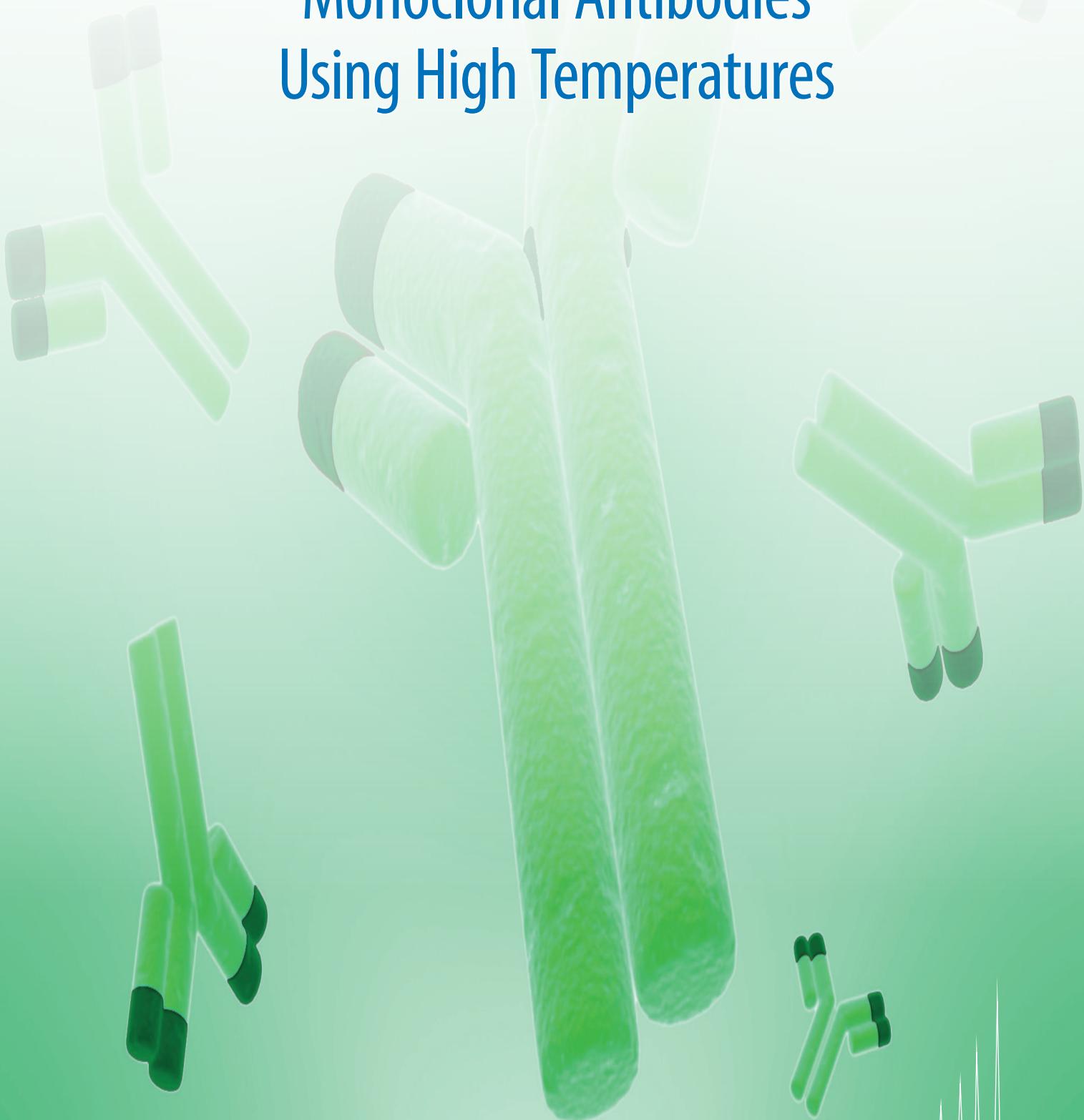


RP Analyses of Monoclonal Antibodies Using High Temperatures





RP Analyses of Monoclonal Antibodies Using High Temperatures

Monoclonal antibodies (MAbs) are immunological active proteins, which bind specifically to certain cells or proteins. This will stimulate the immune system to attack those targets.

MAbs are very important for the treatment of different kinds of cancer and autoimmune diseases. Nowadays, a broad variety of therapeutical antibodies are available on the market and several more are in research and development.

Due to their molecular weight of about 150 kDa, intact antibodies are usually analysed by IEX, SEC or HIC. In addition, RP methods are an easy tool as well.

However, lack of sensitivity and resolution has been a hurdle in the past. With modern RP phases, addressing the requirements of these analytes, it is easy to find a suitable method.

Successful analysis in RP mode for MAbs is enhanced

by employing a temperature stable ($>60^{\circ}\text{C}$), widepore stationary phase.

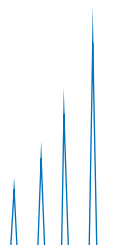
This application note shows how to achieve robust chromatographic results for two commercially available MAbs: Adalimumab (e.g. Humira[®]) and Bevacizumab (Avastin[®]).

The increase in temperatures leads to higher sensitivity and a sharper peak of Adalimumab at temperatures $>60^{\circ}\text{C}$ (figure 1). Bevacizumab shows robust results starting from 70°C , whereas at lower temperatures no peak was detected (figure 2).

With YMC-Triart Bio C4 elevated temperatures can easily be applied, due to stability up to 90°C . In addition, the surface comprising $30\text{nm}/300\text{\AA}$ is beneficial for resolution. In combination with a wide pH range of 1–10 YMC-Triart Bio C4 is a well-suited tool for any MAb (U) HPLC method.

Table 1: Method details

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:	Time [min]	Eluent B [%]
	0	30
	15	60
	30	90
Flow rate:	0.4 mL/min	
Temperature:	25 $^{\circ}\text{C}$	
Detection:	UV at 220 nm	
Injection:	4 μL (0.5 mg/mL)	



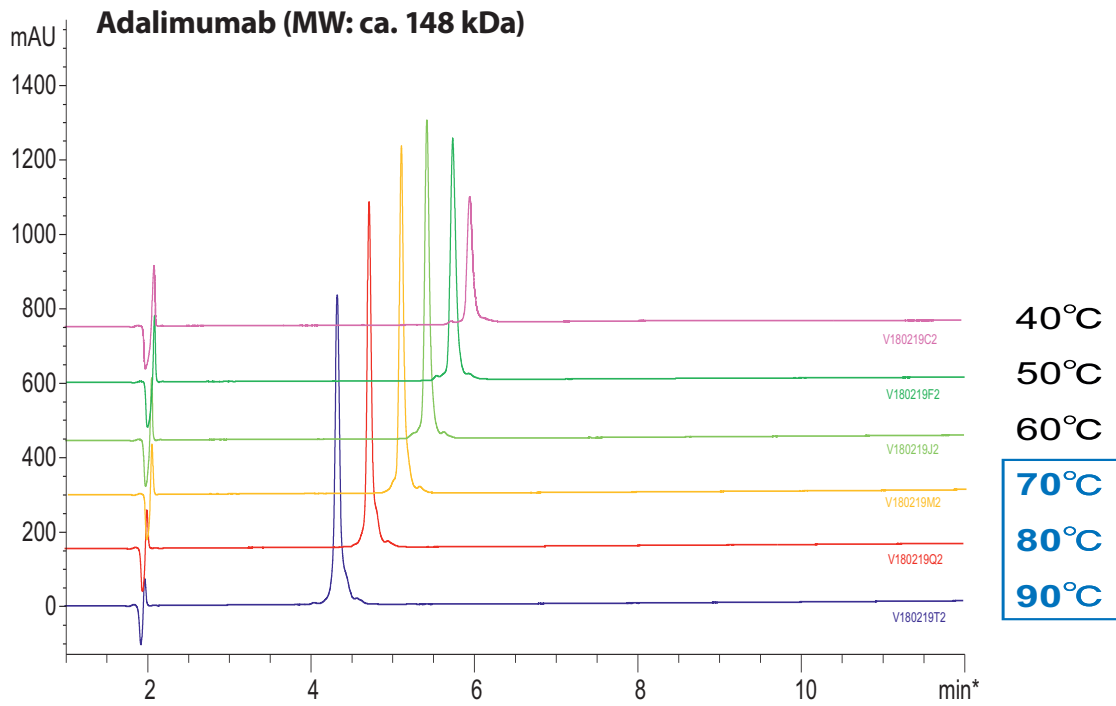


Figure 1: RP Analysis of Adalimumab.

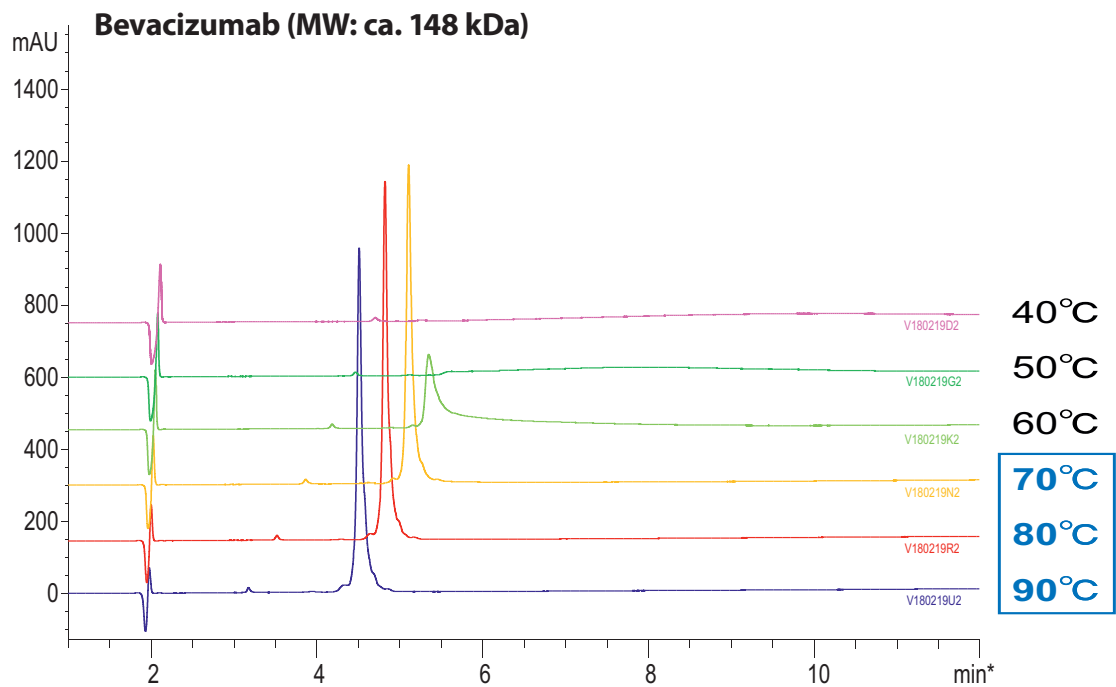
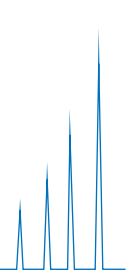


Figure 2: RP Analysis of Bevacizumab.



Ordering Information YMC-Triart Bio C4



YMC-Triart Bio C4, 1.9 µm UHPLC columns (max. pressure 1,000 bar)

Column ID (mm)	Column length (mm)						Guard cartridges* with 5 mm length (pack of 3)
	20	30	50	75	100	150	
2.0	TB30SP9-0202PT	TB30SP9-0302PT	TB30SP9-0502PT	TB30SP9-L502PT	TB30SP9-1002PT	TB30SP9-1502PT	TB30SP9-E5Q1CC**
2.1	TB30SP9-02Q1PT	TB30SP9-03Q1PT	TB30SP9-05Q1PT	TB30SP9-L5Q1PT	TB30SP9-10Q1PT	TB30SP9-15Q1PT	TB30SP9-E5Q1CC**
3.0	—	—	TB30SP9-0503PT	TB30SP9-L503PT	TB30SP9-1003PT	TB30SP9-1503PT	TB30SP9-E503CC

*Guard cartridge holder required, part no. XPCHUHP

**Guard cartridge: 2.1 mm ID

YMC-Triart Bio C4, 3 µm HPLC columns (max. pressure 450 bar)

Column ID (mm)	Column length (mm)							Guard cartridges* with 10 mm length (pack of 5)
	20	33	50	75	100	150	250	
2.1	TB30S03-02Q1PTH	TB30S03-H3Q1PTH	TB30S03-05Q1PTH	TB30S03-L5Q1PTH	TB30S03-10Q1PTH	TB30S03-15Q1PTH	—	TB30S03-01Q1GC
3.0	—	—	TB30S03-0503PTH	TB30S03-L503PTH	TB30S03-1003PTH	TB30S03-1503PTH	—	TB30S03-0103GC
4.6	—	TB30S03-H346PTH	TB30S03-0546PTH	TB30S03-L546PTH	TB30S03-1046PTH	TB30S03-1546PTH	TB30S03-2546PTH	TB30S03-0104GC

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

YMC-Triart Bio C4, 5 µm HPLC columns (max. pressure 450 bar)

Column ID (mm)	Column length (mm)							Guard cartridges* with 10 mm length (pack of 5)
	20	33	50	75	100	150	250	
2.1	TB30S05-02Q1PTH	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

*Guard cartridge holder required, part no. XPGCH-Q1 (2.1, 3, 4 mm ID)

XPCHSPW1 (10 mm ID)

**Max. pressure 100 bar

YMC-Triart Bio C4, 5 µm in YMC-Actus high-throughput semipreparative hardware (max. pressure 300 bar)

Column ID (mm)	Column length (mm)					Guard cartridges* with 10 mm length (pack of 5)
	50	75	100	150	250	
20	TB30S05-0520WX	TB30S05-L520WX	TB30S05-1020WX	TB30S05-1520WX	TB30S05-2520WX	TB30S05-0120CC
30	TB30S05-0530WX	TB30S05-L530WX	TB30S05-1030WX	TB30S05-1530WX	TB30S05-2530WX	TB30S05-0130CC
50	TB30S05-0553DX	—	TB30S05-1053DX	TB30S05-1553DX	TB30S05-2553DX	TB30S05-0553DXG**

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

**No holder required for 50 x 50 mm ID guard columns (no cartridge)

Several other dimensions or particle sizes available.

Check <https://ymc.de/rp-bioseparation.html>

