

# Comparative Elucidation of Cetuximab Heterogeneity on the Intact Protein Level by Cation Exchange Chromatography and Capillary Electrophoresis Coupled to Mass Spectrometry

Fussl F, Trappe A, Carillo S, Jakes C, & Bones J.

*Repligen Corp. now owns the life sciences PAT product portfolio of 908 Devices Inc. Please contact Repligen for further inquiries.*

*Anal Chem*, In-depth analysis of monoclonal antibodies using microfluidic capillary electrophoresis and native mass spectrometry.

DOI: 10.1021/acs.analchem.0c00185.

## Contact

Repligen Corporation  
685 Route 202/206  
Bridgewater, NJ, USA 08807  
analytics-support@repligen.com  
(908) 707-1009

Technical Note

## Abstract

Charge sensitive separation methods such as ion exchange chromatography (CEX) and capillary electrophoresis (CE) have recently been coupled to mass spectrometry to facilitate high resolution profiling of proteoforms present within the charge variant profile of complex biopharmaceuticals. Here we apply pH gradient cation exchange chromatography and microfluidic capillary electrophoresis using the PATsmart™ ZipChip® platform for comparative characterization of the monoclonal antibody cetuximab. Cetuximab harbors 4 glycans per molecule, two on each heavy chain, of which the Fab glycans have been reported to be complex and multiply sialylated. The presence of these extra glycosylation sites in the variable region of the molecules causes significant charge variant and glycan heterogeneity, which makes comprehensive analysis on the intact protein level challenging. Both pH gradient CEX-MS and CE-MS were found to be powerful for the separation of cetuximab charge variants with eight major peaks being base-line resolved using both separation platforms. Informative native mass spectra were collected for each charge variant peak using both platforms that facilitated deconvolution and further analysis. The total proteoform coverage was exceptionally high with >100 isoforms identified and relatively quantified with CEX-MS. In the case of CE-MS the proteoform coverage was >200. A deep insight into the heterogeneity of cetuximab was unveiled, the high levels of sensitivity achievable enable the implementation of the presented technologies even at early stages of the biopharmaceutical development platform, such as developability assessment, process development, and monitoring process consistency.

[Click here](#) to view the entire article.