Generic Analyses of Different Monoclonal Antibodies with RP-(U)HPLC

Monoclonal antibodies (MAbs) are immunologically active proteins, which bind specifically to certain cells or proteins. This stimulates 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 therapeutically 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 have become an easy tool which is compatible with mass spectrometry (MS). 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 now easier to find a suitable method.

Successful analysis in RP mode for MAbs is enhanced by employing a widepore, temperature-stable stationary phase, such as YMC-Triart Bio C4. As a result of its extended stability, it is possible to use temperatures up to 90 °C. This application note shows how to achieve robust chromatographic results for commercially available MAbs: Trastuzumab (Herceptin®), Rituximab (MabThera®), Adalimumab (Humira®), Bevacizumab (Avastin®) and Nist mAB.

This generic analysis is carried out in less than 7 min using 1.9 µm particles for UHPLC. The eluents used are water, acetonitrile and TFA. The elevated temperature of 80 °C results in higher sensitivity and sharper peaks for all the MAbs analysed as shown in figure 1. With YMC-Triart Bio C4 elevated temperatures can easily be used, due to its stability up to 90 °C. In combination with the wide pore size of 30 nm / 300 Å YMC-Triart Bio C4 is a well-suited tool for any MAb (U)HPLC method.

Table 1: Method details

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

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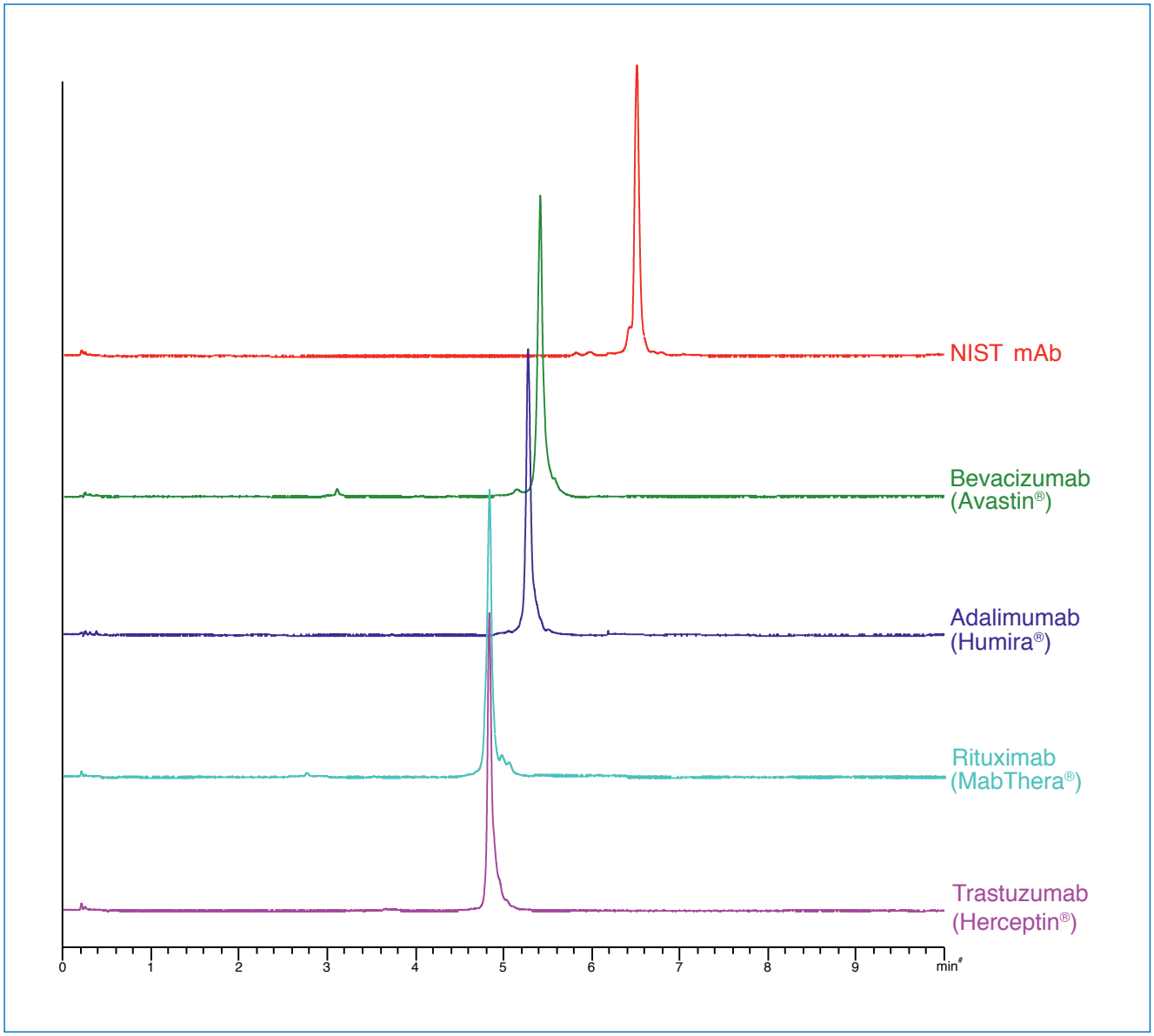

Figure 1. RP analysis of different monoclonal antibodies using YMC-Triart Bio C4.

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