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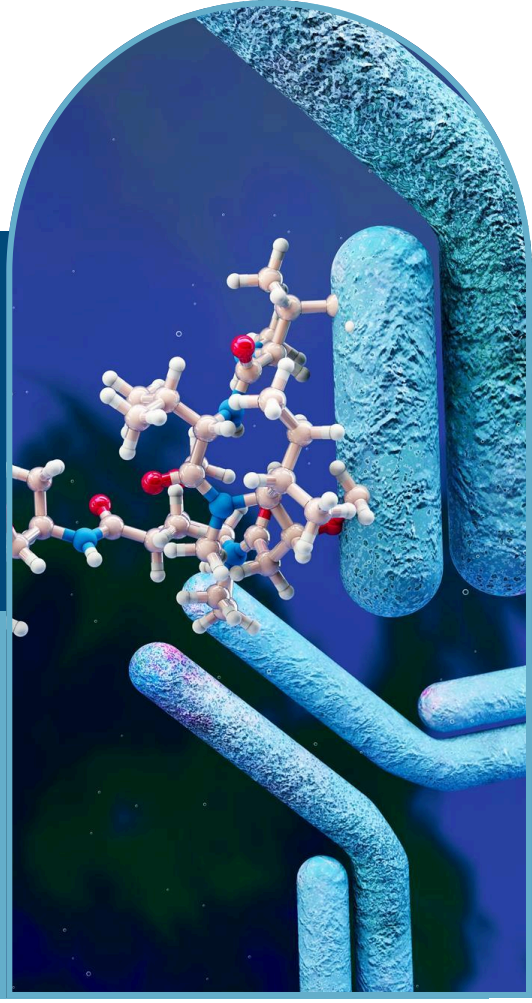

The Right Ratio:

BUILDING AN END-TO-END DAR STRATEGY FOR ADC SUCCESS

Orthogonal LC-MS and HIC Approaches for Robust Drug-to-Antibody Characterization

A developer advancing a novel antibody-drug conjugate (ADC) needed to establish a robust, reliable approach for characterizing drug-to-antibody ratio (DAR). With multiple batches in development and regulatory interactions approaching, they sought not only to support their IND, but also to build a platform that would serve them across long-term release and stability testing. Understanding that DAR can influence everything from potency to pharmacokinetics, they turned to Solvias for a multi-pronged analytical approach that could characterize not just the average DAR — but the full distribution and conjugation landscape of their molecule.

THE CHALLENGES

- **Beyond the Average:** The customer recognized that knowing the average DAR wasn't enough. ADCs with identical averages can behave differently depending on how that payload is distributed across the population of molecules. Without insight into this distribution, the team risked missing critical performance signals.
- **Conjugation Site Uncertainty:** It was unclear where on the antibody the payload was attaching. Site-specific conjugation yields a more homogenous, stable product, while random conjugation may affect biological function or stability — especially as the product moves into late-stage development.
- **Regulatory and Lifecycle Demands:** DAR would be relevant not just for early filings, but also for long-term quality control — including release testing, stability monitoring, and comparability assessments during future scale-up or manufacturing changes.

OUR COLLABORATIVE APPROACH

- **Hydrophobic Interaction Chromatography (HIC):** Solvias applied HIC to separate ADC species based on hydrophobicity, which increases with drug loading. Because each DAR species (e.g., DAR 0, 2, 4, 6) elutes at a different time, the area under each peak provides a precise, quantitative distribution profile. This allowed the customer to assess batch consistency, detect outliers, and understand how minor changes in conjugation chemistry might affect the drug profile.

- **Intact and Reduced LC-MS:** Solvias performed both intact mass and reduced chain analysis using LC-MS. This approach confirmed the average DAR by measuring the exact mass shifts caused by drug addition — both across the full antibody and its individual heavy/light chains. This orthogonal data confirmed the HIC results and revealed any mass deviations suggesting linker instability or unexpected modifications.
- **Conjugation Site Mapping via LC-MS/MS:** To go deeper, Solvias performed peptide mapping to identify specific lysines or cysteines where the drug payload had been attached. This was critical in confirming the conjugation chemistry and understanding whether any non-targeted sites were being modified. Mapping provided a structural fingerprint the team could carry forward for comparability and process control.
- **Strategic Data Reporting:** Solvias delivered a modular reporting package that could serve multiple purposes — from IND submission to release testing setup. This ensured consistency of DAR data interpretation across departments —scientific, regulatory, and quality.

THE RESULTS

- Average DAR and distribution confirmed across all batches using orthogonal methods.
- Conjugation site patterns matched expectations, with no off-target modifications detected.
- Customer established a validated analytical toolkit suitable for IND, ongoing release, and long-term stability monitoring.

CONCLUSION

Drug-to-antibody ratio is more than a statistic — it's a critical lens through which ADC developers understand identity, consistency, and function. By combining HIC, LC-MS, and LC-MS/MS, Solvias helped the customer transform a basic measurement into a robust analytical platform. This solution delivered far more than an IND-ready data package: it enabled confident release testing, informed stability strategy, and provided a structural foundation to support future comparability needs. Whether at batch 1 or batch 100, the customer now has a DAR strategy built to scale — with clarity that regulatory authorities expect and product safety demands.

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- 800+ team members
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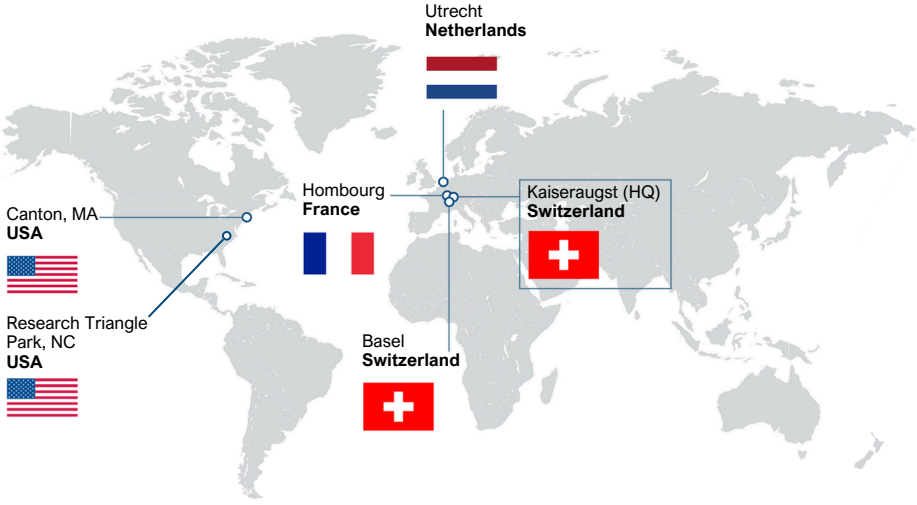
- 700+ customers worldwide
- 6 centers of excellence



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