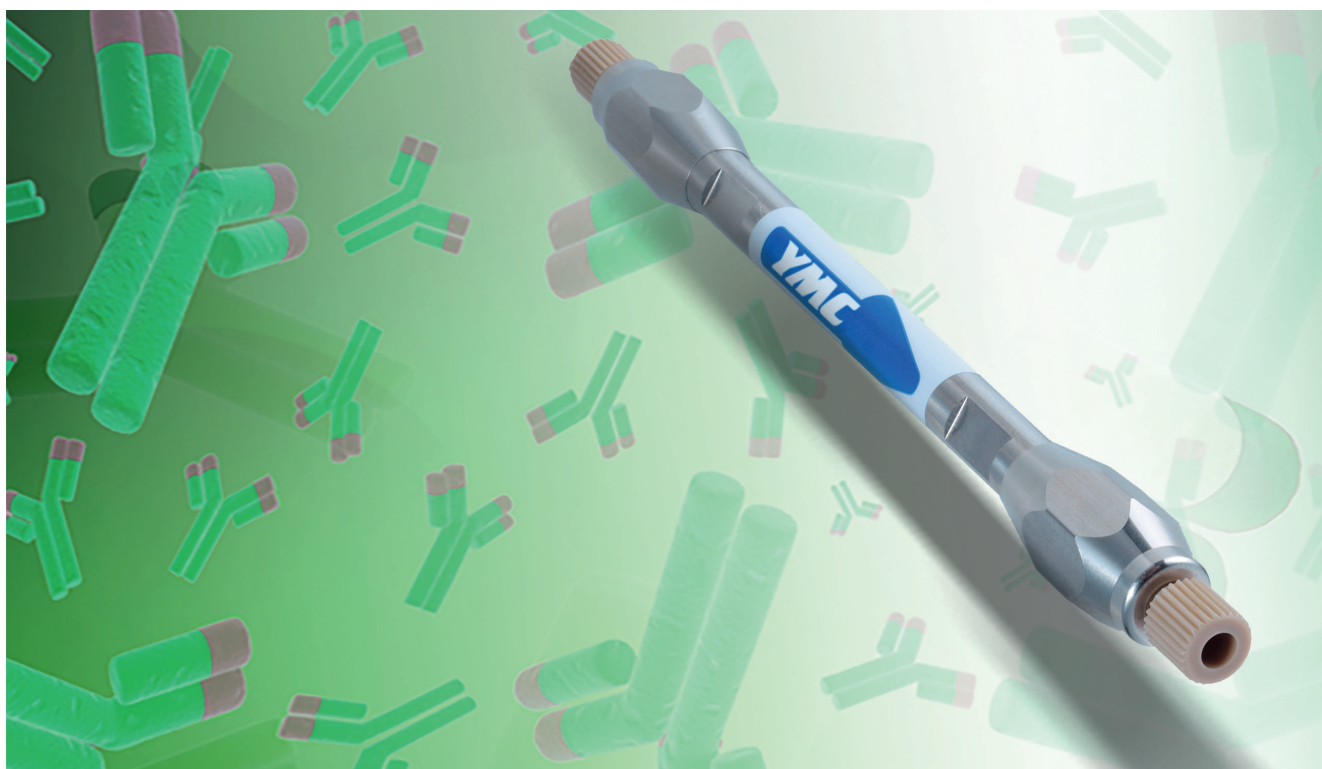




## Analysis of Bevacizumab (Avastin<sup>®</sup>) and its aggregates and fragments

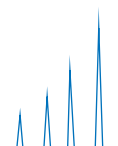
**D**ue to the sizes of monoclonal antibodies (MAbs, about 150 kDa), size exclusion chromatography (SEC) is a standard technique for analysing MAbs such as Bevacizumab (Avastin<sup>®</sup>). It is also a standard separation mode used in quality control to obtain information about aggregation and/or fragmentation of the MAbs.

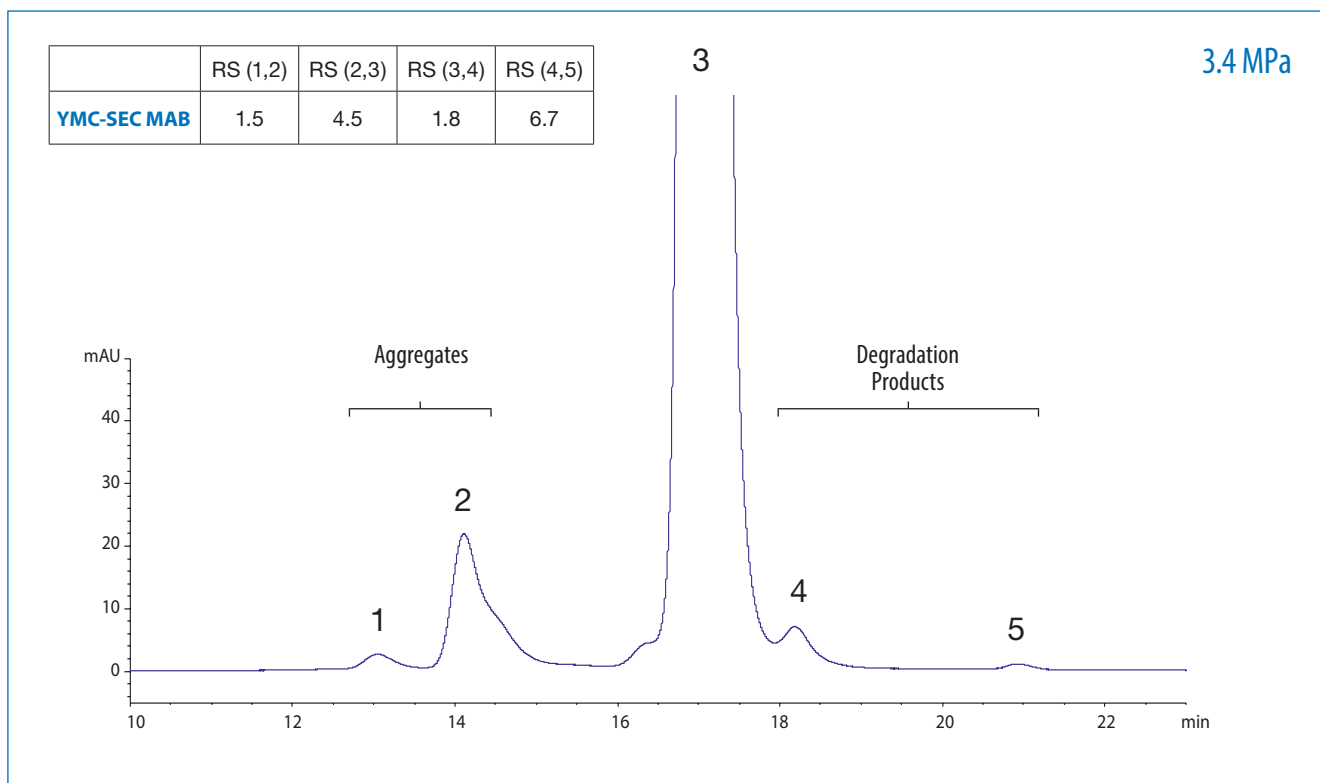


In this application note YMC's dedicated SEC column for antibodies, YMC-SEC MAB, is used to separate the monomer antibody from its aggregates as well as from degradation products in a single run. A neutral phosphate buffer containing 0.2 M NaCl is used as eluent at a flow rate of 0.165 mL/min. UV detection is carried out at 280 nm. The resulting separation provides high resolutions for both the high molecular weight range (peaks 1 and 2) as well as for the low molecular weight range (peaks 4 and 5). The resulting backpressure is only 34 bar/3.4 MPa, while the maximum pressure rating for the column is 140 bar/14 MPa.

Table 1: Chromatographic conditions

|              |  |
|--------------|--|
| 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) cont. 0.2 M NaCl |
| Flow rate:   | 0.165 mL/min                                     |
| Temperature: | 25 °C  |
| Detection:   | UV at 280 nm                                     |
| Injection:   | 10 $\mu$ L (5 mg/mL)                             |
| Sample:      | Bevacizumab (Avastin <sup>®</sup> )              |





**Ordering information for YMC-SEC MAB**

| Particle size<br>[μm] | Pore size<br>[nm] | ID<br>[mm] | Column Length<br>[mm] |                 | Guard cartridges*<br>With 10 mm length |
|-----------------------|-------------------|------------|-----------------------|-----------------|--|
|                       |                   |            | 150                   | 300             |  |
| 3                     | 25                | 4.6        | DLM25S03-1546WT       | DLM25S03-3046WT | DLM25S03-0104GC                        |
|                       |                   | 8          | –                     | DLM25S03-3008WT | –                                      |

\*Holder required, part no XPGCH-Q1

