A Simple Quantitative Method for Monoclonal Antibody Coformulations

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Overview

Monoclonal antibodies (mAbs) are well-established therapeutics for biopharmaceutical work. However, recent trends have investigated the administration of multiple mAbs for immunotherapy and passive immunization strategies. Although characterization may present unique challenges, quantitation of each individual mAb tend to rely on standard analytical workflows, but methods for drug substance of the mAb coformulation often consist of a more complex analysis.

One such consideration is a simple quantitative method for the mAb coformulation. Although this might seem like a straightforward analytical method, the similarities in physicochemical properties between any two mAbs may complicate the analysis. Many mAbs chosen for coformulation are standard format, i.e. IgG1 humanized or chimeric, and expressed from Chinese Hamster Ovary (CHO) cell lines. Consequently, coformulated mAbs will be very have similar molecular weight, glycosylation, and isoelectric point, limiting chromatographic method options.

As such, the analytical method must have the selectivity to separate these highly similar biomolecules. Intact reversed phase then would be an appropriate separation modality, as hydrophobic differences should be sufficient between most mAb samples. Here, we present a proof-of-concept, simple and quantitative method for mAb coformulations by intact protein reversed phase LC using the bioZen™ 2.6 µm WidePore C4 column.

Using a superficially porous, widepore particle morphology not only gives high efficiency separations but enables higher flow rates, e.g. 0.8 mL/min for a 2.1 mm ID. This ensures as shallow a gradient slope possible, which is critical for selectivity of intact proteins. The initial scouting method used was 30-40% B in 5 minutes; with a 150 mm length column this results in a gradient slope of approximately 0.6% B per column volume. Resulting chromatogram overlays for two mAbs- NIST and Trastuzumab- are shown in **Figure 1.** The retention time difference visually show a favorable result.

As seen in **Figure 2**, injecting a 1 mg/mL solution containing both mAbs shows baseline separation, with USP resolution being 3.2. Further optimization of the method by modifying the gradient from 40-50% B (**Figure 3**), using the same gradient slope, yields a marginal improvement of resolution of 4.

Finally, temperature was investigated to improve peak shape, with the intent of maximizing resolution. As observed in **Figure 4,** increasing temperature to 80°C yields an increase of resolution to 4.4.

In summary, a simple reversed phase method can be used for quantitation of coformulated mAbs. A scouting gradient can be used to confirm differences in retention time between two samples, with further optimization including gradient program optimization and modulation of temperature. Limitations of this method include discrepancies in concentration between two mAb samples. Further optimization then would have to be made to ensure applicable linearity for the method.

LC Conditions

Column:	bioZen 2.6 µm WidePore C4, 400 Å
Dimensions:	150 x 2.1 mm
Part No.:	00F-4786-AN
Mobile Phase A:	0.1 % TFA in Water
Mobile Phase B:	0.1 % TFA in Acetonitrile
Gradient Program:	30-40% B in 5 minutes (Fig. 1-2) 40-50% B in 5 minutes (Fig. 3-4)
Flow Rate:	0.8 mL/min
Temperature:	60°C (Fig. 1-3) 80°C (Fig. 4)
Detection:	UV-Vis @ 280 nm
Injection:	1 µL
Sample:	Trastuzumab (1 mg/mL) NIST mAb (1 mg/mL)

Figure 1. Overlay of Trastuzumab and NIST mAb

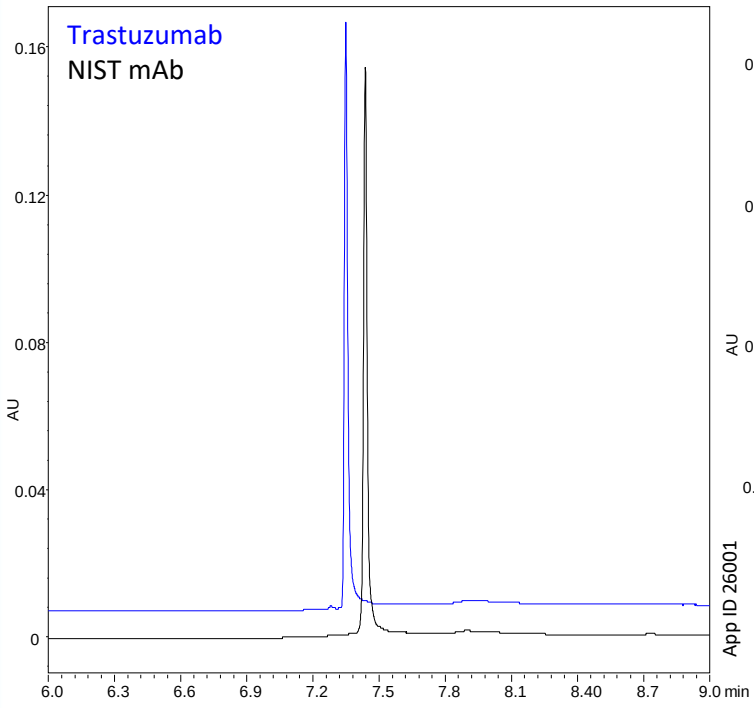


Figure 2. Trastuzumab and NIST, 30-40% B Gradient, , 60 °C

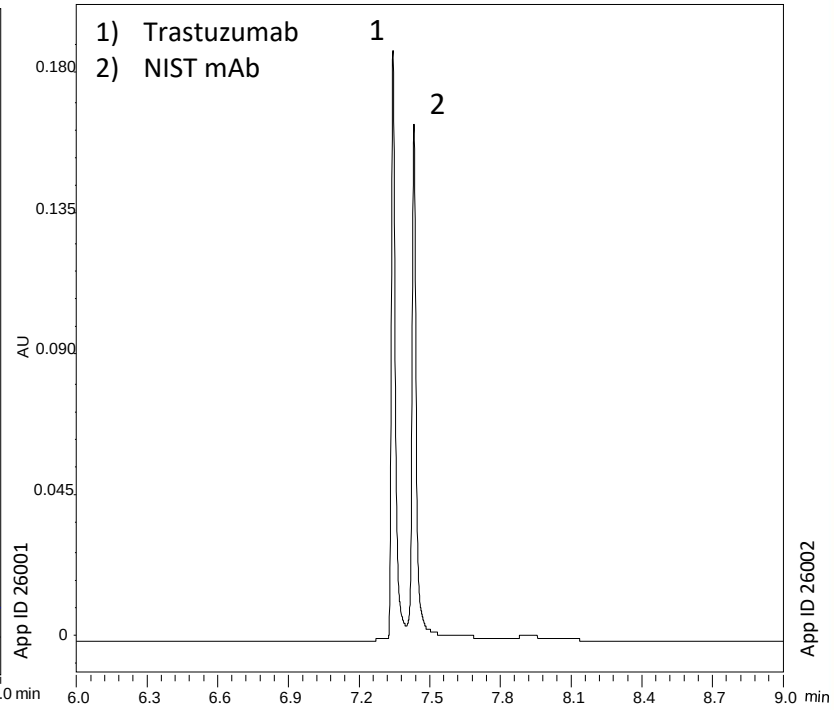


Figure 3. Trastuzumab and NIST, 40-50% B Gradient, 60 °C

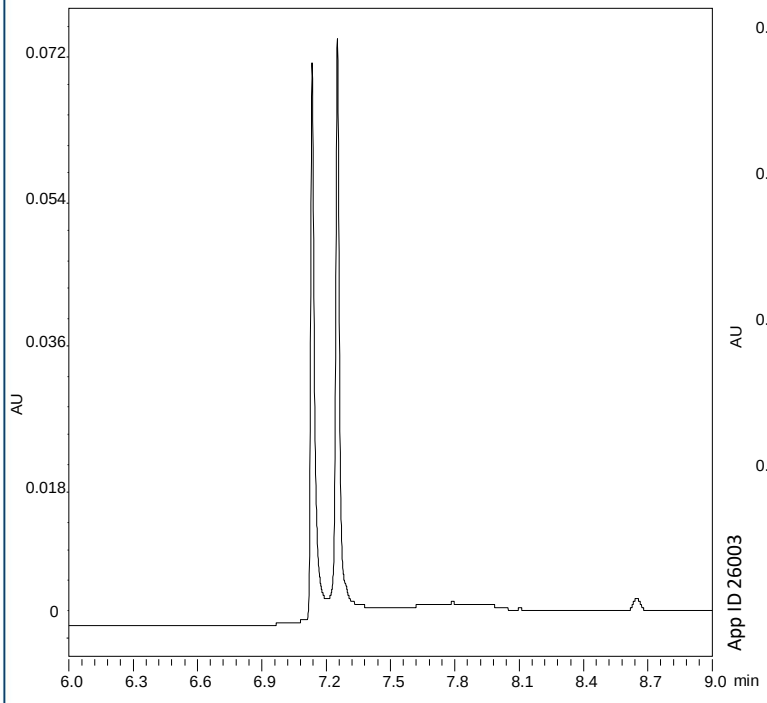
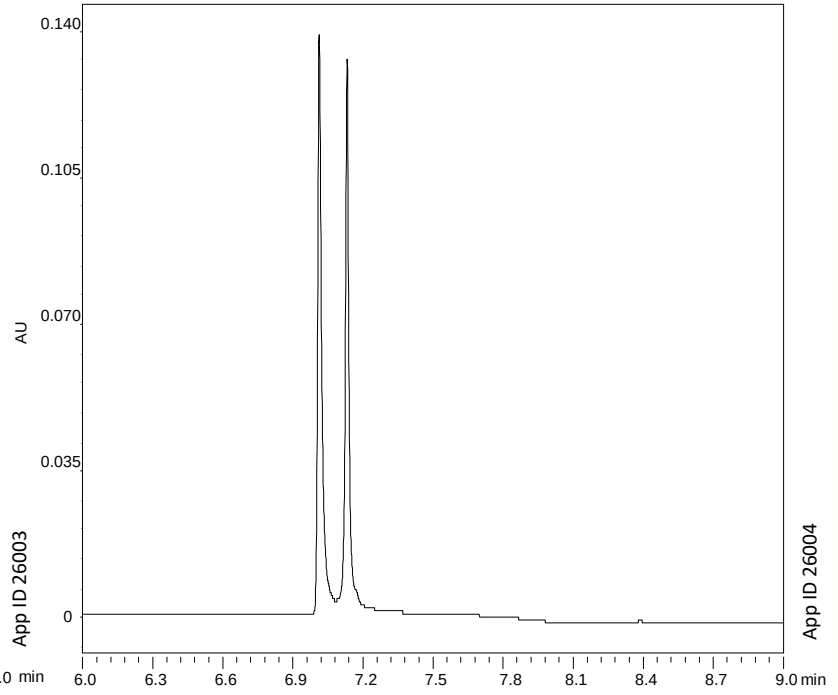


Figure 4. Trastuzumab and NIST, 40-50% B Gradient, 80 °C



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