SUMMARY

New methods for strong cation exchange (SCX) ultra-performance liquid chromatography (UPLC) have allowed effective resolution and characterization of charge variants throughout the ADC manufacturing process.

Retention time reproducibility after SCX UPLC column equilibration enabled monitoring of important changes in product quality.

SCX UPLC-MS performed with novel MS friendly mobile phases allowed for the confirmation of linker position, number of attachments, and glycoform heterogeneity. The method also provided high mass accuracy MS information for the verification of linker/drug incorporation.

INTRODUCTION

Charge variants have gained considerable attention, especially due to their potential effect on biological activity and stability of mAbs. Modification of isoelectric values in manufacturing arises due to enzymatic or degradation processes, such as deamidation, oxidation, isomerization, and fragmentation. Heterogeneity and the dynamic nature of mAbs demand monitoring by various analytical approaches. Cation exchange chromatography, coupled to UV and mass spectrometry, is quickly becoming the method of choice in characterization of charge variants.

Typical cation exchange separations require high concentrations of salts, the gradients are based either on varying the concentration of salts or pH; traditional recipes are not compatible with MS detection. Recently, volatile salts have been successfully used for MS based characterization of charge variants.

In this study, we performed strong cation exchange (SCX) ultra-performance liquid chromatography (UPLC) coupled to UV with a non-volatile salt/pH gradient as well as LC-UV-MS using volatile MS compatible salts. In depth analyses were performed on AJICAP™ mAbs and ADC charge variants.

METHODS

SCX mAb Column Specifications

Strong cation exchange, 3 µm non-porous particle, sulfonic acid functional group ligand, counter ion Na+, pH range 3 -11, max pressure 10,000 psi (74.5 kPa), minimum salt concentration 15 mM, maximum organic concentration 50%, column temperature 10 - 60°C

ANALYTICAL RESULTS

SCX UPLC-UV Experiment 1

Samples: Waters NIST Charge Variant Standard, Naked (Trastuzumab) Antibody, AJICAP™ AJ2 ADC

Strong cation exchange Ultra-Performance Liquid Chromatography Coupled to UV and MS Detectors Allows Robust and Reproducible Characterization of mAb and ADC Charge Variants

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