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SPECIAL REPORT

Early Stage Analytical Considerations for Late Stage Biologics Success

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Analytical methods can be broken down into two categories: those for quality control (QC) release testing and those for product characterization. The former must be performed according to good manufacturing practice (GMP); the latter do not. But every method should be aligned with a drug product's life cycle. That comprises three periods: preclinical work toward an investigational new drug (IND) application, early clinical phases that ensure drug safety, and late clinical work that supports a biologics license application (BLA) and launches commercial efforts. Companies should consider goals, requirements, and documents during each period.

Quality Control Methods

Before IND submission, the goal is to produce a scientifically sound method. It should be fit for purpose based on an analytical target profile (ATP), and good documentation practice is the expectation. During phase 1–2 clinical trials, your goal shifts to gaining process and product knowledge. Analytical methods should be qualified as per GMP standards. Validation can be excessive considering that processes have not been locked down yet. Moving into phase 3 trials and commercialization, a company should have a well-characterized method with proven performance. Analytical methods at this stage require full validation with extensive evaluation of precision and robustness as well as ongoing assurance of quality and control. Documents still must adhere to GMP guidelines.

Method Development Elements: Analytical quality by design (QbD) always should go hand in hand with product QbD (**1**). Thus, analytical method development should begin with identification of critical product attributes and appropriate monitoring techniques. You should map out what to measure and when to measure it. For instance, you might consider using high-performance liquid chromatography (HPLC) to get a bird's-eye view of several product attributes (e.g., deamidation and oxidation levels) in one method. You might decide to measure one quality attribute independently (protein aggregation) depending upon process needs. Regardless, you should develop a risk-assessment strategy based on available clinical data and distinguish between which methods you "must have" and which ones are only "nice to have."

Method development also requires consideration of several interconnected activities (**1**):

Understanding ATPs: Building critical quality attributes (CQAs) into your product/process can yield information that helps define analytical method CQAs.

Considering Existing Methods: Modifying or replacing an existing method requires a bridging or comparability study.

Defining a Technique: It is critical to decide not only what to use (e.g., HPLC, capillary electrophoresis (CE), or mass spectrometry (MS)), but also how to monitor it to ensure appropriate outcomes.

Performing "Scouting" Experiments: You should evaluate different materials (chromatography buffers and columns) and parameters to build a method that survives the test of time. A method will be put in place throughout a product life cycle.

Selecting a Target: Determine where and how a method will be used in a manufacturing process (e.g., in-process or release, drug substance or product).

Qualifying or Validating a Method: Risk assessment is needed to identify method variables and parameters that might influence your ATP.

Defining Controls: Setting reference and control standards helps not only to monitor assays over time, but also to ensure that entire processes are consistent with current International Council for Harmonisation (ICH) guidelines.

Mitigating Time and Cost: All development activities should be weighed alongside these two considerations, and striking the right balance is key for successful development program.

The Avid Bioservices Approach: When a client asks us to develop a new method, we define a target and create an ATP based on attributes such as product identity, purity, potency, and quantitation. Then we select appropriate available platforms, accounting for what to measure and when (e.g., in-process or release testing). When clients request adaptation of their methods, we begin with risk assessment and product-knowledge transfer to anticipate gaps. It helps to know as much as possible about a molecule's structure, mechanism of action (MoA), heterogeneity, and potential impurities, including their bearing on clinical evaluation.

Such preparation leads into method development, qualification, and validation — or, in some cases, a combined qualification–validation approach based on predefined acceptance criteria. During development, we identify a method's strengths and weaknesses, identify key variables and operable regions, understand sample matrices (e.g., buffer and excipients), and establish a control strategy, including acceptance and method-performance criteria. Development of an HPLC method might involve sample considerations (target molecule stability, solubility, and pH), technical factors (the importance of specific columns, buffer gradients, and solvents as well as limitations in quantifying or resolving a critical impurity), and data-processing concerns (resolution criteria, acceptance ranges, and information integrability).

Robustness should be built into an analytical method early in development. Methods that lack robustness might pass initial testing but could fail when transferred to QC. That makes it critical to understand method limitations and parameters, often by testing them case by case. Again, setting proper controls and using reference standards appropriately helps you to understand method variations. Without appropriate controls, assays can lack in quality and require a lengthy, complex validation process.

Toward Validation: Method development and validation are inseparable, but often they are handled by different units. Research and development (R&D) teams establish methods and send them to QC units that sometimes work at other sites and have different expertise. All personnel must work as one unit during this process, and communication is key for success.

At Avid, our analytical development team performs both method development and validation activities, and we start training/transfer activities with QC during the prevalidation stage. That ensures clear knowledge transfer of methods and prevents loss of critical information. If development/validation activities are from different teams or sites, then we recommend using method transfer protocols. Such documents should define transfer scope, objectives, roles, and responsibilities across teams and sites. Protocols also should list and describe transferred materials, including lot numbers, storage conditions, and information about who is doing what with those materials. Transitioning from R&D to QC also can include comparability studies based on risk assessments performed before method transfer. At Avid, we are flexible and adapt to other approaches, including bridging studies between transfer partners, partial validation, and revalidation based on outcomes of risk assessments.

Understanding Method Limitations: Qualification and validation demonstrate that an analytical method is fit for purpose. Thus, it is important to understand a method's strengths and weaknesses during development. That helps a company develop controls that ensure the reliability of past and future data.

Another important study parameter to consider is the stability-indicating capability of an analytical method. Many samples are sensitive to room temperature during method execution. We must track that information, minimize sample exposure to room temperature, and set appropriate controls. Adding method instructions about how, where, and when to remove samples from a freezer can accomplish that.

Significant variations in assay results also come from intermediate precision, so it is critical to understand what is causing variations in a method. Differences in equipment across sites can be resolved by selecting one instrument type from a single vendor, if possible. Variations that come from analysts can be eliminated with additional training.

Validation Timelines: It is a common industry practice to categorize analytical methods based on their complexity. For example, Category 1 methods such as pH and UV measurements usually are simple and require a short timeline for development and/or verification and validation (roughly two to four weeks).

Category 2 assays are considered to be moderately complex in terms of scientific and technical expertise. For example, assays used for determining purity/impurity by HPLC and CE require a certain level of training and experience to execute. They usually take longer times for development and validation than Category 1 assays do (two to three months).

Cell-based potency assay methods, including those for antibody-dependent cellular cytotoxicity (ADCC) and cytokine release, are highly complex and commonly fall under Category 3. Such assays require specialized, method-specific cell banks and critical

reagents that are customized based on a target's MoA. Development and validation activities for these assays usually take a significant amount of time if started from scratch (one to two years). To overcome such limitations, laboratories are adapting to vendor-supplied prequalified assays (thaw-and-use kits).

Characterization Methods

Orthogonal analytical approaches often are applied during clinical testing to demonstrate the consistency of drug product used in preclinical and clinical studies. It is critical to demonstrate that a product's CQAs have been consistent throughout its life cycle. Characterization testing reveals a product's molecular heterogeneity and helps us understand the pattern recognition — something analogous to fingerprinting. Early adoption of comprehensive characterization techniques and deep understanding of product/process will ensure drug safety, purity, and potency profiles.

For instance, investigating a protein's higher-order structures yields important information about its interaction mechanisms. Being wrong about a drug's MoA will have potential impact on its efficacy and on patient safety. Below are some techniques for molecular characterization.

Primary Structure: electrospray ionization (ESI)-MS for intact mass, LC-MS for peptide mapping, and CE-SDS (sodium dodecyl sulfate)

Secondary Structure: circular dichroism (CD) and Fourier-transform infrared (FT-IR)

Higher-Order Structure: hydrogen–deuterium exchange (HDX)-MS, X-ray powder diffraction, fluorescence (FL) spectroscopy.

Functional assessments could include tests of equilibrium dissociation constants (using surface plasmon resonance, FL enzyme-linked immunosorbent assays (ELISAs), or kinetic-exclusion assays), ligand-binding assays (e.g., in vitro potency assays and competitive ELISAs), and cell-based assays.

The Evolution of Characterization: The focus of characterization efforts will change over a product's life cycle. Laboratory-scale viral clearance and toxicity studies are common before IND submission. At the preclinical stage, primary-structure analysis is required to determine the presence of possible sequence variants and amino acid misincorporation. In addition, limited R&D lot stability tests and forced-degradation studies can help reveal limitations in analytical methods. Sample availability could be a challenge at this early stage, and cost will prevent application of cutting-edge, high-resolution analytical methods. However, these early characterization assays are some of the most important ones to perform because they can mitigate risks incurred by future variations — and these phase-appropriate characterization studies expand in scope as you move further into a product's life cycle.

Lastly, extractables/leachables (E&L) studies often start during phase 1–2 clinical trials but become critical by phase 3. At this stage, your process should be locked down, and you should have gained enough knowledge about your product and process.

Consistency Is Key

The goal of manufacturing should be to consistently deliver high-quality product that reflects the materials used since early clinical trials. Kozlowski and Swann (**2**) showed that product quality depends on a triad of release testing, product/process characterization, and process control. If we imagine a product's life cycle as an iceberg, then QC forms just the tip — the most visible part. The middle of the iceberg reflects process/product attributes, which are revealed by extended characterization. Thorough characterization of product/process physiochemical attributes establishes a solid foundation for release testing and increases process control for consistent manufacturing of drugs. Process control is the iceberg's base. Improved controls can shrink that base and minimize unknowns. There will be unknowns learned over time, which will get updated into the product cycle by appropriate control strategies. Good understanding of Kozlowski and Swann's iceberg analogy will help in risk-mitigation strategies.

References

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About Avid Bioservices

Avid Bioservices is a dedicated contract development and manufacturing organization with nearly three decades of experience developing and manufacturing biopharmaceutical drug substances derived from mammalian cell culture. Avid's services include CGMP clinical and commercial drug substance manufacturing, bulk packaging, release and stability testing, and regulatory submission support. Avid also offers process development activities such as upstream and downstream development and optimization and analytical methods development, testing and characterization.

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