Purity Analysis for Intact Monoclonal Antibodies and Fragments

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Overview

Monoclonal antibodies (mAbs) are well-established therapeutics, with a variety of different analytical methods associated with purity analysis. An analytical technique common for mAb purity is reversed phase LC (RPLC). This is a primary technique utilized for mAbs because of its relatively short analysis time, with high resolution, and its ability to separate hydrophobic variants such as oxidation, glycoforms, and lysine variants. As such, intact reversed phase methods can be implemented during lead selection and optimization, and on to downstream, including stability-indicating methods and quality control lot release.

One particularly appealing aspect of analysis by intact reversed phase, is the capability of implementing a "platform" method. In the context of this analysis, the platform method is capable of being used as a starting point for analytical method development for a purity method. Additionally, the method can be used as an effective screening method, if different analytes (e.g. different leads or candidates) are being analyzed. **Figure 1** shows the same method being used to analyze several different humanized and chimeric mAbs. Although each mAb varies somewhat in physicochemical properties, retention times for each mAb elute in the narrow window of organic solvent associated to the gradient slope (i.e. 30-40% acetonitrile). **Figure 2** shows purity analysis of NIST mAb, with a prominent pre-peak separated well enough for facile integration using typical parameters.

Another utility of reversed phase is to analyze mAb fragments. This technique is especially useful as the same analytical method used for intact mAb might also work for fragments. **Figure 3** highlights the separation of reduced NIST, showing mAb heavy chain and light chain purity. The sample, being somewhat degraded, shows quite a few variants, especially associated to the heavy chain, with the main peak being only 30.2% peak area.

Although the analysis of heavy and light chains is useful in that reducing antibodies is straightforward and well understood, the use of site-specific cysteine proteases is also another approach. These proteases can be particularly important if one region of the antibody (e.g. Fc or Fab) is necessary for more specific characterization or quantitation of variants.

Figure 4 shows the analysis of NIST digested with IdeS, a cysteine protease that cleaves below the hinge region of a human IgG1, yielding three fragments, a (Fab')2 fragment with both Fab arms, and two Fc fragments. This allows for characterization specifically of the Fc, which might give more insight into lysine variants, glycoforms, and oxidations that are common in the CH2 region of the mAb.

In summary, intact RPLC is a simple yet high resolution analytical technique that can be used for the purity analysis of monoclonal antibodies. The method can be used for intact protein as well as fragments generated by chemical reduction or enzymatic digestion.

LC Conditions

Column:	bioZen 2.6 µm WidePore C4, 400 Å
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:	30-40% B in 5 minutes (Fig 1-2)
Flow Rate:	0.8 mL/min
Temperature:	80°C
Detection:	UV @ 280 nm
Injection:	2 µL NIST mAb, as indicated (0.5 mg/mL)

Figure 1. Intact mAb Analysis, Chromatogram Stack

Four different monoclonal antibodies analyzed using the bioZen™ 2.6 µm WidePore C4, using the same analytical method and conditions.

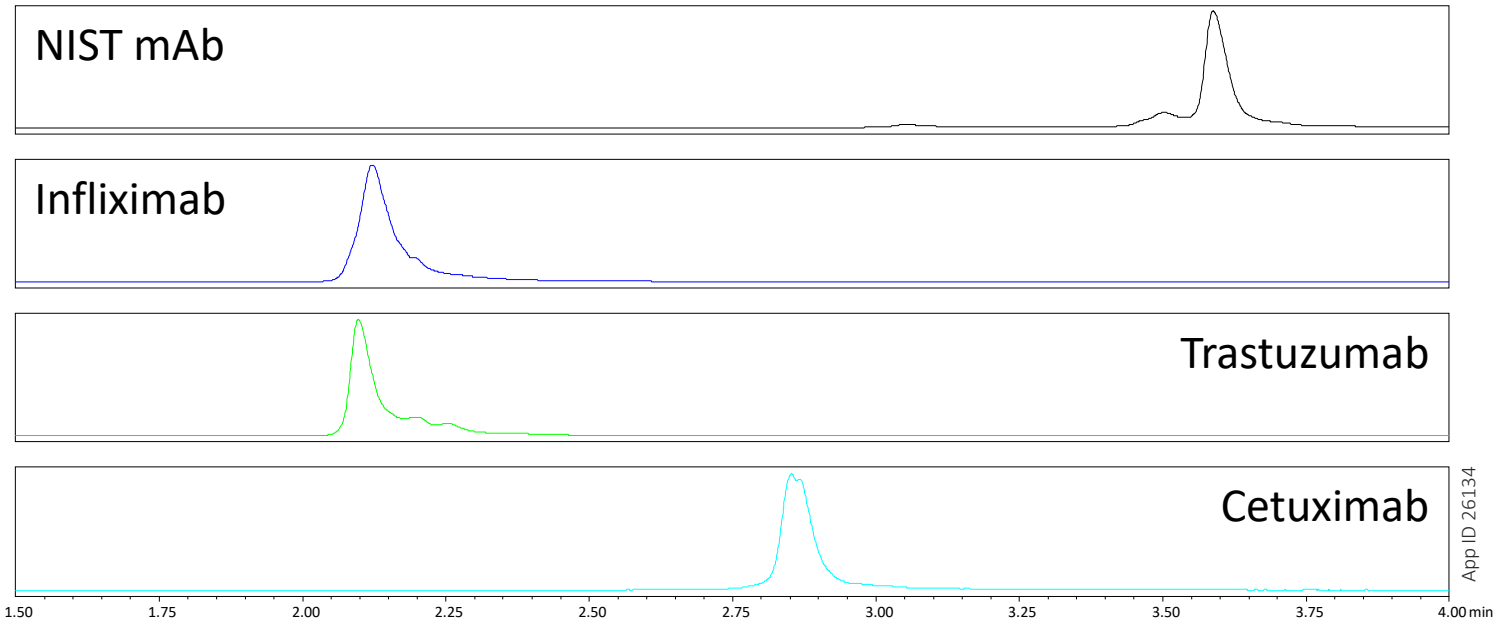


Figure 2. Purity Analysis of Intact mAb, NIST

Purity analysis of NIST mAb (RM 8671), using the reversed phase bioZen WidePore C4 platform method. Table shows relative percent areas for pre- and post-peak impurities, relative to main peak.

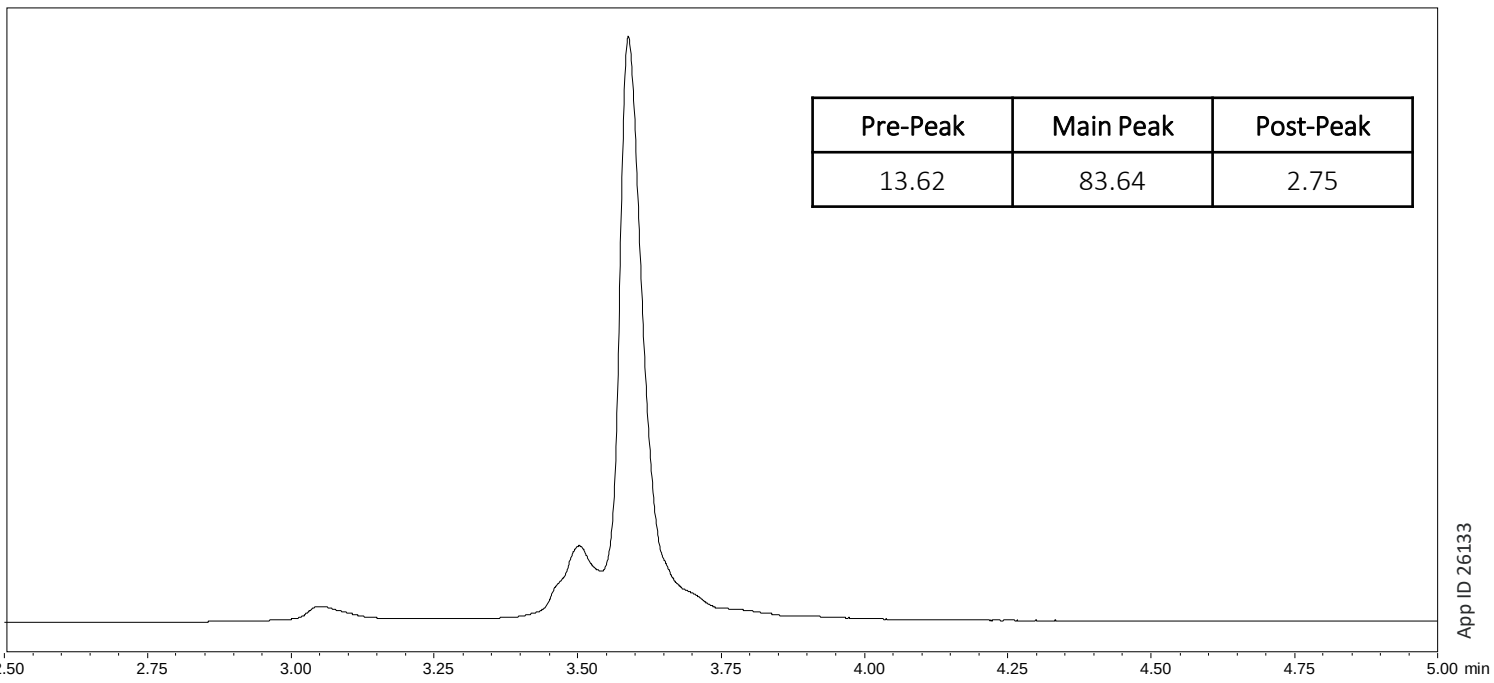


Figure 3. Purity Analysis of Heavy Chain and Light Chain, NIST mAb

Reduced NIST mAb analyzed using reversed phase bioZen™ WidePore C4 platform method. Light chain and heavy chain impurities shown as peak area relative percentage.

Fragment	Pre-Peak	Main Peak	Post-Peak
LC	0.3	74.7	25.0
HC	18.2	30.2	51.8

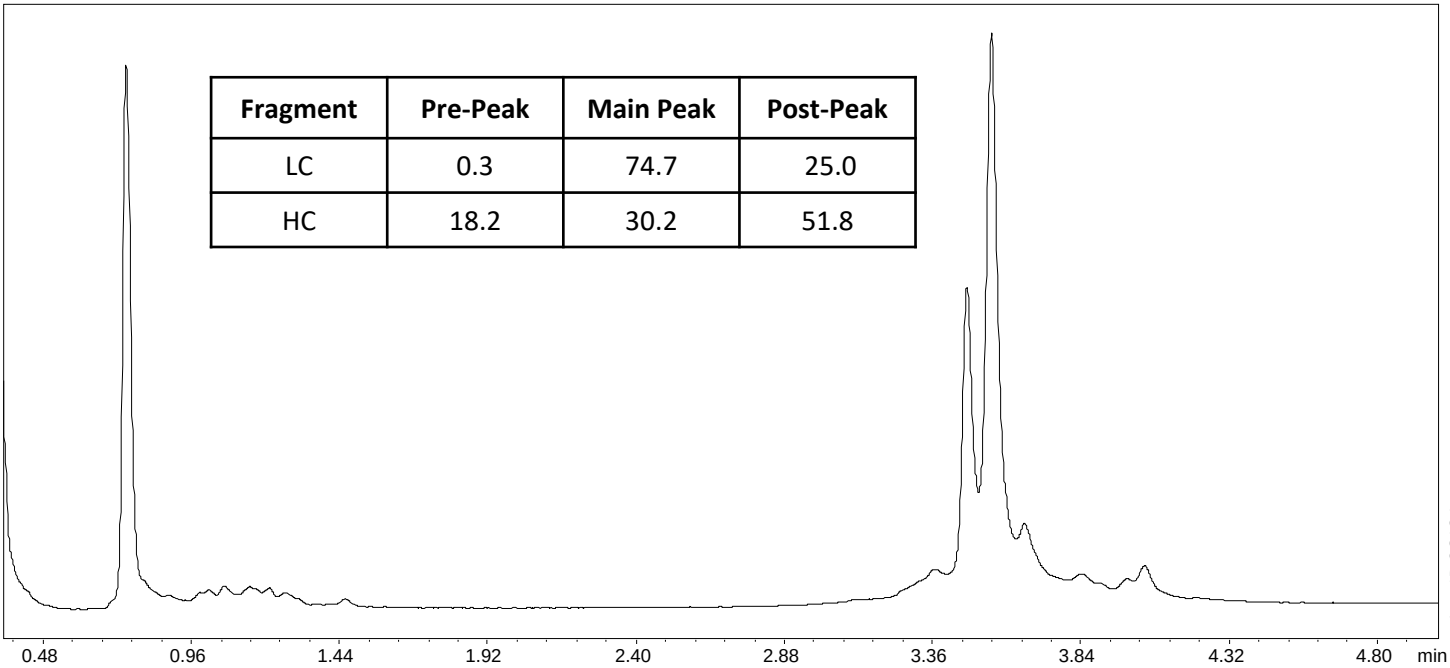
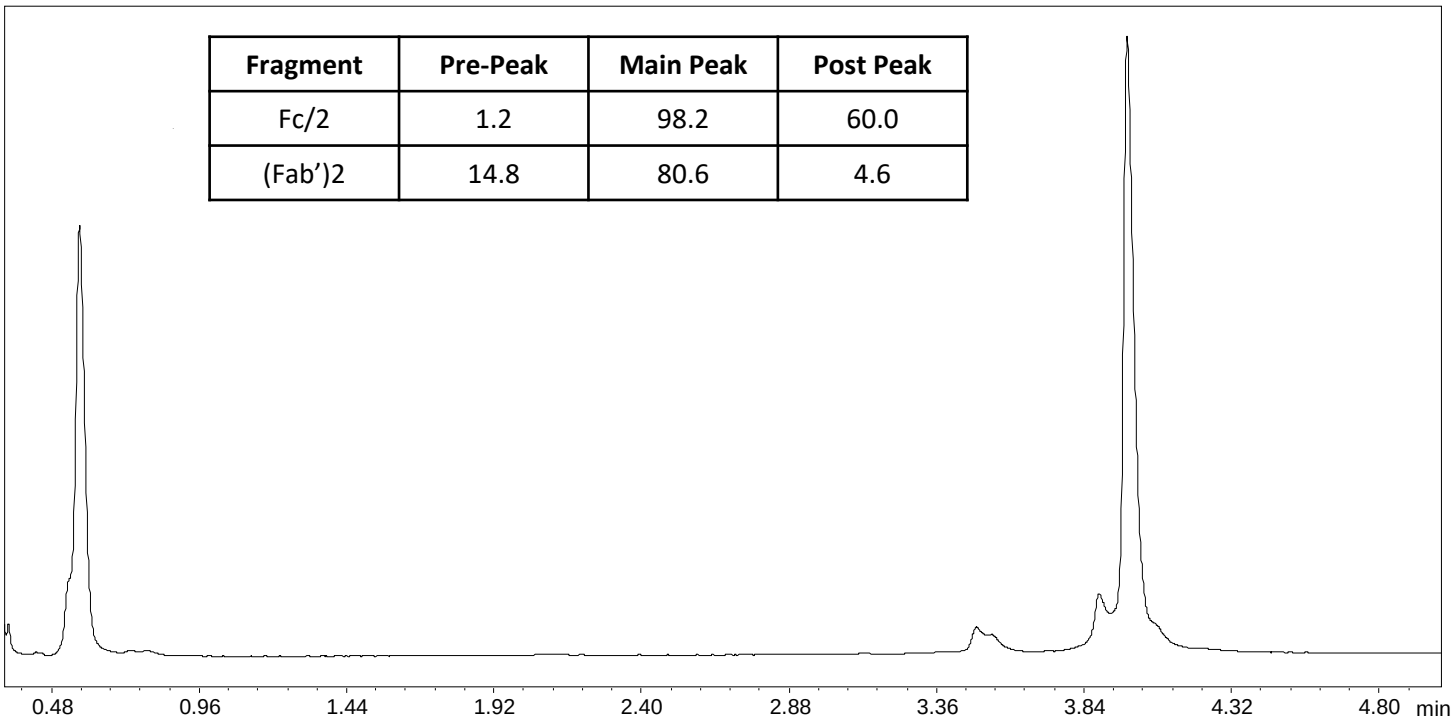


Figure 4. Purity Analysis of IdeS Generated Fragments, NIST mAb

NIST mAb fragments Fc/2, (Fab')2 analyzed using reversed phase bioZen WidePore C4 platform method. Fragment impurities shown as peak area relative percentage.

Fragment	Pre-Peak	Main Peak	Post Peak
Fc/2	1.2	98.2	60.0
(Fab')2	14.8	80.6	4.6



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