

Detect slight differences in hydrophobicity

Hydrophobic Interaction Chromatography Column

Shim-pack™ BIO HIC

Antibody-drug conjugates (ADCs) couple antibodies with a drug to more directly target cancer cells, making it possible to kill the cancer cells selectively and effectively. For this reason, ADCs are viewed as next-generation treatments with a more powerful and extensive therapeutic range than existing chemotherapeutic agents. To ensure quality control of ADCs, analysis of the drug-to-antibody ratio (DAR) is performed using hydrophobic interaction chromatography (HIC).

Shim-pack BIO HIC columns are ideal for DAR analysis, which indicates ADC efficacy and safety, by determining slight differences in hydrophobicity without denaturing biopolymers.



Need for DAR Analysis in ADCs

ADCs consist of lysine and cysteine residues of a monoclonal antibody (mAb) and small-molecule drugs (Fig. 1). However, since mAbs contain multiple amino acid residues involved in their binding, the mAbs are known to form heterogeneous ADCs with different binding numbers and binding sites.

These heterogeneities can affect a drug's efficacy and safety, making analysis of the drug-antibody ratio (DAR) essential to determine the quality characteristics of ADCs. In addition, maintaining the distribution of DAR within a certain range enables quality assurance in ADC manufacturing.

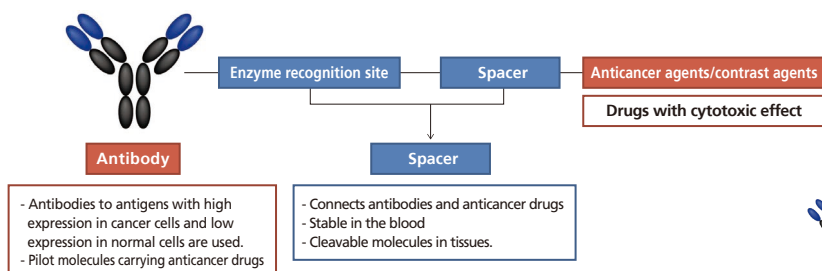


Fig. 1 Structure of ADCs

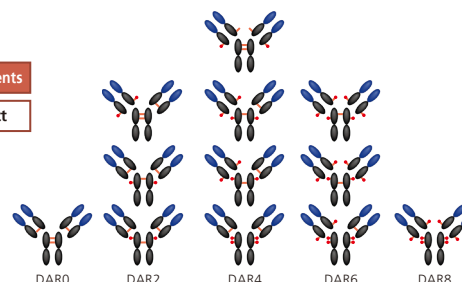


Fig. 2 Polydisperse Distribution of DAR of a Cysteine-Conjugated ADC

DAR Analysis using Shim-pack BIO HIC

Fig. 3 shows the result of an analysis of cysteine-conjugated ADCs (Fig. 2) using the Shim-pack BIO HIC and an a biocompatible HPLC system with PDA detection. In the cysteine-conjugated ADCs, small-molecule drugs bind to thiol groups formed by the reduction of four disulfide bonds involved in protein conformation, resulting in the distribution of DAR0, DAR2, DAR4, DAR6, and DAR8. In HIC, the more drug that is bound, the more the conjugate is retained on the column.

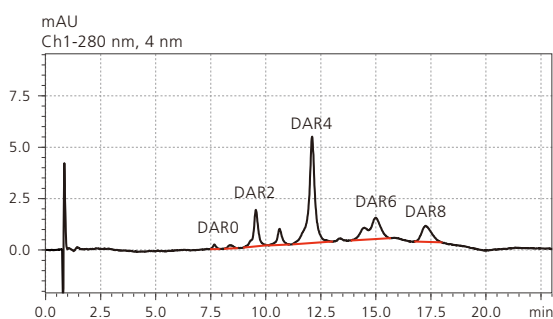


Fig. 3 DAR Analysis of a Cysteine-Conjugated ADC using Shim-pack BIO HIC

Analytical Conditions

System	: Inert LC (1.6 mL mixer)
Workstation	: LabSolutions™ LC/GC
Column	: Shim-pack Bio HIC (100 mm. x 4.6 mm.I.D., 4 μm)
Mobile Phase	: A) 50 mM NaH ₂ PO ₄ -Na ₂ HPO ₄ (pH 7.0) containing 1.5 M (NH ₄) ₂ SO ₄ /2-propanol (95/5) B) 50 mM NaH ₂ PO ₄ -Na ₂ HPO ₄ (pH 7.0)/2-propanol (80/20)
Gradient	: 0%B (0-1 min), 0-100%B (1-18 min), 100%B (18-23 min)
Flow Rate	: 1.0 mL/min
Injection Volume	: 5 μL
Column Temperature	: 25 °C
Detection	: 280 nm (PDA)
Sample	: Cysteine-liked ADC Mimic (5 mg/mL)

*Automatic waveform processing is performed with i-peak finder (LabSolutions' automatic waveform processing algorithm).

■ Excellent Reproducibility to Help Calculate DAR

Reproducibility of area values obtained from repeat injections (n=6) of cysteine-conjugated ADC samples and an example of an automated DAR calculation report are shown below. (DAR can be calculated automatically using LabSolutions DB/CS custom reporting.) The Shim-pack BIO HIC, developed for biopharmaceutical analysis, makes it possible to detect slight differences in hydrophobicity of small-molecule drugs and perform rapid DAR analysis with high resolution.

Reproducibility of area values (n=6)

	%RSD
DAR0	5.98
DAR2	2.57
DAR4	1.62
DAR6	2.23
DAR8	2.87

◆ How to Calculate DAR

$$DAR = \sum_{n=0}^8 \frac{\text{Peak area values} \times \text{Number of drug binding}}{\text{Total amount of the peak area values}}$$

Automatic DAR Calculation Report						
測定開始日	2020/03/25 19:09:56(+09:00)					
分析者	Shimadzu Taro					
分析装置	Inert LC					
サンプル名	ADC					
分析結果						
ID#	Name	Ret. time(min)	Area	Area%	Height	DAR calculated value
1	DAR0	7.661	2440	1.241	229	0
2	DAR2	9.551	28019	14.255	1790	0.285
3	DAR4	12.102	98518	50.123	5114	2.005
4	DAR6	15.013	43209	21.983	1013	1.319
5	DAR8	17.26	24367	12.397	769	0.992
Total			196553	100	8915	4.601

Example of automated DAR calculation report

■ Shim-pack Bio HIC

P/N	Description
227-31174-01	Shim-pack BIO HIC 4 μm, 4.6 mm × 100 mm

■ Shim-pack Series Columns Supporting Biopharmaceutical Analysis

Size exclusion chromatography column	Shim-pack Bio Diol Series
Ion exchange chromatography column	Shim-pack Bio IEX Series
Reversed phase chromatography column for protein analysis	Shim-pack Scepter™ C4-300

For details, please refer to the following website.
<https://www.shimadzu.com/an/hplc/column/index.html>

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