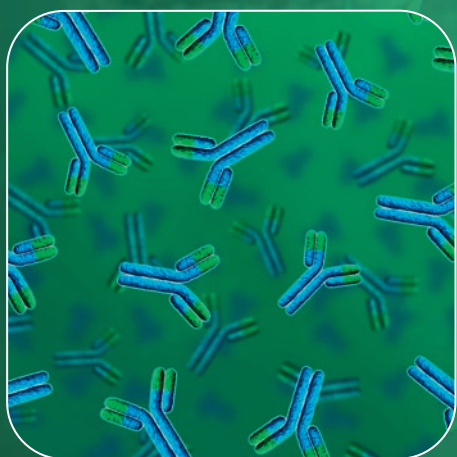
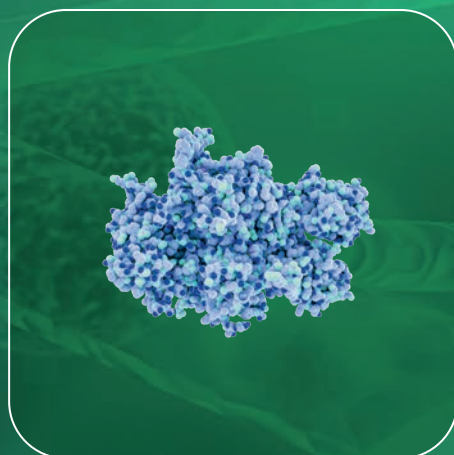


# YMC

## 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 size                                                                                                              | 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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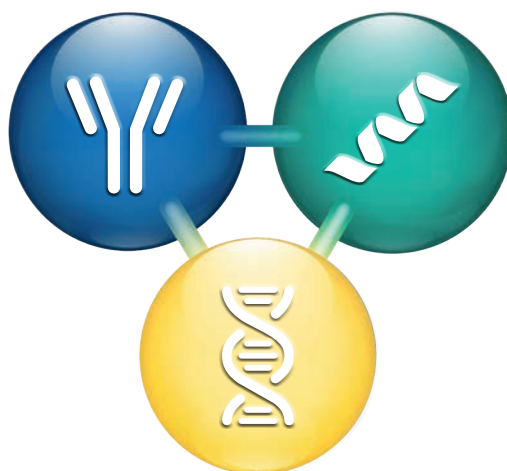
AdvanceBio is a trademark of Agilent Technologies Inc.

BioAssist, NPR, TSKgel are trademarks of Tosoh Corp.

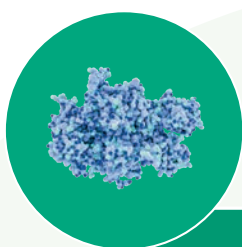
Mono Q, Mono S are trademarks of Cytiva.

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

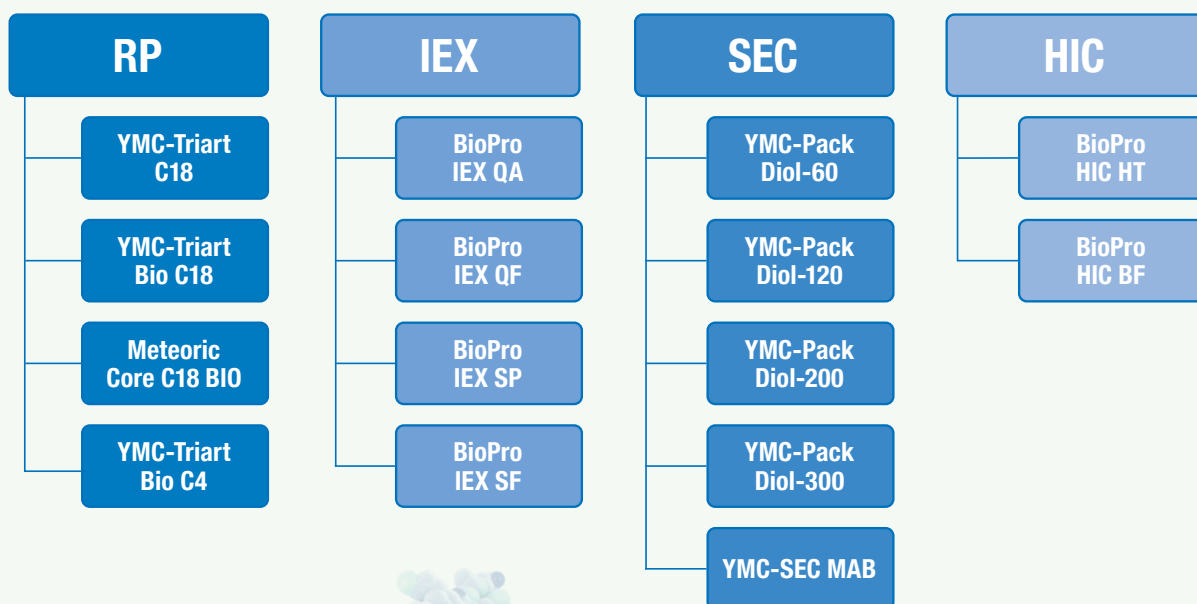
|                                        | Page        |
|----------------------------------------|-------------|
| Phase selection guide .....            | 4–5         |
| Method Validation Kits for BioLC ..... | 6           |
| <b>BioLC applications</b> .....        | <b>7–22</b> |
| Antibodies / ADCs .....                | 8–14        |
| Proteins / Peptides .....              | 15–17       |
| Oligonucleotides / Plasmids .....      | 18–22       |
| (Bioinert) Reversed Phase .....        | 23–44       |
| Size Exclusion (SEC) .....             | 45–54       |
| Ion Exchange (IEX) .....               | 55–66       |
| Hydrophobic Interaction (HIC) .....    | 67–76       |
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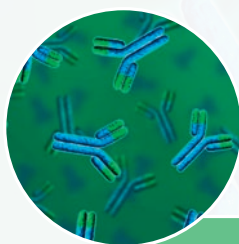


## Phase selection guide

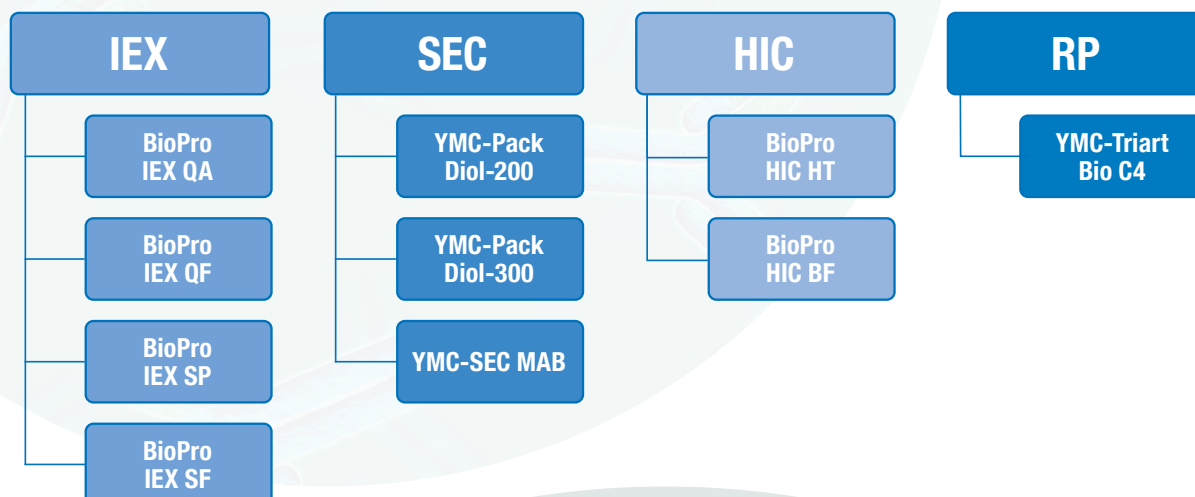


### Proteins / Peptides

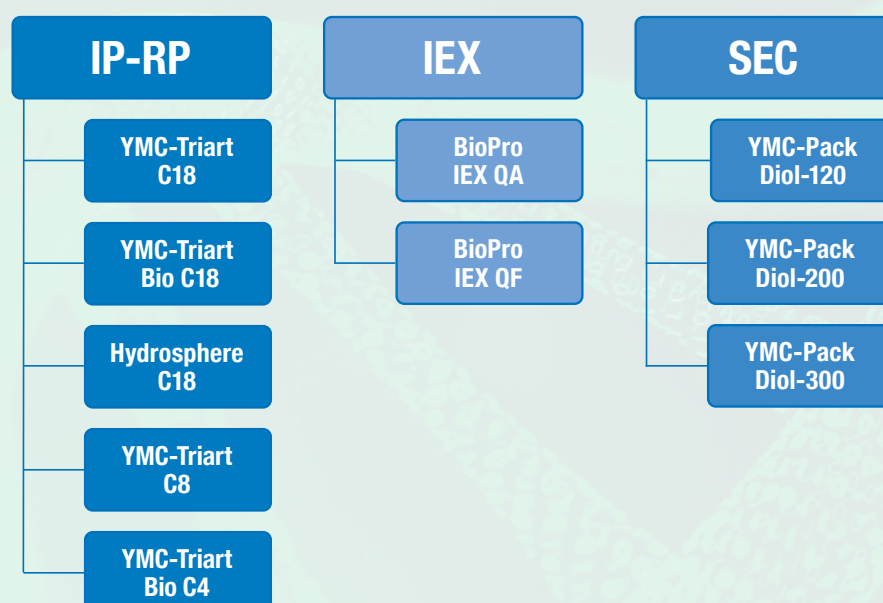




## (Monoclonal) Antibodies



## Oligonucleotides / Nucleic Acids



## Bio QC – Validation kit

### Method Validation Kits for BioLC

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

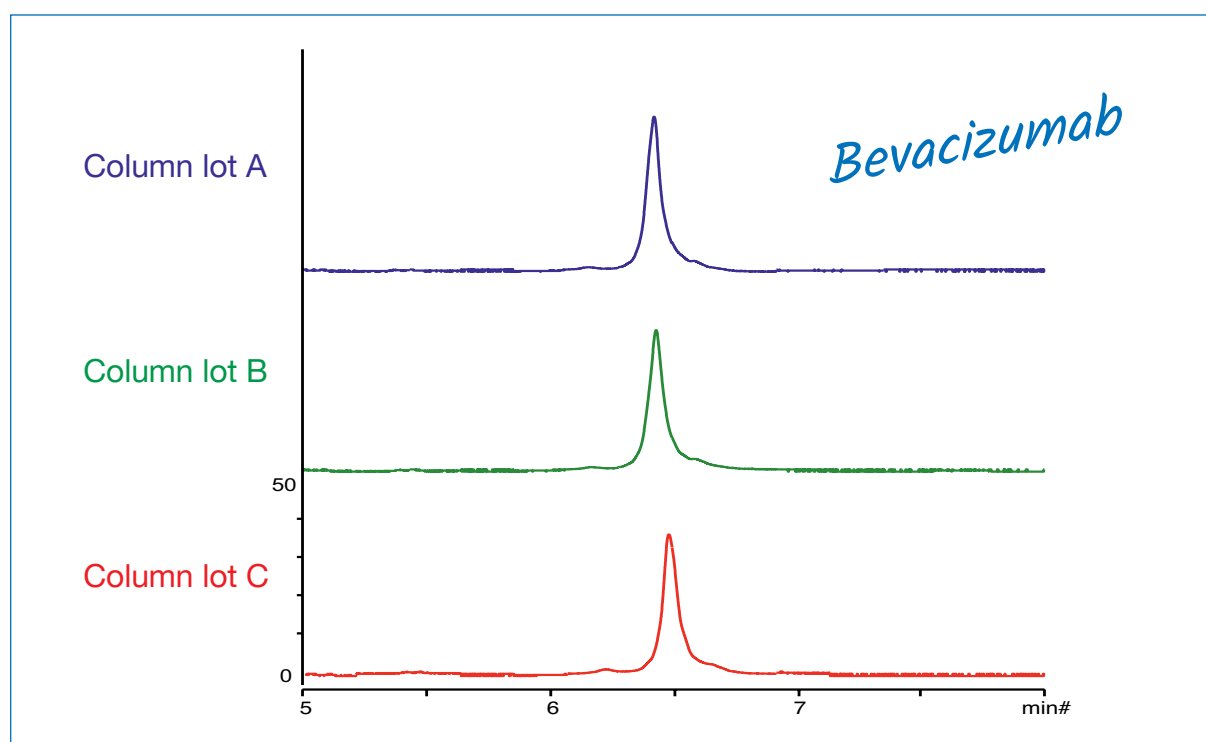
#### Validation kit:

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



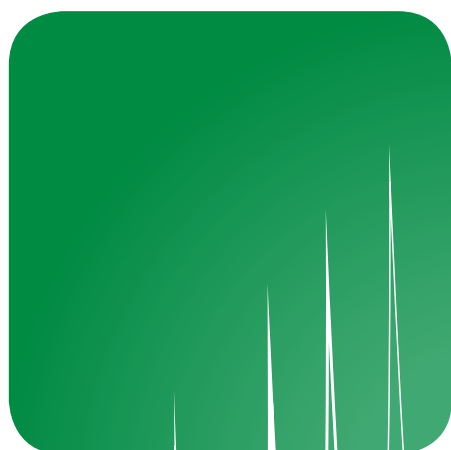
To order a validation kit simply use the ordering number for the column of interest, e.g. **TB30SP9-05Q1PT** and add V1: **TB30SP9-05Q1PTV1**.

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

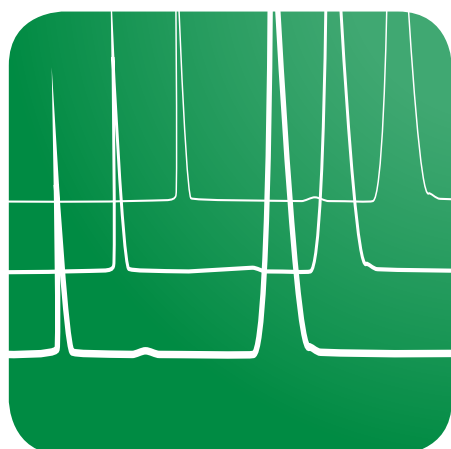
#### Batch Reservation Service

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 preppacked columns for release according to a pre-arranged schedule.

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



## BioLC Applications

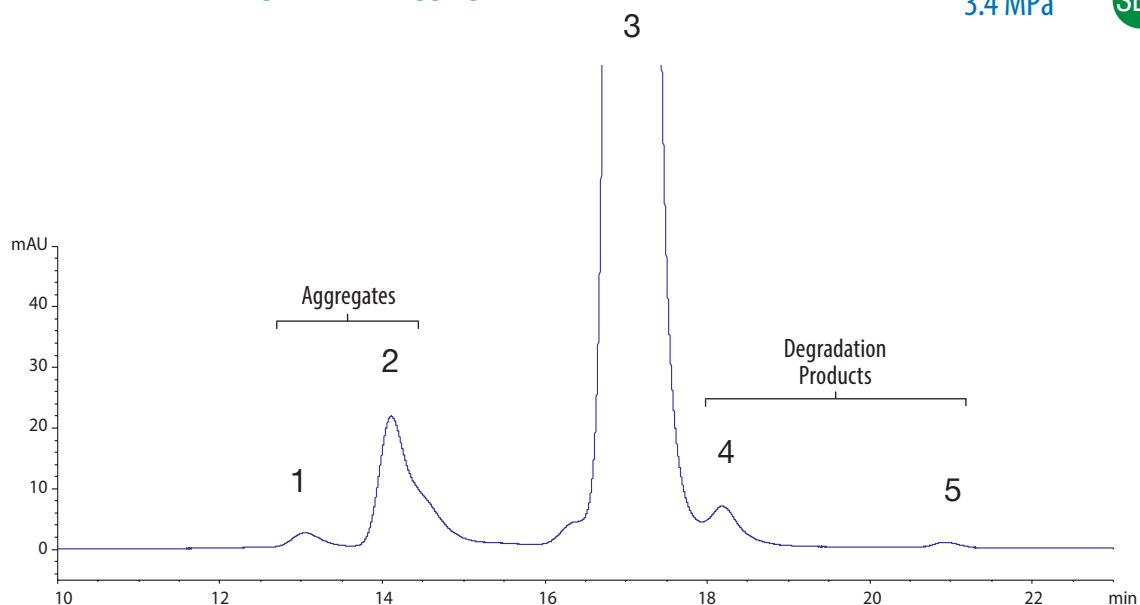


# BioLC applications – Antibodies

## Bevacizumab and its fragments and aggregates

3.4 MPa

SEC



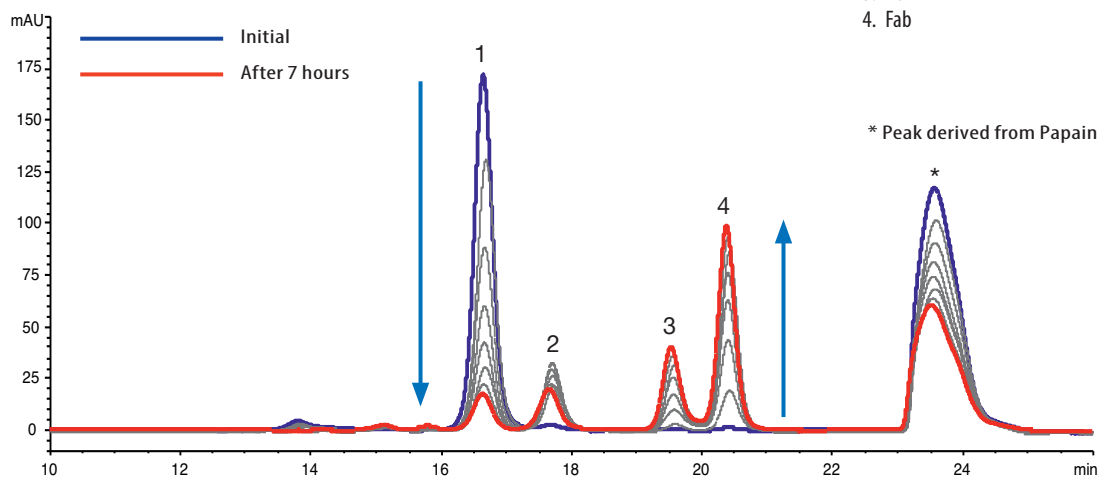
Column: YMC-SEC MAB (3  $\mu$ m, 25 nm) 300 x 4.6 mm ID  
 Part No.: DLM25S03-3046WT  
 Eluent: 0.1 M phosphate buffer (pH = 7) containing 0.2 M NaCl  
 Flow rate: 0.165 mL/min  
 Temperature: 25°C

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

## Analysis of digested antibody

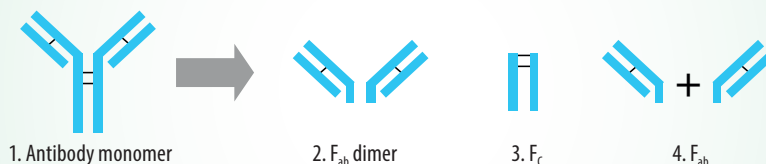
1. Antibody monomer
2. Fab dimer
3. FC
4. Fab

SEC



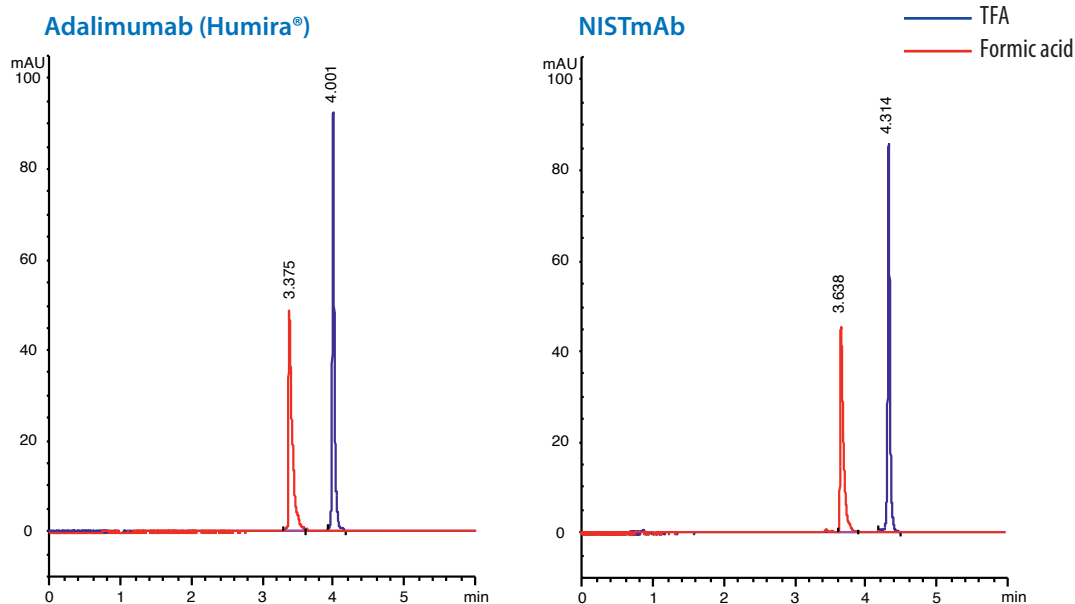
Column: YMC-SEC MAB (3  $\mu$ m, 25 nm) 300 x 4.6 mm ID  
 Part No.: DLM25S03-3046WT  
 Eluent: 0.1 M phosphate buffer (pH = 7) containing 0.2 M NaCl  
 Flow rate: 0.165 mL/min

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



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

RP

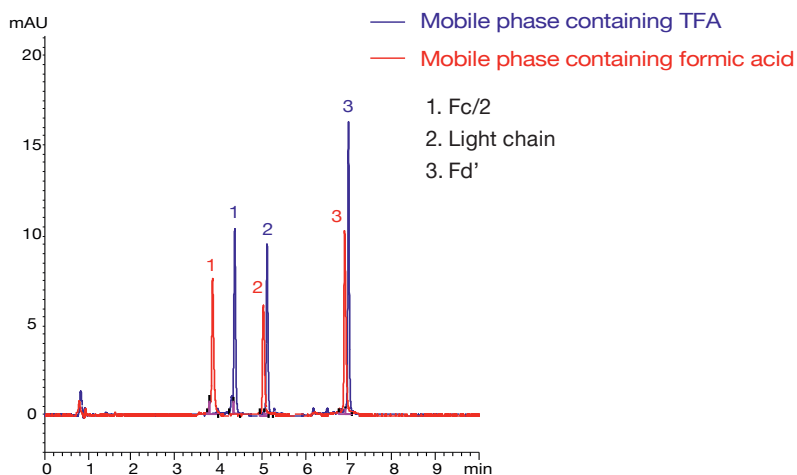


Column: YMC-Triart Bio C4 (1.9  $\mu$ m, 30 nm) 150 x 2.1 mm ID  
Part No.: TB30SP9-15Q1PT  
Eluent: A) water/TFA or formic acid (100/0.1)  
B) acetonitrile/TFA or formic acid (100/0.1)  
Gradient: 10–95%B (0–10 min)

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

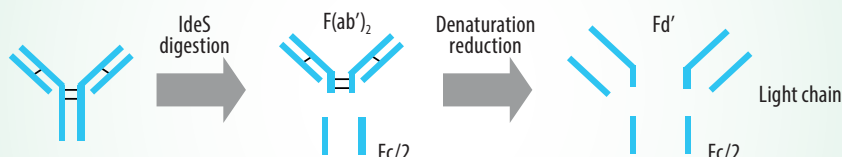
## LC/MS compatible analysis of monoclonal antibody fragments

RP



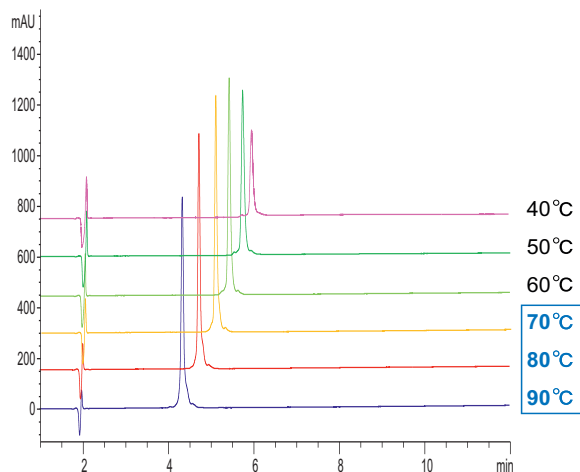
Column: YMC-Triart Bio C4 (1.9  $\mu$ m, 30 nm) 150 x 2.1 mm ID  
Part No.: TB30SP9-15Q1PT  
Eluent [TFA]: A) water/TFA (100/0.1)  
B) acetonitrile/TFA (100/0.1)  
Gradient [TFA]: 25–50%B (0–10 min), 90%B (10–12.5 min)  
Eluent [formic acid]: A) water/formic acid (100/0.1)  
B) acetonitrile/formic acid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

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



# BioLC applications – Antibodies

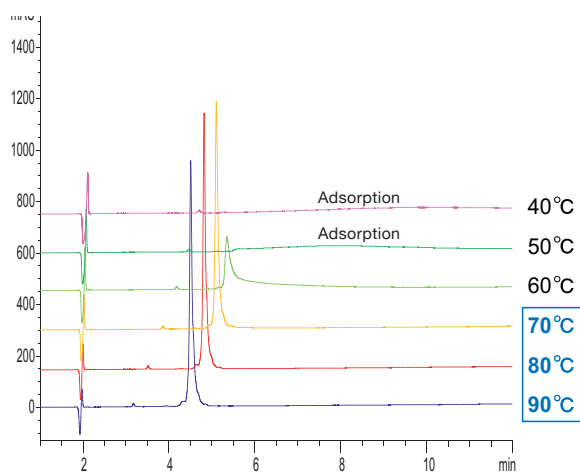
## Adalimumab (Humira®, MW: ca. 148 kDa)



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

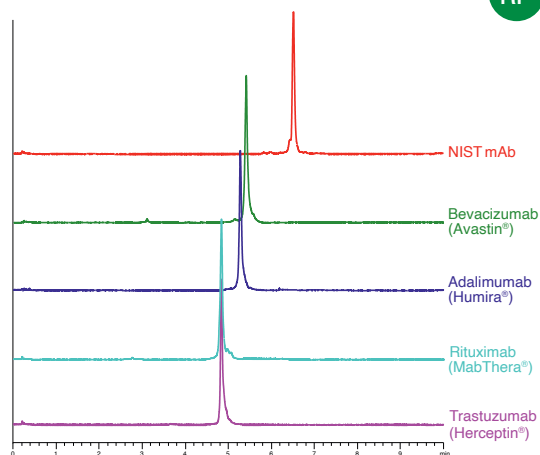
## Bevacizumab (Avastin®, MW: ca. 148 kDa)

RP



## Analysis of different monoclonal antibodies

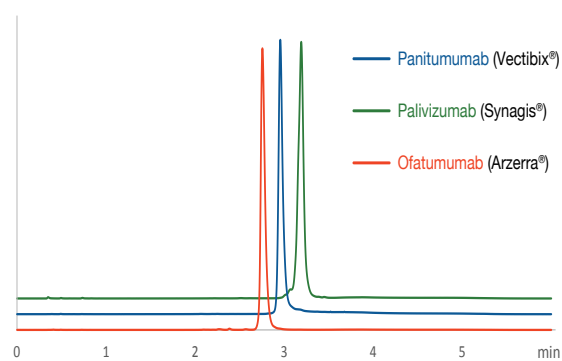
RP



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

## Analysis of challenging monoclonal antibodies

RP



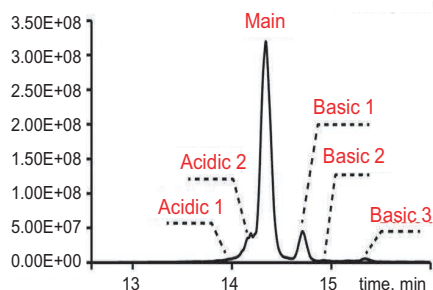
Column: YMC-Triart Bio C4 (1.9 µm, 30 nm) 50 x 2.1 mm ID  
Part No.: TB30SP9-05Q1PT  
Eluent: A) water/TFA (100/0.1)  
B) acetonitrile/TFA (100/0.1)  
Gradient: 25–50%B (0–4 min)  
Flow rate: 0.4 mL/min  
Temperature: 90 °C  
Detection: Fluorescence: ex 280 nm, em 350 nm  
Injection: 0.5 µL

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

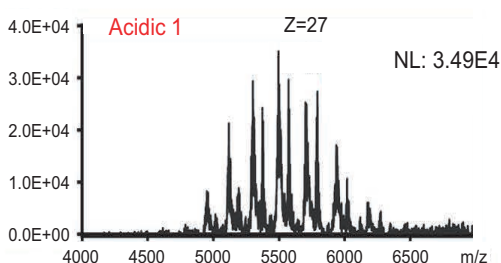
## Native online SCX-MS analysis of monoclonal antibodies

IEX

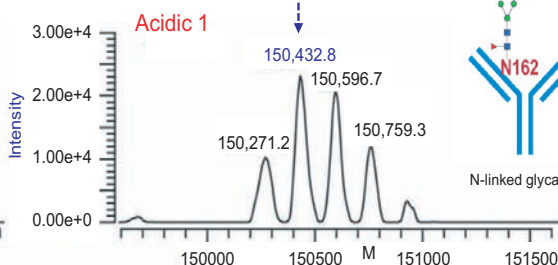
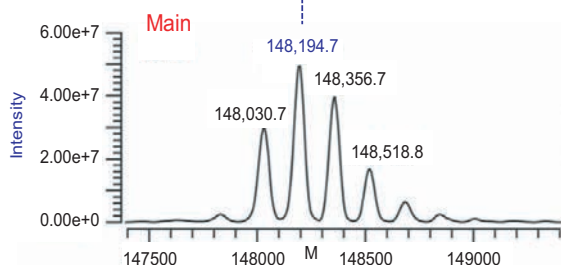
[TIC, native NISTmAb]



[Raw mass spectrum]



[Deconvoluted mass spectra]



Column: BioPro IEX SF (5  $\mu$ m) 100 x 4.6 mm ID  
 Part No.: SF00S05-1046WP  
 Eluent: A) 20 mM  $\text{CH}_3\text{COONH}_4$ - $\text{CH}_3\text{COOH}$  (pH 5.6)  
 B) 140 mM  $\text{CH}_3\text{COONH}_4$ -10 mM  $\text{NH}_4\text{HCO}_3$  (pH 7.4)  
 Gradient: 0%B (0–2 min), 0–100%B (2–18 min), 100%B (18–22 min)  
 Flow rate: 0.4 mL/min  
 (To enable online simultaneous UV and MS detection, a post-column analytical splitter (~400:1 ratio) was connected)

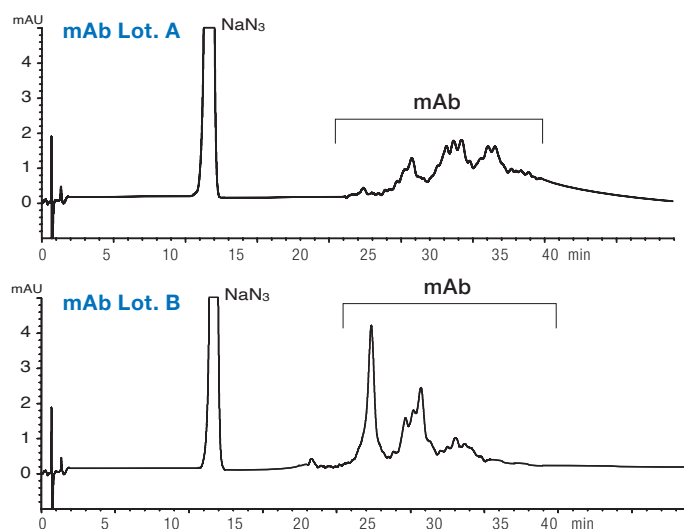
Temperature: 45°C  
 Detection: nanospray ionization-mass spectrometry (NSI-MS)  
 Load: 50  $\mu$ g  
 System: LC) ACQUITY UPLC I-Class system (Waters)  
 MS) ExactiveTM Plus EMR mass spectrometer (Thermo Fisher Scientific)

By courtesy of S. Wang, Regeneron Pharmaceuticals Inc.

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

## Different production batches of IgG1

IEX

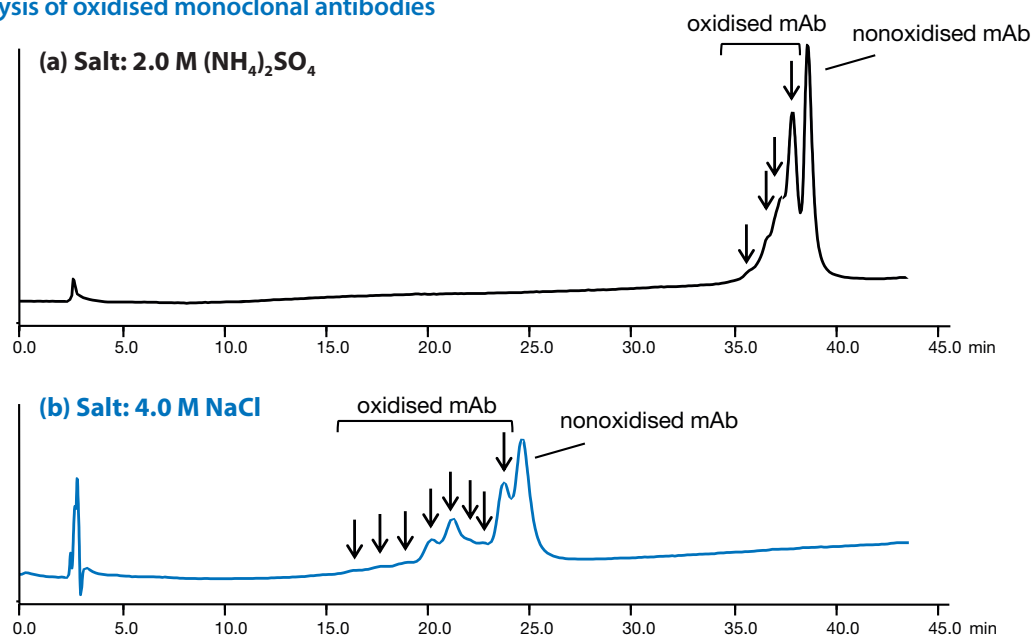


Column: BioPro IEX QF (5  $\mu$ m) 100 x 4.6 mm ID  
 Part No.: QF00S05-1046WP  
 Eluent: A) 20 mM Tris-HCl (pH 8.1)  
 B) 20 mM Tris-HCl (pH 8.1) containing 0.5 M NaCl  
 Gradient: 10–25%B (0–60 min)  
 Flow rate: 1.0 mL/min (360 cm/h)

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  $\text{NaN}_3$ )

# BioLC applications – Antibodies

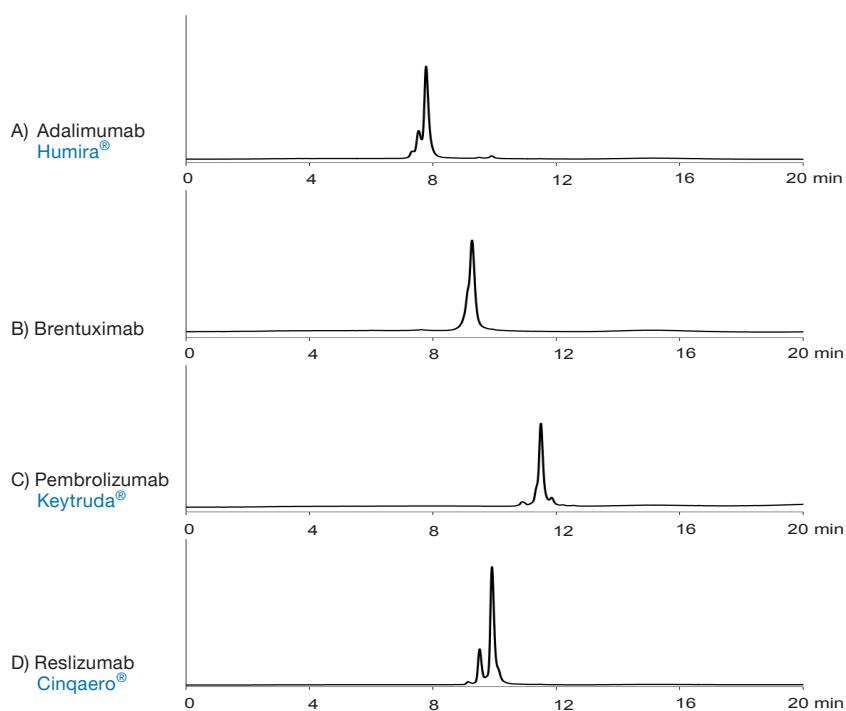
## Analysis of oxidised monoclonal antibodies



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 salt  
 B) 100mM NaH<sub>2</sub>PO<sub>4</sub>-Na<sub>2</sub>HPO<sub>4</sub> (pH 7.0)  
 Gradient: 40–80%B (0–40 min), 80%B (40–45 min)

Flow rate: 0.3 mL/min  
 Temperature: 25 °C  
 Detection: UV at 280 nm  
 Injection: 5 μL (1.0 mg/mL)  
 Sample: oxidised NISTmAb

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



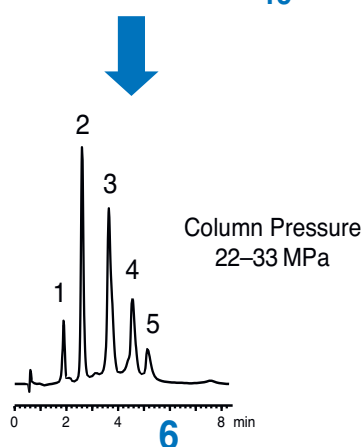
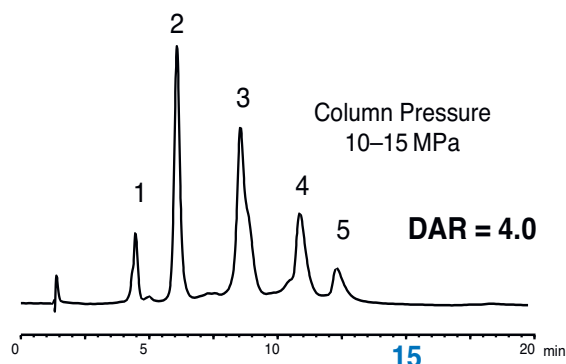
Column: BioPro HIC BF (4 μm) 100 x 4.6 mm ID  
 Part No.: BHB00S04-1046WT  
 Eluent: A) 20 mM NaH<sub>2</sub>PO<sub>4</sub>-Na<sub>2</sub>HPO<sub>4</sub> (pH 7.4) containing 1.5 M (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>  
 B) 20 mM NaH<sub>2</sub>PO<sub>4</sub>-Na<sub>2</sub>HPO<sub>4</sub> (pH 7.4) / 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 μL (2 mg/mL)

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

## High throughput DAR determination by shortening analysis time

HIC



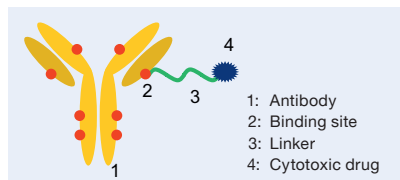
Flow rate  
0.5 mL/min

2.5x faster

Flow rate  
1.2 mL/min

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

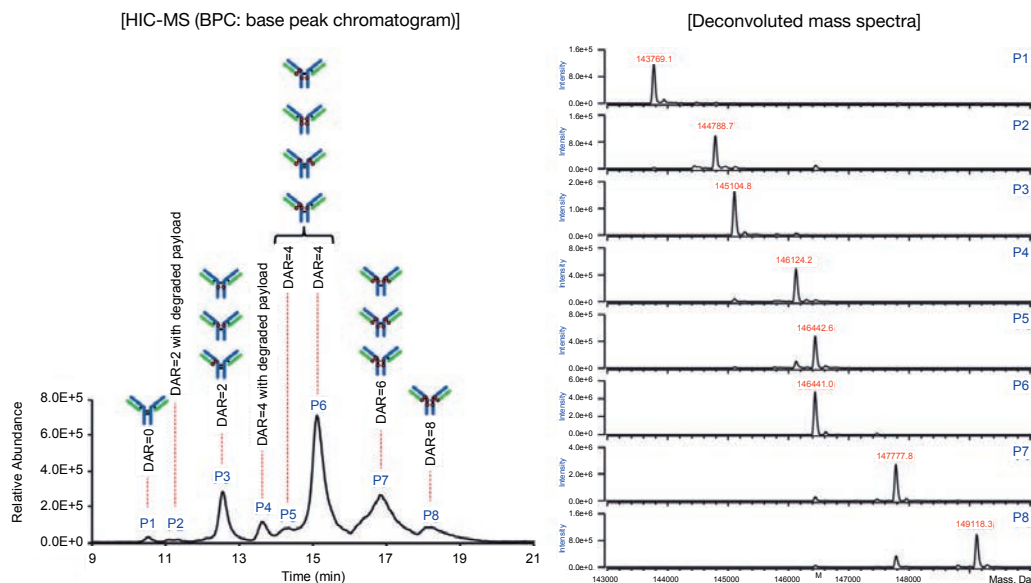
Column: BioPro HIC HT (2.3  $\mu$ m) 100 x 4.6 mm ID  
 Part No.: BHH00SQ3-1046PTH  
 Eluent: A) 20 mM  $\text{NaH}_2\text{PO}_4$ - $\text{Na}_2\text{HPO}_4$  (pH 7.0) containing 1.0 M  $(\text{NH}_4)_2\text{SO}_4$   
 B) 20 mM  $\text{NaH}_2\text{PO}_4$ - $\text{Na}_2\text{HPO}_4$  (pH 7.0)/2-propanol (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°C  
 Detection: UV at 280 nm  
 Injection: 10  $\mu$ L  
 Sample: Brentuximab vedotin (Adcetris®) (2.5 mg/mL)



# BioLC applications – Antibody-Drug-Conjugates

## Native online HIC-MS analysis of cys-linked ADCs

HIC



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

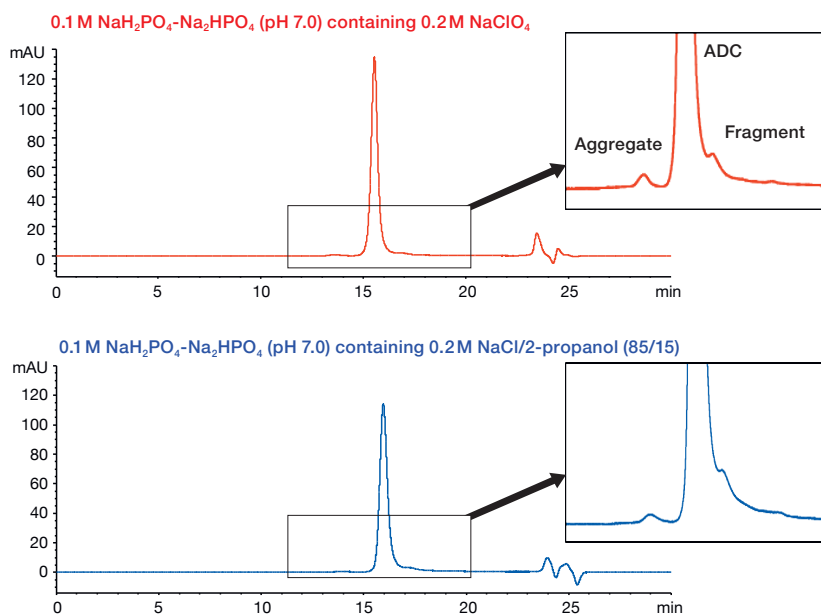
Injection: 10  $\mu$ g  
 Sample: SigmaMAb ADC-mimic  
 Setup: Post-column makeup flow:  
 100% water at 1.5 mL/min (reducing salt conc. 6-fold)  
 Splitter to reduce the flow rate to 1–5  $\mu$ L/min

By courtesy of S. Wang, Regeneron Pharmaceuticals Inc.

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

## Separation of Brentuximab vedotin from its aggregates and fragments

SEC



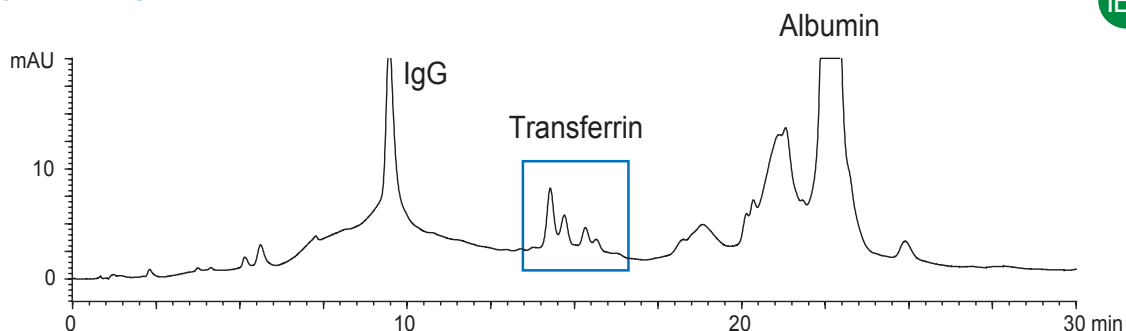
Column: YMC-SEC MAB (3  $\mu$ m, 25 nm) 300 x 4.6 mm ID  
 Part No.: DLM25S03-3046WT  
 Eluent: 0.1 M phosphate buffer (pH = 7) containing 0.2 M NaClO4  
 0.1 M phosphate buffer (pH = 7) containing 0.2 M NaCl/2-propanol (85/15)  
 Flow rate: 0.165 mL/min

Temperature: 25  $^{\circ}$ C  
 Detection: UV at 280 nm  
 Injection: 4  $\mu$ L (2.5 mg/mL)  
 Sample: Brentuximab vedotin (Adcetris<sup>®</sup>) for injection

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

## Separation of proteins in human serum

IEX



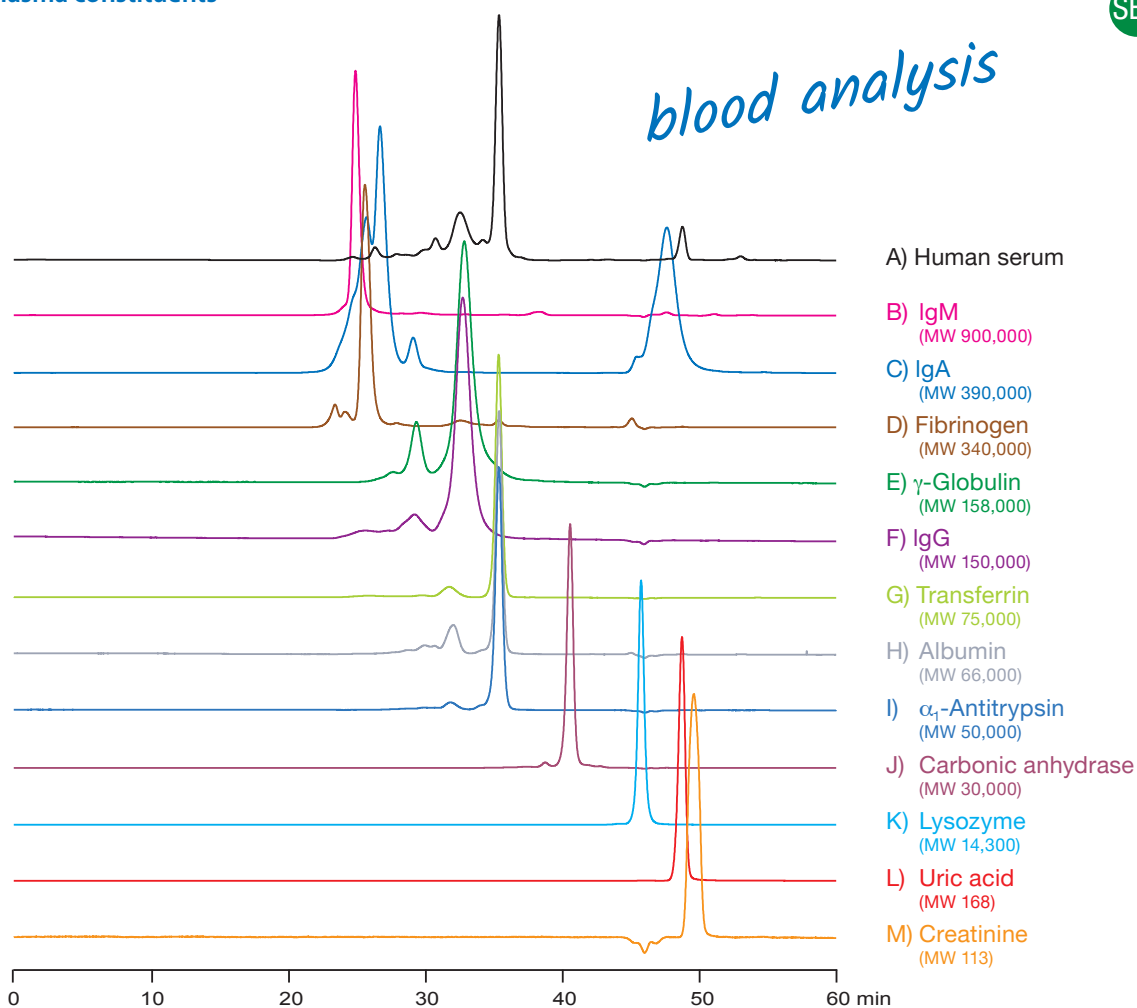
Column: BioPro IEX QA (5  $\mu$ m) 50 x 4.6 mm ID  
 Part No.: QAA0S05-0546WP  
 Eluent: A) 20 mM Tris-HCl (pH 8.6)  
 B) 20 mM Tris-HCl (pH 8.6) containing 0.5 M NaCl  
 Gradient: 0–30%B (0–15 min), 30–100%B (15–30 min)

Flow rate: 0.5 mL/min  
 Temperature: 25°C  
 Detection: UV at 280 nm  
 Injection: 20  $\mu$ L  
 Sample: Human serum (100  $\mu$ L/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

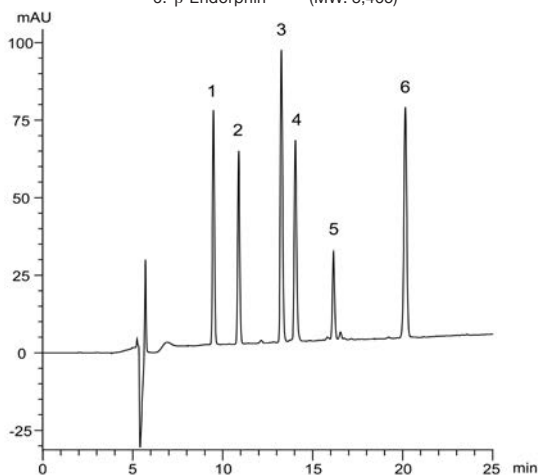
Temperature: ambient (25°C)  
 Detection: UV at 280 nm  
 Injection: 20  $\mu$ L (L: 1  $\mu$ L)  
 Sample: A) 100  $\mu$ L/mL; B-M) 1.0 mg/mL

# BioLC applications – Peptides

## Peptides covering different MW

RP

1. Oxytocin (MW: 1,007)
2. Met-Enkephalin (MW: 574)
3. Leu-Enkephalin (MW: 556)
4. Neurotensin (MW: 1,673)
5.  $\gamma$ -Endorphin (MW: 1,859)
6.  $\beta$ -Endorphin (MW: 3,465)

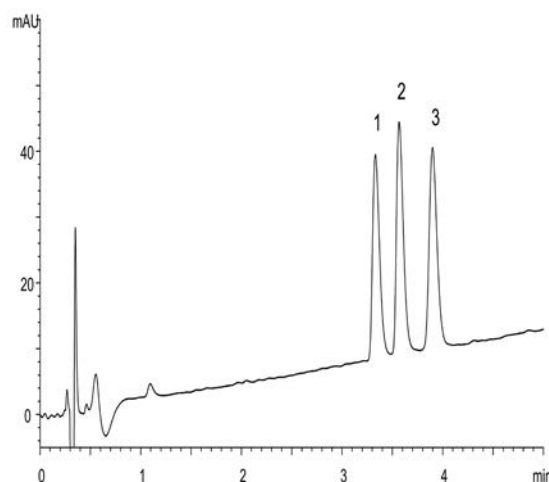


Column: YMC-Triart C18 (5  $\mu$ m, 12 nm) 150 x 2.0 mm ID  
Part No.: TA12S05-1502WT  
Eluent: A) water/TFA (100/0.1)  
B) acetonitrile/TFA (100/0.1)  
Gradient: 20%–45%B (0–25 min)  
Flow rate: 0.2 mL/min  
Temperature: 37 °C  
Detection: UV at 220 nm  
Injection: 2  $\mu$ L (0.075  $\approx$  0.25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

## Antimicrobial peptides

RP

1.  $\alpha$ -Defensin-1 (Human) (MW: 3,442)
2.  $\alpha$ -Defensin-2 (Human) (MW: 3,371)
3.  $\alpha$ -Defensin-3 (Human) (MW: 3,486)

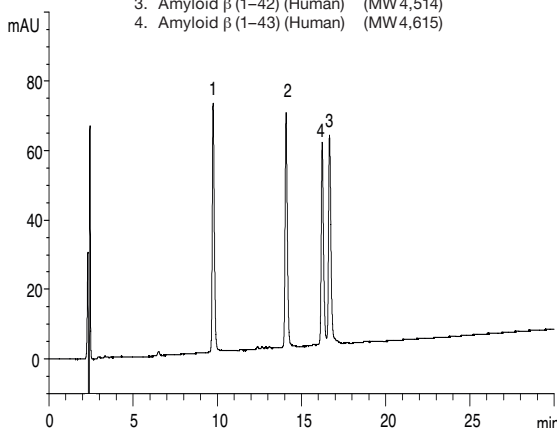


Column: YMC-Triart C18 (1.9  $\mu$ m, 12 nm) 50 x 2.0 mm ID  
Part No.: TA12SP9-0502PT  
Eluent: A) water/formic acid (100/0.1)  
B) 2-propanol/acetonitrile/formic acid (50/50/0.08)  
Gradient: 10%–25%B (0–10 min)  
Flow rate: 0.4 mL/min  
Temperature: 70 °C  
Detection: UV at 220 nm  
Injection: 1 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

## Amyloid $\beta$ -peptides

RP

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



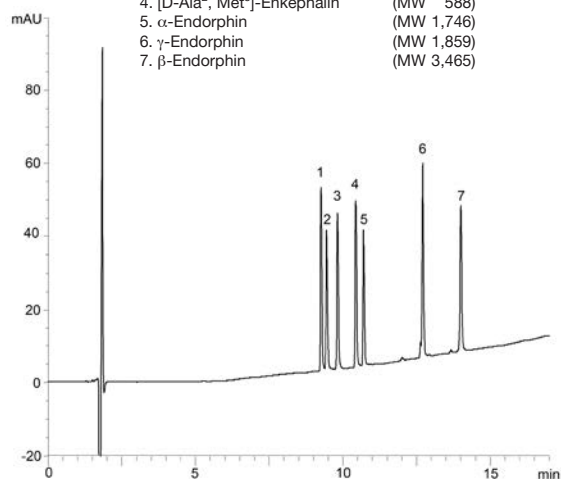
Amyloid  $\beta$  (1–43): Asp-Ala-Glu-Phe-Arg-His-Asp-Ser-Gly-Tyr-Glu-Val-His-His-Gln-Lys-Leu-Val-Phe-Phe-Ala-Glu-Asp-Val-Gly-Ser-Asn-Lys-Gly-Ala-Ile-Ile-Gly-Leu-Met-Val-Gly-Gly-Val-Val-Ile-Ala-Thr

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 °C  
Detection: UV at 220 nm  
Injection: 4  $\mu$ L (each 0.1 mg/mL)

## Peptides

RP

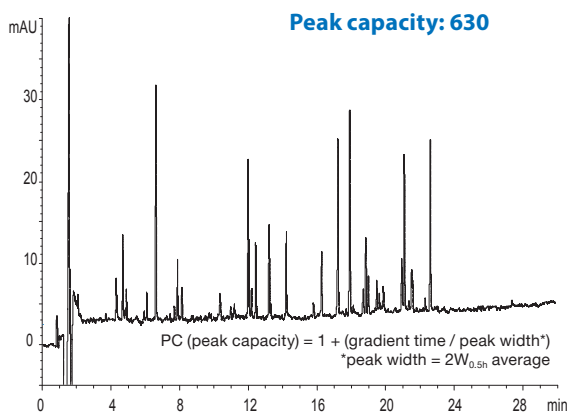
1. BAM-12P (MW 1,425)
2. [D-Ala<sup>2</sup>, Met<sup>5</sup>]-Enkephalinamide (MW 587)
3. Met-Enkephalin (MW 574)
4. [D-Ala<sup>2</sup>, Met<sup>5</sup>]-Enkephalin (MW 588)
5.  $\alpha$ -Endorphin (MW 1,746)
6.  $\gamma$ -Endorphin (MW 1,859)
7.  $\beta$ -Endorphin (MW 3,465)



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 °C  
Detection: UV at 220 nm  
Injection: 2  $\mu$ L (0.02–0.5 mg/mL)  
Pressure: 14.9–16.1 MPa (2,160–2,330 psi)

## Tryptic digest of Hemoglobin

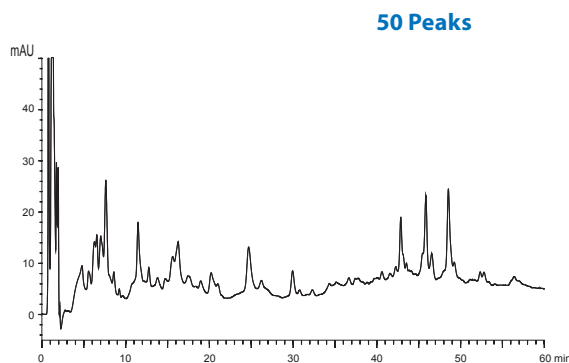
RP



Column: YMC-Triart C18 (1.9  $\mu$ m, 12 nm) 200 x 2.0 mm ID  
(Two coupled 100 x 2.0 mm ID)  
Part No.: TA12SP9-1002PT (2x)  
Eluent: A) water/TFA (100/0.1)  
B) acetonitrile/TFA (100/0.08)  
Gradient: 5–40%B (0–30 min)  
Flow rate: 0.4 mL/min  
Temperature: 70°C  
Detection: UV at 220 nm  
Injection: 20  $\mu$ 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

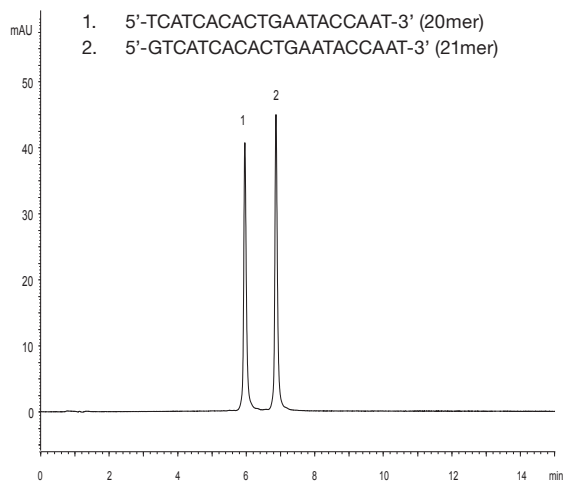
IEX



Column: BioPro IEX QA (5  $\mu$ m) 50 x 4.6 mm ID  
Part No.: QAA0S05-0546WP  
Eluent: A) 20 mM Tris-HCl (pH 8.6)  
B) 20 mM Tris-HCl (pH 8.6) containing 0.5 M NaCl  
Gradient: 0–15%B (0–30 min), 15–60%B (30–60 min)  
Flow rate: 0.5 mL/min  
Temperature: 25°C  
Detection: UV at 220 nm  
Injection: 20  $\mu$ L  
Sample: Tryptic digest of BSA

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

IEX

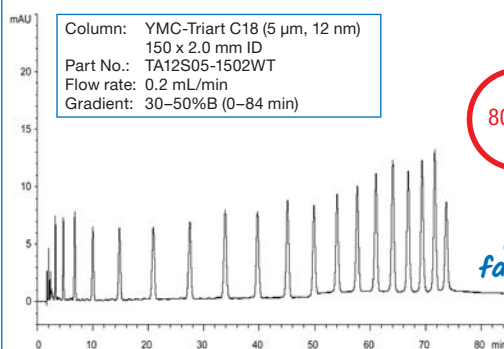


Column: BioPro IEX QF (5  $\mu$ m) 100 x 4.6 mm ID  
Part No.: QF00S05-1046WP  
Eluent: A) 10 mM NaOH  
B) 10 mM NaOH containing 1.0 M NaClO<sub>4</sub>  
Gradient: 25–55%B (0–15 min), 100%B (15–20 min)  
Flow rate: 1.0 mL/min  
Temperature: 25°C  
Detection: UV at 260 nm  
Injection: 4  $\mu$ L (5 nmol/L)

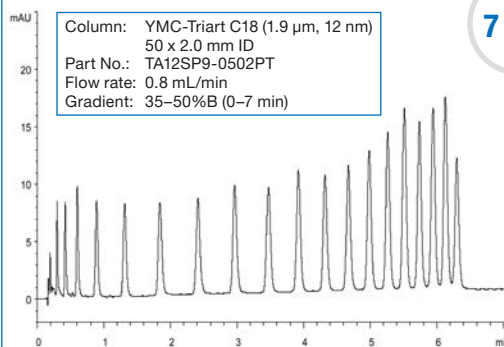
## Oligonucleotides d(T)2-20 method transfer from HPLC to UHPLC

RP

### Conventional LC method



### UHPLC method

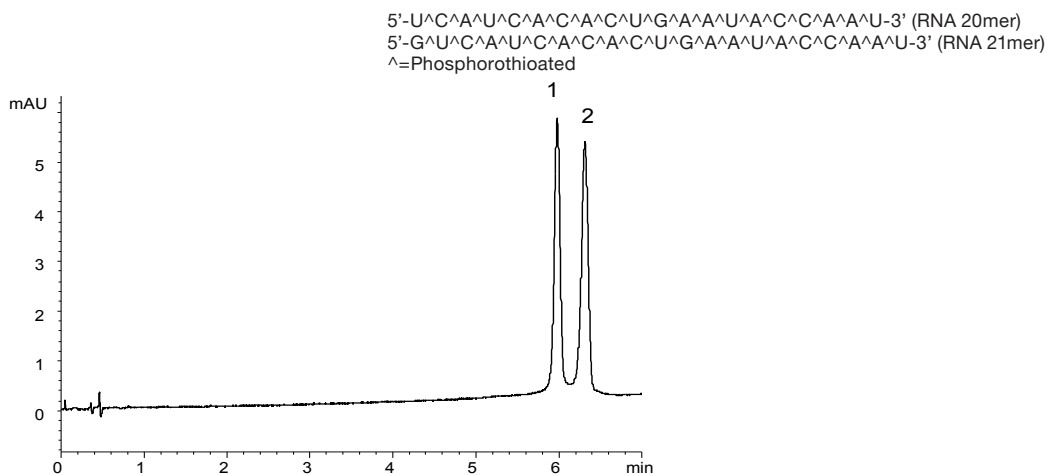


Eluent: A) 10 mM di-n-butylamine-acetic acid (pH 6.0)  
B) methanol  
Temperature: 37°C  
Detection: UV at 269 nm  
Injection: 1  $\mu$ L (5 nmol/mL)

# BioLC applications – Oligonucleotides

## Challenging phosphorothioate oligonucleotides

RP



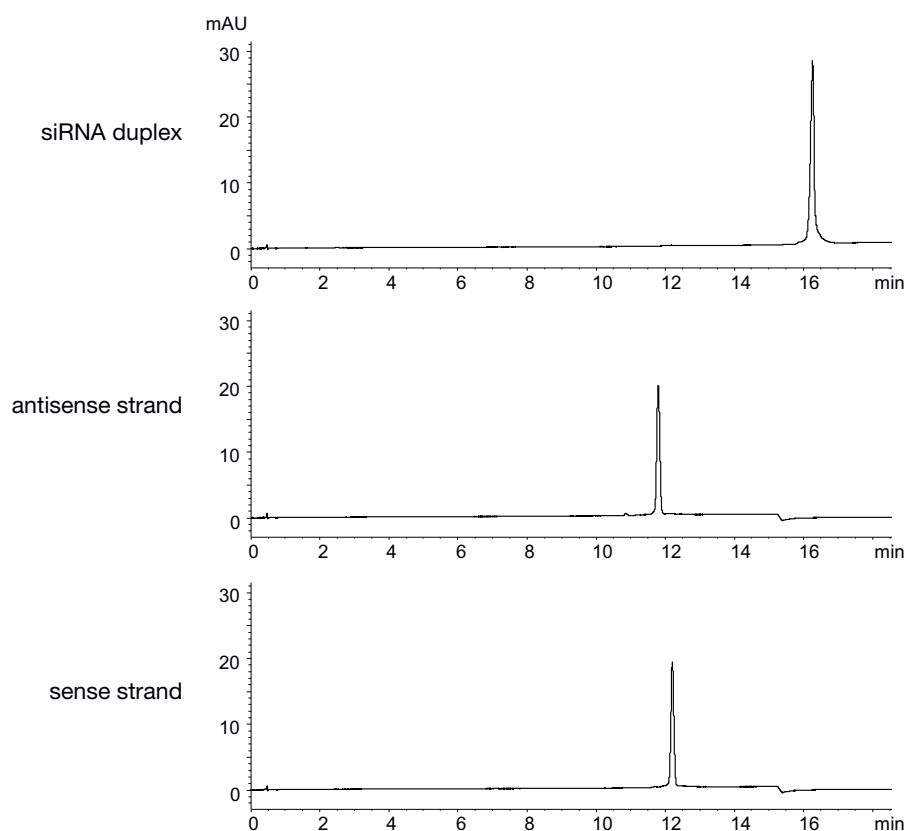
Column: YMC-Accura Triart Bio C18 (1.9  $\mu$ m, 30 nm) 50 x 2.1 mm ID  
Part No.: TA30SP9-05Q1PTC  
Eluent: A) 15 mM triethylamine - 400 mM HFIP\*  
B) methanol  
Gradient: 10–20%B (0–10 min)

Flow rate: 0.42 mL/min  
Temperature: 65 °C  
Detection: UV at 260 nm  
Injection: 1  $\mu$ L (each 1.0 nmol/mL)

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

## siRNA under non-denaturing conditions

RP



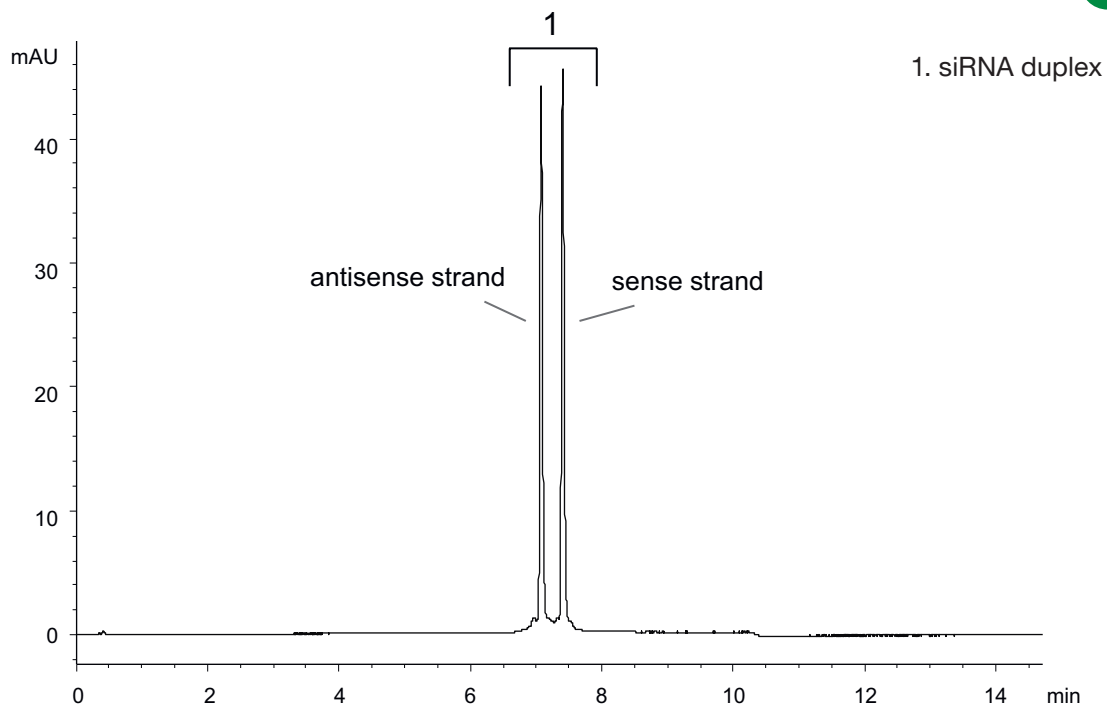
Column: YMC-Accura Triart Bio C18 (1.9  $\mu$ m, 30 nm) 50 x 2.1 mm ID  
Part No.: TA30SP9-05Q1PTC  
Eluent: A) 15 mM triethylamine - 400 mM HFIP\* (pH 8)  
B) methanol  
Gradient: 10%–28%B (0–18 min)

Flow rate: 0.42 mL/min  
Temperature: 25 °C  
Detection: UV at 260 nm  
Injection: 1  $\mu$ L (5 nmol/mL)  
Sample: siRNA duplex & single strands

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

## siRNA duplex under denaturing conditions

RP



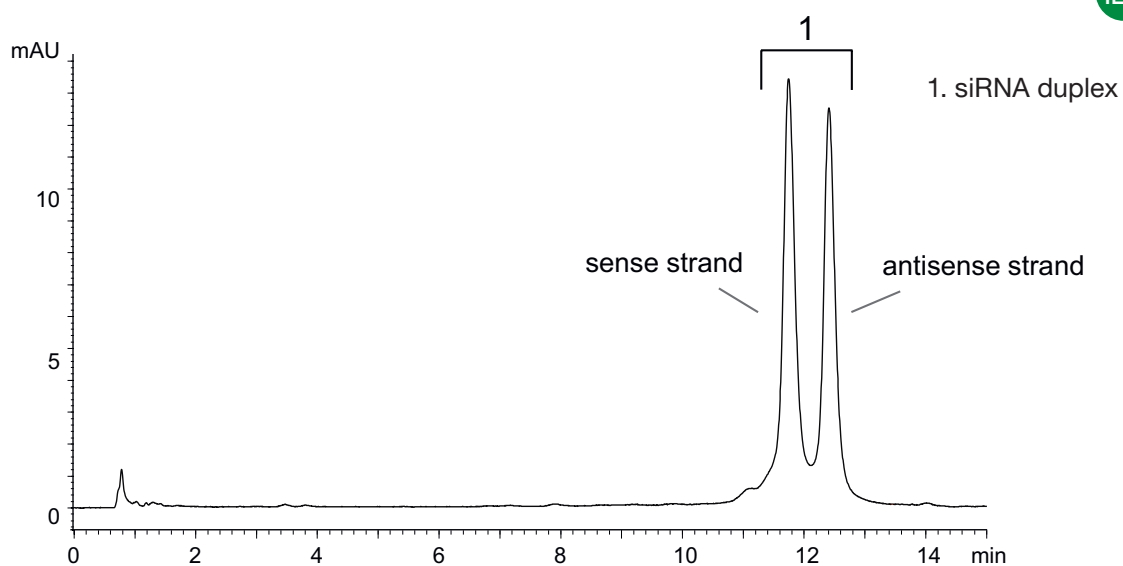
Column: YMC-Accura Triart Bio C18 (1.9  $\mu$ m, 30 nm) 50 x 2.1 mm ID  
 Part No.: TA30SP9-05Q1PTC  
 Eluent: A) 15 mM TEAA\* (pH 8)  
 B) methanol  
 Gradient: 5%–20%B (0–15 min)

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

\*triethylammonium acetate

## siRNA duplex under denaturing conditions

IEX



Column: BioPro IEX QF (5  $\mu$ m) 100 x 4.6 mm ID  
 Part number: QF00S05-1046WP  
 Eluent: A) 10 mM NaOH  
 B) 10 mM NaOH containing 1 M NaClO<sub>4</sub>  
 Gradient: 30%–37%B (0–15 min)

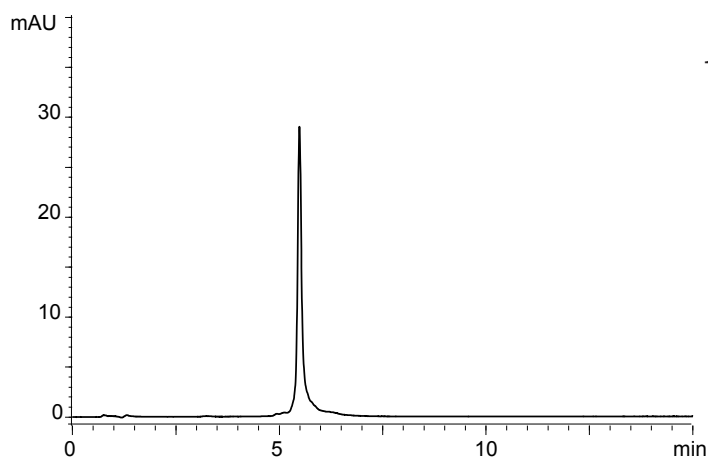
Flow rate: 1.0 ml/min  
 Temperature: 25 °C  
 Detection: UV at 260 nm  
 Injection: 4  $\mu$ l (5 nmol/ml)  
 Sample: siRNA duplex

# BioLC applications – Oligonucleotides

## AEX analysis of siRNA duplex under non-denaturing conditions

IEX

### 1. siRNA duplex



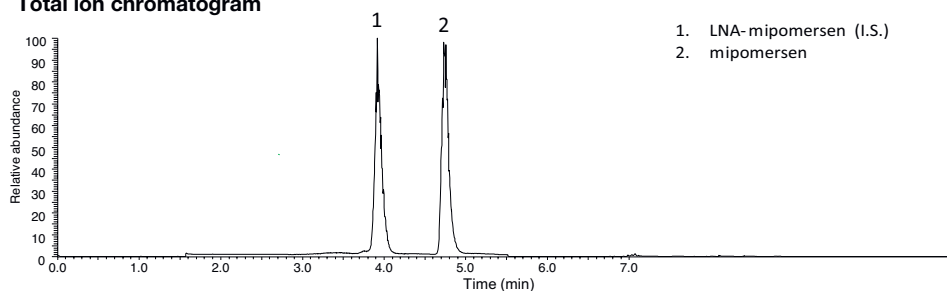
Column: BioPro IEX QF (5µm) 100 x 4.6 mm ID  
Part number: QF00S05-1046WP  
Eluent: A) 20mM Tris-HCl (pH 8.1)  
B) 20mM Tris-HCl (pH 8.1) containing 1 M NaClO<sub>4</sub>  
Gradient: 25%–40%B (0–15 min)

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

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

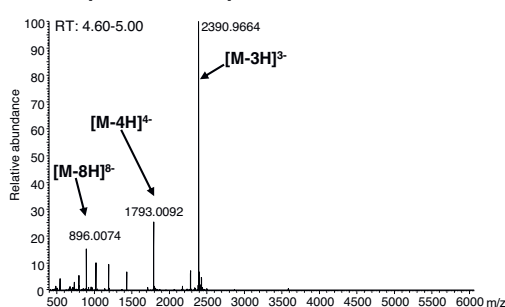
RP

### Total ion chromatogram

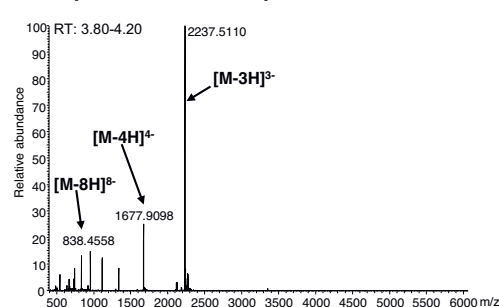


1. LNA-mipomersen (I.S.)  
2. mipomersen

### Mass spectrum of mipomersen



### Mass spectrum of LNA-mipomersen



Column: **YMC-Triart C8 metal-free** (1.9µm, 12nm)\* 100 x 2.1 mm ID  
Part No.: TO12SP9-10Q1PTP  
Eluent: A) water/triethylamine/HFIP\*\*  
(100/0.4/2; triethylamine 28.0mM, HFIP 135.8mM)  
B) methanol/triethylamine/HFIP (100/0.4/2)  
Gradient: [Sample separation step]  
10–40%B (0–5.0 min)

[Column wash steps]  
40–70%B (5.0–5.1 min), 70%B (5.1–7.0 min), 70–10%B (7.0–7.1 min),  
10%B (7.1–8.0 min), 10–90%B (8.0–8.1 min), 90%B (8.1–9.0 min),  
90–10%B (9.0–9.1 min), 10%B (9.1–10.0 min),  
10–90%B (10.0–10.1 min), 90%B (10.1–11.0 min),  
90–10%B (11.0–11.1 min)  
Flow rate: 0.3mL/min  
Temperature: 50 °C  
Injection: 10µL (1000ng/mL)  
System : LC) Vanquish Binary Pump H system  
HRMS) Orbitrap HRMS Q Exactive Plus

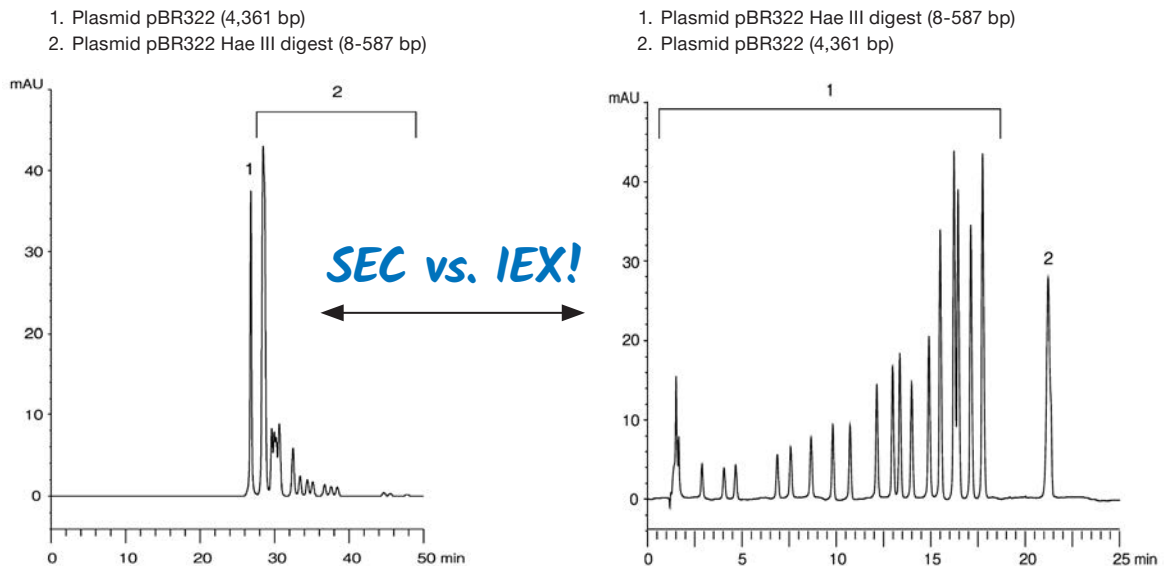
\* Prewash the column prior to the first use with water/methanol/phosphoric acid (70/30/0.1) for 1 hour

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

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

## Plasmid pBR322 restriction and pBR322 Hae III restriction fragments

SEC IEX

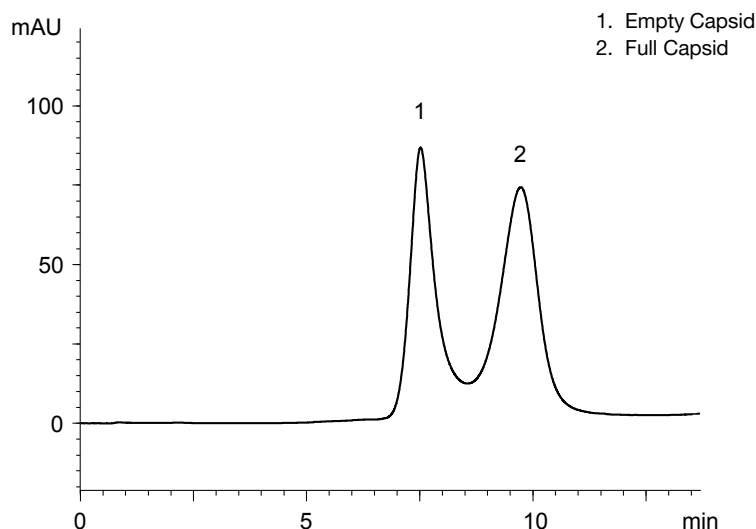


Columns: YMC-Pack Diol-300 + Diol-200 (5  $\mu$ m) 500 x 8.0 mm ID  
Part Nos.: DL30S05-5008WT + DL20S05-5008WT  
Eluent: 0.1 M  $\text{KH}_2\text{PO}_4$ - $\text{K}_2\text{HPO}_4$  (pH 7.0) containing 0.2 M NaCl  
Flow rate: 0.7 mL/min  
Temperature: ambient (25°C)  
Detection: UV at 260 nm  
Injection: 10  $\mu$ L

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

## Intact adeno-associated virus

IEX



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

This research was supported by AMED under Grant Number JP18ae0201001.

# BioLC applications – AAVs

## Denatured adeno-associated viruses

RP

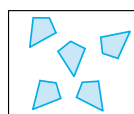
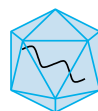
### Sample preparation

AAV2  
1.46 x 10<sup>12</sup> vg/mL  
AAV5  
3.95 x 10<sup>12</sup> vg/mL

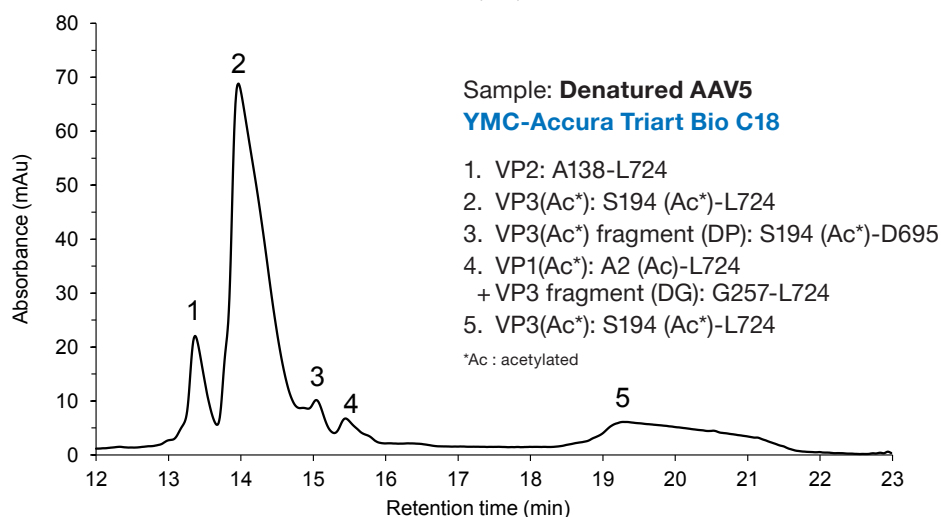
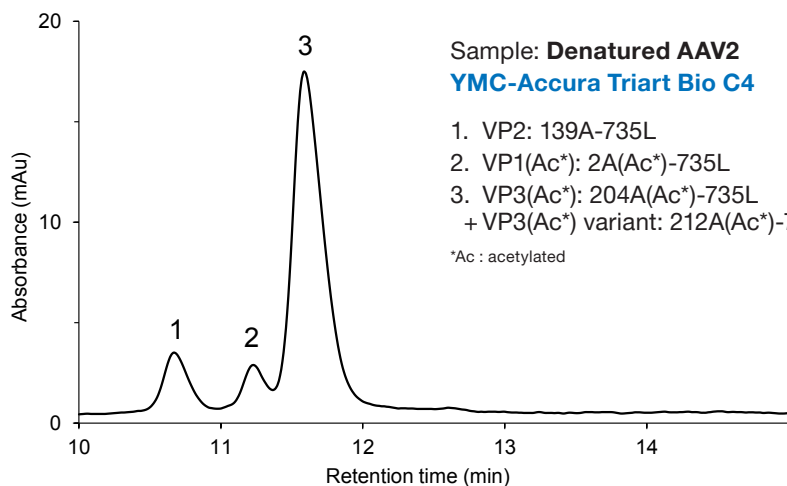
10% acetic acid treatment  
RT, 15 min

Centrifuged at 12,000 rpm  
5 min

analysis



VPs: 59–81 kDa



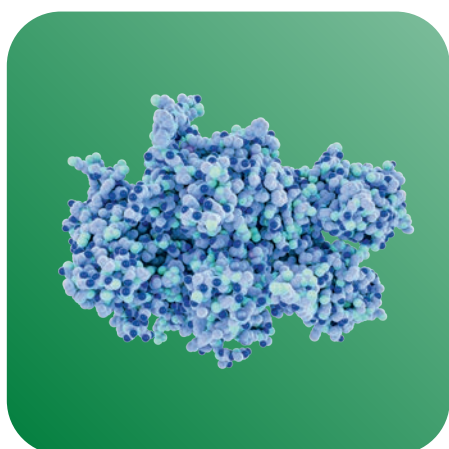
Columns: **YMC-Accura Triart Bio C4** (1.9  $\mu$ m, 30 nm) 150 x 2.1 mm ID  
**YMC-Accura Triart Bio C18** (1.9  $\mu$ m, 30 nm) 150 x 2.1 mm ID  
Part Nos.: TB30SP9-15Q1PTC  
TA30SP9-15Q1PTC  
Eluent: A) water/difluoroaceticacid (100/0.1)  
B) acetonitrile/difluoroaceticacid(100/0.1)  
Gradient: 20–32%B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

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

This research was supported by AMED under Grant Number JP18ae0201001.



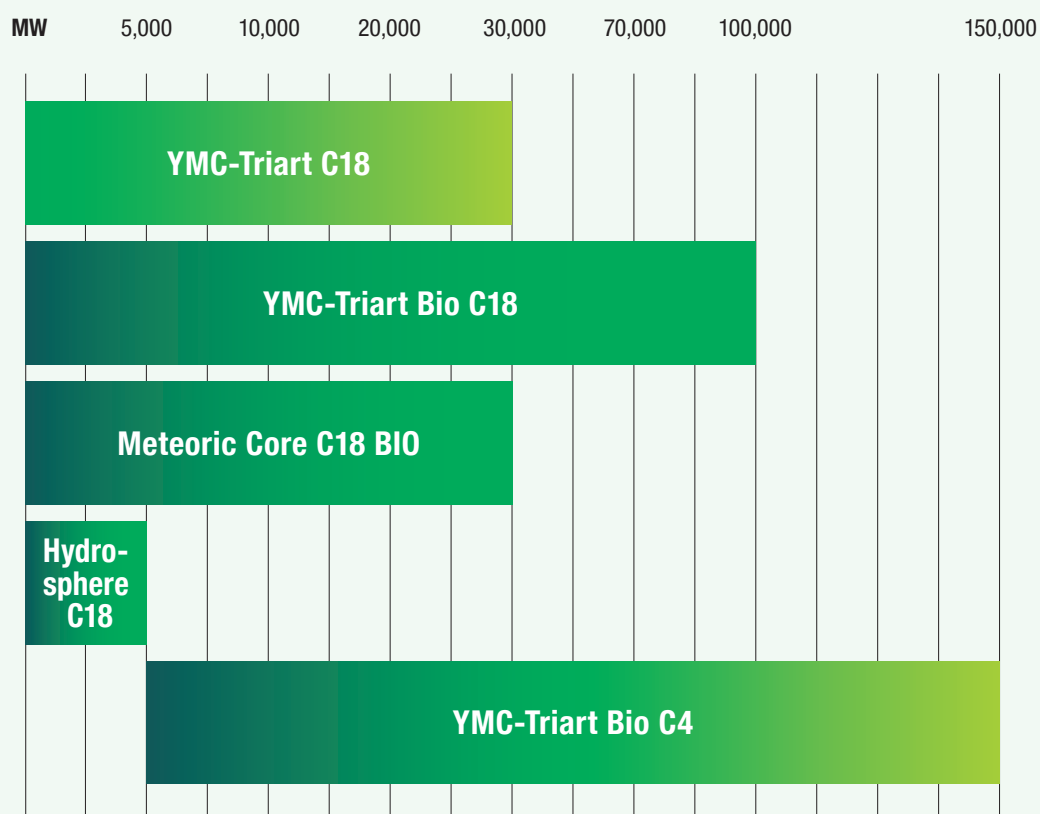
(Bioinert)  
RP



## 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



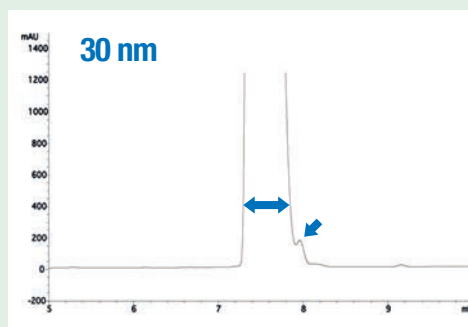
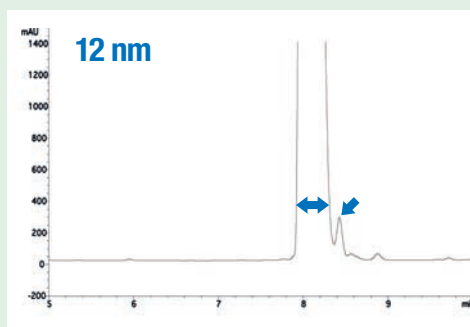
- most appropriate MW range
- extended MW range by elevated temperature
- appropriate MW range

## Influence of pore size

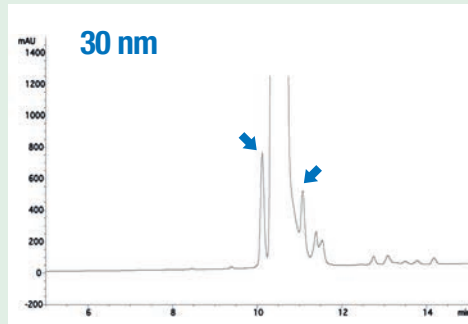
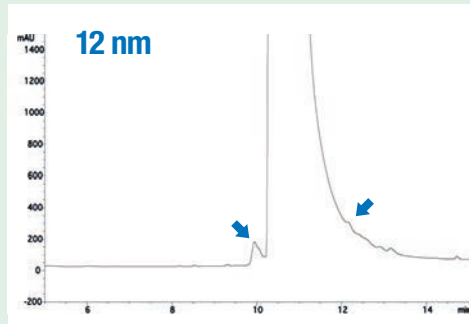
As shown in the table (on the left), the C18 column with 12 nm pore size is suitable for small peptides up to a MW of 5,000 Da. The highest efficiency for large peptides or small proteins can be obtained by using a wide pore C8 phase with 20 nm porosity. Most proteins can be eluted efficiently with a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

Angiotensin II  
(MW 1,046)



BSA  
(MW 67,000)



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

## RP – UHPLC / HPLC Selectivities

### Selectivities for proteins/peptides and antibodies

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

### Selectivities for oligonucleotides

|                               | YMC-Triart C18                  | YMC-Triart Bio C18             | YMC-Triart C8                  | Hydrosphere C18 |
|-------------------------------|---------------------------------|--------------------------------|--------------------------------|-----------------|
| Base particle                 | organic/inorganic hybrid silica |                                |                                | silica          |
| Modification                  | C18 (USP L1)                    | C18 (USP L1)                   | C8 (USP L7)                    | C18 (USP L1)    |
| Particle Size / $\mu\text{m}$ | 1.9, 3, 5                       | 1.9, 3, 5                      | 1.9, 3, 5                      | 2, 3, 5         |
| Pore Size / nm                | 12                              | 30                             | 12                             | 12              |
| pH range                      | 1.0 – 12.0                      | 1.0–12.0                       | 1.0–12.0                       | 2.0–8.0         |
| Temperature range             | pH < 7: 90 °C<br>pH > 7: 50 °C  | pH < 9: 90 °C<br>pH > 9: 50 °C | pH < 7: 90 °C<br>pH > 7: 50 °C | 50 °C           |

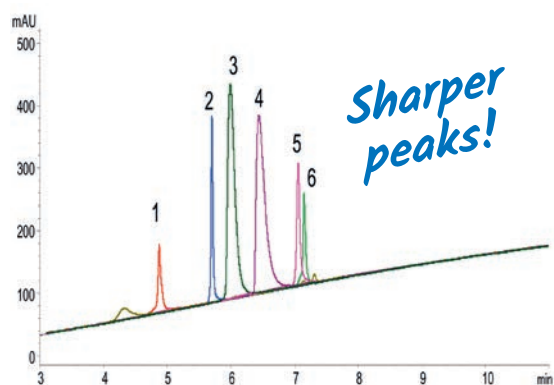


**B**ioinert YMC-Triart columns are available for improved sensitivity, peak shape and recovery of coordinating compounds such as nucleotides, oligonucleotides or phosphorylated proteins/peptides.

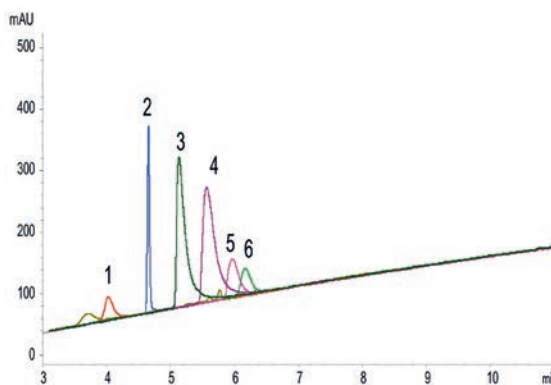
## Better performance using YMC-Triart Bio C4

High sensitivity and sharp peaks under LC/MS compatible conditions

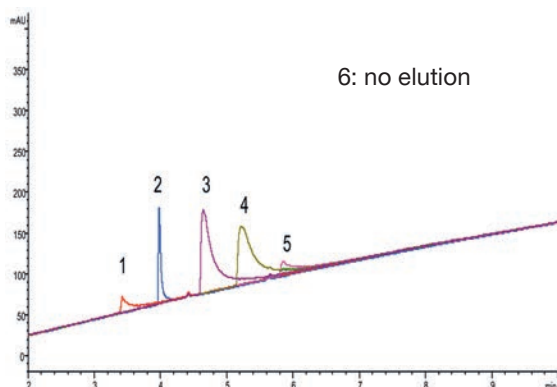
YMC-Triart Bio C4 (3  $\mu$ m, 30 nm)



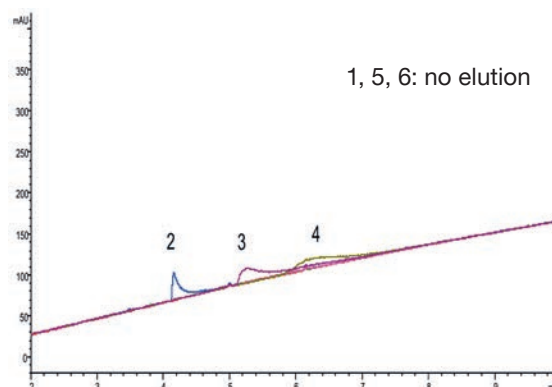
XBridge Protein BEH C4 (3.5  $\mu$ m, 30 nm)



AdvanceBio RP-mAb C4 (3.5  $\mu$ m, 45 nm)



Aeris widepore C4 (3.6  $\mu$ m, 20 nm)



Column: 150 x 3.0 mm ID  
Part No.: TB30S03-1503PTH  
Eluent: A) water/formic acid (100/0.1)  
B) acetonitrile/formic acid (100/0.1)  
Gradient: 10–95%B (0–15 min)  
Flow rate: 0.4 mL/min (for 3.0 mm ID)  
1.0 mL/min (for 4.6 mm ID)  
Temperature: 40 °C

Detection: UV at 220 nm  
Sample: 1. Cytochrome c (Horse heart)  
2. Insulin (Bovine pancreas)  
3. Transferrin (Human)  
4. BSA  
5.  $\beta$ -Lactoglobulin (Bovine)  
6.  $\alpha$ -Chymotrypsinogen A (Bovine pancreas)

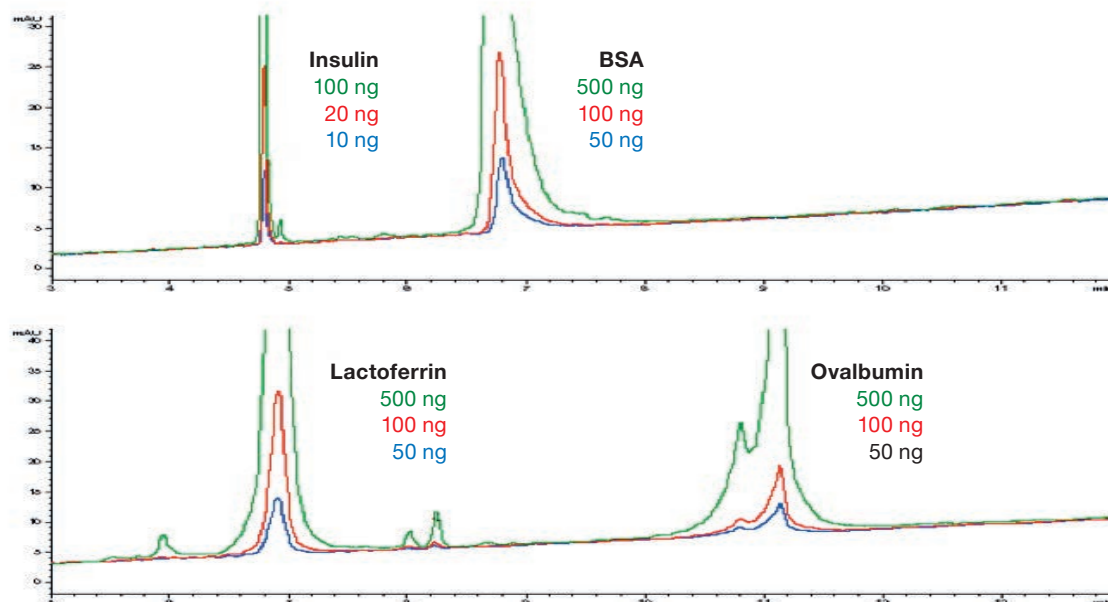
YMC-Triart Bio C4 shows better peak shape and recovery with a mobile phase containing formic acid, which is commonly used for LC/MS analysis. Therefore, YMC-Triart Bio C4 is ideal for high sensitivity analysis of proteins.

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

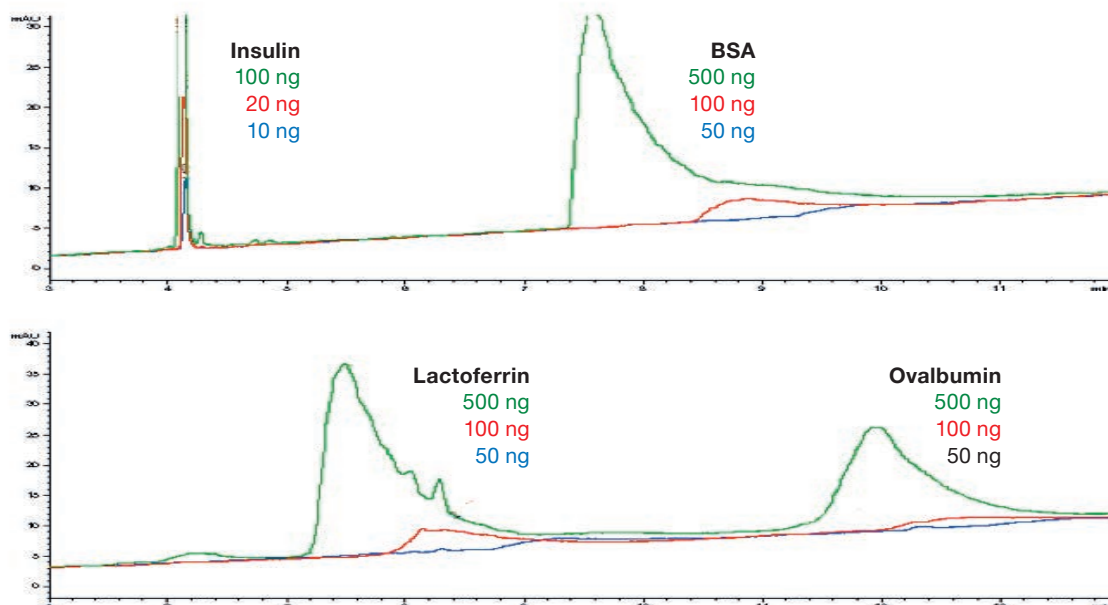
### No sample adsorption by YMC-Triart Bio C4 columns

#### Ideal for Microanalysis

##### YMC-Triart Bio C4 (1.9 $\mu$ m, 30 nm)



##### Aeris widepore C4 (3.6 $\mu$ m, 20 nm)

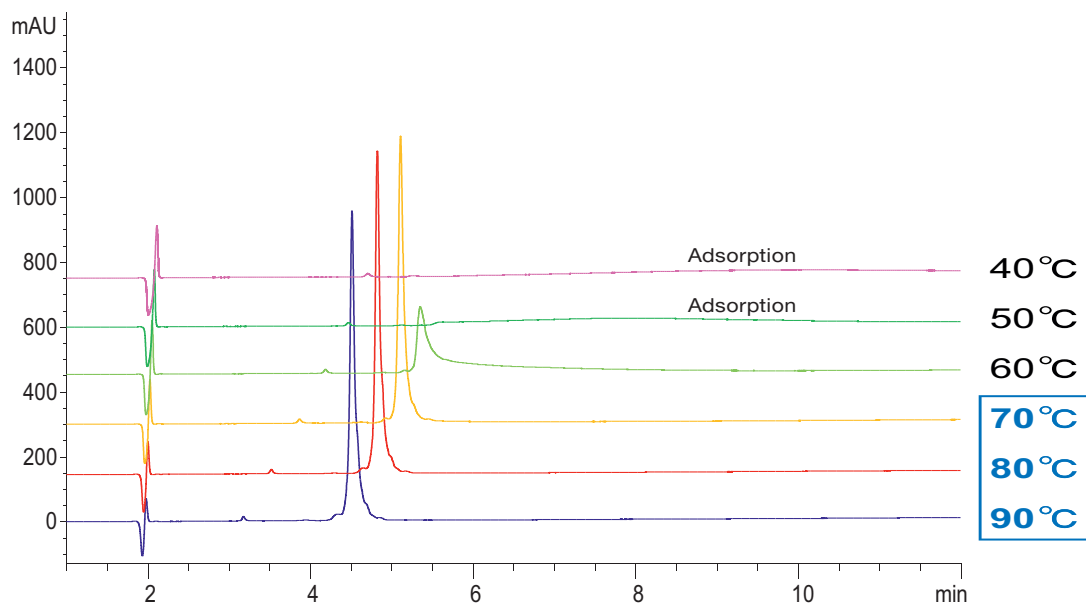


Column: 150 x 2.1 mm ID  
 Part No.: TB30SP9-15Q1PT  
 Eluent: A) water/TFA (100/0.05)  
 B) acetonitrile/TFA (100/0.05)  
 Gradient: 25-60%B (0-15 min), 90%B (15-20 min), 25%B (20-35 min)  
 Flow rate: 0.2 mL/min  
 Temperature: 40°C  
 Detection: UV at 220 nm

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

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



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

“

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

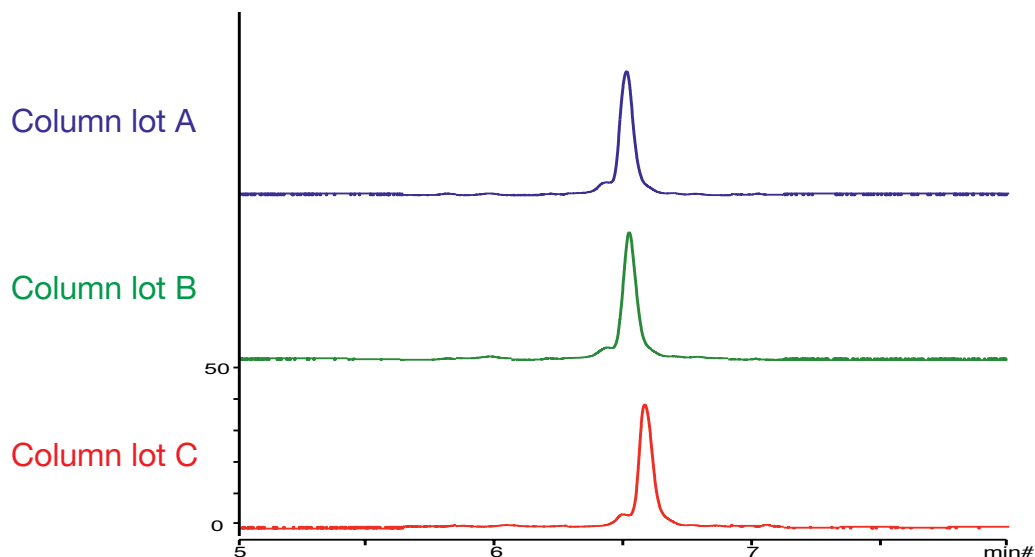
*Lars M. H. Reinders, Institute for Energy and Environmental Technology e. V. (IUTA, DE)*

”

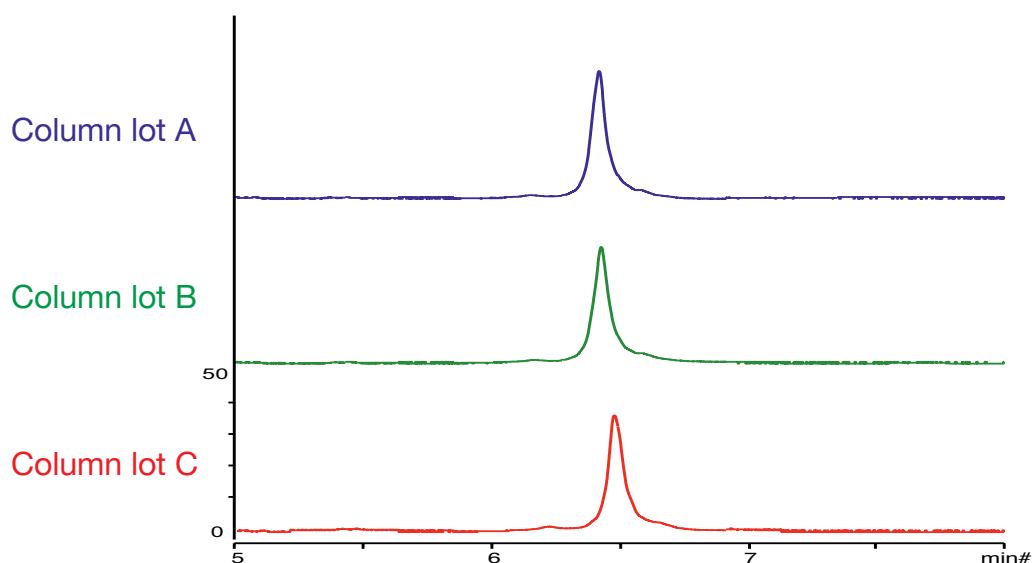
## RP – YMC-Triart Bio C4: Reproducibility

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

NISTmAb, 8671



Bevacizumab (Avastin®)



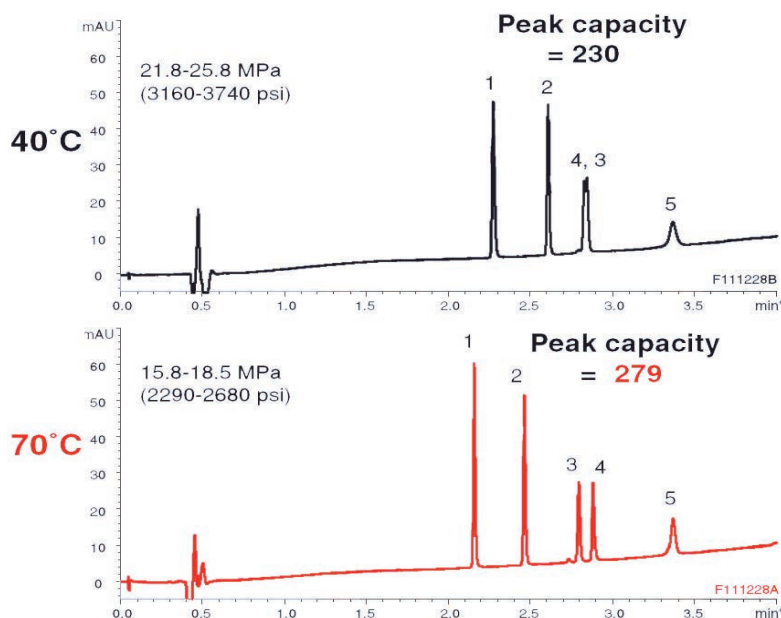
Column: YMC-Triart Bio C4 (1.9  $\mu$ m, 30 nm) 50 x 2.1 mm ID  
Part No.: TB30SP9-05Q1PT  
Eluent: A) water/TFA (100/0.1), B) acetonitrile/TFA (100/0.1)  
Gradient: 25–45%B (0–10 min)  
Flow rate: 0.4 mL/min  
Temperature: 80 °C  
Detection: UV at 280 nm  
Injection: 2  $\mu$ L (0.5 mg/mL)

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.

## More temperature flexibility using YMC-Triart

### Highly efficient RP-HPLC separation of proteins

#### Mixture A (MW 500–18,400)



| Analytes | MW | Peak width 1/2h (min) |      |
|----------|----|-----------------------|------|
|          |    | 40°C                  | 70°C |

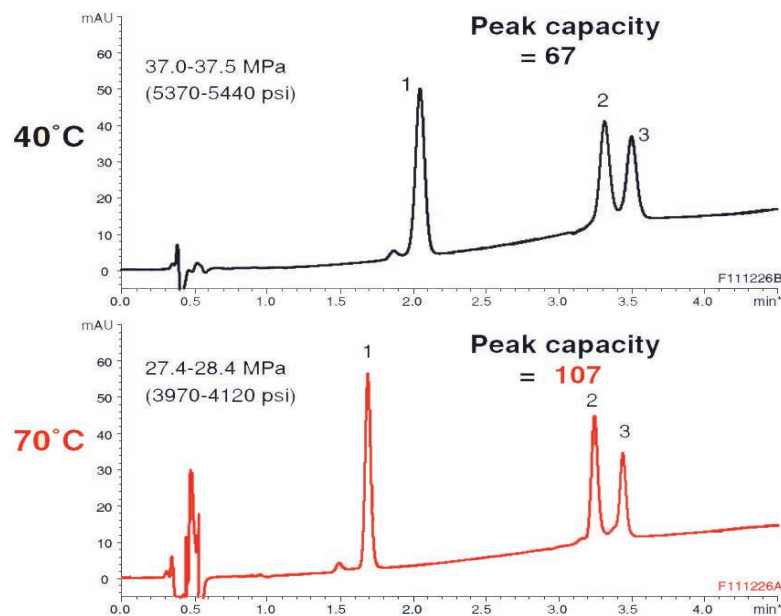
#### Mixture A

|                      |        |       |       |
|----------------------|--------|-------|-------|
| 1. Oxytocin          | 1,007  | 0.017 | 0.014 |
| 2. Leu-Enkephalin    | 556    | 0.015 | 0.015 |
| 3. β-Endorphin       | 3,465  | —     | 0.016 |
| 4. Insulin           | 5,733  | —     | 0.015 |
| 5. β-Lactoglobulin A | 18,400 | 0.043 | 0.030 |

#### Mixture B

|                       |        |       |       |
|-----------------------|--------|-------|-------|
| 1. Lysozyme           | 14,300 | 0.069 | 0.044 |
| 2. α-Chymotrypsinogen | 25,700 | 0.080 | 0.049 |
| 3. β-Lactoglobulin A  | 18,400 | 0.080 | 0.048 |

#### Mixture B (MW 14,300–25,700)



*High temperatures only possible with YMC-Triart*

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

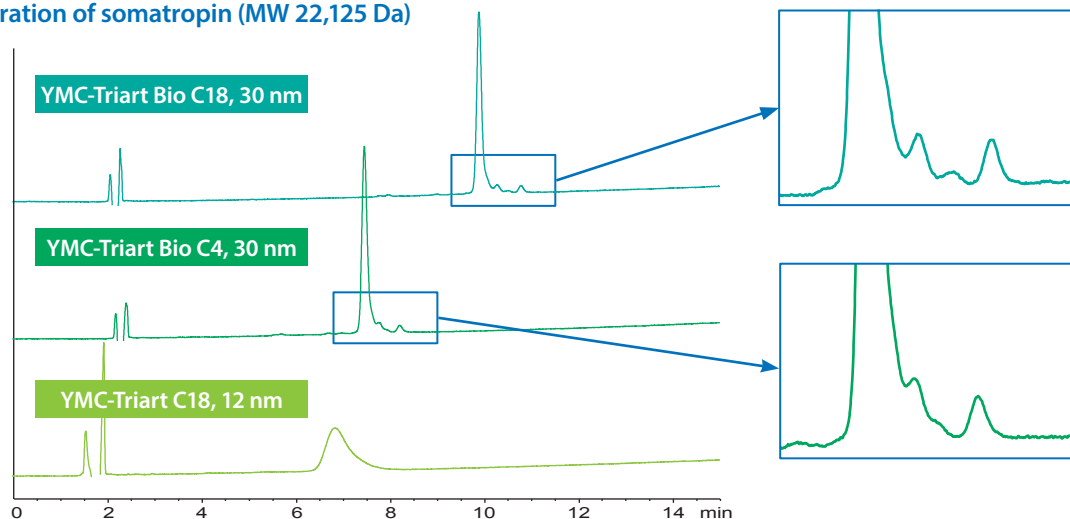
Flow rate: 0.4 mL/min  
Detection: UV at 220 nm  
Injection: 1 μL (50 μg/mL) - condition A  
1 μL (250 μg/mL) - condition B  
System: Agilent 1200SL

PC (peak capacity) = 1 + (gradient time / peak width\*)  
\*peak width = 2W<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)



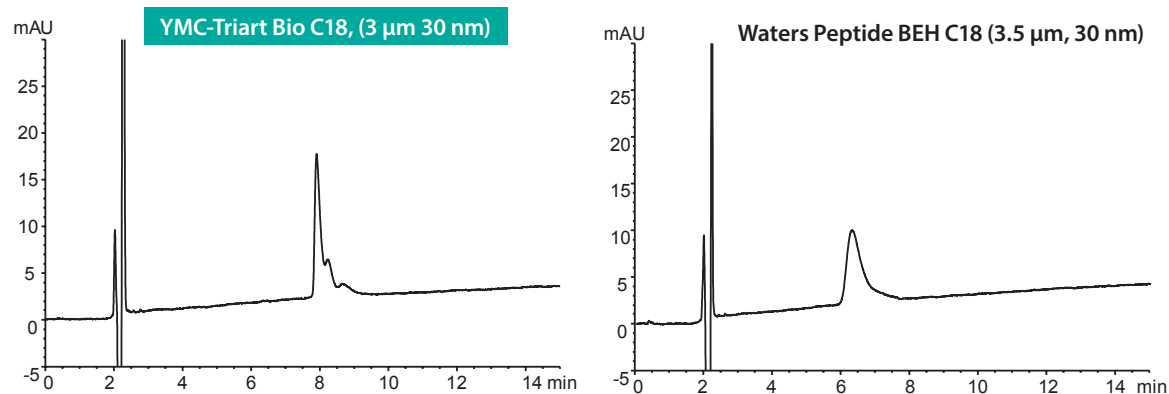
Columns: 150 x 3.0 mm ID (3  $\mu$ m)  
 Part Nos.: TA30S03-1503PTH  
 TB30S03-1503PTH  
 TA12S03-1503PTH  
 Eluent: A) water/TFA (100/0.1)  
 B) acetonitrile/TFA (100/0.08)

Gradient: 50–70%B (0–15 min)  
 Flow rate: 0.425 mL/min  
 Temperature: 40 °C  
 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)

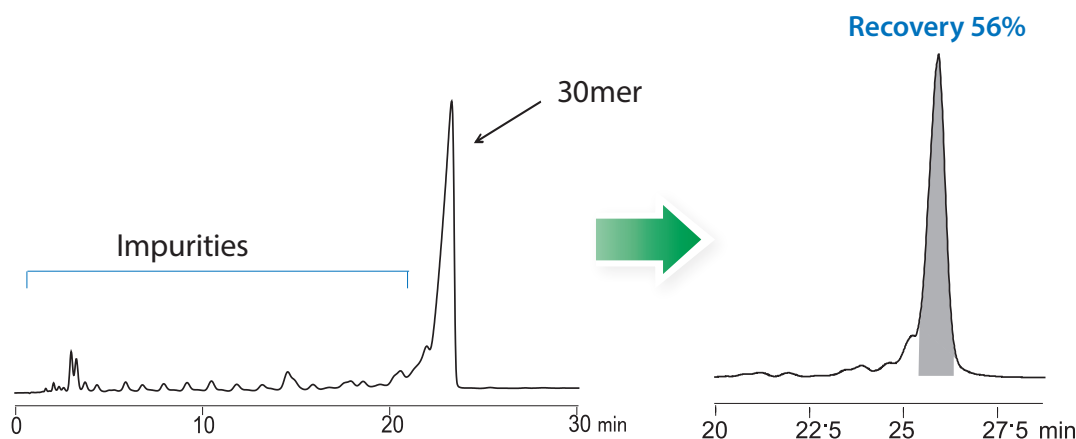
YMC-Triart Bio C18 is suitable for highly sensitive analysis and structural analysis of proteins using LC/MS since good peak shapes in mobile phase containing formic acid can be achieved.

## Easy purification of oligonucleotides with YMC-Actus semiprep 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

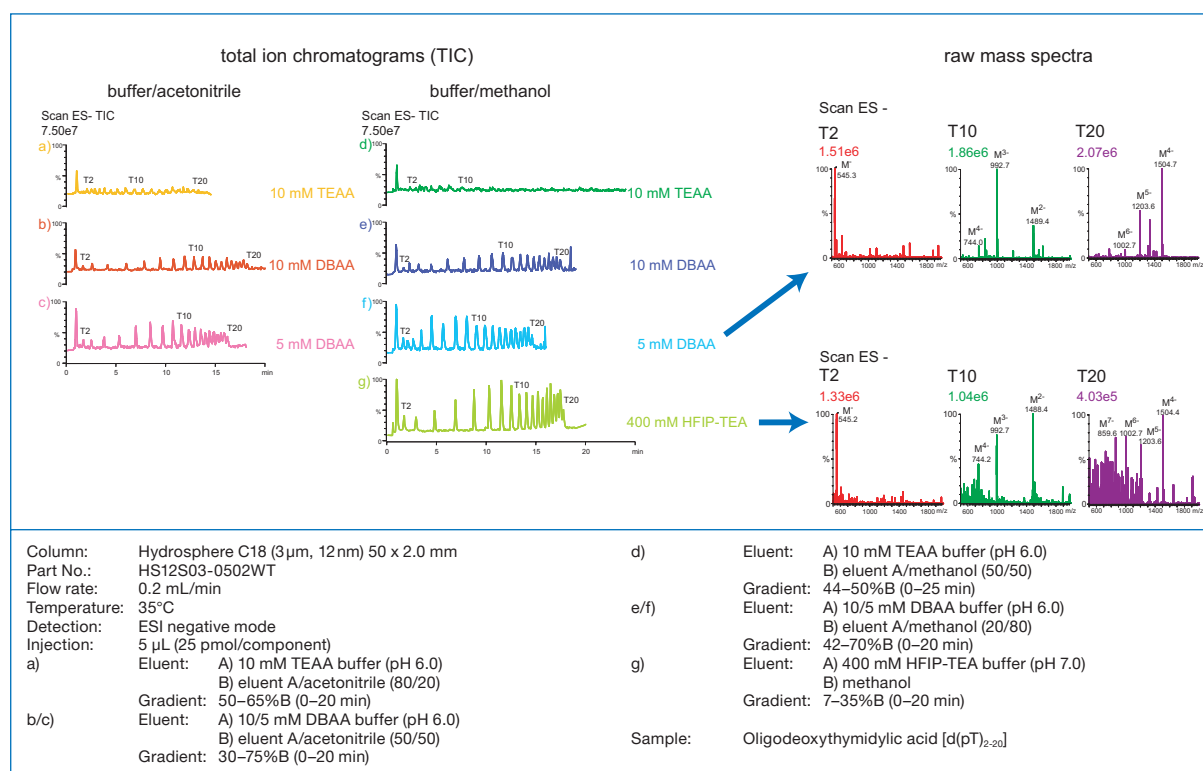
**Purification** 19 mL/min, 100  $\mu$ L injection  
**YMC-Actus Hydrosphere C18**  
 50 x 20 mm ID, 5  $\mu$ m



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%

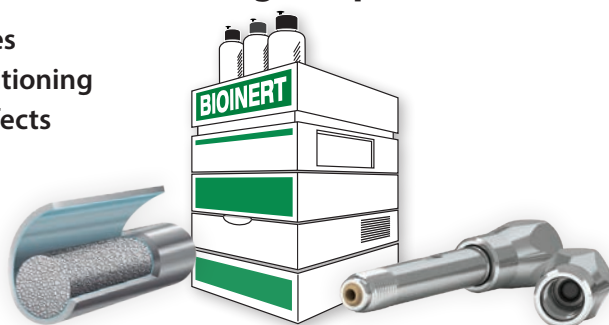
## Influences of mobile phase conditions on intensity of ESI-MS



## RP – YMC-Triart: Bioinert hardware

### Bioinert columns for bioseparations and coordinating compounds

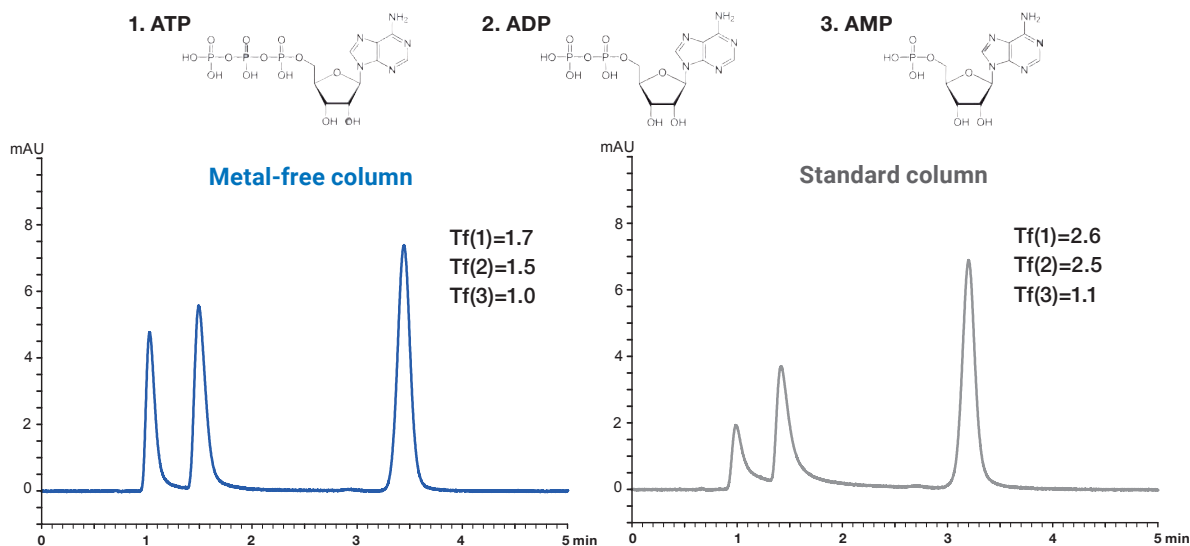
- Exceptional peak shapes with high sensitivities
- Excellent recoveries without column preconditioning
- Superior reproducibility and no carry-over effects
- Ideal for highly sensitive LC/MS analyses
- Different bioinert hardware options



#### Specification

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

#### Improved sensitivity for coordination compounds



Column: YMC-Triart C18 (3 µm, 12nm) 50 x 2.1 mm ID  
 Part Nos.: TA12S03-05Q1PTP (metal-free) or TA12S03-05Q1PTH (standard hardware)  
 Eluent: 5 mM HCOONH<sub>4</sub>  
 Flow rate: 0.21 mL/min

Temperature: 25 °C  
 Detection: UV at 265 nm  
 Injection: 1 µL (10 mg/mL)  
 System: bioinert/"metal-free" HPLC system

Metal coordinating compounds, which have a phosphate group in their structure, tend to show poor peak shape due to interactions with metals, such as the stainless steel in column bodies and frits. By using a bioinert column hardware, better peak shapes can be expected.

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

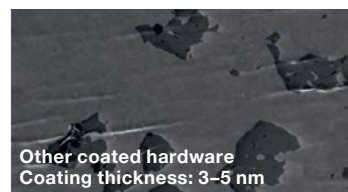
## YMC-Accura Triart: durable bioinert coating



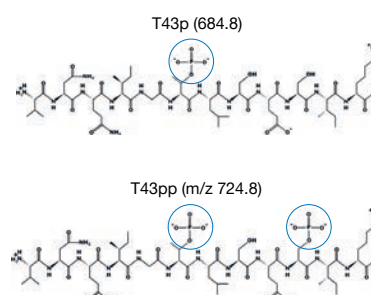
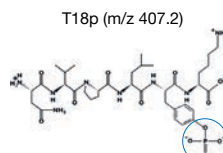
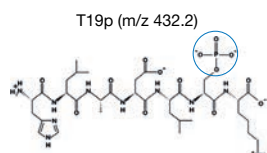
The robust bioinert coating used on YMC-Accura hardware is 130 to 320-fold thicker making it more durable than other similar hardware concepts. A long-term inertness against sensitive substances is ensured. In order to demonstrate its robustness, a YMC-Accura column was packed multiple times. Even though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

\*Scanning Electron Microscope

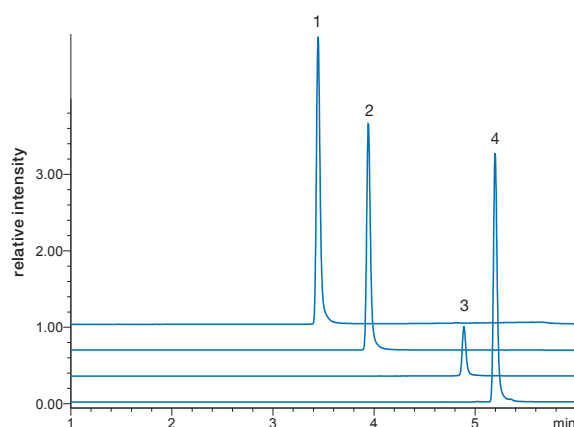
Other coated columns can lose their inertness over time. This will again lead to adsorption of sensitive compounds on the uncovered metallic surfaces. Peak tailing, loss of recovery and sample carry-over are typical results of the delamination of the coating. After only unpacking a coated competitor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



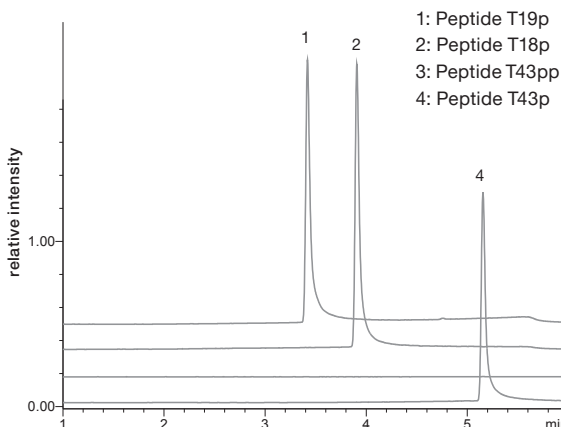
### Full recovery of phosphorylated peptides



#### YMC-Accura Triart C18



#### Standard column



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

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

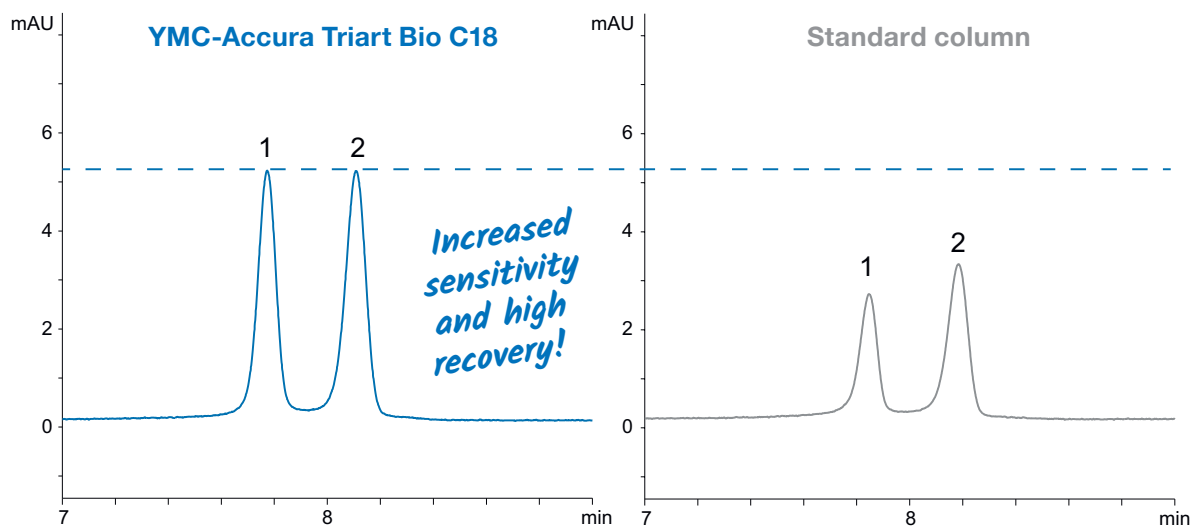
By courtesy of Shimadzu Europa.

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

## RP – YMC-Triart: Bioinert hardware

### Significantly higher sensitivity and recovery

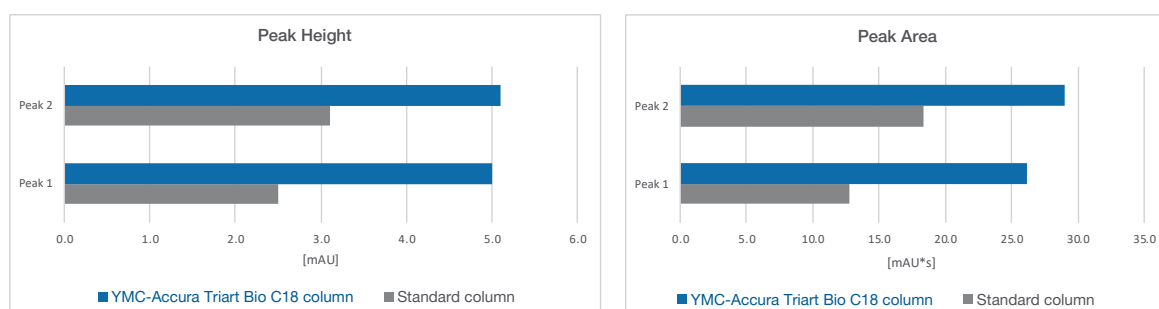
Ideal choice for challenging analytes such as phosphorothioate oligonucleotides



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

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

### High sensitivity and recovery

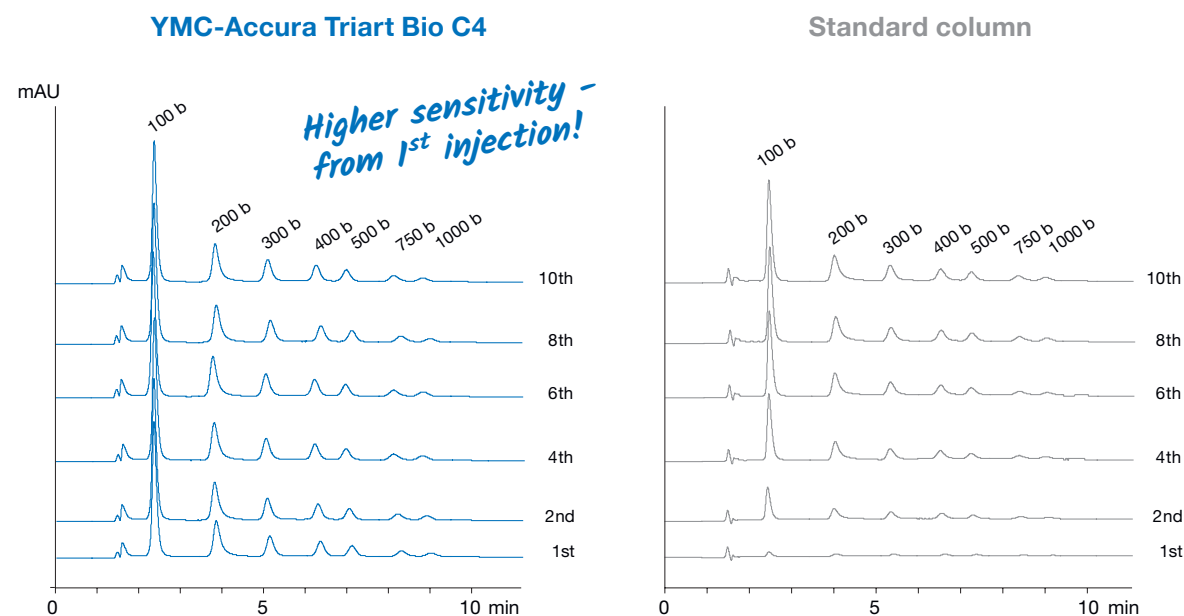


*Doubled peak height and area!*

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

## Reliable results from the first injection

No preconditioning required for reliable results

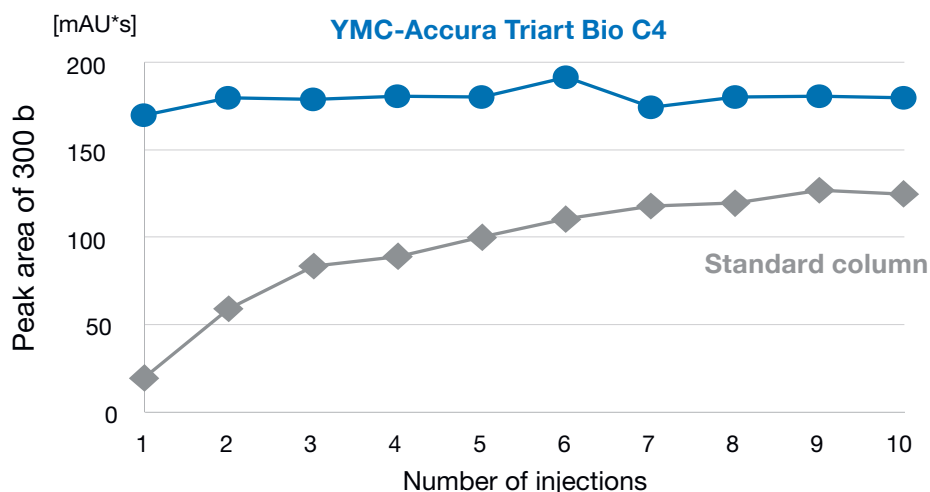


Column: YMC-Accura Triart Bio C4 (1.9  $\mu$ m, 30 nm) 100 x 2.1 mm ID  
Part No.: TA30SP9-10Q1PTC  
Eluent: A) 50 mM TEAA\* (pH 7.0)/acetonitrile (95/5)  
B) 50 mM TEAA (pH 7.0)/acetonitrile (50/50)  
Gradient: 9–14%B (0–10 min), 80%B (10–15 min)

Flow rate: 0.2 mL/min  
Temperature: 80°C  
Detection: UV at 254 nm  
Injection: 1  $\mu$ L (0.25 mg/mL)  
Sample: 100–1,000 bases (Century™-Plus RNA Markers)

\* Triethylammonium acetate

Constantly higher peak areas and therefore recoveries

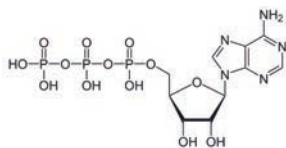


The YMC-Accura Triart Bio C4 column shows stable peak areas from the first injection, while the standard stainless-steel column provides only 10% of the peak area (for the 300 base marker) with the first injection. Even after the tenth injection, the peak areas of the stainless-steel column are considerably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

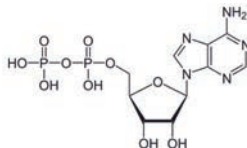
## RP – Expert Tips: (Oligo)nucleotides

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

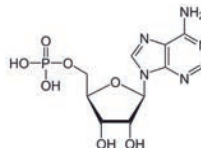
1 ATP



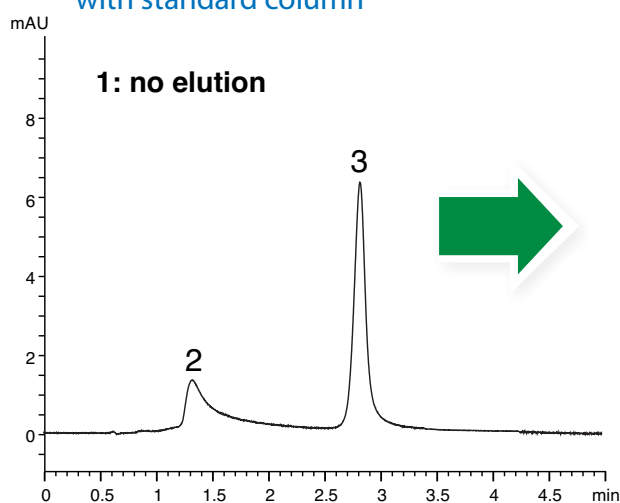
2 ADP



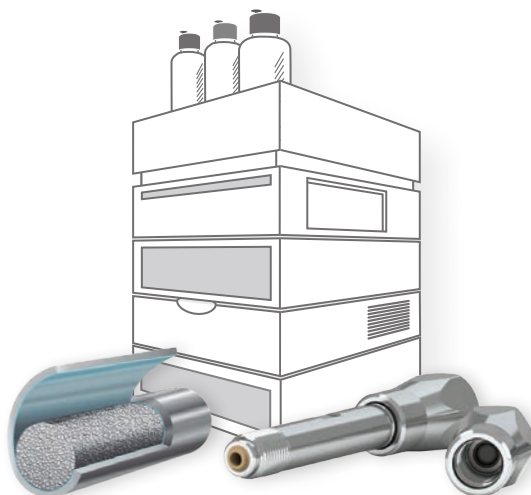
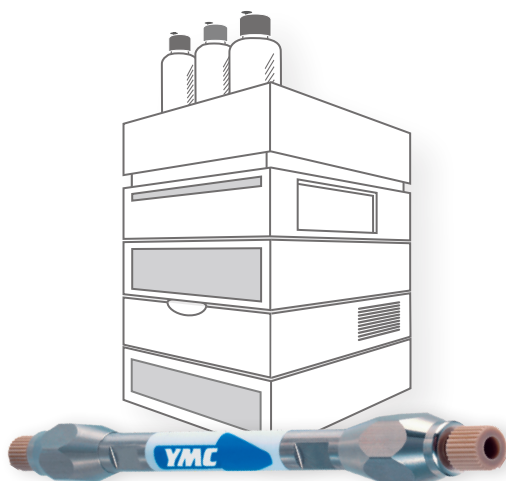
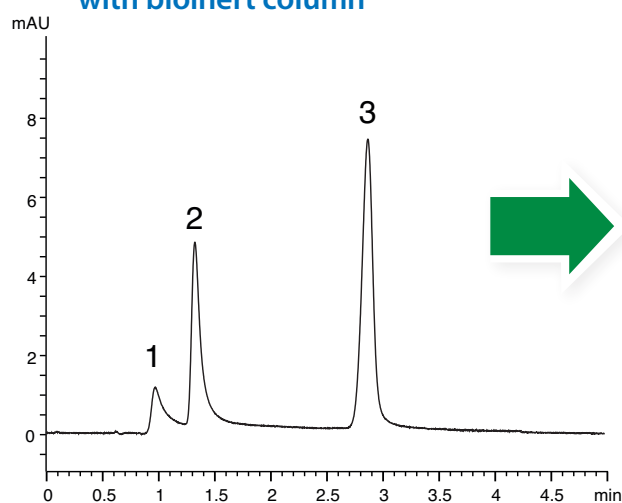
3 AMP



Ordinary HPLC system  
with standard column



Ordinary HPLC system  
with bioinert column

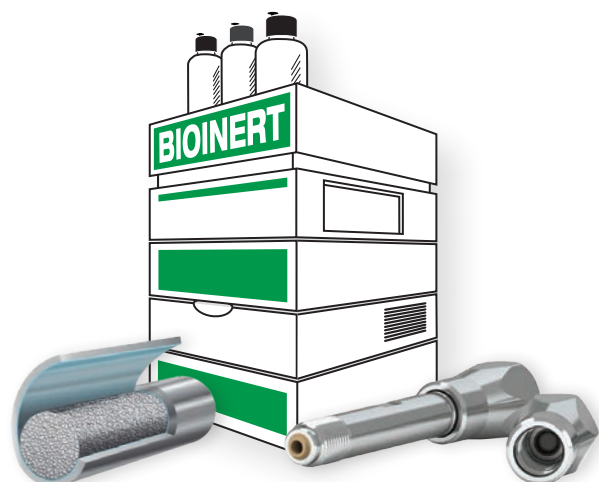
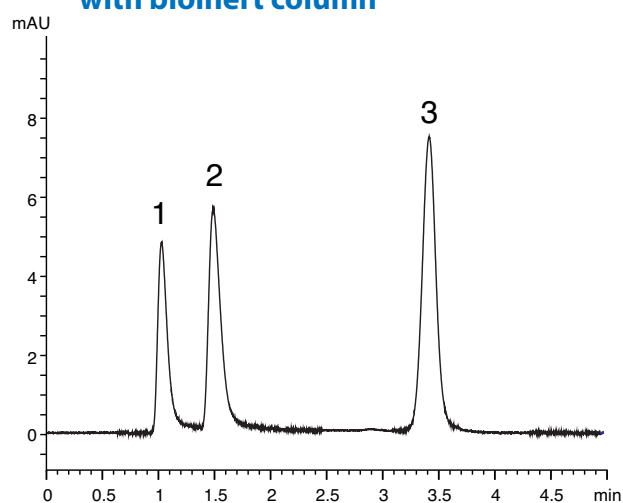


Column: YMC-Triart C18 (3  $\mu$ m, 12 nm) 50 x 2.1 mm ID  
 Part Nos: TA12S03-05Q1PT (standard hardware)  
 TA12S03-05Q1PTP (bioinert hardware)  
 Eluent: 5 mM HCOONH<sub>4</sub>  
 Flow rate: 0.21 mL/min  
 Temperature: 25°C  
 Detection: UV at 265 nm  
 Injection: 1  $\mu$ L (10  $\mu$ g/mL)

\*Bioinert HPLC system: PEEK sample loop, PEEK injector port, and PEEK tubing are used.

ATP peak is detected, and peak shape of ADP is improved as a result of using a bioinert column.

### Bioinert HPLC system\* with bioinert column



“

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

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

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

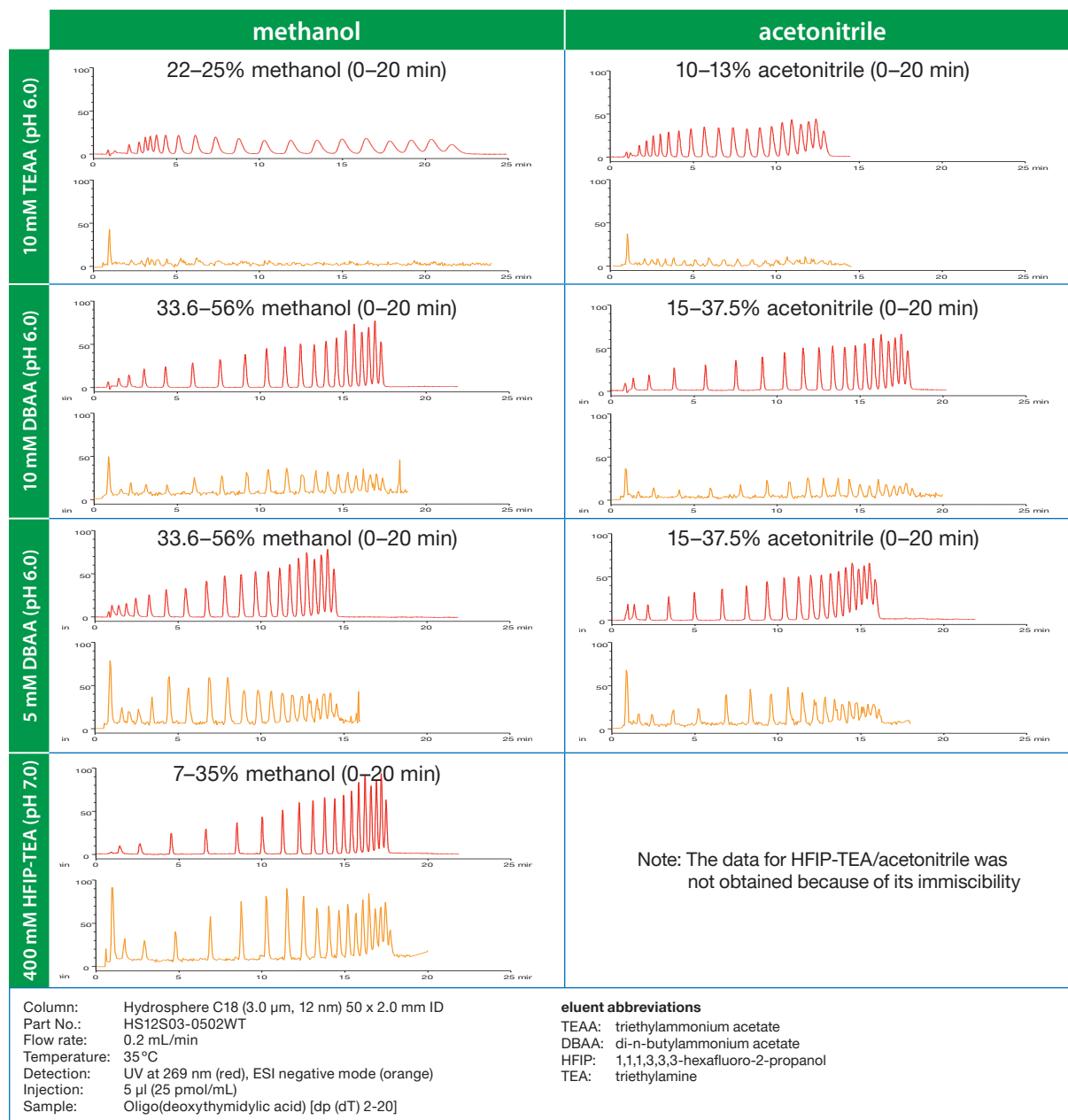
”

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

## RP – Expert Tips: Oligonucleotides

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

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



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

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

### 1.9 µm UHPLC columns (max. pressure 100 MPa)

| Phase              | Column ID [mm] | Column length [mm] |                |                |                |                | Guard cartridges* with 5 mm length |
|--------------------|----------------|--------------------|----------------|----------------|----------------|----------------|------------------------------------|
|                    |                | 30                 | 50             | 75             | 100            | 150            |                                    |
| YMC-Triart C18     | 1.0            | —                  | TA12SP9-0501WT | —              | TA12SP9-1001WT | TA12SP9-1501WT | TA12SP9-E5Q1CC**                   |
|                    | 2.0            | TA12SP9-0302PT     | TA12SP9-0502PT | TA12SP9-L502PT | TA12SP9-1002PT | TA12SP9-1502PT | TA12SP9-E5Q1CC**                   |
|                    | 2.1            | TA12SP9-03Q1PT     | TA12SP9-05Q1PT | TA12SP9-L5Q1PT | TA12SP9-10Q1PT | TA12SP9-15Q1PT | TA12SP9-E5Q1CC**                   |
|                    | 3.0            | —                  | TA12SP9-0503PT | TA12SP9-L503PT | TA12SP9-1003PT | TA12SP9-1503PT | TA12SP9-E503CC                     |
| YMC-Triart Bio C18 | 2.0            | TA30SP9-0302PT     | TA30SP9-0502PT | TA30SP9-L502PT | TA30SP9-1002PT | TA30SP9-1502PT | TA30SP9-E5Q1CC**                   |
|                    | 2.1            | TA30SP9-03Q1PT     | TA30SP9-05Q1PT | TA30SP9-L5Q1PT | TA30SP9-10Q1PT | TA30SP9-15Q1PT | TA30SP9-E5Q1CC**                   |
|                    | 3.0            | —                  | TA30SP9-0503PT | TA30SP9-L503PT | TA30SP9-1003PT | TA30SP9-1503PT | TA30SP9-E503CC                     |
| YMC-Triart C8      | 2.0            | T012SP9-0302PT     | T012SP9-0502PT | T012SP9-L502PT | T012SP9-1002PT | T012SP9-1502PT | T012SP9-E5Q1CC**                   |
|                    | 2.1            | T012SP9-03Q1PT     | T012SP9-05Q1PT | T012SP9-L5Q1PT | T012SP9-10Q1PT | T012SP9-15Q1PT | T012SP9-E5Q1CC**                   |
|                    | 3.0            | —                  | T012SP9-0503PT | T012SP9-L503PT | T012SP9-1003PT | T012SP9-1503PT | T012SP9-E503CC                     |
| YMC-Triart Bio C4  | 2.0            | TB30SP9-0302PT     | TB30SP9-0502PT | TB30SP9-L502PT | TB30SP9-1002PT | TB30SP9-1502PT | TB30SP9-E5Q1CC**                   |
|                    | 2.1            | TB30SP9-03Q1PT     | TB30SP9-05Q1PT | TB30SP9-L5Q1PT | TB30SP9-10Q1PT | TB30SP9-15Q1PT | TB30SP9-E5Q1CC**                   |
|                    | 3.0            | —                  | TB30SP9-0503PT | TB30SP9-L503PT | TB30SP9-1003PT | TB30SP9-1503PT | TB30SP9-E503CC                     |

\*Guard cartridge holder required, part no. XPCUHP

\*\*Guard cartridge: 2.1 mm ID

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

| Phase                     | Column ID [mm] | Column length [mm] |                  |                 |
|---------------------------|----------------|--------------------|------------------|-----------------|
|                           |                | 50                 | 100              | 150             |
| YMC-Accura Triart C18     | 2.1            | TA12SP9-05Q1PTC    | TA12SP9-100Q1PTC | TA12SP9-15Q1PTC |
| YMC-Accura Triart Bio C18 | 2.1            | TA30SP9-05Q1PTC    | TA30SP9-10Q1PTC  | TA30SP9-15Q1PTC |
| YMC-Accura Triart C8      | 2.1            | T030SP9-05Q1PTC    | T012SP9-10Q1PTC  | T012SP9-15Q1PTC |
| YMC-Accura Triart Bio C4  | 2.1            | TB30SP9-05Q1PTC    | TB30SP9-10Q1PTC  | TB30SP9-15Q1PTC |

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

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

Special column connectors required.

## RP – Ordering information

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

| Phase              | Column ID [mm] | Column length [mm] |                 |                 |                 |                 |                 | Guard cartridges* with 10 mm length<br>(pack of 5) |
|--------------------|----------------|--------------------|-----------------|-----------------|-----------------|-----------------|-----------------|----------------------------------------------------|
|                    |                | 30/33              | 50              | 75              | 100             | 150             | 250             |                                                    |
| YMC-Triart C18     | 2.1            | TA12S03-H3Q1PTH    | 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-H3Q1PTH    | 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-H3Q1PTH    | 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            | TB30S03-03Q1PTH    | TB30S03-05Q1PTH | TB30S03-L5Q1PTH | TB30S03-10Q1PTH | TB30S03-15Q1PTH | –               | TB30S03-01Q1GC                                     |
|                    | 3.0            | –                  | TB30S03-05Q3PTH | TB30S03-L5Q3PTH | TB30S03-10Q3PTH | TB30S03-15Q3PTH | –               | TB30S03-01Q3GC                                     |
|                    | 4.6            | TB30S03-0346PTH    | TB30S03-0546PTH | TB30S03-L546PTH | TB30S03-1046PTH | TB30S03-1546PTH | TB30S03-2546PTH | TB30S03-01Q4GC                                     |
| Hydrosphere C18    | 2.1            | HS12S03-03Q1WT     | HS12S03-05Q1WT  | HS12S03-L5Q1WT  | HS12S03-10Q1WT  | HS12S03-15Q1WT  | HS12S03-25Q1WT  | HS12S03-01Q1GC                                     |
|                    | 3.0            | HS12S03-03Q3WT     | HS12S03-05Q3WT  | HS12S03-L5Q3WT  | HS12S03-10Q3WT  | HS12S03-15Q3WT  | HS12S03-25Q3WT  | HS12S03-01Q3GC                                     |
|                    | 4.6            | HS12S03-0346WT     | HS12S03-0546WT  | HS12S03-L546WT  | HS12S03-1046WT  | HS12S03-1546WT  | HS12S03-2546WT  | HS12S03-01Q4GC                                     |

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

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

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

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

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

Special column connectors required.

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

| Phase                 | Column ID [mm] | Column length [mm] |                 |                 |                 |                 | Precolumn filter 0.5 µm |
|-----------------------|----------------|--------------------|-----------------|-----------------|-----------------|-----------------|-------------------------|
|                       |                | 30                 | 50              | 75              | 100             | 150             |                         |
| Meteoric Core C18 BIO | 2.1            | CAW16SQ7-03Q1PT    | CAW16SQ7-05Q1PT | CAW16SQ7-L5Q1PT | CAW16SQ7-10Q1PT | CAW16SQ7-15Q1PT | XRPRCS35                |
|                       | 3.0            | CAW16SQ7-0303PT    | CAW16SQ7-0503PT | CAW16SQ7-L503PT | CAW16SQ7-1003PT | CAW16SQ7-1503PT |                         |
|                       | 4.6            | CAW16SQ7-0346PT    | CAW16SQ7-0546PT | CAW16SQ7-L546PT | CAW16SQ7-1046PT | CAW16SQ7-1546PT |                         |

\*Holder required, part no. XRPRCS03

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

| 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            | TA12S05-H3Q1PTH    | TA12S05-05Q1PTH | TA12S05-L5Q1PTH | TA12S05-10Q1PTH | TA12S05-15Q1PTH | –               | TA12S05-01Q1GC                      |
|                    | 3.0            | –                  | TA12S05-0503PTH | TA12S05-L503PTH | TA12S05-1003PTH | TA12S05-1503PTH | –               | TA12S05-0103GC                      |
|                    | 4.6            | TA12S05-H346PTH    | TA12S05-0546PTH | TA12S05-L546PTH | TA12S05-1046PTH | TA12S05-1546PTH | TA12S05-2546PTH | TA12S05-0104GC                      |
| YMC-Triart Bio C18 | 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                      |
| YMC-Triart C8      | 2.1            | TO12S05-H3Q1PTH    | TO12S05-05Q1PTH | TO12S05-L5Q1PTH | TO12S05-10Q1PTH | TO12S05-15Q1PTH | –               | TO12S05-01Q1GC                      |
|                    | 3.0            | –                  | TO12S05-0503PTH | TO12S05-L503PTH | TO12S05-1003PTH | TO12S05-1503PTH | –               | TO12S05-0103GC                      |
|                    | 4.6            | TO12S05-H346PTH    | TO12S05-0546PTH | TO12S05-L546PTH | TO12S05-1046PTH | TO12S05-1546PTH | TO12S05-2546PTH | TO12S05-0104GC                      |
| YMC-Triart Bio C4  | 2.1            | TB30S05-H3Q1PTH    | TB30S05-05Q1PTH | TB30S05-L5Q1PTH | TB30S05-10Q1PTH | TB30S05-15Q1PTH | –               | TB30S05-01Q1GC                      |
|                    | 3.0            | –                  | TB30S05-0503PTH | TB30S05-L503PTH | TB30S05-1003PTH | TB30S05-1503PTH | –               | TB30S05-0103GC                      |
|                    | 4.6            | TB30S05-H346PTH    | TB30S05-0546PTH | TB30S05-L546PTH | TB30S05-1046PTH | TB30S05-1546PTH | TB30S05-2546PTH | TB30S05-0104GC                      |
| Hydrosphere C18    | 2.1            | HS12S05-03Q1WT     | HS12S05-05Q1WT  | HS12S05-L5Q1WT  | HS12S05-10Q1WT  | HS12S05-15Q1WT  | HS12S05-25Q1WT  | HS12S05-01Q1GC                      |
|                    | 3.0            | HS12S05-0303WT     | HS12S05-0503WT  | HS12S05-L503WT  | HS12S05-1003WT  | HS12S05-1503WT  | HS12S05-2503WT  | HS12S05-0103GC                      |
|                    | 4.6            | HS12S05-0346WT     | HS12S05-0546WT  | HS12S05-L546WT  | HS12S05-1046WT  | HS12S05-1546WT  | HS12S05-2546WT  | HS12S05-0104GC                      |

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

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

| Phase                     | Column ID [mm] | Column length [mm] |                 |                 |
|---------------------------|----------------|--------------------|-----------------|-----------------|
|                           |                | 50                 | 100             | 150             |
| YMC-Accura Triart C18     | 2.1            | TA12S05-05Q1PTC    | TA12S05-10Q1PTC | TA12S05-15Q1PTC |
|                           | 4.6            | TA12S05-0546PTC    | TA12S05-1046PTC | TA12S05-1546PTC |
| YMC-Accura Triart Bio C18 | 2.1            | TA30S05-05Q1PTC    | TA30S05-10Q1PTC | TA30S05-15Q1PTC |
|                           | 4.6            | TA30S05-0546PTC    | TA30S05-1046PTC | TA30S05-1546PTC |
| YMC-Accura Triart C8      | 2.1            | TO12S05-05Q1PTC    | TO12S05-10Q1PTC | TO12S05-15Q1PTC |
|                           | 4.6            | TO12S05-0546PTC    | TO12S05-1046PTC | TO12S05-1546PTC |
| YMC-Accura Triart Bio C4  | 2.1            | TB30S05-05Q1PTC    | TB30S05-10Q1PTC | TB30S05-15Q1PTC |
|                           | 4.6            | TB30S05-0546PTC    | TB30S05-1046PTC | TB30S05-1546PTC |

## RP – Ordering information

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

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

Special column connectors required.

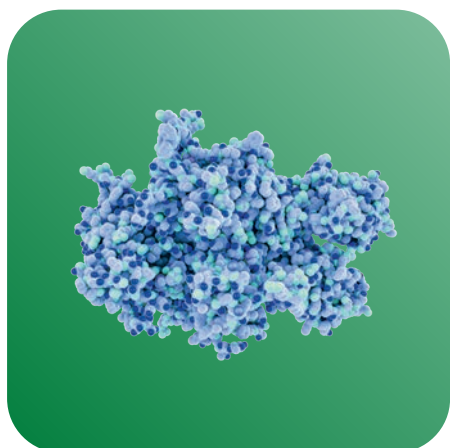
### 5 µm YMC-Actus high-throughput (semi)preparative columns (max. pressure 20–30 MPa)

| Phase                     | Column ID [mm] | Column length [mm] |                |                |                |                | Guard cartridges* with 10 mm length |
|---------------------------|----------------|--------------------|----------------|----------------|----------------|----------------|-------------------------------------|
|                           |                | 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 | –                                   |

\*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 µm for UHPLC**
- **Cost effective**

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

“

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

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

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**Column Selection Tool**

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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

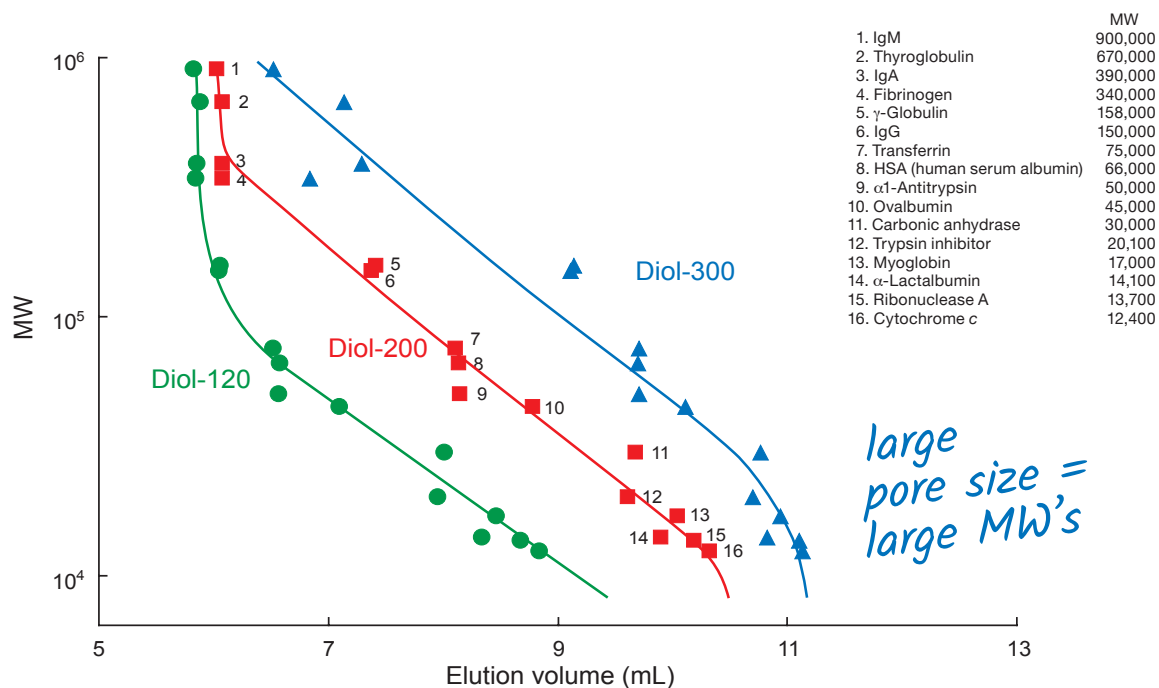
● YMC-Pack Diol-300

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

## Phases for different MW ranges

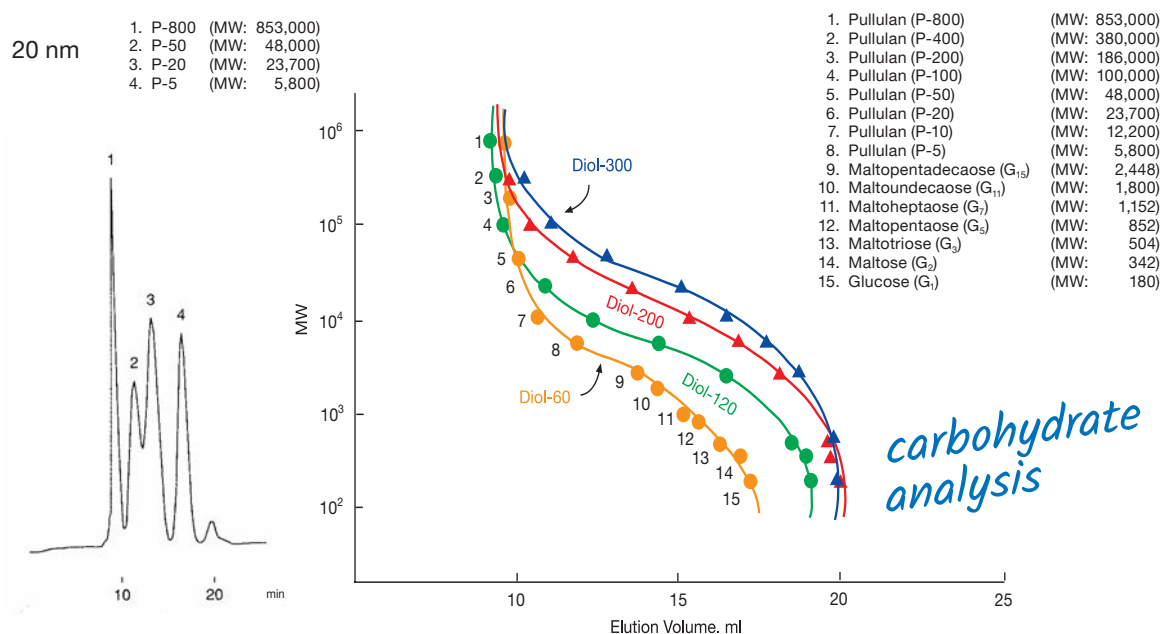
For separation of proteins with molecular weights from 10,000 to several 100,000 Da



Column: YMC-Pack Diol, 300 x 8.0 mm ID  
 Part Nos.: DL12S05-3008WT, DL20S05-3008WT, DL30S05-3008WT  
 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: 25°C  
 Detection: UV at 280 nm

For molecular weight determination of oligosaccharides and polysaccharides

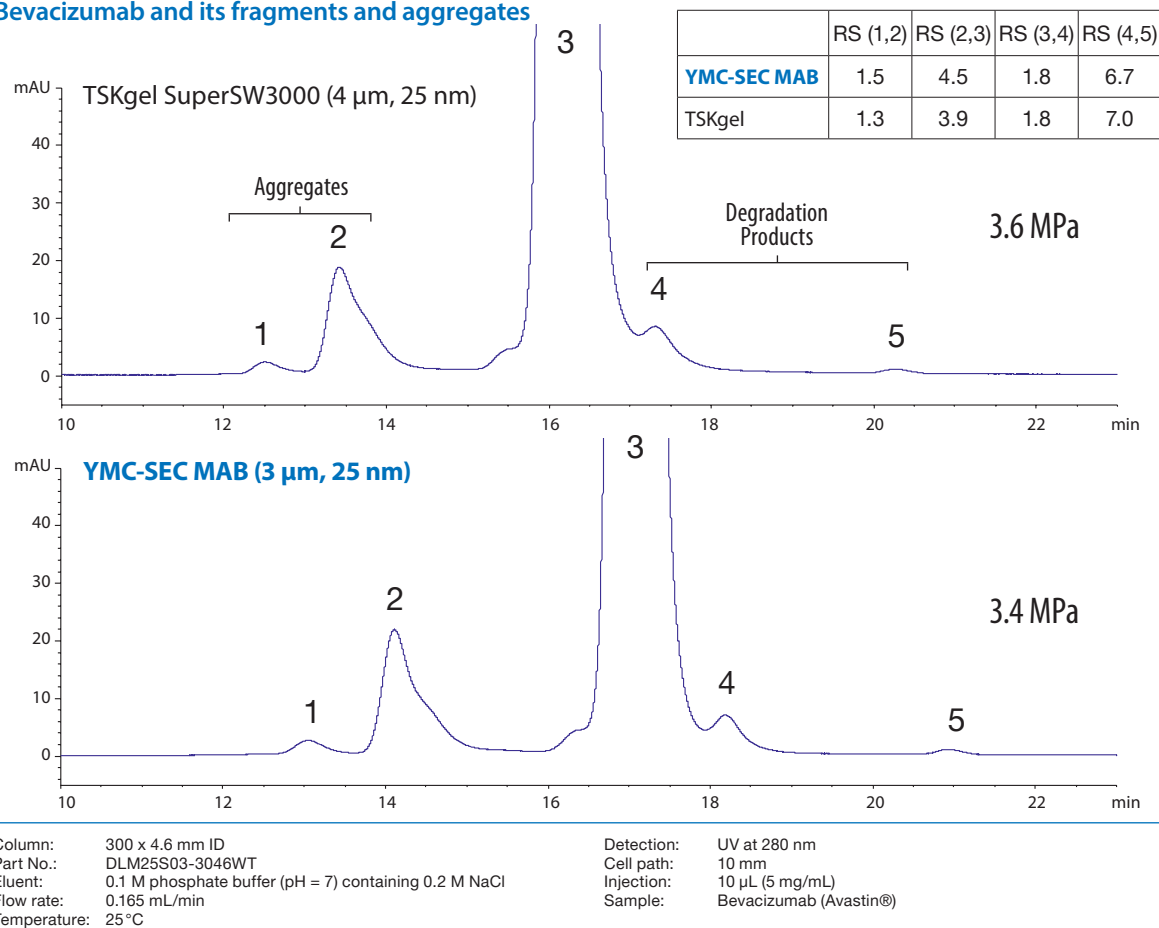


Column: YMC-Pack Diol (20 nm) 500 x 8.0 mm ID  
 Part No.: DL20S05-5008WT  
 Eluent: water

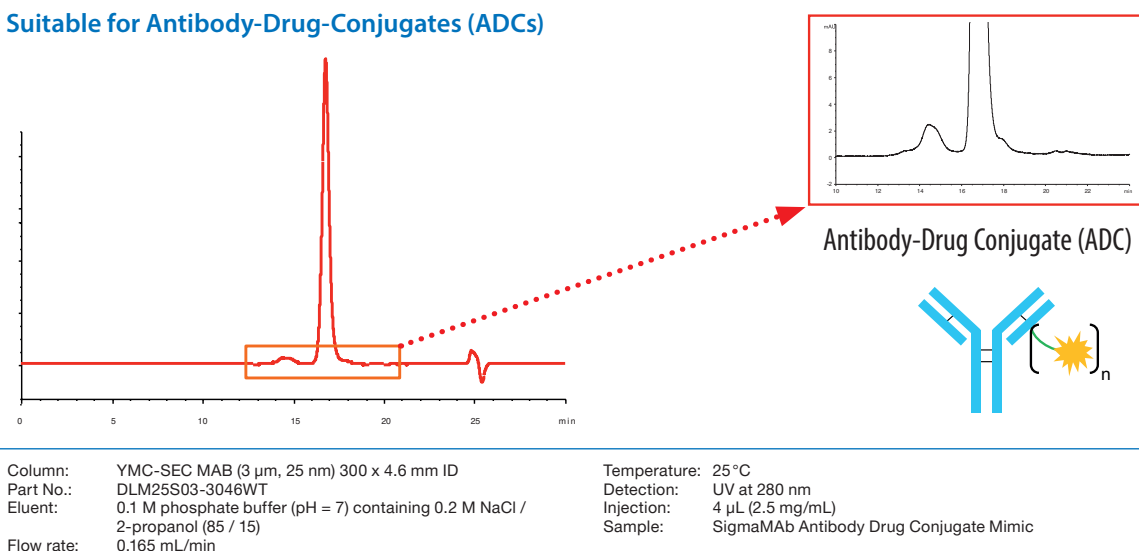
Flow rate: 1.0 mL/min  
 Temperature: ambient  
 Detection: RI

## Ideal choice for monoclonal antibodies

### Bevacizumab and its fragments and aggregates



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

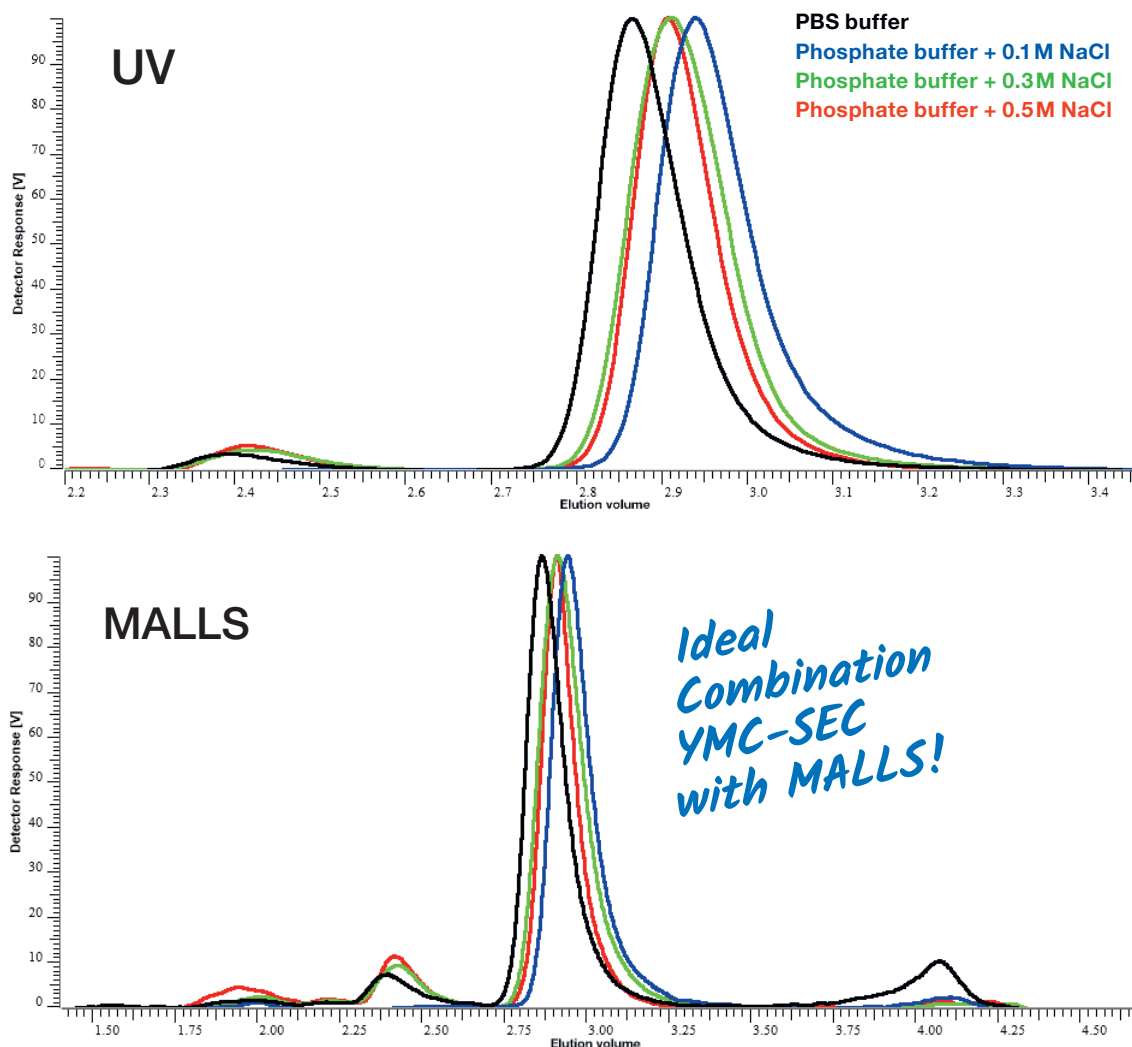


**YMC-SEC MAB is also suitable for the analysis of Antibody-Drug Conjugates (ADCs). The addition of an organic solvent to the mobile phase can improve the results obtained for ADC analysis.**

## SEC – YMC-SEC MAB: MALLS

## YMC-SEC columns ideally combined with light scattering detection

## Detection of higher molar mass species by MALLS



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

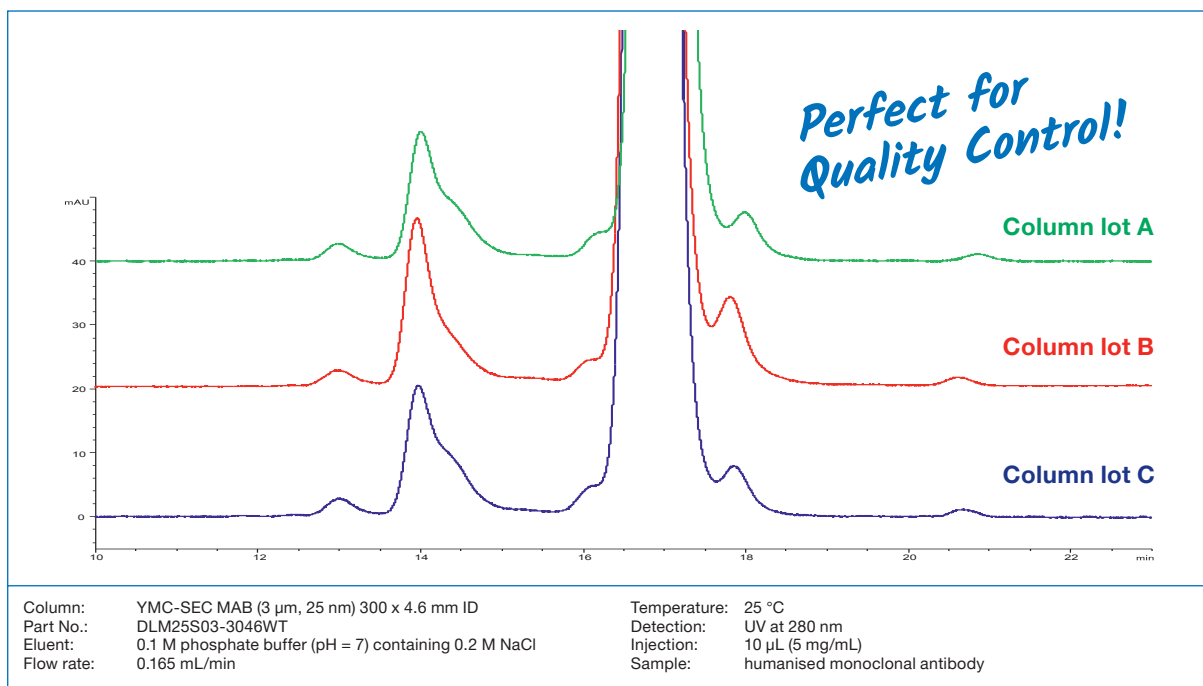
Courtesy of Thorsten Hofe, PSS Polymer Standards Service GmbH, Mainz, Germany.

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

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

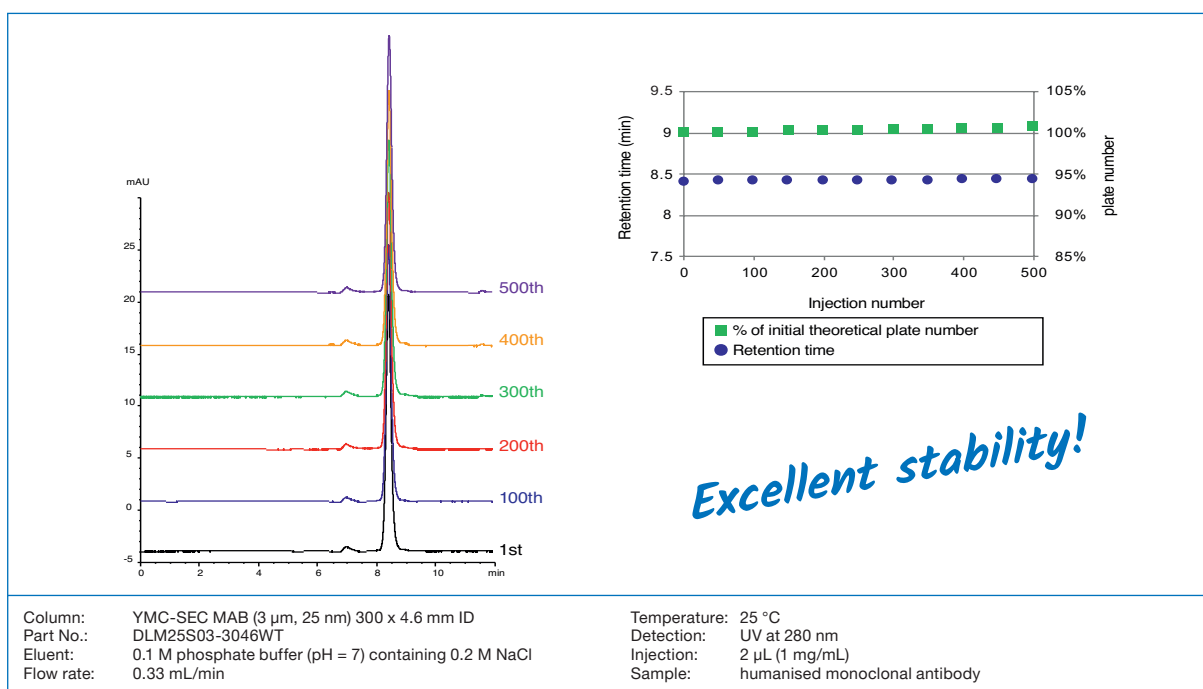
Compared to UV detection, the MALLS signal shows 2 higher molar mass species, aggregates of Bevacizumab, at about 2.0 mL and 2.3 mL elution volume.

## Excellent lot-to-lot reproducibility



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

## High column stability



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

## SEC – YMC-Pack Diol: Resolution & throughput

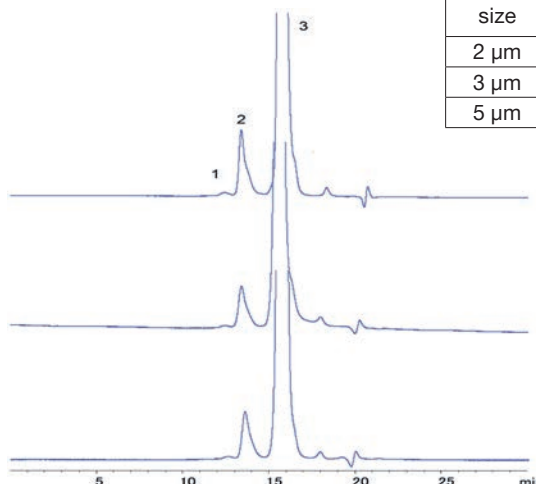
### Benefits of using smaller particles

#### Higher resolution for analysis of monoclonal antibodies

(A) YMC-Pack Diol-300 (2  $\mu\text{m}$ )  
300 x 4.6 mm ID

(B) YMC-Pack Diol-300 (3  $\mu\text{m}$ )  
300 x 4.6 mm ID

(C) YMC-Pack Diol-300 (5  $\mu\text{m}$ )  
300 x 4.6 mm ID



| Particle size   | Rs (1,2) | Rs (2,3) | N (3)  |
|-----------------|----------|----------|--------|
| 2 $\mu\text{m}$ | 1.17     | 4.15     | 16,200 |
| 3 $\mu\text{m}$ | 1.03     | 3.18     | 10,400 |
| 5 $\mu\text{m}$ | 0.88     | 2.67     | 8,500  |

Columns: YMC-Pack Diol-300, 300 x 4.6 mm ID  
Part Nos.: (A) DL30S02-3046PTH  
(B) DL30S03-3046WT  
(C) DL30S05-3046WT  
Eluent: 0.1 M  $\text{KH}_2\text{PO}_4$ - $\text{K}_2\text{HPO}_4$  (pH 7.0) containing 0.2 M NaCl

Flow rate: 0.2 mL/min  
Temperature: ambient  
Detection: UV at 280 nm  
Sample: Humanised monoclonal antibody (IgG1)

All three particle sizes show identical separation patterns for monoclonal antibody analysis. This allows easy method transfer between HPLC and UHPLC.

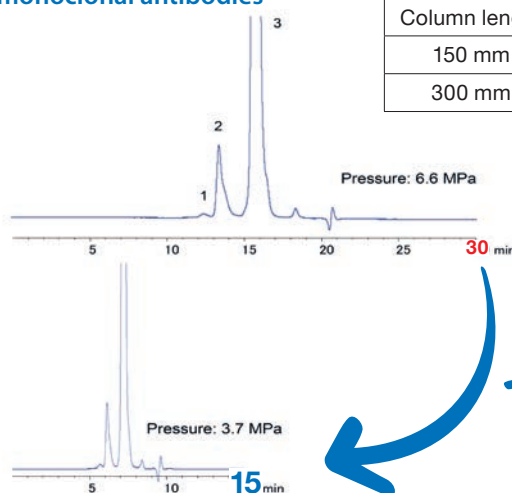
A method developed using conventional HPLC can be directly transferred to UHPLC using a 2  $\mu\text{m}$  YMC-Pack Diol

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  $\mu\text{m}$  column.

#### High throughput analysis of monoclonal antibodies

YMC-Pack Diol-300 (2  $\mu\text{m}$ )  
300 x 4.6 mm ID

YMC-Pack Diol-300 (2  $\mu\text{m}$ )  
150 x 4.6 mm ID



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

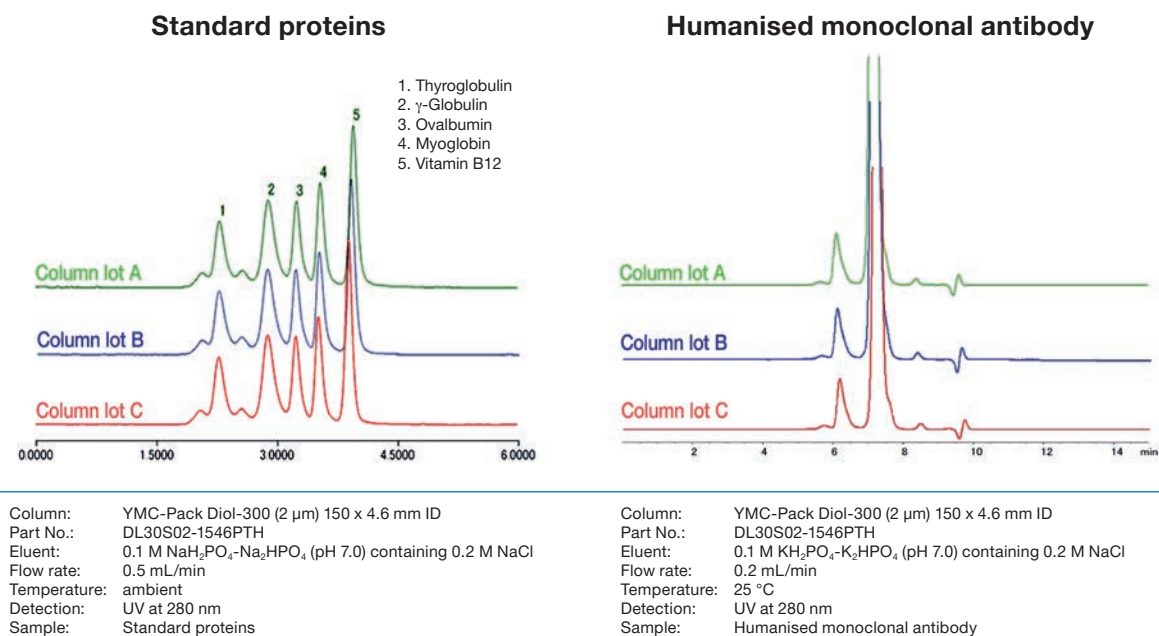
Columns: YMC-Pack Diol-300, 150 or 300 x 4.6 mm ID  
Part Nos.: DL30S02-3046PTH / DL30S02-1546PTH  
Eluent: 0.1 M  $\text{KH}_2\text{PO}_4$ - $\text{K}_2\text{HPO}_4$  (pH 7.0) containing 0.2 M NaCl  
Flow rate: 0.2 mL/min

Temperature: ambient  
Detection: UV at 280 nm  
Sample: Humanised monoclonal antibody (IgG1)

By using a 150 mm length column, 50% shorter run times can be achieved with the good resolution as for a 300 mm length column (compare upper and lower chromatograms). This allows an increase in throughput to be achieved.

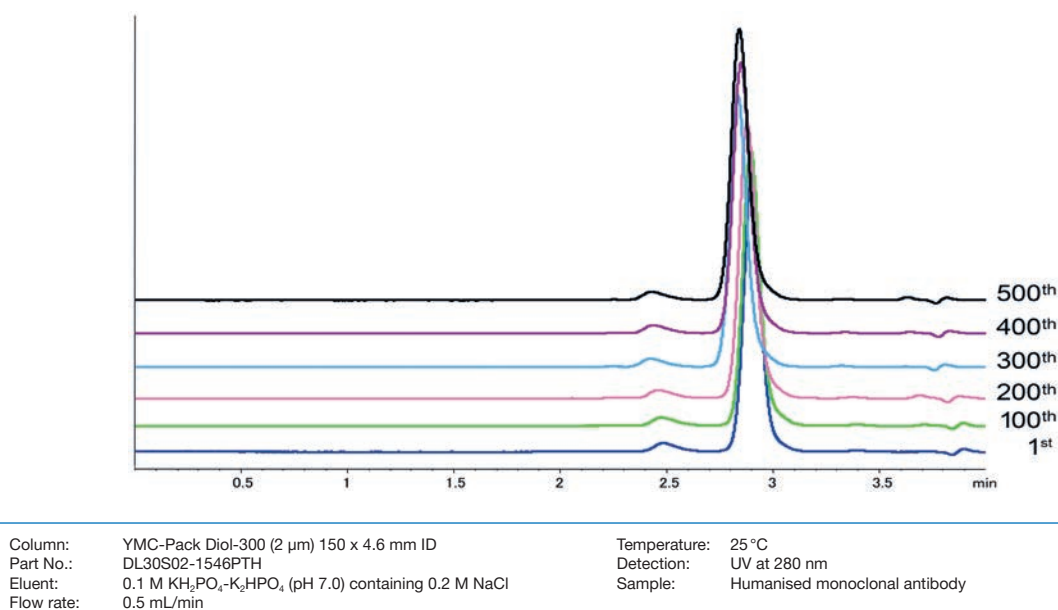
## Reproducibility and stability data

### Excellent batch-to-batch reproducibility



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

### Long-term stability



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

## SEC – Ordering information

## 2 µm UHPLC columns (max. pressure 45 MPa)

| Phase             | Column ID [mm] | Column length [mm] |                 | Guard cartridges* with 10 mm length |
|-------------------|----------------|--------------------|-----------------|-------------------------------------|
|                   |                | 150                | 300             | (pack of 5)                         |
| 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 (max. pressure 14–20 MPa)

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

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

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

## 5 µm HPLC columns (max. pressure 20 MPa)

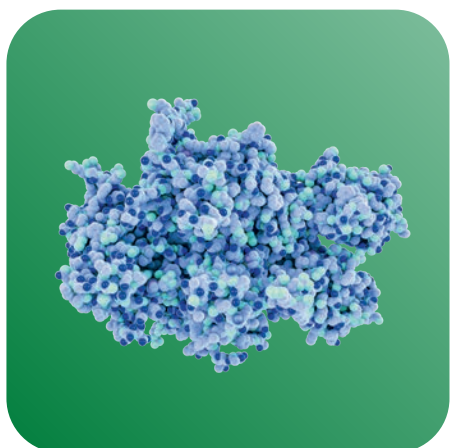
| Phase             | Column ID [mm] | Column length [mm] |                |                | Guard cartridges* with 10/30 mm length |
|-------------------|----------------|--------------------|----------------|----------------|----------------------------------------|
|                   |                | 250                | 300            | 500            | (pack of 5)                            |
| 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 guard columns (1 piece)  
recommended column coupler part no. XRCP1602 (for 8 mm ID) and XRCP1605 (for 10 mm ID)

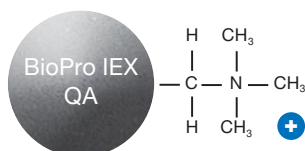


IEX

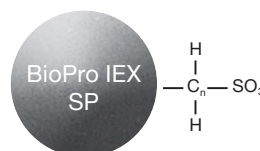


# IEX – Bio Pro Series

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



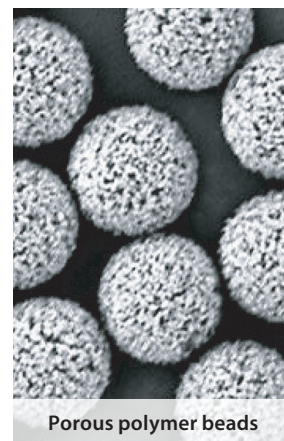
**strong anion  
exchanger**



**strong cation  
exchanger**

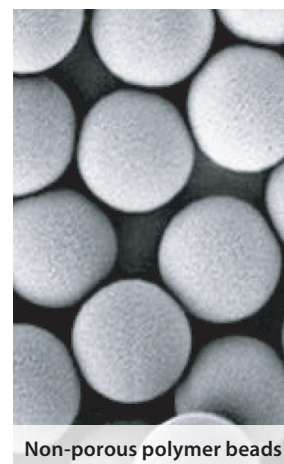
|                               | BioPro IEX QA                           | BioPro IEX SP                          |
|-------------------------------|-----------------------------------------|----------------------------------------|
| Matrix                        | hydrophilic polymer (polymethacrylate)  | 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 $\mu\text{m}$ : 18–25 MPa (2,610–3,625 psi)<br>5 $\mu\text{m}$ : 6–12 MPa (870–1,740 psi) |                                        |
| Column hardware               | PEEK                                                                                        |                                        |



Non-porous polymer beads

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.

## 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

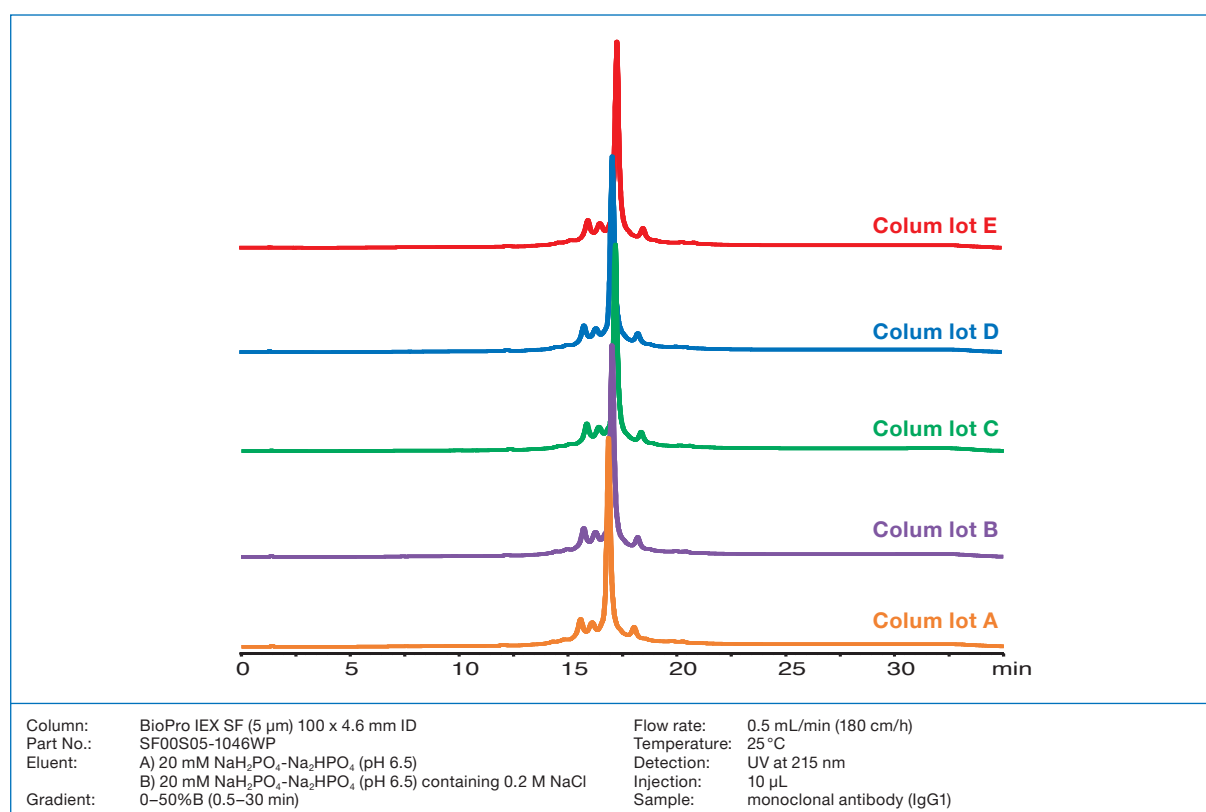
|                    | Dynamic binding capacity<br>(mg/mL-gel,<br>10% breakthrough) | Eluted amount<br>(mg/mL-gel) | Recovery*<br>(%) |
|--------------------|--------------------------------------------------------------|------------------------------|------------------|
| BioPro IEX QA      | 126  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

*High  
recovery  
rates for  
BioPro IEX*

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

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

## Excellent batch-to-batch reproducibility



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

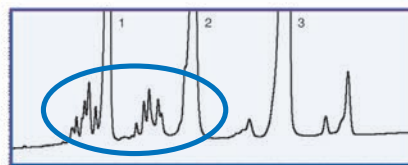
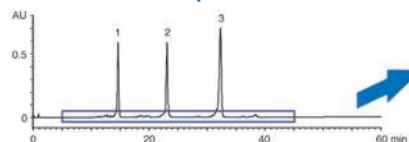
BioPro IEX columns are the best choice for the quality control of mAbs and other biopharmaceuticals.

# IEX – BioPro IEX: Resolution & throughput

## Superior resolution

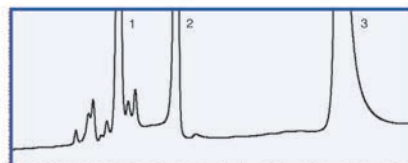
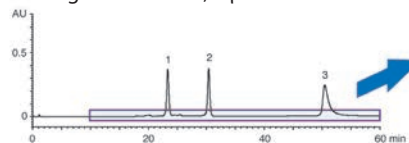
Comparison of standard protein separation on BioPro IEX SP and commercial S type products

**BioPro IEX SP, 5  $\mu$ m**

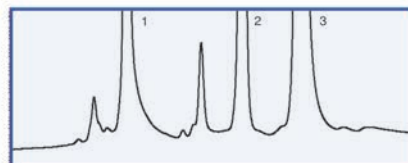
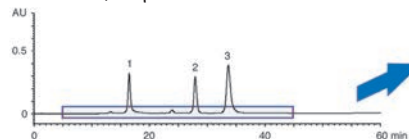


*Superior resolution*

**TSKgel BioAssist S, 7  $\mu$ m**



**Mono S, 10  $\mu$ m**



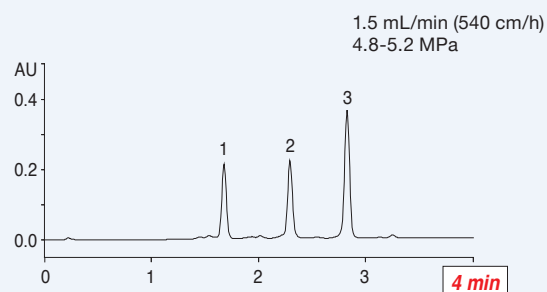
Eluent: A) 20 mM  $\text{KH}_2\text{PO}_4$ - $\text{K}_2\text{HPO}_4$  (pH 6.8)  
B) 20 mM  $\text{KH}_2\text{PO}_4$ - $\text{K}_2\text{HPO}_4$  (pH 6.8) containing 0.5 M NaCl  
Gradient: 0–100%B (0–60 min)  
Flow rate: BioPro IEX SP, TSKgel BioAssist S 0.5 mL/min  
Mono S 0.59 mL/min  
Temperature: 25 °C

Detection: UV at 220 nm  
Injection: BioPro IEX SP, TSKgel BioAssist S 20  $\mu$ L  
Mono S 23.6  $\mu$ L  
Sample: 1. Ribonuclease A (0.5 mg/mL)  
2. Cytochrome c (0.5 mg/mL)  
3. Lysozyme (0.5 mg/mL)

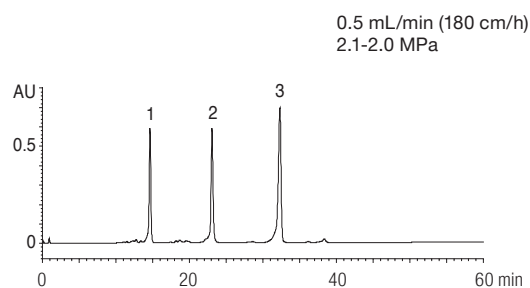
Only BioPro IEX is available in the smaller particle size and is therefore able to provide superior resolution.

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

**Non-porous type**  
**BioPro IEX SF (5  $\mu$ m) 30 x 4.6 mm ID**



**Porous type**  
**BioPro IEX SP (5  $\mu$ m) 50 x 4.6 mm ID**



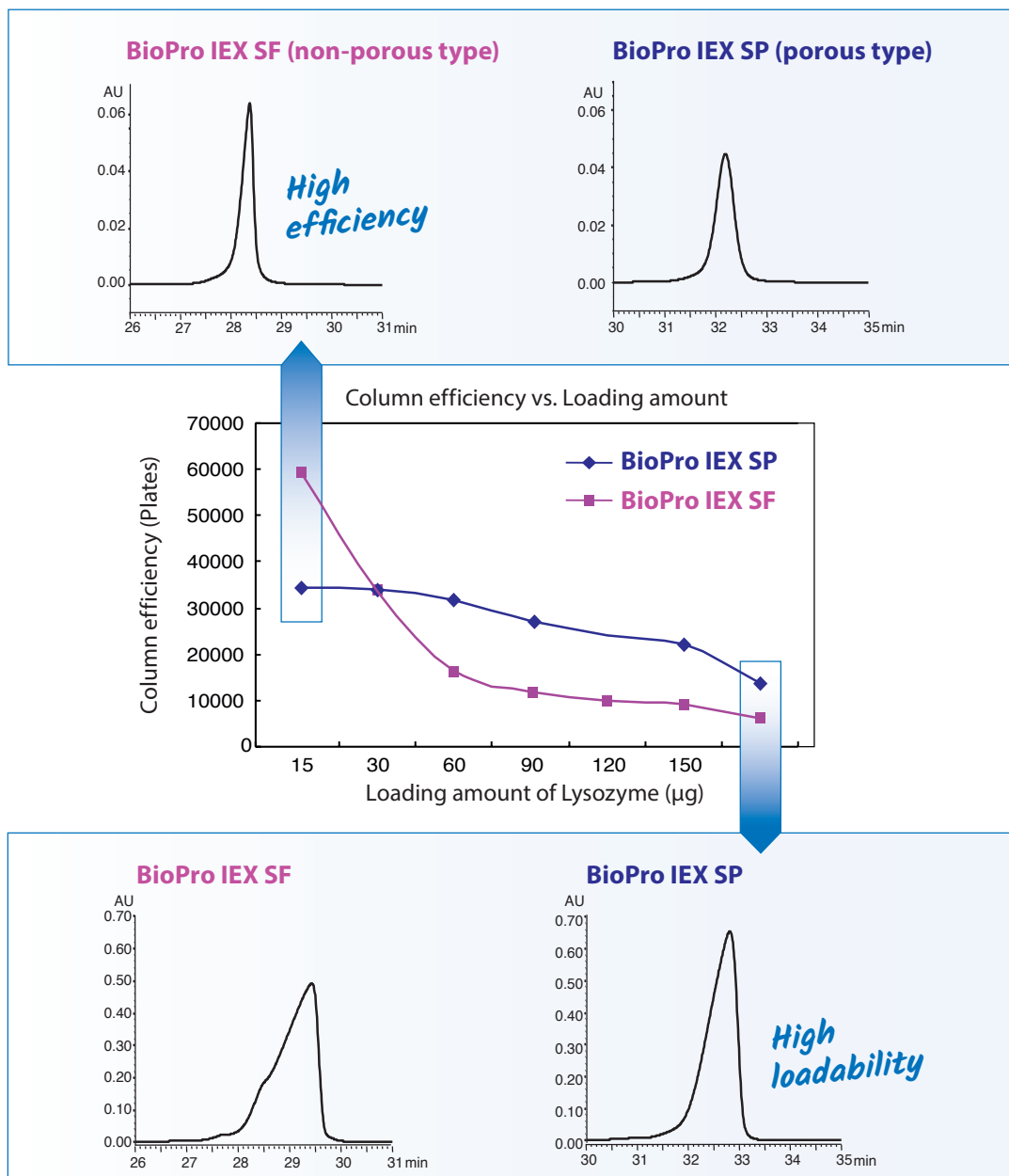
Eluent: A) 20 mM  $\text{KH}_2\text{PO}_4$ - $\text{K}_2\text{HPO}_4$  (pH 6.8)  
B) 20 mM  $\text{KH}_2\text{PO}_4$ - $\text{K}_2\text{HPO}_4$  (pH 6.8) containing 0.5 M NaCl  
Part Nos.: SF00S05-0346WP (non-porous)  
SPA0S05-0546WP (porous)  
Gradient: 0–100%B (0–4 min) for BioPro IEX SF  
0–100%B (0–60 min) for BioPro IEX SP

Temperature: 25 °C  
Detection: UV at 220 nm  
Injection: 20  $\mu$ L  
Sample: 1. Ribonuclease A (0.5 mg/mL)  
2. Cytochrome c (0.5 mg/mL)  
3. Lysozyme (0.5 mg/mL)

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

## Column efficiency and loadability

### When to use porous and non-porous BioPro IEX



Columns: (5 µm) 50 x 4.6 mm ID  
 Part Nos.: SF00S05-0546WP  
 SPA0S05-0546WP  
 Eluent: A) 20 mM  $\text{NaH}_2\text{PO}_4$ - $\text{Na}_2\text{HPO}_4$  (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)

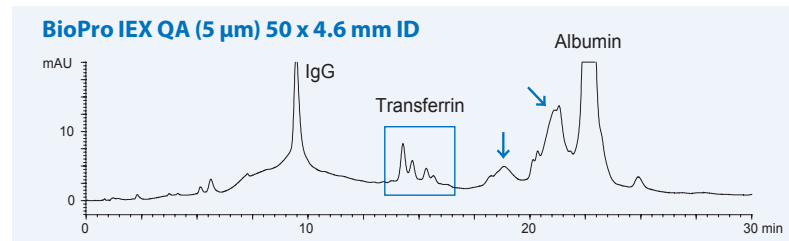
Flow rate: 0.5 mL/min  
 Temperature: 25°C  
 Detection: UV at 280 nm  
 Injection: 100 µL  
 Sample: Lysozyme

**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.**

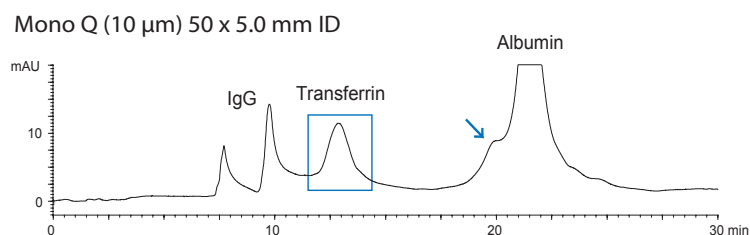
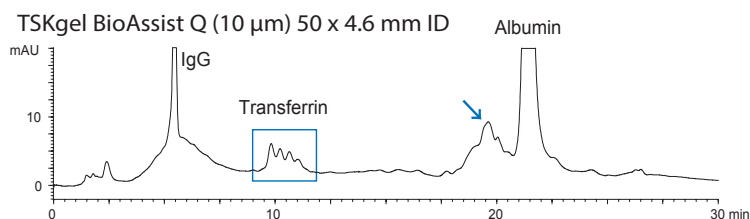
# IEX – BioPro IEX: Challenging separations

## Protein separation in challenging matrices

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



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



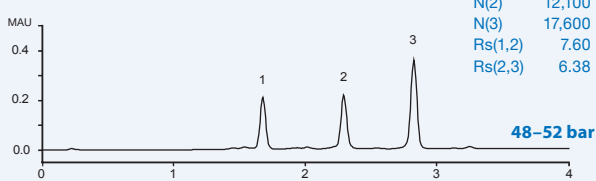
Part No.: QAA0S05-0546WP  
Eluent: A) 20 mM Tris-HCl (pH 8.6)  
B) 20 mM Tris-HCl (pH 8.6) containing 0.5 M NaCl  
Gradient: 0–30%B (0–15 min), 30–100%B (15–30 min)  
Flow rate: 0.5 mL/min

Temperature: 25 °C  
Detection: UV at 280 nm  
Injection: 20 µL  
Sample: Human serum (100 µL/mL)

## Better performance at lower backpressure

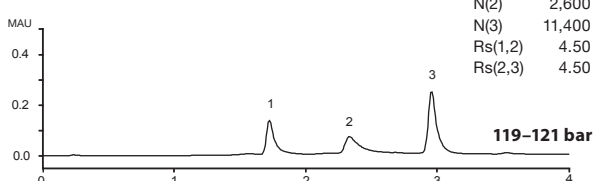
### Comparison of standard protein separation on BioPro IEX SF and a commercial SP-type product

#### BioPro IEX SF (5 µm) 30 x 4.6 mm ID



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

#### TSKgel SP-NPR (2.5 µm) 35 x 4.6 mm ID



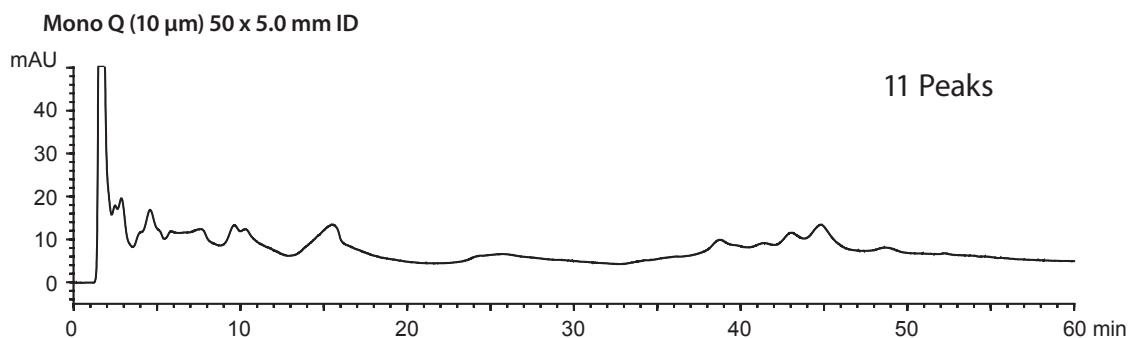
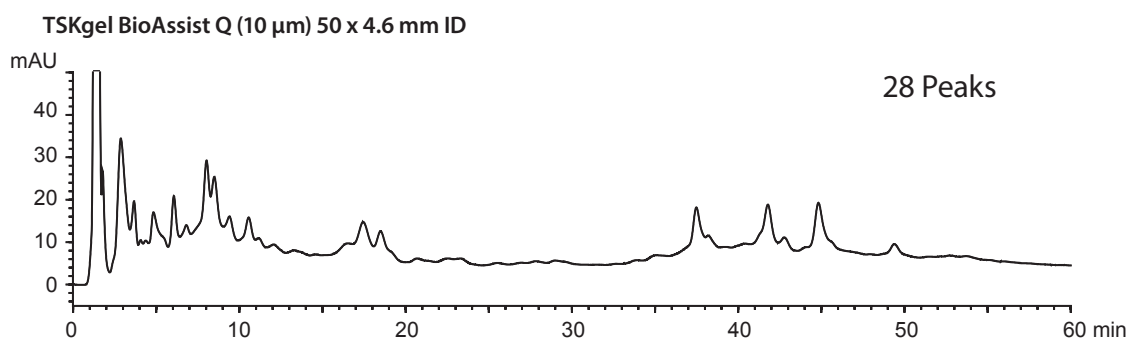
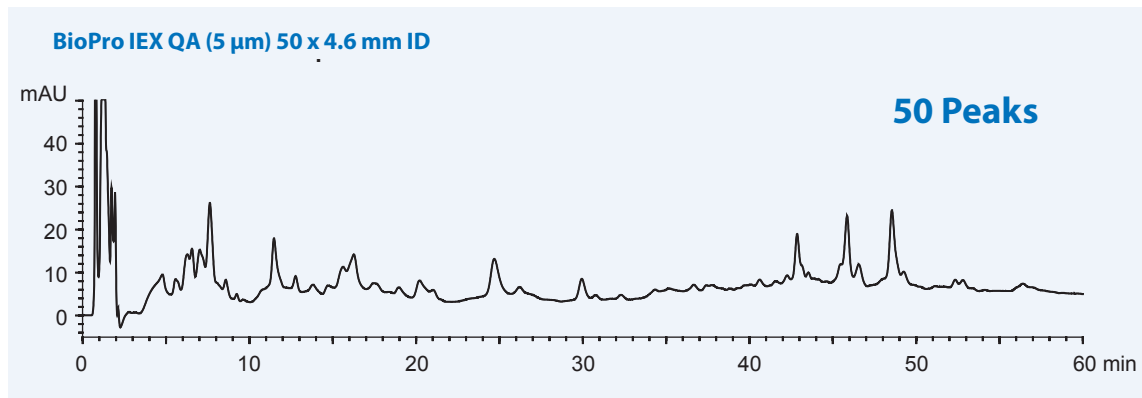
Part No.: SF00S05-0346WP  
Eluent: A) 20 mM  $\text{KH}_2\text{PO}_4$ - $\text{K}_2\text{HPO}_4$  (pH 6.8)  
B) 20 mM  $\text{KH}_2\text{PO}_4$ - $\text{K}_2\text{HPO}_4$  (pH 6.8) containing 0.5 M NaCl  
Gradient: BioPro IEX SF 0–100%B (0–4 min)  
TSKgel SP-NPR 0–100%B (0–4.67 min)  
Flow rate: 1.5 mL/min  
Temperature: 25 °C  
Detection: UV at 220 nm  
Injection: 20 µL  
Sample: 1. Ribonuclease A (0.1 mg/mL)  
2. Cytochrome c (0.1 mg/mL)  
3. Lysozyme (0.1 mg/mL)

*higher plate count*

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

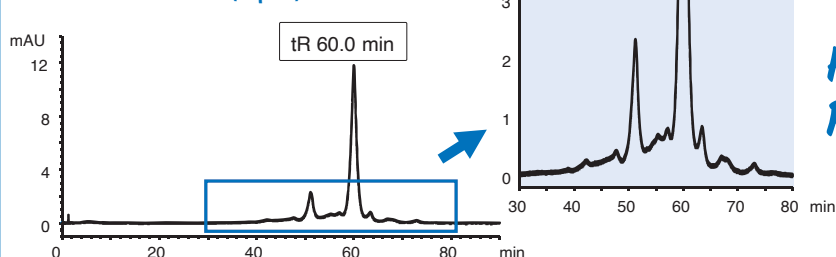


Part No.: QAA0S05-0546WP  
 Eluent: A) 20 mM Tris-HCl (pH 8.6)  
 B) 20 mM Tris-HCl (pH 8.6) containing 0.5 M NaCl  
 Gradient: 0–15%B (0–30 min), 15–60%B (30–60 min)  
 Flow rate: 0.5 mL/min  
 Temperature: 25 °C  
 Detection: UV at 220 nm  
 Injection: 20  $\mu$ L  
 Sample: Tryptic digest of BSA

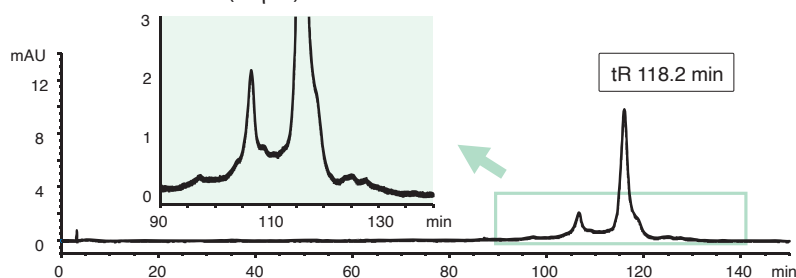
# IEX – BioPro IEX: Antibody analysis

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

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



### WCX: ProPac WCX-10 (10 µm) 250 x 4.0 mm ID

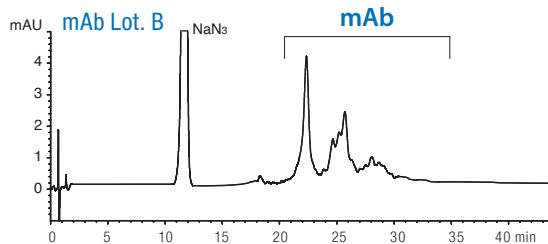
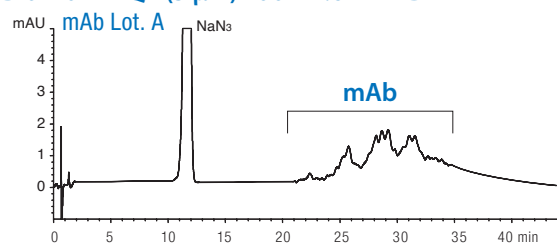


|                 |                                                                                |                                                                       |
|-----------------|--------------------------------------------------------------------------------|-----------------------------------------------------------------------|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

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

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

### 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)<br>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/h)                                                          | Sample: Mouse monoclonal IgG1 anti-human IgG4<br>(Purified by DEAE chromatography, containing Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> ) |

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

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

## Optimisation of oligonucleotide separations on ion exchange chromatography

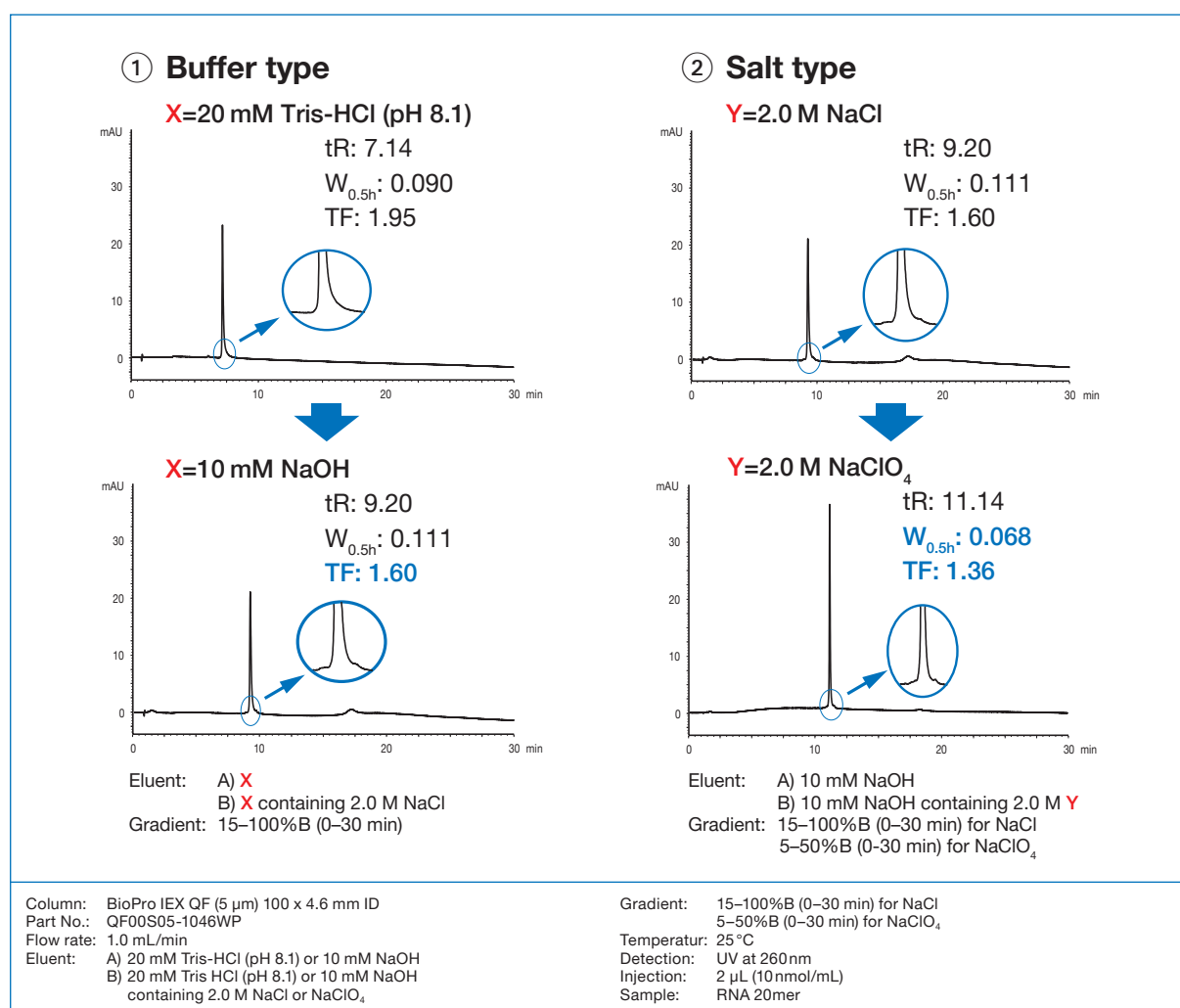
Non-porous anion exchange column is generally suitable for analysis of oligonucleotides. For optimisation of single-stranded DNA and RNA of about 20mer some conditions such as type of mobile phase and column temperature can be changed.

### 1 Improvement of peak tailing

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

Single-stranded RNA (ssRNA) 5'-UCAUCACACUGAAUACCAU-3' (RNA 20mer)

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

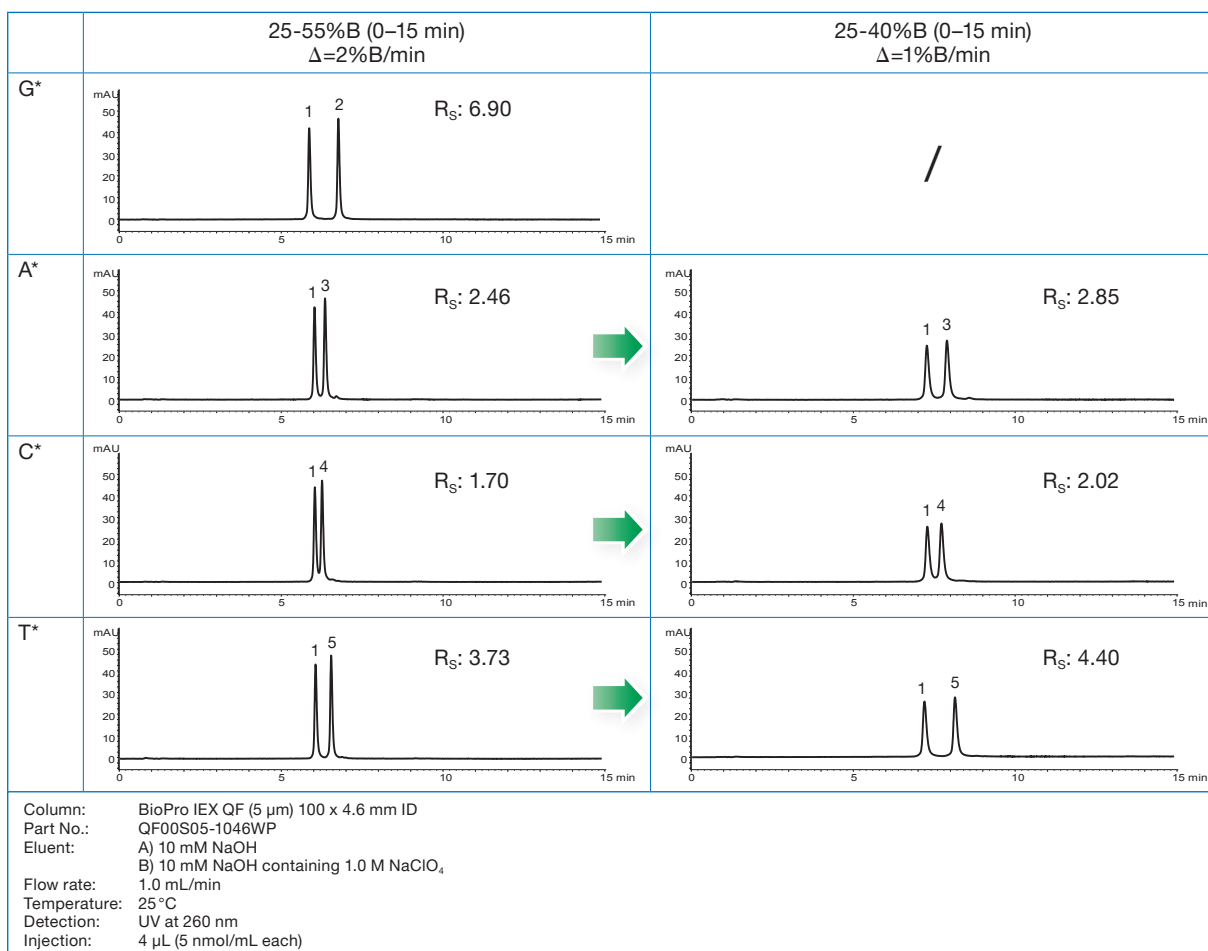
When the initial gradient concentration of NaCl is low (ex. 50 mM), carry-over is observed. By increasing the initial gradient concentration of NaCl up to 400 carry-over can be avoided with good reproducibility.

## IEX – Expert Tips: Oligonucleotides

### 3 Improvement of ssDNA separation with single-base differences (differing in the type of base of 5' end of DNA 21mer)

When ssDNAs with single-base differences (differing in the type of base of 5' end of DNA 21mer) is analysed the degree of separation varies depending on the type of base at the 5' end. If the separation is difficult, it can be improved by making the gradient steeper.

|   |                     |                                         |
|---|---------------------|-----------------------------------------|
| 1 | Single-stranded DNA | 5'-TCATCACACTGAATACCAAT-3' (DNA 20mer)  |
| 2 |                     | 5'-GTCATCACACTGAATACCAAT-3' (DNA 21mer) |
| 3 |                     | 5'-ATCATCACACTGAATACCAAT-3' (DNA 21mer) |
| 4 |                     | 5'-CTCATCACACTGAATACCAAT-3' (DNA 21mer) |
| 5 |                     | 5'-TTCATCACACTGAATACCAAT-3' (DNA 21mer) |



\*base of 5' end of DNA 21mer

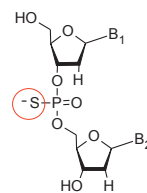
### 4 Improvement of the separation of phosphorothioate oligonucleotides with single-base differences in length

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 2<sup>19</sup> (524,288) stereoisomers. A steeper gradient curve, increasing column temperature and adding organic solvent can improve peak separation.

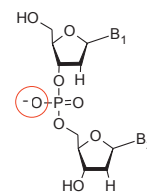
## Sample Group 2 (Phosphorothioate oligonucleotides; PS)

|   |                     |                                                                    |
|---|---------------------|--------------------------------------------------------------------|
| 1 | Single-stranded RNA | 5'-U~C~A~U~C~A~C~A~C~U~G~A~A~U~A~C~C~A~A~U-3' (RNA 20mer All PS)   |
| 2 | Single-stranded RNA | 5'-G~U~C~A~U~C~A~C~A~C~U~G~A~A~U~A~C~C~A~A~U-3' (RNA 21mer All PS) |

~ = Phosphorothioated



Phosphorothioate Linkage (PS)



Phosphodiester Linkage (PO)

X/Y=100/0  
32–80%B (0–24 min)  
= Δ20 mM NaClO<sub>4</sub>/min  
25°C



**Steep gradient curve**  
X/Y=100/0  
32–80%B (0–8 min)  
= Δ60 mM NaClO<sub>4</sub>/min  
25°C



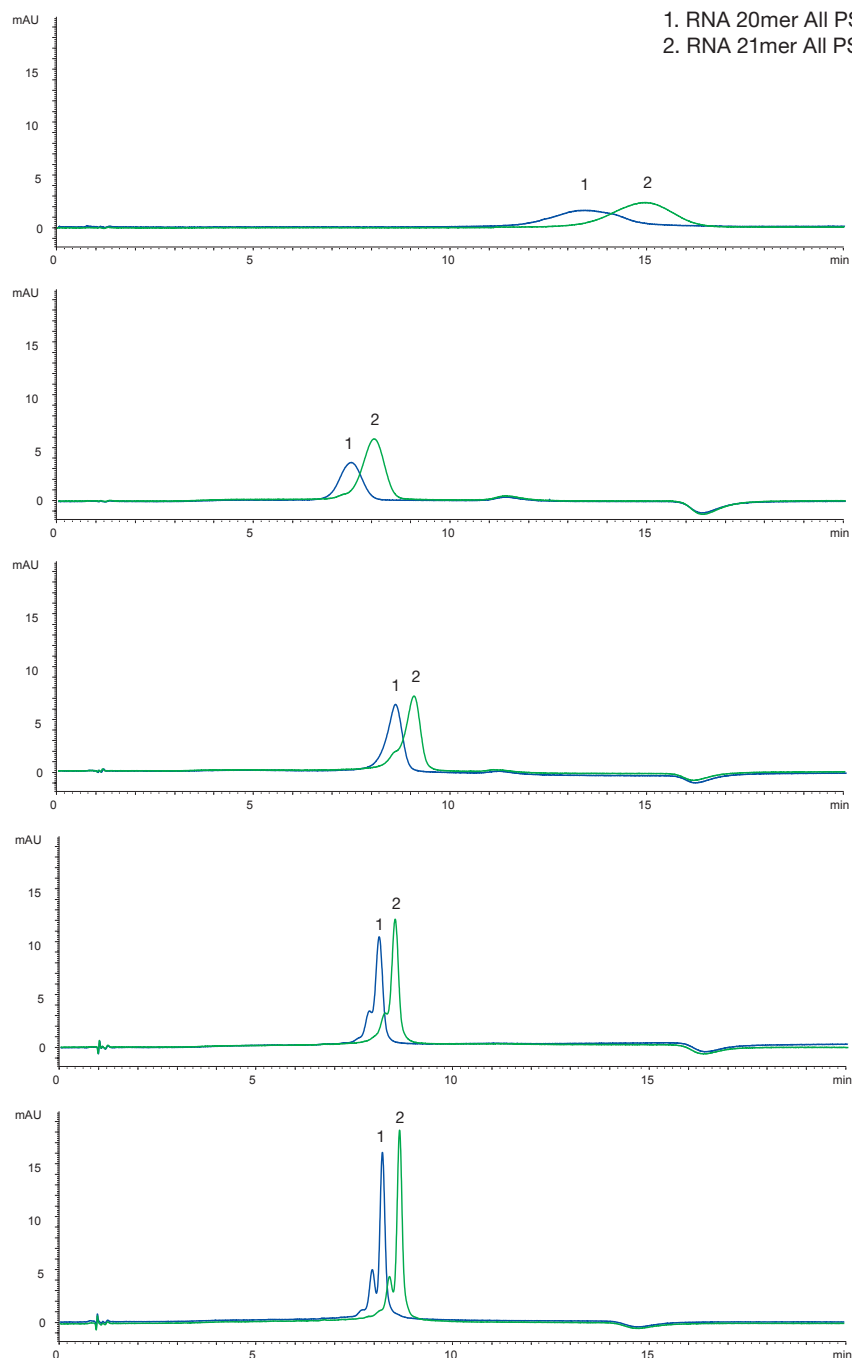
**Raising column temperature**  
X/Y=100/0  
32–80%B (0–8 min)  
= Δ60 mM NaClO<sub>4</sub>/min  
60°C



**Addition of organic solvent**  
X/Y=80/20  
40–100%B (0–8 min)  
= Δ60 mM NaClO<sub>4</sub>/min  
60°C



**Increasing ratio of organic solvent**  
X/Y=70/30  
40–100%B (0–6.3 min)  
= Δ60 mM NaClO<sub>4</sub>/min  
60°C



Column: BioPro IEX QF (5 μm) 100 x 4.6 mm ID  
Part No.: QF00S05-1046WP  
Eluent: A) 10 mM NaOH/methanol (X/Y)  
B) 10 mM NaOH containing 1.0M NaClO<sub>4</sub>/methanol (X/Y)

Flow rate: 1.0 mL/min  
Detection: UV at 260 nm  
Injection: 2 μL (10 nmol/mL)

# IEX – Ordering information

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

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

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

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

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

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

\* Holder required, part no. XRPRCP02

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

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

\*\* optionally bioinert coated stainless steel hardware available

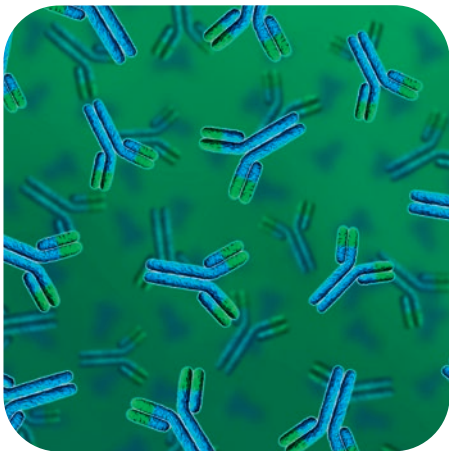
## 6 µm porous semiprep. columns, stainless steel hardware (max. pressure 4 MPa)

| Phase         | Column ID [mm] | Column length [mm] |
|---------------|----------------|--------------------|
|               |                | 100                |
| BioPro IEX QA | 10             | QAA0S06-1010WT     |
|               | 20             | QAA0S06-1020WT     |
| BioPro IEX SP | 10             | SPA0S06-1010WT     |
|               | 20             | SPA0S06-1020WT     |

Other dimensions on demand



HIC



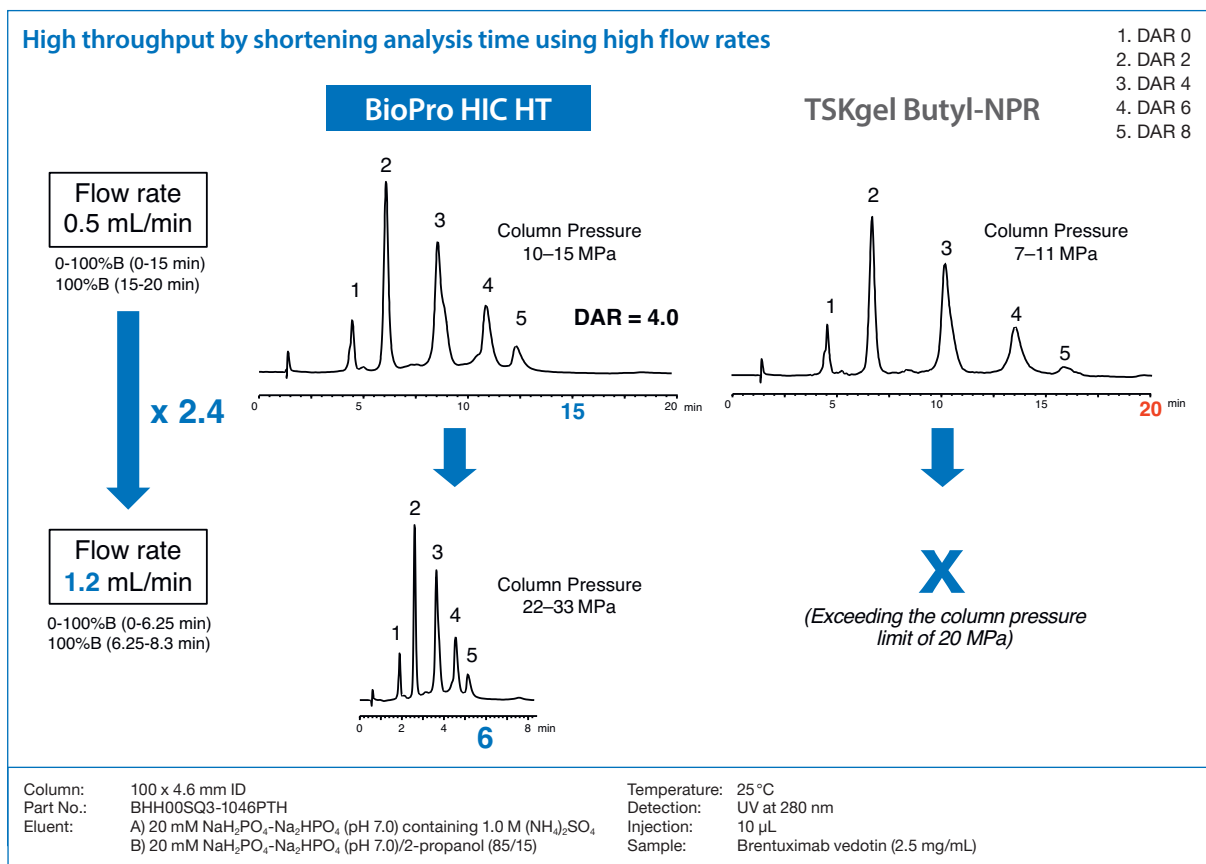
## HIC – BioPro Series

- 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                               |

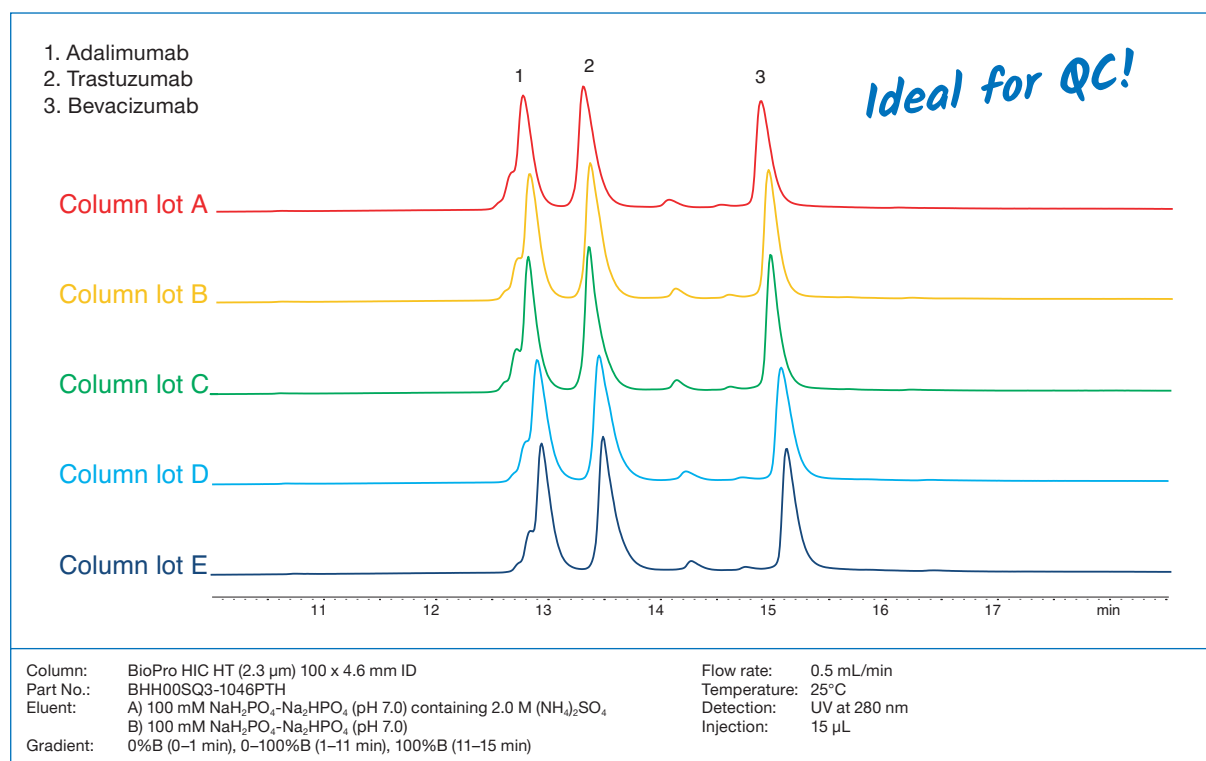
### High column stability

High throughput by shortening analysis time using high flow rates



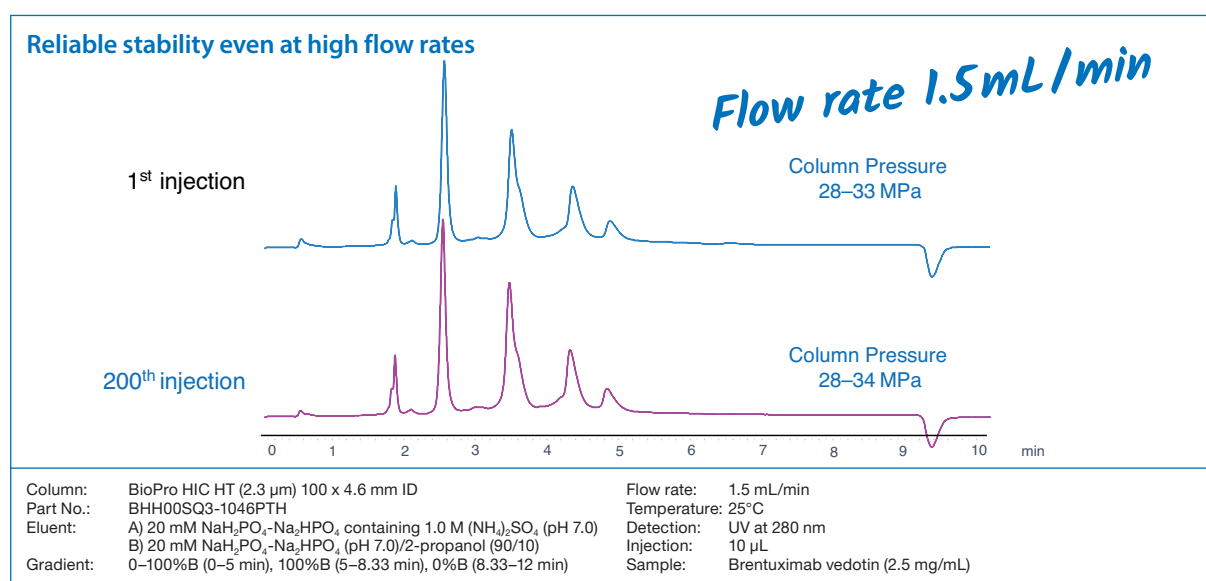
**BioPro HIC HT improves analysis throughput of ADCs by 2–3 times with an excellent Drug-to-Antibody Ratio (DAR). The rapid analysis is possible without loss of resolution. Competitor HIC columns fail under these conditions.**

## Excellent batch-to-batch reproducibility



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

## Exceptional stability



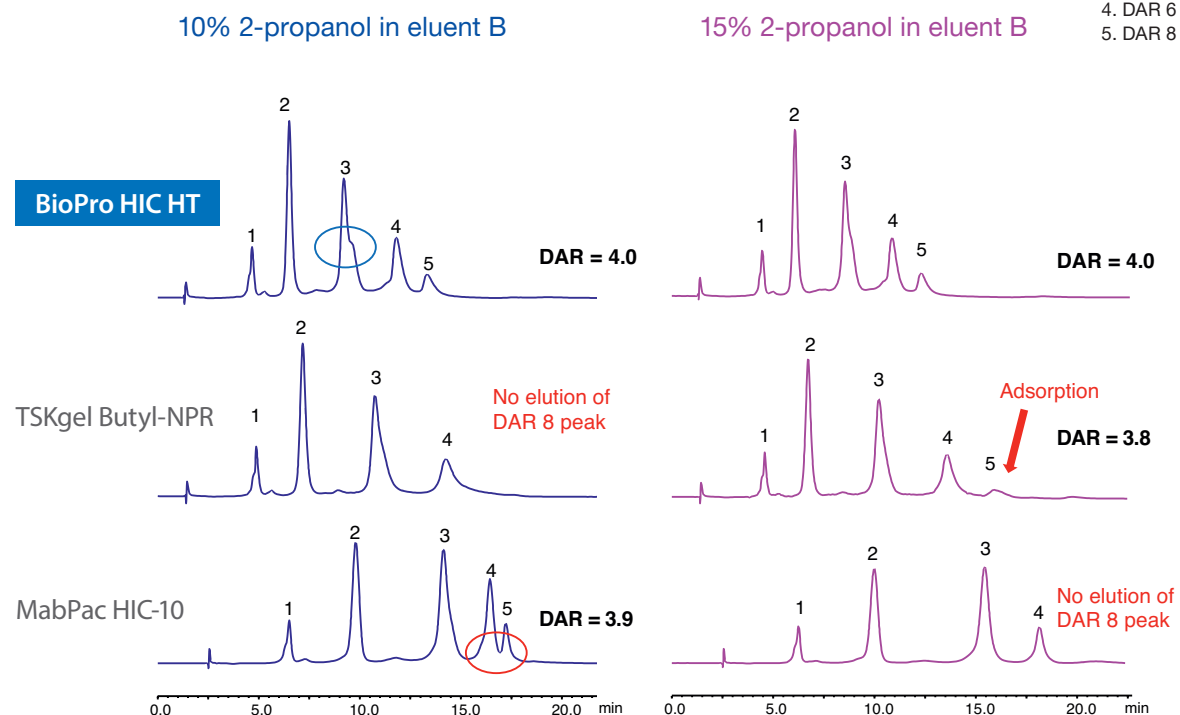
BioPro HIC HT offers excellent stability under high flow rates/high pressure conditions due to its unique rigid particle and optimised column packing technology.

## HIC – BioPro HIC: ADC analysis

### Designed for analysis of ADCs

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

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



Column: 100 x 4.6 mm ID  
 Part No.: BHH00SQ3-1046PTH  
 Eluent: A) 20 mM  $\text{NaH}_2\text{PO}_4$ - $\text{Na}_2\text{HPO}_4$  (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 (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  $\mu\text{L}$   
 Sample: Brentuximab vedotin (2.5 mg/mL)

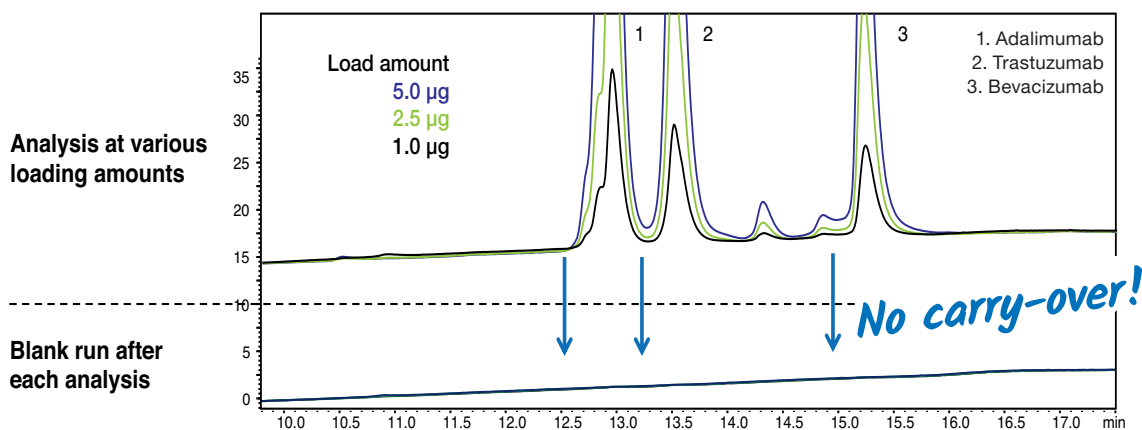
BioPro HIC HT offers higher resolution than conventional HIC columns. Its surface modification suppresses excessive or too strong adsorption of ADCs and results in highly reliable quantification. With varying 2-propanol content, all peaks are completely eluted from the BioPro HIC HT column with high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

#### BioPro HIC HT offers:

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

## Excellent recovery and virtually no carry-over

### Highly accurate quantification of ADCs and antibodies



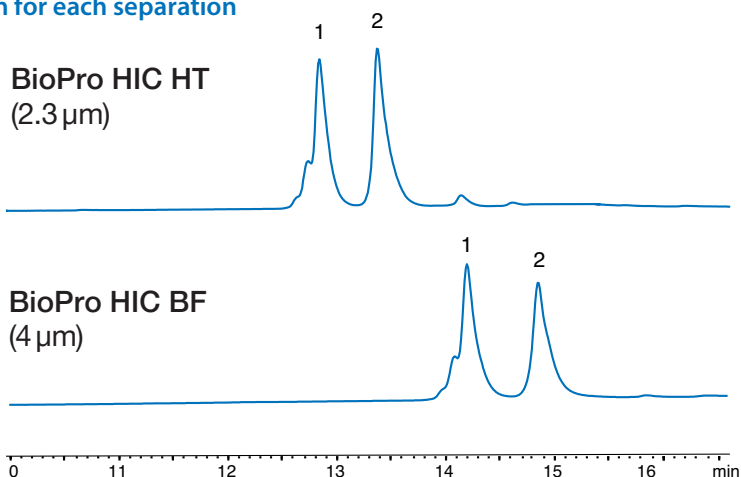
Column: BioPro HIC HT (2.3 µm) 100 x 4.6 mm ID  
Part No.: BHH00SQ3-1046PTH  
Eluent: A) 100 mM NaH<sub>2</sub>PO<sub>4</sub>-Na<sub>2</sub>HPO<sub>4</sub> (pH 7.0) containing 2.0 M (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>  
B) 100 mM NaH<sub>2</sub>PO<sub>4</sub>-Na<sub>2</sub>HPO<sub>4</sub> (pH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

## Different hydrophobicity

### The right column for each separation



Column: 100 x 4.6 mm ID  
Part Nos.: BHH00SQ3-1046PTH  
BHB00S04-1046WT  
Eluent: A) 100 mM 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)

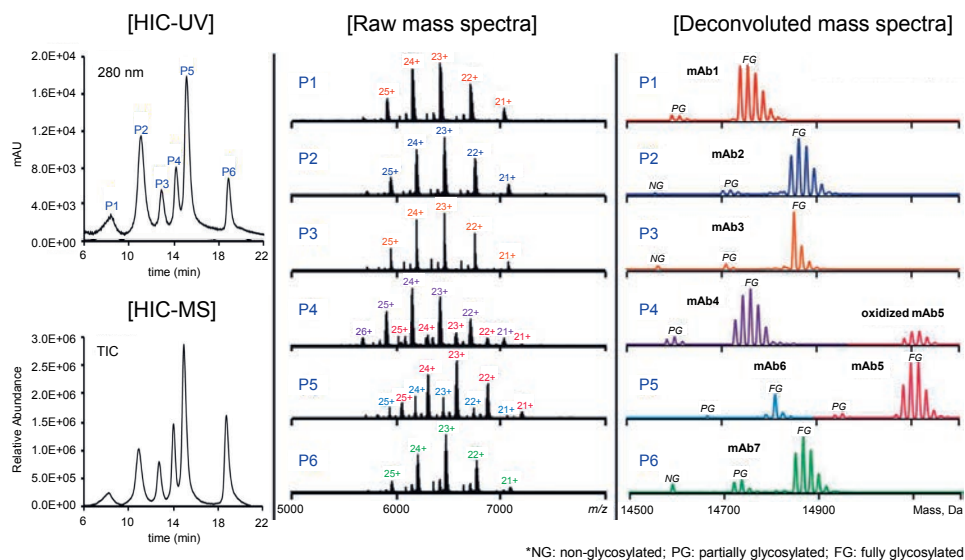
Flow rate: 0.5 mL/min  
Temperature: 25 °C  
Detection: UV at 280 nm  
Injection: 15 µL  
Sample: 1. Adalimumab (Humira®; 0.5 mg/mL)  
2. Trastuzumab (Herceptin®; 0.5 mg/mL)

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

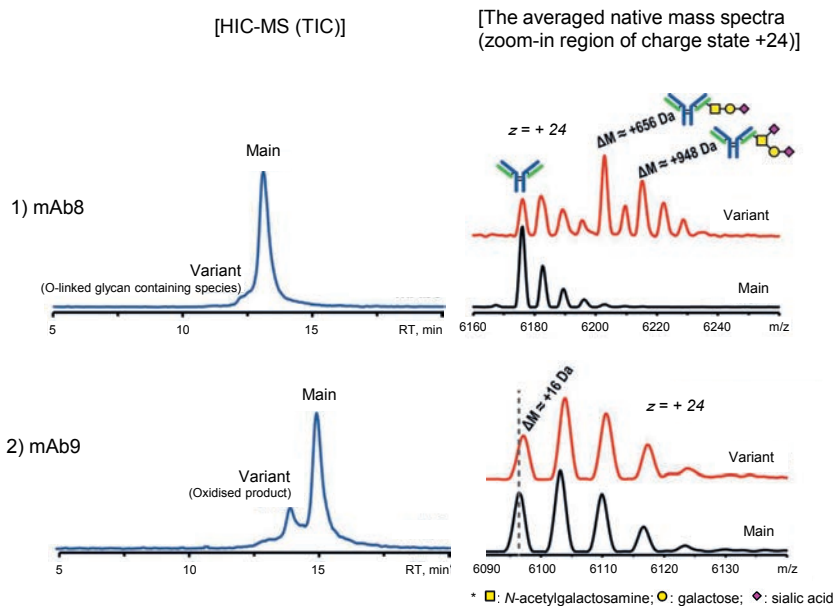
# HIC – BioPro HIC: Direct HIC-MS coupling

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

### Separation of an antibody mixture of seven different mAbs



### Separation of two mAbs from their molecular variants



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

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

Courtesy by S. Wang, Regeneron Pharmaceuticals Inc.

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

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

## The influence of salts in HIC separations

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

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

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

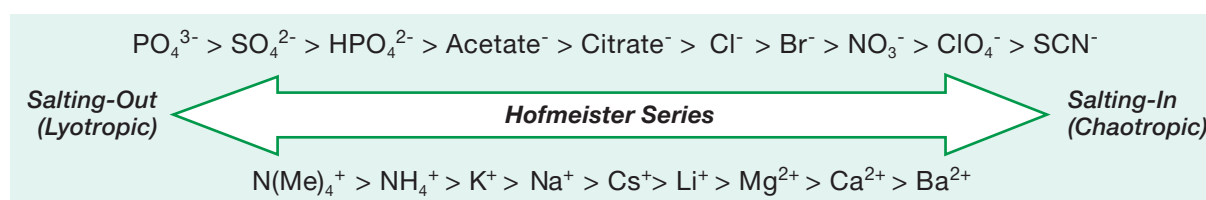


Fig. 1: The Hofmeister Series of lyotropic and chaotropic ions.

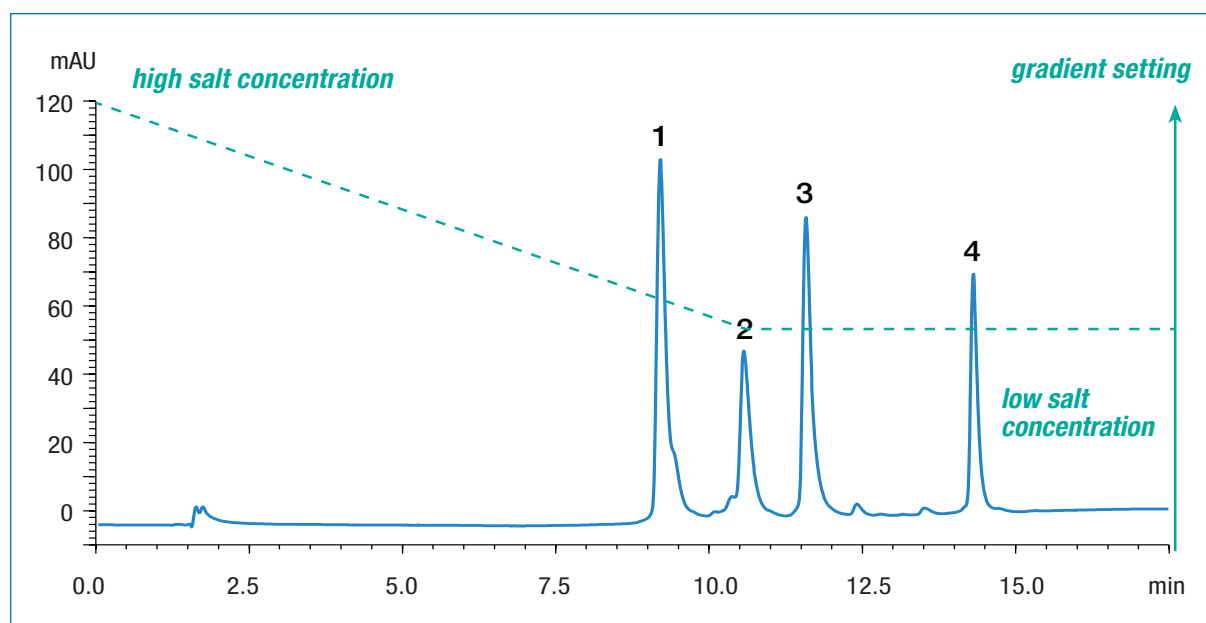


Fig. 2: Method with decreasing salt gradient.

Column: BioPro HIC BF (100 x 4.6 mm ID)  
Part No.: BHB00S04-1046WT  
Eluent: A) 100 mM  $\text{NaH}_2\text{PO}_4$ - $\text{Na}_2\text{HPO}_4$  (pH 7.0) containing 2.0 M  $(\text{NH}_4)_2\text{SO}_4$   
B) 100 mM  $\text{NaH}_2\text{PO}_4$ - $\text{Na}_2\text{HPO}_4$  (pH 7.0)  
Flow rate: 0.5 mL/min  
Gradient: 0–100%B (0–11 min), 100%B (11–15 min)  
Temperature: 25°C  
Detection: UV at 280 nm  
Injection: 15 µL

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

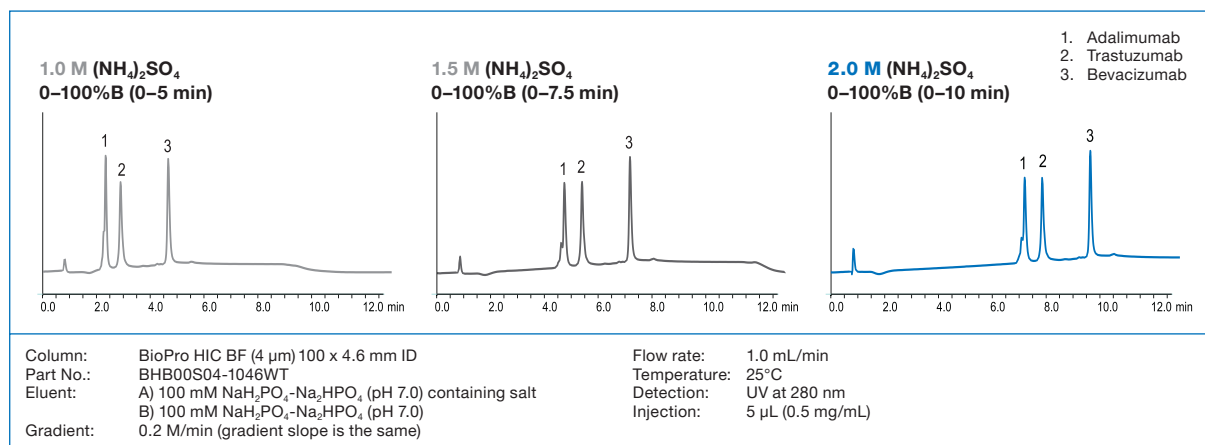
HIC is particularly effective when used to separate proteins and monoclonal antibodies. The separation of monoclonal antibodies, mAb aggregates and glycosylated mAbs can be achieved due to their specific hydrophobic properties. It also provides an excellent method for determination of drug-to-antibody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

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

## HIC – Expert Tips: Separation factors

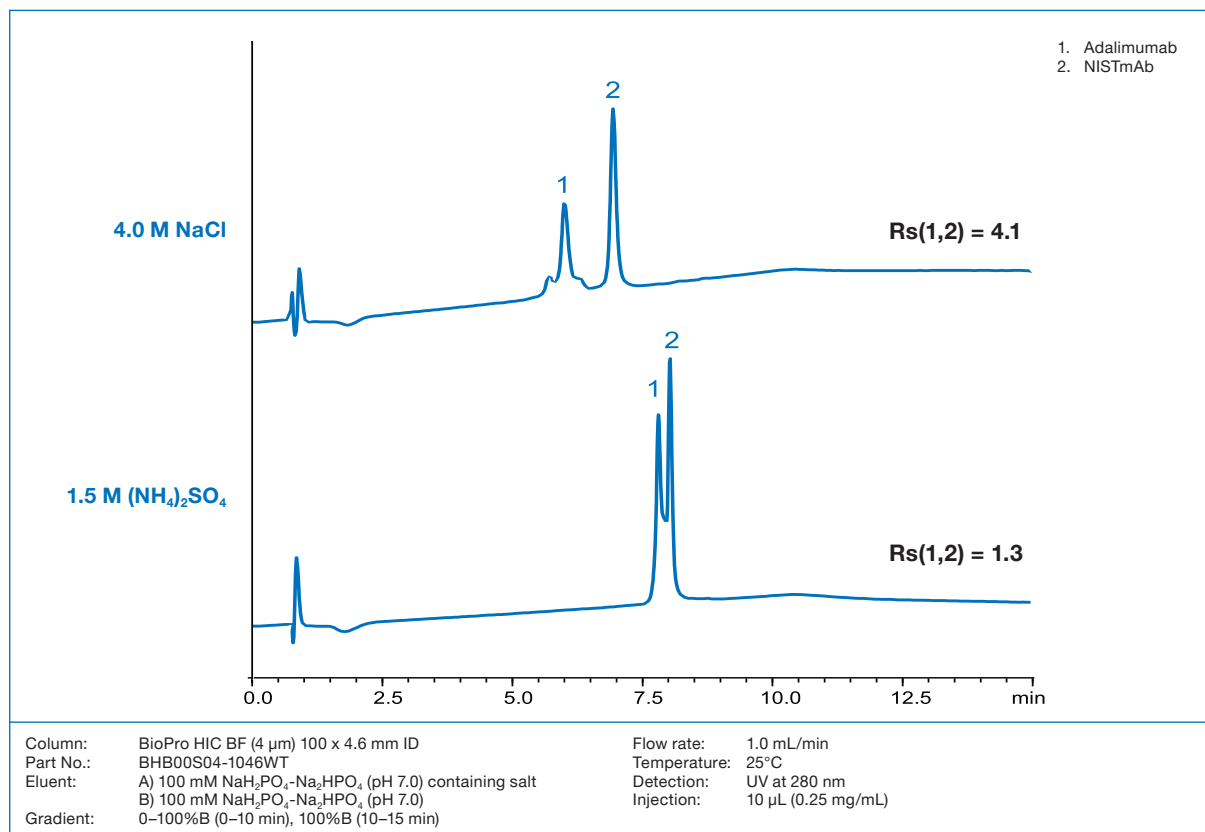
### Effect of initial salt concentration

**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.



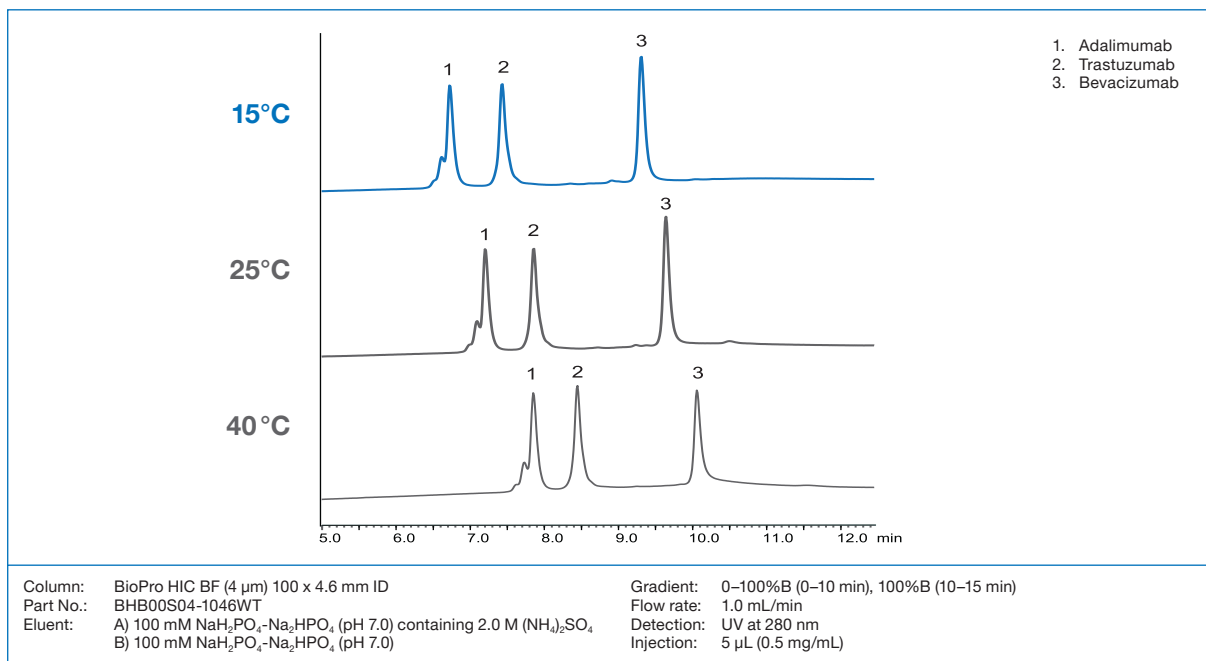
### Influence of the type of salt

**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.



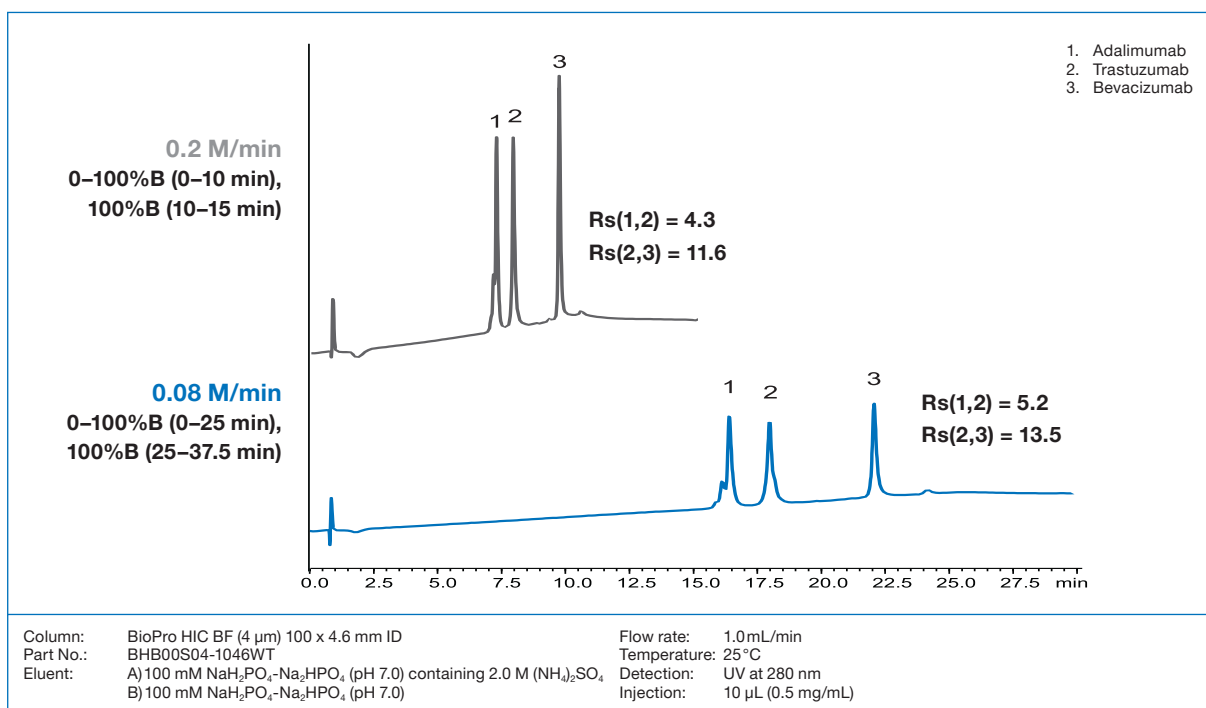
## Temperature influence

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



## Variation of gradient slope

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

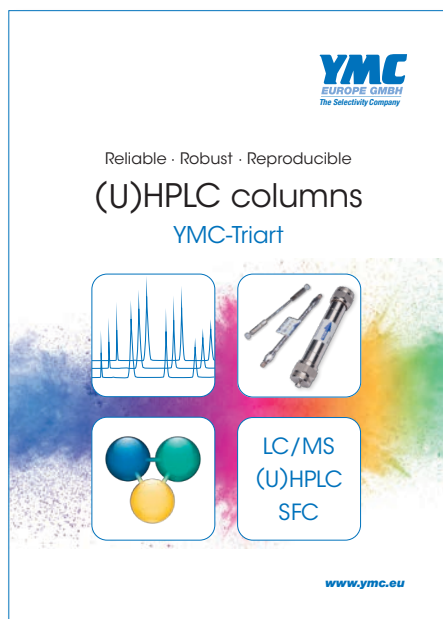


# HIC – Ordering information

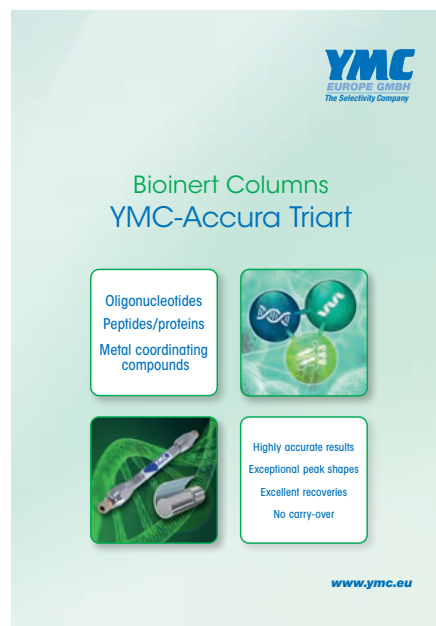
## Ordering information

| Phase                                      | Particle size<br>[μm] | Column ID<br>[mm] | Column length<br>[mm] | Part number      | Precolumn filter<br>2 μm* |
|--------------------------------------------|-----------------------|-------------------|-----------------------|------------------|---------------------------|
|                                            |                       |                   |                       |                  | (pack of 5)               |
| BioPro HIC HT<br>(max. pressure<br>40 MPa) | 2.3                   | 4.6               | 100                   | BHH00SQ3-1046PTH | XRPRCS35                  |
|                                            | 2.3                   | 4.6               | 33                    | BHH00SQ3-H346PTH | XRPRCS35                  |
| BioPro HIC BF<br>(max. pressure<br>20 MPa) | 4                     | 4.6               | 100                   | BHB00S04-1046WT  | XRPRCS35                  |

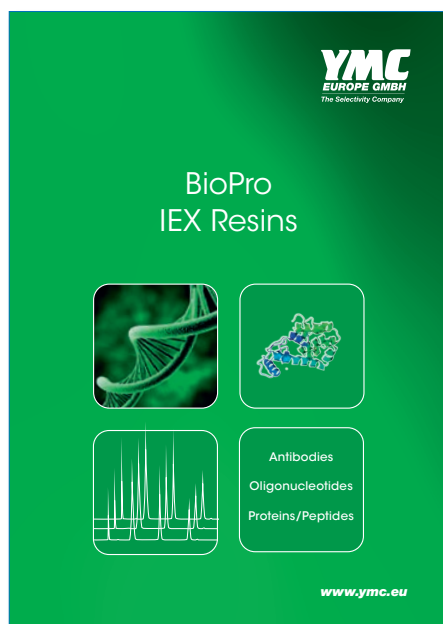
\*Holder required, part no XRPRCS03  
Other dimensions on demand



YMC-Triart



YMC-Accura Triart



BioPro IEX Resins



YMC Oligonucleotide Columns

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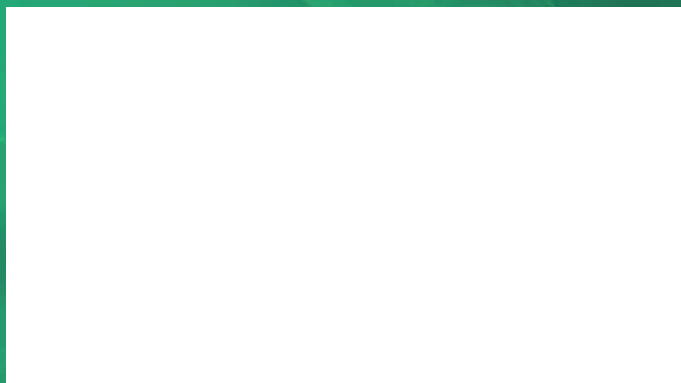
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