Method Optimization of Purity Analysis of a Fc-Fusion Protein by Reversed Phase HPLC

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Overview

A primary analytical technique for purity for recombinant proteins is reversed phase LC (RPLC). This is a simple, robust methodology with a relatively short analysis time, with high resolution, its ability to separate hydrophobic variants such as oxidation, glycoforms, and clipping. As such, RPLC is a common analytical approach for monoclonal antibodies (mAbs), especially considering it can be implemented at both intact and subunit levels without extensive method optimization when combined with a bioZen™ 2.6 µm WidePore C4 LC column.

Fc-fusion proteins are another, well established recombinant protein modality, similar to mAbs in that they contain the conserved region of a humanized IgG1 which extends their half-life. One might expect these proteins to behave similar chromatographically, however, Fc-fusion proteins present many different analytical challenges. Depending on the polypeptide the Fc is conjugated to, glycosylation can vary considerably, with both N-Linked and O-Linked glycans effecting protein function, and thus structure. From a physicochemical perspective, this makes the analysis of Fc-fusions at the intact level quite difficult.

However, there is still utility in an RPLC analytical method of Fc-fusion proteins, either for extended characterization and identification for impurities or simply for use as a simple purity method. As the starting point for method development, a standard approach to intact and subunit analysis of mAbs; i.e. a shallow gradient slope across a narrow range of organic, utilizing a high temperature, exceeding 60°C. When analyzing etanercept by running a standard mAb platform method, as shown in **Figure 1**, the profile is acceptable, however, elution occurs very early in the profile. As such, any subsequent analysis, such as chemical reduction or partial hydrolysis with a cysteine protease, might be limited. To optimize capacity factor, a simple adjustment of the starting and ending conditions of the gradient while maintaining the gradient slope can be employed. That is, the change in organic over the same time interval should give similar profiles, and indeed this result is observed when adjusting the gradient to 25-35% B over 5 minutes.

This adjustment in capacity factor allows for the analysis of the reduced etanercept. As seen in **Figure 2**, the resulting reduced fragments of etanercept elute earlier; with the previous gradient program of 30-40% B, these fragments would elute too early in the gradient for proper quantitation and integration. Further, robustness of the method might be a concern with systems that have varying dwell volumes.

In summary, purity analysis by reversed phase LC is a primary method for the analysis of recombinant proteins. Fc-fusions are a common recombinant protein therapeutic that are related to monoclonal antibodies but can behave quite differently chromatographically. However, by simply adjusting gradient programs to optimize capacity factor or retention time, one can develop a purity method in a similar manner that one would develop a method for mAbs.

LC Conditions

Column:	bioZen 2.6 µm WidePore C4
Dimensions:	100 x 2.1 mm
Part No.:	00D-4786-AN
Mobile Phase A:	0.1 % TFA in Water
Mobile Phase B:	0.1 % TFA in Acetonitrile
Gradient Program:	As indicated (Figure 1)
	25-35% in 5 minutes (Figure 2)
Flow Rate:	0.8 mL/min
Temperature:	80°C
Detection:	UV-Vis @ 214 nm
Injection:	2 µL Etanercept, as indicated (0.5 mg/mL)

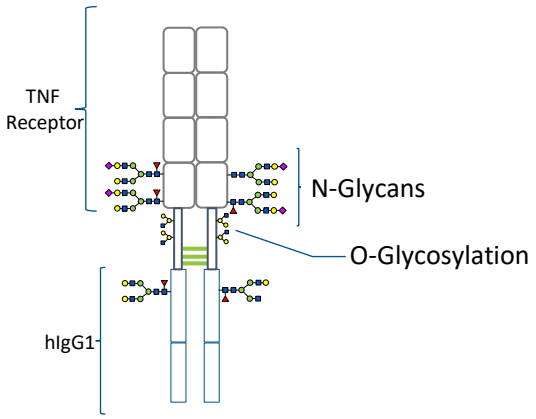


Figure 1. Etanercept Gradient Program Optimization

Overlay of etanercept using two different gradient programs. Blue trace shows a standard intact mAb method. Adjusting the gradient program to a lower starting organic concentration, while maintaining the gradient slope, yields a favorable separation that allows for the separation and retention of any earlier eluting variants or fragments.

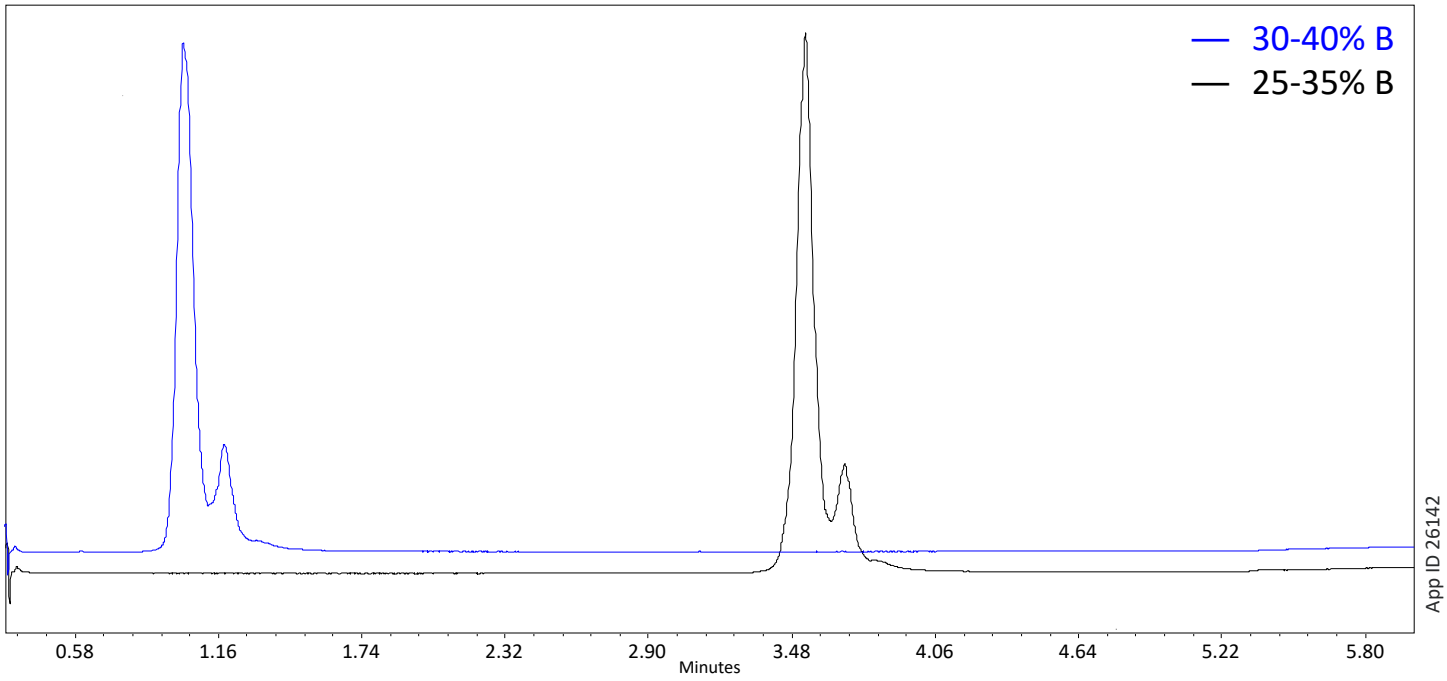
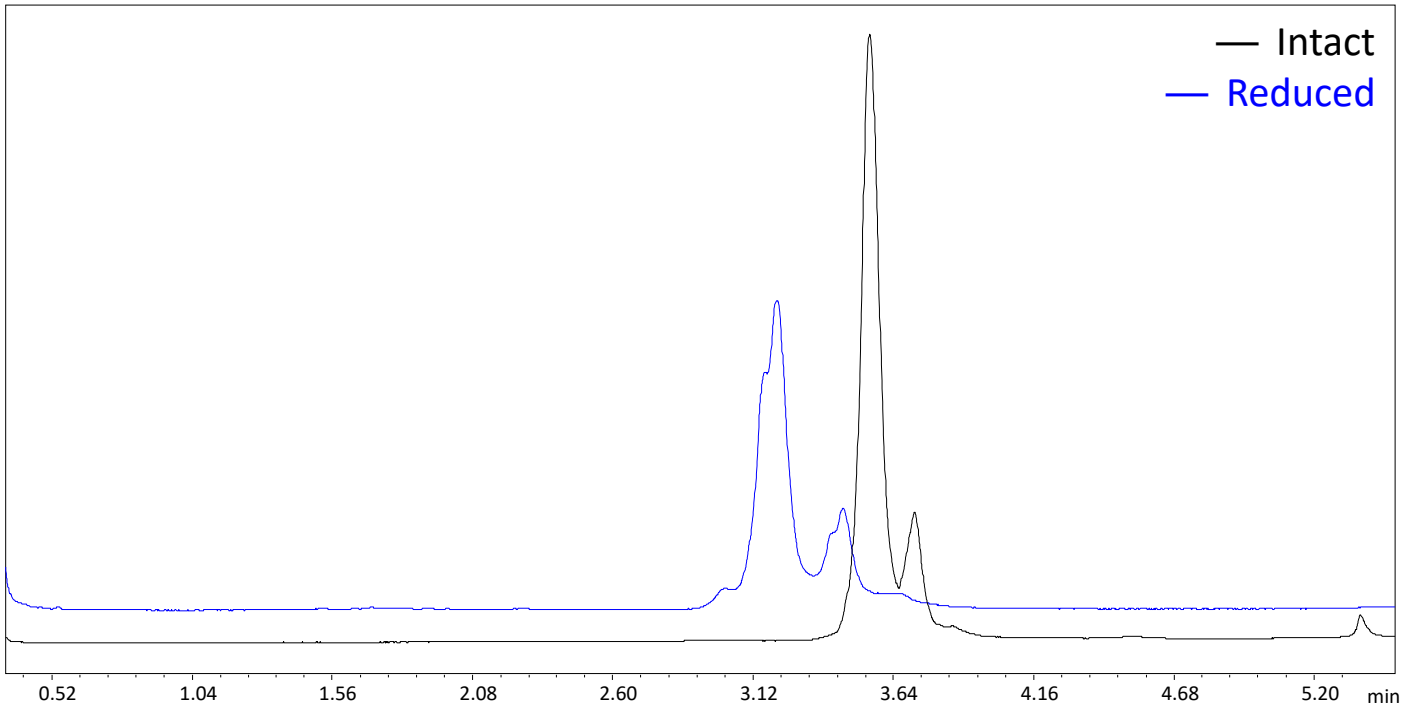


Figure 2. Etanercept, Intact and Reduced

Overlay of intact and reduced etanercept. The adjustment in gradient program allows for sufficient retention to ensure proper quantitation and integration of peaks.



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