Assessment of Disulfide Variants of IgG2 Monoclonal Antibodies by Intact Reversed Phase

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Overview

A primary analytical technique for purity for recombinant proteins is reversed phase LC (RPLC). This simple, robust methodology has a relatively short analysis time, as well being high resolution, capable of separation of hydrophobic variants such as oxidation, glycoforms, and clipping. As such, it 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.

One application where intact reversed phase is commonly used is in the assessment of disulfide heterogeneity for IgG2 isotypes. Because their Fc-mediator effector function differ when compared to IgG1s, IgG2s may be a more appropriate therapeutic platform depending on the biotherapeutic mechanism of action. As it pertains to reversed phase chromatography, IgG2s uniquely have 4 interchain disulfides, which consequently yields unique arrangements with different structural properties. Each variant behaves differently chromatographically, and although orthogonal methods such as disulfide mapping are necessary for full characterization, intact reversed phase is still a useful tool for monitoring and assessing overall disulfide variant isoforms.

When developing a platform method for IgG1s, implementing a relatively shallow gradient and short run times is more feasible. Since physicochemical properties for most IgG1s are very similar, a gradient slope of 0.4 % B per column volume and a gradient length of 5 minutes might yield optimal results on a wide variety of different molecules. However, platform methods for IgG2 antibodies requires a wider window of organic to accommodate the differences in physicochemical properties between them. To ensure shallow gradient slope, the gradient length subsequently must be extended.

The resulting method uses a 0.6 % B per column volume gradient slope, with a 20-45 % B gradient program over 10 minutes. Of particular importance for this method is the maintenance of the high flow-rate; ensuring that the linear velocity is high allows for the shallowing of gradient slope without compromising the method run time. This is important for intact reversed phase methods, as temperature is a method that is optimized to ensure optimal separation, and aside from gradient slope, is the most critical method parameter to optimize for intact reversed phase methods.

Figure 1 shows the separation of panitumumab, a relatively hydrophobic IgG2. The inset shows different isoforms, putative disulfide variants, for panitumumab. Figure 2 shows a similar profile for denosumab, another IgG2 monoclonal antibody. Previous studies suggest that earlier eluting variants are IgG2-B variants, with IgG-A/B and IgG-A being more hydrophobic thus retaining longer[1]. These would need to be confirmed via disulfide mapping.

In summary, purity analysis by reversed phase LC is a primary method for the analysis of recombinant proteins. For intact analysis of IgG2 isotypes, intact reversed phase is a relatively simple and high throughput method to understand the overall disulfide heterogeneity of disulfide isoforms.

HPLC Conditions

Column:	bioZen 2.6 µm WidePore C4
Dimension:	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:	20-45 % B in 10 minutes, 0.6 % B/CV
Flow-rate:	0.8 mL/min
Temperature:	80° C
Detection:	UV-Vis @ 280 nm
Injection:	2 µL
Samples:	As indicated (0.5 mg/mL)

Figure 1. Disulfide Variants of Panitumumab

Intact reversed phase analysis of panitumumab. The shallow 0.6% B per column volume gradient slope facilitates the separation. Putative variants include IgG2-B, IgG2-A/B, and IgG2-A, in order of elution. Shallowing of the gradient further might improve the separation for this relatively hydrophobic IgG2.

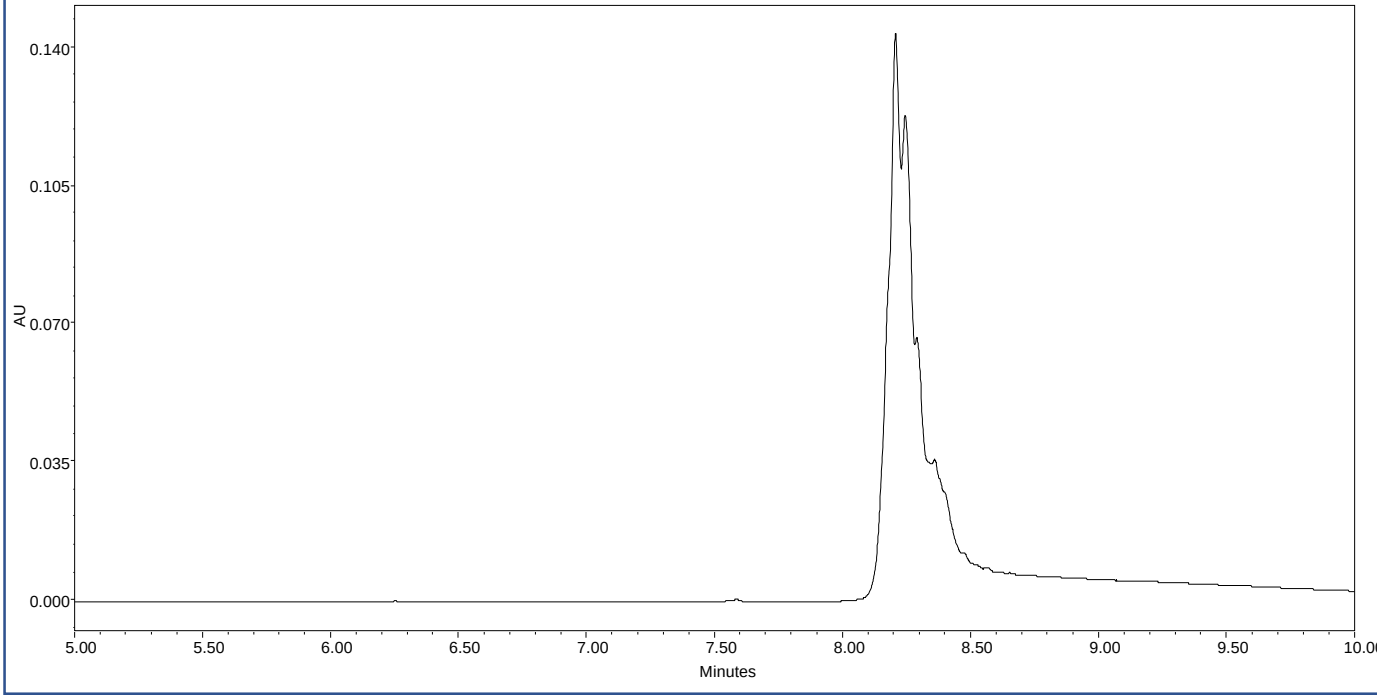
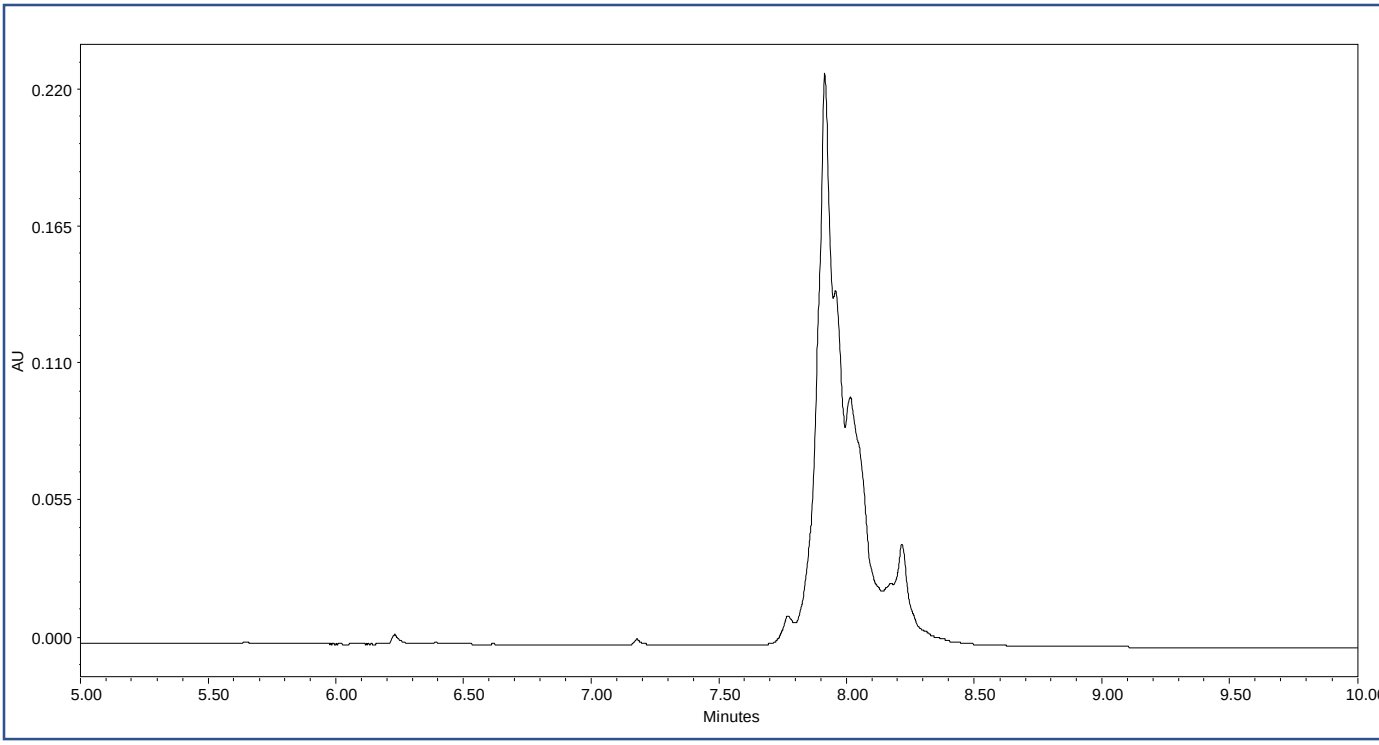


Figure 2. Disulfide Variants of Denosumab

Intact reversed phase analysis of denosumab. Slightly earlier eluting than panitumumab, showing similar variants. These include IgG2-B, IgG2-A/B, and IgG2-A.



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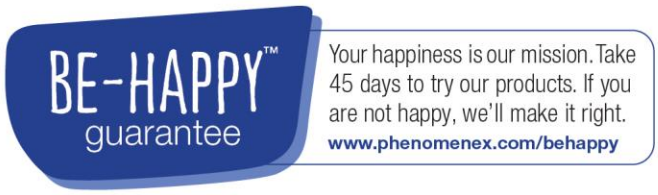

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