Mastering ADC Bioconjugation:
From DAR Control to Efficient Free Payload Removal

Antibody-Drug Conjugates (ADCs) represent one of the most advanced classes of targeted therapies in oncology, combining the specificity of monoclonal antibodies with the potency of cytotoxic agents. Yet their development remains highly complex, particularly during bioconjugation, where both efficacy and safety are defined. Through two case studies, this article illustrates how a combination of process optimisation and tailored purification strategies enabled precise control of Drug-to-Antibody Ratio (DAR) and efficient removal of residual free payload. Despite tight timelines and stringent specifications, robust and scalable solutions were implemented, demonstrating how integrated expertise can accelerate ADC development.

Bioconjugation: A Critical Step in ADC Development

ADCs are sophisticated constructs built from three components: a monoclonal antibody, a linker and a cytotoxic payload. Each plays a distinct role, but it is the bioconjugation step that ultimately determines how effectively these components work together. By controlling how many payload molecules are attached to each antibody molecule, bioconjugation directly impacts the Drug-to-Antibody Ratio (DAR), a key driver of therapeutic efficacy. At the same time, it must ensure that any unreacted payload, highly cytotoxic by nature, is reduced to trace levels to guarantee patient safety.

Balancing these two dimensions is not straightforward. Increasing payload loading can improve potency but often comes at the cost of higher levels of residual free drug or linker-payload species. Conversely, aggressive purification can affect yield or product quality. In practice, success depends on the ability to align conjugation efficiency with an effective and scalable purification strategy. The following case studies highlight how these challenges were addressed in real development programs.

Reducing Residual Free Drug: A Combined Process Strategy

In the first case, the main obstacle emerged immediately after conjugation. Significant levels of free drug remained in the reaction mixture, and standard purification methods were unable to reduce them sufficiently. Given the inherent toxicity of the payload, this represented a critical safety concern and a potential barrier to clinical progression.

Rather than relying solely on downstream processing, the team adopted a dual strategy. The first step focused on improving the conjugation reaction itself. Using a Design of Experiment approach, key parameters such as buffer composition, reaction time and reagent concentrations were systematically evaluated. This work led to a better-controlled reaction and a noticeable reduction in free drug levels even before purification.

The second step involved the development of a dedicated tangential flow filtration (TFF) process. Different membrane materials and buffer conditions were screened to identify the most effective combination for separating the ADC from the free drug. Beyond empirical testing, diafiltration performance was modelled to determine the optimal volume required to achieve the lowest possible residual levels.

The result was a robust and efficient process in which residual free drug was reduced to levels three times below the required specification. Just as importantly, the solution was implemented within the project timeline, allowing preclinical and clinical activities to proceed without delay. This outcome underscored the importance of addressing upstream and downstream challenges in parallel, rather than treating purification as an isolated step.

Fast-Tracking Bioconjugation Under Tight Constraints

The second case study presented an even broader challenge, combining ambitious technical targets with a highly constrained timeline. The objective was to develop a process capable of delivering a DAR of 8, while ensuring that residual free linker-payload remained below 0.02% and that the final product was fully monomeric. At the same time, the process needed to remain as simple as possible, ideally avoiding chromatography, and had to be completed within four months.

The work began with optimisation of the reduction step, which is essential for exposing the antibody's conjugation sites. Selecting the appropriate reducing agent proved critical. While dithiothreitol offered cost advantages, tris(2-carboxyethyl) phosphine (TCEP) was ultimately preferred due to its greater stability, lower interaction with proteins and compatibility with a wider pH range. Rather than implementing a full Design of Experiments, the team relied on a simplified design and prior expertise to rapidly identify robust conditions, including the choice of buffer system.

Attention then shifted to the conjugation reaction itself. Here, the interplay between process parameters became evident. The ratio of linker-payload to antibody and the duration of the reaction both had a strong influence on DAR, while reaction time also affected the proportion of monomeric species. By contrast, factors such as stirring and small variations in temperature showed limited impact under the selected conditions.

After exploring the experimental space, a set of conditions emerged that consistently delivered the desired DAR of 8 while maintaining product quality. These included a reaction time of one and a half hours, an antibody concentration of 15 g/L and a linker-payload/antibodyratio of 11. Interestingly, this ratio not only supported the target DAR but also reduced raw material consumption compared to higher ratios, providing a tangible cost benefit.

However, these conditions did not fully minimise residual free linker-payload. Faced with a trade-off, the team made a deliberate decision to prioritise control of DAR and address the remaining impurity through purification. This choice proved to be pivotal in meeting both performance and timeline objectives.

Designing an Efficient Purification Strategy

Following conjugation, approximately 5% of free linker-payload remained in solution, far above the acceptable limit. The challenge was therefore to develop a purification step capable of reducing this level to below 0.02% without introducing unnecessary complexity.

A tangential flow filtration approach was again selected, with a systematic evaluation of key parameters. Membrane material emerged as a critical factor, with clear differences observed between cellulose- and polyethersulfone-based systems. Membrane cut-off also required careful consideration, as a 100 kDa threshold led to unacceptable product losses. Buffer composition, particularly ionic strength, showed a more moderate but still measurable effect on separation performance.

Through iterative testing, a set of conditions was identified that enabled efficient removal of free linker-payload using a single TFF step. Although a chromatographic alternative was explored in parallel as a risk mitigation measure, it ultimately proved unnecessary.

The final process successfully met all quality targets, reducing residual linker-payload to below 0.02% while preserving product integrity. Moreover, it was scaled up to 100 litres without issue, confirming its suitability for clinical manufacturing. Importantly, the entire development was completed within the four-month timeframe, demonstrating the effectiveness of a focused and pragmatic approach.

From Process Optimisation to Integrated Development

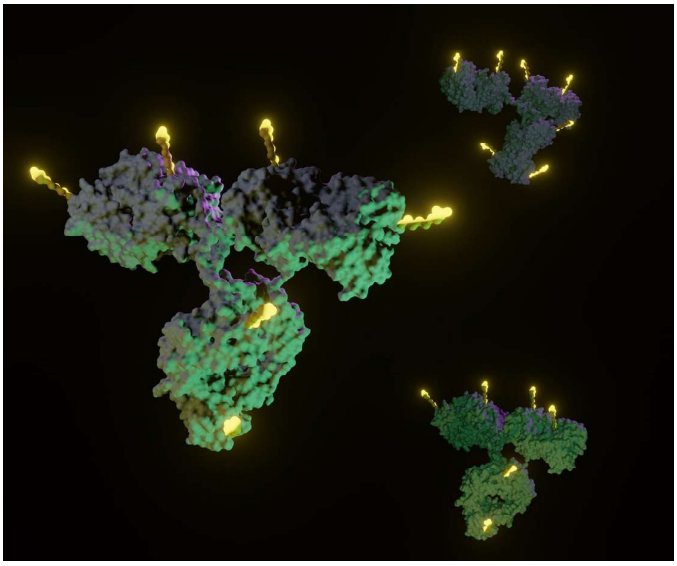
Taken together, these case studies highlight several broader lessons for ADC development. First, bioconjugation and purification should not be considered as separate challenges. Decisions made during conjugation, such as targeting DAR or selecting reaction conditions, have a direct impact on downstream processing requirements. Aligning these steps early can significantly improve overall efficiency.

Second, while Design of Experiments remains a powerful tool, it is not always the most appropriate approach under tight timelines. In both cases, a combination of structured experimentation and expert judgment enabled faster progress without compromising robustness.

Finally, these examples illustrate the value of integration across disciplines. Successful ADC development requires not only expertise in biologics, chemistry and analytics, but also the ability to connect these domains within a coherent process strategy. By doing so, it becomes possible to simplify workflows, reduce development risks and accelerate the path to clinical evaluation.

Conclusion

Bioconjugation lies at the core of ADC performance, defining



both therapeutic efficacy and patient safety. As these case studies demonstrate, achieving the right balance between DAR control and residual payload removal requires more than incremental optimisation. It calls for a holistic approach in which conjugation and purification are developed in tandem, guided by both data and experience.

Even under significant time pressure, it is possible to design processes that are robust, scalable and aligned with stringent quality requirements. In this context, the ability to make informed trade-offs and to integrate expertise across scientific domains emerges as a key driver of success in modern ADC development.

An Integrated Approach to ADC Bioconjugation

As the ADC field continues to evolve, the increasing complexity of these molecules calls for stronger integration between biologics, chemistry, analytics and process engineering. The examples presented here illustrate how aligning expertise across these domains can simplify development strategies, reduce risks and accelerate timelines.

In this context, integrated models that combine monoclonal antibody production, linker-payload chemistry and conjugation capabilities offer a clear advantage. By streamlining the transition from early development to clinical manufacturing, such approaches contribute to more efficient process design and more reliable scale-up, ultimately supporting the faster delivery of innovative ADC therapies to patients.

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