

# TSKgel® HIC-ADC Series

Columns Designed for Antibody-Drug Conjugates

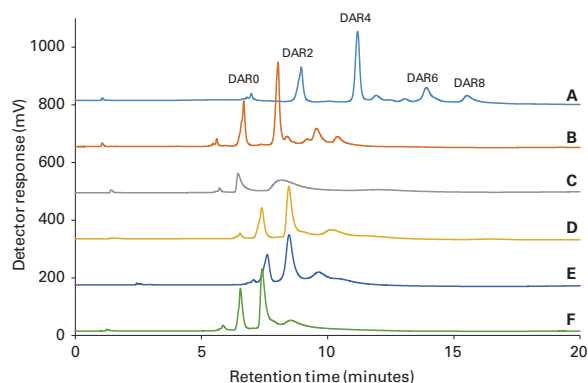
The name says it all: TSKgel HIC-ADC columns are Hydrophobic Interaction Chromatography columns designed for the analysis of antibody-drug conjugates (ADCs). Tosoh has constructed a new particle for HIC separations that achieves exceptional resolution among variably conjugated ADC variants. This new industry standard in non-porous particle technology ensures exceptional performance, efficiency, and robustness in the DAR analysis.

An expanding TSKgel HIC-ADC toolbox now offers broadened selectivity for ADCs built using novel therapies and conjugation techniques. The TSKgel HIC-ADC series simplifies method development, validation, and tech-transfer for DAR quantitation by enhancing DAR variant separation setting a new bar for ADC analysis.

## Achieve unmatched performance in DAR quantification for ADCs

The most important factor in determining the Drug-Antibody-Ratio (DAR) in ADCs is maximum resolution among different payload variants. Individual DAR peak areas can then be used to calculate the average DAR. While both TSKgel HIC-ADC columns yield suitable separation profiles for DAR quantitation, the TSKgel HIC-ADC Phenyl column excels for conjugates rich in heterocyclic content. When benchmarked against other commercially available HIC columns, the TSKgel HIC-ADC columns demonstrate superior selectivity for the separation of Phospholipase-C conjugated antibody isoforms (Figure 1).

Figure 1. Drug-to-Antibody Variant Separation Profiles of Various HIC Stationary Phases.



Columns: A. TSKgel HIC-ADC Phenyl (4.6 mm ID x 10 cm)  
 B. TSKgel HIC-ADC Butyl (4.6 mm ID x 10 cm)  
 C. TSKgel Butyl-NPR (4.6 mm ID x 10 cm)  
 D. Commercial HIC column A (4.6 mm ID x 10 cm)  
 E. Commercial HIC column B (4.6 mm ID x 10 cm)  
 F. Commercial HIC column C (4.6 mm ID x 10 cm)

Mobile phase A: 0.1 mol/L Sodium Phosphate Buffer + 1 mol/L Ammonium Sulfate  
 Mobile phase B: 0.1 mol/L Sodium Phosphate Buffer +25% Isopropanol

Gradient: 0-15 min., 0-100% Mobile Phase B  
 Flow rate: 0.50 mL/min  
 Detection: UV @ 215 nm  
 Temperature: 25 °C  
 Injection vol.: 20 µL  
 Sample: 20 µL U-73122-conjugated mAb

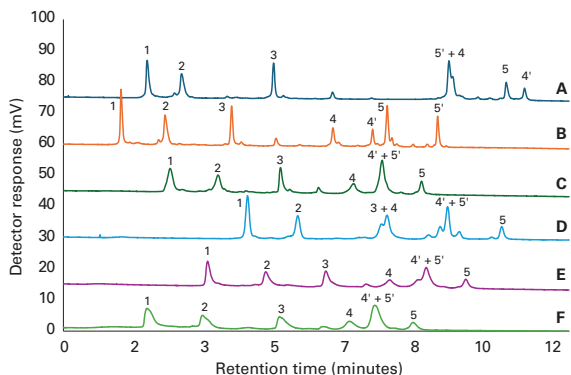
### Short profile

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

While TSKgel HIC-ADC columns facilitate HIC-based ADC separations, **Figure 2** additionally demonstrates their suitability for protein mixtures. Both TSKgel HIC-ADC columns exhibit superior separation performance relative to alternative HIC columns.

When comparing the TSKgel HIC-ADC Phenyl and Butyl profiles, the Phenyl column better resolves less hydrophobic proteins including myoglobin, RNaseA, and lysozyme (Peaks 1-3) and increases retention for all molecules tested in the mix. On the other hand, the more hydrophobic proteins are eluted with superior peak shapes using the TSKgel HIC-ADC Butyl column.

**Figure 2.** Comparing Protein Mix Separations among HIC Stationary Phases.



**Columns:**  
 A. TSKgel HIC-ADC Phenyl (4.6 mm ID x 10 cm)  
 B. TSKgel HIC-ADC Butyl (4.6 mm ID x 10 cm)  
 C. TSKgel Butyl NPR (4.6 mm ID x 10 cm)  
 D. Commercial HIC column A (4.6 mm ID x 10 cm)  
 E. Commercial HIC column B (4.6 mm ID x 10 cm)  
 F. Commercial HIC column C (4.6 mm ID x 10 cm)

**Mobile phase A:** 0.1 mol/L Sodium Phosphate Buffer + 2.3 mol/L Ammonium Sulfate

**Mobile phase B:** 0.1 mol/L Sodium Phosphate Buffer

**Gradient:** 0-15 min., 0-100% Mobile Phase B

**Flow rate:** 1 mL/min

**Detection:** UV @ 280 nm

**Temperature:** 25 °C

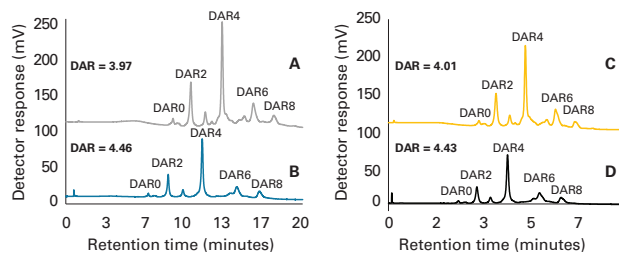
**Injection vol.:** 13 µL

**Sample:** Protein mixture  
 1. Myoglobin (17 kDa)  
 2. Ribonuclease A (13.7 kDa)  
 3. Lysozyme (14.3 kDa)  
 4. α-Chymotrypsin (25 kDa)  
 5. α-Chymotrypsinogen A (25.7 kDa)

## Enable Faster Throughput for DAR analysis

With the 3.5 cm TSKgel HIC-ADC column, DAR determinations can be reduced to a 7-minute run time while preserving performance analyzed with its 10 cm 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 TSKgel HIC-ADC Columns.



**Columns:**  
 A: TSKgel HIC-ADC Phenyl (4.6 mm ID x 10 cm)  
 B: TSKgel HIC-ADC Butyl (4.6 mm ID x 10 cm)  
 C: TSKgel HIC-ADC Phenyl (4.6 mm ID x 3.5 cm)  
 D: TSKgel HIC-ADC Butyl (4.6 mm ID x 3.5 cm)

**Mobile phase A:** 0.1mol/L sodium phosphate buffer + 1.0mol/L ammonium sulfate (pH 7.0)

**Mobile phase B:** 0.1mol/L sodium phosphate buffer + Isopropanol = 70 / 30

**Gradient:** A & C: 0 - 100 % Mobile Phase B (0 - 15 min; Linear)  
 B & D: 0 - 100 % Mobile Phase B (0 - 6 min; Linear)

**Flow rate:** 1 mL/min

**Detection:** UV @ 215 nm

**Temperature:** 25 °C

**Injection vol.:** 10 µL

**Sample:** SigmaMAb Antibody Drug Conjugate (ADC) Mimic

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## Ordering Information

Part #	Description	Particle size	Dimensions
0023536	TSKgel HIC-ADC Phenyl	5 µm	4.6 mm ID x 3.5 cm L
0023537	TSKgel HIC-ADC Phenyl	5 µm	4.6 mm ID x 10 cm L
0023542	TSKgel guardcolumn HIC-ADC Phenyl	5 µm	4.6 mm ID x 0.5 cm L
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 guardcolumn HIC-ADC Butyl	5 µm	4.6 mm ID x 0.5 cm L



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