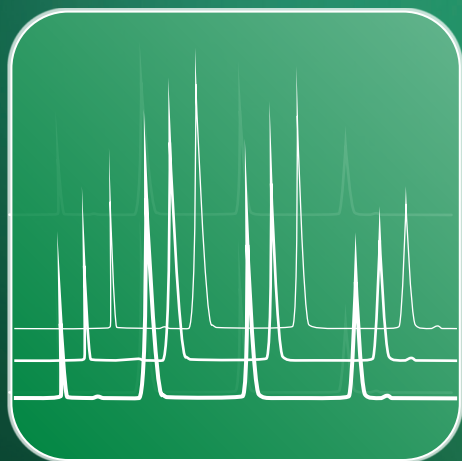


YMC

Size Exclusion Columns



5 Pore Sizes
3 Particle Sizes
(U)HPLC
SEC-MALLS



SEC – UHPLC / HPLC Selectivities

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

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

“

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

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

”

Column Selection Tool

for MW < 10,000

• YMC-Pack Diol-60

for MW 1,000 to 100,000

• YMC-Pack Diol-120

for MW 5,000 to 300,000

• YMC-Pack Diol-200

for MW 10,000 to 700,000

• YMC-SEC MAB

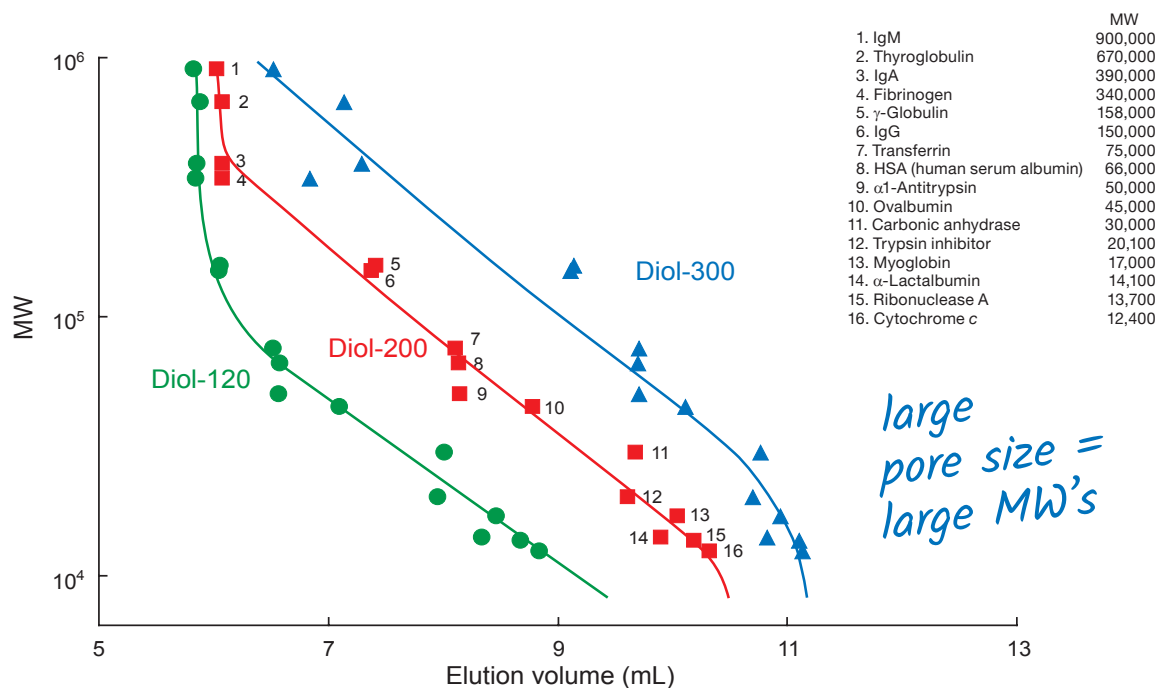
for MW 20,000 to 1,000,000

• YMC-Pack Diol-300

SEC – YMC-Pack Diol: Phase selection for proteins

Phases for different MW ranges

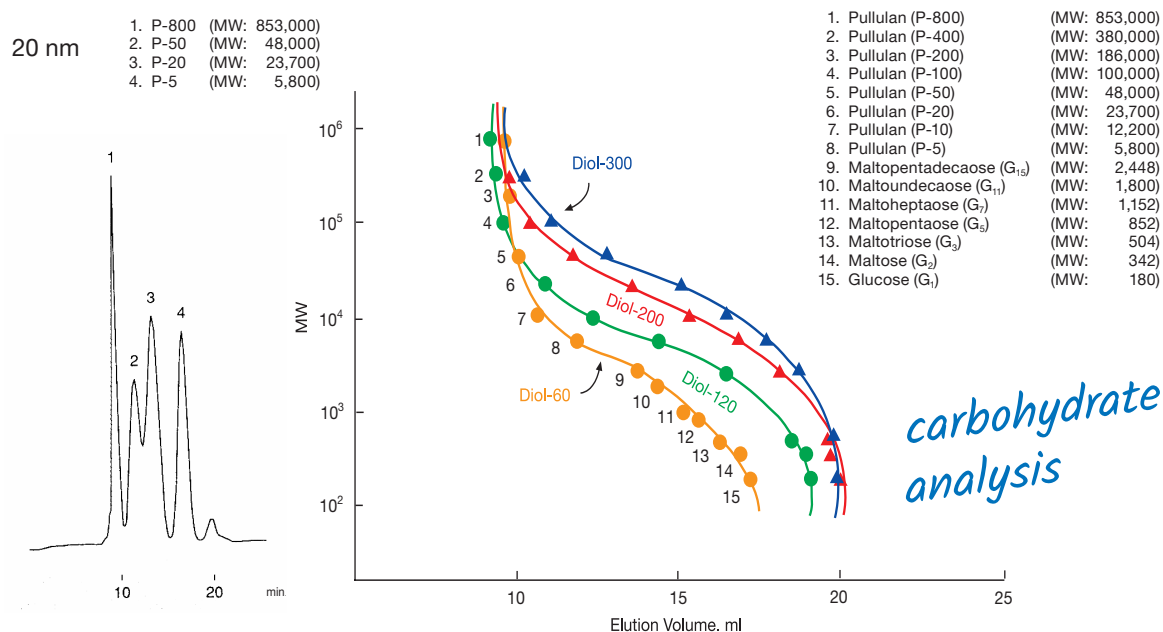
For separation of proteins with molecular weights from 10,000 to several 100,000 Da



Column: YMC-Pack Diol, 300 x 8.0 mm ID
Part Nos.: DL12S05-3008WT, DL20S05-3008WT, DL30S05-5008WT
Eluent: 0.1 M KH_2PO_4 - K_2HPO_4 (pH 7.0) containing 0.2 M NaCl

Flow rate: 0.5 mL/min
Temperature: 25 °C
Detection: UV at 280 nm

For molecular weight determination of oligosaccharides and polysaccharides

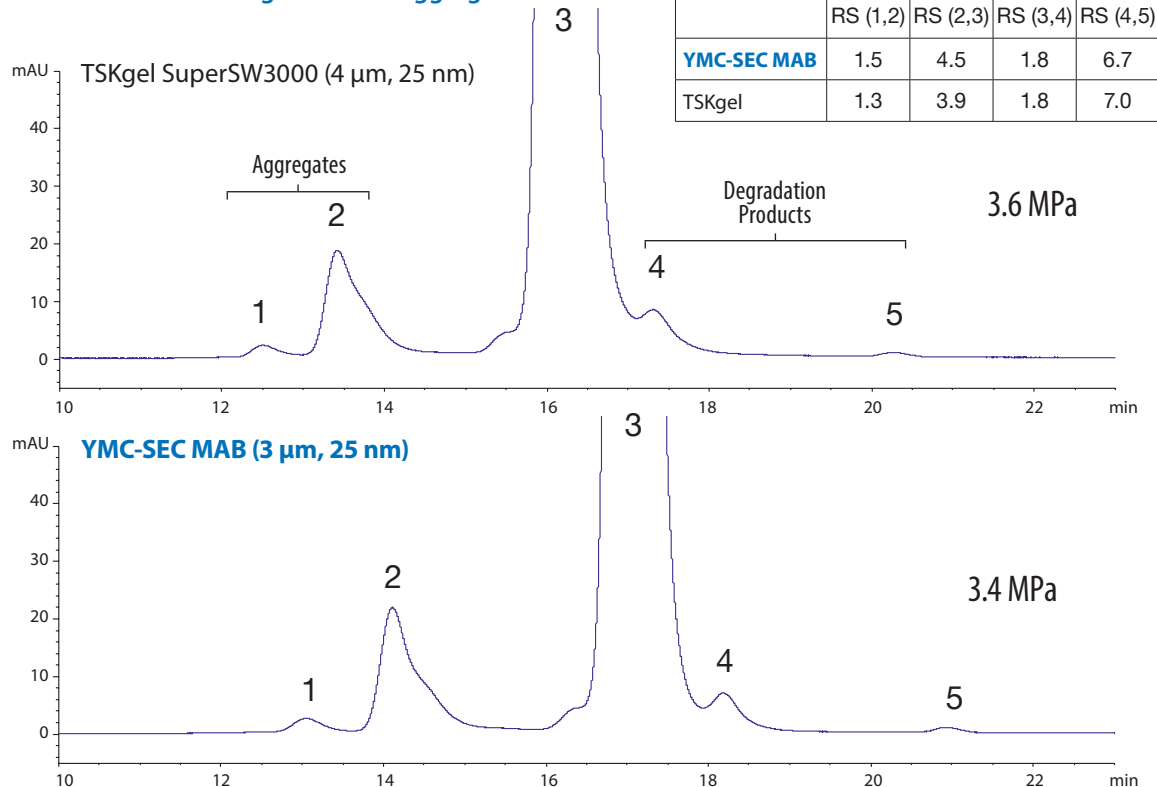


Column: YMC-Pack Diol (20 nm) 500 x 8.0 mm ID
Part No.: DL20S05-5008WT
Eluent: water

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

Ideal choice for monoclonal antibodies

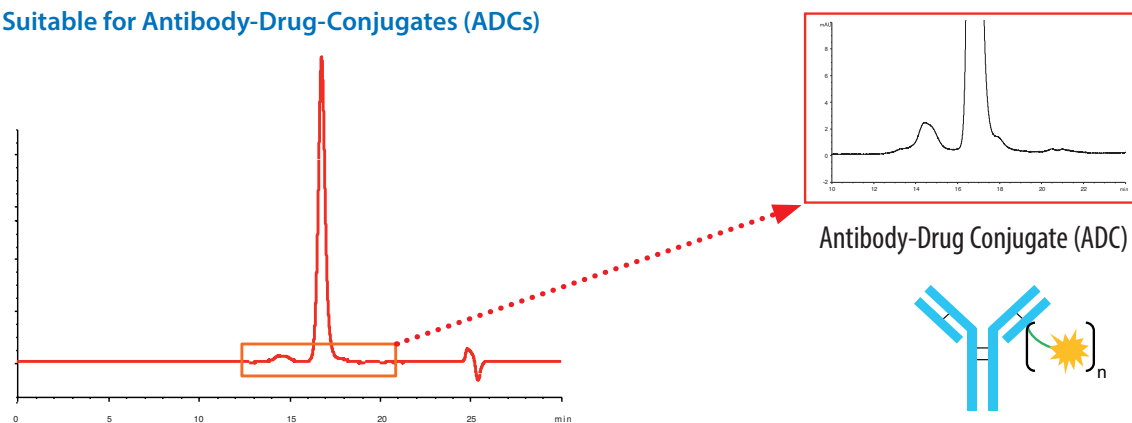
Bevacizumab and its fragments and aggregates



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

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

Suitable for Antibody-Drug-Conjugates (ADCs)



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

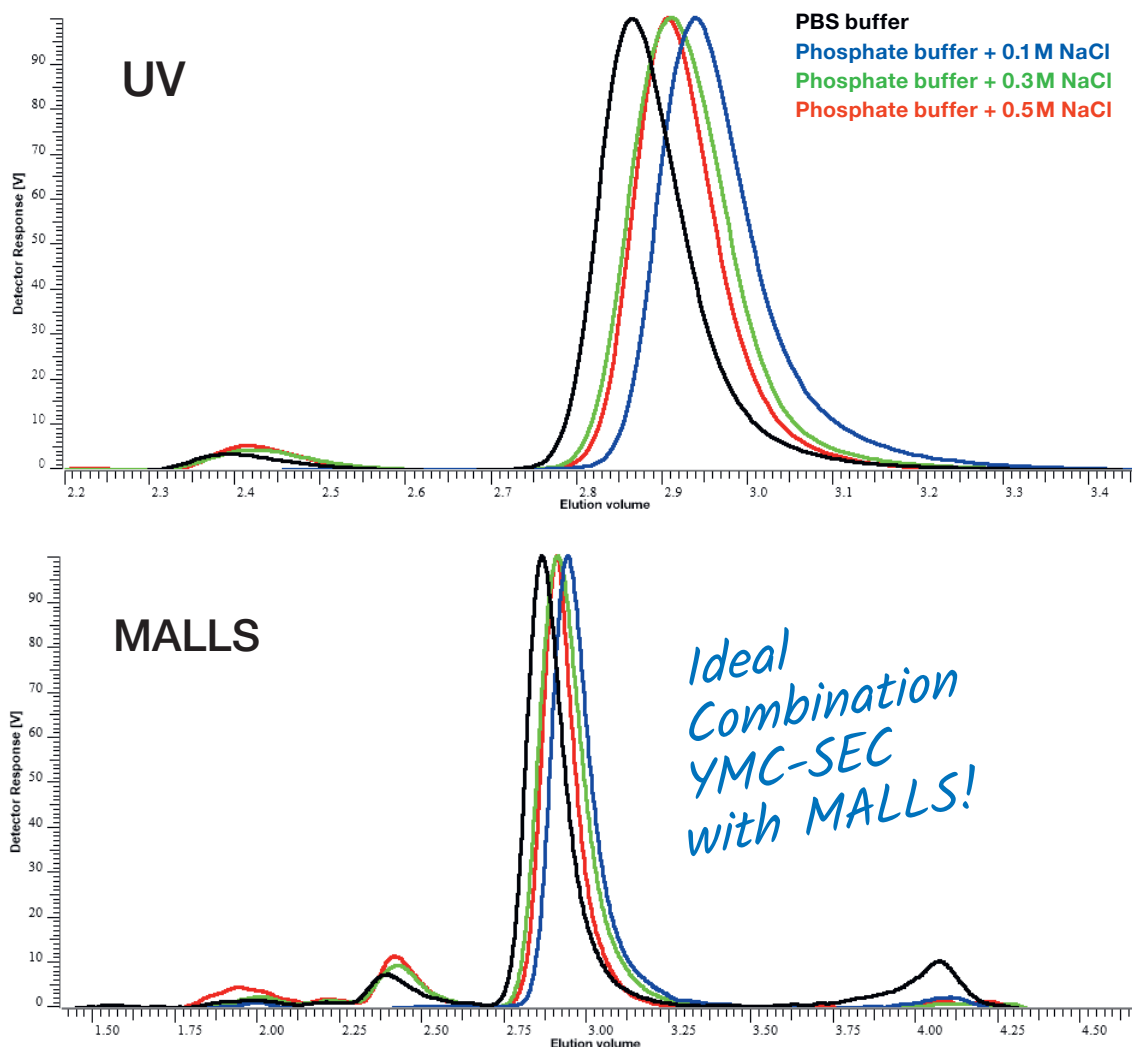
Temperature: 25 °C
 Detection: UV at 280 nm
 Injection: 4 µL (2.5 mg/mL)
 Sample: SigmaMAb Antibody Drug Conjugate Mimic

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

SEC – YMC-SEC MAB: MALLS

YMC-SEC columns ideally combined with light scattering detection

Detection of higher molar mass species by MALLS



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

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

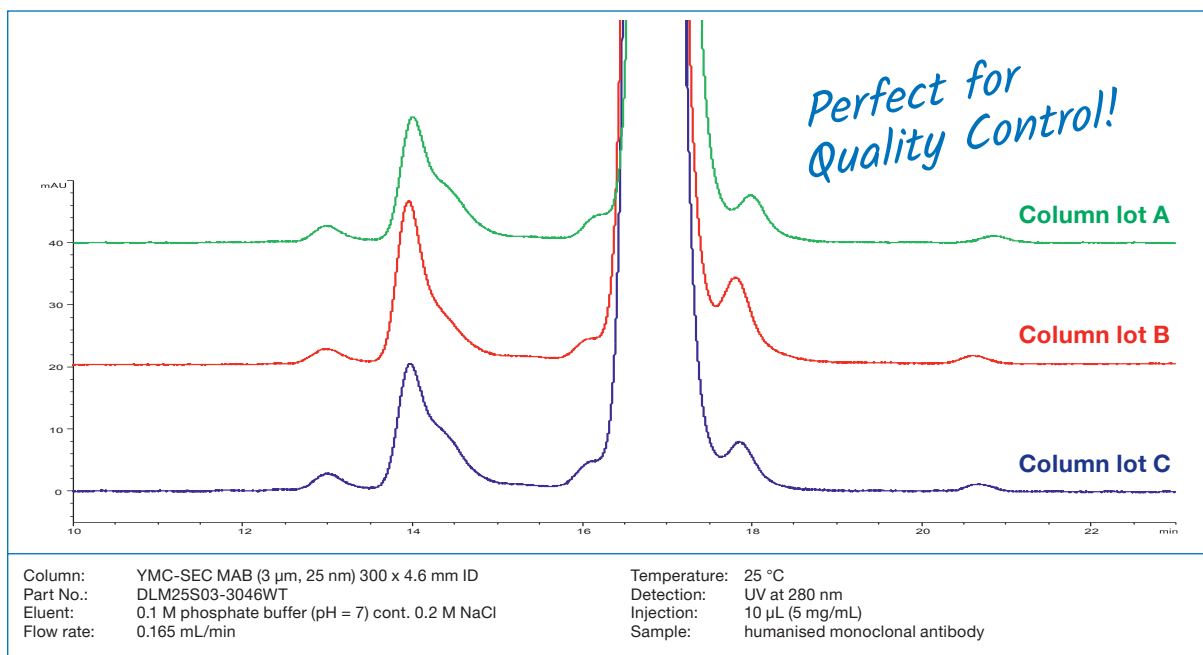
Four different buffers, a phosphate buffered saline (PBS) pH 7.4 and phosphate buffers pH 6.6 with varying concentrations of NaCl, were used to develop a suitable MALLS detection method for MABs.

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

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

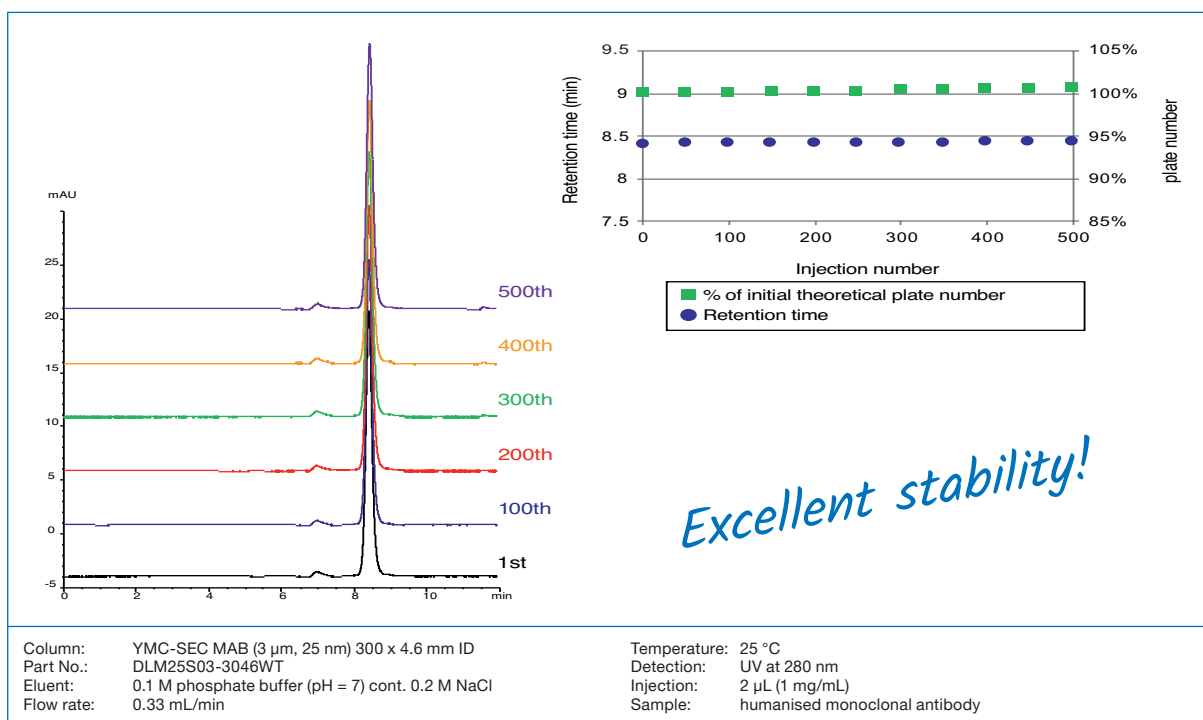
SEC – YMC-SEC MAB: Reproducibility & stability

Excellent lot-to-lot reproducibility



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

High column stability



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

SEC – YMC-Pack Diol: Resolution & throughput

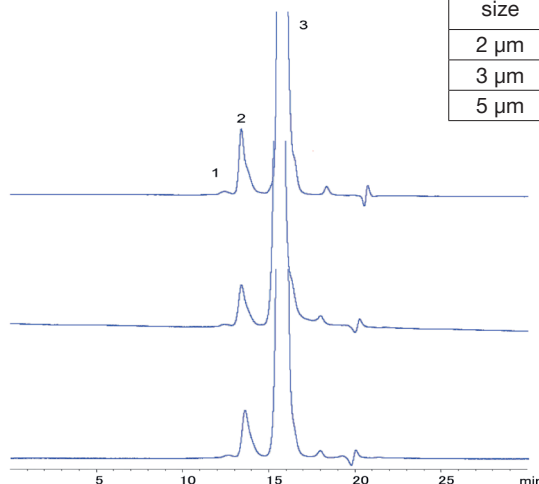
Benefits of using smaller particles

Higher resolution for analysis of monoclonal antibodies

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

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

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



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

Columns: YMC-Pack Diol-300, 300 x 4.6 mm ID
Part Nos.: (A) DL30S02-3046PTH
(B) DL30S03-3046WT
(C) DL30S05-3046WT
Eluent: 0.1 M KH_2PO_4 - K_2HPO_4 (pH 7.0) containing 0.2 M NaCl

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

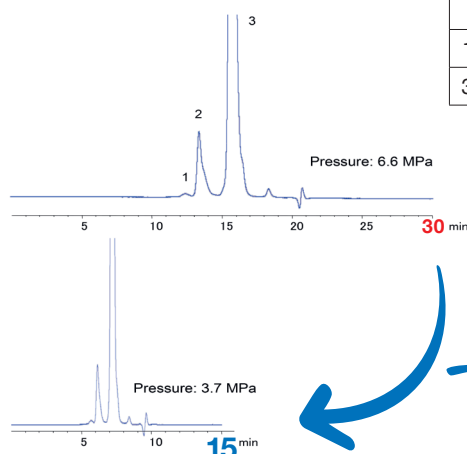
All three particle sizes show identical separation patterns for monoclonal antibody analysis. This allows easy method transfer between HPLC and UHPLC. A method developed using conventional HPLC can be directly transferred to UHPLC using a 2 μm YMC-Pack Diol

column. YMC-Pack Diol UHPLC columns greatly improve the resolution between aggregates and the monomer peak. In addition, a shoulder peak which can be observed after the monomer peak can be partially separated using the 2 μm column.

High throughput analysis of monoclonal antibodies

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

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



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

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

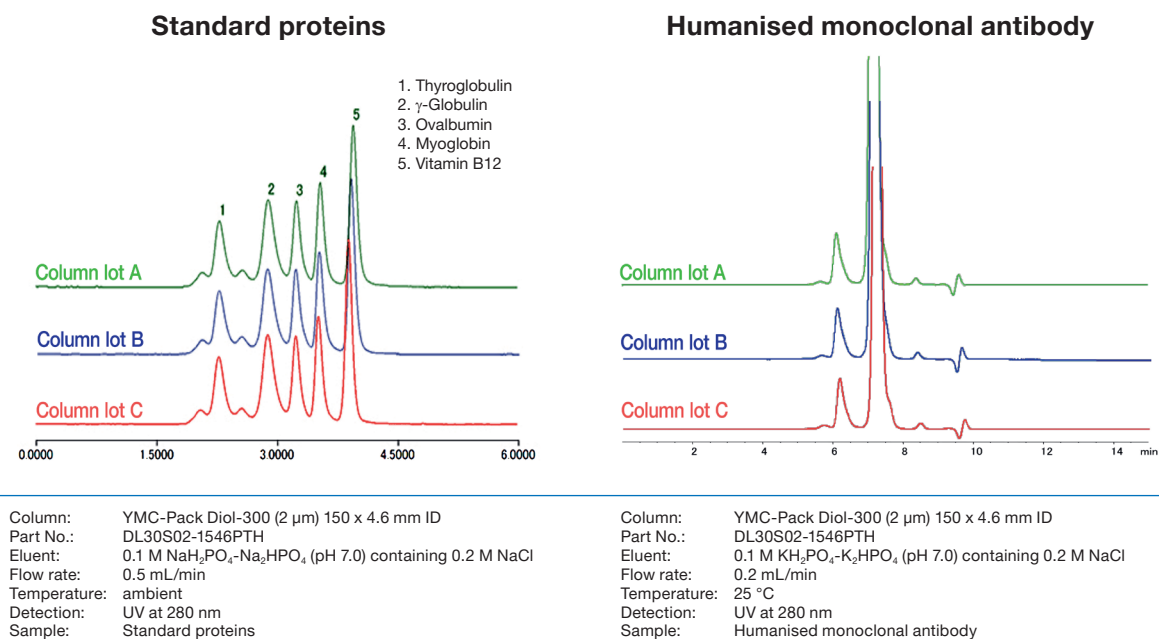
Temperature: ambient
Detection: UV at 280 nm
Sample: Humanised monoclonal antibody (IgG1)

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

SEC – YMC-Pack Diol: Reproducibility & stability

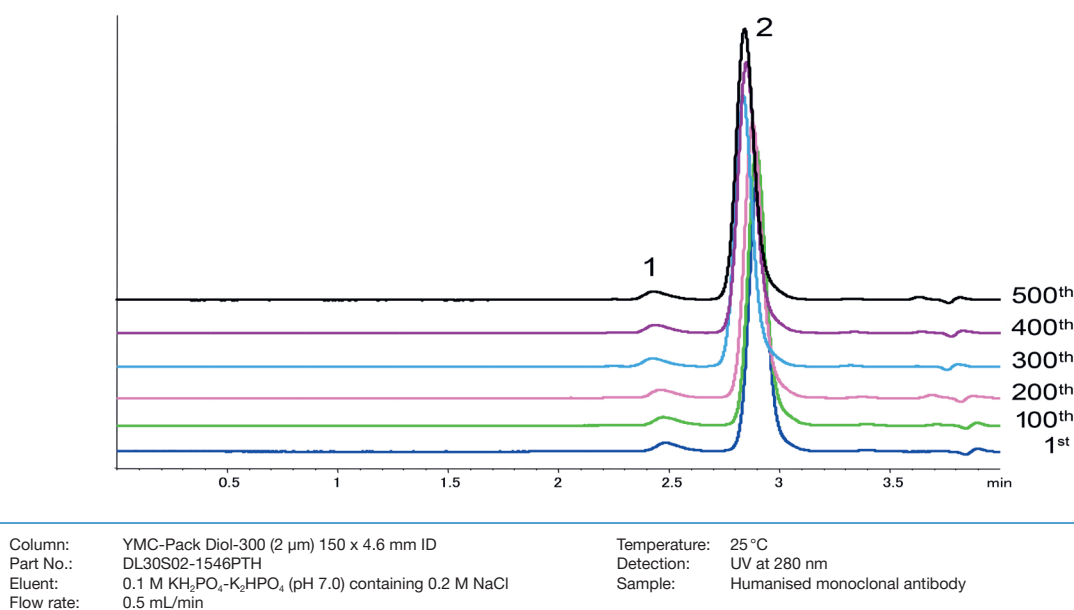
Reproducibility and stability data

Excellent batch-to-batch reproducibility



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

Long-term stability



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

SEC – Ordering information

2 µm UHPLC columns

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

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

3 µm HPLC columns

Phase	Column ID [mm]	Column length [mm]			Guard cartridges* with 10/30 mm length
		150	250	300	
YMC-SEC MAB	4.6	DLM25S03-1546WT	–	DLM25S03-3046WT	DLM25S03-0104GC
	6.0	–	–	–	–
	8.0	–	–	DLM25S03-3008WT	–
YMC-Pack Diol-60	4.6	DL06S03-1546WT	DL06S03-2546WT	DL06S03-3046WT	DL06S03-0104GC
	6.0	–	–	DL06S03-3006WT	–
	8.0	DL06S03-1508WT	–	DL06S03-3008WT	DL06S03-0308WTG**
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 guards

SEC – Ordering information

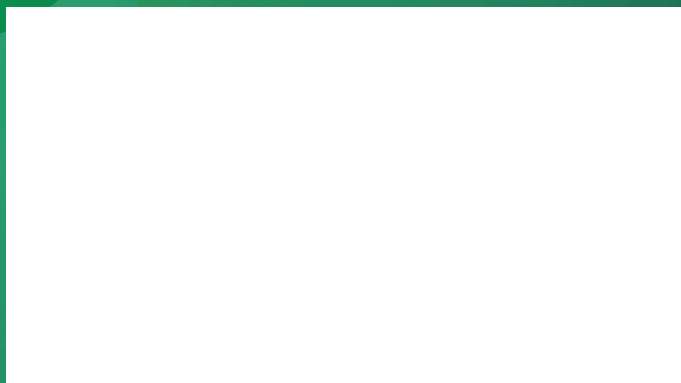
5 µm HPLC columns

Phase	Column ID [mm]	Column length [mm]			Guard cartridges* with 10/30 mm length
		250	300	500	(pack of 5)
YMC-Pack Diol-60	4.6	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 8/10 mm ID guards

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