

Analytical Characterization of NIST MAb by Hydrophobic Interaction Chromatography (HIC)

Sepax Technologies, Inc.

Proteomix HIC Butyl-NP5, 5um, NP 4.6 x 100 mm
(PN:431NP5-4610)



NIST MAb on Proteomix HIC Butyl-NP5 – Gradient Optimization

HICM1010

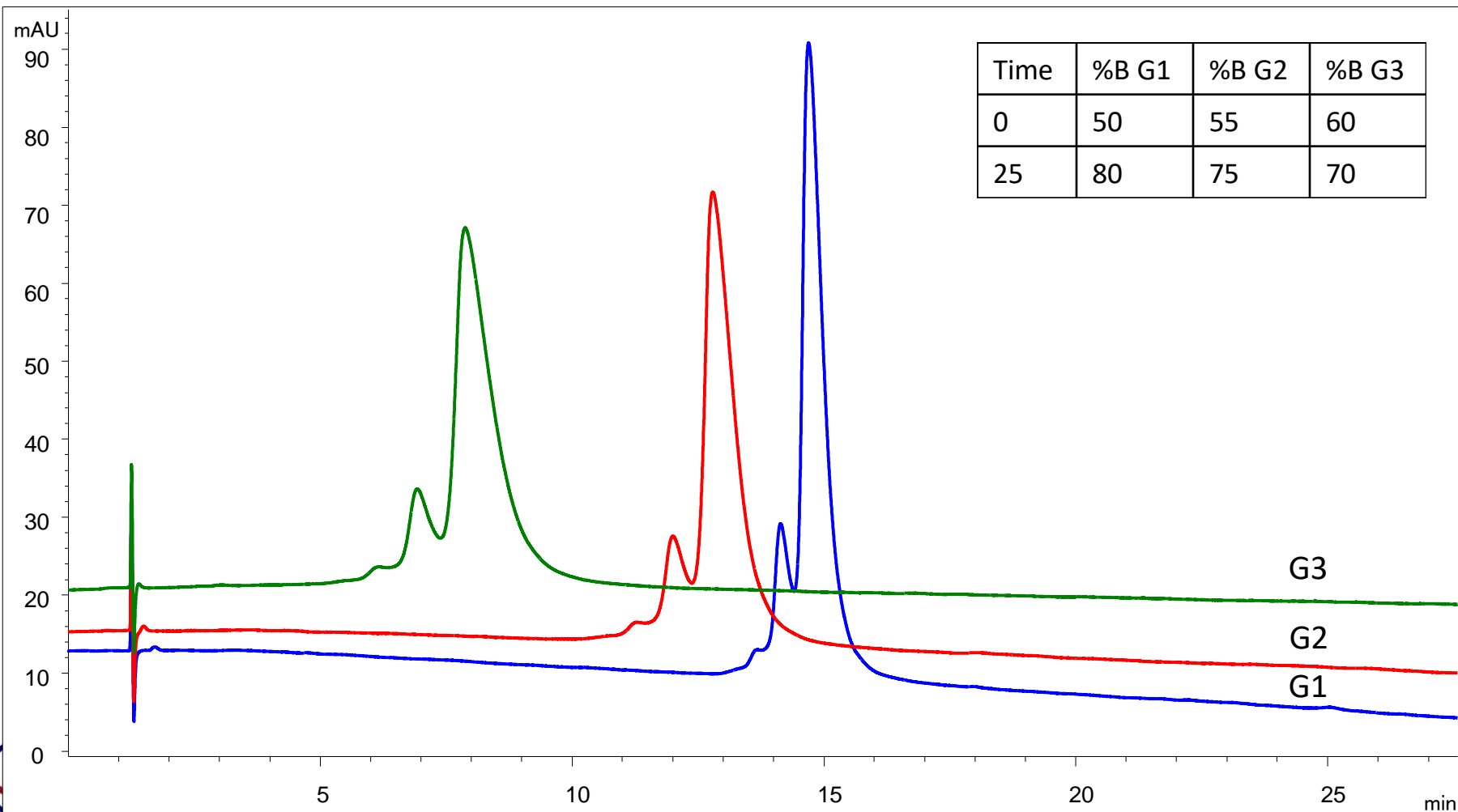
Column: Proteomix HIC Butyl-NP5, 5 μ m, NP 4.6 x 100 mm (PN:431NP5-4610)

Mobile phase: A: 2 M Ammonium Sulfate, in 100 mM Sodium Phosphate pH 7.0,

B: 100 mM Sodium Phosphate pH 7.0

Flow rate: 0.5 mL/min; Detector: UV 280 nm; Column temperature: 30°C

Sample: NIST MAb 10 mg/mL (pI 9.18, in 12.5 mM histidine, pH 6.0), Injection volume: 2 μ L



NIST MAb on Proteomix HIC Butyl-NP5 – Gradient 2 Zoom In

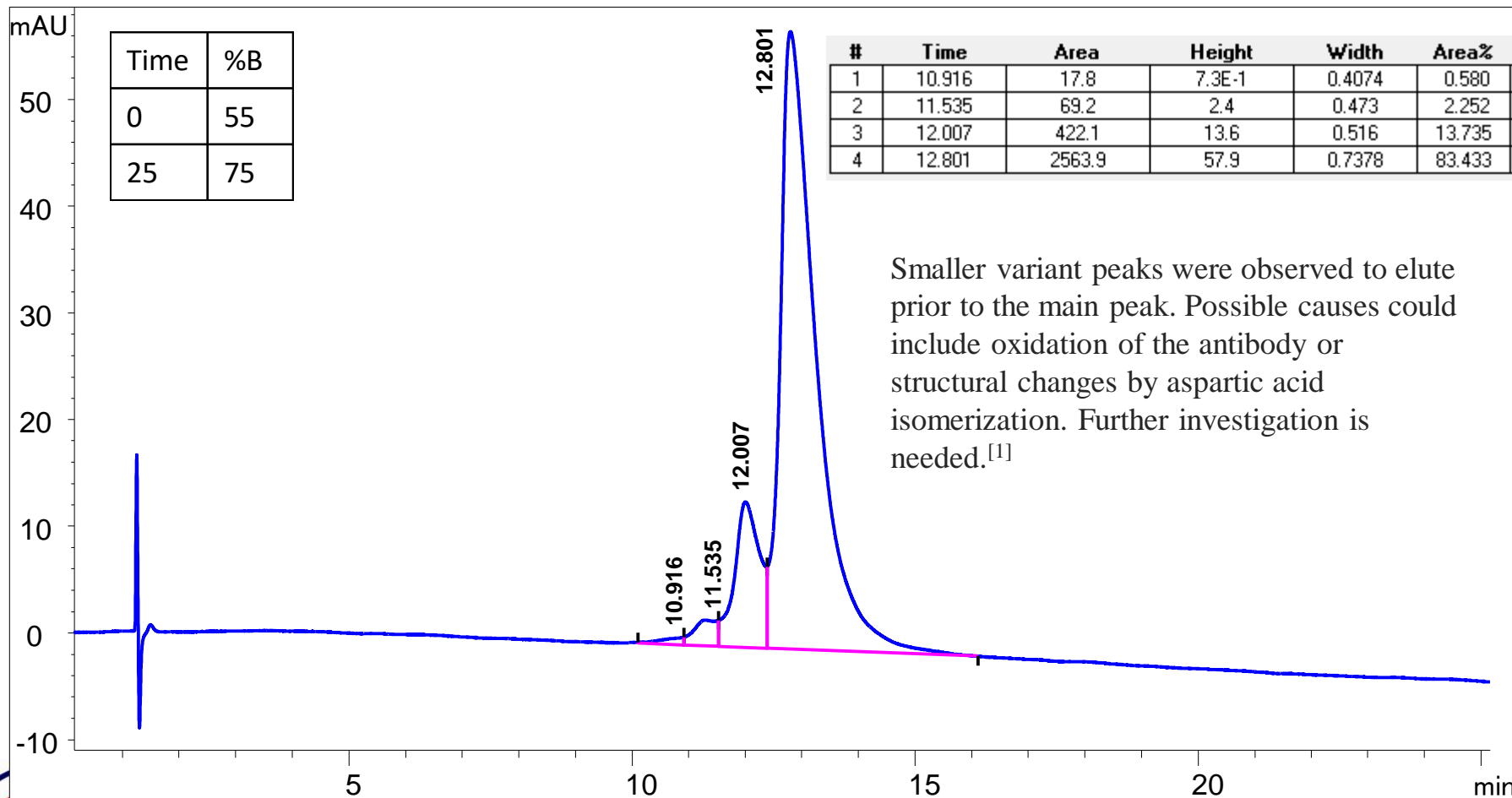
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Smaller variant peaks were observed to elute prior to the main peak. Possible causes could include oxidation of the antibody or structural changes by aspartic acid isomerization. Further investigation is needed.^[1]

^[1] Separation Methods and Orthogonal Techniques

David A. Michels, Anna Y. Ip, Thomas M. Dillon, Kurt Brorson, Scott Lute, Brittany Chavez, Ken M. Prentice, Lowell J. Brady, and Karen J. Miller
 State-of-the-Art and Emerging Technologies for Therapeutic Monoclonal Antibody Characterization Volume 2. Biopharmaceutical Characterization: The NISTmAb Case Study. January 1, 2015. 237-284
 DOI:10.1021/bk-2015-1201.ch005