TOSOH

Application Note

Antibody Drug Conjugate Purification using TOYOPEARL® PPG-600M Resin

HIC Resin for DAR Separation

Introduction

Antibody drug conjugates (ADCs) are constructed of three components: a monoclonal antibody (mAb) which is specific to a cell-surface cancer antigen, a highly potent cytotoxic drug (payload), and a linker that covalently attaches the cytotoxic drug to the mAb. ADCs are one of the highest annual growth rate drugs to treat cancers and other diseases that require chemotherapeutic methods. Due to the highly toxic payload, high safety standards should be implemented during method and process development.

The purification process of ADCs is complex due to the heterogeneity of the conjugates. The main challenges are in the isolation of the unconjugated antibody and free drug, and the determination of the distribution of the average number of drugs conjugated to the antibodies, which correlates with the potency of the ADC and is referred to as the Drug-to-Antibody Ratio (DAR). High DARs are associated with high cytotoxic levels and can cause aggregation, affecting the stability of the ADC. On the other hand, low DARs affect the efficacy of the therapeutic.

An ADC mimic contains a non-toxic payload with similar structure and physicochemical properties as the toxic payload of an ADC. Therefore, it can be used as a model to develop a suitable purification process or an analytical method. The ADC mimic developed at Tosoh Bioscience used in this study consists of Adalimumab bound to Fluorescein 5-isocyanate (FITC). It displays a similar DAR as real ADCs, as shown in mass spectrometric evaluation, and therefore is a useful tool for analytical and preparative method development.

Due to the very hydrophobic payload, ADCs are more hydrophobic than normal monoclonal antibodies. An increasing DAR results in an increase in the hydrophobicity of the ADC, which can be used for the separation of different DAR. A relatively hydrophilic HIC resin, TOYOPEARL PPG-600M, is used in the purification study of an ADC mimic. This resin has the benefits of high recovery due to the relatively hydrophilic ligand, together with a high binding capacity and a wide working pH range.

Materials and Methods

The antibody used in this study is Adalimumab, a biosimilar of Humira®. The ADC mimic consists of a heterogenic, randomized coupling of fluorescein-5-isothiocyanate through the lysine group to the antibody.

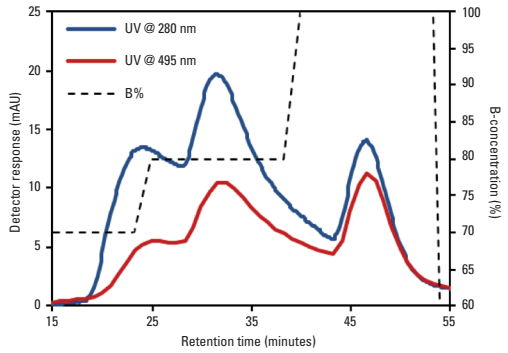
TOYOPEARL PPG-600M, 65 µm, 50 nm hydrophobic interaction resin was used in this study. The resin was packed into an Omnifit® Benchmark column (6.6 mm ID × 10 cm).

A TSKgel® Butyl-NPR analytical HIC column, 2.5 µm, 4.6 mm ID × 3.5 cm was used for analyzing collected ADC mimic fractions.

Purifying ADC mimic using TOYOPEARL PPG-600M

The ADC mimic was injected onto a TOYOPEARL PPG-600M column to separate the ADC mimic in fractions of low, medium and high DAR *(Figure 1)*.

Figure 1. Separation of the ADC mimic by a three-step gradient from 70-80-100% B in TOYOPEARL PPG-600M



The concentration of low salt buffer in each step can be adjusted to modify the separation:

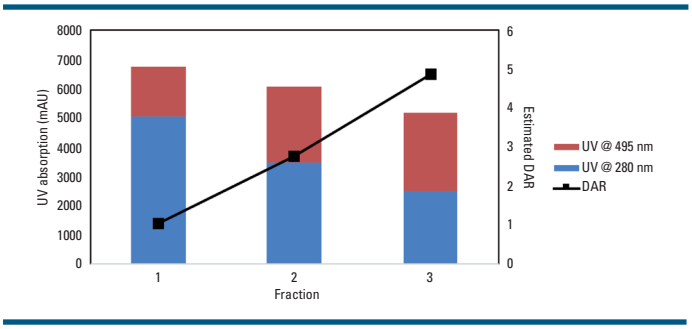
1. Equilibrate (5 CV, 250 cm/h): 100 mmol/L sodium phosphate, 1.5 mol/L ammonium sulfate, pH 6.5
2. Load (5 mg/mL-resin, 150 cm/h): FITC-Adalimumab-mimic
3. Wash (5 CV, 250 cm/h): 100 mmol/L sodium phosphate, 1.5 mol/L ammonium sulfate, pH 6.5
4. Elute 1 (5 CV, 175 cm/h): 30% 100 mmol/L sodium phosphate, 1.5 mol/L ammonium sulfate, pH 6.5 + 70% 100 mmol/L sodium phosphate, pH 6.5
5. Elute 2 (5 CV, 175 cm/h): 20% 100 mmol/L sodium phosphate, 1.5 mol/L ammonium sulfate, pH 6.5 + 80% 100 mmol/L sodium phosphate, pH 6.5
6. Elute 3 (5 CV, 175 cm/h): 100 mmol/L sodium phosphate, pH 6.5
7. Sanitize (5 CV, 250 cm/h): 500 mmol/L sodium hydroxide
8. Equilibrate (5 CV, 250 cm/h): 100 mmol/L sodium phosphate, 1.5 mol/L ammonium sulfate, pH 6.5

DAR analysis using TSKgel Butyl-NPR

The eluate for each elution step was fractionated and analyzed on a TSKgel Butyl-NPR column. Due to the different absorption maxima between FITC (495 nm) and antibody (280 nm), it is possible to calculate an UV-estimated DAR according to the following equation *(Figure 2)*:

$$DAR_{estimated} = \frac{2.77 * A_{495}}{A_{280} - (0{,}35 * A_{495})}$$

Figure 2. Absorbance differences of the ADC mimic and estimated DAR for each elution step on TOYOPEARL PPG-600M resin



Each bar represents the absorbance of the ADC mimic in blue for the antibody and red for FITC. The fractions from the step gradient were analyzed on a TSKgel Butyl NPR column.

Conclusions

Due to randomized and heterogenic coupling, the purification process of an ADC is more complex than the separation of a site-directed ADC, since we see a variation on DAR from 0 to 6.

Nevertheless, the TOYOPEARL PPG-600M resin offers sufficient selectivity in step gradient to separate the ADC-conjugate into groups with low, medium and high DARs. The low DAR fraction has an average DAR of 1, the medium DAR fraction of 3 and the high DAR fraction a medium DAR of 5.

All approved ADCs exhibit DARs between 2 and 4. By slight adjustments of the concentration during the step gradient, the process can be adjusted to isolate ADCs within target DAR ranges.