High-Resolution Mapping of Drug Conjugated Peptides in an ADC Digest

Peptide map comparison of mAb and drug conjugated mAb

Application Note

Biologics and Biosimilars

**Introduction**

Currently, antibody drug conjugates (ADCs) are prime protein drugs for biotherapeutic use. When a cytotoxic drug is conjugated to a biotherapeutic monoclonal antibody (mAb), there are several options for the conjugation site. As part of the characterization of ADCs, it is important to be able to identify these conjugation sites. This can be done using peptide mapping. The specificity of the enzyme to cleave the mAb into peptide fragments results in different cleavage patterns, and, hence, peptide fragments, around the conjugation site. High-resolution peptide mapping can be used to identify peptides that are produced as a result of conjugation of the cytotoxic drug. This Application Note demonstrates the use of the Agilent AdvanceBio Peptide Mapping Column and an Agilent 1290 Infinity LC system for ADC peptide mapping analysis. For analysis using UV, it is important to have high resolution to identify the individual peptides, therefore, the method was developed with an optimized flow rate and gradient time for increased peak capacity. Comparison of a peptide map of Trastuzumab biotherapeutic mAb and its cytotoxic drug conjugate, ADC, revealed the peptide map differences corresponded to drug-conjugated peptides. These hydrophobic peptides were resolved on the AdvanceBio Peptide Mapping Column.
The peak capacity values were calculated by dividing the gradient time by the average peak width of five peptide peaks at baseline (5σ). Figure 1 depicts the effect of gradient time and flow rate on peak capacity. The results suggested that a 0.5 mL/min flow rate and 60 minutes gradient time gave the highest peak capacity values for the 2.1 x 250 mm, 2.7 μm column. These would, therefore, be the optimum conditions for identifying the peptides that have cytotoxic drug conjugation with high resolution.

Results and Discussion

Peak capacity is often used as an evaluation criterion to measure the performance of a column under given chromatographic conditions. mAb digestion produces many peptides for analysis. Therefore, it is necessary to develop a method that can increase peak capacity. Also, peak capacity is essential in a peptide mapping study so small impurity peaks or sample heterogeneity can be addressed. Due to the heterogeneous nature of ADC with glycosylation and cytotoxic drug conjugates, tryptic-digested ADC will generate more complex peptides than unconjugated mAb. To monitor the tryptic-digested ADC peptide mixture, optimization of gradient time and flow rates is critical to achieve high peak capacity.

Figure 1. Effect of flow rate and gradient time on peak capacity.
Figure 2 shows the peptide map of the tryptic digested ADC. The peptide map shows excellent performance with baseline separation and resolution across the entire gradient profile. There was a significant improvement in separation time with the 250-mm column (60 minutes) compared to a 150-mm column (220 minutes) as previously reported in the literature for peptide mapping of ADC. A peak capacity value of 354 was obtained and the RSD values demonstrate the excellent reproducibility of retention time and peak area and, thus, the precision of the system (Table 1).

To identify peptides that have the cytotoxic drug attached, the peptide digests of the mAb and its conjugate, ADC, were analyzed by monitoring the UV trace at 252 nm (Figure 3). Peptide maps of ADC are different from those of Trastuzumab. It is clearly evident that the more hydrophobic drug-bonded peptides in ADC are eluted later (~ 40 to 60 minutes). Comparing the two peptide maps shows a group of later-eluting peptides identified in the ADC digest that are not present in the digest of the mAb. These hydrophobic peptides are the ones with the cytotoxic drug conjugation.

Table 1. RSD of retention time and area (n = 5) of peaks shown in Figure 2.

<table>
<thead>
<tr>
<th>Peak</th>
<th>Mean RT (min)</th>
<th>RSD RT (%)</th>
<th>Mean area (mAU/min)</th>
<th>RSD Area (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak 1</td>
<td>5.37</td>
<td>0.13</td>
<td>369.2</td>
<td>0.76</td>
</tr>
<tr>
<td>Peak 2</td>
<td>14.27</td>
<td>0.06</td>
<td>106.1</td>
<td>1.66</td>
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<tr>
<td>Peak 3</td>
<td>28.84</td>
<td>0.02</td>
<td>202.61</td>
<td>0.09</td>
</tr>
<tr>
<td>Peak 4</td>
<td>35.86</td>
<td>0.02</td>
<td>193.83</td>
<td>0.58</td>
</tr>
</tbody>
</table>
Conclusions

High-resolution peptide maps are obtained when the 250-mm Agilent AdvanceBio Peptide Mapping Column is used with an Agilent 1290 Infinity LC System. By comparing the peptide maps of the mAb and its conjugate, ADC, it is possible to identify the peptides conjugated with a cytotoxic drug in the ADC digest. Additionally, we demonstrated that the AdvanceBio Peptide Mapping Column provided resolution across the range of peptide types. Good peak shapes and reproducibility were obtained for the analysis of the more hydrophobic conjugated peptides, enabling identification and quantitation.

References
