Tips from our Protein Separation ZenMasters

Drug-Antibody Ratio (DAR)

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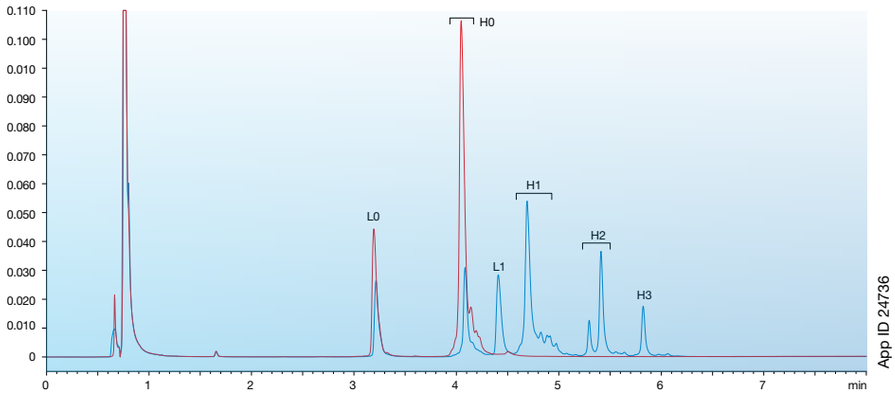
Why do average DAR for cysteine conjugates use Reversed Phase HPLC?

Average DAR is a critical quality attribute for Antibody-Drug Conjugate (ADC), as it gives indications of toxicity, efficacy, and pharmacokinetics.[1] As such, DAR is determined very early, even during early development research.

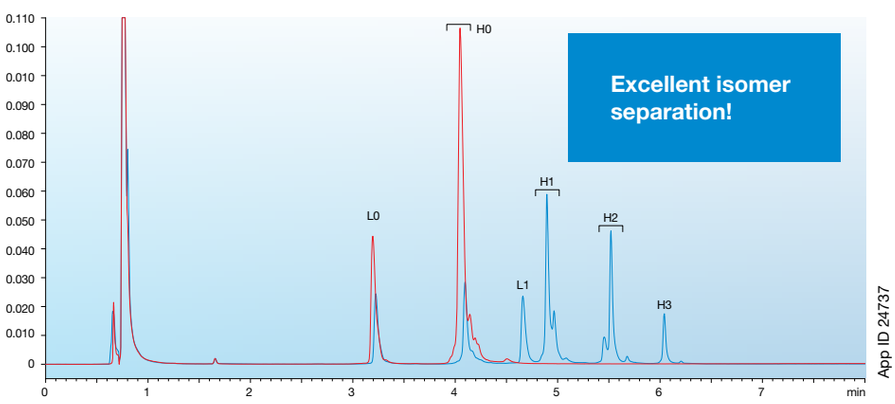
Although Hydrophobic Interaction Chromatography (HIC) might give both average DAR and drug distribution, this approach requires rather extensive mobile phase optimization and method development, and might be more appropriate further downstream of drug development. Another option to assess DAR for cysteine conjugates is Native MS. Although this approach gives assessment of drug distribution and DAR, it requires a high-resolution instrument such as a Time-of-Flight (TOF)-MS, not to mention expertise in large molecule MS.

Given the above challenges, reversed phase HPLC is a preferred route for average DAR. This approach can be done with cysteine conjugates simply by fully reducing the protein and summing together the total peak areas for conjugated heavy and light chains, as indicated below. With optimized conditions, there is also partial separation of different positional isomers, though again, drug distribution would need to be assessed with an orthogonal method.

Average DAR (Trastuzumab mc-PAB-MMAF Cysteine Conjugate)



Average DAR (Trastuzumab vc-PAB-MMAE Cysteine Conjugate)



1. Peters, C.; Brown, S. “Antibody-Drug conjugates as novel anti-Cancer chemotherapeutics.” Bioscience Reports, vol. 35, no. 4, Dec. 2015, doi:10.1042/bsr20150089.