MYCENAX

WhitePaper

Understanding Process Development of Antibody-Drug Conjugates in Preclinical and Early Phase Clinical Trials

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Chapter 01

Understanding Process Development of Antibody-Drug Conjugates in Preclinical and Early Phase Clinical

01 Understanding Process Development of Antibody-Drug Conjugates in Preclinical and Early Phase Clinical Trials

Key Takeaways

- ADCs have attracted significant interest as highly targeted and potent medicines in many therapeutic areas.
- Development of ADCs have been complicated by the need to balance drug efficacy and safety, requiring extensive investigations into conjugation technologies and the resulting critical quality attributes of ADCs.
- Mycenax offers a simplified and reliable platform for the development of ADC manufacturing processes, enabling accelerated preclinical and clinical development.

Introduction

The unique challenge of scaling-out patient-specific cell therapy manufacturing processes is achieving a reduction in the cost per dose, given that there are currently few economies of scale to exploit. Therefore, minimizing the cost of idle capacity will be critical when ramping up towards commercial production. The construction and validation of additional manufacturing capacity must be carefully managed and aligned with projected patient accrual or product sales, to avoid incurring a high cost per dose caused by the large overhead cost of these idle facilities.

Process Overview

Despite the diversity of conjugation technologies, the ADC manufacturing process can generally be divided into 4 stages (Figure 1) that are consistent across most chemical and enzymatic conjugation strategies.

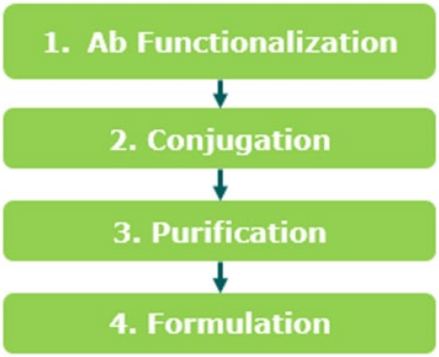


Figure 1. A generalized 4-stage manufacturing process of ADC

Stage 1. Antibody (Ab) functionalization

Apart from conventional lysine conjugation, antibodies that use other conjugation strategies need to be functionalized to generate suitable conjugation sites. The functionalization reactions are categorized as follows: reduction of interchain disulfide bonds for conventional cysteine conjugation, reduction and re-oxidation of interchain disulfide bonds for engineered cysteine conjugation, glycan modification/remodeling, and enzymatic modification of specific amino acids. As such, additional adjustments of process variables, such as concentration, temperature, reaction time, pH and buffer, may be necessary to accommodate required conditions for the next stage.

Stage 2. Conjugation

The antibody containing suitable conjugation sites is subject to conditions conducive to conjugation reactions with the drug. Generally, the linker-payload is dissolved in an organic solvent (e.g., DMSO, DMA) or an aqueous solvent and then added to an aqueous solution containing the functionalized antibody to form a crude mixture of the targeted ADC. The linker-payload is charged in excess relative to the available conjugation sites on the antibody to ensure complete conjugation. To manage the excess unconjugated linker-payload, a quenching step may be necessary after the conjugation reaction based on the chemistry of conjugation reactions.

Stage 3. Purification

After the above manipulations, there are usually certain small-molecule and biological impurities in the crude mixture of the targeted ADC. Typical small-molecule impurities include unconjugated payload, other payload-related substances derived from process-related byproducts and degradation, and solvent. They can be removed by ultrafiltration and diafiltration (UF/DF). Typical biologic impurities consist of aggregates, fragments, undesired DAR species and non-antibody-related impurities (e.g., endotoxins) can be removed with size exclusion, hydrophobic interaction or ion exchange chromatographic procedures.

Stage 4. Formulation

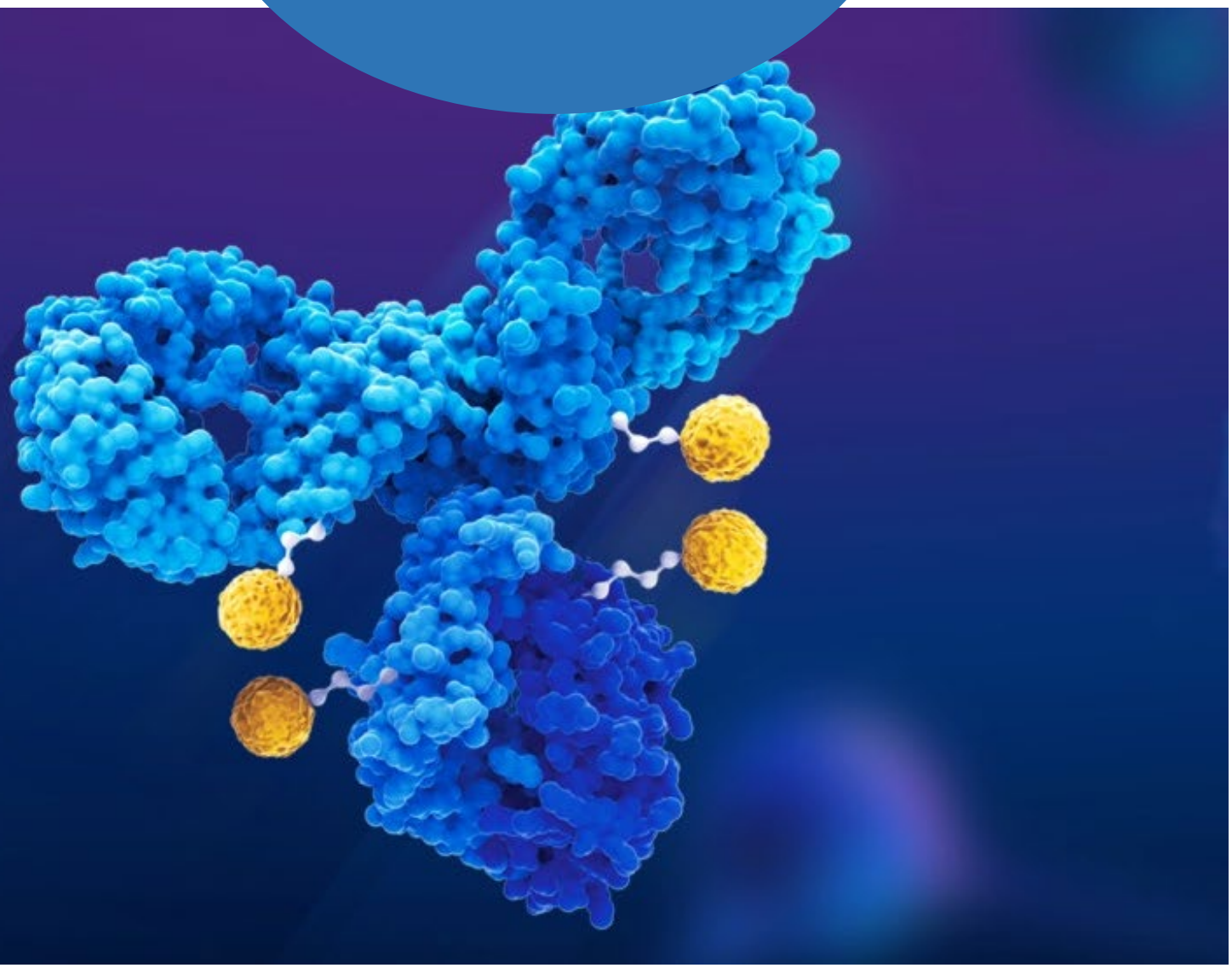
The purified ADC solution tends to be unstable due to the hydrophobicity of the linker-payload. Lyophilization formulation is commonly employed to ensure long-term stability of an ADC. The ADC is formulated with suitable excipients by taking into consideration the effect of pH, ionic strength and surfactant interactions on solubility and stability.

The following sections focus on important considerations related to the ADC manufacturing process stages 1 to 3. They include critical quality attributes (CQAs), potential challenges and possible solutions during process development, along with some case examples. Information regarding formulation will be presented in a separate white paper to be published in the future.

Chapter 02

Critical Quality Attributes

Critical quality attributes related to the ADC manufacturing process (stage 1 to stage 3) are identified through prior knowledge and experimental findings including the following:



DAR DAR holds critical importance as it has a direct impact on the balance of efficacy and toxicity. To maintain ADC product consistency, precise control over DAR within stringent specifications is essential and can be challenging.

DAR distribution The DAR distribution of an ADC with the same average DAR can be different with varying drug loads. Different DAR species may have different efficacy, toxicity and pharmacokinetic profiles. Hence, the distribution of DAR species is critically important.

Unconjugated payload An excess of unconjugated payload can impact the balance of toxicity and efficacy. Moreover, it is possible that unconjugated payload may undergo further degradation, complicating the characteristics of cytotoxicity. Although there are no specific guidelines for specification of the payload-related impurities in ADCs, ICH Q3A, Q3B, and M7 should be taken into consideration.

Aggregates and/or fragments They also may have a potential impact on safety and efficacy (including immunogenicity and hyper-potency).

Residual solvent Organic solvent may be involved in the conjugation stage to dissolve the linker-payload; therefore, solvent removal to acceptable levels is necessary according to ICH Q3C.

Chapter 03

Challenges and Solutions of ADC Process development

03 Challenges and Solutions of ADC Process development

Efficient process development of ADC involving numerous process variables.

There are multiple process variables affecting ADC quality. As well, there are cross interactions and interdependencies between the different manufacturing stages. A complete study of all variables is time-consuming and impractical. Mycenax platform offers simplification of process variables based on proprietary know how to enable efficient process development. Take as an example an ADC with conventional cysteine-based conjugation, stages 1 and 2 of the manufacturing process determine the most crucial CQA' s of ADC, DAR and DAR distribution. The stage 3 purification process serves as a corrective step aiming to remove undesired DAR species. Let' s focus on stages 1 and 2, as shown in **Table 1**. In our experience, ADCs produced based on an initial plan usually meet the quality requirements for proof-of-concept stages. Several process variables can be reduced to just the molar equivalent (Mol. Eq.) of reducing agent to antibody and that of linker-payload to antibody, which are highly correlated to CQAs. In addition, our platform employs in-process controls (IPCs) to ensure proper completion of both oxidation and reduction reactions, substituting for arbitrary fixation of reaction time, with the goal of achieving consistency across batches. As drug development progresses, an optimized plan with more variables can be executed to further improve product quality, increase yield, and reduce overall process time and cost.

Table 1. Simplified process development of Cysteine-based conjugation

Step	Process Variable	Main Effect	Initial Plan	Optimized Plan
Ab Reduction	Reaction Buffer	**Reactivity**	Fix	V
	pH	**Reactivity**	Fix	V
	Conc. (mg/mL)	**Reactivity**	Fix	V
	Reducing Reagent (Mol. Eq.)	**DAR**	V	V
	Reaction Temp. (℃)	**DAR, DAR distribution, Aggregate**	Fix	V
	Reaction Time (H)		By IPC	By IPC
Conjugation	Linker-Payload (Mol. Eq.)	**DAR, Unconjugated payload**	V	V
	Conc.	**Reactivity**	Follow	Follow
	pH	**Reactivity**	Fix	Fix
	Solvent %	**Reactivity, Residual solvent**	Fix	V
	Reaction Temp. (℃)	**DAR, DAR distribution, Aggregate**	Fix	V
	Reaction Time		By IPC	By IPC
	Quencher (Mol. Eq.)	**Process-related byproducts**	Fix	V

Precise control of DAR

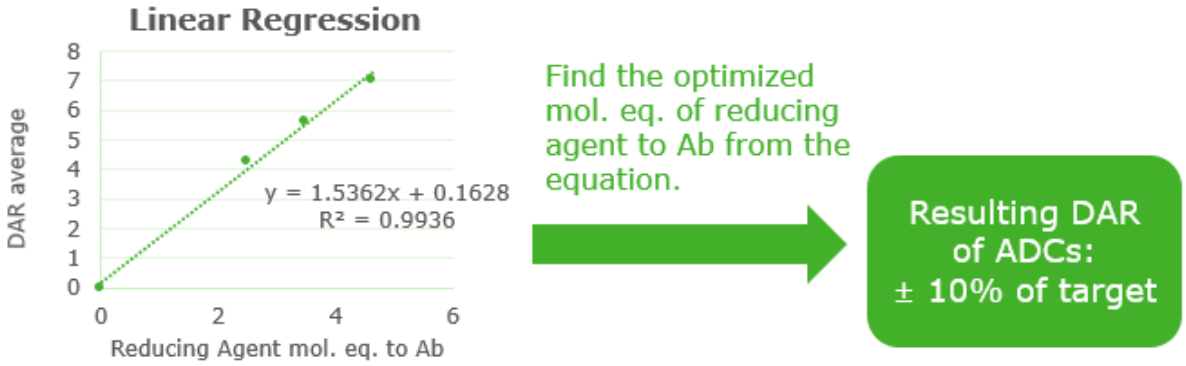


Figure 2. Precise DAR control

As mentioned above, a stringent DAR requires precise control of the number of conjugation sites generated in stage 1 and complete conjugation in stage 2. Take as an example an ADC with conventional cysteine-based conjugation, the conjugation sites are cysteines generated by reduction of interchain disulfide bonds. DAR is directly related to the reduced level of the antibody, which relates to the molar equivalent of

reducing agent to antibody, and hence the completion level of conjugation. Our control strategy is established based on a linear regression between the molar equivalent of reducing agent to antibody, the excess molar equivalent of linker-payload to conjugation sites, and IPCs to ensure complete reaction. IPCs instead of control based on reaction times alone can improve batch-to-batch consistencies because reaction times often differ from batch to batch due to different antibody batches, different production scales, and/or different mixing conditions. We have demonstrated that the average DAR can be precisely controlled based on molar equivalents derived from the linear regression approach mentioned above, as shown in **Figure 2**. Numerous batches of an ADC targeting DAR 2, 4, 6 or 8 are shown to be well-controlled in terms of DAR with variabilities within± 10%.

Improvement of DAR distribution during stages 1 & 2

DAR distribution can be optimized based on the amount of linker-payload and reaction temperature. A reduction of odd DAR species is shown in **Figure 3** as an illustration. Insufficient linker-payload results in odd DAR species. After a study of the molar equivalent of linker-payload to antibody, the optimized molar equivalent has been shown to minimize odd DAR species. An optimization of major DAR species is shown in **Figure 4**. Interestingly the DAR 4 species increased by about 7%, which likely results from the effect of low temperature.

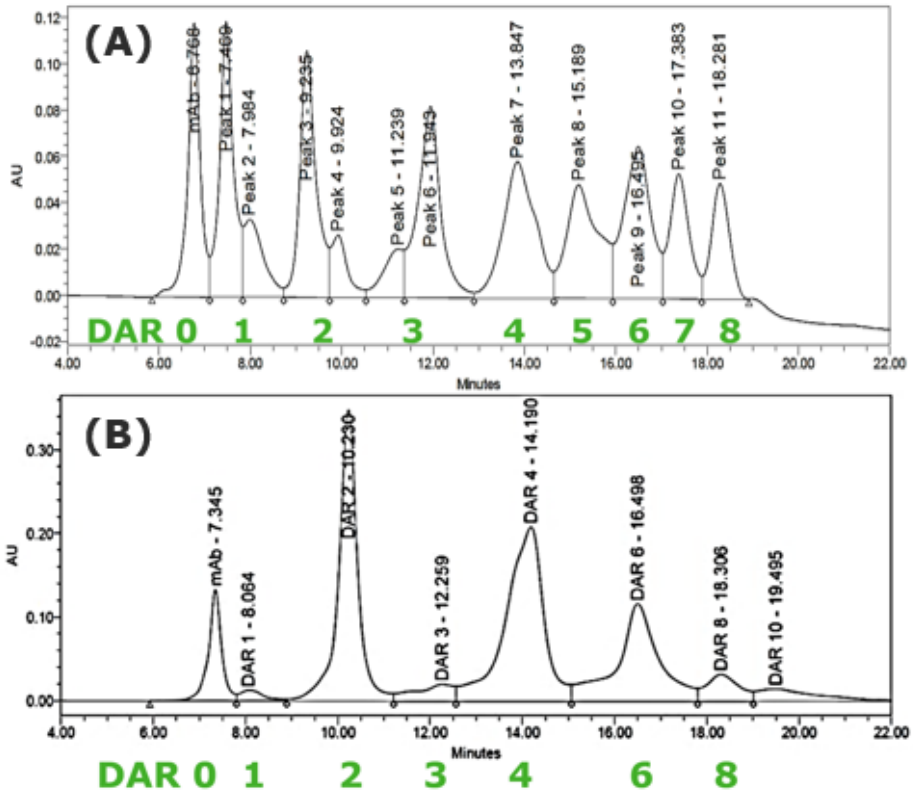
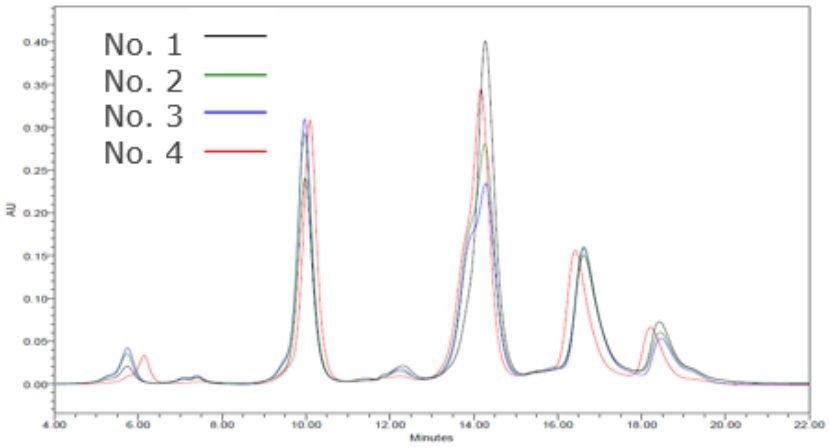


Figure 3. Minimize heterogeneity of odd-DAR distribution. (A) Insufficient linker-payload; (B) Sufficient linker-payload.



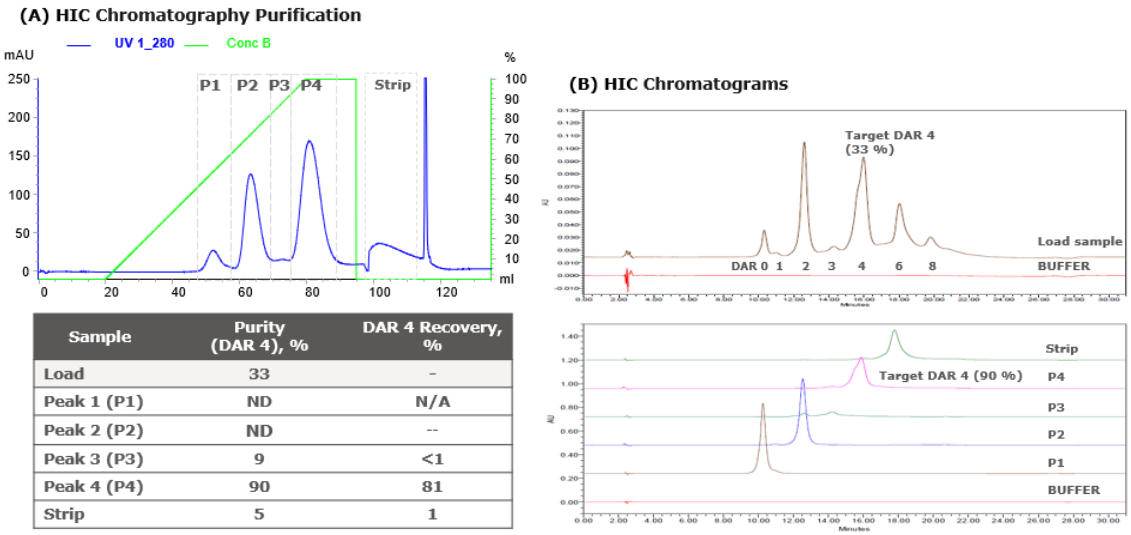
EXP No.	Temperature	DAR Distribution					DARavg.
		0	2	4	6	8	
1.	Low ↓ High	1.95	19.99	43.01	21.95	8.60	4.4
2.		2.80	24.46	42.20	21.24	7.61	4.2
3.		3.32	24.41	39.15	22.57	6.97	4.2
4.		4.35	27.75	35.92	21.62	6.09	4.0
Reduction and Conjugation were monitored by IPCs to ensure complete reaction.							

Figure 4. Optimization of major DAR specie of ADC targeting DAR 4.

Improvement of DAR distribution in stage 3

Both non-site-specific and site-specific conjugations can produce undesired DAR species. To improve homogeneity of ADCs, hydrophobic interaction chromatography (HIC) is typically employed. We have established a platform with demonstrated performance for DAR purification providing fast development of chromatography purification. The platform entails initial screening for suitable salt type and concentrations, followed by a high throughput screening for performance, and evaluation using scaled-down models. In salt screening, salt buffer for chromatography

and the range of salt concentrations are evaluated and established based on the turbidity of salt buffer mixed with the crude ADC. Suitable salt buffers with acceptable maximum concentrations are applied to the high throughput screening; and conditions with better recovery yields are selected for scaled-down-model testing and optimization. As shown in **Figure 5**, our platform successfully achieves purification of DAR 4 from an ADC sample, which is derived from conventional cysteine conjugation and contains only 33% of targeting DAR 4 content. There are 4 peaks observed during elution. Fractions are collected and analyzed. The HIC analysis shows successful removal of undesired DAR species (DAR 0 1, 2, 3, 6 and 8); and as a result, purity of the target species (DAR 4) is improved from 33% up to 90%. The overall recovery of DAR 4 is up to 81%. In this case, recovery of DAR 4 specie is better than expected.

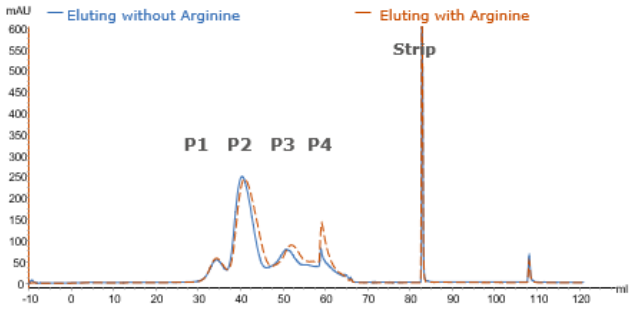


Sample	Purity (DAR 4), %	DAR 4 Recovery, %
Load	33	-
Peak 1 (P1)	ND	N/A
Peak 2 (P2)	ND	--
Peak 3 (P3)	9	<1
Peak 4 (P4)	90	81
Strip	5	1

Figure 5. Purification of ADC targeting DAR 4 via hydrophobic interaction chromatography

Purification results for another ADC with DAR 2 as the target species are shown in **Figure 6**. Two experiments using elution buffers with or without arginine were conducted. The results of DAR 2 purity in both experiments are similar and both are higher than 98%. The experiment with arginine achieves a higher recovery yield possibly due to the inhibition of aggregation by arginine.

(A) HIC Chromatography Purification



(B) Eluting buffer without Arginine

Sample	Purity (DAR 2), %	DAR 2 Recovery, %	Overall Recovery, %
Load	55	N/A	N/A
Peak 1 (P1)	6	3	N /A
Peak 2 (P2)	99	71	39
Peak 3 (P3)	ND	N/A	N/A

(C) Eluting buffer with Arginine

Sample	Purity (DAR 2), %	DAR 2 Recovery, %	Overall Recovery, %
Load	55	N/A	N/A
Peak 1 (P1)	2	0	0
Peak 2 (P2)	98	78	42
Peak 3 (P3)	6	0	0

Figure 6. Purification of ADC targeting DAR 2 via hydrophobic chromatography

Product Consistency in scaled-up batches

Due to the complexity of ADC manufacturing, a well-designed process is crucial to ensure process and product consistencies. Mycenax' s simplified process development platform has demonstrated superior performance with conventional cysteine-based conjugations. In **Table 2**, a stepwise scale-up verification shows that product quality derived from small-scale batches is consistent with that from large-scale batches. As shown in **Figure 7**, the DAR distributions from these batches are highly similar.

Batch Size* (mL)	IPC Controls		DARavg. (HIC-LC) 4.0±0.3	Purity (SE-LC)	TFF (UF/DF) purification at large scale	
	-SH/Ab in Red.	-SH/Ab in Conj.			DMSO ≦ 5000 ppm	Small-molecular Impurities
1.5	4.0	0.0	4.12	98.87%		
10-40	4.0~4.4	<0.2	3.9~4.2	>98.5%		
100	4.3	0.1	4.11	98.48%	3699	N.D.
1000	4.3	0.1	4.11	98.88%	1347	N.D.

Table 2. Analysis result of stepwise scale-up batches

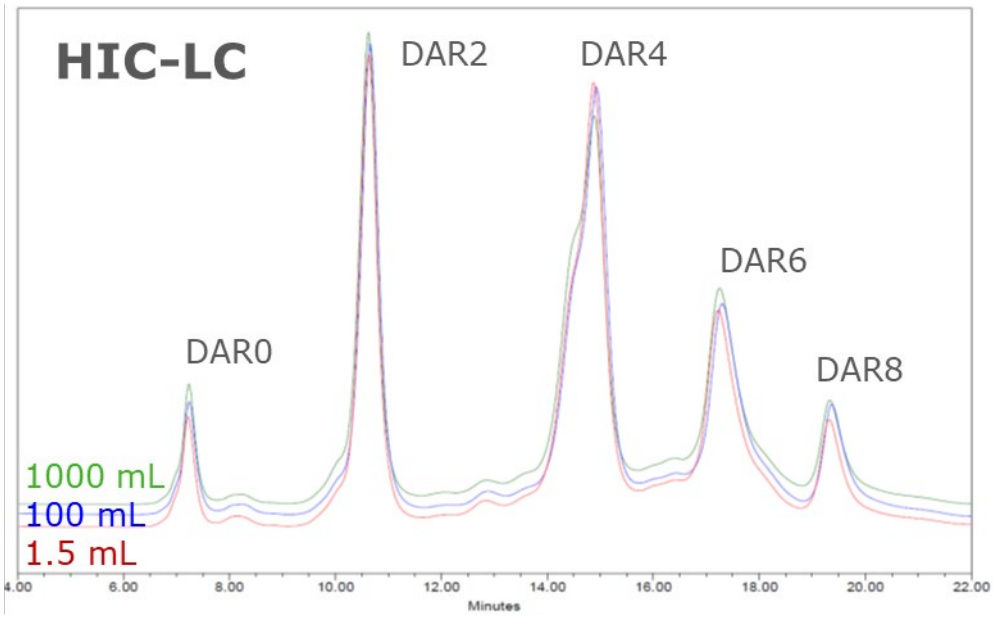


Figure 7. DAR Distribution of stepwise scale-up batches

Chapter 04

Conclusion

“Conclusion

A reliable and scalable ADC manufacturing process can be developed by using Mycenax’ s simplified process development platform based on understanding of the relationship between the process and CQAs. The simplified process development has the potential to enable accelerated development of new ADCs. In this article, we describe that antibody functionalization and conjugation stages are the most critical steps in determining DAR and DAR distributions; and their optimization is a top priority in ADC process development. Post-conjugation purification procedures are necessary to remove unconjugated payload and payload-related substances, and, in some cases, to enrich the desired DAR species, for which Mycenax’ s technology platform has demonstrated superior performance.

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Glossary

Ab	Antibody
ADC	Antibody-Drug Conjugate
CQA	Critical Quality Attribute
DAR	Drug-to-Antibody Ratio
DMA	N, N -dimethylacetamide
DMSO	Dimethyl Sulfoxide
FDA	Food and Drug Administration
HIC	Hydrophobic Interaction Chromatography
ICH	International Council for Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
IPC	In-Process Control
Mol. Eq.	Molar Equivalent
UF/DF	Ultrafiltration and diafiltration

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