TOSOH

APPLICATION NOTE

ANTIBODY-DRUG CONJUGATE MIMIC PURIFICATION WITH TOYOPEARL® PPG-600M HIC RESIN FOR DAR-SEPARATION

INTRODUCTION

Antibody-drug conjugates (ADCs) are promising anti-cancer biopharmaceuticals with one of the highest annual growth rates. Four ADCs received market approval to this point. They combine the high selectivity and affinity of an antibody to cancer cells with the toxicity of chemotherapeutics in one molecule.

ADCs consist of a monoclonal antibody, covalently bound via a linker to a highly potent cytotoxic drug. Due to the highly toxic payload, very high safety standards should be implemented during method and process development. ADC-mimic contain a non-toxic payload with similar structure and physicochemical properties as the toxic payload of an ADC. Therefore, they can be used as a model to develop a suitable purification process or analytical method. The ADC-mimic in this work consists of Adalimumab bound to Fluorescein 5-isocyanate (FITC).

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 separation in different Drug-Antibody-Ratio (DAR), which correlates with the potency of the ADC. 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 therapeutics.

The mass spectrometric evaluation shows, that the ADC-mimic developed at Tosoh Bioscience displays a similar drug-to-antibody ratio as real ADCs. Thus, this ADC-mimic 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 this study. This resin has the benefits of a high recovery due to the relatively hydrophilic ligand together with a high binding capacity, and a wide working pH range.

TOYOPEARL PPG-600M: the HIC resin for the purification of ADCs with specific DARs

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 x 10 cm).

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

PURIFYING ADC-MIMIC USING TOYOPEARL PPG-600M

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

CHROMATOGRAM OF THE ADC-MIMIC BY A THREE-STEP GRADIENT FROM 70-80-100 % B IN TOYOPEARL PPG-600M (6.6 MM ID X 10 CM)

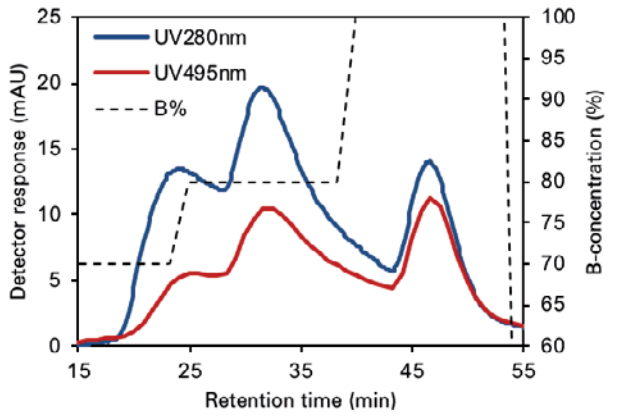

Figure 1

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-Miimic
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 TSKgel Butyl-NPR. 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):

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

ABSORBANCE DIFFERENCE OF THE ADC-MIMIC AND ESTIMATED DAR FOR EACH ELUTION STEP

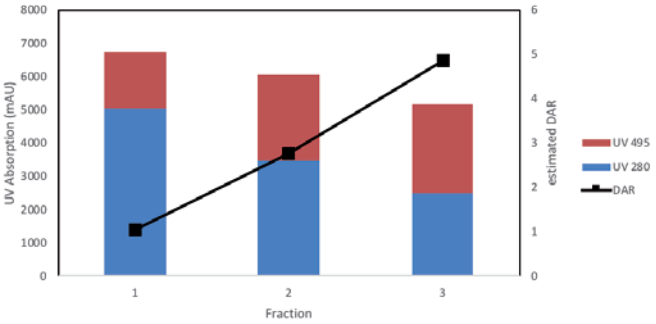

Figure 2

Absorbance difference of the ADC-mimics and estimated DAR for each elution step on PPG-600M. Each bar represents the absorbance of the ADC-mimics in blue for the antibody and red for FITC. The fractions from the step gradient were analyzed on TSKgel Butyl NPR (4.6 mm ID x 3.5 cm)

CONCLUSIONS

Due to the randomized and heterogenic coupling, the purification process is more complex than the separation of 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.