APPLICATIONS

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Analysis of Antibody Drug Conjugates using High Efficiency Gel Filtration Columns

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Gel filtration chromatography is a common method used for the analysis of macromolecules, including antibody drug conjugates (ADCs). This presents unique challenges since ADCs are more hydrophobic than their parent monoclonal antibodies (mAbs). In this study, we investigate the feasibility of using a non-toxic ADC model with a Yarra™ 3 µm SEC-3000 column for aggregate analysis.

Introduction

Antibody drug conjugates (ADCs) are immunoconjugates designed for delivery of a cytotoxic agent, or payload, directly to tumor cells. ADCs are composed of a payload covalently bonded to the monoclonal antibody via lysine, cysteine, and unnatural amino acid residues. Site-specific linker chemistries generally produce homogenous conjugate species. However, depending on the hydrophobicity of the cytotoxic drug and the linker chemistry, heterogeneous species can still form and may have more of a propensity for aggregation[1].

The most common analytical method for aggregate analysis of ADCs and other monoclonal antibody therapeutics is Gel Filtration Chromatography (GFC). Adjustments to existing GFC methods, such as the minor addition of IPA to the mobile phase, are typically made for acceptable peak shape and chromatography[2].

As reported by Wagner-Rousset and colleagues[3], an antibody fluorophore conjugate can be used as an alternative to ADCs for demonstrating analytical method development, including GFC. In this study, we used a non-toxic ADC model and investigated the feasibility of GFC method development using Yarra 3 µm SEC-3000 HPLC columns.

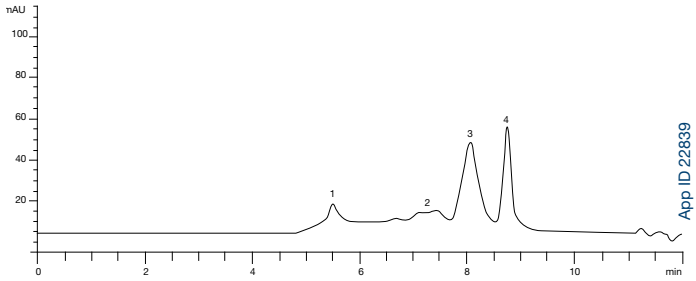
Materials and Methods

FITC-IgG was purchased from Fitzgerald Industries International (Acton, MA, USA). The instrument used for all GFC separations was an Agilent® 1290 LC system (Agilent Technologies, Palo Alto, CA, USA) with an upper pressure limit of 1200 bar, equipped with a binary pump, autosampler, and UV-Vis Detector. All separations used a Yarra 3 µm SEC-3000 300 x 7.8 mm HPLC column (p/n 00H-4513-K0) obtained from Phenomenex (Torrance, CA, USA). Mobile phases consisted of low salt buffer solutions with or without an IPA modifier (noted in each chromatogram).

Results and Discussion

Using a low salt buffer (100 mM Sodium Phosphate, pH 6.8), Yarra 3 µm SEC-3000 gave acceptable peak shape of monomer ADC and separation of dimer and HMW aggregate is observed (**Figure 1**).

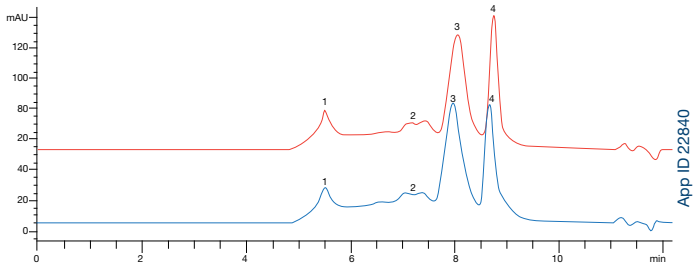
Figure 1. hIgG-FITC in Low Salt Buffer



Column: Yarra 3 µm SEC-3000
Dimensions: 300 x 7.8 mm
Part No.: 00H-4513-K0
Mobile Phase: 100 mM Sodium Phosphate, pH 6.8
Flow Rate: 1.0 mL/min
Injection Volume: 5 µL
Temperature: Ambient
Detection: UV @ 214 nm
Sample: 1. HMW Aggregate
2. Dimer
3. FITC-IgG
4. LMW Fragment

The addition of 10 % IPA did not significantly change the profile, and with this non-toxic ADC model it was not necessary for acceptable chromatography (**Figure 2**). This also indicates that the surface of the Yarra particles is highly inert and the addition of IPA is not necessary to reduce adsorption or obtain sharper peak shape of the larger aggregates.

Figure 2. hIgG-FITC in Low Salt Buffer + 10 % IPA



Column: Yarra 3 µm SEC-3000
Dimensions: 300 x 7.8 mm
Part No.: 00H-4513-K0
Mobile Phase: 100 mM Sodium Phosphate, pH 6.8 (blue trace)
100 mM Sodium Phosphate, pH 6.8 + 10 % IPA (red trace)
Flow Rate: 1.0 mL/min
Injection Volume: 5 µL
Temperature: Ambient
Detection: UV @ 214 nm
Sample: 1. HMW Aggregate
2. Dimer
3. FITC-IgG
4. LMW Fragment

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Conclusion

GFC for aggregate analysis is a common application for monoclonal antibodies and is particularly necessary for ADC analysis since conjugation can cause a propensity for aggregation. Typically, modifications must be made to existing GFC methods because of the hydrophobic nature of ADCs.

In this study, we use a non-toxic ADC model to demonstrate that aggregate analysis could be performed on Yarra™ 3 µm SEC-3000 HPLC column. No modification to the existing GFC method running conditions was necessary to obtain acceptable results. This might prove useful for applications requiring screening of various ADC isoforms.

References

1. Junutula, et al. “Site-specific Conjugation of a Cytotoxic Drug to an Antibody Improves the Therapeutic Index.” Nature Biotechnology 26.8 (2008): 925-32
2. Wakankar, Aditya, Yan Chen, Yatin Gokarn, and Fredric S. Jacobson. “Analytical Methods for Physicochemical Characterization of Antibody Drug Conjugates.” MAbs 3.2 (2011): 161-72
3. Wagner-Rousset, et al. “Antibody-drug Conjugate Model Fast Characterization by LC-MS following IdeS Proteolytic Digestion.” MAbs 6.1 (2014)

Yarra Column Ordering Information

Yarra 3 µm SEC Columns (mm)	Narrow Bore	Analytical	Analytical	SecurityGuard™ Cartridges (mm)
Phases	300 x 4.6	150 x 7.8	300 x 7.8	4 x 3.0*
Yarra 3 µm SEC-2000	00H-4512-E0	00F-4512-K0	00H-4512-K0	AJ0-4487
Yarra 3 µm SEC-3000	00H-4513-E0	00F-4513-K0	00H-4513-K0	AJ0-4488
Yarra 3 µm SEC-4000	00H-4514-E0	00F-4514-K0	00H-4514-K0	AJ0-4489

*SecurityGuard™ Analytical Cartridges require holder, Part No.: KJ0-4282 for ID: 4.6 - 7.8 mm

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guarantee

If Yarra analytical columns do not provide at least an equivalent or better separation as compared to competing column with similar dimension, phase, and dimensions, return the column with comparative data within 45 days for a FULL REFUND.

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