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Analysis of monoclonal antibodies and antibody-drug-conjugates using new hydrophobic interaction chromatography (HIC) columns

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Various types of Monoclonal Antibody (mAb) products including intact mAbs, mAb fragments, engineered variants, and Antibody-Drug Conjugates (ADCs) are being developed for the treatment of cancer and other diseases due to their excellent biocompatibility and high selectivity. The proliferation of monoclonal antibody therapeutics and their susceptibility to various biochemical modifications has highlighted the importance of characterizing these highly heterogeneous products for their safety and efficacy. Hydrophobic Interaction Chromatography (HIC) is a technique for separation of proteins and has been widely used as an orthogonal method to size exclusion chromatography and ion exchange chromatography for the characterization of mAb variants. MAbs, mAb fragments and antibody-drug conjugate samples were diluted with mobile phase A and injected on to MAbPac HIC columns. MAb fragment was prepared by papain digestion and oxidized mAb was prepared by adding hydrogen peroxide or 2,2'-azobis (2-amidinopropane) dihydrochloride (AAPH). Antibody-drug conjugate mimic sample was prepared by reduction of disulfide bonds followed by addition of thiol-reactive drug mimics. Here, we introduce a new family of HIC columns designed for mAb analysis. Three different ligand chemistries-polyamide, amide and butyl-were developed for the analysis of a wide range of mAb samples. MAb aggregates, mAb fragment, oxidized mAbs, and antibody-drug conjugates were investigated using these new HIC columns. Using the polyamide column, mAb aggregates as well as hydrophilic variants of the mAb were separated. For the mAb fragment analysis, two mAb samples were digested with papain and were separated with all three HIC columns. The amide column gave the best separation of Fab and Fc fragments of both mAbs. For oxidized mAb variant analysis, a mAb sample was oxidized using hydrogen peroxide and was injected without further processing. The amide column was able to detect two oxidation variants. For the final application, a cysteine-linked antibody-drug conjugate mimic sample was analyzed. The novel butyl column baseline separated all the drug-to-antibody ratio (DAR) species with a 20 minute gradient. Together, these results demonstrate the complementary selectivity of these columns which is critical for the characterization of various types of mAb products including ADCs.

Biography

Robert van Ling has been working in the field of protein characterization and proteomics. Currently acting in his role as European Support Expert, he focuses on the chromatographic workflows and chemistries for mAb and protein therapeutics, such aggregation studies, charge variant and oxidation monitoring.

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