Tips from our Protein Separation ZenMasters

Drug-Antibody Ratio (DAR)

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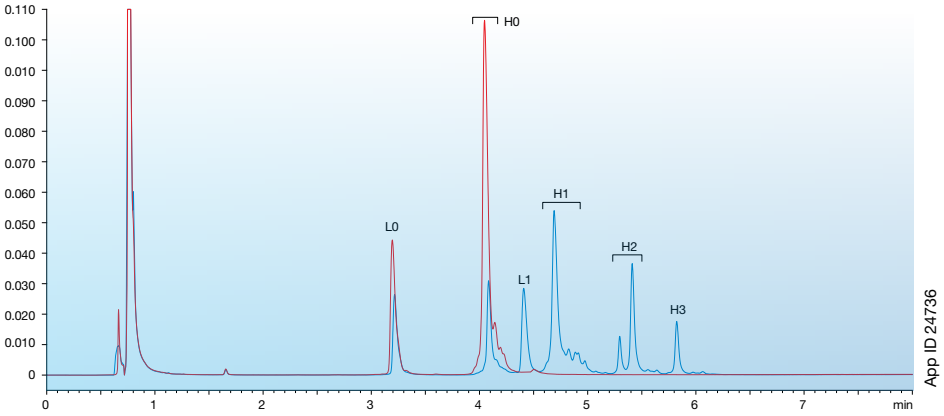
Why do average DAR for cysteine conjugates use Reversed Phase HPLC?

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.

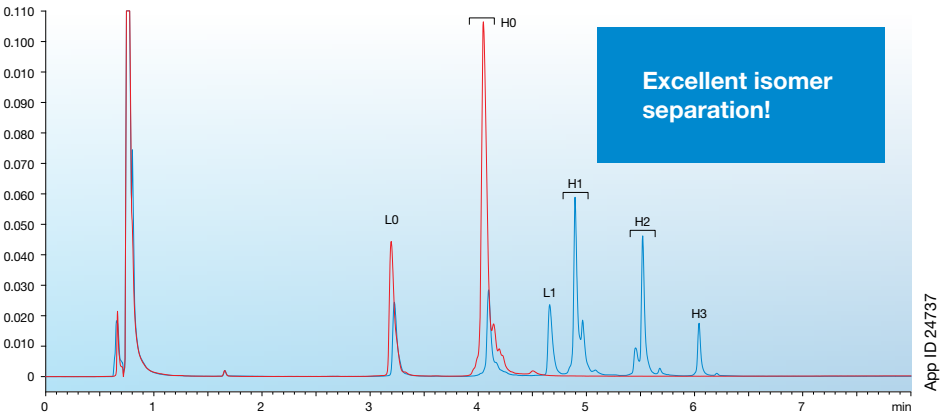
Although Hydrophobic Interaction Chromatography (HIC) might give both average DAR and drug distribution, this approach requires rather extensive mobile phase optimization and method development, and might be more appropriate further downstream of drug development. Another option to assess DAR for cysteine 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.

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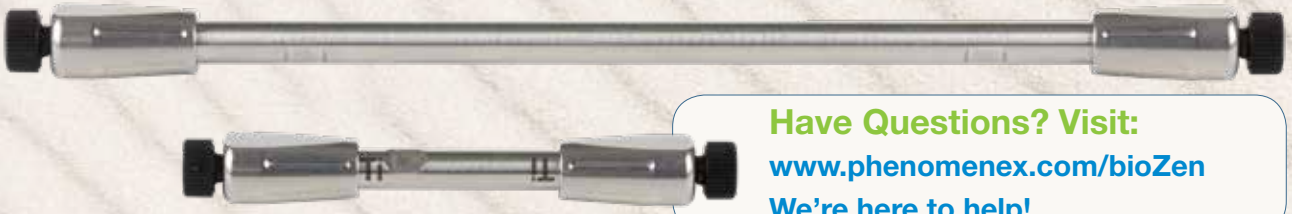
Product Ordering Information

bioZen™ Products - Powered by Biocompatible Hardware

bioZen Columns (mm)								Biocompatible Guard Cartridges*	
Phases	**50 x 2.1**	**100 x 2.1**	**150 x 2.1**	**250 x 2.1**	**50 x 4.6**	**150 x 4.6**	**300 x 4.6**	**for 2.1 mm**	**for 4.6 mm**
bioZen 2.6 µm Glycan	—	00D-4773-AN	00F-4773-AN	—	—	—	—	AJ0-9800	—
bioZen 1.6 µm Peptide PS-C18	00B-4770-AN	—	00F-4770-AN	—	—	—	—	AJ0-9803	—
bioZen 3 µm Peptide PS-C18	—	—	—	—	00B-4771-E0	00F-4771-E0	—	—	AJ0-7606
bioZen 1.7 µm Peptide XB-C18	00B-4774-AN	—	00F-4774-AN	—	—	—	—	AJ0-9806	—
bioZen 2.6 µm Peptide XB-C18	00B-4768-AN	—	00F-4768-AN	00G-4768-AN	00B-4768-E0	00F-4768-E0	—	AJ0-9806	AJ0-9808
bioZen 3.6 µm Intact C4	00B-4767-AN	—	00F-4767-AN	—	00B-4767-E0	00F-4767-E0	—	AJ0-9809	AJ0-9811
bioZen 3.6 µm Intact XB-C8	00B-4766-AN	—	00F-4766-AN	—	00B-4766-E0	00F-4766-E0	—	AJ0-9812	AJ0-9814
bioZen 1.8 µm SEC-2	—	—	—	—	—	00F-4769-E0	00H-4769-E0	—	AJ0-9850
bioZen 1.8 µm SEC-3	—	—	—	—	—	00F-4772-E0	00H-4772-E0	—	AJ0-9851

*AJ0-7606 requires guard holder KJ0-4282. All other guard cartridges require guard holder AJ0-9000.



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guarantee

If bioZen columns in this technical note do not provide at least equivalent separations as compared to a competing column of the same phase, particle size, and dimensions, return the column with the comparative data within 45 days for a FULL REFUND.

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