



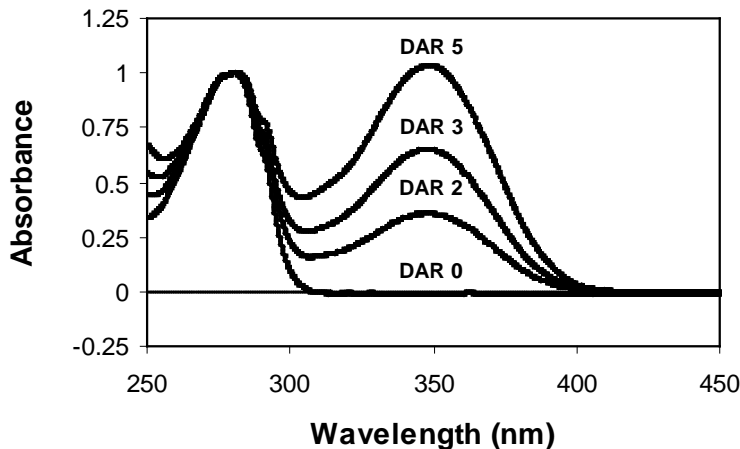
Analytical Characterization of Antibody-Drug Conjugates

Summary

Antibody-drug conjugates (ADCs) represent a rapidly growing class of therapeutics undergoing clinical development. Wolfe Laboratories provides expert analytical characterization, which plays an important role during discovery, process scale-up, manufacturing, and release/stability testing of ADCs. Selection of appropriate analytical techniques depends on the properties of the ADC linker, the drug and the choice of attachment site.

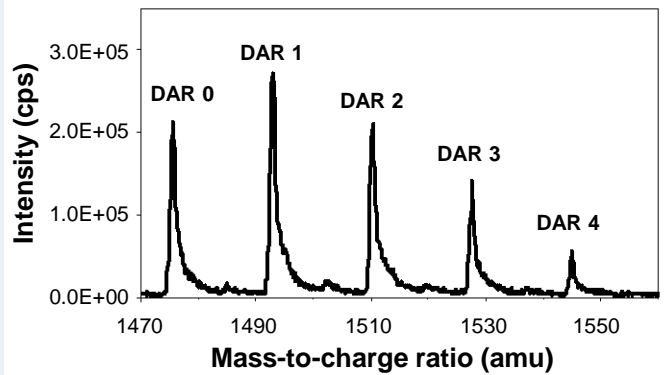
Because ADCs are highly heterogeneous, Wolfe Laboratories employs multiple orthogonal methods to fully characterize them. The below figures illustrate how Wolfe utilized UV/VIS spectroscopy, size exclusion (SE)-UPLC, dynamic light scattering, and mass spectrometry to characterize the average drug-to-antibody ratio (DAR), percent free drug, aggregation state, and DAR heterogeneity of an immunoconjugate system.

Average DAR by UV/VIS Spectroscopy



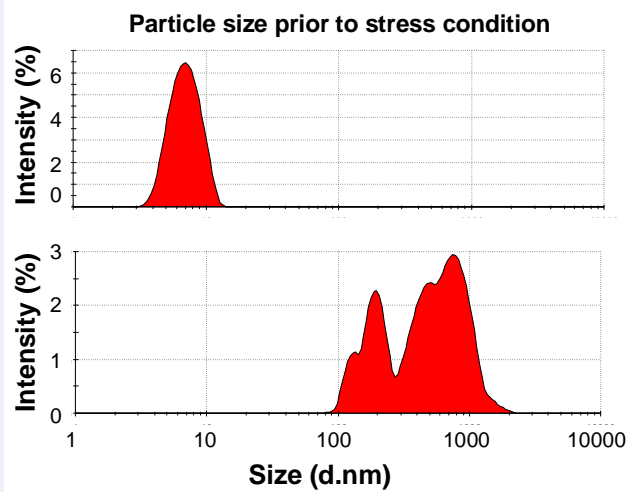
Average drug-to-antibody ratios (DARs) determined by direct measurement of protein absorbance at 280 nm and drug absorbance at 346 nm.

DAR Distribution by Mass Spectroscopy



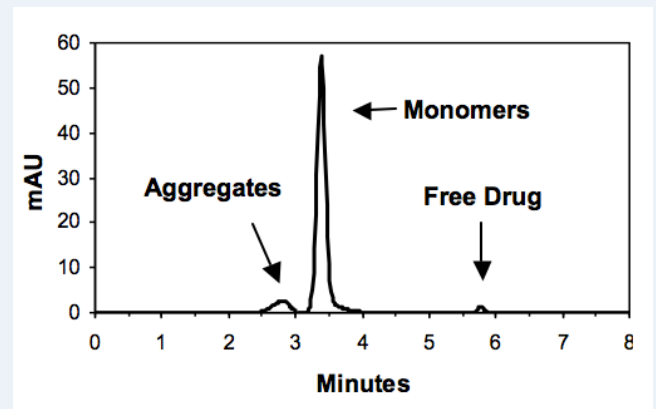
Mass spectroscopic analysis of individual DAR species comprising an immunoconjugate with an average DAR of ~ 2.

Aggregation Pattern assessed by Dynamic Light Scattering



Detection of irreversible aggregation of immunoconjugates by dynamic light scattering during stress testing.

Percent Free Drug and Aggregation State by SE-UPLC



Aggregation state and average DAR determined by size exclusion (SE)-UPLC with UV detection at 346 nm.