

# Powerful HIC Columns for ADC Characterization



Welch Materials

## Overview:

Welch Advanchrom HIC-Butyl column is a high-performance hydrophobic interaction column (HIC), designed using advanced large-pore silica microspheres combined with proprietary surface bonding technology, making it ideal for antibody and ADC characterization.

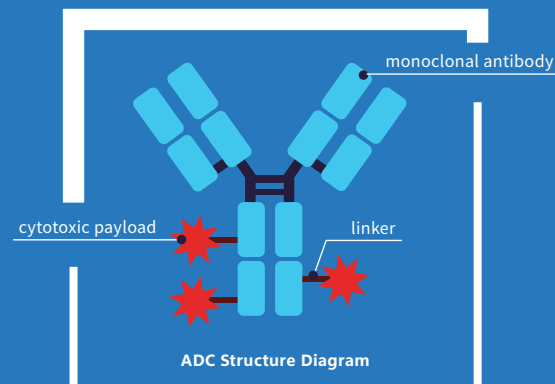
## Introduction:

Biopharmaceuticals like antibody-drug conjugates (ADCs) play an important role in oncology treatments. ADCs are drug molecules formed by conjugating antibodies with cytotoxic payloads via linkers.

The drug-to-antibody ratio (DAR) directly reflects the payload quantity per antibody and impacts both therapeutic efficacy and safety. Therefore, accurate determination and monitoring of DAR are essential throughout ADC development and commercial production, and HIC is a widely used technique in this process.

## Product Features:

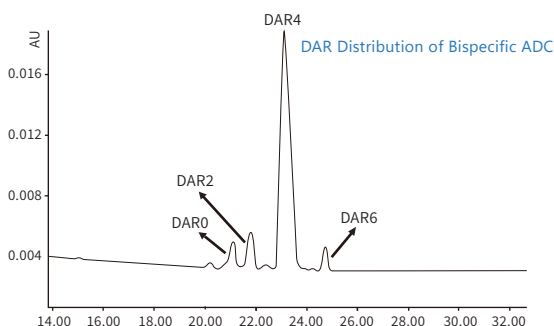
- Unique chemical design offering excellent selectivity for ADC molecules
- Ultra-high purity large-pore silica microsphere matrix for superior column efficiency
- Low non-specific adsorption ensuring high recovery rates
- Excellent pressure tolerance and durability
- Outstanding batch-to-batch reproducibility
- Effective retention and separation using lower salt concentrations



## Product Information:

Product Name	Advanchrom HIC-Butyl Column	Column Tube	Stainless Steel
Bonded Phase	Butyl	Dimensions	4.6 x 50 mm, 4.6 x 35 mm
Matrix	Ultra-high purity large-pore silica	Max. Pressure	6000 psi
Particle Size	3 $\mu$ m	Max. Temperature	60 $^{\circ}$ C
Pore Size	1000 $\text{\AA}$	pH Range	2 - 8

### Application 1: DAR Analysis of Bispecific ADC



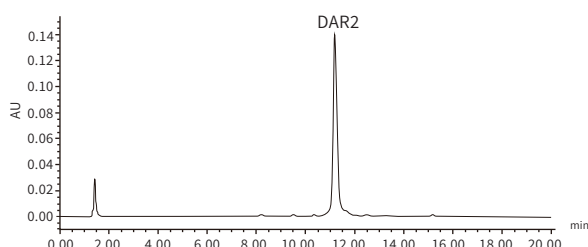
Column:	Advanchrom HIC-Butyl
Specification:	3 $\mu$ m, 4.6 x 50 mm
Mobile Phases:	A: 100 mM PB Solution + 1.0 M $(\text{NH}_4)_2\text{SO}_4$ , pH 7.0 B: 100 mM PB Solution, pH 7.0 / IPA = 80/20
Flow Rate:	0.5 mL/min
Wavelength:	280 nm
Temperature:	25 $^{\circ}$ C
Injection Volume:	10 $\mu$ L
Sample:	ADC

Elution Program:		
T (min)	A%	B%
0	90	10
3	90	10
35	0	100
38	0	100
39	90	10
45	90	10

Under ammonium sulfate conditions, ADC molecules with different DAR values show effective retention and separation on HIC columns.

Although ammonium sulfate is widely used for DAR determination, it is non-volatile and incompatible with mass spectrometry (MS). To address this, we also tested a volatile ammonium acetate system, achieving excellent retention and peak shape for both DAR2 ADC and rituximab, providing an MS-compatible solution for future DAR characterization.

#### Application 2: DAR2 Analysis of Monoclonal ADC

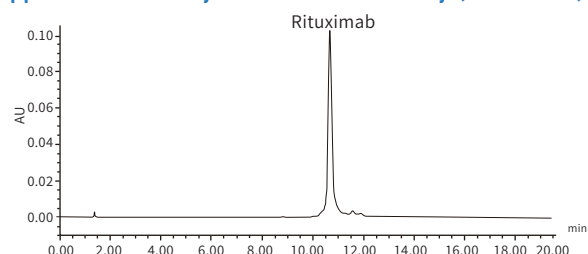


Column:	Advanchrom HIC-Butyl
Specification:	3 $\mu$ m, 4.6 x 50 mm
Mobile Phases:	A): 1.0 M CH <sub>3</sub> COONH <sub>4</sub> B): 25 mM CH <sub>3</sub> COONH <sub>4</sub> / ACN = 70/30
Flow Rate:	0.5 mL/min
Wavelength:	280 nm
Temperature:	30 °C
Injection Volume:	10 $\mu$ L
Sample:	ADC (Application 2) / Rituximab (Application 3)

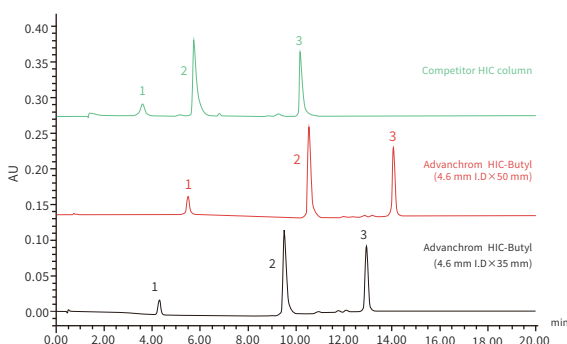
#### Elution Program:

T (min)	A%	B%
0	100	0
2	100	0
15	0	100
20	0	100
22	100	0
30	100	0

#### Application 3: Analysis of Intact Antibody (Rituximab)



#### Application 4: Separation of Standard Proteins



Column:	Advanchrom HIC-Butyl
Specification:	3 $\mu$ m, 4.6 x 50 mm; 3 $\mu$ m, 4.6 x 35 mm
Mobile Phases:	A): 100 mM PB Solution + 2.0 M (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> , pH 7.0; B): 100 mM PB Solution, pH 7.0 / ACN = 90/10
Flow Rate:	1.0 mL/min
Wavelength:	280 nm
Temperature:	30 °C
Injection Volume:	10 $\mu$ L
Sample:	(~1 mg/mL dissolved in Mobile Phase A) 1. RNase A; 2. Lysozyme; 3. $\alpha$ -Chymotrypsinogen

#### Elution Program:

T (min)	A%	B%
0	100	0
1	100	0
15	0	100
20	0	100
22	100	0
30	100	0

- Both Welch HIC columns provided excellent retention and separation, delivering superior peak shape and theoretical plates compared to competitor columns.

#### Recommended Mobile Phases

For HIC-UV applications, both ammonium sulfate and ammonium acetate systems can be used to achieve optimal results. For HIC-MS applications, use ammonium acetate, as ammonium sulfate is not compatible.

#### Quality Assurance

Each Advanchrom HIC-Butyl column is manufactured with a mature packing process and strict quality control to ensure superior performance.

#### Ordering Information

Part No.	Product	Specification
0001-01037	Advanchrom HIC-Butyl	3 $\mu$ m, 4.6x50 mm
0001-01036		3 $\mu$ m, 4.6x35 mm