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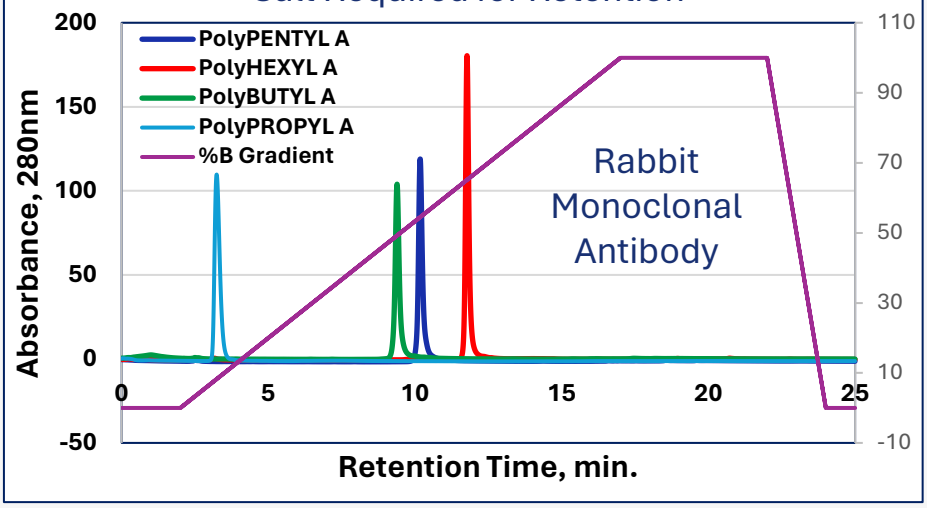
HPLC SUPPLIES FOR THE LIFE SCIENCES

HIC Application Note

Hydrophobic Interaction Chromatography (HIC) of Proteins on a New Series of HIC Ligands that Retain Samples with Less Salt for Direct Analysis via Mass Spectrometry (MS) with Native Structures Intact.

Increased HIC Ligand Size Reduces Salt Required for Retention

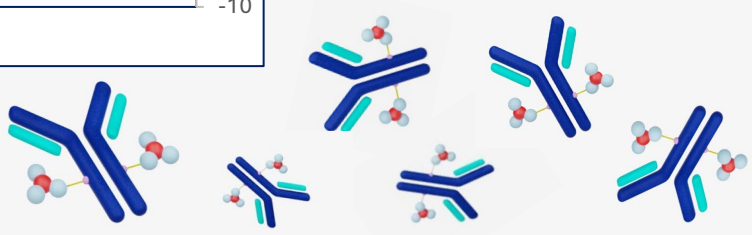


Overview

Our new series of materials for HIC permit the retention of proteins using concentrations of ammonium acetate compatible with direct analysis via MS. Proteins can be separated and eluted with their native structures intact. This is useful for top-down proteomics and permits the analysis of some proteins that are not compatible with conditions of reversed-phase chromatography. The example to the left illustrates the reduction in salt achieved by increasing the ligand size in our new materials. Here we effectively reduced the salt required for retention from 1M to 0.4M Am-OAc.

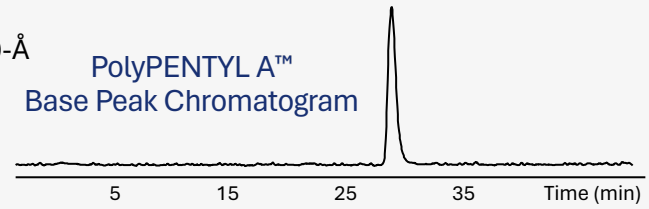
Parameters:

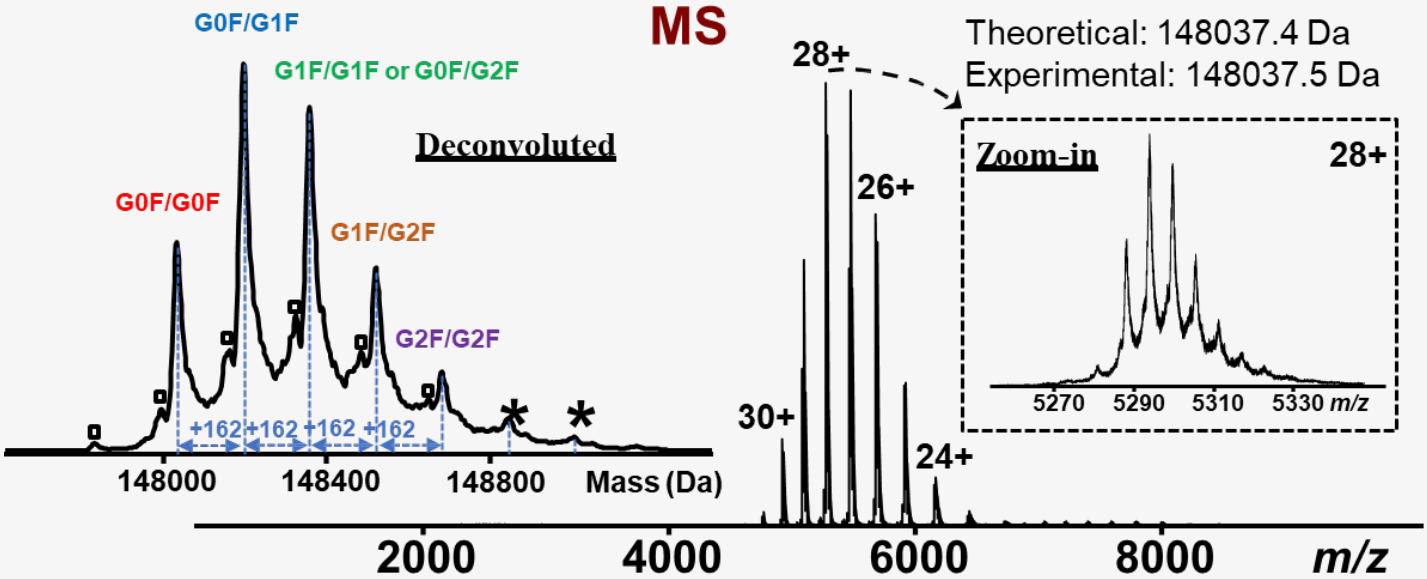
Buffer A: 1.0M Ammonium Acetate
Buffer B: 25mM Ammonium Acetate, 50% ACN
Gradient: 0-100%B, 15min



HIC-MS of Antibodies

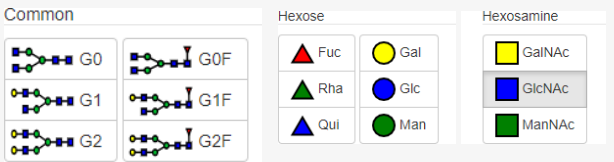
Sample: NIST mAb standard 8671 (IgG1κ)
Column: PolyPENTYL A™ capillary, 100x0.2-mm, 2-µm, 1000-Å
Mobile Phase: Decreasing gradient of NH4-Oac
Flow rate: 2.6 µl/min
MS: maXis II QTOF (Bruker)





The composition of this antibody's glycoform variants is consistent with the literature: M. Hilliard *et al.*, *mAbs 9* (2017) 1349.

The mass spectrum is that of a protein with native structure.



-203 Da (-GlcNAc)
* +162 Da

(data courtesy of Bifan Chen, Ziqing Lin, & Ying Ge, Univ. Wisconsin-Madison)