ABZENA

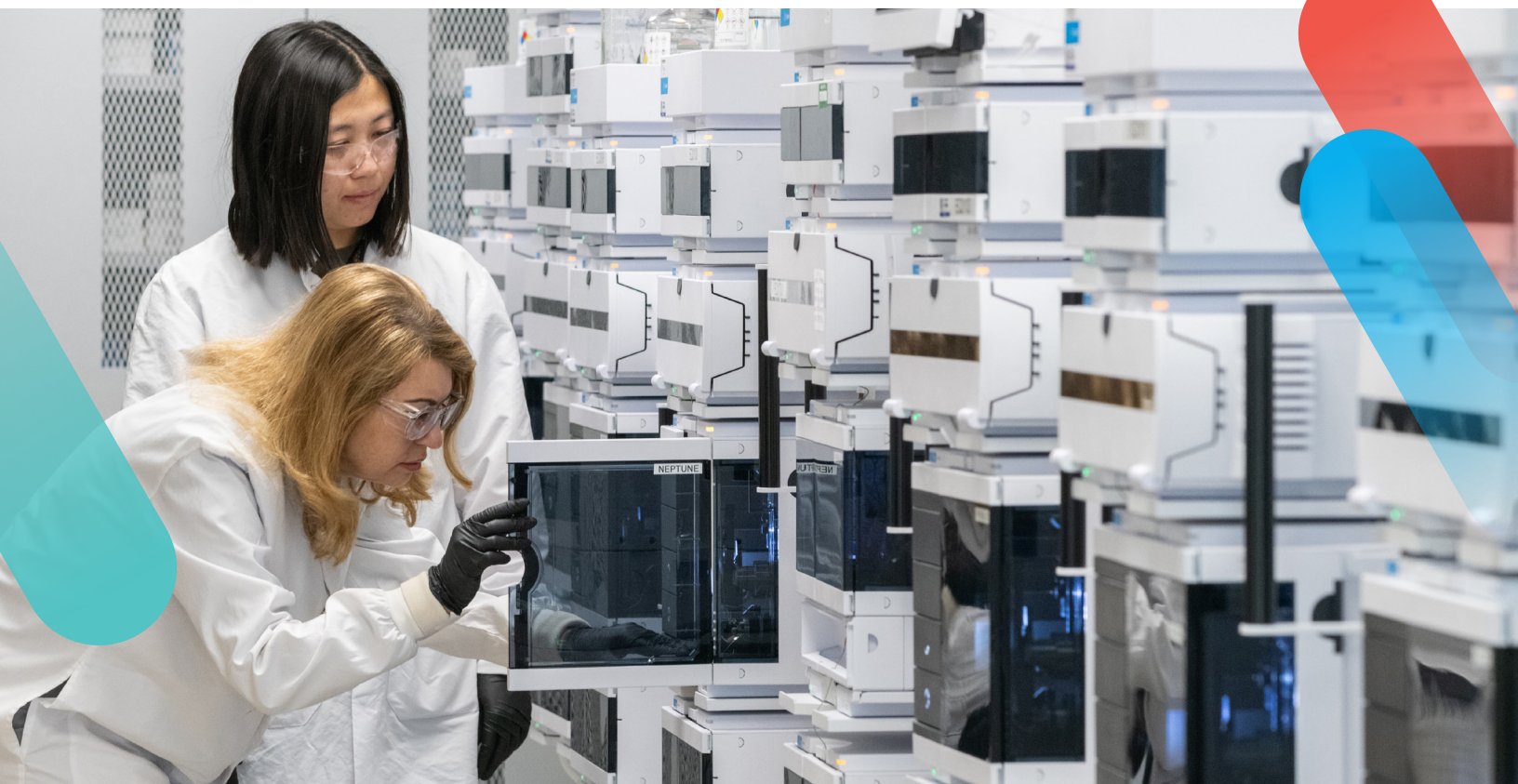
Analytical Method Development for Biologics & Bioconjugates

Developing robust analytical methods in the pre-clinical setting are often complicated by limited information about a therapeutic's properties and indistinct regulatory expectations. Unlike later stages of drug development, where a more comprehensive understanding of a molecule's key characteristics exists, early-stage programs typically face significant data gaps.

This uncertainty makes it challenging to design analytical methods that are sensitive enough to detect critical attributes yet specific enough to provide reliable results. Compounding this difficulty is the ambiguity surrounding early-phase method validation requirements, which can differ by therapeutic class, geographic region, and intended clinical application. Without clear guidelines, sponsors risk misalignment with regulatory expectations, which can cause setbacks that delay clinical trials and increase costs.

Establishing scientifically sound analytical methods early in development is vital to accelerating process optimization and meeting regulatory milestones. A robust set of phase-appropriate assays not only aids in identifying critical quality attributes and formulation challenges but also ensures a smoother transition into later development stages. Having high-quality, accurate data from the start drives more informed decision-making, reduces rework, and positions your therapeutic for success in subsequent toxicological and clinical studies.

At Abzena, we understand that efficient and compliant early-phase method validation can make or break a program. We excel in crafting targeted analytical strategies that align with current regulatory expectations, whether you're working with small molecules, biologics, or complex bioconjugates like ADCs. Our decades of experience allow us to tailor methods to the specific needs of your therapeutic to ensure each assay is fit-for-purpose and primed to evolve alongside your program.



Technical Expertise

Abzena provides a robust suite of analytical method development services designed to support every phase of your therapeutic program—from early discovery through clinical manufacturing and beyond. Our scientific rigor and deep expertise enable us to develop, qualify, and validate assays tailored to the unique requirements of biologics, ADCs, and other bioconjugates. By integrating state-of-the-art methodologies with a thorough understanding of regulatory expectations, we help ensure data accuracy, accelerate process optimization, and facilitate smooth transitions across development milestones.

Pre-Clinical Support

Analytical methods are essential to all aspects of pre-clinical drug development, informing critical decisions and de-risking subsequent development phases. Abzena's methods support:

- **Cell Line Development:** We use advanced screening assays to identify and select top-performing clones for robust protein expression. This includes measuring product titers, evaluating growth characteristics, and performing early-stage quality checks—such as glycosylation profiling or charge heterogeneity—to ensure each clone meets specific project goals based on the target product profile (TPP).
- **Upstream Process Development:** Our team applies real-time monitoring strategies to optimize cell culture performance. Using targeted analytical assays, we evaluate parameters such as nutrient utilization, metabolite accumulation, product yield, and process consistency.

- **Downstream Process Development:** We design analytical methods to assess purification efficiency (e.g., host cell protein quantification, aggregation analysis) and product purity at each downstream step. This ensures that any process adaptations—such as changes in chromatography conditions—are supported by reliable and actionable data.
- **Linker-Payload Synthesis (ADCs):** Analytical methods verify the linker's identity, monitor payload stability, and ensure optimal process conditions during ADC conjugation steps. Our advanced characterization capabilities help confirm the final product and detect potentially harmful impurities or byproducts.
- **Conjugation Process Development (ADCs and Bioconjugates):** We measure drug-to-antibody ratio (DAR), assess conjugation efficiency, determine conjugation site and evaluate product homogeneity through specialized chromatographic methods and advanced mass spectrometry. This data drives process refinements that maintain consistent yields, potency, and overall product safety, supporting a robust and scalable conjugation strategy.
- **Formulation Development:** Through stress testing, forced degradation studies, and excipient compatibility assays, we identify optimal formulation conditions to maintain stability, extend shelf life, and that are appropriate for the desired route of administration. Particle size analysis and charge variant profiling further confirm that the product remains within defined quality attributes.
- **Toxicological Batch Manufacturing, Release, and Stability:** We produce toxicology-ready batches under controlled conditions to support pre-clinical studies, ensuring consistent product characterization and safety. Specialized analytical methods monitor product integrity and stability, providing reliable data that informs early-stage development decisions and mitigates risk prior to clinical trials.
- **Clinical Manufacturing, Release, and Stability:** Abzena's GMP manufacturing infrastructure uses validated release and stability methods to support clinical trial material production. Routine testing of critical quality attributes (CQAs) ensures each batch meets stringent regulatory standards throughout clinical development.

By establishing stage-appropriate and fit-for-purpose analytical methods early, drug developers gain the data-driven insights needed to refine and scale processes efficiently - minimizing risk, reducing rework, and positioning programs for future success.

State-of-the-Art Analytical Techniques

Abzena employs cutting-edge instrumentation and methodologies to deliver comprehensive, high-quality data for manufacturing, release, and stability studies and in-depth characterization. Each technique is selected and customized based on the unique requirements of your product and the development stage.

Technique	Instrumentation	Capabilites
Chromatography	• (u)HPLC-UV • (u)HPLC-MALS • (u)HPLC-ELSD • (u)HPLC-FLR • (u)HPLC-RI	• Characterize molecular weight distribution, quantify product and process-related impurities, and confirm batch-to-batch consistency. • MALS (Multi-Angle Light Scattering) helps accurately determine molecular weight and oligomeric states without relying on calibration standards. • ELSD (Evaporative Light Scattering Detection) and RI (Refractive Index) are particularly useful for components with weak chromophores, but they are also broader in their applicability to different sample types. • Examples of analysis include SEC, HIC, RP-HPLC, peptide map, IEX, glycans, excipients (i.e. PS20/PS80), DAR, free drug, etc.
Mass Spectrometry Detection	• HPLC-SQM • HPLC-QQQ • HPLC-QTOF	• Offer sensitive and specific mass spectrometric analysis to detect minor variants, quantify payloads in ADCs, and identify post-translational modifications. • QTOF (Quadrupole Time-of-Flight) systems enable high-resolution mass measurements, which are critical for detailed product characterization and for verifying product integrity (e.g., clipped species, glycoforms, sequencing).
Binding and Function	• Flow cytometry and Biacore • ELISA • Multiple detection platforms	• Evaluate the potency, binding affinity, and functional activity of your biologic or bioconjugate, providing real-time data on how modifications or process changes affect therapeutic performance. • ELISA-based methods and SPR analysis using a Biacore help to quantify target binding or detect specific impurities (e.g., host cell protein, protein A), ensuring confidence in the therapeutic's safety and efficacy profile.
Molecular Biology	• qPCR	• Quantitation of the amount of residual host cell DNA present in a sample
Capillary Electrophoresis	• R/NR CE-SDS • icIEF	• Enables robust quantification of molecular weight variants and aggregates under reducing (R) and non-reducing (NR) conditions. • Aids in detecting fragmentation, host cell protein contamination, and other structural changes. • Profiles charge variants accurately, supporting batch release specifications and highlighting any unintended modifications. • Critical for monitoring post-translational modifications (e.g., sialylation) and ensuring product consistency.

Gas Chromatography (HS and Direct Injection)	• Headspace (HS) Analysis • Direct Injection	• Identifies and quantifies residual solvents, a critical safety attribute for small molecules and complex bioconjugates. • Facilitates in-depth analysis of volatile and semi-volatile impurities, ensuring product compliance with regulatory guidelines.
Spectroscopy	• FTIR (Fourier-Transform Infrared) • Raman • UV (ultraviolet)/SoloVPE	• Support raw material identity testing and rapid QC decisions. • Provide insights into secondary and tertiary structures, which help confirm protein folding integrity and detect chemical modifications. • UV analysis can be used to perform protein concentration determination and is also an option for DAR analysis.
Particle Characterization	• Dynamic Light Scattering (DLS)	• Measures particle size and polydispersity index, detecting early signs of aggregation or instability. • Invaluable for formulation optimization and stability testing across multiple stress conditions.
Compendial Testing	• Endotoxin • Bioburden • Appearance • pH • Osmolality	• Performed in accordance with USP/EP guidelines to confirm product sterility and safety. • Ensures that each batch meets essential microbial and physical quality requirements throughout development.

Summary

Throughout every stage of therapeutic development - from cell line selection to final release - robust analytical methods are vital to ensuring product quality, de-risking decisions, and meeting regulatory milestones. Abzena's integrated approach combines deep scientific expertise with advanced instrumentation to develop, qualify, and validate methods tailored to your molecule's unique needs.

By aligning these methods with ICH Q14 and ICH Q2(R2) guidelines, we generate data-driven insights that guide process optimization, mitigate risk, and facilitate smooth progress toward clinical and commercial success. Whether you are refining an upstream process, verifying payload attachment in ADCs, or conducting final release testing, Abzena provides the specialized support and technical rigor necessary for high-quality therapeutics that meet the evolving regulatory expectations.