APPLICATION NOTE

Fast UHPLC analysis of commercial monoclonal antibodies at elevated temperatures using YMC-Triart Bio C4

Monoclonal antibodies (MAbs) are immunologically active proteins, which bind specifically to certain cells or proteins. This stimulates the immune system to attack those targets. The importance of MAbs is becoming increasingly important for the treatment of different types of cancer and autoimmune diseases. Nowadays, a wide variety of therapeutic antibodies are available on the market and several more are in research and development.

Intact antibodies are usually analysed by IEX, SEC or HIC because of their high molecular weight (about 150 kDa). Mass spectrometry (MS) compatible RP methods have also become a useful tool. In the past, a lack of sensitivity and resolution has been a hurdle. With modern RP phases addressing the requirements of these analytes, it is now easier to find a suitable method.

Using a wide pore, temperature stable stationary phase such as YMC-Triart Bio C4, MAbs can be successfully analysed in RP mode. As a result of its extended stability, it is possible to use temperatures up to 90 °C.

This application note developed by Department of Analytical Pharmaceutical Chemistry, the University of Geneva demonstrates the robust chromatographic method using the commercially available MAbs: Ofatumumab (Arzerra®), Panitumumab (Vectibix®) and Palivizumab (Synagis®).

The analysis is carried out in 4 min and demonstrates that elevated temperatures up to 90 °C result in higher recovery and sharper peaks for all three MAbs; a high temperature of 90 °C is absolutely necessary for Panitumumab in order to achieve high recovery.

Table 1: Chromatographic conditions.

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

YMC Europe GmbH · Schöttmannshof 19 · 46539 Dinslaken · Phone +49 (0)2064 427-0 · Fax +49 (0) 2064 427-222 · Email: info@ymc.de · www.ymc.de

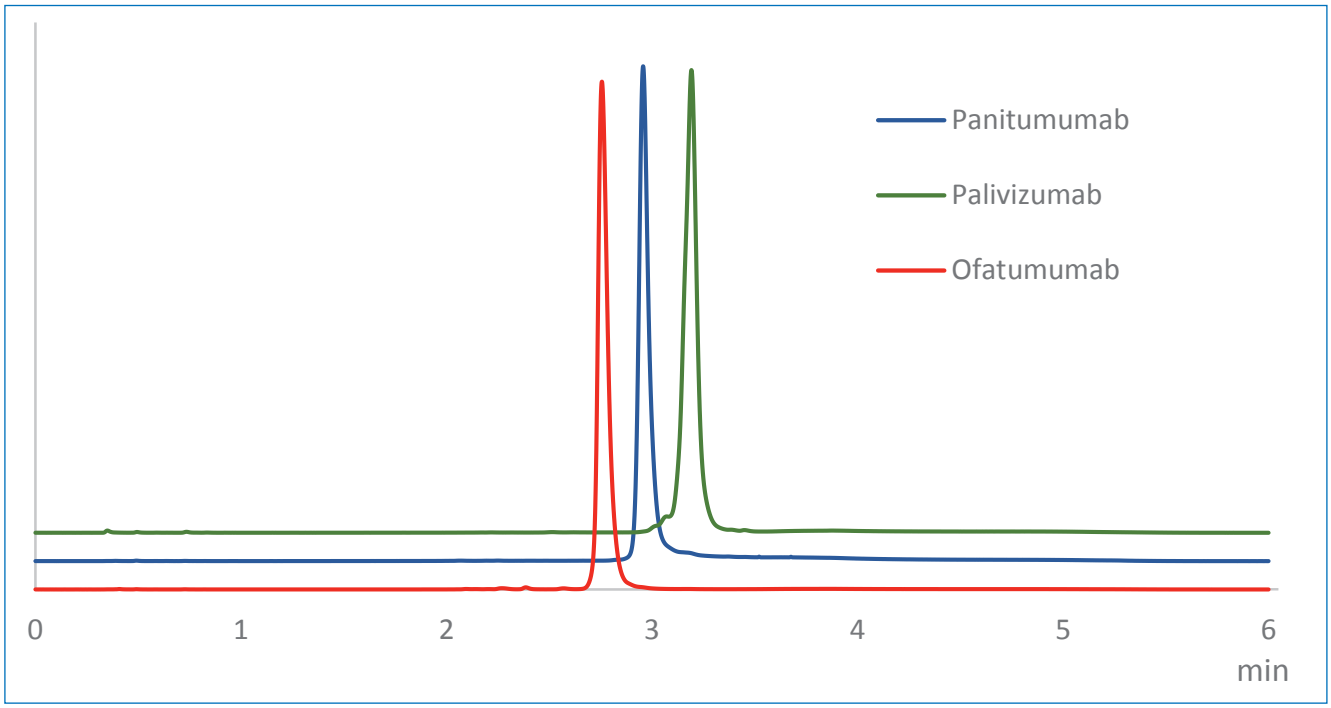

Figure 1: UHPLC analysis of different monoclonal antibodies using YMC-Triart Bio C4.

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