

TSKgel® HIC-ADC Butyl Columns

Designed for Antibody-Drug Conjugate Analysis

The name says it all: the TSKgel HIC-ADC Butyl column is a hydrophobic interaction chromatography (HIC) column designed specifically for Antibody-Drug Conjugates (ADCs). Tosoh has developed a new particle construction to achieve exceptional separation of variably conjugated ADC variants. This next-generation breakthrough in non-porous particle technology makes DAR analysis more reliable and improves separation efficiency. The TSKgel HIC-ADC Butyl column sets a new industry standard, ensuring exceptional performance and robustness.

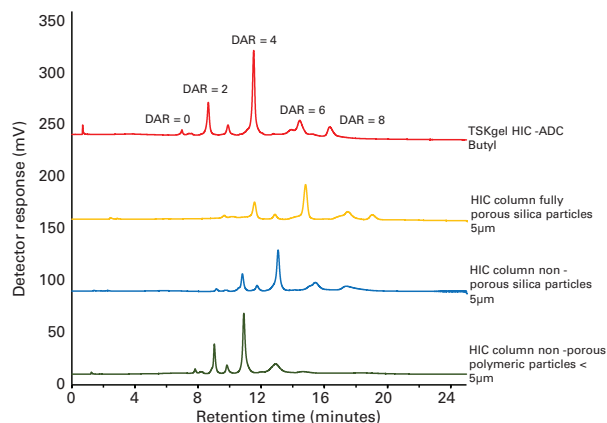
Beyond technical performance, these columns simplify method development and tech-transfer processes for DAR analyses. In addition, the TSKgel HIC-ADC Butyl columns enhance both the accuracy and efficiency of Quality Control processes and enable the highest quality standards in ADC product analysis.

Performance of the TSKgel HIC-ADC Butyl columns

Achieve unmatched precision in DAR quantification for ADCs

The most critical aspect of quantifying DAR in ADCs is maximizing resolution among payload variants. Individual DAR peak areas can then be used to calculate the average DAR. When benchmarked against competitor HIC columns, the TSKgel HIC-ADC Butyl column showed superior separation of conjugated DAR isoforms (*Figure 1*).

Figure 1. TSKgel HIC-ADC Butyl performance measured against competitor HIC columns for ADCs.



Column: a) TSKgel HIC-ADC Butyl (4.6 mm ID × 10 cm)
 b) Commercial HIC column A (4.6 mm ID × 10 cm)
 c) Commercial HIC column B (4.6 mm ID × 10 cm)
 d) Commercial HIC column C (4.6 mm ID × 10 cm)
 Buffer A: 0.05 mol/L sodium phosphate buffer + 1.2 mol/L ammonium sulfate (pH 7.0)
 Buffer B: 0.05 mol/L sodium phosphate buffer (pH 7.0) / 2-propanol = 80 / 20
 Gradient: 0 – 100% B (0 – 15 min., linear)
 Flow rate: 0.5 mL/min
 Detection: UV @ 215 nm
 Temperature: 25 °C
 Injection vol.: 5 µL
 Sample: SigmaMAb™ Antibody-Drug Conjugate (ADC) Mimic

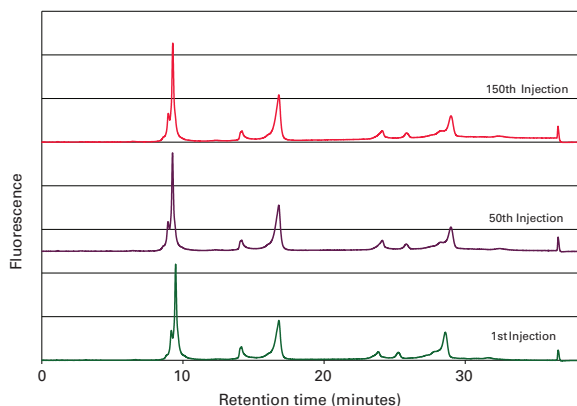
Short profile

| | |
|-------------------------|---|
| ➤ Separation mechanism: | Hydrophobic interaction chromatography |
| ➤ Base material: | Butyl-functionalized polymethacrylate |
| ➤ Particle size: | 5 µm |
| ➤ Pore size: | Non-porous |
| ➤ Dimensions: | 4.6 mm ID × 3.5 cm L and 4.6 mm ID × 10 cm L, guard column available (4.6 mm ID × 5 mm L) |
| ➤ Applications: | ADC analysis, Drug-antibody ratio analysis, intact protein separations |

Optimize efficiency with robust and reproducible results

TSKgel HIC-ADC-Butyl columns have been engineered to enhance robustness and reproducibility. They consistently deliver reproducible retention times with 150 injections of Adcetris®, a widely-used ADC therapeutic (Figure 2). Consequently, TSKgel HIC-ADC-Butyl columns ensure reduced downtime and reliable performance, making them the columns of choice for any ADC DAR analytical challenge.

➤ **Figure 2.** Column longevity for HIC analyses on TSKgel HIC-ADC-Butyl

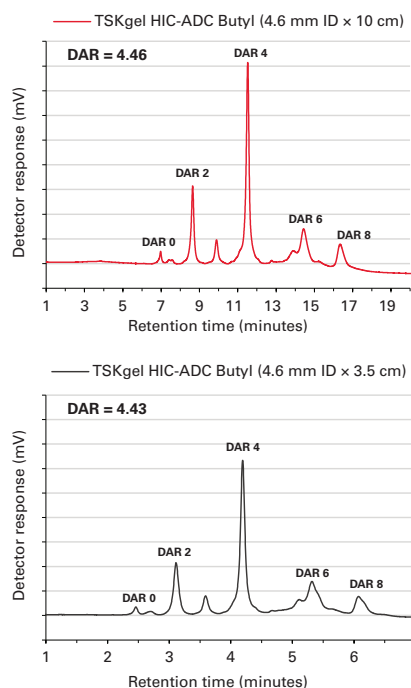


Column: TSKgel HIC-ADC Butyl (4.6 mm ID x 10 cm)
 Buffer A: 0.1 mol/L sodium phosphate buffer + 1.5 mol/L ammonium sulfate (pH 7.0) + 5 % IPA
 Buffer B: 0.1 mol/L sodium phosphate buffer (pH 7.0)/ +20 % IPA
 Gradient: 0 – 100% B , linear 0 - 30 min
 Flow rate: 0.5 mL/min
 Detection: FLD Ex: 280 nm, Em 348 nm
 Temperature: 25 °C
 Injection vol.: 10 µL
 Sample: ADC Adcetris

Increase throughput for DAR analysis

With the TSKgel HIC-ADC Butyl 3.5 cm column, DAR determinations can be reduced to a 7-minute run time while preserving relative resolution analyzed with its 10cm equivalent (Figure 3). Now run times can be accelerated without compromising on accuracy or resolution, ensuring faster results without sacrificing quality.

➤ **Figure 3.** Comparison of ADC run times between the TSKgel HIC-ADC Butyl 10cm column (top) and the 3.5cm column (bottom).



Separation conditions: identical to Figure 1 except flow rate. Top: 0.8 mL/min. Bottom: 2 mL/min.

Ordering Information

| Part # | Description | Particle size | Dimensions |
|---------|-----------------------------------|---------------|----------------------|
| 0023538 | TSKgel HIC-ADC Butyl | 5 µm | 4.6 mm ID x 3.5 cm L |
| 0023539 | TSKgel HIC-ADC Butyl | 5 µm | 4.6 mm ID x 10 cm L |
| 0023543 | TSKgel guard column HIC-ADC Butyl | 5 µm | 4.6 mm ID x 0.5 cm L |

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