temperature: 90 °C  
 Injection volume: 0.5  $\mu$ L  
 Detection: Fluorescence: ex 280nm, em 350nm

By courtesy of University of Geneva, School of Pharmaceutical Sciences,  
 Department of Analytical Pharmaceutical Chemistry

# BioLC applications – Antibodies

Separation of different charge heterogenic mAbs via SCX-MS

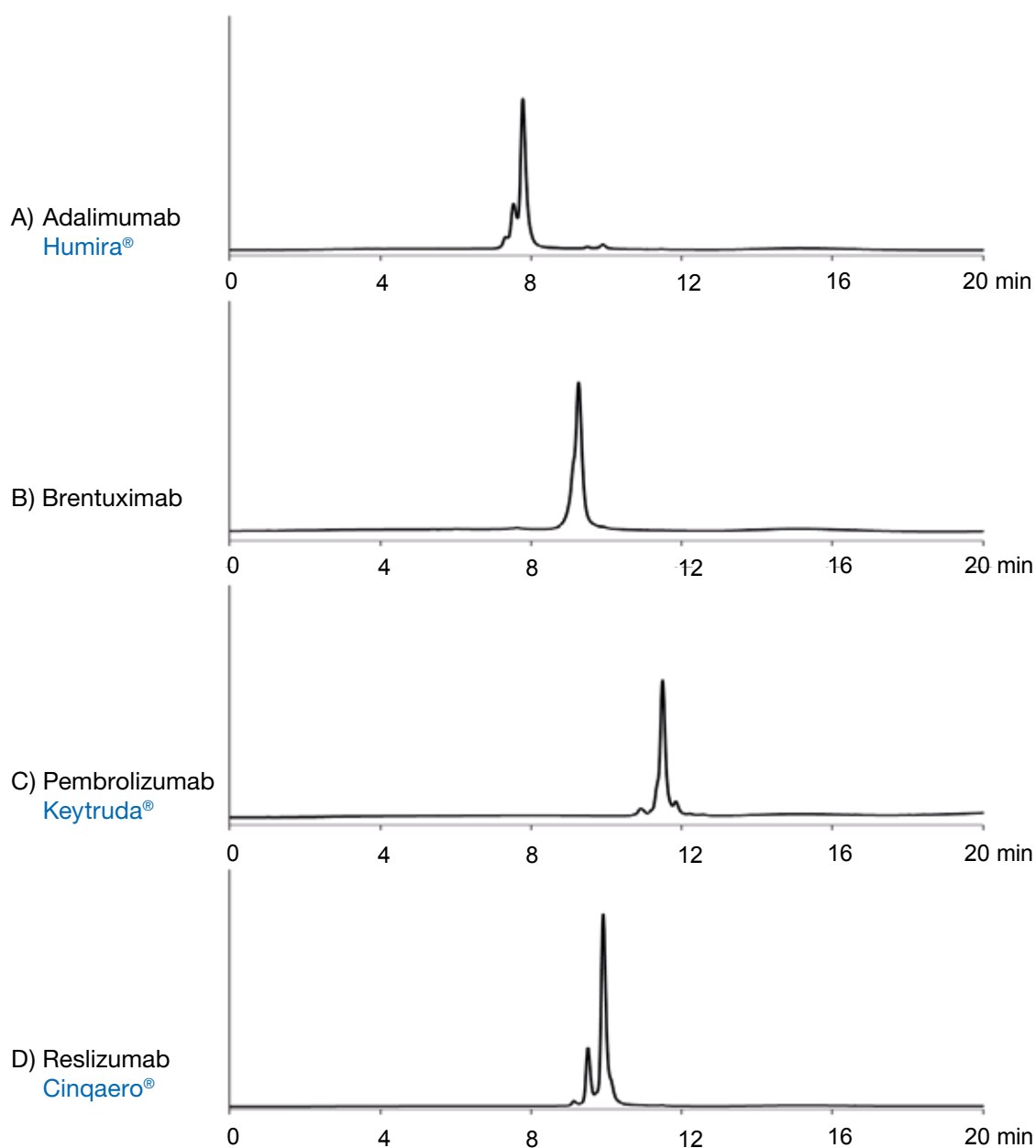


Column: BioPro IEX SF (5  $\mu$ m) 100 x 4.6 mm ID  
 Part. No.: SF00S05-1046WP  
 Eluent: A) 20 mM  $\text{CH}_3\text{COONH}_4$ , pH 5.6 adjusted with 20 mM  $\text{CH}_3\text{COOH}$   
 B) 140 mM  $\text{CH}_3\text{COONH}_4$ , 10 mM  $(\text{NH}_4)_2\text{CO}_3$ , pH 7.6  
 Gradient: 0% B (0–2 min), 0–100% B (2–16 min), 100% B (16–20 min), 0% B (20–27 min)  
 Flow rate: 0.4 mL/min  
 Temperature: 45  $^\circ\text{C}$   
 Equipment: Post column analytical splitter (~ 400:1) to reduce analytical flow to ca. 1  $\mu\text{L}/\text{min}$   
 Detector: PDA (UV)  
 PicoTip Emitter (NSI-MS)

Application data taken from 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, 13013-13020.

## HIC Analysis of different monoclonal antibodies using isopropanol as modifier

HIC

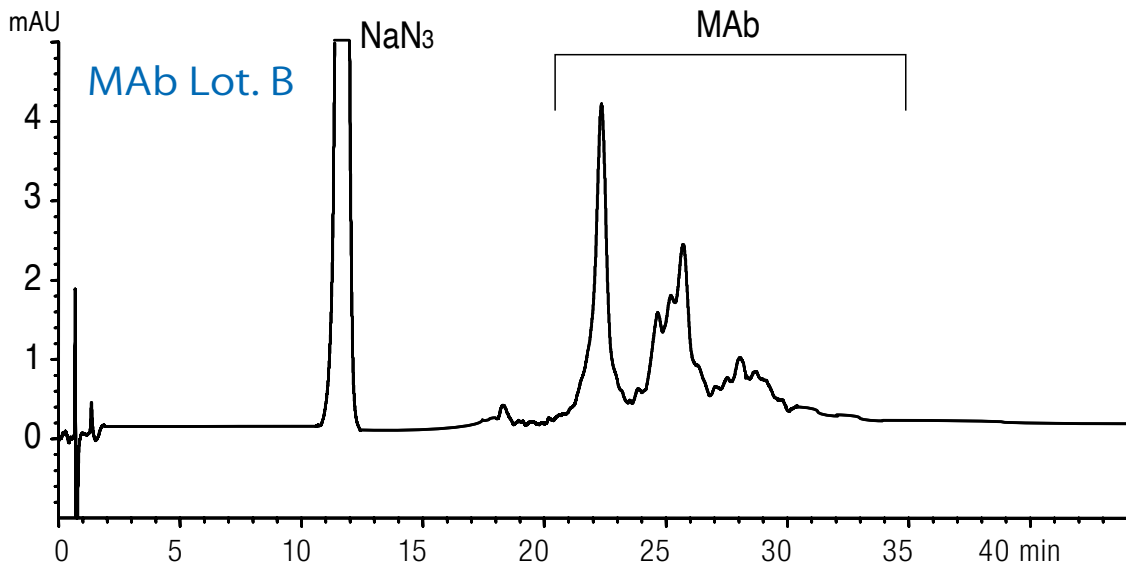
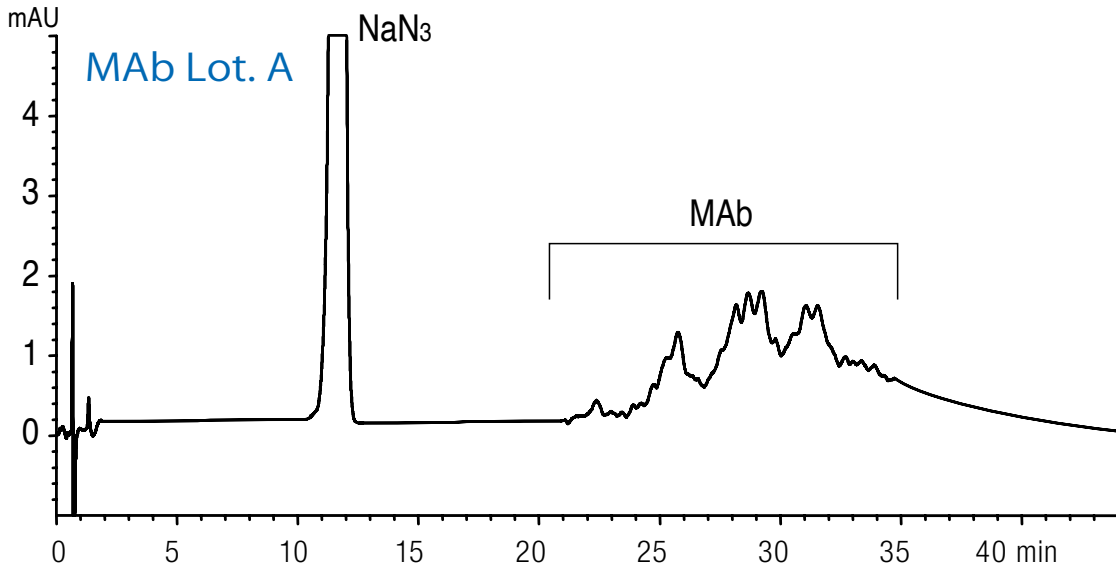


Column: BioPro HIC BF (4  $\mu$ m) 100 x 4.6 mm ID  
 Part No.: BHB00S04-1046WT  
 Eluent: A) 20 mM  $\text{NaH}_2\text{PO}_4$ - $\text{Na}_2\text{HPO}_4$  (pH 7.4) containing 1.5 M  $(\text{NH}_4)_2\text{SO}_4$   
 B) 20 mM  $\text{NaH}_2\text{PO}_4$ - $\text{Na}_2\text{HPO}_4$  (pH 7.4) / 2-propanol (85/15)  
 Gradient: 0–100 % B (0–20 min)  
 Flow rate: 1.0 mL/min  
 Temperature: 20 °C  
 Detection: fluorescence: ex 280 nm, em 360 nm  
 Injection: 3  $\mu$ L (2 mg/mL)

by courtesy of University of Geneva, School of Pharmaceutical Sciences,  
 Department of Analytical Pharmaceutical Chemistry

# BioLC applications – Antibodies

Different production batches of IgG1

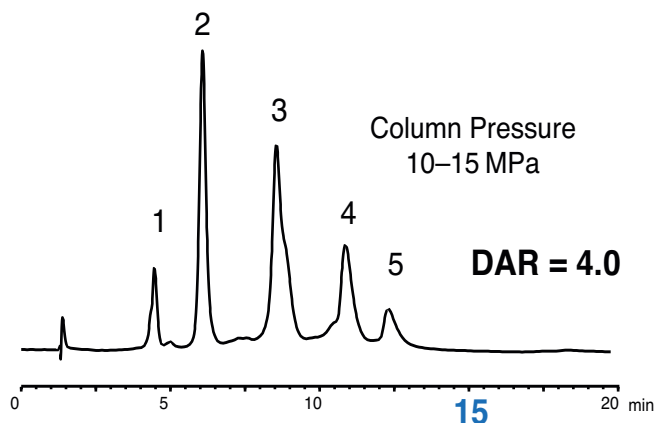
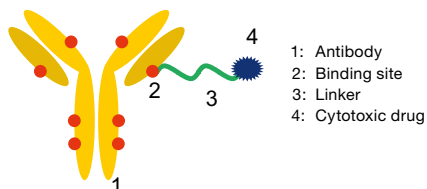


Column: BioPro IEX QF (5  $\mu$ m) 100 x 4.6 mm ID  
 Part No.: QF00S05-1046WP  
 Eluent: A) 20 mM Tris-HCl (pH 8.1)  
 B) 20 mM Tris-HCl (pH 8.1) containing 0.5 M NaCl  
 Gradient: 10-25%B (0-60 min)  
 Flow rate: 1.0 mL/min (360 cm/hr)  
 Temperature: 25 °C  
 Detection: UV at 220 nm  
 Injection: 14  $\mu$ L (0.1 mg/mL)  
 Sample: Mouse monoclonal IgG1 anti-human IgG4  
 (Purified by DEAE chromatography, containing NaN<sub>3</sub>)

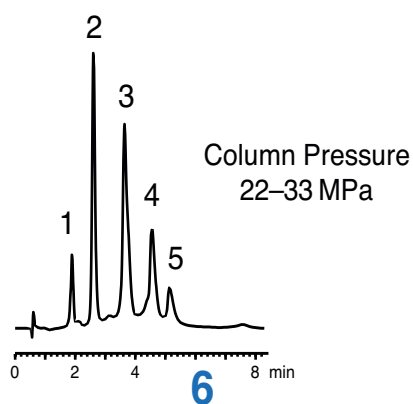
# BioLC applications – Antibody-Drug-Conjugates

## High throughput DAR determination by shortening analysis time

HIC



2.5 x faster

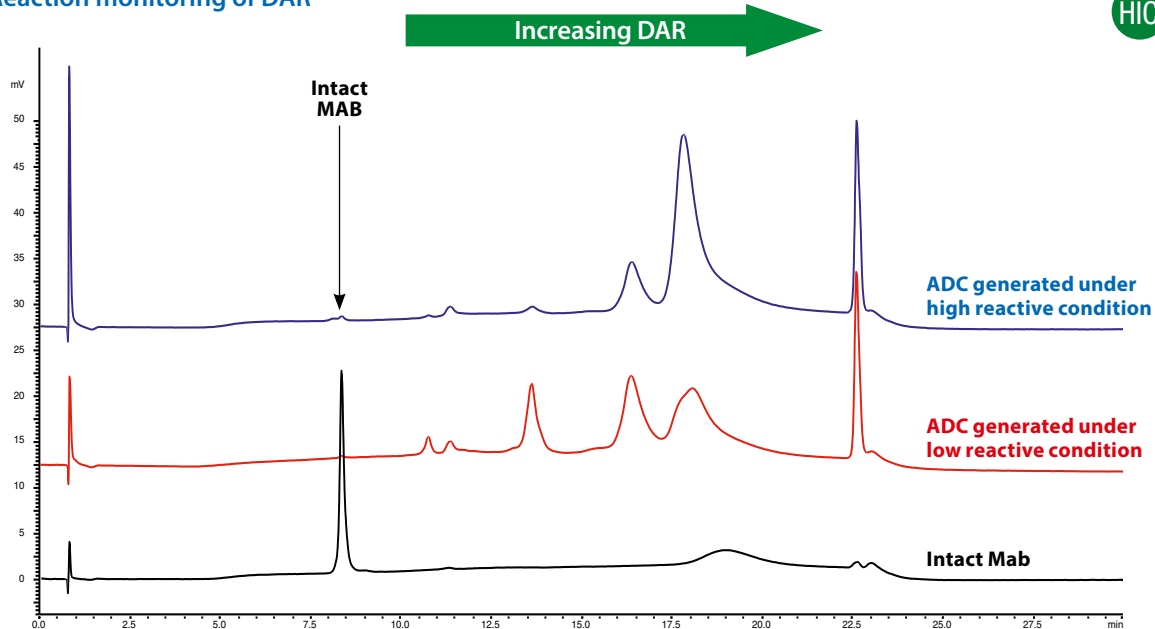


1. DAR 0
2. DAR 2
3. DAR 4
4. DAR 6
5. DAR 8

Column: BioPro HIC HT (2.3  $\mu$ m) 100 x 4.6 mm ID  
 Part No: BHH00SQ3-1046PTH  
 Eluent: A) 20 mM  $\text{NaH}_2\text{PO}_4$ - $\text{Na}_2\text{HPO}_4$  (pH 7.0) containing 1.0 M  $(\text{NH}_4)_2\text{SO}_4$   
 B) 20 mM  $\text{NaH}_2\text{PO}_4$ - $\text{Na}_2\text{HPO}_4$  (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  $^\circ$ C  
 Detection: UV at 280 nm  
 Injection: 10  $\mu$ L  
 Sample: Brentuximab vedotin (Adcetris<sup>®</sup>) (2.5 mg/mL)

# BioLC applications – Antibody-Drug-Conjugates

## Reaction monitoring of DAR

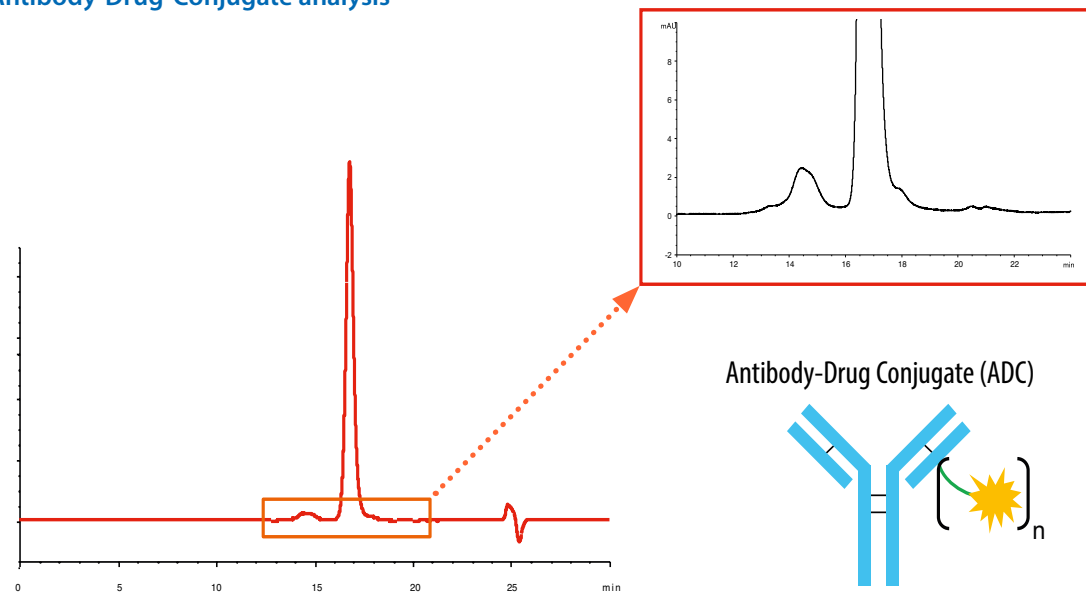


HIC

Column: BioPro HIC BF (4  $\mu$ m) 100 x 4.6 mm ID  
 Part No.: BHB00S04-1046WT  
 Eluent: A) 50 mM  $\text{NaH}_2\text{PO}_4$ - $\text{Na}_2\text{HPO}_4$  (pH 7.0) containing 1.5 M  $(\text{NH}_4)_2\text{SO}_4$ /2-propanol (95/5)  
 B) 50 mM  $\text{NaH}_2\text{PO}_4$ - $\text{Na}_2\text{HPO}_4$  (pH 7.0)/2-propanol (80/20)  
 Gradient: 0%B (0-1 min), 0-100%B (1-15 min), 100%B (15-20 min), 0%B (20-30 min)  
 Flow rate: 1.0 mL/min  
 Temperature: 25  $^\circ\text{C}$   
 Detection: UV at 280 nm  
 Sample: Antibody Drug Conjugate\*

\*Courtesy of RIKEN

## Antibody-Drug-Conjugate analysis



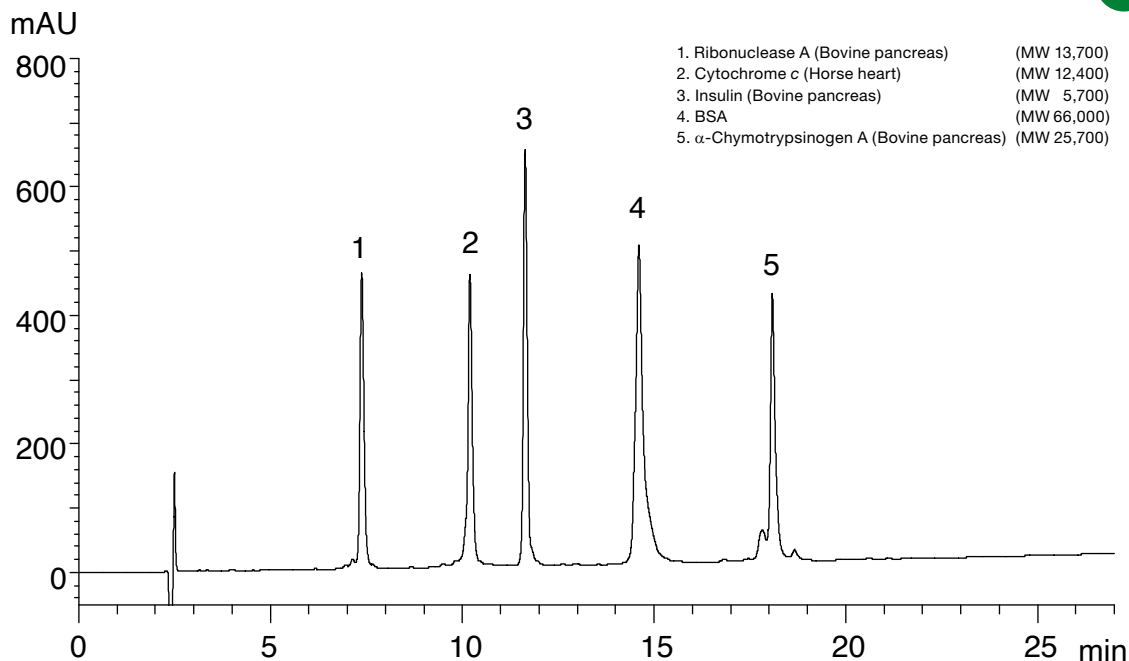
SEC

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



## Proteins (MW 5,700 ~ 66,000)

RP

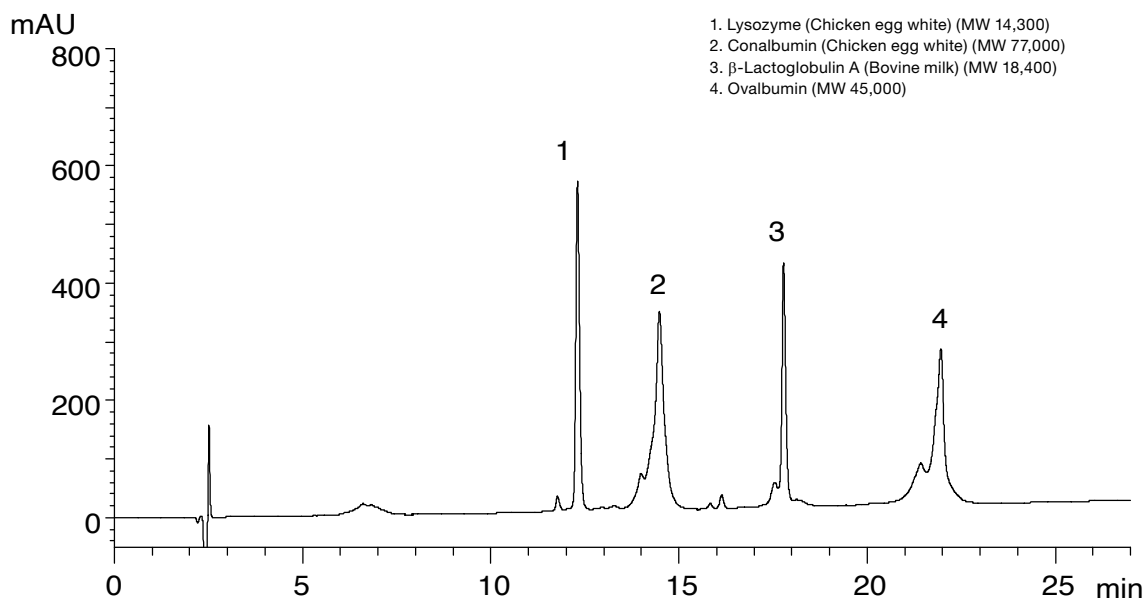


Column: YMC-Triart Bio C4 (5  $\mu$ m, 30 nm) 150 x 3.0 mm ID  
 Part No.: TB30S03-1503PTH  
 Eluent: A) water/TFA (100/0.1)  
 B) acetonitrile/TFA (100/0.1)  
 Gradient: 20-60%B (0-27 min), 90%B (27-35 min)

Flow rate: 0.4 mL/min  
 Temperature: 70 °C  
 Detection: UV at 220 nm  
 Injection: 10  $\mu$ L (0.25 ~ 0.50 mg/mL)

## Proteins (MW 14,300 ~ 77,000)

RP



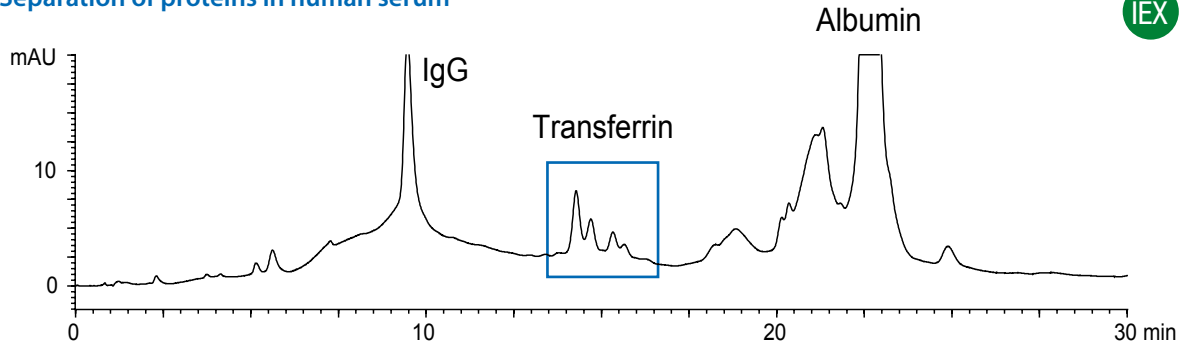
Column: YMC-Triart Bio C4 (5  $\mu$ m, 30 nm) 150 x 3.0 mm ID  
 Part No.: TB30S03-1503PTH  
 Eluent: A) water/TFA (100/0.1)  
 B) acetonitrile/TFA (100/0.1)  
 Gradient: 20-60%B (0-27 min), 90%B (27-35 min)

Flow rate: 0.4 mL/min  
 Temperature: 70 °C  
 Detection: UV at 220 nm  
 Injection: 10  $\mu$ L (0.25 ~ 0.50 mg/mL)

# BioLC applications – Proteins

## Separation of proteins in human serum

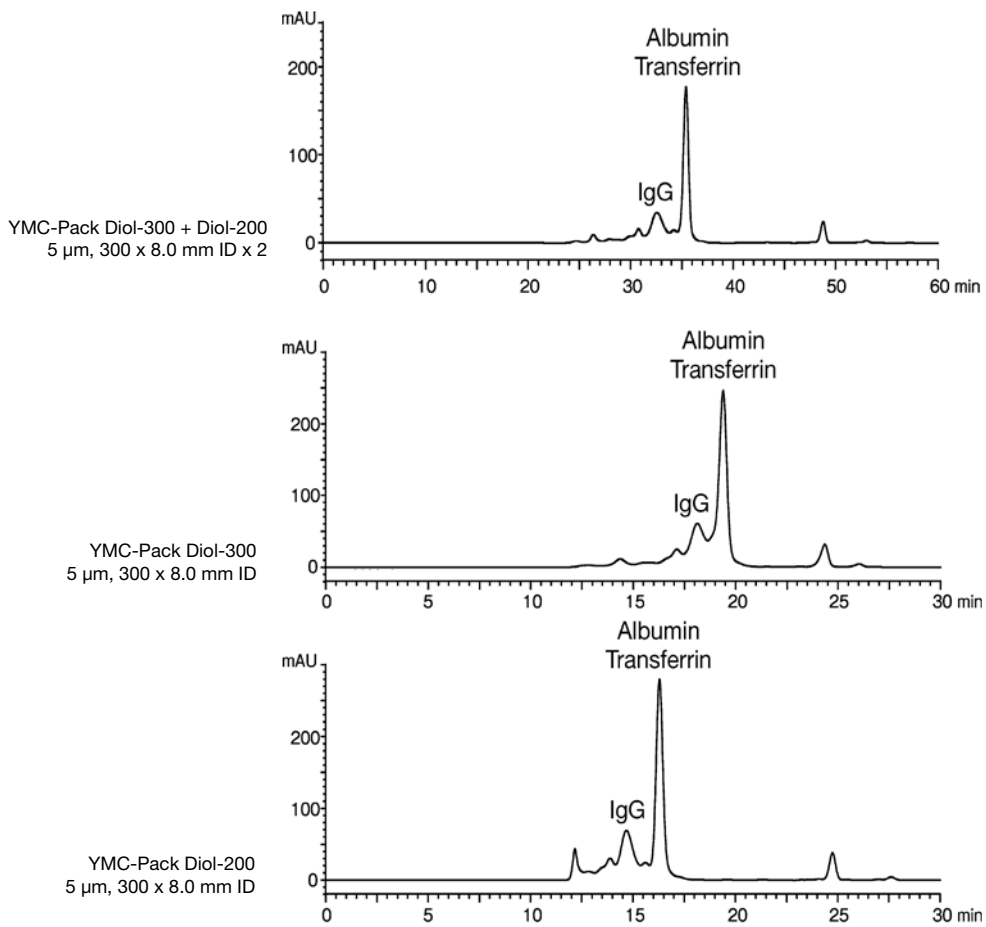
IEX



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

## Proteins in human serum

SEC

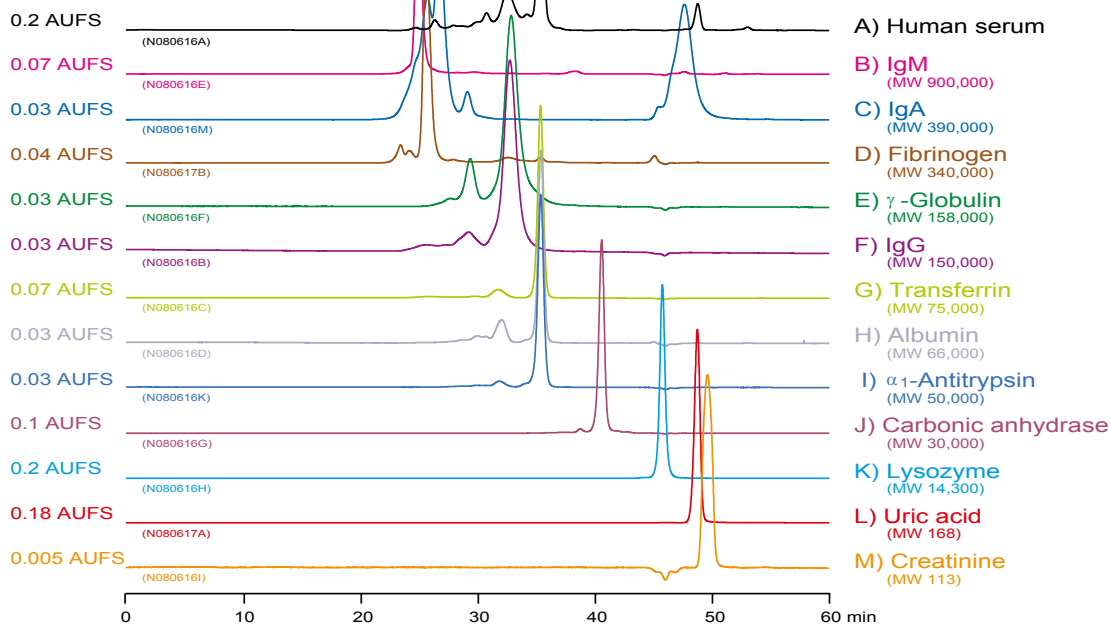


Eluent:	0.1 M KH <sub>2</sub> PO <sub>4</sub> -K <sub>2</sub> HPO <sub>4</sub> (pH 7.0) containing 0.2 M NaCl
Part Nos.:	DL30S05-3008WT + DL20S05-3008WT
Flow rate:	0.5 mL/min
Temperature:	ambient (25 °C)
Detection:	UV at 280 nm
Injection:	20 µL
Sample:	Human serum (100 µL/mL)

## Plasma constituents

SEC

*blood analysis*

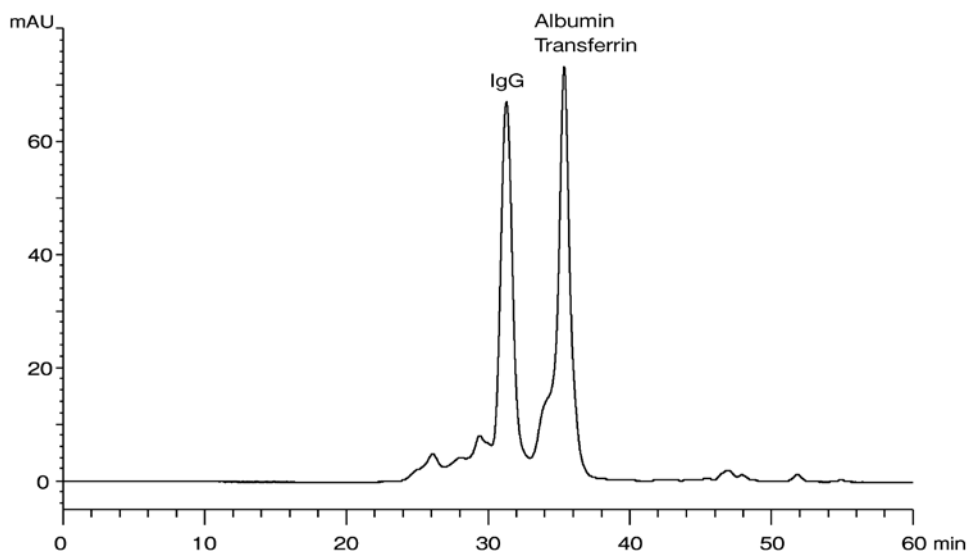


Columns: YMC-Pack Diol-300 + Diol-200 (5  $\mu$ m) 300 x 8.0 mm ID x 2  
 Part Nos.: DL30S05-3008WT + DL20S05-3008WT  
 Eluent: 0.1 M  $\text{KH}_2\text{PO}_4$ - $\text{K}_2\text{HPO}_4$  (pH 7.0) containing 0.2 M NaCl  
 Flow rate: 0.5 mL/min

Temperature: ambient (25  $^\circ\text{C}$ )  
 Detection: UV at 280 nm  
 Injection: 20  $\mu\text{L}$  (L: 1  $\mu\text{L}$ )  
 Sample: A) 100  $\mu\text{L}/\text{mL}$ ; B-M) 1.0 mg/mL

## Proteins in mouse ascites fluid

SEC



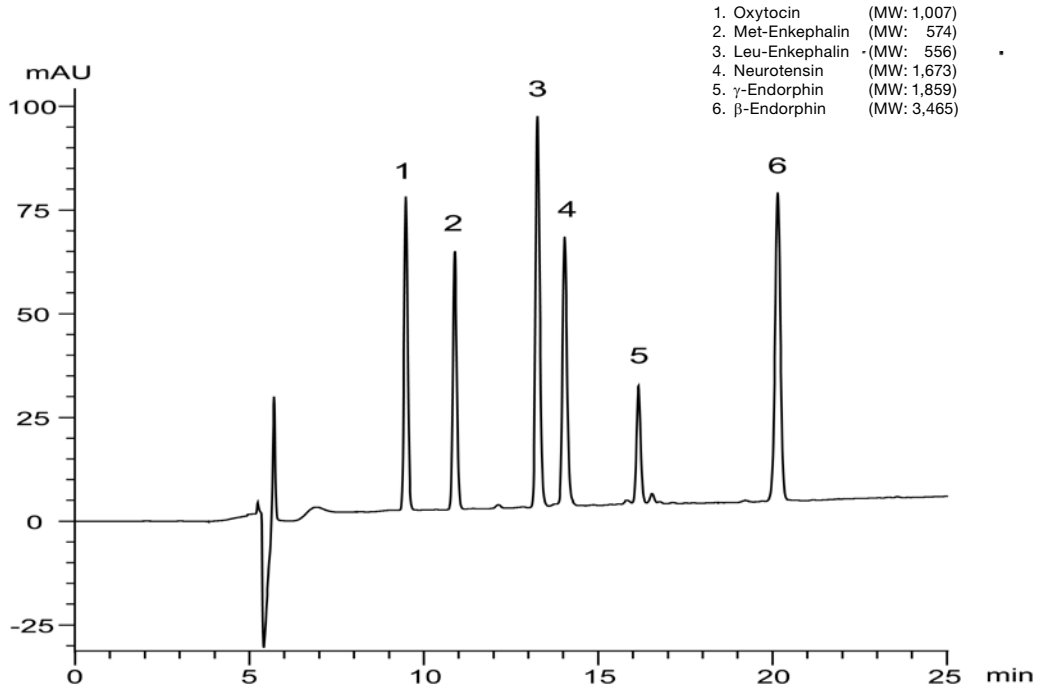
Columns: YMC-Pack Diol-300 + Diol-200 (5  $\mu$ m) 300 x 4.6 mm ID  
 Part Nos.: DL30S05-3046WT + DL20S05-3046WT  
 Eluent: 0.1 M  $\text{KH}_2\text{PO}_4$ - $\text{K}_2\text{HPO}_4$  (pH 7.0)  
 Flow rate: 0.17 mL/min

Temperature: ambient (25  $^\circ\text{C}$ )  
 Detection: UV at 220 nm  
 Injection: 10  $\mu\text{L}$  (60 times dilution with water)

# BioLC applications – Peptides

## Peptides

RP

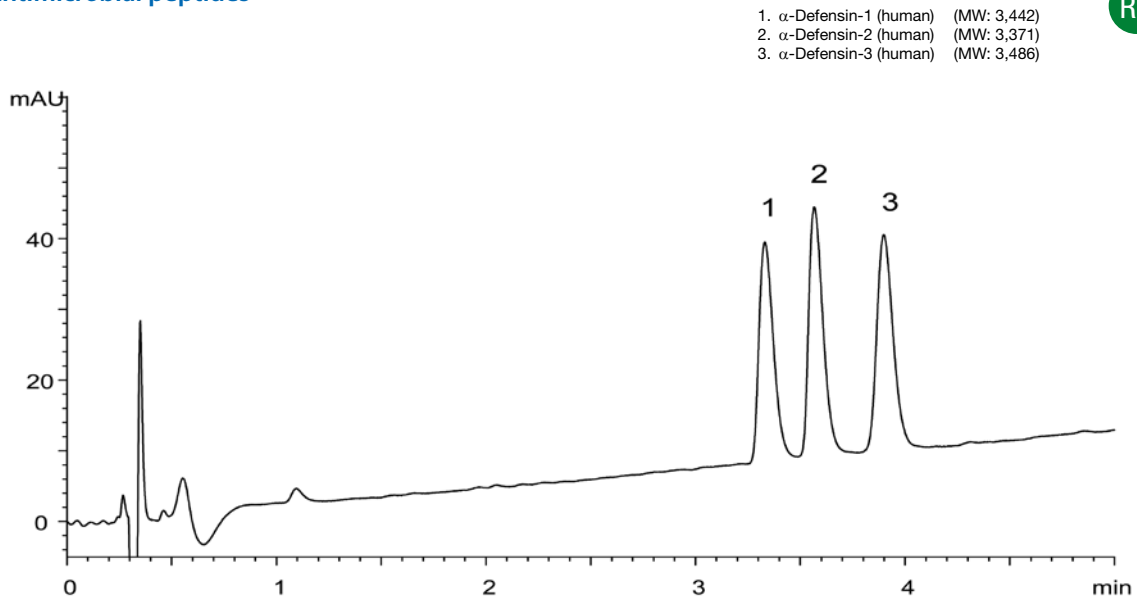


Column: YMC-Triart C18 (5  $\mu$ m, 12 nm) 150 x 2.0 mm ID  
 Part No.: TA12S05-1502WT  
 Eluent: A) water / TFA (100/0.1)  
 B) acetonitrile / TFA (100/0.1)  
 Gradient: 20%-45% B (0-25 min)

Flow rate: 0.2 mL/min  
 Temperature: 37  $^{\circ}$ C  
 Detection: UV at 220 nm  
 Injection: 2  $\mu$ L (0.075  $\approx$  0.25 mg/mL)

## Antimicrobial peptides

RP

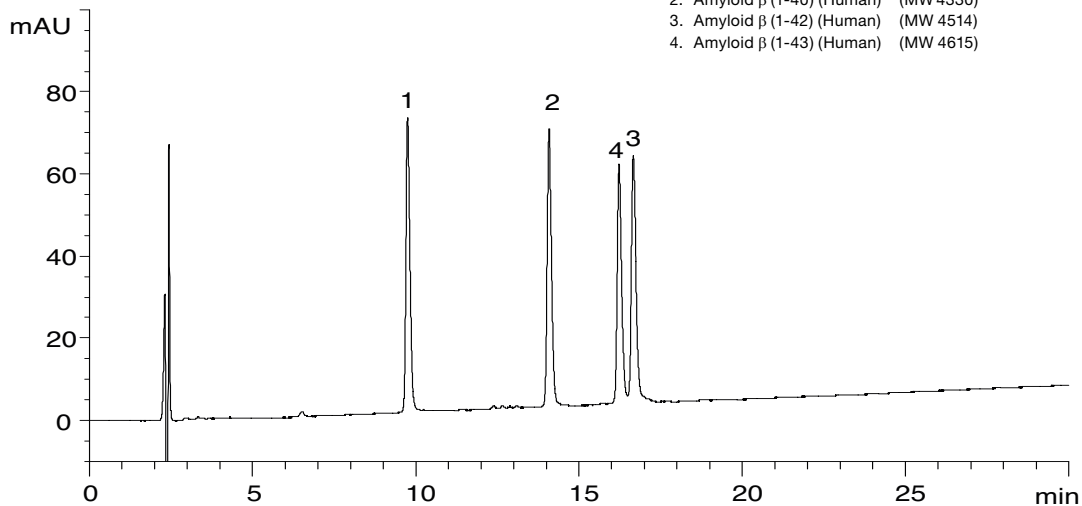


Column: YMC-Triart C18 (1.9  $\mu$ m, 12 nm) 50 x 2.0 mm ID  
 Part No.: TA12SP9-0502PT  
 Eluent: A) water / formic acid (100/0.1)  
 B) 2-propanol / acetonitrile / formic acid (50/50/0.08)  
 Gradient: 10%-25% B (0-10 min)

Flow rate: 0.4 mL/min  
 Temperature: 70  $^{\circ}$ C  
 Detection: UV at 220 nm  
 Injection: 1  $\mu$ L (50  $\mu$ g/mL)

## Amyloid $\beta$ -peptides

RP



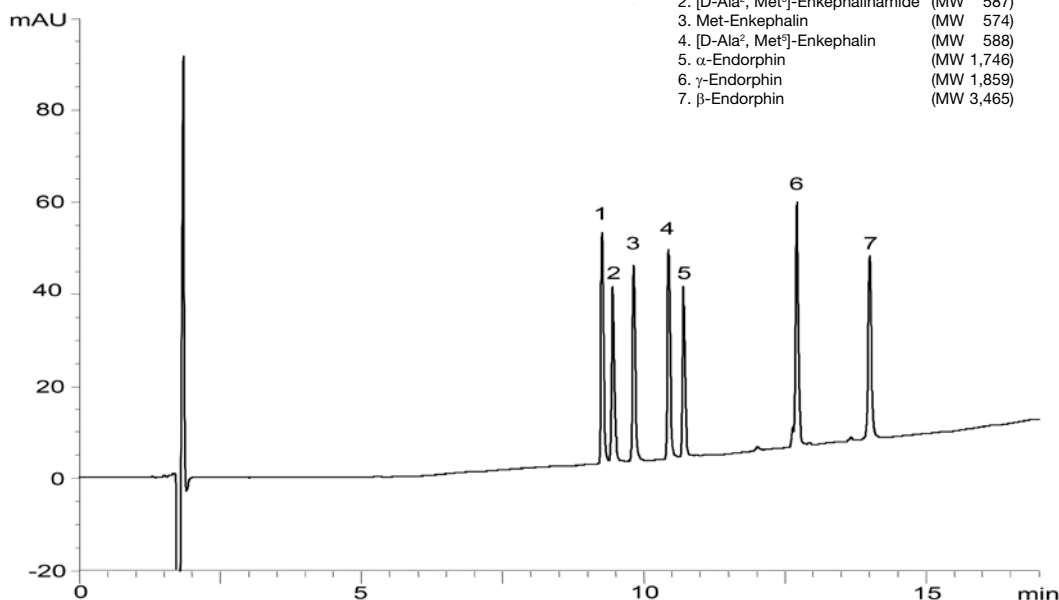
Amyloid  $\beta$  (1-43) : Asp-Ala-Glu-Phe-Arg-His-Asp-Ser-Gly-Tyr-Glu-Val-His-His-Gln-Lys-Leu-Val-Phe-Phe-Ala-Glu-Asp-Val-Gly-Ser-Asn-Lys-Gly-Ala-Ile-Ile-Gly-Leu-Met-Val-Gly-Gly-Val-Val-Ile-Ala-Thr

Column: YMC-Triart Bio C4 (3  $\mu$ 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  $^{\circ}$ C  
 Detection: UV at 220 nm  
 Injection: 4  $\mu$ L (each 0.1 mg/mL)

## Peptides

RP



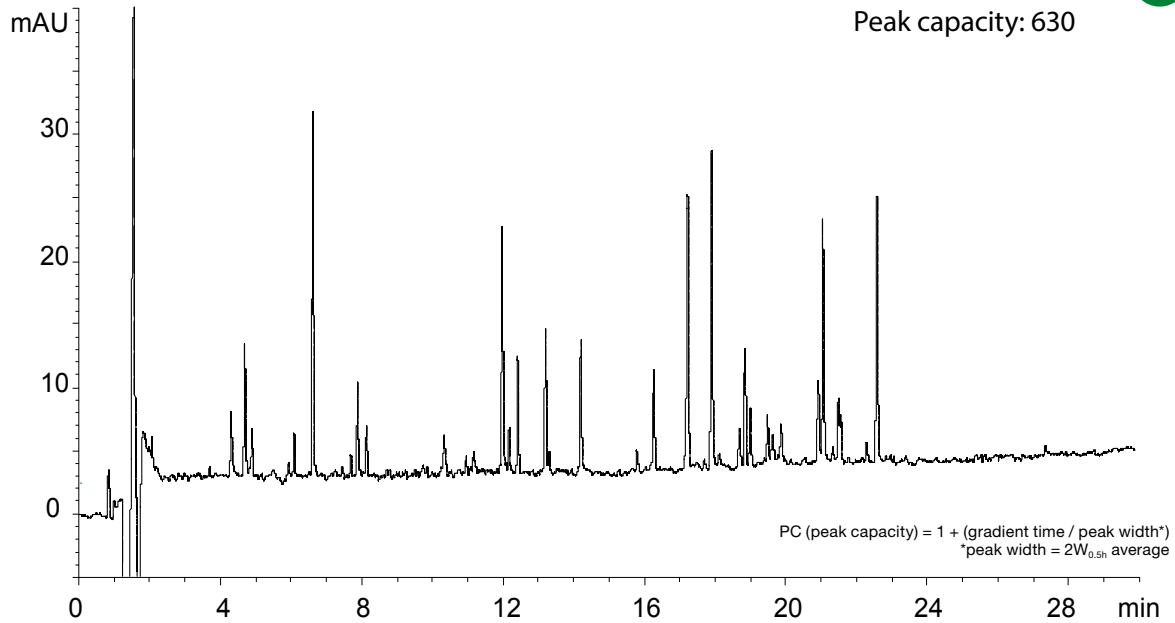
Column: Meteoric Core C18 BIO (2.7  $\mu$ m, 16 nm) 150 x 2.1 mm ID  
 Part No.: CAW16SQ7-15Q1PT  
 Eluent: A) water/TFA (100/0.1)  
 B) acetonitrile/TFA (100/0.1)  
 Gradient: 15-55% B (0-15 min), 55% B (15-17 min)

Flow rate: 0.2 mL/min  
 Temperature: 40  $^{\circ}$ C  
 Detection: UV at 220 nm  
 Injection: 2  $\mu$ L (0.02-0.5 mg/mL)  
 Pressure: 14.9-16.1 MPa (2160-2330 psi)

# BioLC applications – Peptide Mapping

## Tryptic digest of Hemoglobin

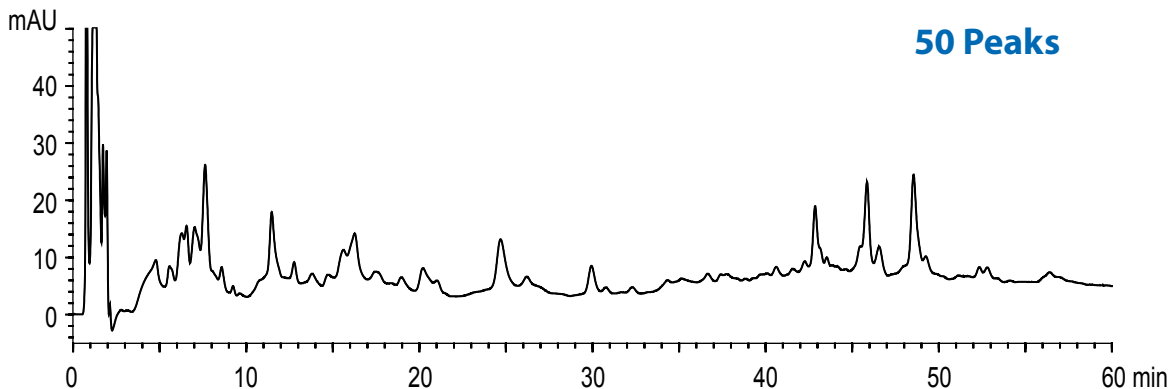
RP



Column: YMC-Triart C18 (1.9  $\mu\text{m}$ , 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  $^{\circ}\text{C}$   
 Detection: UV at 220 nm  
 Injection: 20  $\mu\text{L}$   
 Sample: Tryptic digest of Bovine Hemoglobin (2.5 nmol/mL)  
 Pressure: 58.1-61.6 MPa (8,430-8,930 psi)

## Peptide mapping of tryptic digests of BSA with highest sensitivity

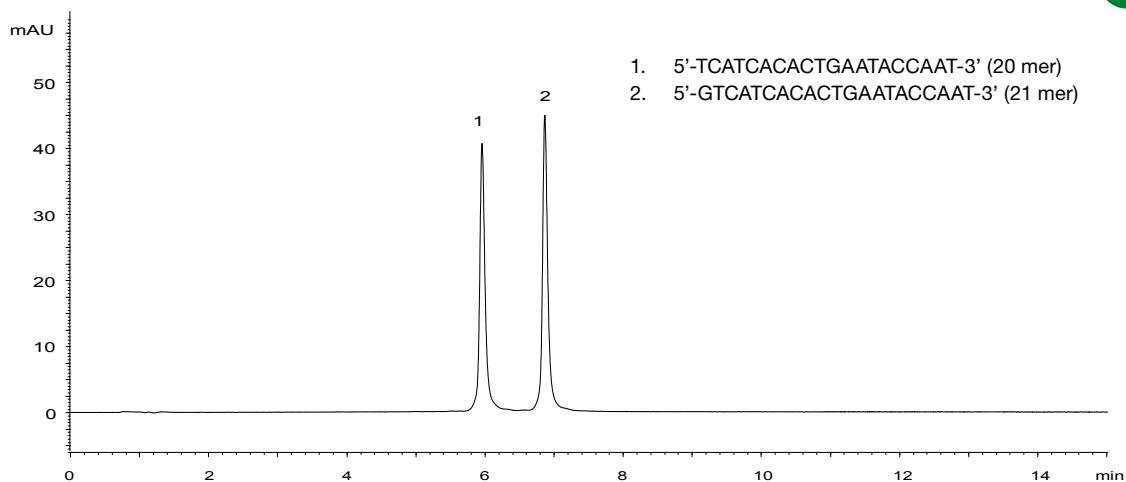
IEX



Column: BioPro IEX QA (5  $\mu\text{m}$ ) 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  $^{\circ}\text{C}$   
 Detection: UV at 220 nm  
 Injection: 20  $\mu\text{L}$   
 Sample: Tryptic digest of BSA

## Separation of synthetic oligonucleotides (single-strand DNA)

IEX

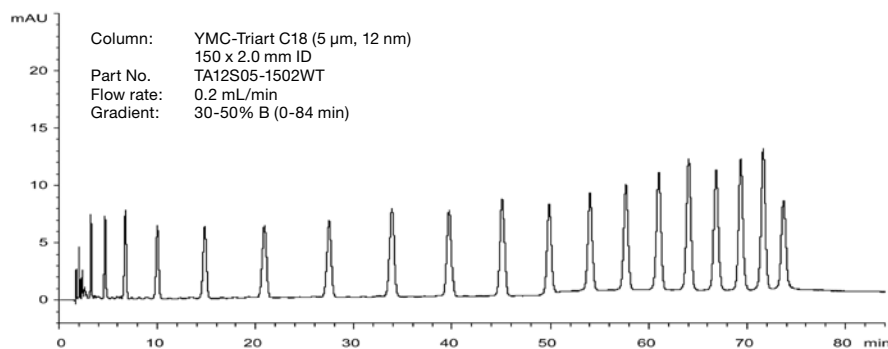


Column: BioPro IEX QF (5  $\mu$ m) 100 x 4.6 mm ID  
 Part no.: QF00S05-1046WP  
 Eluent: A) 10 mM NaOH  
 B) 10 mM NaOH containing 1.0 M NaClO<sub>4</sub>  
 Gradient: 25-55%B (0-15 min), 100%B (15-20 min)  
 Flow rate: 1.0 mL/min  
 Temperature: 25 °C  
 Detection: UV at 260 nm  
 Injection: 4  $\mu$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

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

RP

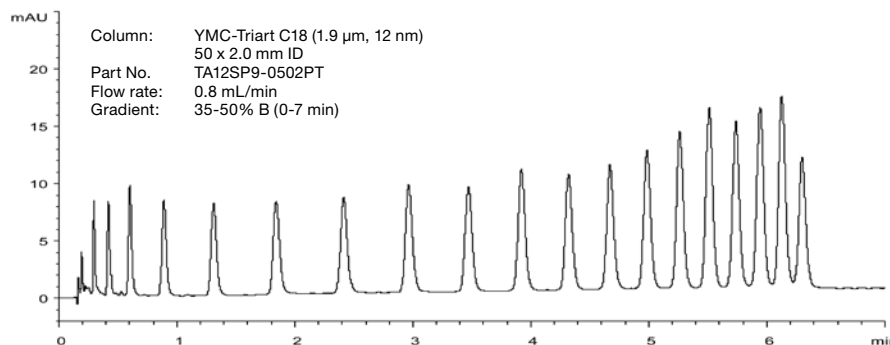
### Conventional LC method



80 min

11x faster

### UHPLC method



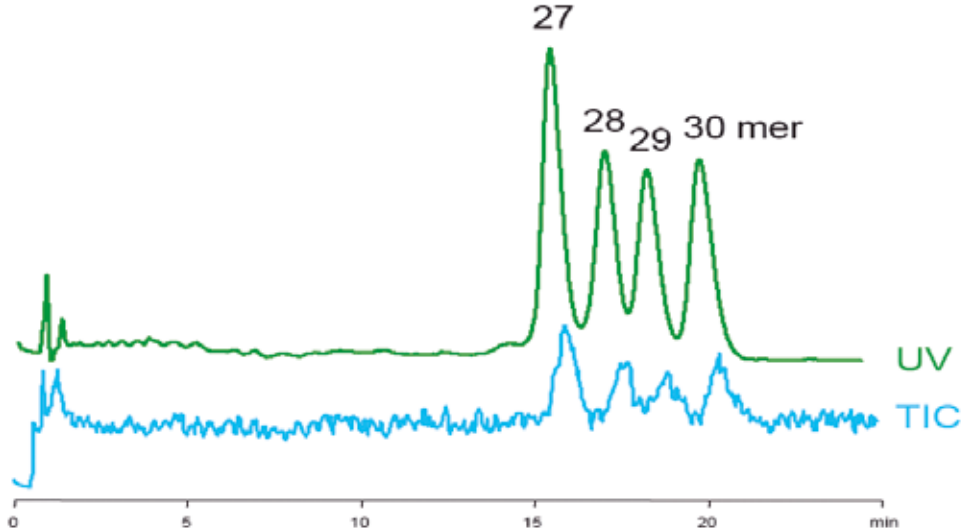
7 min

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

# BioLC applications – Oligonucleotides

## LC-MS analysis of synthetic 27-30 mer oligonucleotides

RP



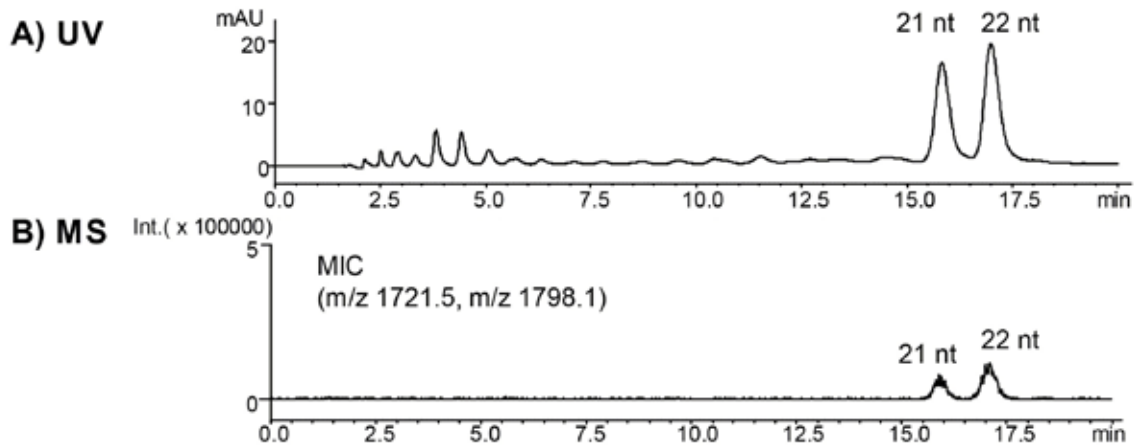
Sample: Primer of DNA sequencing

5'-CCGCTCGAGCTAAAAAAGCCTGTGTTACC-3' (30 mer)

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

## LC/MS analysis of miRNA

RP



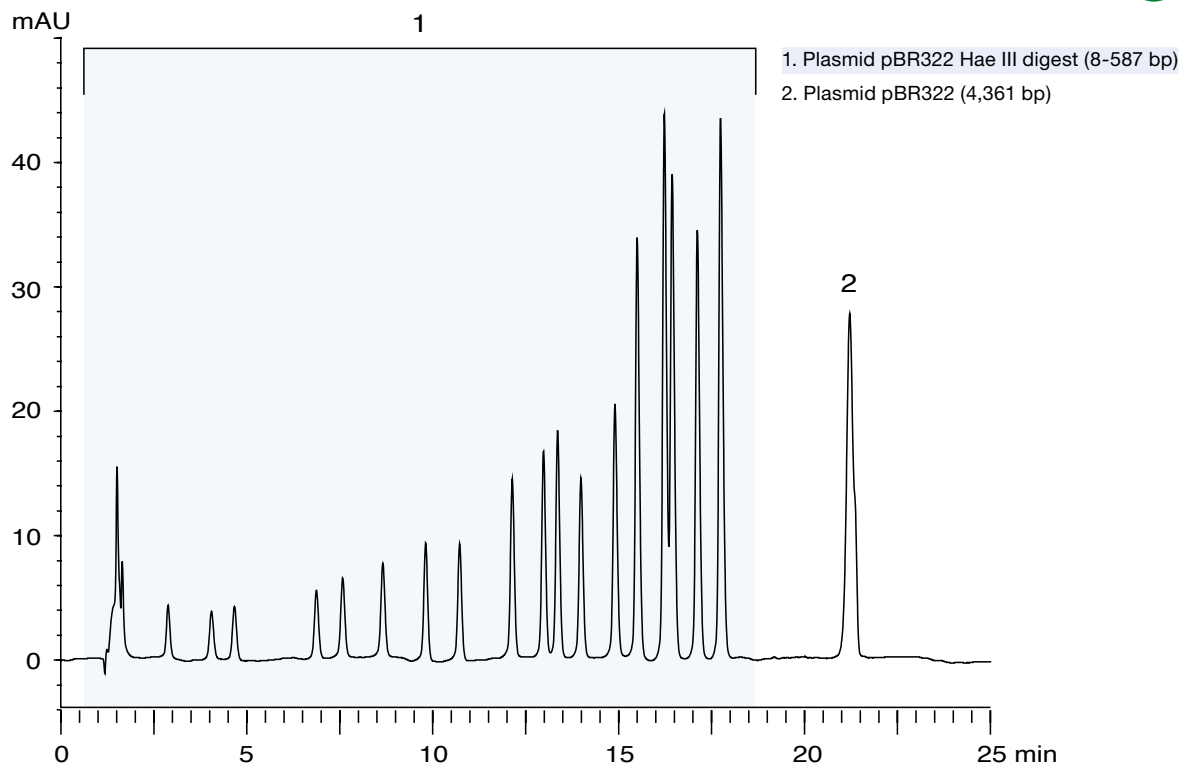
Courtesy of M. Yamada, SHIMADZU CORPORATION

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

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



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



Column: BioPro IEX QF (5  $\mu$ 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

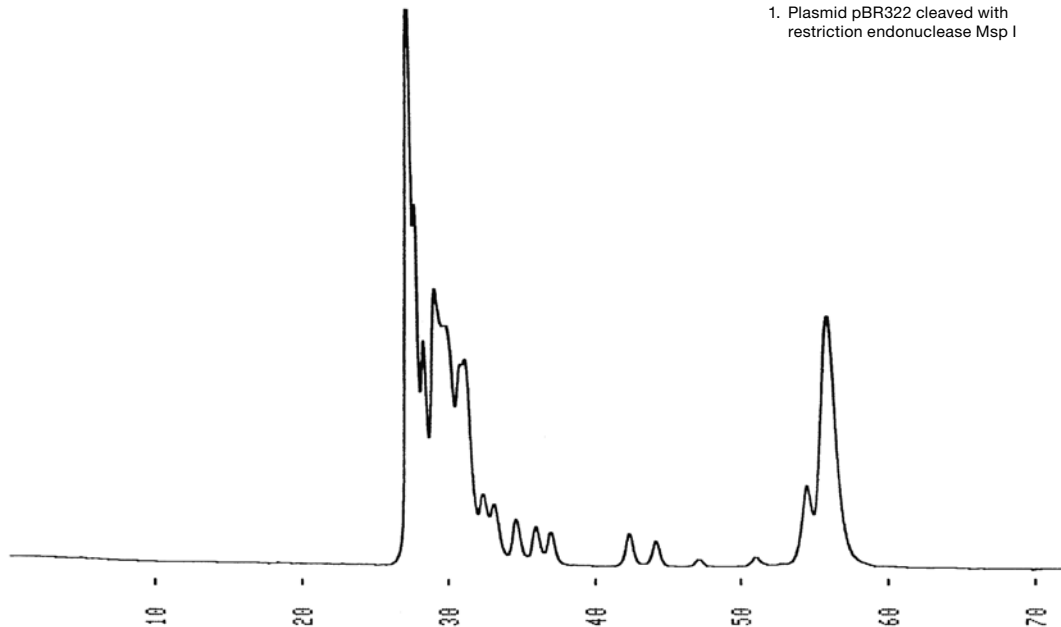
Temperature: 35 °C  
 Detection: UV at 260 nm  
 Injection: 10  $\mu$ L  
 Sample: Plasmid pBR322 Hae III digest (0.13 mg/mL)  
 Plasmid pBR322 (0.03 mg/mL)

# BioLC applications – Plasmids

## Plasmid pBR322 restriction fragment

SEC

1. Plasmid pBR322 cleaved with restriction endonuclease Msp I



Columns: YMC-Pack Diol-300 + Diol-200 (5 µm) 500 x 8.0 mm ID  
 Part Nos.: DL30S05-5008WT + DL20S05-5008WT  
 Eluent: 0.1 M KH<sub>2</sub>PO<sub>4</sub>-K<sub>2</sub>HPO<sub>4</sub> (pH 7.0) containing 0.2M NaCl  
 Flow rate: 0.7 mL/min

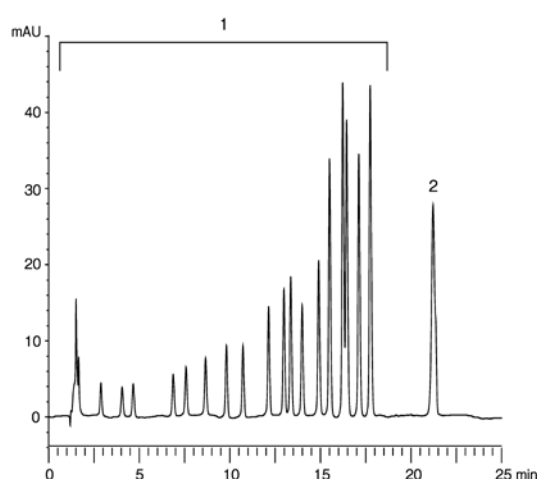
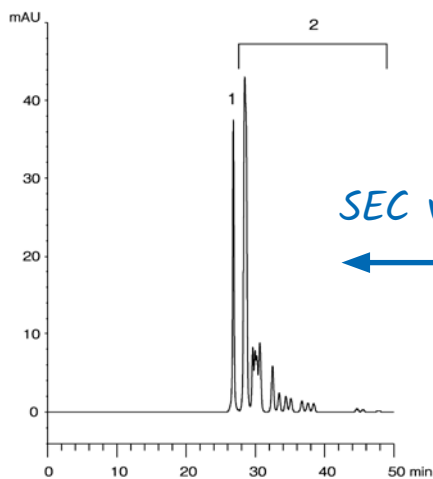
Temperature: ambient (26 °C)  
 Detection: UV at 260 nm, 0.01 AUFS  
 Injection: 3 µL (0.49 mg/mL)  
 Sample: Plasmid pBR322 cleaved with restriction endonuclease Msp I

## Plasmid pBR322 restriction and pBR322 Hae III restriction fragment

SEC IEX

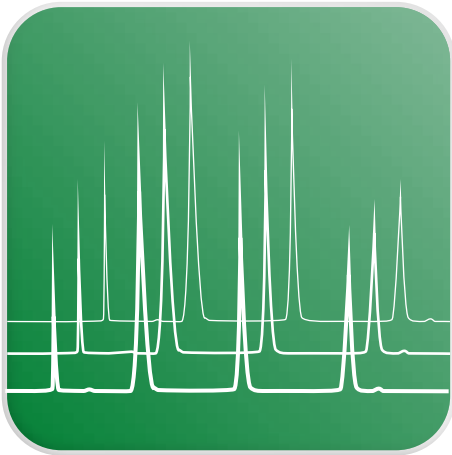
1. Plasmid pBR322 (4,361 bp)  
 2. Plasmid pBR322 Hae III digest (8-587 bp)

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

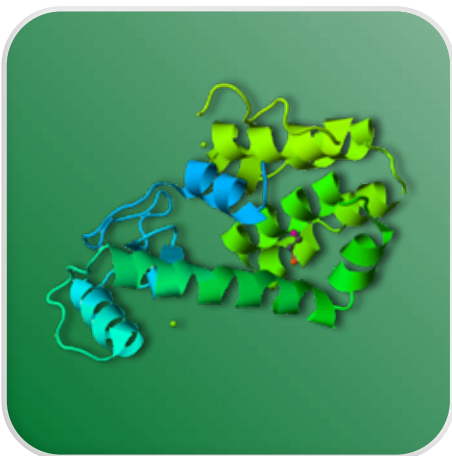


Columns: YMC-Pack Diol-300 + Diol-200 (5 µm) 500 x 8.0 mm ID  
 Part Nos.: DL30S05-5008WT + DL20S05-5008WT  
 Eluent: 0.1 M KH<sub>2</sub>PO<sub>4</sub>-K<sub>2</sub>HPO<sub>4</sub> (pH 7.0) containing 0.2 M NaCl  
 Flow rate: 0.7 mL/min  
 Temperature: ambient (25 °C)  
 Detection: UV at 260 nm  
 Injection: 10 µL

Column: BioPro IEX QF (5 µm) 100 x 4.6 mm ID  
 Part No.: QF00S05-1046WP  
 Eluent: A) 20 mM Tris-HCl (pH 8.1)  
 B) 20 mM Tris-HCl (pH 8.1) containing 0.1 M NaCl  
 Gradient: 70-85% B (0-20 min), 85% B (20-25 min)  
 Flow rate: 0.5 mL/min  
 Temperature: 35 °C  
 Detection: UV at 260 nm  
 Injection: 10 µL



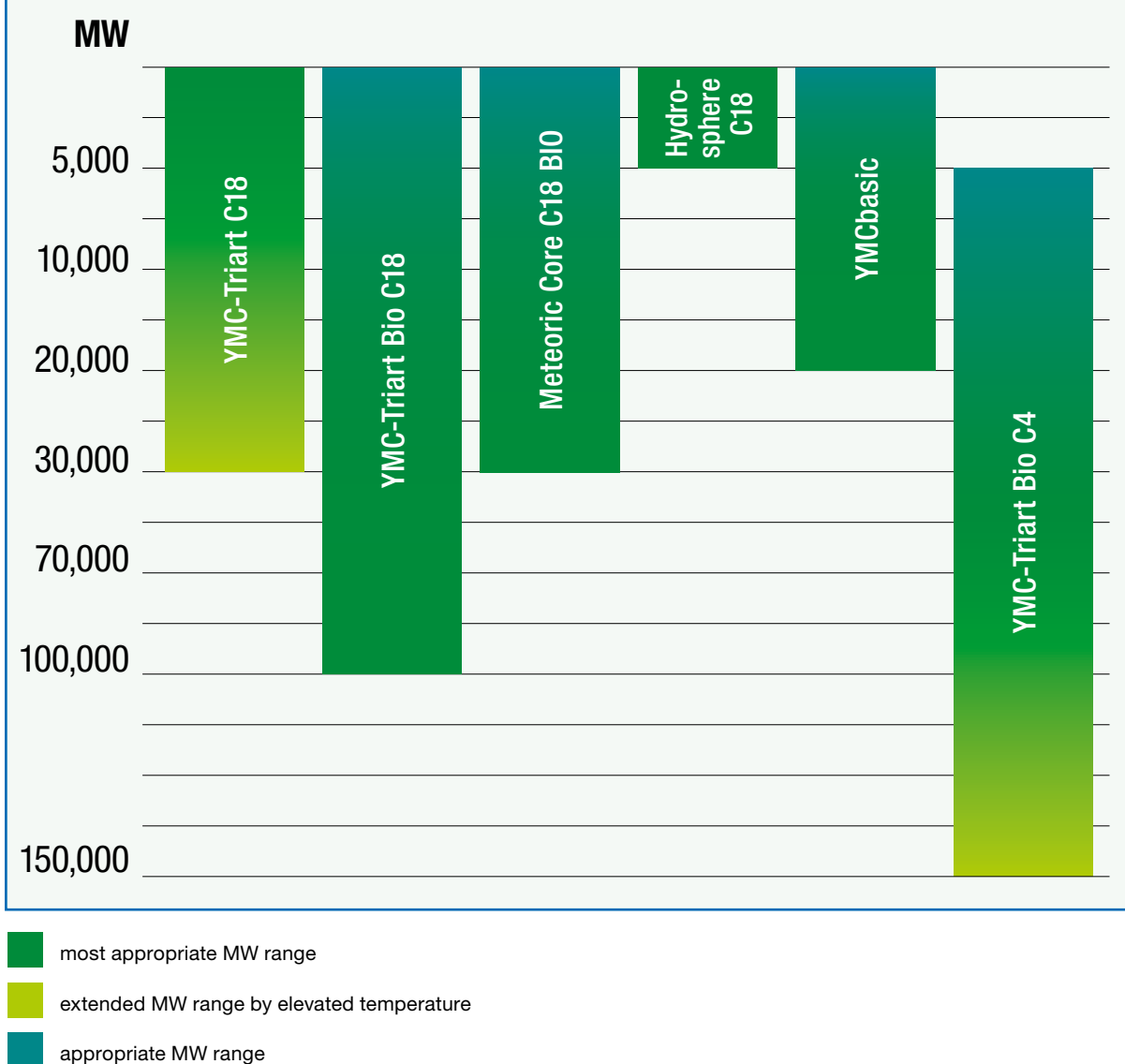
RP



## RP – Bioseparation Columns

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

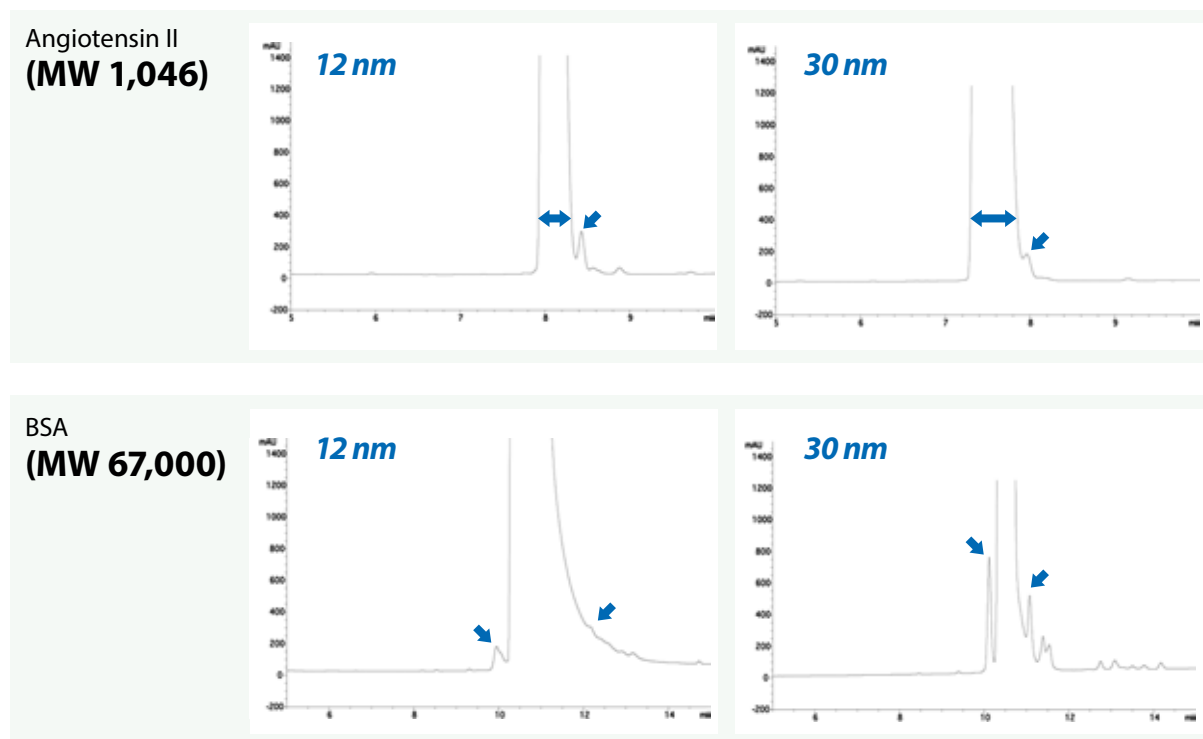
### Column Selection Tool according to molecular weight



## Influence of pore size

**A**s shown in the table (on the left), the C18 column with 12 nm pore size is suitable for small peptides up to a MW of 5,000 Da. The highest efficiency for large peptides or small proteins can be obtained by using a wide pore C8 phase with 20 nm porosity. Most proteins can be eluted efficiently with a wide pore C4 column with 30 nm porosity.

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



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

## RP – UHPLC / HPLC Selectivities

### C4 and C8 selectivities for proteins (and peptides) and antibodies

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

### C18 selectivities for peptides (and proteins)

	YMC-Triart C18	YMC-Triart Bio C18	Meteoric Core Bio C18
<b>Modification</b>	C18 (USP L1)	C18 (USP L1)	C18 (USP L1)
<b>Particle Size / <math>\mu\text{m}</math></b>	1.9, 3, 5	1.9, 3, 5	2.7
<b>Pore Size / nm</b>	12	30	8 (16)
<b>pH range</b>	1.0 – 12.0	1.0 – 12.0	1.5 – 10.0
<b>Temperature range</b>	pH < 7: 90 °C pH > 7: 50 °C	pH < 7: 90 °C pH > 7: 50 °C	pH < 7: 70 °C pH > 7: 50 °C

### C18 and C8 selectivities for oligonucleotides

	YMC-Triart C18	YMC-Triart Bio C18	Hydrosphere C18	YMC-Triart C8
<b>Modification</b>	C18 (USP L1)	C18 (USP L1)	C18 (USP L1)	C8 (USP L7)
<b>Particle Size / <math>\mu\text{m}</math></b>	1.9, 3, 5	1.9, 3, 5	2, 3, 5	1.9, 3, 5
<b>Pore Size / nm</b>	12	30	12	12
<b>pH range</b>	1.0 – 12.0	1.0 – 12.0	2.0 – 8.0	1.0 – 12.0
<b>Temperature range</b>	pH < 7: 90 °C pH > 7: 50 °C	pH < 7: 90 °C pH > 7: 50 °C	50 °C	pH < 7: 90 °C pH > 7: 50 °C



*Biocompatible hardware available!*

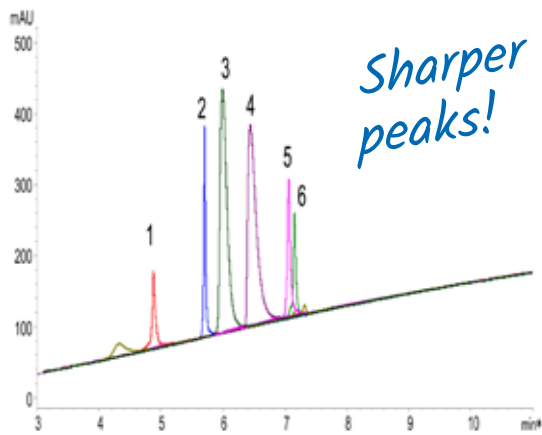
YMC-Triart metal-free columns are available for improved sensitivity and peak shape of coordinating compounds such as nucleotides or oligonucleotides, see page 47.

# RP – YMC-Triart Bio C4: Sharper peaks

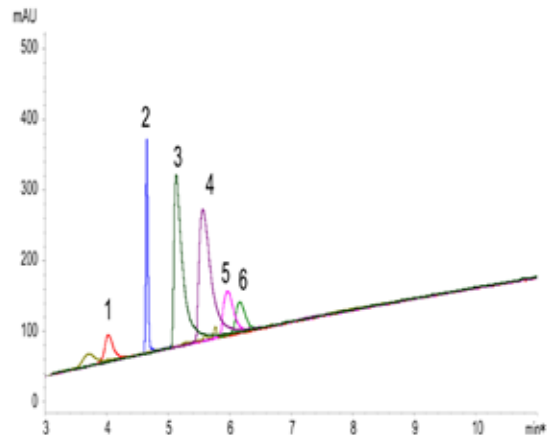
## Better performance using YMC-Triart Bio C4

High sensitivity and sharp peaks under LC/MS compatible conditions

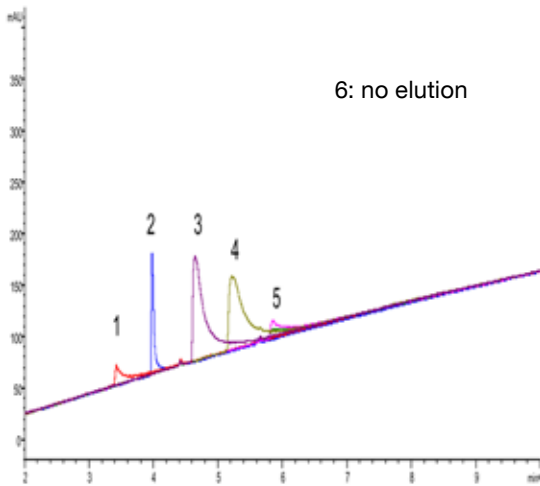
YMC-Triart Bio C4 (3 µm, 30 nm)



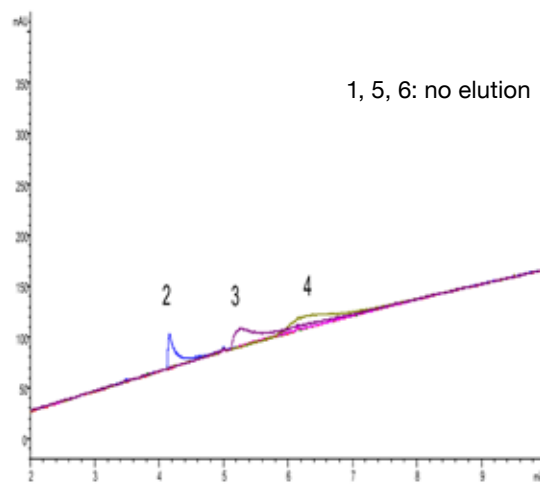
XBridge Protein BEH C4 (3.5 µm, 30 nm)



AdvanceBio RP-mAb C4 (3.5 µm, 45 nm)



Aeris widepore C4 (3.6 µm, 20 nm)



Column: 150 x 3.0 mm ID  
Eluent: A) water/formic acid (100/0.1)  
          B) acetonitrile/formic acid (100/0.1)  
Gradient: 10-95%B (0-15 min)  
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)

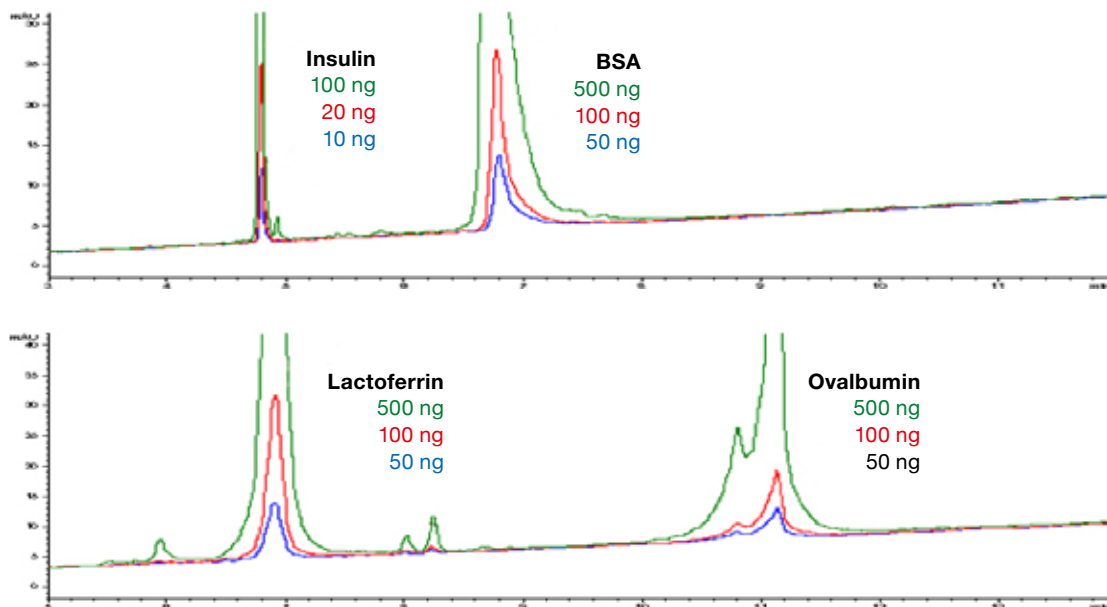
YMC-Triart Bio C4 shows better peak shape and recovery with a mobile phase containing formic acid, which is commonly used for LC/MS analysis. Therefore, YMC-Triart Bio C4 is ideal for high sensitivity analysis of proteins.

# RP – YMC-Triart Bio C4: No column adsorption

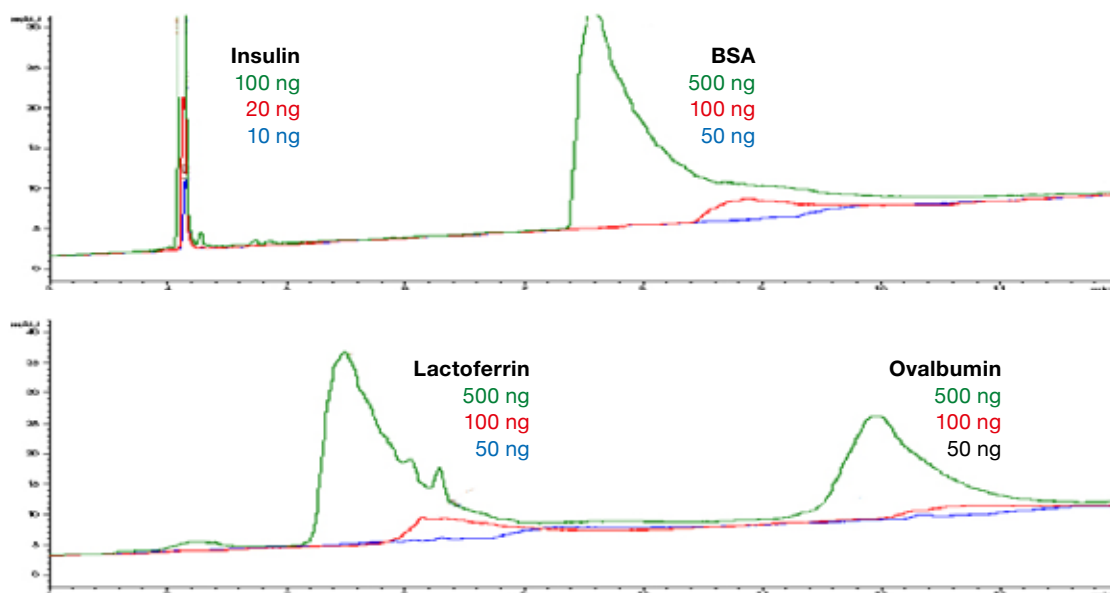
## No sample adsorption by YMC-Triart Bio C4 columns

Ideal for Microanalysis

YMC-Triart Bio C4 (1.9 μm, 30 nm)



Aeris widepore C4 (3.6 μm, 20 nm)



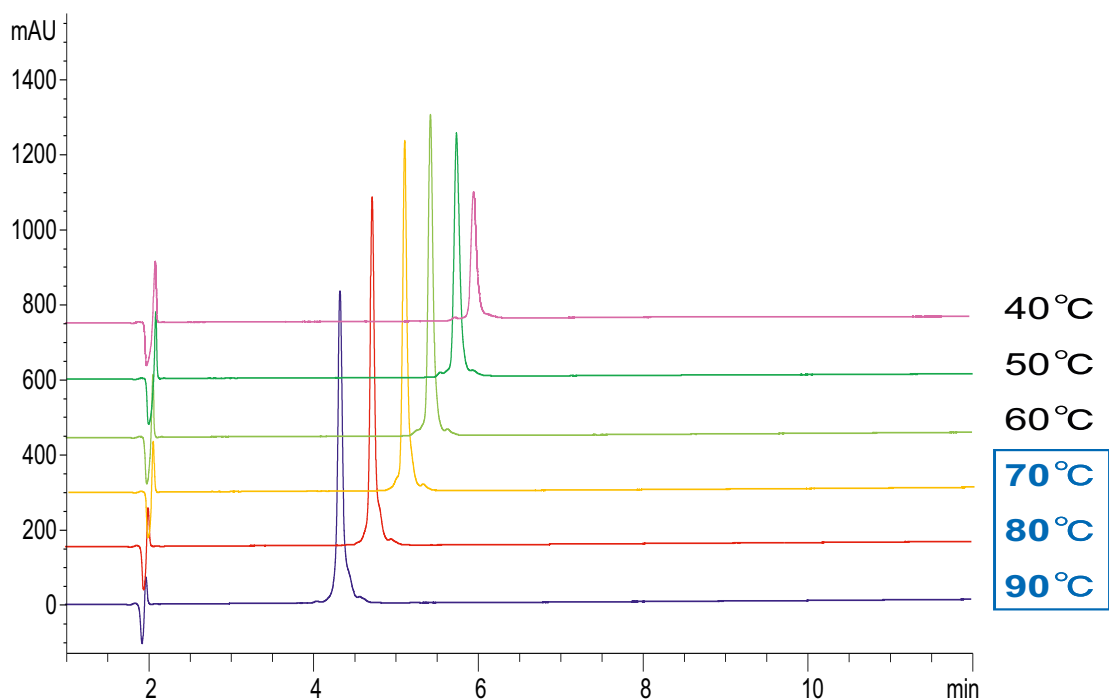
Column: 150 x 2.1 mm ID  
 Eluent: A) water/TFA (100/0.05)  
 B) acetonitrile/TFA (100/0.05)  
 Gradient: 25-60%B (0-15 min), 90%B (15-20 min), 25%B (20-35 min)  
 Flow rate: 0.2 ml/min  
 Detection: UV at 220 nm  
 Temperature: 40 °C

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

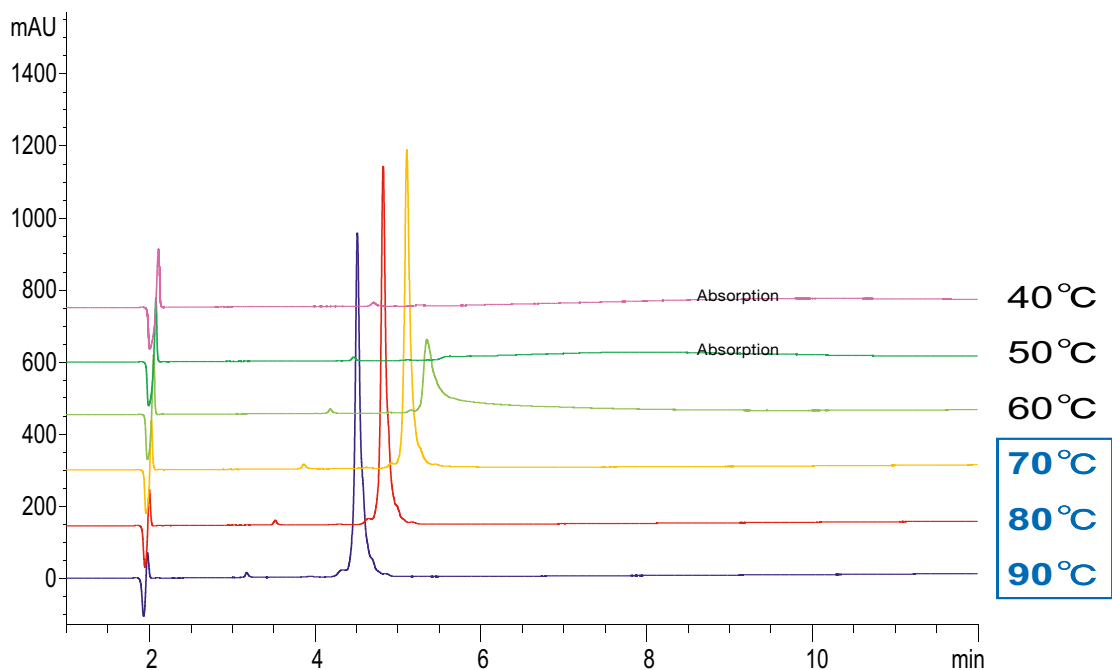


## High temperature tolerance allows antibody analysis

Adalimumab (Humira®, MW: ca. 148 kDa)



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

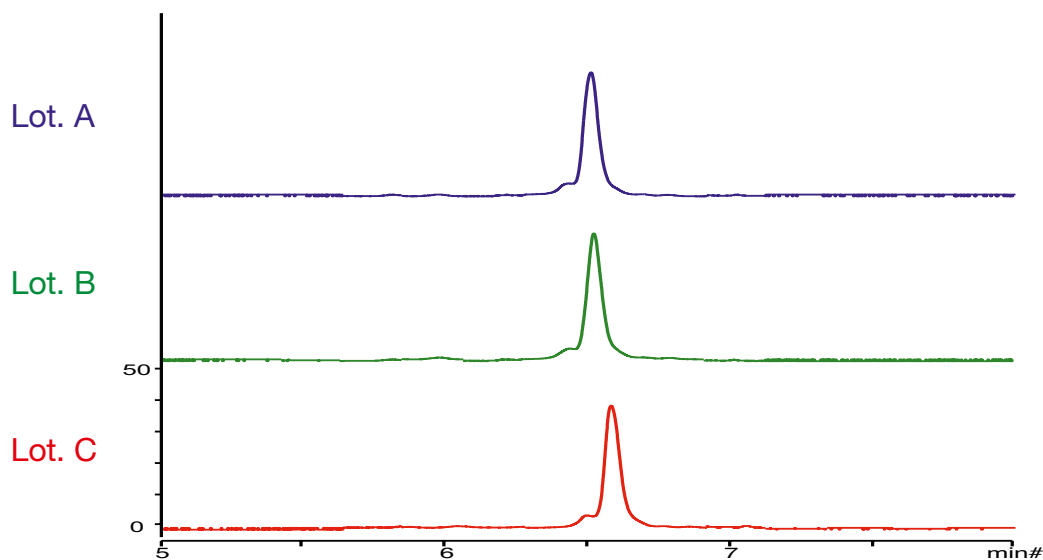


Column: YMC-Triart Bio C4 (3  $\mu$ m, 30 nm) 150 x 3.0 mm ID  
 Part No: TB30S03-1503PTH  
 Eluent: A) water/TFA (100/0.1).  
 B) acetonitrile/TFA (100/0.1)  
 Gradient: 30-60%B (0-15 min), 90%B (15-30min)  
 Detection: UV at 220 nm  
 Flow rate: 0.4 mL/min  
 Sample: Adalimumab (0.5 mg/mL) or Bevacizumab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

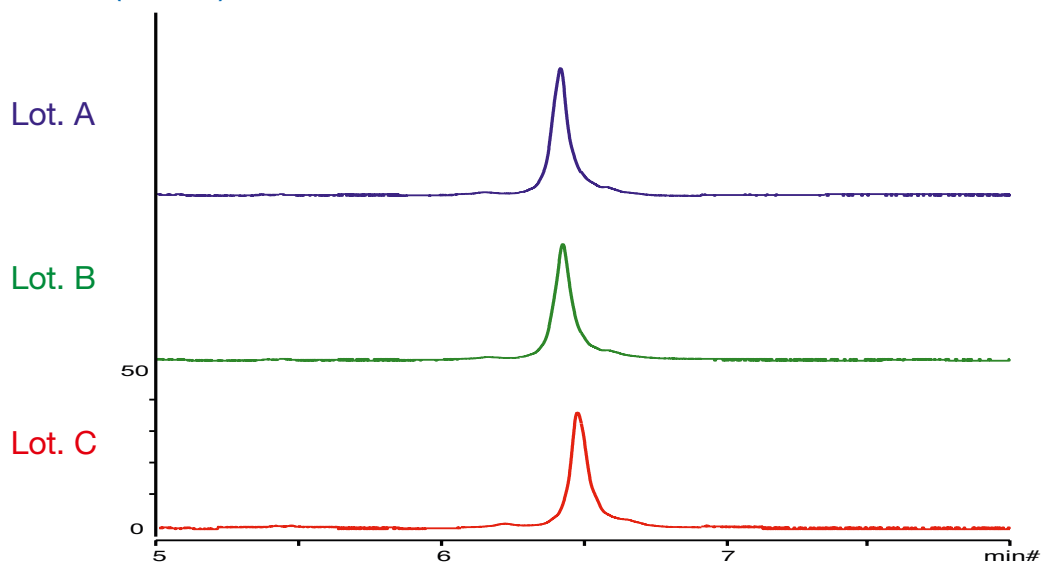
## RP – YMC-Triart Bio C4: Reproducibility

## Excellent Batch-to-batch reproducibility for antibody analysis

NIST mAb, 8671



Bevacizumab (Avastin®)



Column: YMC-Triart Bio C4 (1.9  $\mu$ 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)  
Detection: UV at 280 nm  
Flow rate: 0.4 mL/min  
Temperature: 80 °C  
Injection: 2  $\mu$ L (0.5 mg/mL)

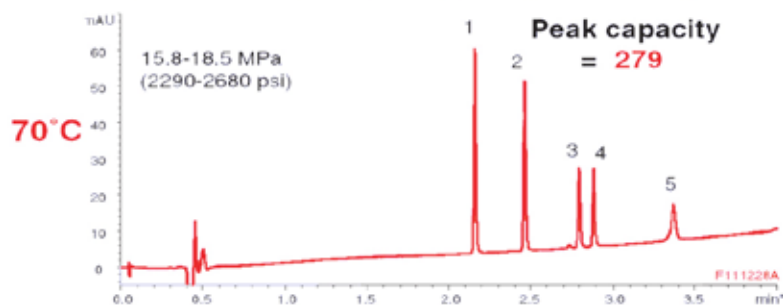
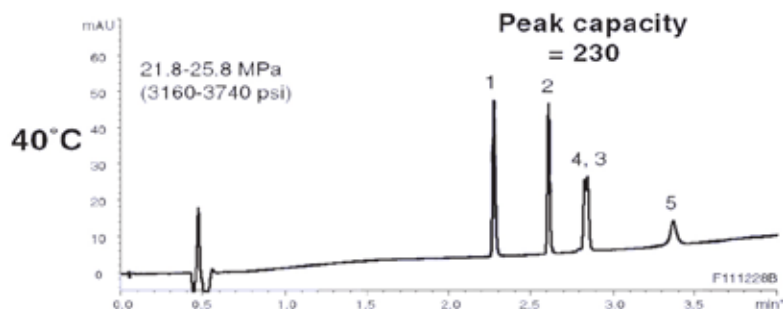
YMC-Triart Bio C4 shows excellent lot-to-lot reproducibility for antibodies. Not only is retention time highly reproducible, but also the resolution of minor impurity peaks. This makes YMC-Triart Bio C4 ideal for quality control of biopharmaceuticals.

# RP – YMC-Triart C18: Temperature stability

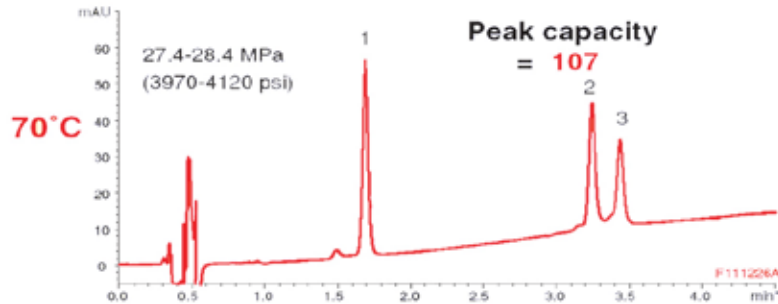
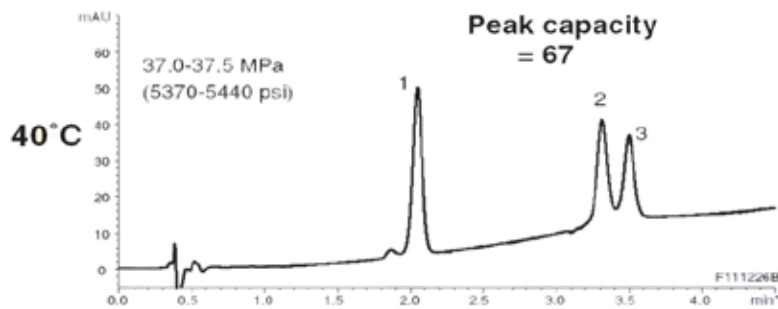
## More temperature flexibility using YMC-Triart

### Highly efficient RP-HPLC separation of proteins

Mixture A (MW 500–18,400)



Mixture B (MW 14,300–25,700)



Analytes	MW	Peak width 1/2h (min)	
		40 °C	70 °C
<b>Mixture A</b>			
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
<b>Mixture B</b>			
1. Lysozyme	14,300	0.069	0.044
2. α-Chymotrypsinogen	25,700	0.080	0.049
3. β-Lactoglobulin A	18,400	0.080	0.048

*High temperatures only possible with YMC-Triart*

Column: YMC-Triart C18 (1.9 μm, 12 nm) 50 x 2.0 mm ID  
 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

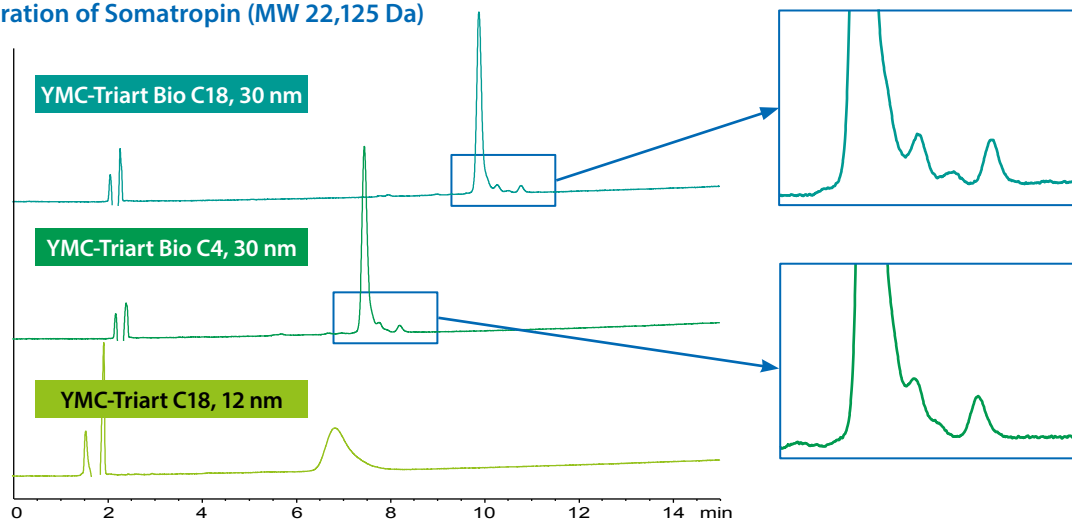
Flow rate: 0.4 mL/min  
 Detection: UV at 220 nm  
 Injection: 1 μL (50 μg/mL) - condition A  
 1 μL (250 μg/mL) - condition B  
 System: Agilent 1200SL

PC (peak capacity) = 1 + (gradient time / peak width\*)  
 \*peak width = 2W<sub>0.5h</sub> average

# RP – YMC-Triart Bio C18: Great peak shapes

## Ideal solutions for any kind of biomolecule

Separation of Somatropin (MW 22,125 Da)



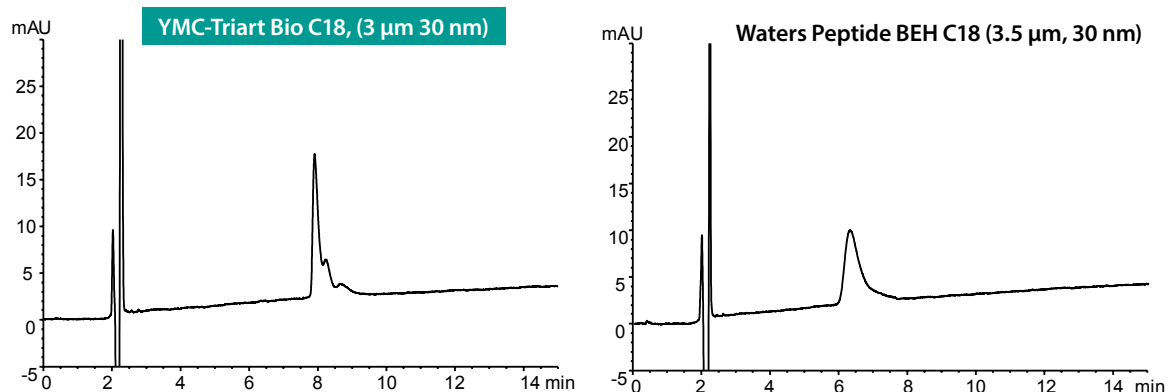
Columns: 150 x 3.0 mm ID (3  $\mu$ m)  
 Part Nos.: TA30S03-1503PTH  
 TB30S03-1503PTH  
 TA12S03-1503PTH  
 Eluent: A) water/TFA (100/0.1)  
 B) acetonitrile/TFA (100/0.08)

Gradient: 50-70%B (0-15 min)  
 Flow rate: 0.425 mL/min  
 Temperature: 40  
 Detection: UV at 220 nm  
 Injection: 4  $\mu$ 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.

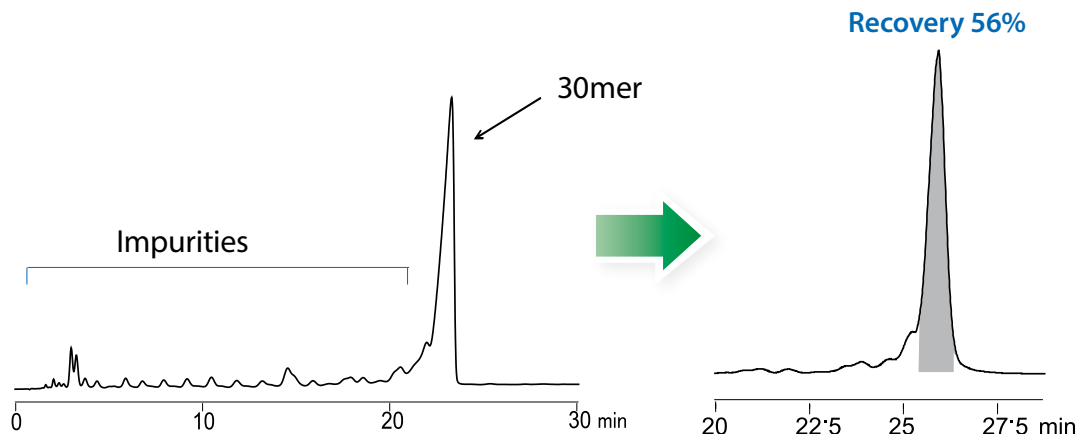
# RP – Hydrosphere C18: Oligonucleotide purification

## Easy purification of Oligonucleotides with YMC-Actus semi prep columns

### Purification of synthetic 30mer oligonucleotide

**Analysis** 1.0 mL/min, 5  $\mu$ L injection  
**Hydrosphere C18**  
 50 x 4.6 mm ID, 5  $\mu$ m

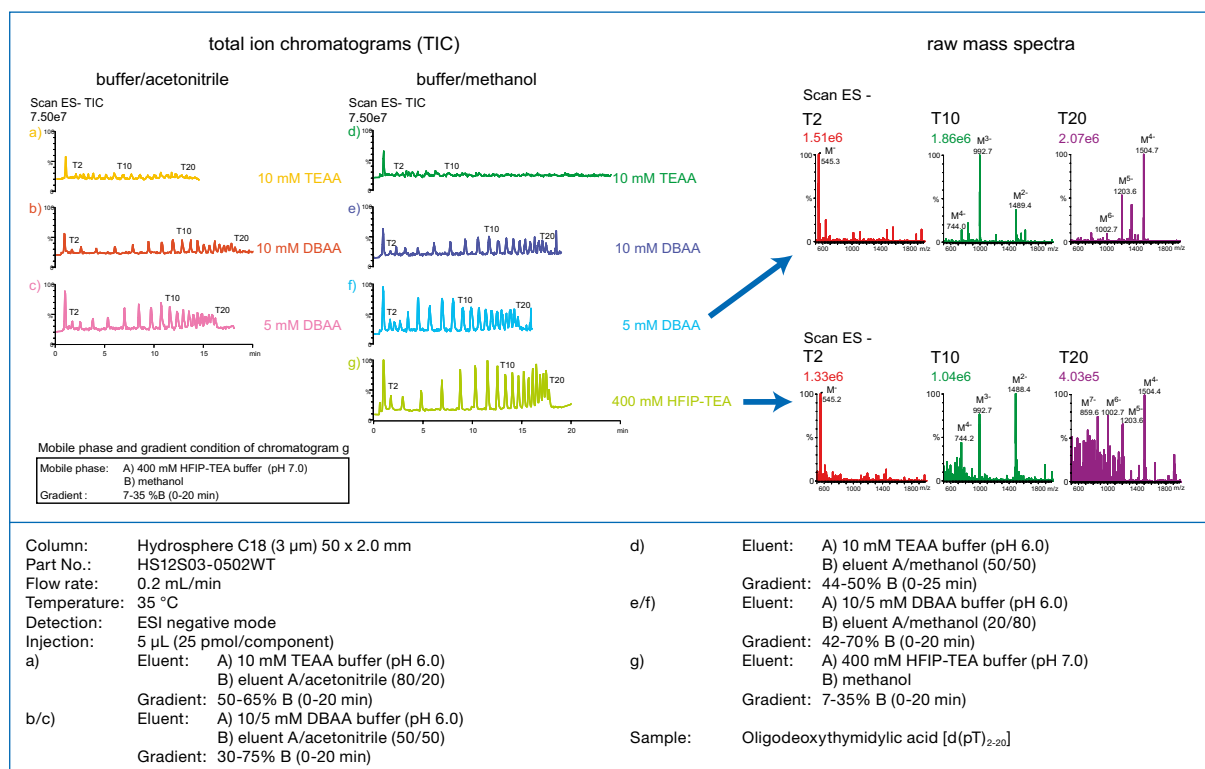
**Purification** 19 mL/min, 100  $\mu$ L injection  
**YMC-Actus Hydrosphere C18**  
 50 x 20 mm ID, 5  $\mu$ m



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  $\mu$ M)

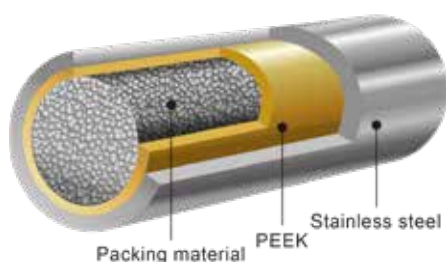
**purity > 99%**

## Influences of mobile phase conditions on intensity of ESI-MS



# RP – YMC-Triart: Biocompatible hardware

## Metal-free column hardware ideal for oligonucleotide analysis



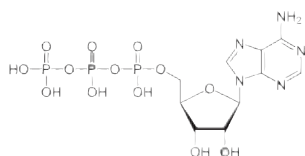
### Specifications

YMC-Triart Phases	C18, Bio C18, C8, Bio C4
Particle Size	1.9, 3, 5 $\mu\text{m}$
Inner layer	PEEK
Outer layer	Stainless steel
Frit	PEEK
Pressure limit	1.9 $\mu\text{m}$ : 100 MPa (15,000 psi) 3/5 $\mu\text{m}$ : 45 MPa (6,525 psi)

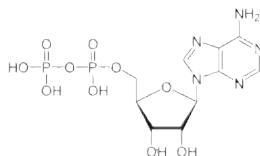
Special column connectors required.  
See ordering information recommendations.

### Improved sensitivity for coordination compounds

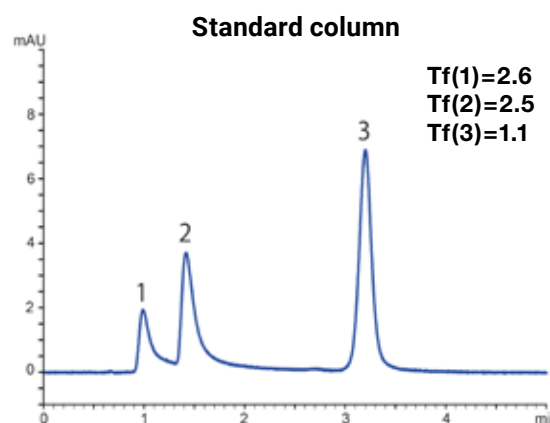
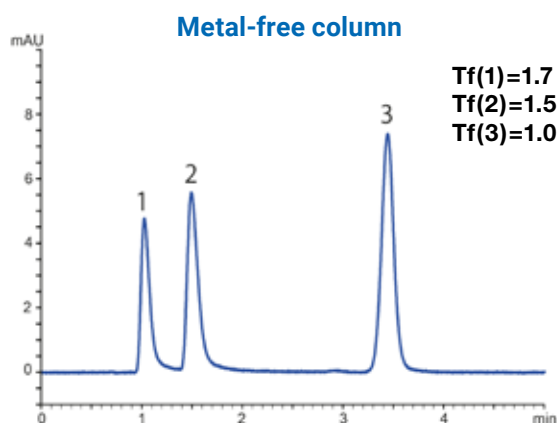
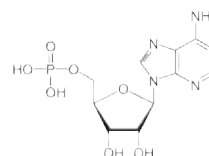
1. ATP



2. ADP



3. AMP



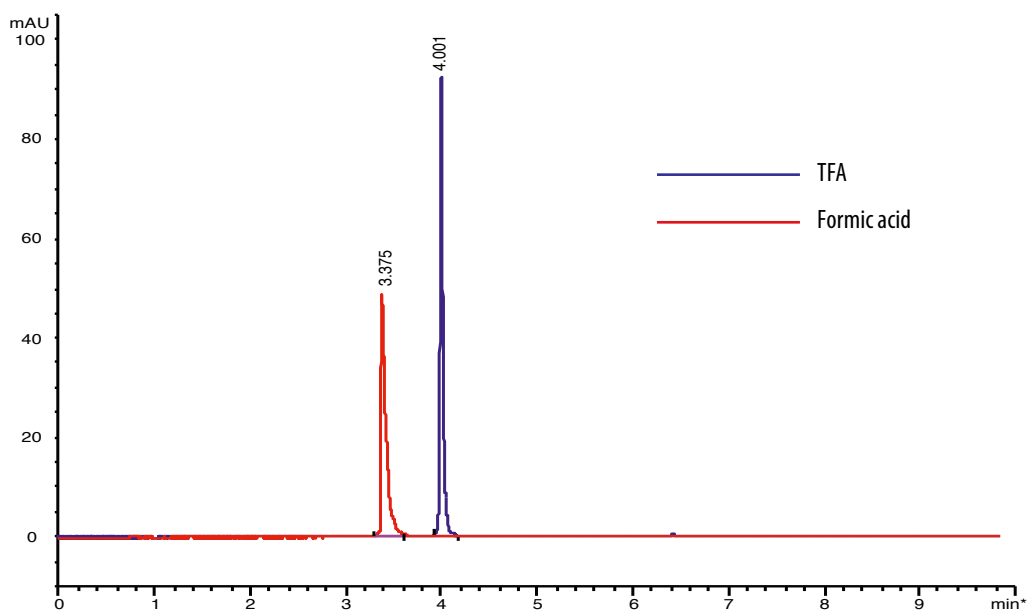
Column: YMC-Triart C18 (3  $\mu\text{m}$ ) 50 x 2.1 mm ID  
Part Nos.: TA12S03-05Q1PTP (metal-free) or  
TA12S03-05Q1PTH (regular hardware)  
Eluent: 5 mM  $\text{HCOONH}_4$   
Flow rate: 0.21 mL/min  
Temperature: 25  $^\circ\text{C}$   
Detection: UV at 265 nm  
Injection: 1 mL (10 mg/mL)  
System: bioinert/"non-metal" HPLC system

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

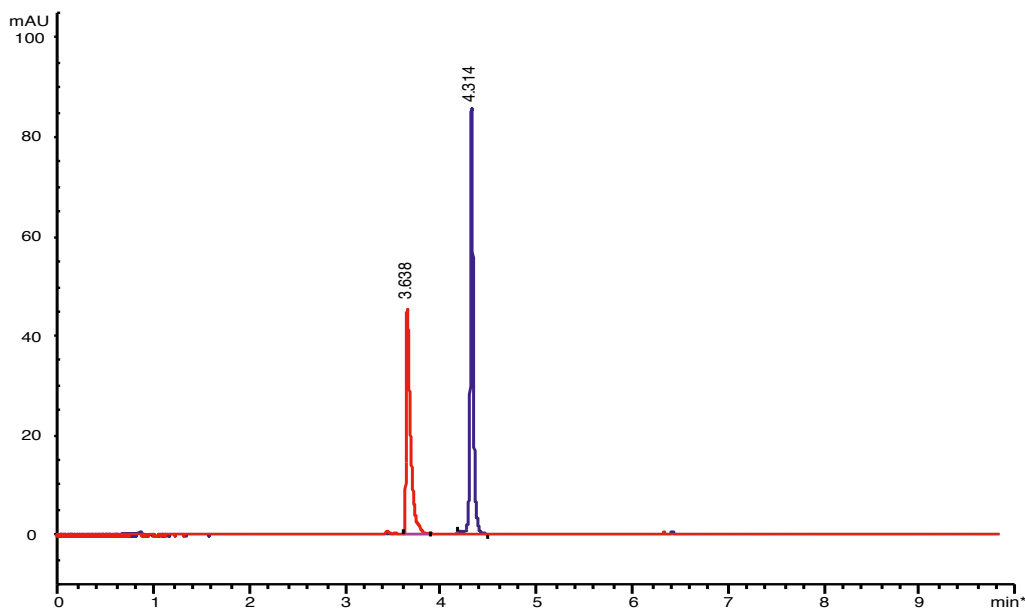
## Use of MS compatible conditions for antibody analysis by RP

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

### Adalimumab (Humira®)



### NIST Mab

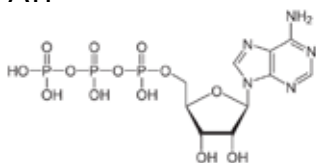


Column: YMC-Triart Bio C4 (1.9  $\mu$ m, 30 nm) 150 x 2.1 mm ID  
 Part No: TB30SP9-15Q1PT  
 Eluent: A) water/TFA or Formic acid (100/0.1)  
 B) acetonitrile/TFA or Formic acid (100/0.1)  
 Gradient: 10-95%B (0-10 min)  
 Detection: UV at 280 nm (0.13s, 40Hz)  
 Flow rate: 0.4 mL/min  
 Temperature: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

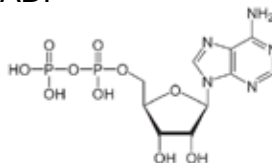
# RP – Expert Tips: (Oligo)nucleotides

## Influence of system and column hardware on the analysis of nucleotides

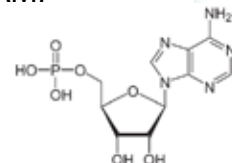
1 ATP



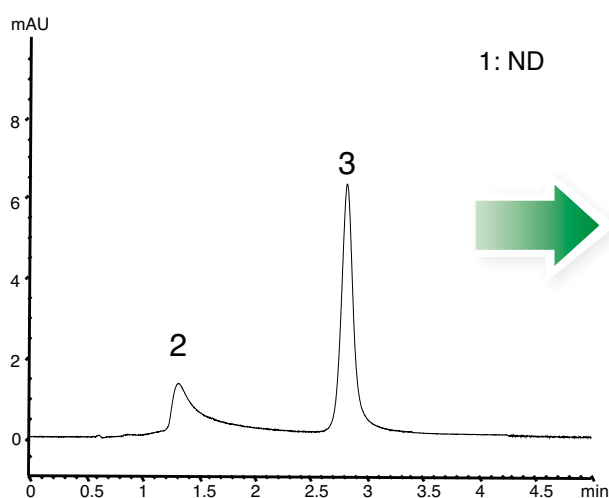
2 ADP



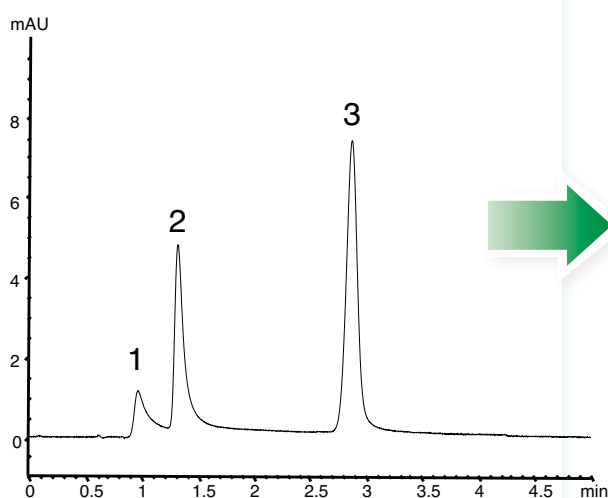
3 AMP



Ordinary HPLC system with standard column

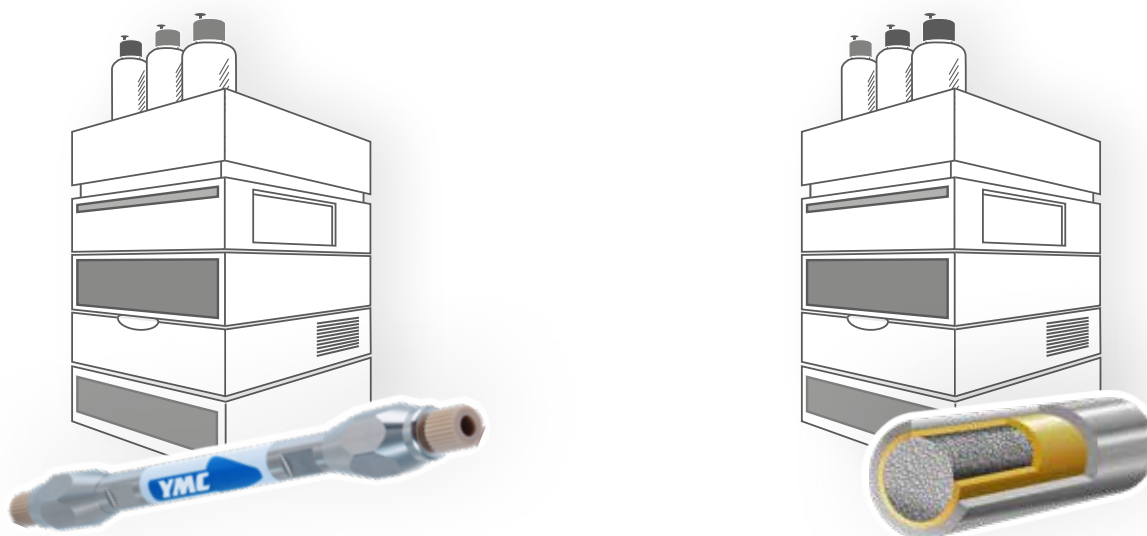


Ordinary HPLC system with metal-free column



Column: YMC-Triart C18 (3  $\mu$ m, 12 nm) 50 x 2.1 mm ID  
 Part Nos: TA12S03-05Q1PT (standard hardware)  
 TA12S03-05Q1PTP (metal-free hardware)  
 Eluent: 5 mM HCOONH<sub>4</sub>  
 Flow rate: 0.21 mL/min  
 Temperature: 25 °C  
 Detection: UV at 265 nm  
 Injection: 1  $\mu$ L (10  $\mu$ g/mL)

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

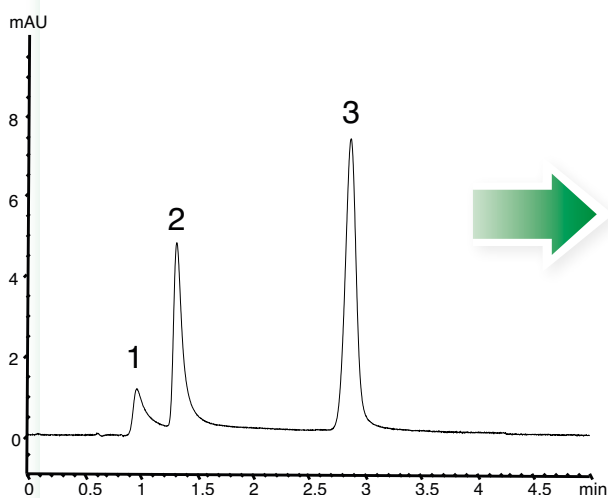


ATP peak is detected, and peak shape of ADP is improved as a result of using the metal-free column.

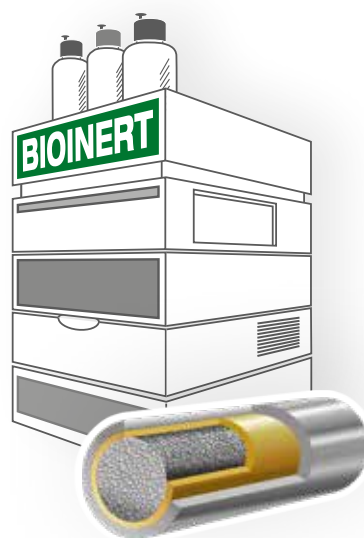
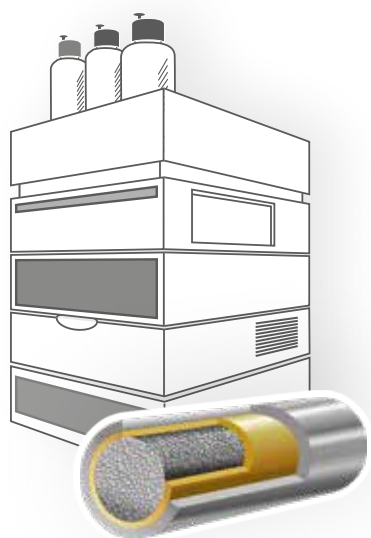
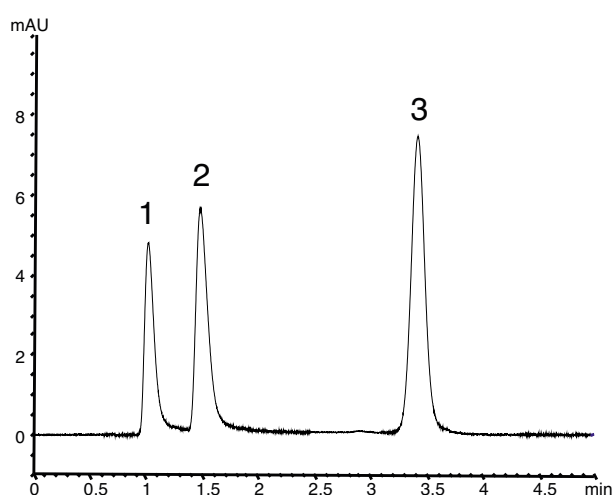


# RP – Expert Tips: (Oligo)nucleotides

Ordinary HPLC system  
with metal-free column



“Non-metal” HPLC system\*  
with metal-free column

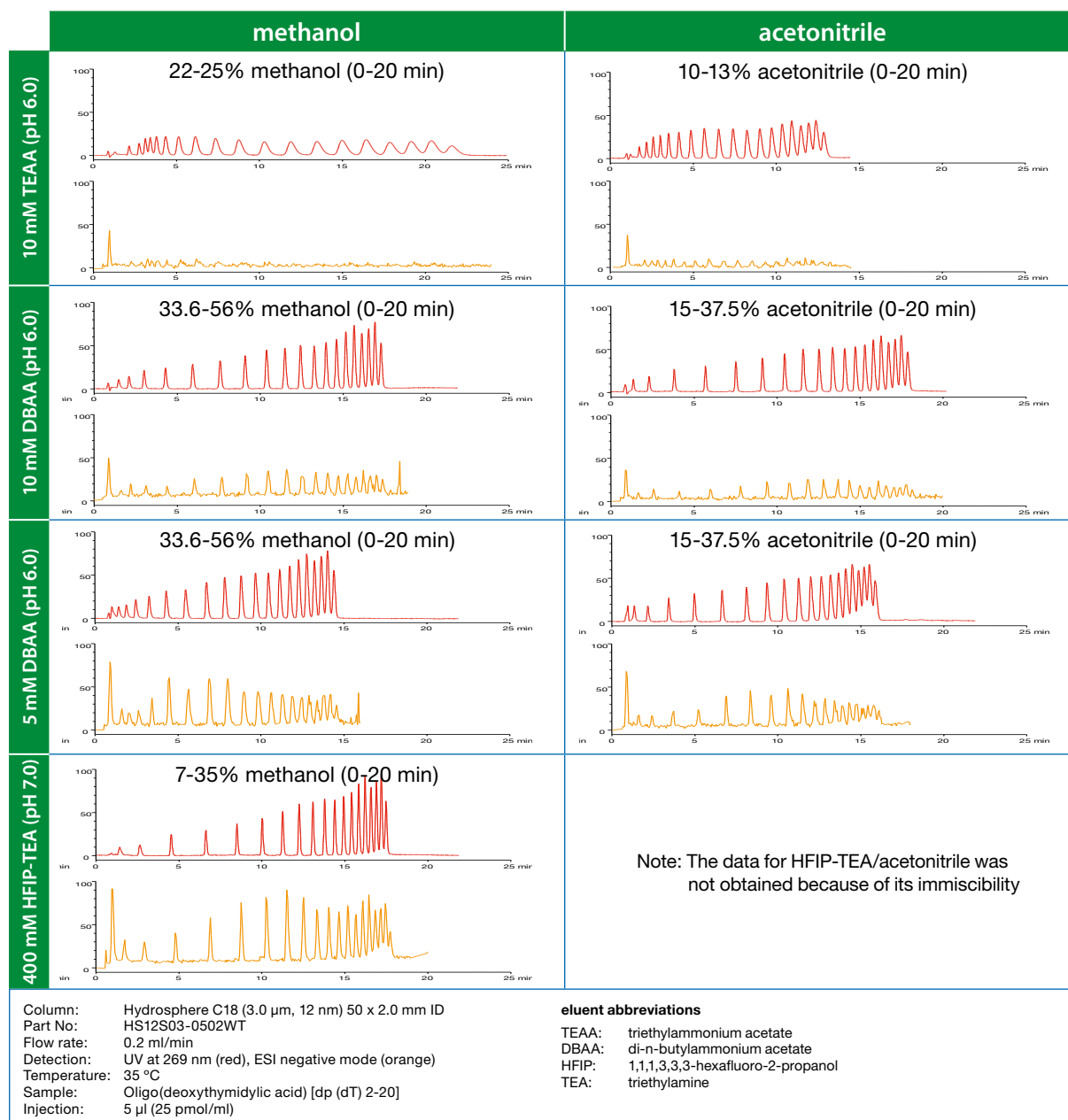


Peak shape is greatly improved as a result of using “non-metal” HPLC system

# RP – 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



**T**he mobile phase composition has different effects on the separation and signal intensity in electrospray ionisation mass spectrometry (ESI-MS) of oligonucleotides. Using different gradient conditions, acceptable retention and resolution can be achieved (upper UV chromatograms; red trace) for each separation by optimising the gradient slope of the organic solvent regardless of the type of mobile phase. The ESI-MS intensity is significantly influenced

by the type and concentration of ion-pairing buffer as shown in the lower MS chromatograms (orange trace). HFIP-TEA buffer/methanol systems provide the maximum MS intensity. Enhanced retention and MS intensity are obtained using 10 mM DBAA buffer compared to 10 mM TEAA buffer, and the lower DBAA concentration results in approximately 1.5–3 times increase in the intensity without any change in the concentration of organic solvent.

# RP – Ordering information

## 1.9 µm UHPLC columns

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

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

Special column connectors required.

## Column connectors

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

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

# RP – Ordering information

## 3 µm HPLC columns

Phase	Column ID [mm]	Column length [mm]						Guard cartridges* with 10 mm length
		30/33	50	75	100	150	250	
YMC-Triart C18	2.1	TA12S03-H301PTH	TA12S03-05Q1PTH	TA12S03-L5Q1PTH	TA12S03-10Q1PTH	TA12S03-15Q1PTH	–	TA12S03-01Q1GC
	3.0	–	TA12S03-05Q3PTH	TA12S03-L5Q3PTH	TA12S03-10Q3PTH	TA12S03-15Q3PTH	–	TA12S03-01Q3GC
	4.6	TA12S03-H346PTH	TA12S03-0546PTH	TA12S03-L546PTH	TA12S03-1046PTH	TA12S03-1546PTH	TA12S03-2546PTH	TA12S03-01Q4GC
YMC-Triart Bio C18	2.1	TA30S03-H301PTH	TA30S03-05Q1PTH	TA30S03-L5Q1PTH	TA30S03-10Q1PTH	TA30S03-15Q1PTH	–	TA30S03-01Q1GC
	3.0	–	TA30S03-05Q3PTH	TA30S03-L5Q3PTH	TA30S03-10Q3PTH	TA30S03-15Q3PTH	–	TA30S03-01Q3GC
	4.6	TA30S03-H346PTH	TA30S03-0546PTH	TA30S03-L546PTH	TA30S03-1046PTH	TA30S03-1546PTH	TA30S03-2546PTH	TA30S03-01Q4GC
YMC-Triart C8	2.1	T012S03-H301PTH	T012S03-05Q1PTH	T012S03-L5Q1PTH	T012S03-10Q1PTH	T012S03-15Q1PTH	–	T012S03-01Q1GC
	3.0	–	T012S03-05Q3PTH	T012S03-L5Q3PTH	T012S03-10Q3PTH	T012S03-15Q3PTH	–	T012S03-01Q3GC
	4.6	T012S03-H346PTH	T012S03-0546PTH	T012S03-L546PTH	T012S03-1046PTH	T012S03-1546PTH	T012S03-2546PTH	T012S03-01Q4GC
YMC-Triart Bio C4	2.1	TB30S03-H301PTH	TB30S03-05Q1PTH	TB30S03-L5Q1PTH	TB30S03-10Q1PTH	TB30S03-15Q1PTH	–	TB30S03-01Q1GC
	3.0	–	TB30S03-05Q3PTH	TB30S03-L5Q3PTH	TB30S03-10Q3PTH	TB30S03-15Q3PTH	–	TB30S03-01Q3GC
	4.6	TB30S03-H346PTH	TB30S03-0546PTH	TB30S03-L546PTH	TB30S03-1046PTH	TB30S03-1546PTH	TB30S03-2546PTH	TB30S03-01Q4GC
Hydrosphere C18	2.1	HS12S03-H3Q1QT	HS12S03-05Q1QT	HS12S03-L5Q1QT	HS12S03-10Q1QT	HS12S03-15Q1QT	HS12S03-25Q1QT	HS12S03-01Q1GC
	3.0	HS12S03-H3Q3QT	HS12S03-05Q3QT	HS12S03-L5Q3QT	HS12S03-10Q3QT	HS12S03-15Q3QT	HS12S03-25Q3QT	HS12S03-01Q3GC
	4.6	HS12S03-0346WT	HS12S03-0546WT	HS12S03-L546WT	HS12S03-1046WT	HS12S03-1546WT	HS12S03-2546WT	HS12S03-01Q4GC
YMCbasic (eq. C8)	2.1	BA99S03-H3Q1QT	BA99S03-05Q1QT	BA99S03-L5Q1QT	BA99S03-10Q1QT	BA99S03-15Q1QT	BA99S03-25Q1QT	BA99S03-01Q1GC
	3.0	BA99S03-H3Q3QT	BA99S03-05Q3QT	BA99S03-L5Q3QT	BA99S03-10Q3QT	BA99S03-15Q3QT	BA99S03-25Q3QT	BA99S03-01Q3GC
	4.6	BA99S03-0346WT	BA99S03-0546WT	BA99S03-L546WT	BA99S03-1046WT	BA99S03-1546WT	BA99S03-2546WT	BA99S03-01Q4GC

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

## 3 µm metal-free HPLC columns

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

Special column connectors required.

# RP – Ordering information

## 2.7 µm Core-Shell columns

Phase	Column ID [mm]	Column length [mm]					Precolumn filter 0.5 µm
		30	50	75	100	150	
							(pack of 3)
<b>Meteoric Core C18 BIO, 16 nm</b>	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

Phase	Column ID [mm]	Column length [mm]						Guard cartridges* with 10 mm length
		30/33	50	75	100	150	250	
								(pack of 5)
<b>YMC-Triart C18</b>	2.1	TA12S05-H3Q1PTH	TA12S05-05Q1PTH	TA12S05-L5Q1PTH	TA12S05-10Q1PTH	TA12S05-15Q1PTH	–	TA12S05-01Q1GC
	3.0	–	TA12S05-0503PTH	TA12S05-L503PTH	TA12S05-1003PTH	TA12S05-1503PTH	–	TA12S05-0103GC
	4.6	TA12S05-H346PTH	TA12S05-0546PTH	TA12S05-L546PTH	TA12S05-1046PTH	TA12S05-1546PTH	TA12S05-2546PTH	TA12S05-0104GC
<b>YMC-Triart Bio C18</b>	2.1	TA30S05-H3Q1PTH	TA30S05-05Q1PTH	TA30S05-L5Q1PTH	TA30S05-10Q1PTH	TA30S05-15Q1PTH	–	TA30S05-01Q1GC
	3.0	–	TA30S05-0503PTH	TA30S05-L503PTH	TA30S05-1003PTH	TA30S05-1503PTH	–	TA30S05-0103GC
	4.6	TA30S05-H346PTH	TA30S05-0546PTH	TA30S05-L546PTH	TA30S05-1046PTH	TA30S05-1546PTH	TA30S05-2546PTH	TA30S05-0104GC
<b>YMC-Triart C8</b>	2.1	T012S05-H3Q1PTH	T012S05-05Q1PTH	T012S05-L5Q1PTH	T012S05-10Q1PTH	T012S05-15Q1PTH	–	T012S05-01Q1GC
	3.0	–	T012S05-0503PTH	T012S05-L503PTH	T012S05-1003PTH	T012S05-1503PTH	–	T012S05-0103GC
	4.6	T012S05-H346PTH	T012S05-0546PTH	T012S05-L546PTH	T012S05-1046PTH	T012S05-1546PTH	T012S05-2546PTH	T012S05-0104GC
<b>YMC-Triart Bio C4</b>	2.1	TB30S05-H3Q1PTH	TB30S05-05Q1PTH	TB30S05-L5Q1PTH	TB30S05-10Q1PTH	TB30S05-15Q1PTH	–	TB30S05-01Q1GC
	3.0	–	TB30S05-0503PTH	TB30S05-L503PTH	TB30S05-1003PTH	TB30S05-1503PTH	–	TB30S05-0103GC
	4.6	TB30S05-H346PTH	TB30S05-0546PTH	TB30S05-L546PTH	TB30S05-1046PTH	TB30S05-1546PTH	TB30S05-2546PTH	TB30S05-0104GC
<b>Hydrosphere C18</b>	2.1	HS12S05-H3Q1QT	HS12S05-05Q1QT	HS12S05-L5Q1QT	HS12S05-10Q1QT	HS12S05-15Q1QT	HS12S05-25Q1QT	HS12S05-01Q1GC
	3.0	HS12S05-H303QT	HS12S05-0503QT	HS12S05-L503QT	HS12S05-1003QT	HS12S05-1503QT	HS12S05-2503QT	HS12S05-0103GC
	4.6	HS12S05-0346WT	HS12S05-0546WT	HS12S05-L546WT	HS12S05-1046WT	HS12S05-1546WT	HS12S05-2546WT	HS12S05-0104GC
<b>YMCbasic (eq. C8)</b>	2.1	BA99S05-H3Q1QT	BA99S05-05Q1QT	BA99S05-L5Q1QT	BA99S05-10Q1QT	BA99S05-15Q1QT	BA99S05-25Q1QT	BA99S05-01Q1GC
	3.0	BA99S05-H303QT	BA99S05-0503QT	BA99S05-L503QT	BA99S05-1003QT	BA99S05-1503QT	BA99S05-2503QT	BA99S05-0103GC
	4.6	BA99S05-0346WT	BA99S05-0546WT	BA99S05-L546WT	BA99S05-1046WT	BA99S05-1546WT	BA99S05-2546WT	BA99S05-0104GC

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

# RP – Ordering information

## 5 µm metal-free HPLC columns

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

Special column connectors required.

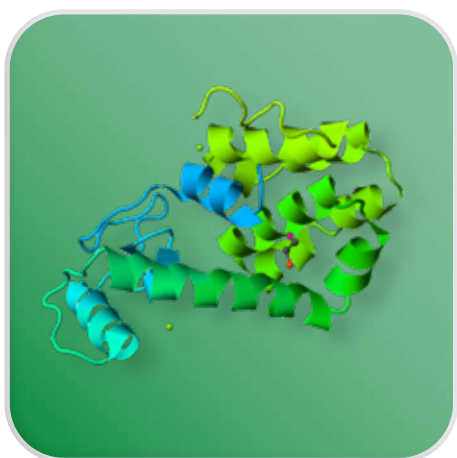
## 5 µm YMC-Actus high-throughput semipreparative columns

Phase	Column ID [mm]	Column length [mm]					Guard cartridges* with 10 mm length (pack of 5)
		50	75	100	150	250	
<b>YMC-Triart C18</b>	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**
<b>YMC-Triart Bio C18</b>	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**
<b>YMC-Triart C8</b>	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**
<b>YMC-Triart Bio C4</b>	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**
<b>Hydrosphere C18</b>	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
	50	HS12S05-0553DX	–	HS12S05-1053DX	HS12S05-1553DX	HS12S05-2553DX	–
<b>YMCbasic (eq. C8)</b>	20	BA99S05-0520WX	BA99S05-L520WX	BA99S05-1020WX	BA99S05-1520WX	BA99S05-2520WX	BA99S05-0120CCN
	30	BA99S05-0530WX	BA99S05-L530WX	BA99S05-1030WX	BA99S05-1530WX	BA99S05-2530WX	BA99S05-0130CCN
	50	BA99S05-0553DX	–	BA99S05-1053DX	BA99S05-1553DX	BA99S05-2553DX	–

\*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 carbohydrates and nucleic acid components
- Excellent reproducibility with minimal secondary interactions
- 2  $\mu\text{m}$  for UHPLC
- Cost effective

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



### Column Selection Tool

for MW < 10,000

● YMC-Pack Diol-**60**

for MW 1,000 to 100,000

● YMC-Pack Diol-**120**

for MW 5,000 to 300,000

● YMC-Pack Diol-**200**

for MW 10,000 to 700,000

● YMC-**SEC MAB**

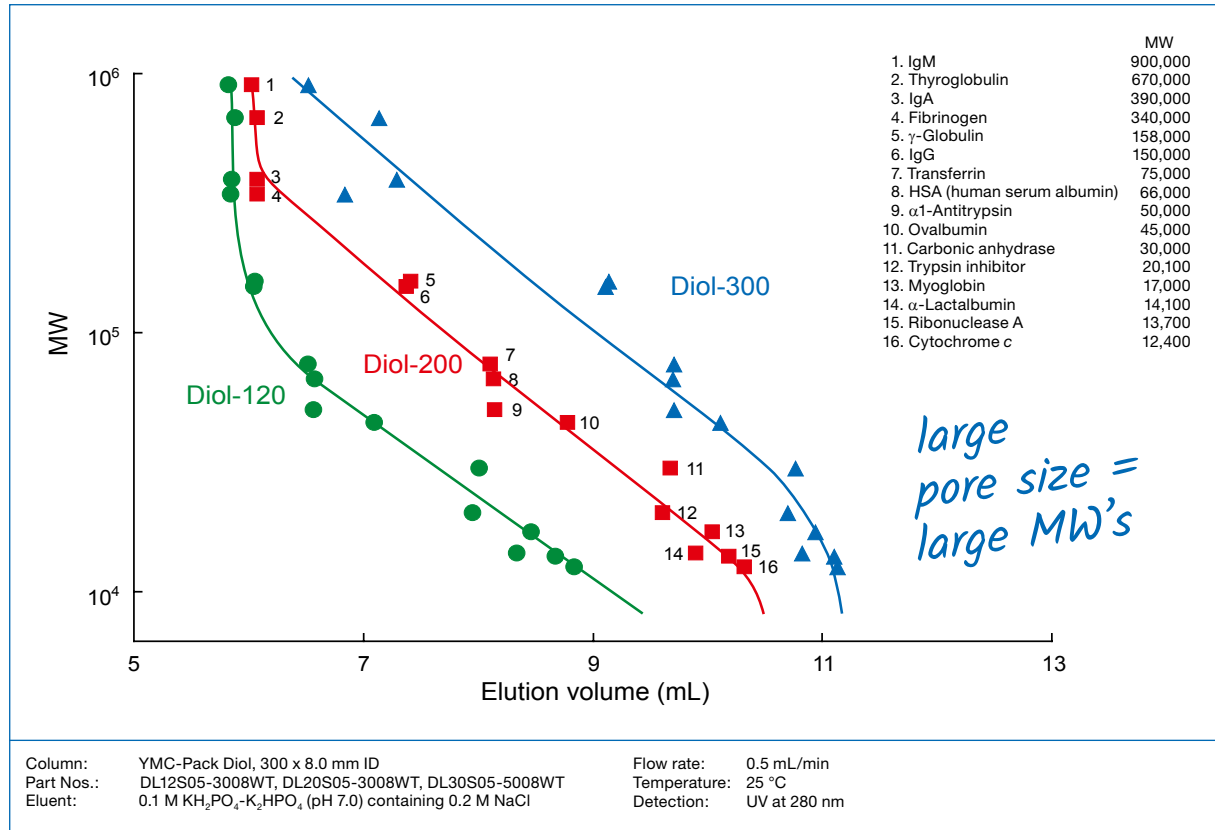
for MW 20,000 to 1,000,000

● YMC-Pack Diol-**300**

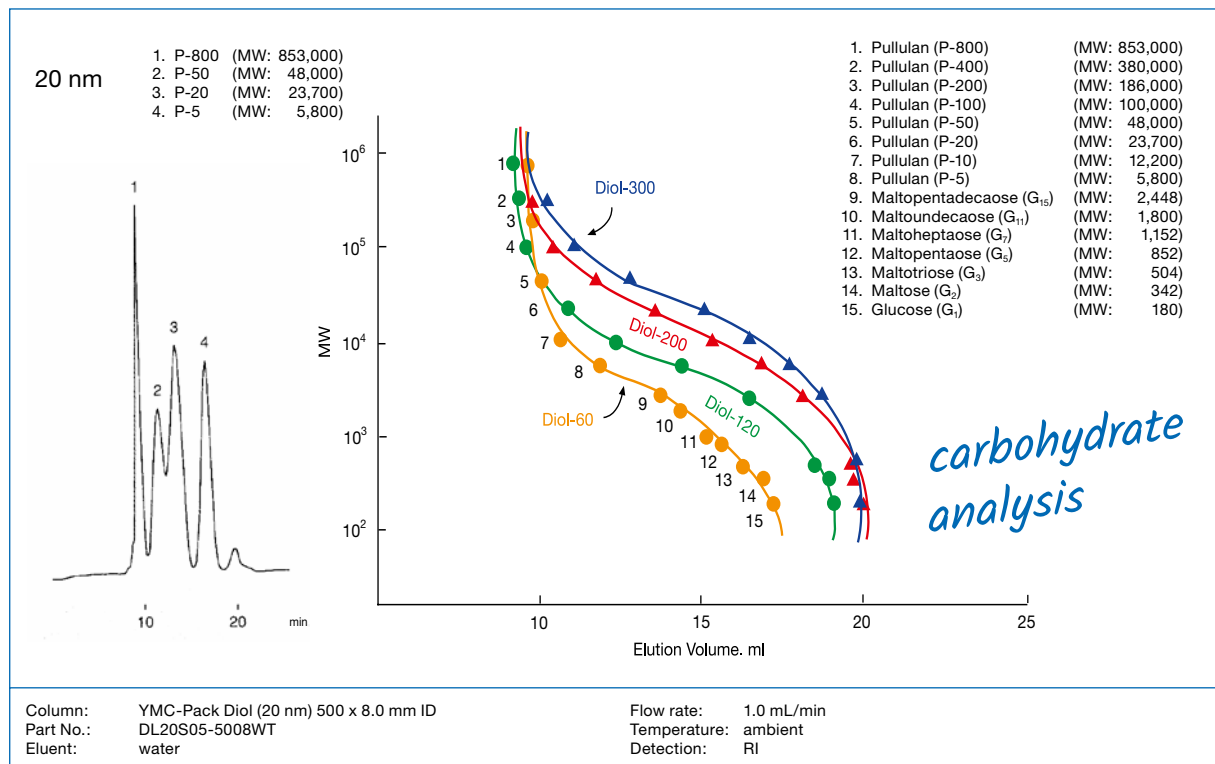
# SEC – YMC-Pack Diol: Phase selection for proteins

## Phases for different MW ranges

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

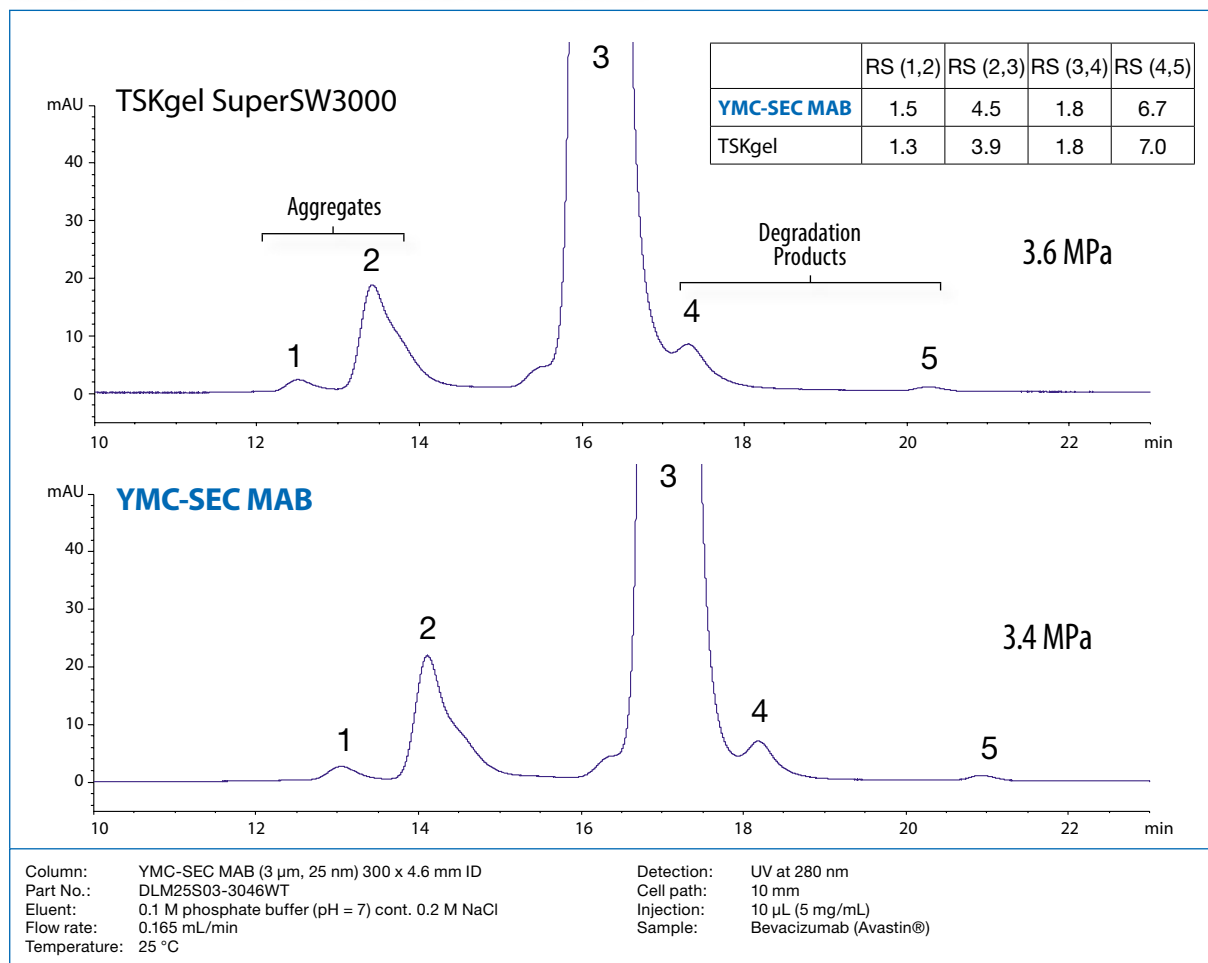


## For molecular weight determination of oligosaccharides and polysaccharides

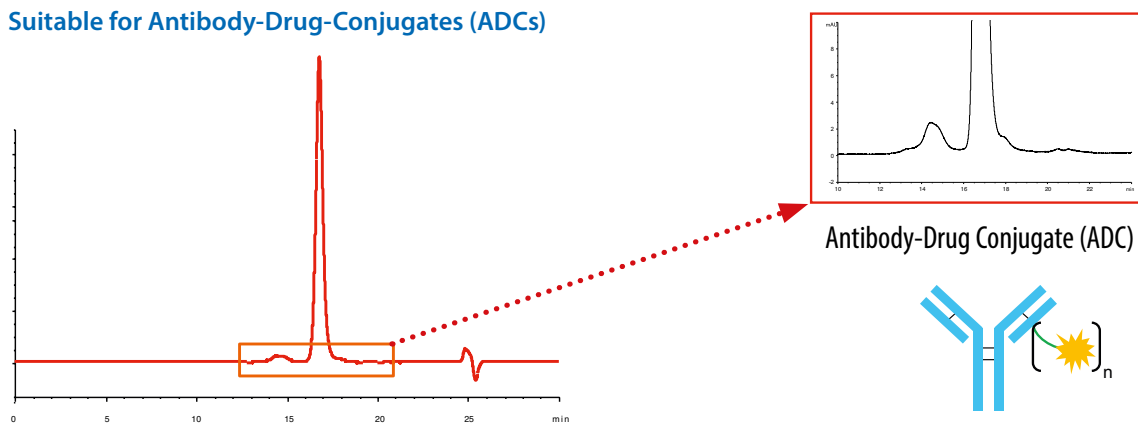


# SEC – YMC-SEC MAB: MAb & ADC analysis

## Ideal choice for monoclonal antibodies



## Suitable for Antibody-Drug-Conjugates (ADCs)

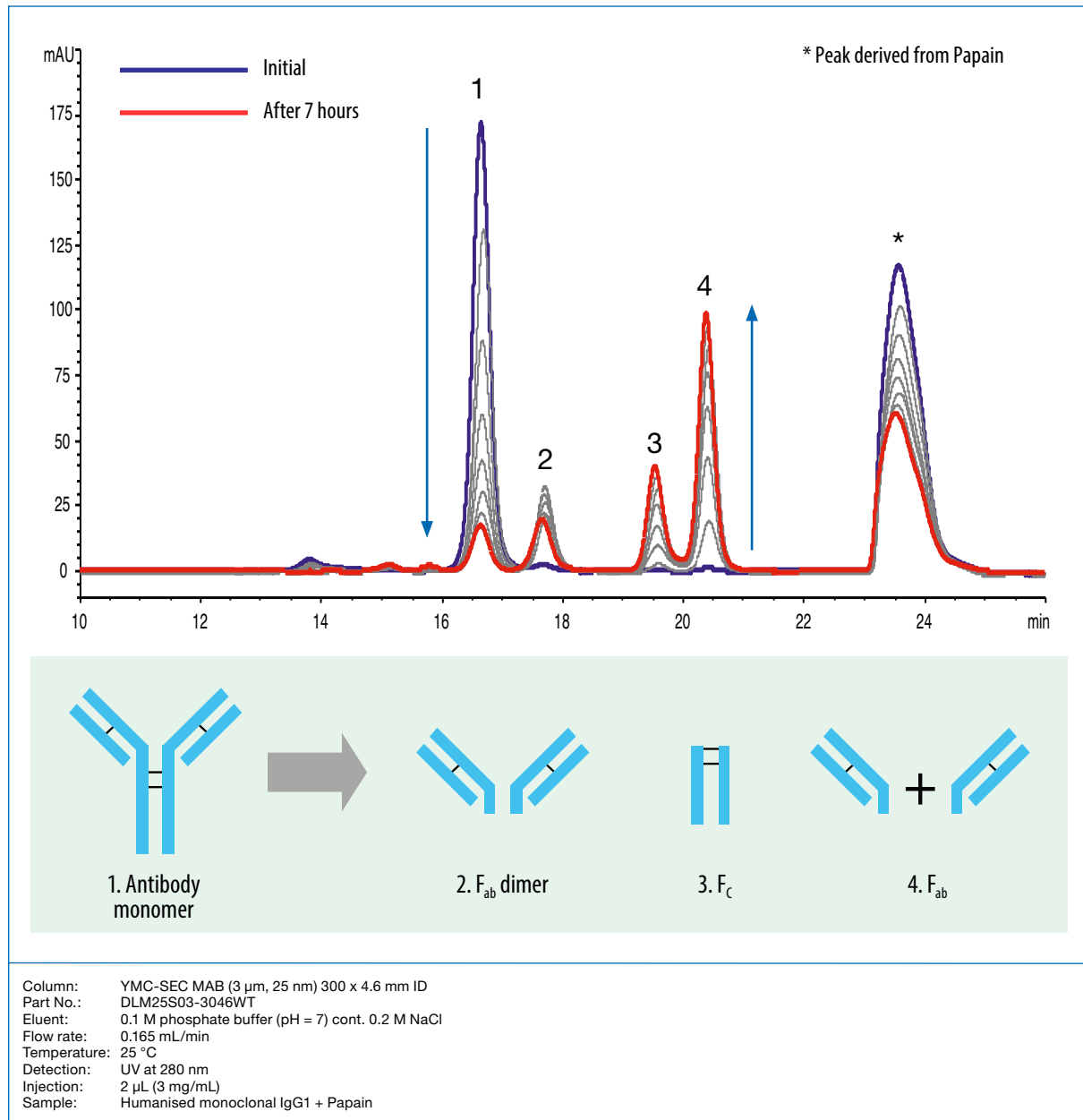


Column: YMC-SEC MAB (3 μm, 25 nm) 300 x 4.6 mm ID  
 Part No.: DLM25S03-3046WT  
 Eluent: 0.1 M phosphate buffer (pH = 7) cont. 0.2 M NaCl / 2-propanol (85 / 15)  
 Flow rate: 0.165 mL/min  
 Temperature: 25 °C  
 Detection: UV at 280 nm  
 Injection: 4 μL (2.5 mg/mL)  
 Sample: SigmaMAb Antibody Drug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

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

# SEC – YMC-SEC MAB: Digest monitoring

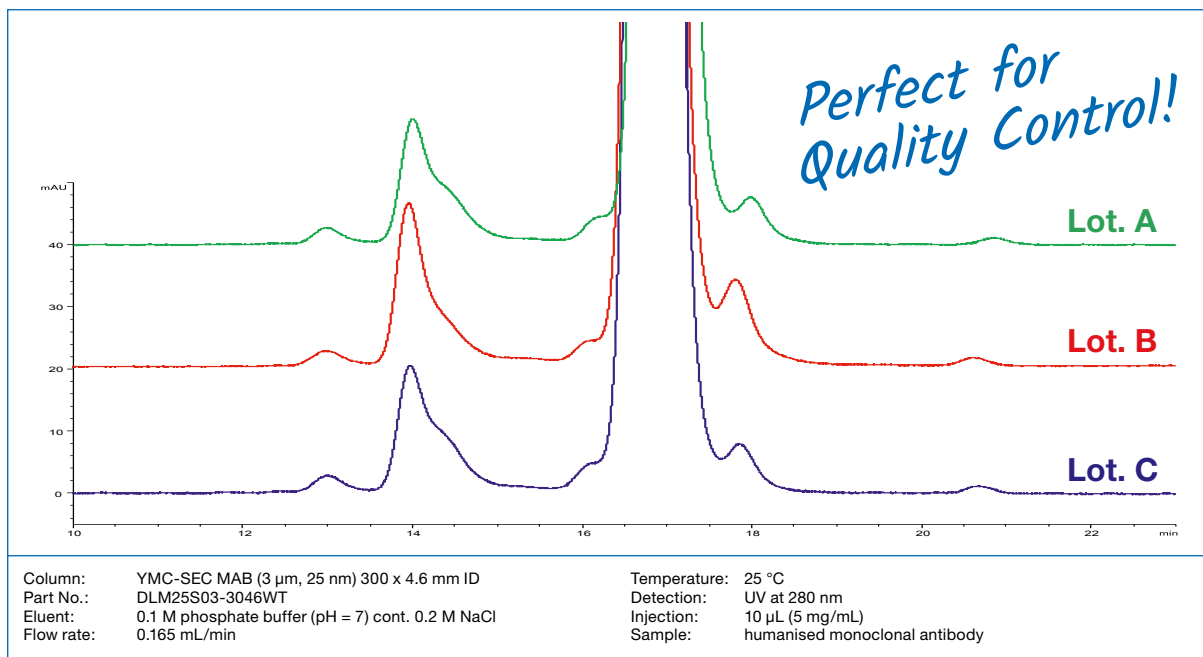
## Ideal for analysis of digested antibodies



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

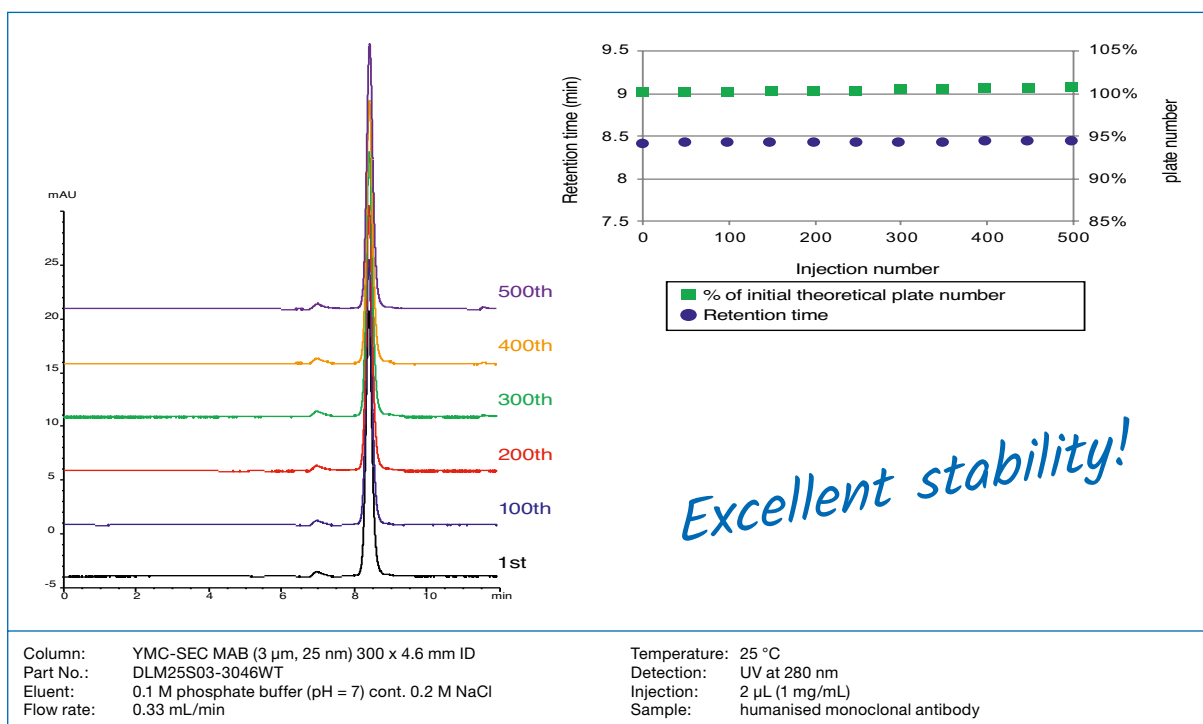
# SEC – YMC-SEC MAB: Reproducibility & Stability

## Excellent lot-to-lot reproducibility



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

## High Column Stability



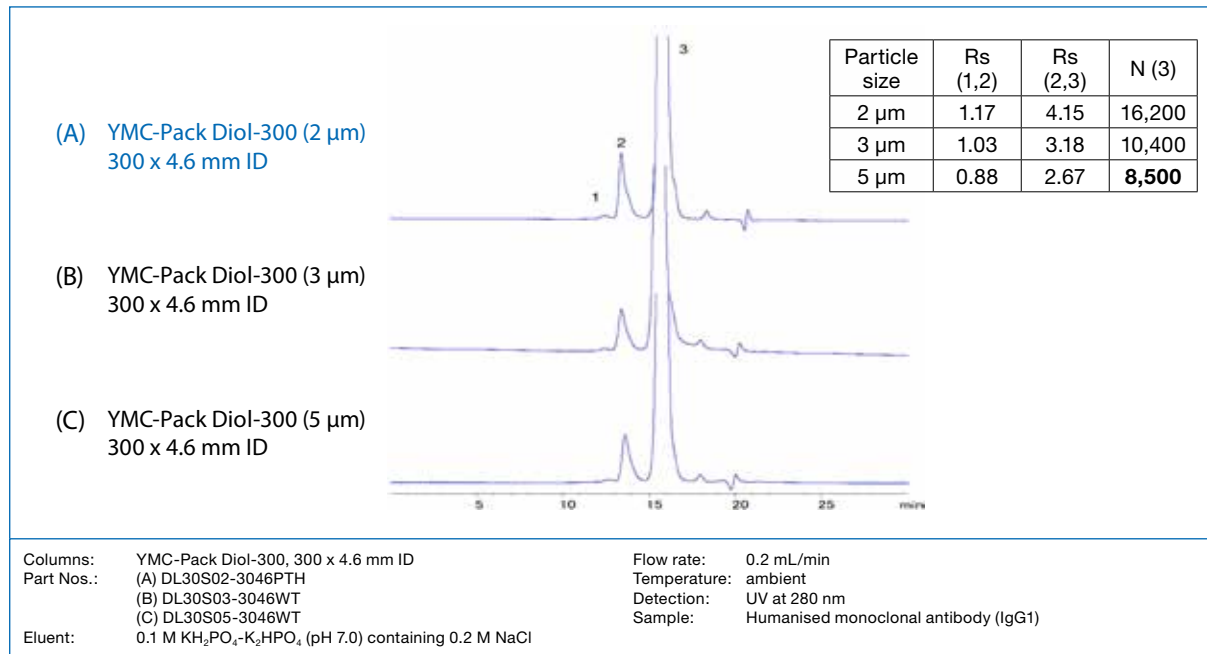
Excellent stability!

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

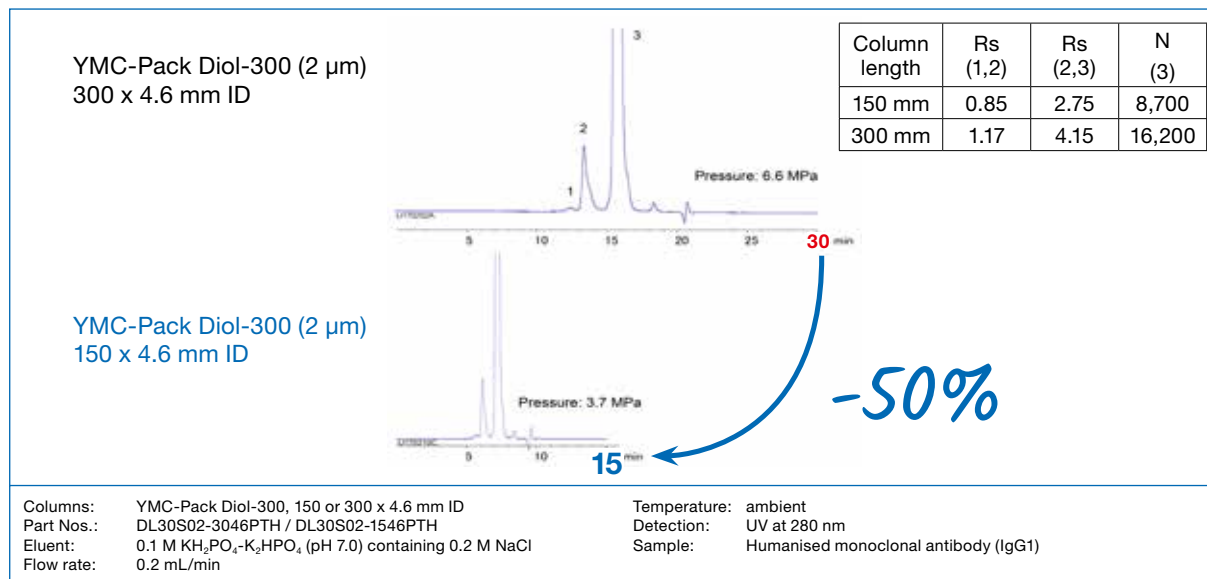
### Higher resolution for analysis of monoclonal antibodies



**A**ll 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

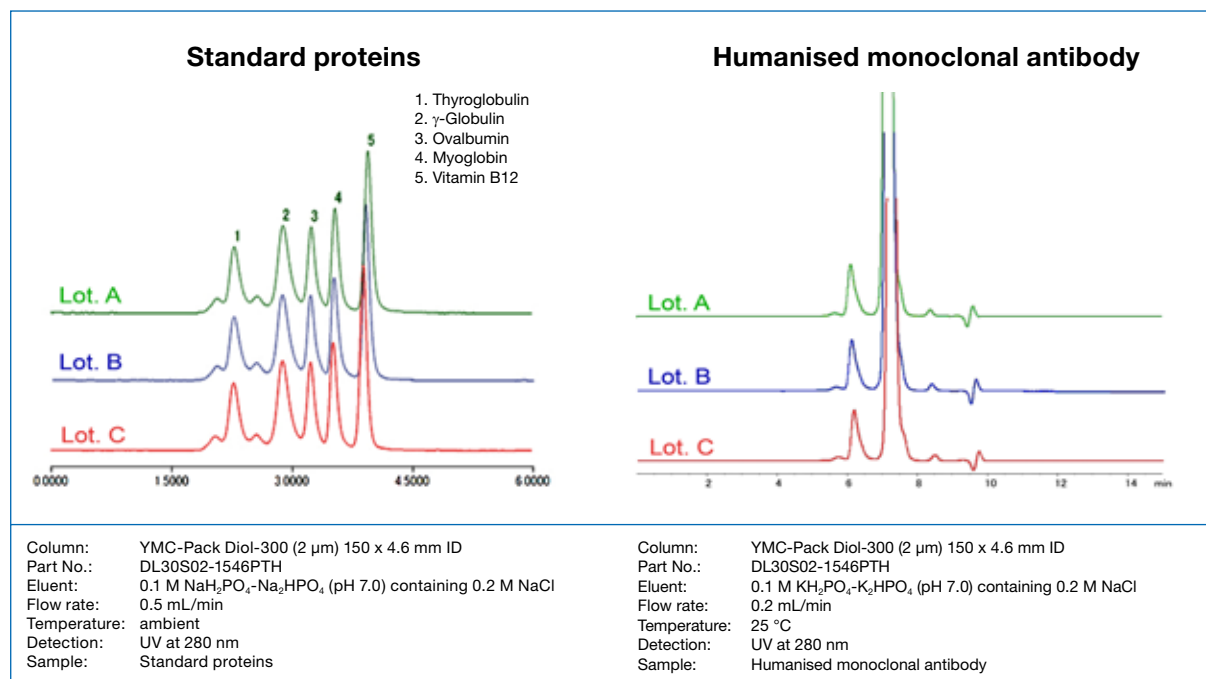


By using a 150 mm length column, 50% shorter run times can be achieved with the same resolution as for a 5 µm 300 mm length column (compare upper and lower chromatograms). This allows an increase in throughput to be achieved. The backpressure is only 6.6 MPa, even for the 300 mm column. Therefore, YMC-Pack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

# SEC – YMC-Pack Diol: Reproducibility & stability

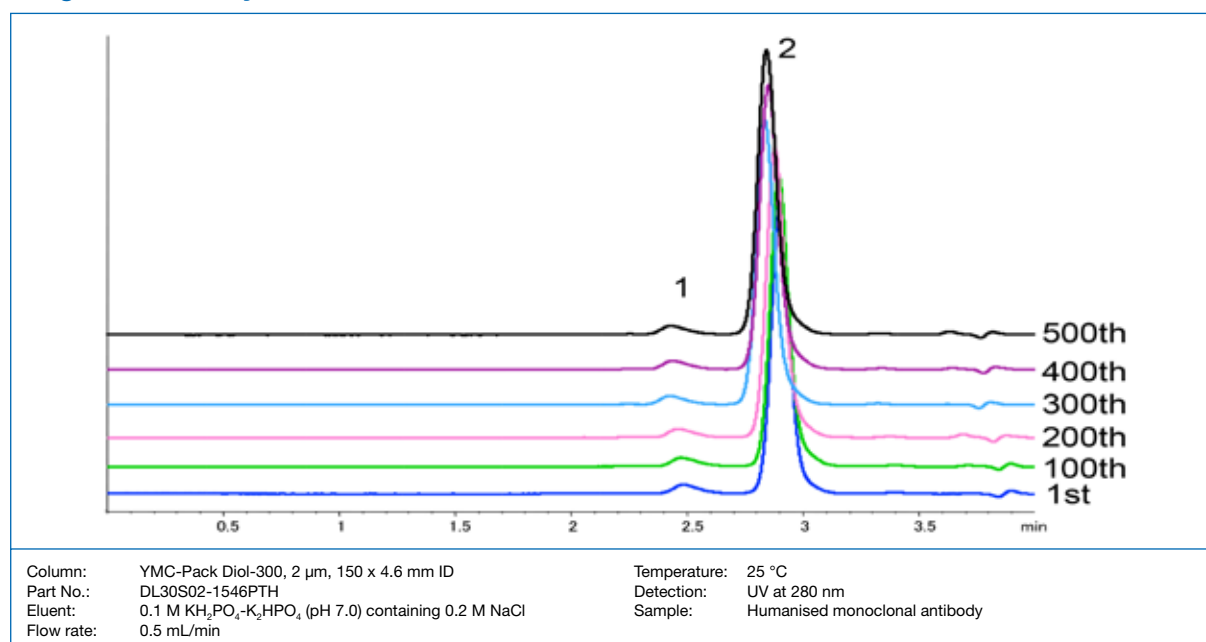
## Reproducibility and stability data

### Excellent batch to batch reproducibility



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

### Long-term stability



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 – YMC-Pack Diol: Reproducibility & stability

## 2 µm UHPLC columns

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

## 3 µm HPLC columns

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

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

\*\*no holder required for 30 x 8 mm ID guards

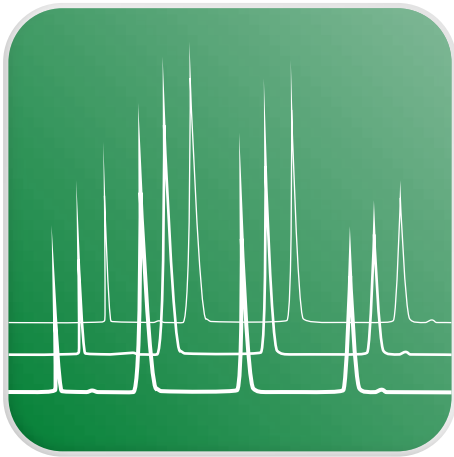
## 5 µm HPLC columns

Phase	Column ID [mm]	Column length [mm]			Guard cartridges* with 10/30 mm length (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**

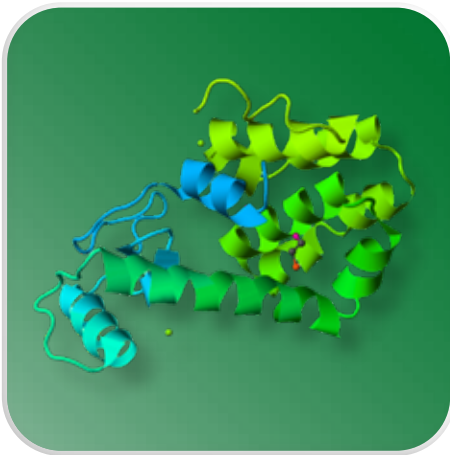
\*Guard cartridge holder required, part no. XPGCH-Q1

\*\*no holder required for 30 x 8 mm ID guards



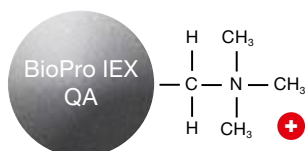


IEX

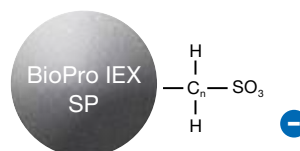


# IEX – Bio Pro Series

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



strong anion exchanger



strong cation exchanger

	BioPro IEX QA	BioPro IEX SP
Matrix	hydrophilic polymer (polymethacrylate)	hydrophilic polymer (polymethacrylate)
Particle size / $\mu\text{m}$	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	$\text{Cl}^-$	$\text{Na}^+$
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  $\mu\text{m}$  for preparative scale



Porous polymer beads

	BioPro IEX QF	BioPro IEX SF
Matrix	hydrophilic polymer (polymethacrylate)	hydrophilic polymer (polymethacrylate)
Particle size / $\mu\text{m}$	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	$\text{Cl}^-$	$\text{Na}^+$
Available pH range	2.0 - 12.0	2.0 - 12.0
Temperature range	4 – 60 °C	
Pressure limit	3 $\mu\text{m}$ : 25 MPa (3,625 psi) 5 $\mu\text{m}$ : 6 – 12 MPa (870 – 1,740 psi)	
Column hardware	PEEK	



Non-porous polymer beads

## General

YMC's BioPro IEX series of ion exchange columns are available in QA and SP chemistries, based on 5  $\mu\text{m}$  porous (QA or SP columns) or on 3 or 5  $\mu\text{m}$  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.

# IEX – BioPro IEX: Reproducibility & DBC

## High binding capacity and high recovery for porous type

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

## Comparison of dynamic binding capacity (DBC) for BSA

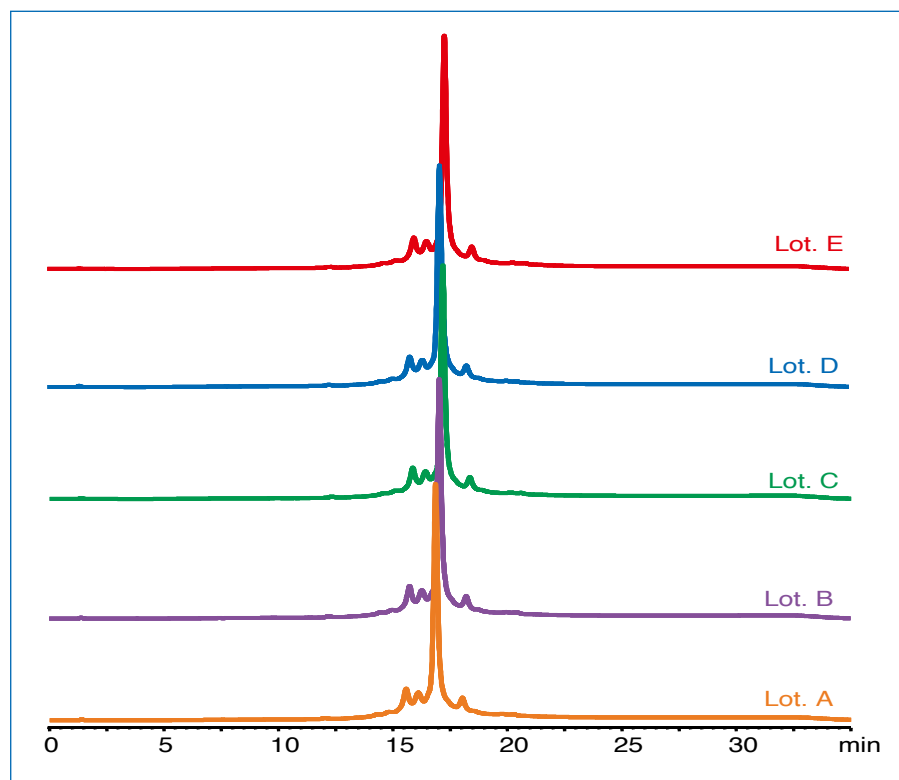
	Dynamic binding capacity (mg/mL-gel, 10% breakthrough)	Eluted amount (mg/mL-gel)	Recovery* (%)
BioPro IEX QA	126	120	95
Mono Q (GE Healthcare)	100	35	35
BioAssist Q (Tosoh Bioscience)	73	58	79

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

*High recovery rates for BioPro IEX*

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

## Excellent batch-to-batch reproducibility



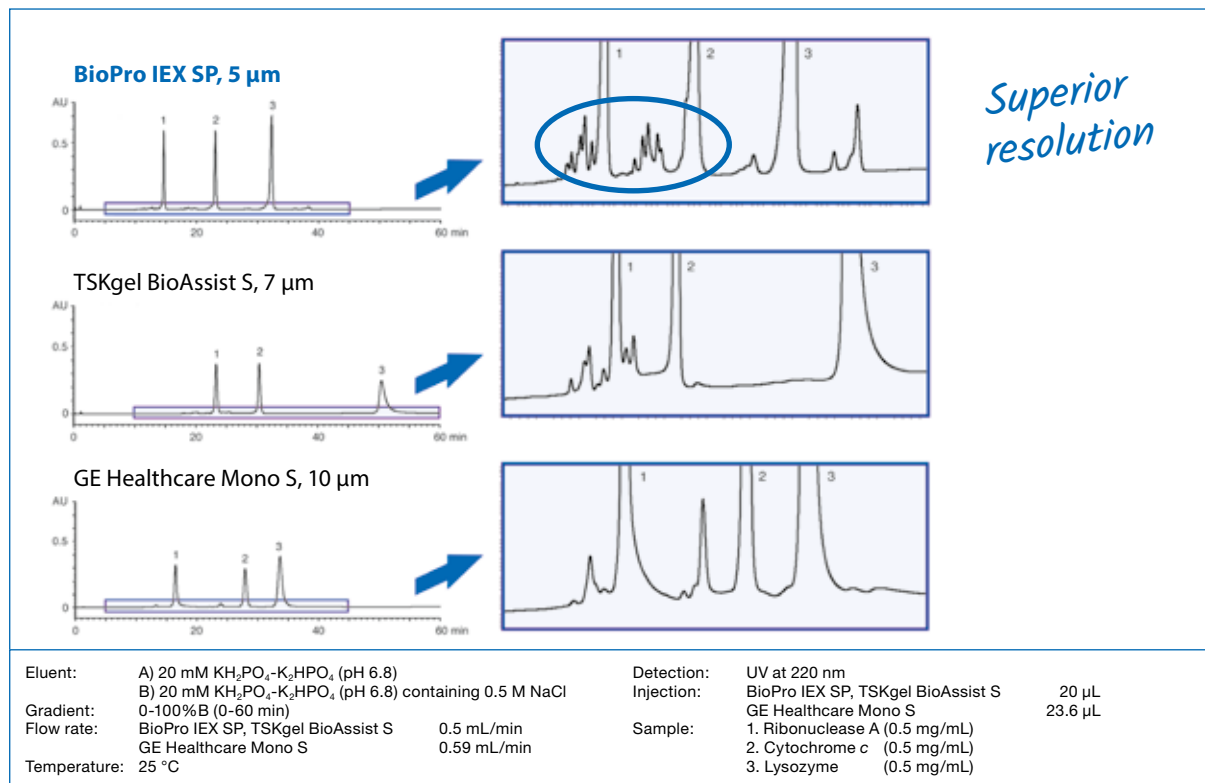
Column: BioPro IEX SF (5 µm) 100 x 4.6 mm ID  
 Part No.: SF00S05-1046WP  
 Eluent: A) 20 mM NaH<sub>2</sub>PO<sub>4</sub>-Na<sub>2</sub>HPO<sub>4</sub> (pH 6.5)  
 B) 20 mM NaH<sub>2</sub>PO<sub>4</sub>-Na<sub>2</sub>HPO<sub>4</sub> (pH 6.5) containing 0.2 M NaCl  
 Gradient: 0-50%B (0.5-30 min)  
 Flow rate: 0.5 mL/min (180 cm/hr)  
 Temperature: 25 °C  
 Detection: UV at 215 nm  
 Injection: 10 µL  
 Sample: monoclonal antibody (IgG1)

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

# IEX – BioPro IEX: Resolution & throughput

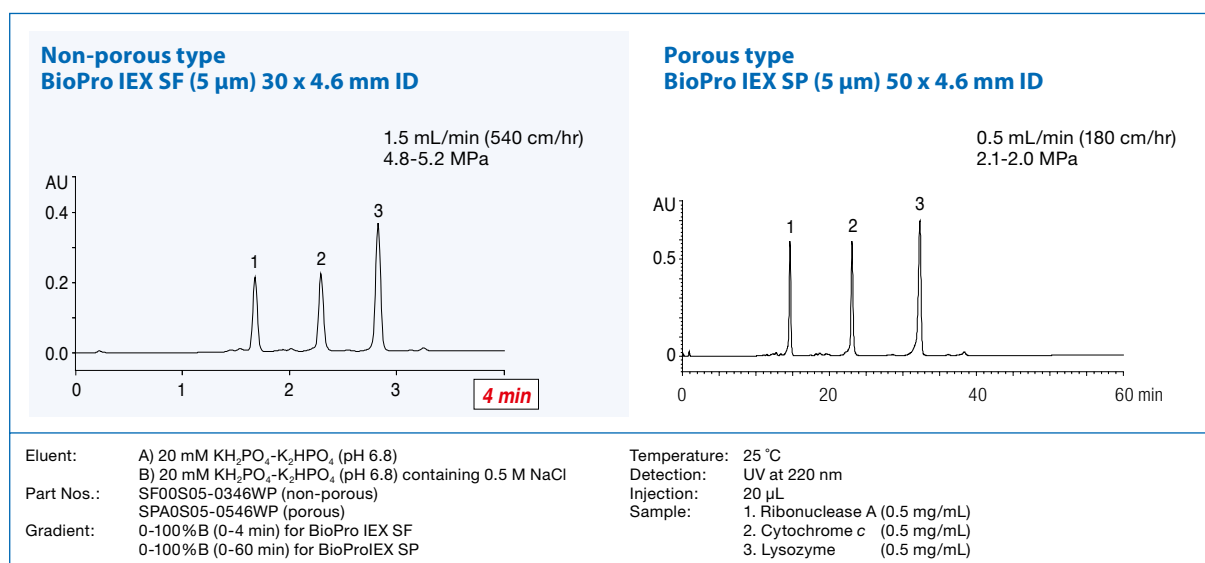
## Superior resolution

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



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

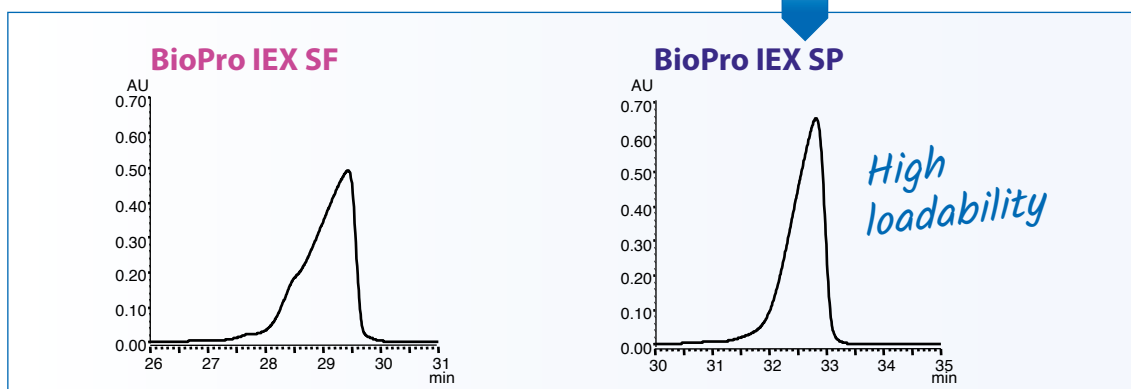
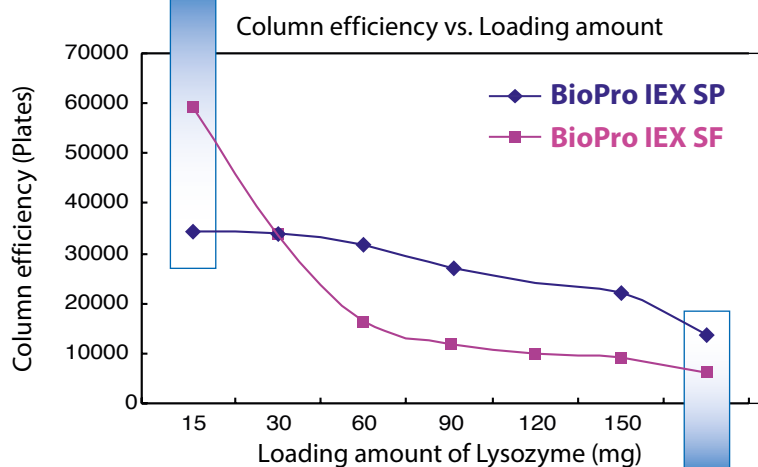
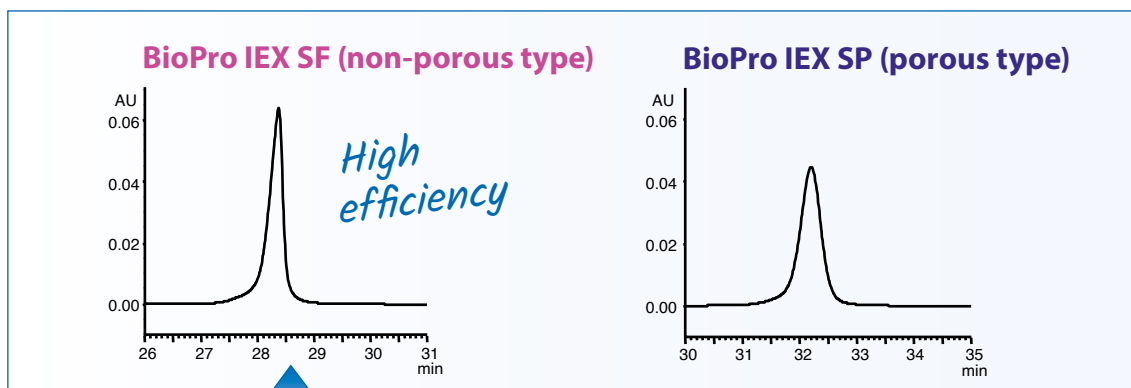
## Ultra-high-throughput analysis with non-porous BioPro IEX



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



Eluent: A) 20 mM  $\text{NaH}_2\text{PO}_4$ - $\text{Na}_2\text{HPO}_4$  (pH 6.8)  
 B) 20 mM  $\text{NaH}_2\text{PO}_4$ - $\text{Na}_2\text{HPO}_4$  (pH 6.8) containing 0.5 M NaCl  
 Gradient: 0–100%B (0–60 min)  
 Flow rate: 0.5 mL/min  
 Temperature: 25°C

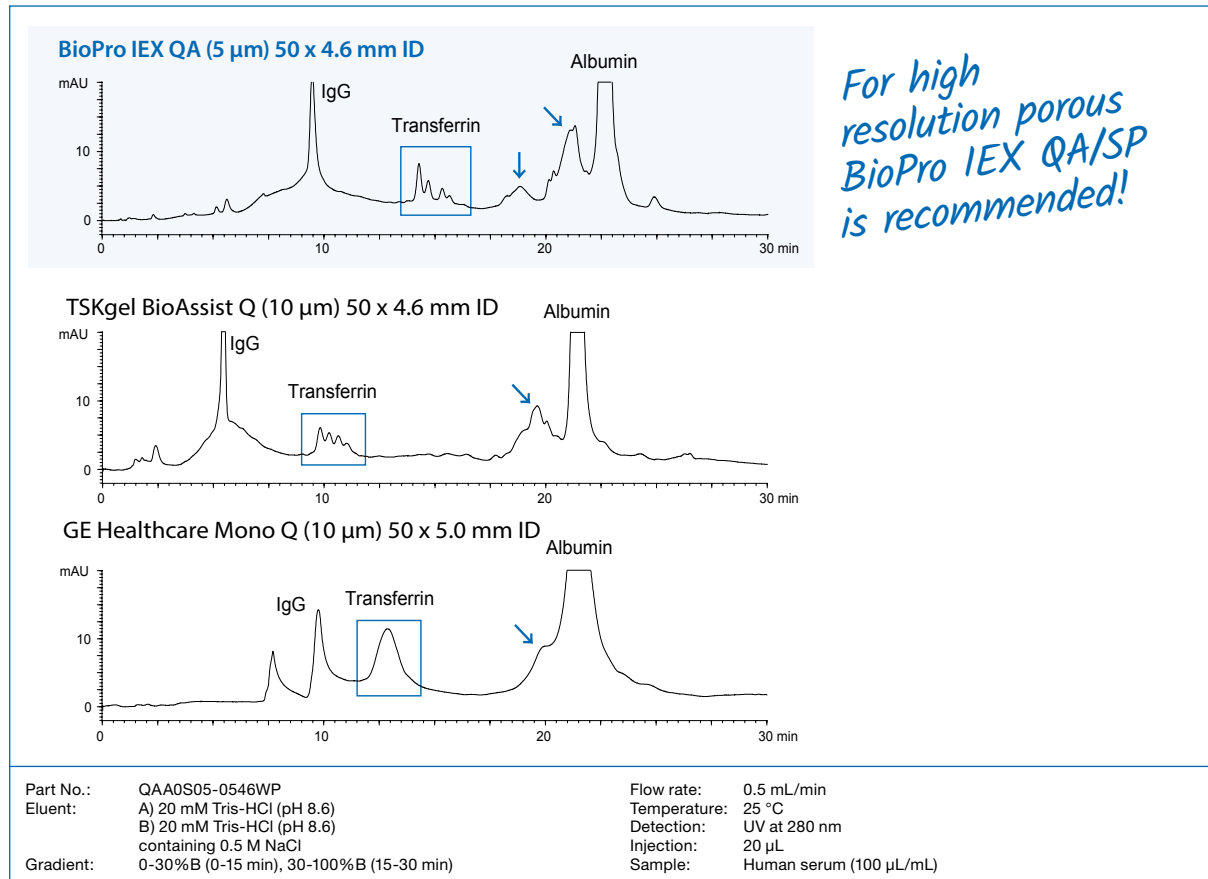
Detection: UV at 280 nm  
 Injection: 100 mL  
 Sample: 1. Ribonuclease A  
 2. Cytochrome c  
 3. Lysozyme

**BioPro IEX SF offers outstanding column efficiency at small amount of sample loading. Non-porous type of BioPro IEX columns are especially suitable for microscale analysis which requires higher resolution. BioPro IEX SP maintains the good peak shape even when the loading amount increases. Porous type BioPro IEX columns with high capacity are useful for high-load analytical separations and laboratory-scale purification.**

# IEX – BioPro IEX: Challenging separations

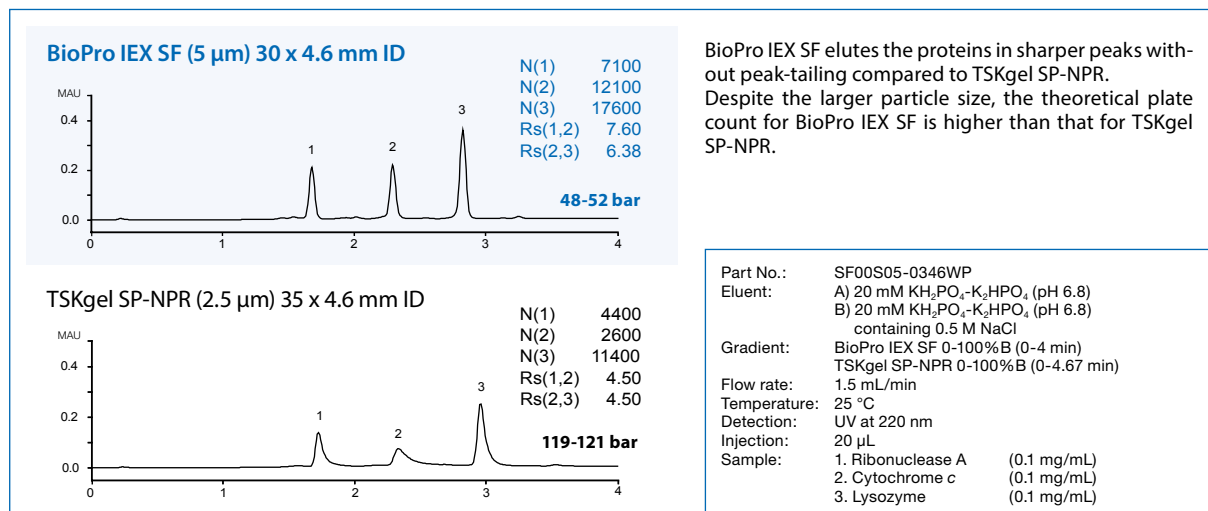
## Protein separation in challenging matrices

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



## Better performance at lower backpressure

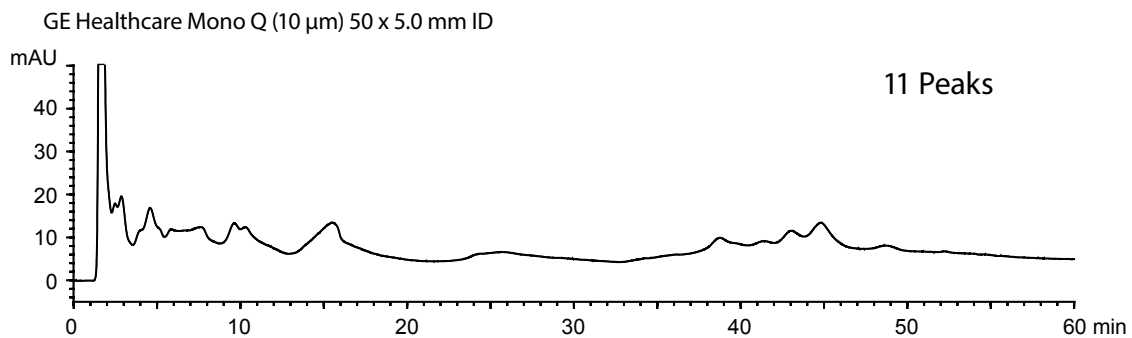
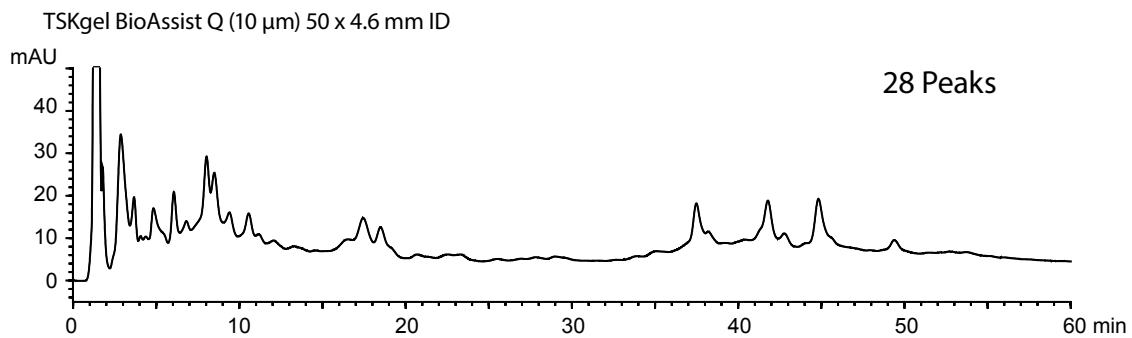
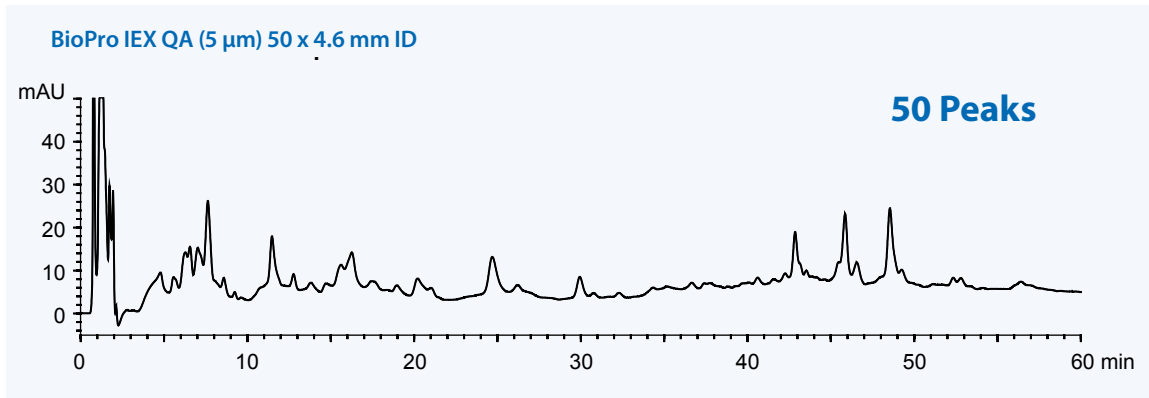
### Comparison of standard protein separation on BioPro IEX SF and a commercial SP-type product



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

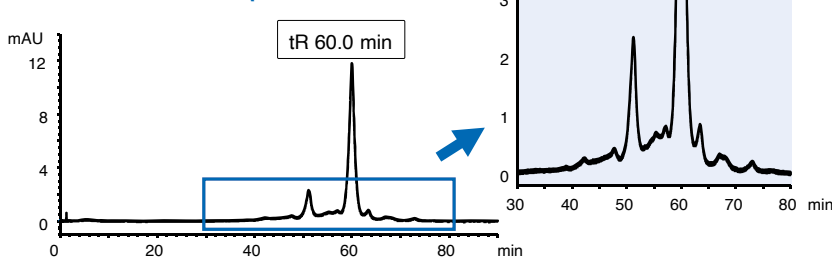


Part No.: QAA0S05-0546WP  
Eluent: A) 20 mM Tris-HCl (pH 8.6)  
B) 20 mM Tris-HCl (pH 8.6) containing 0.5 M NaCl  
Gradient: 0-15%B (0-30 min), 15-60%B (30-60 min)  
Flow rate: 0.5 mL/min  
Temperature: 25 °C  
Detection: UV at 220 nm  
Injection: 20  $\mu$ L  
Sample: Tryptic digest of BSA

# IEX – BioPro IEX: Antibody analysis

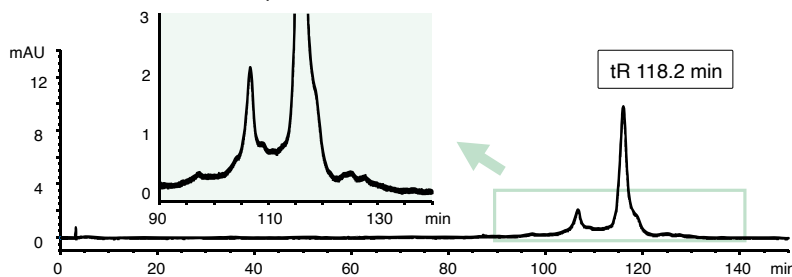
## Monoclonal antibody analysis with non-porous cation exchange columns

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



*Higher Resolution!*

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

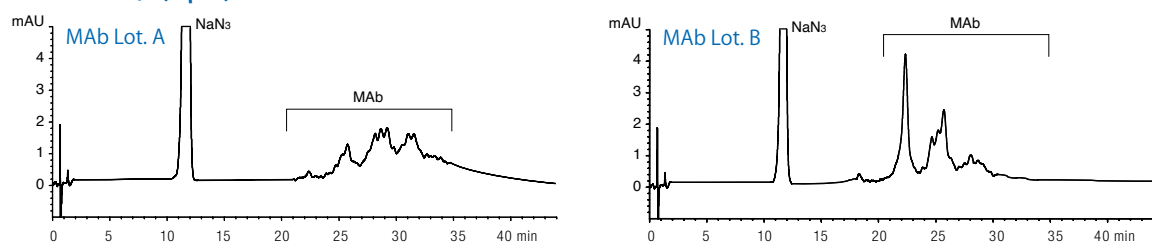


Part No.:	SF00S05-1046WP	Temperature:	30 °C
Eluent:	A) 20 mM MES-NaOH (pH 5.6) B) 20 mM MES-NaOH (pH 5.6) containing 0.2 M NaCl	Detection:	UV at 280 nm
Gradient:	35%B (70 mM NaCl)	Injection:	10 µL
Gradient slope:	0.25%B/min (0.5 mM NaCl)	Sample:	Humanized monoclonal IgG1 (1 mg/mL)
Flow rate:	180 cm/hr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

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

## QC of monoclonal antibodies with non-porous BioPro IEX QF

BioPro IEX QF (5 µm) 100 x 4.6 mm ID



Part No.:	QF00S05-1046WP	Temperature:	25 °C
Eluent:	A) 20 mM Tris-HCl (pH 8.1) B) 20 mM Tris-HCl (pH 8.1) containing 0.5 M NaCl	Detection:	UV at 220 nm
Gradient:	10-25%B (0-60 min)	Injection:	14 µL (0.1 mg/mL)
Flow rate:	1.0 mL/min (360 cm/hr)	Sample:	Mouse monoclonal IgG1 anti-human IgG4 (Purified by DEAE chromatography, containing NaNa <sub>3</sub> )

Two different batches of commercially available MAb purified by DEAE chromatography were analyzed on a BioPro IEX QF column (100 mm length).

The MAb was separated into several peaks, and the batch-to-batch variability is observed. The BioPro IEX QF/SF 100 mm length columns, which have high efficiency, are ideal for characterization of glycoproteins, such as monoclonal antibodies, and for quality control assessment of biopharmaceuticals.



## Optimisation of oligonucleotide separations on ion exchange chromatography

Non-porous anion exchange column is generally suitable for analysis of oligonucleotides. For optimisation of single-stranded DNA and RNA of about 20 mer some conditions such as type of mobile phase and column temperature can be changed.

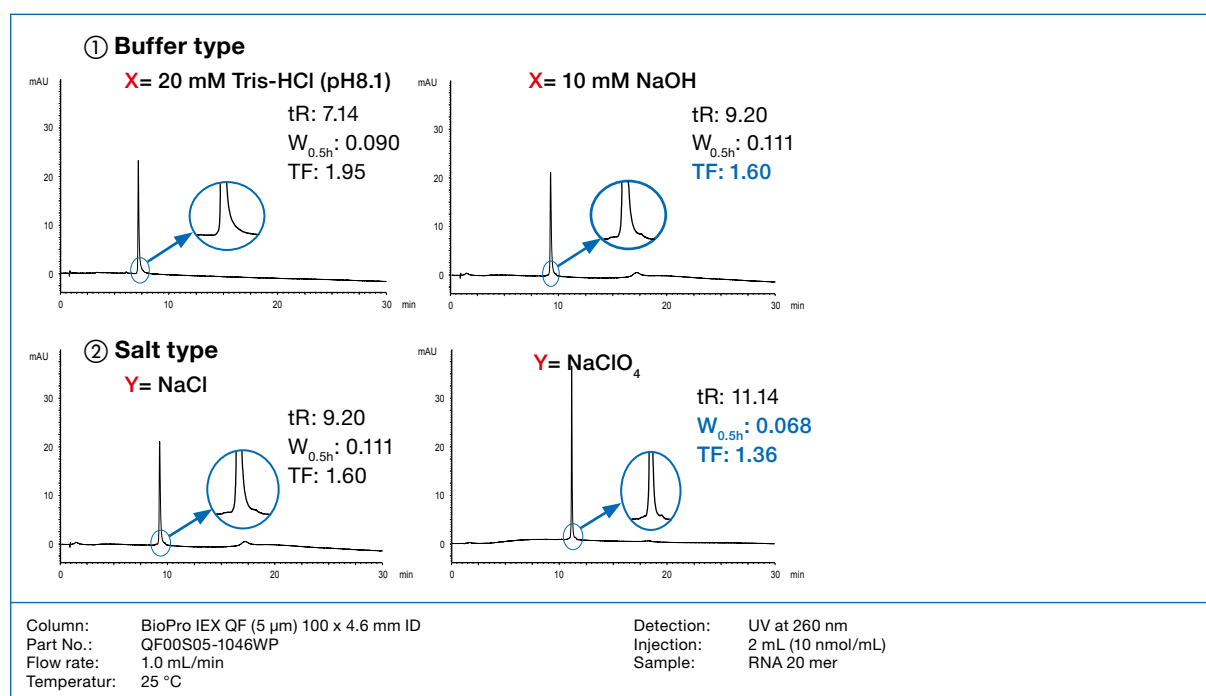
### Sample Group 1 (Phosphodiester oligonucleotides; PO)

1	Single-stranded DNA	5'-TCATCACACTGAATACCAAT-3' (DNA 20 mer)
2	(ssDNA)	5'-GTCATCACACTGAATACCAAT-3' (DNA 21 mer)
3		5'-UCAUCACACUGAAUACCAU-3' (RNA 20 mer)
4	Single-stranded RNA	5'-GUCAUCACACUGAAUACCAU-3' (RNA 21 mer)
5	(ssRNA)	5'-U(M)C(M)A(M)U(M)C(M)A(M)C(M)A(M)C(M)U(M)G(M)A(M)A(M)U(M)A(M)C(M)C(M)A(M)A(M)U(M)-3' (2'-OMe RNA 20 mer)
6		5'-G(M)U(M)C(M)A(M)U(M)C(M)A(M)C(M)A(M)C(M)U(M)G(M)A(M)A(M)U(M)A(M)C(M)C(M)A(M)A(M)U(M)-3' (2'-OMe RNA 21 mer)

N(M)=2'-OMe RNA

## 1 Improvement of peak tailing

By changing the buffer from 20 mM Tris-HCl (pH 8.1) to 10 mM NaOH, the tailing factor for an oligonucleotide is reduced. Furthermore, the peak tailing is further suppressed when NaClO<sub>4</sub> was added to 10 mM NaOH instead of NaCl.



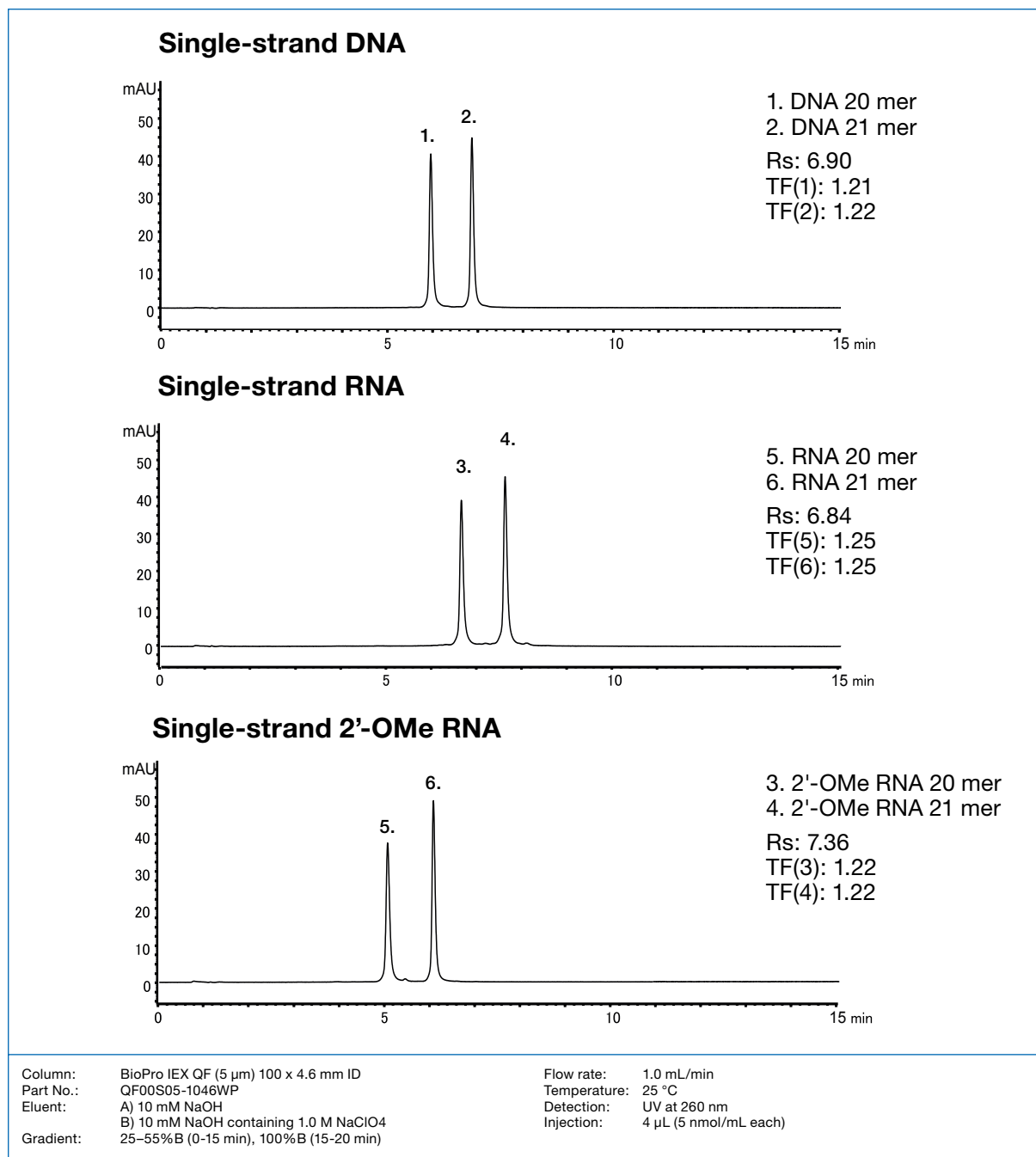
## 2 Improvement of carry over

When the initial gradient concentration of NaCl is low (ex. 50 mM), carryover is observed. By increasing the initial gradient concentration of NaCl up to 400 carryover can be avoided with good reproducibility.

## IEX – Expert Tips: Oligonucleotides

### 3 Examples under optimised conditions

Each ssDNA, ssRNA and 2'-OMe ssRNA with single-base difference in length can be separated under the optimised condition.



### 4 Optimum conditions

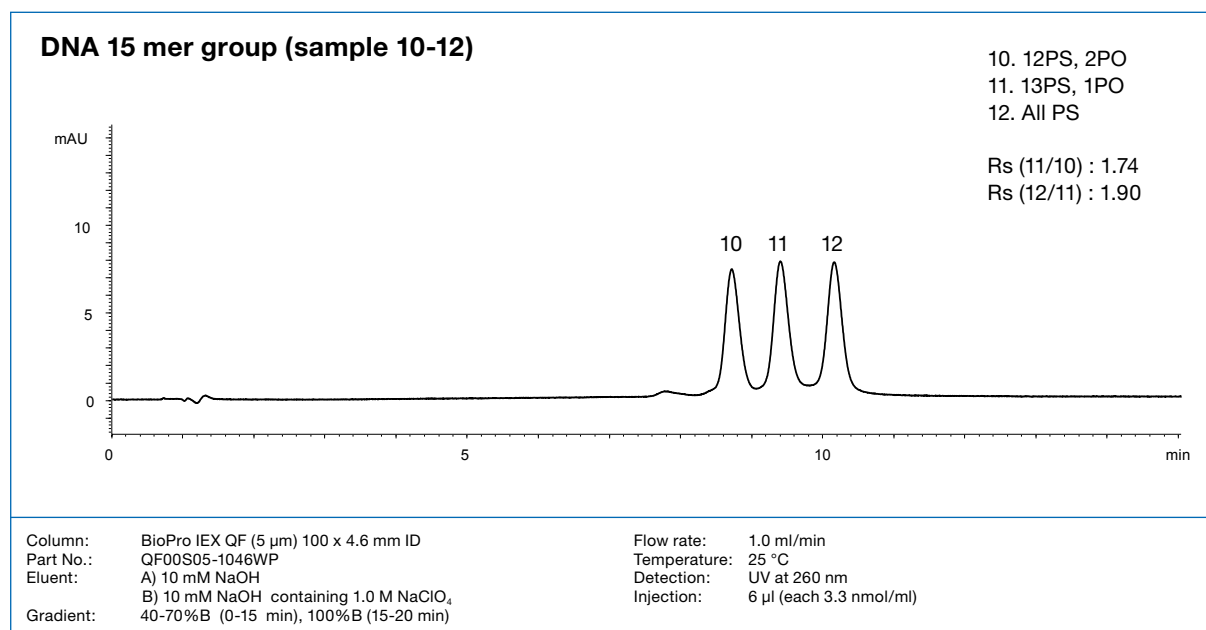
10 mM NaOH (as buffer solution), NaClO<sub>4</sub> (as salt) and higher initial gradient concentration of salt are preferable to suppress carryover and peak tailing.



## IEX – Expert Tips: Oligonucleotides

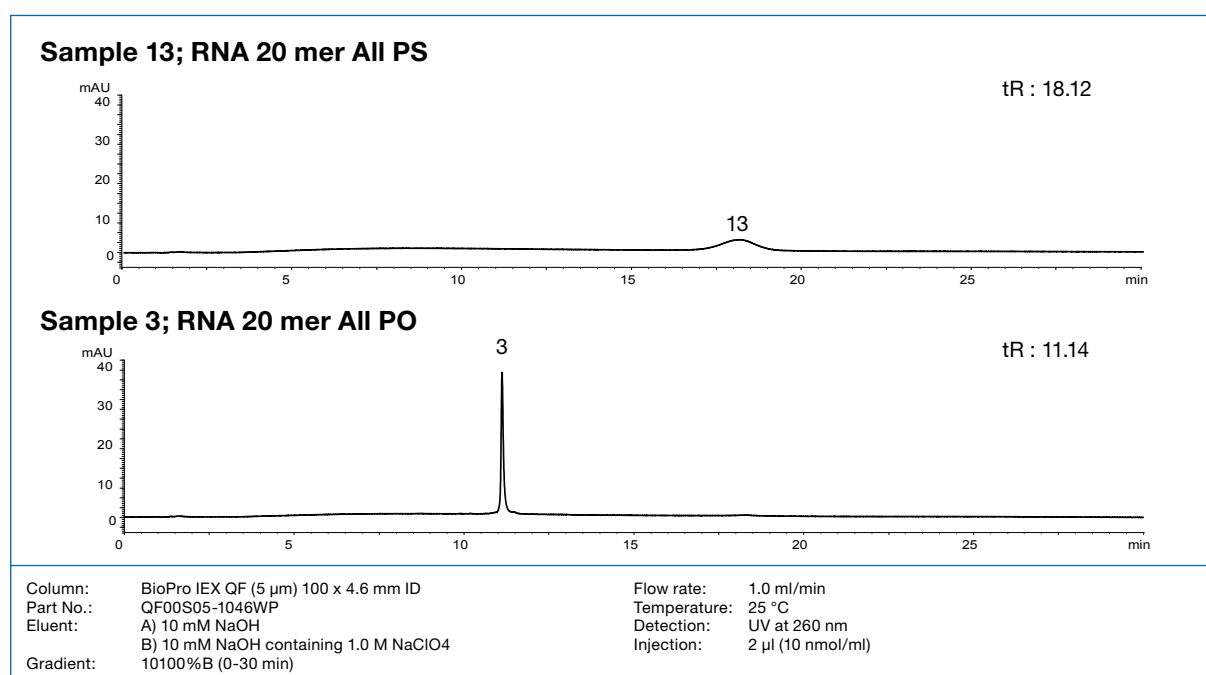
### 6 Resolution of phosphorothioate oligonucleotides with different degrees of thiolation

Using the optimised conditions described, [all PS], [13PS, 1PO] and [12PS, 2PO] of DNA 15mer (samples 10–12) were clearly separated by ion exchange chromatography.



### 7 Difference in required salt concentrations for eluting modified RNA (all PS) and normal RNA (all PO)

The separation of RNA 20mer all PS is compared with RNA 20mer all PO under the same conditions. Since acidity of all PS is much higher than all PO, a higher salt concentration is required for elution. The peak of all PS is much broader because it is thought that all PS contains as many as 219 (524, 288) stereoisomers.



## 8 Separation of optimisation trial of phosphorothioate oligonucleotides with single-base differences in length

A steeper gradient curve, increasing column temperature and adding organic solvent can improve peak separation. However, increase of organic solvent ratio gave little improvement in peak separation.

X/Y=100/0  
32–80%B (0–24 min)  
=  $\Delta 20$  mM NaClO<sub>4</sub>/min  
25 °C



**Steep gradient curve**  
X/Y=100/0  
32–80%B (0–8 min)  
=  $\Delta 60$  mM NaClO<sub>4</sub>/min  
25 °C



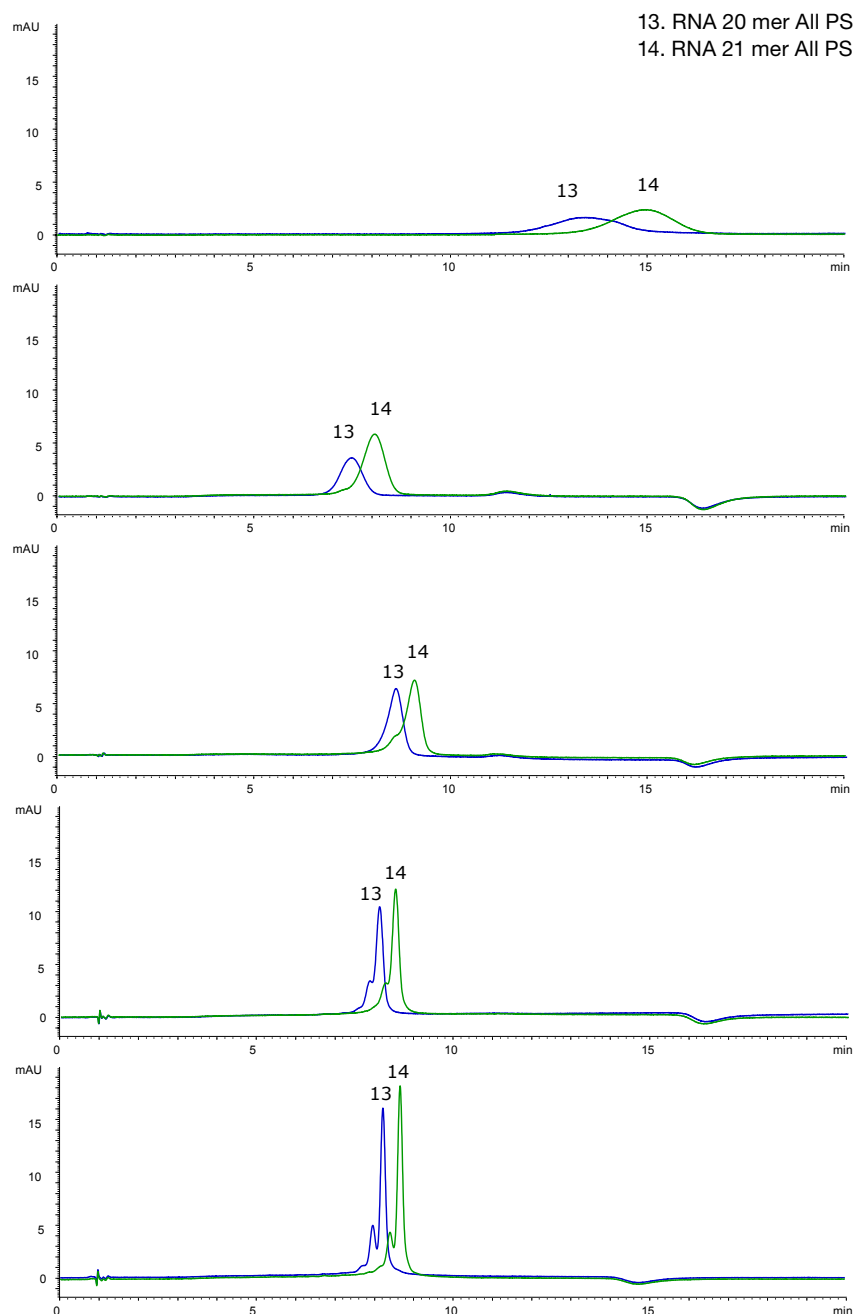
**Raising column temperature**  
X/Y=100/0  
32–80%B (0–8 min)  
=  $\Delta 60$  mM NaClO<sub>4</sub>/min  
60 °C



**Addition of organic solvent**  
X/Y=80/20  
40–100%B (0–8 min)  
=  $\Delta 60$  mM NaClO<sub>4</sub>/min  
60 °C



**Increasing ratio of organic solvent**  
X/Y=70/30  
40–100%B (0–6.3 min)  
=  $\Delta 60$  mM NaClO<sub>4</sub>/min  
60 °C



Column: BioPro IEX QF (5  $\mu$ m) 100 x 4.6 mm ID  
Part No.: QF00S05-1046WP  
Eluent: A) 10 mM NaOH/methanol (X/Y)  
B) 10 mM NaOH containing  
Gradient: 1.0 M NaClO<sub>4</sub>/methanol (X/Y)  
Flow rate: 1.0 ml/min  
Detection: UV at 260 nm  
Injection: 2  $\mu$ l (10 nmol/ml)

# IEX – Ordering information

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

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

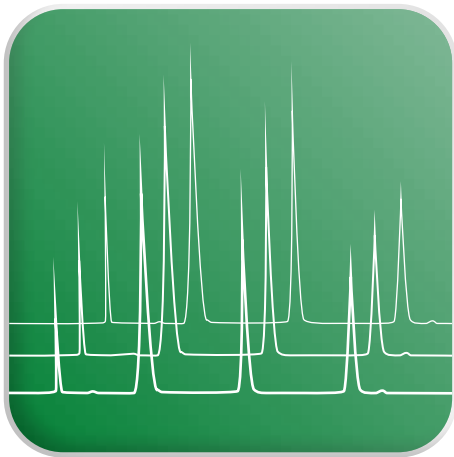
## 5 µm non-porous analytical columns in PEEK hardware (max. pressure 60–120 bar)

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

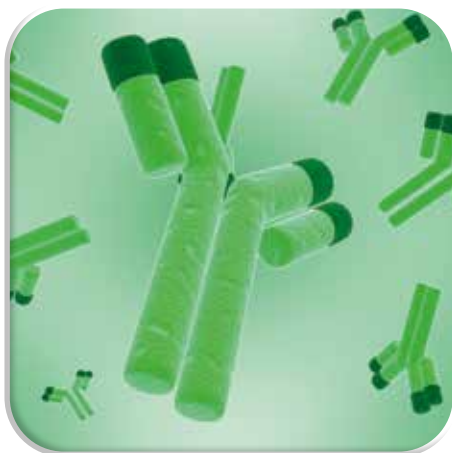
## 5 µm porous analytical columns in PEEK hardware (max. pressure 25–35 bar)

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

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



HIC



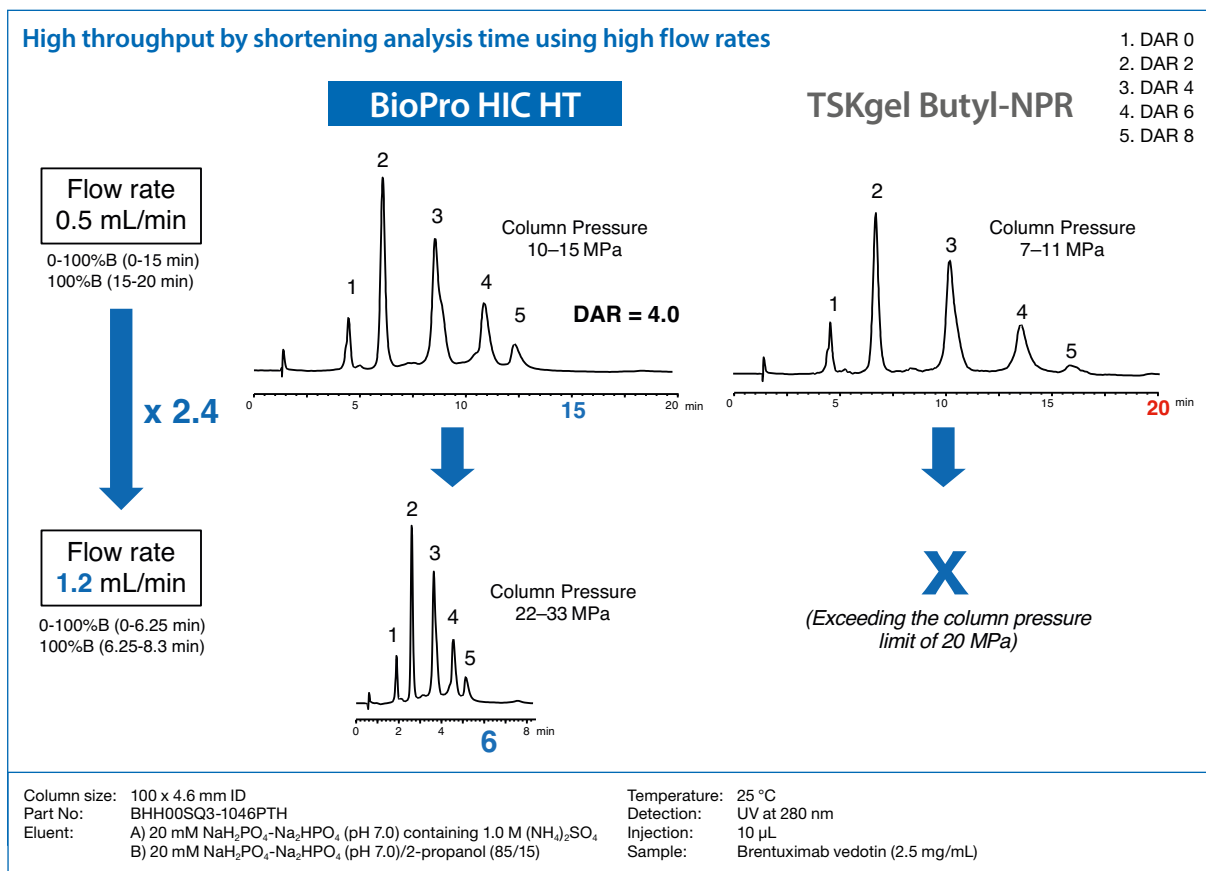
# HIC – BioPro Series

- 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 / $\mu\text{m}$	2.3	4
Pore	non-porous	non-porous
Functional group	butyl	butyl
pH range	2-12	2-12
Pressure limit	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

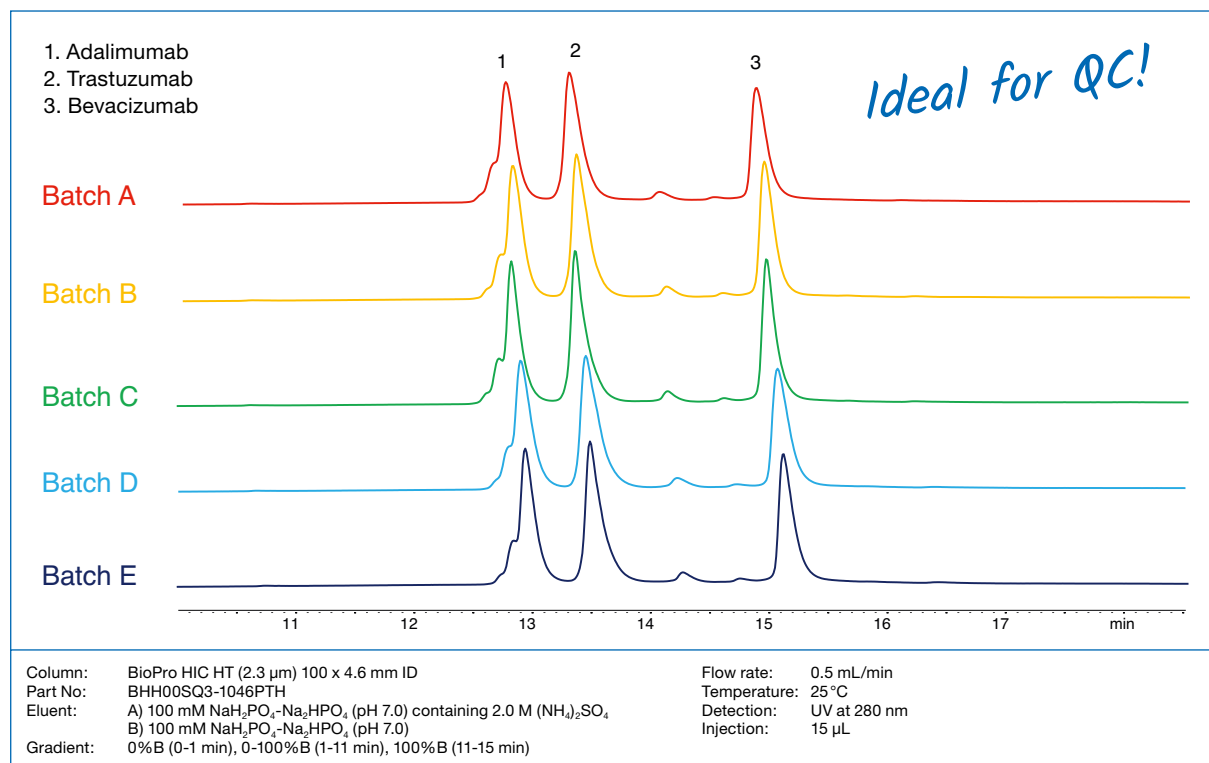


**BioPro HIC HT improves analysis throughput of ADCs by 2–3 times with an excellent Drug-to-Antibody Ratio (DAR). The rapid analysis is possible without loss of resolution. Competitor HIC columns fail under this conditions.**



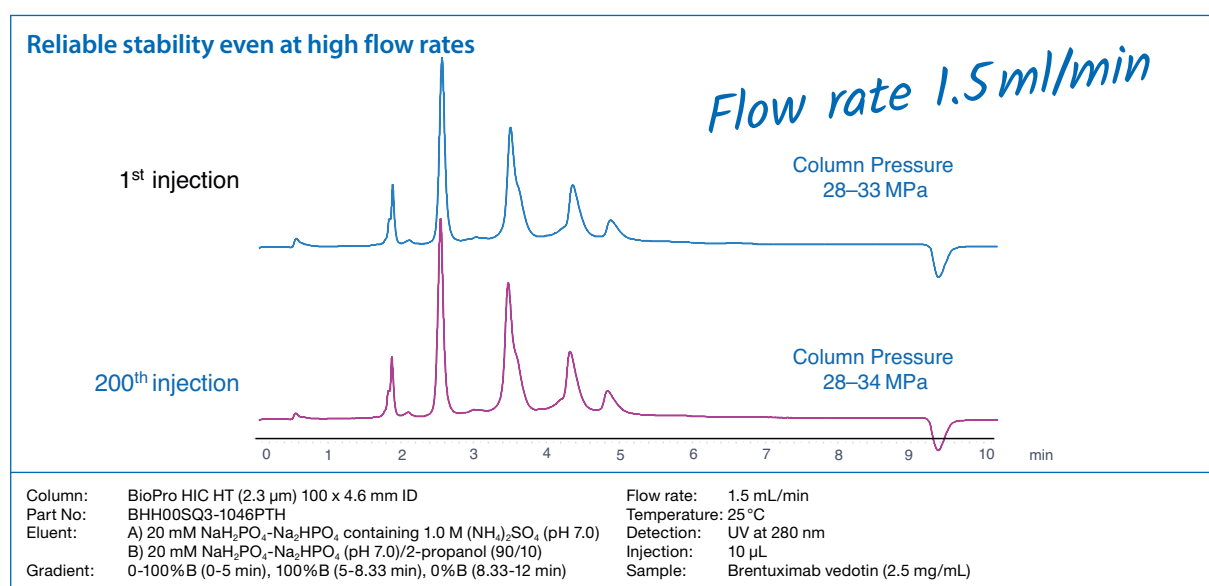
# HIC – BioPro HIC: Reproducibility & stability

## 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



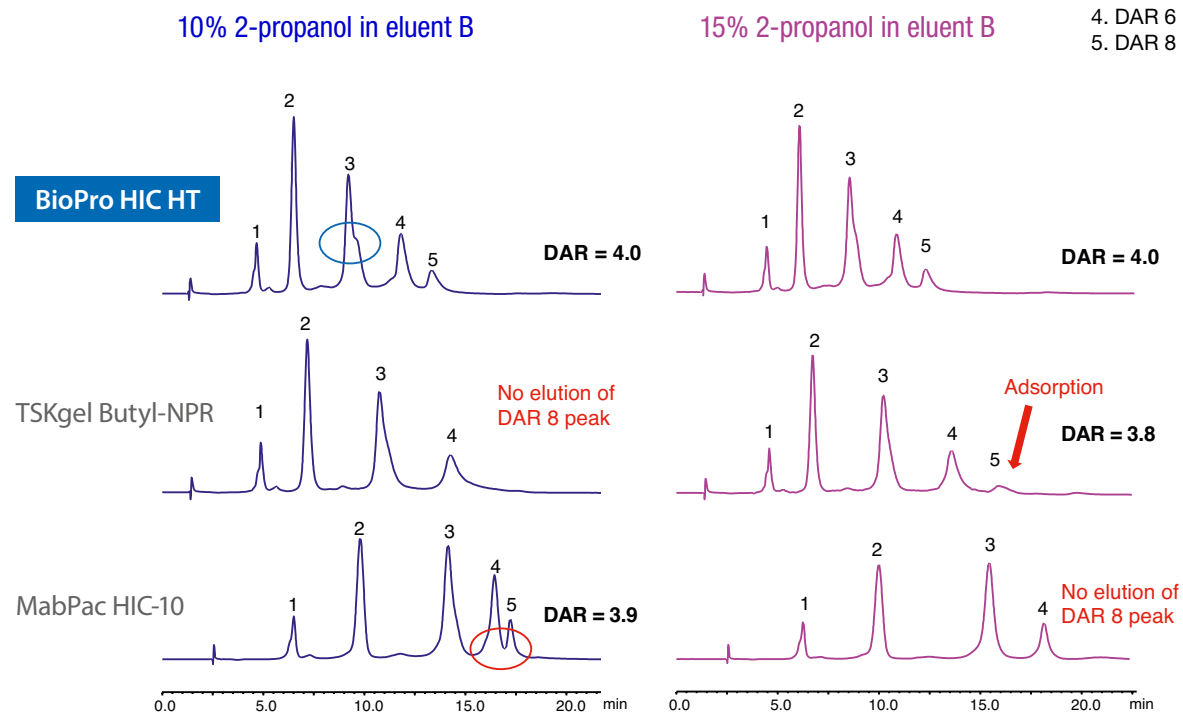
BioPro HIC HT offers excellent stability under high flow rates/high pressure conditions due to its unique rigid particle and optimised column packing technology.

# 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 size: 100 x 4.6 mm ID  
 Part No: BHH00SQ3-1046PTH  
 Eluent: A) 20 mM NaH<sub>2</sub>PO<sub>4</sub>-Na<sub>2</sub>HPO<sub>4</sub> (pH 7.0) containing 1.0 M (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>  
 B) 20 mM NaH<sub>2</sub>PO<sub>4</sub>-Na<sub>2</sub>HPO<sub>4</sub> (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.

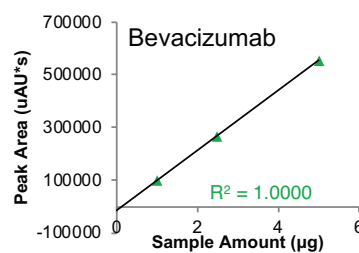
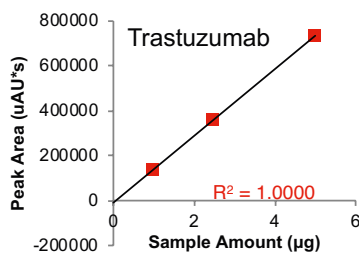
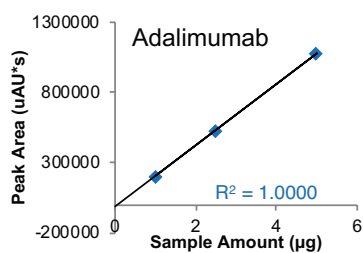
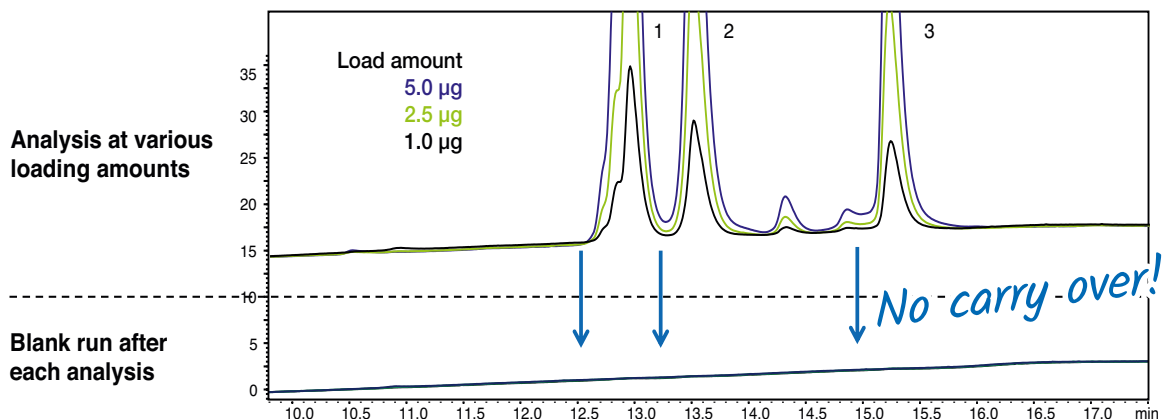
### BioPro HIC HT offers:

- 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

1. Adalimumab
2. Trastuzumab
3. Bevacizumab



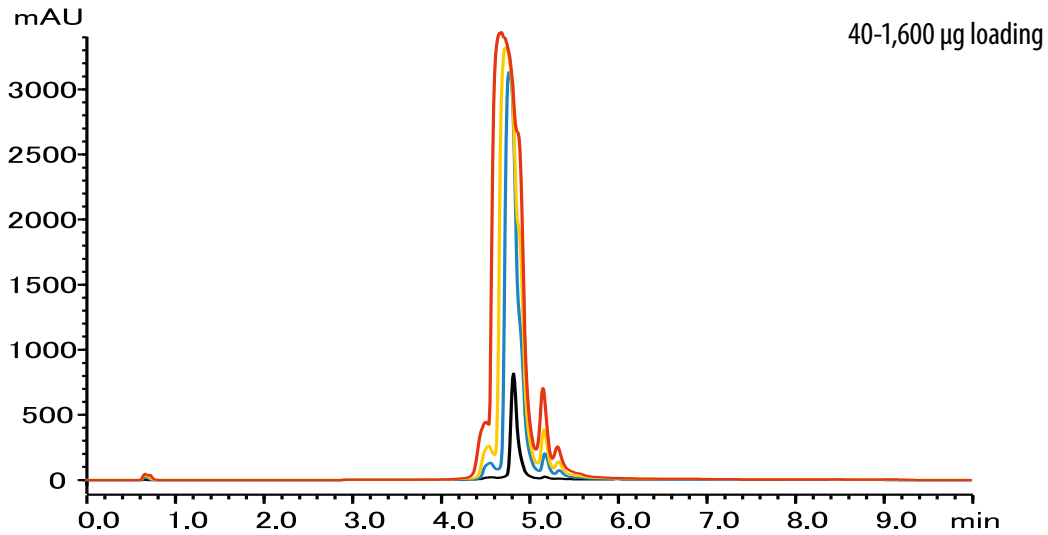
Column: BioPro HIC HT (2.3 µm) 100 x 4.6 mm ID  
 Part No: BHH00SQ3-1046PTH  
 Eluent: A) 100 mM NaH<sub>2</sub>PO<sub>4</sub>-Na<sub>2</sub>HPO<sub>4</sub> (pH 7.0) containing 2.0 M (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>  
 B) 100 mM NaH<sub>2</sub>PO<sub>4</sub>-Na<sub>2</sub>HPO<sub>4</sub> (pH 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 carryover. This contributes to highly accurate quantitation of ADCs and antibodies.**

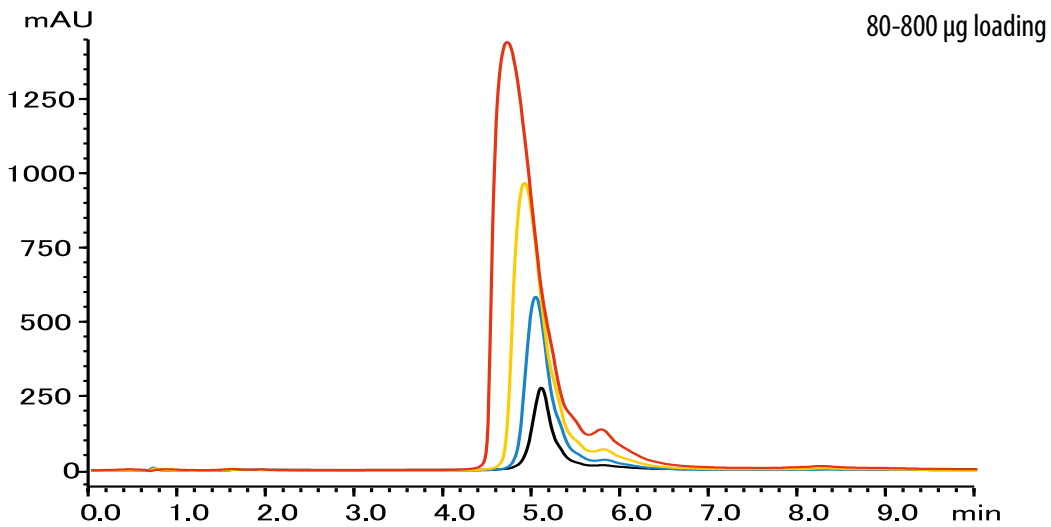
# HIC – BioPro HIC: Loadability

## High loadability

### Lysozyme



### Humanized monoclonal IgG



Column:	BioPro HIC BF (4 µm) 100 x 4.6 mm ID	
Part No.:	BHB00S04-1046WT	
Eluent:	A) 100 mM NaH <sub>2</sub> PO <sub>4</sub> -Na <sub>2</sub> HPO <sub>4</sub> (pH 7.0) containing 2.0 M (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> B) 100 mM NaH <sub>2</sub> PO <sub>4</sub> -Na <sub>2</sub> HPO <sub>4</sub> (pH 7.0)	
Gradient:	0-100%B (0-4.58 min), 100%B (4.58-6.58 min) for lysozyme 60%B(0-0.5 min), 60-100%B (0.5-7.5 min), 100%B (7.5-10 min) for IgG	
Flow rate:	1.2 mL/min	
Temperature:	25 °C for lysozyme 30 °C for IgG	
Detection:	UV at 280 nm	
Injection:	4, 40, 80, 160 µL for lysozyme 32, 80, 160, 320 µL for IgG	Sample: Lysozyme (10 mg/mL) Humanized monoclonal IgG (2.5 mg/mL)

**BioPro HIC BF provides superior peak shapes, even under high loading conditions. This allows detection of very tiny amounts of impurities in the sample.**

**In addition, it can be used for lab-scale purifications e.g. for isolation of variants for various research requirements (e. g. structural analysis).**

## The influence of salts in HIC separations

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

Certain non-denaturing salts are used to improve the hydrophobic interactions between proteins and the stationary phase. The mobile phase is typically an aqueous solution of salts such as ammonium sulfate or sodium chloride and a buffer to control pH (usually phosphate

buffer between pH 6 and 7). The Hofmeister series of lyotropic and chaotropic ions shown below in Fig. 1 provides a template for salt selection. High concentrations of salt, particularly ammonium sulfate, may precipitate proteins; therefore, solubility should be checked under the initial gradient (binding) conditions. The strength of the interaction between the protein and stationary phase decreases with decreasing salt gradient (see Fig. 2). Another option is a change of pH which results in an increase in the charge on the protein due to the ionisation of acidic groups.

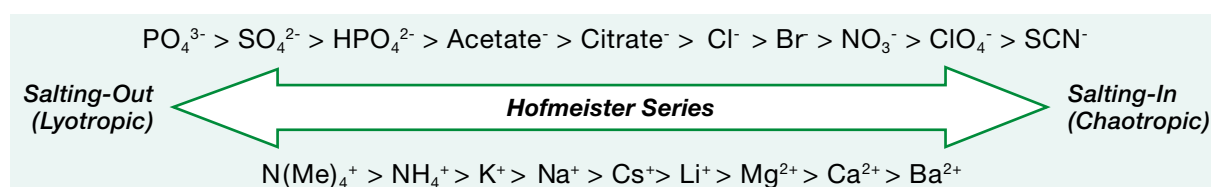


Fig. 1: The Hofmeister Series of lyotropic and chaotropic ions.

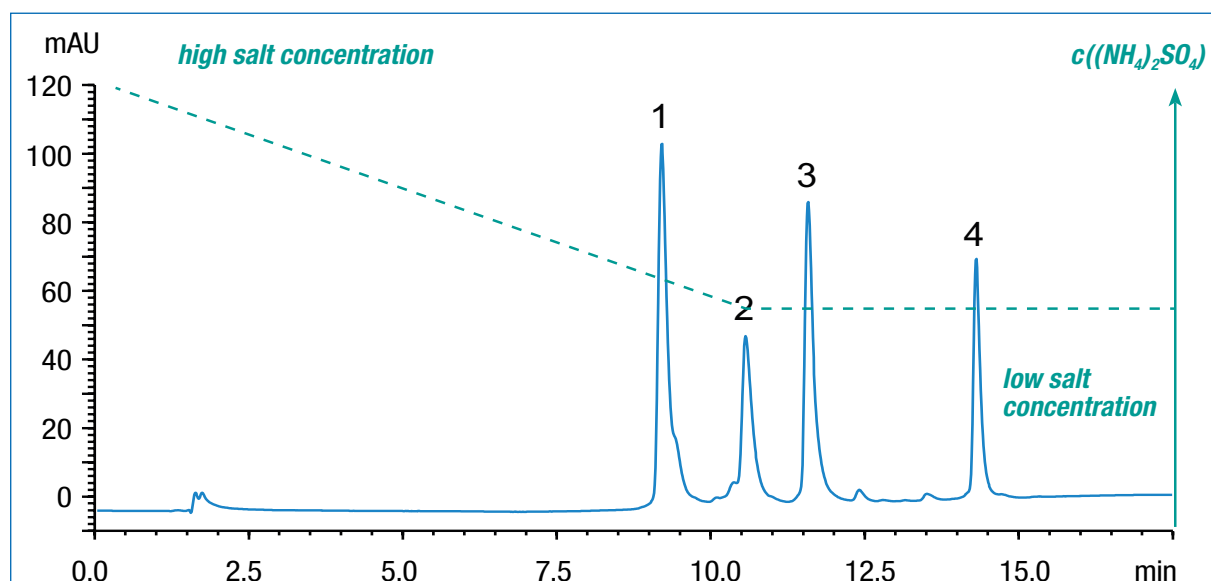


Fig. 2: Method with decreasing salt gradient.

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

HIC is particularly effective when used to separate proteins and monoclonal antibodies. The separation of monoclonal antibodies (MAb), MAb aggregates and glycosylated MAbs can be achieved due to their specific hy-

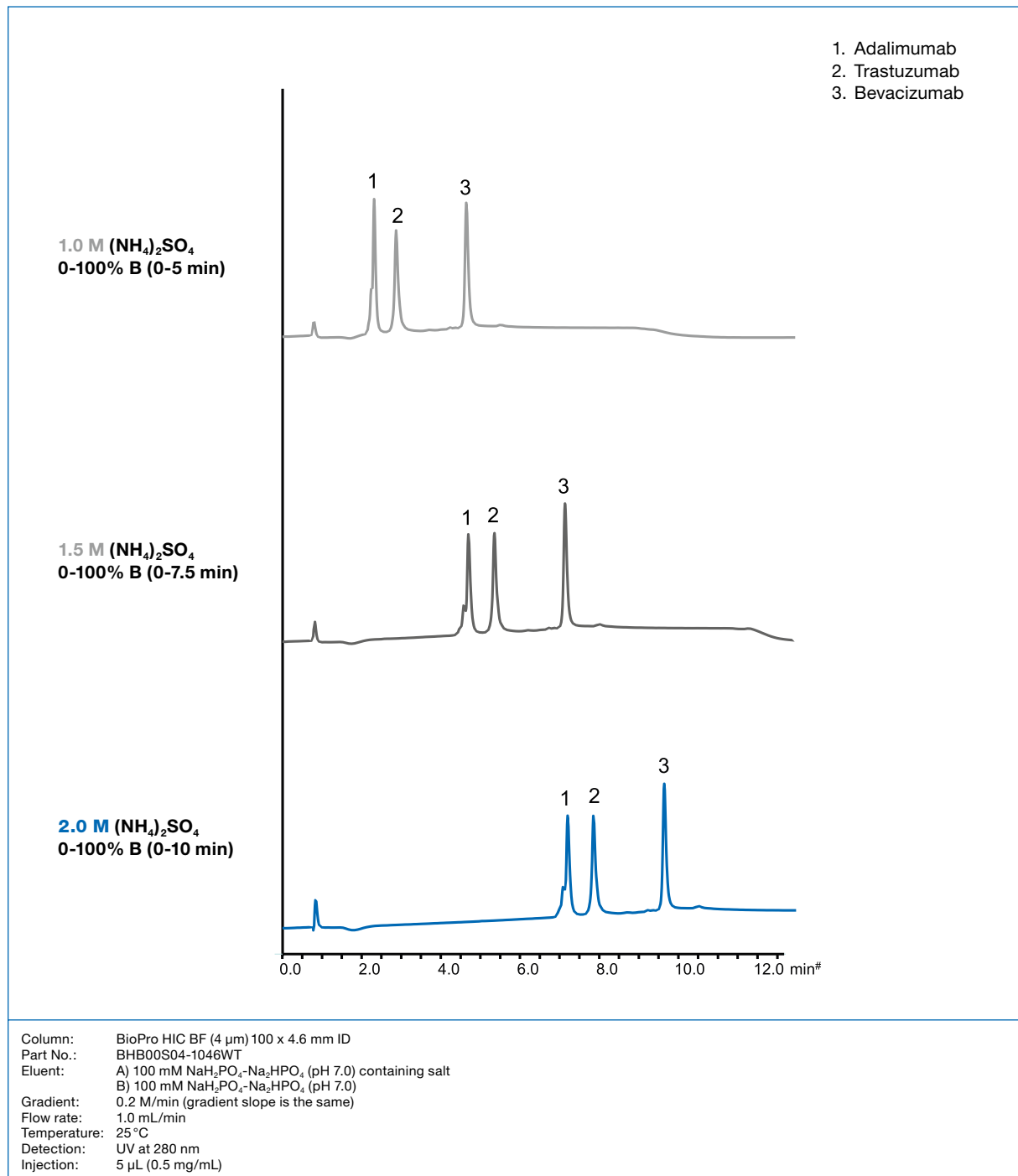
drophobic properties. It also provides an excellent method for determination of drug-to-antibody ratios (DAR) in antibody-drug conjugates (ADCs).

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

# HIC – Expert Tips: Separation factors

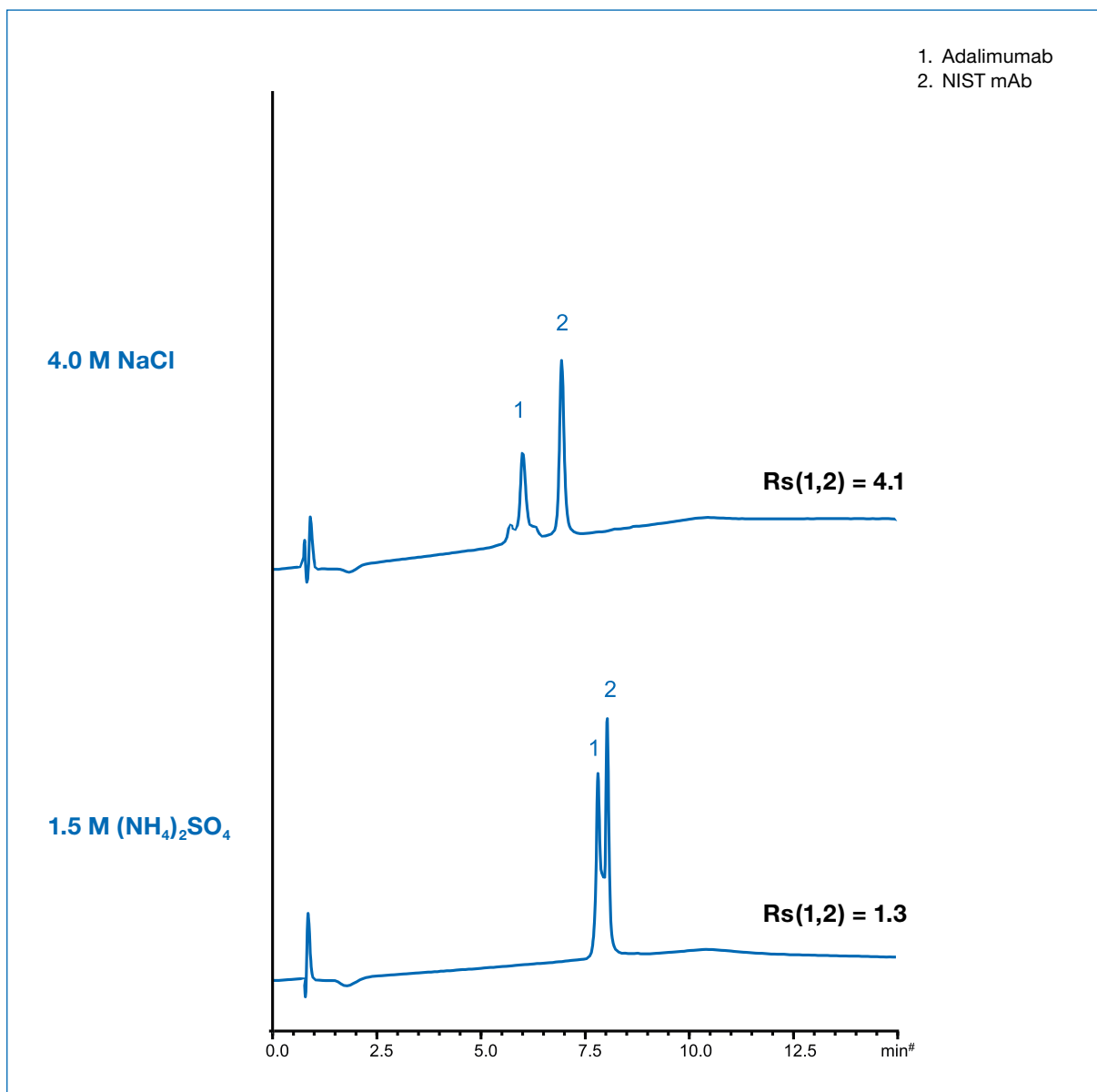
## Effect of initial salt concentration

**B**uffers 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 salt-  
ing-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

**N**aCl 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.

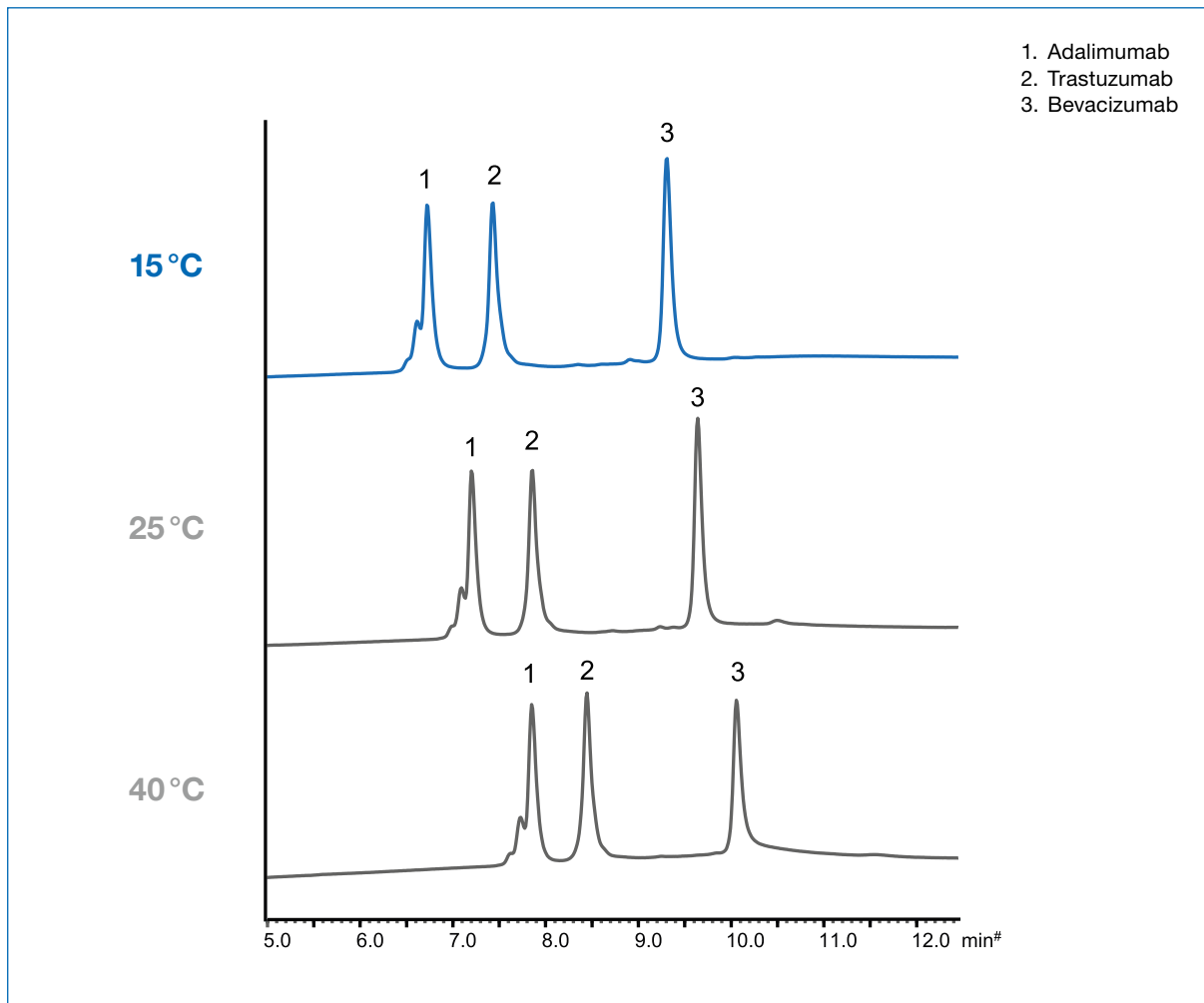


Column: BioPro HIC BF (4  $\mu\text{m}$ ) 100 x 4.6 mm ID  
 Part No.: BHB00S04-1046WT  
 Eluent: A) 100 mM  $\text{NaH}_2\text{PO}_4$ - $\text{Na}_2\text{HPO}_4$  (pH 7.0) containing salt  
 B) 100 mM  $\text{NaH}_2\text{PO}_4$ - $\text{Na}_2\text{HPO}_4$  (pH 7.0)  
 Gradient: 0–100%B (0–10 min), 100%B (10–15 min)  
 Flow rate: 1.0 mL/min  
 Temperature: 25  $^\circ\text{C}$   
 Detection: UV at 280 nm  
 Injection: 10  $\mu\text{L}$  (0.25 mg/mL)

# HIC – Expert Tips: Separation factors

## Temperature influence

**I**n 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.



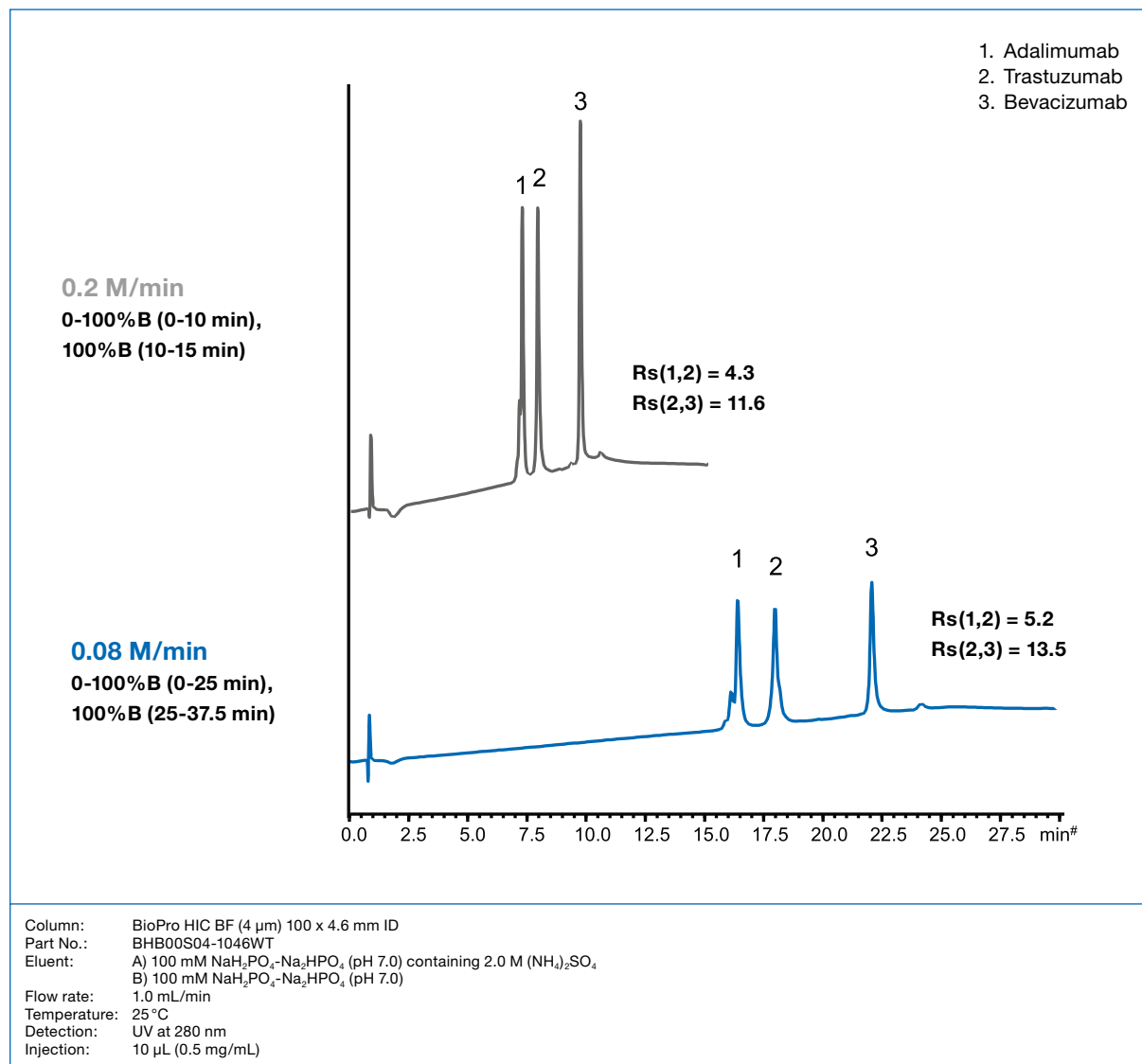
Column: BioPro HIC BF (4 μm) 100 x 4.6 mm ID  
 Part No.: BHB00S04-1046WT  
 Eluent: A) 100 mM NaH<sub>2</sub>PO<sub>4</sub>-Na<sub>2</sub>HPO<sub>4</sub> (pH 7.0) containing 2.0 M (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>  
 B) 100 mM NaH<sub>2</sub>PO<sub>4</sub>-Na<sub>2</sub>HPO<sub>4</sub> (pH 7.0)  
 Gradient: 0–100%B (0–10 min), 100%B (10–15 min)  
 Flow rate: 1.0 mL/min  
 Detection: UV at 280 nm  
 Injection: 5 μL (0.5 mg/mL)



# HIC – Expert Tips: Separation factors / Ordering information

## Variation of gradient slope

**I**n general, shallower gradients improve the separation and the resulting resolution.



## Ordering information

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

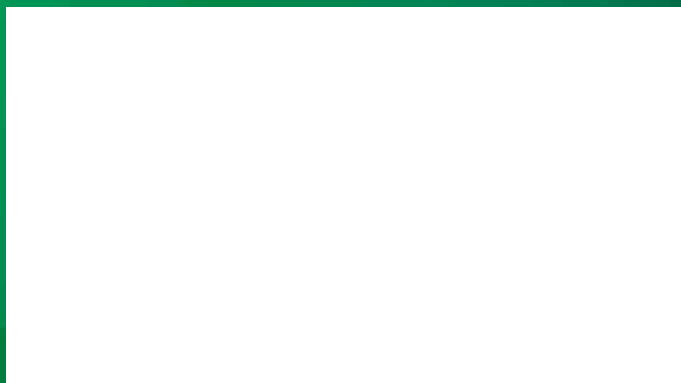
\*Holder required, part no XRPRCS03

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