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Sample Analysis & Method Validation for Bioassays

An integrated approach
powered by analytics

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Introduction

Bioassays are quantitative procedures for measuring a drug's biological activity in biological samples. They are frequently employed in drug development and are essential for assessing the safety and efficacy of biopharmaceutical products such as monoclonal antibodies, vaccines, and gene treatments. Pharmacokinetic analysis and ADA analysis are two critical areas of bioassay creation and validation.

The assessment of medication concentrations in biological samples such as blood, plasma, or serum over time is known as pharmacokinetic analysis. It gives critical information about how a drug is absorbed, distributed, metabolized, and removed from the body. ADA analysis evaluates the immunogenicity of biologics, which refers to a drug's ability to elicit an immune response and create ADA in patients.

The challenge today is that many labs use analysis software that are not fully integrated with LIMS. Hence, there is a need for a lot of manual work for assay-related analysis, which requires significant effort and also remains vulnerable to human errors.

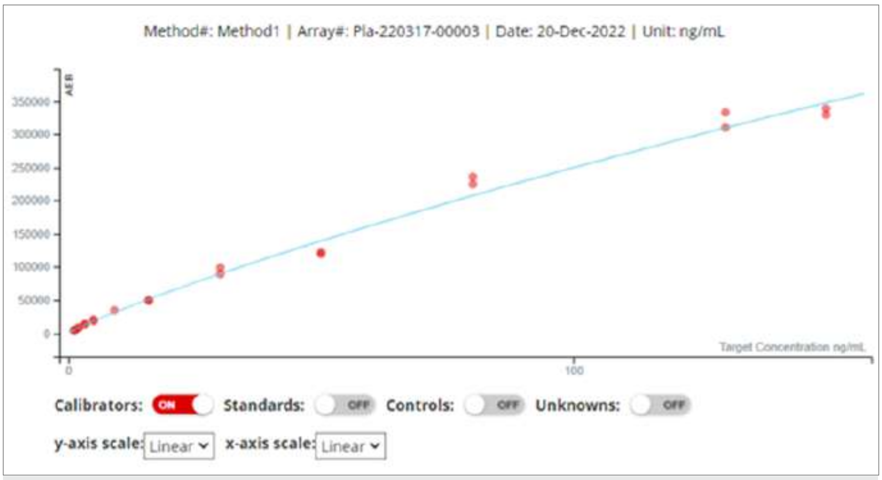
This whitepaper also explores the possibility and feasibility of an integrated solution that interfaces seamlessly with LIMS and provides all the statistical and mathematical models needed, goes a long way in eliminating this challenge, leading to significant cost savings and minimizing the possibility of human errors.

Sample Analysis for Pharmacokinetic Analysis

A calibration curve is a fundamental tool used in pharmacokinetic (PK) analysis to quantify the concentration of a drug in biological samples.

- It is a graphical representation of the relationship between drug concentration and the response of an analytical method, typically measured using a validated analytical technique such as LC-MS or ELISA.
- It uses linear or non-linear regression options (e.g. linear, 4PL, 5PL, quadratic, etc.)
- The residual weightage typically uses options such as 1/x, 1/x2, 1/y, 1/y2, and so on.
- The analyst applies various specifications to the outcome (usually related to CV and RE limits) and tags samples or plates as pass or fail

Standard Curve



A sophisticated analytics tool that offers options for various curve fitting models, allows seamless application of business rules, and integrates easily with LIMS, is of a huge advantage to lab analysts, boosting productivity and reducing the risk of manual errors

Method Development & Validation for Pharmacokinetic Analysis

The development and validation of the calibration curve is a crucial step in PK analysis to ensure accurate and reliable quantification of drug concentrations in biological samples. Method development involves selecting an appropriate analytical technique, optimizing parameters such as sample preparation, chromatographic conditions, and detector settings, and determining the linear range and sensitivity of the method.

The table below shows some of the relevant experiments for method validation for ADA

Accuracy

- The calibration curve should accurately quantify the drug concentration in biological samples.
- Accuracy is assessed by comparing the measured drug concentrations of reference samples to their known concentrations.

Dilution Linearity & Integrity

- To demonstrate that high concentrations of the analyte of interest can be accurately measured within the assay quantitative range upon dilution and multiplying the measured concentration by the dilution factor.
- To ensure that the obtained test results of diluted samples outside the validated range are directly proportional to the concentration of analyte of interest in the sample.
- Integrity of Dilution should cover the maximum anticipated concentration of analyte in sample assessment.

Precision

- The calibration curve should exhibit good precision, which refers to the repeatability and reproducibility of the measured response at different drug concentrations.
- Precision is evaluated by analysing replicates of reference samples and calculating the coefficient of variation (CV %) as a measure of precision.

Selectivity

- Assess the ability of the bio-analytical method to measure and differentiate the analyte in the presence of nonspecific matrix components that may be expected to be present, by analysis of at least 10 independent sources of blank matrix.

Robustness

- The calibration curve should be robust and should not be significantly influenced by small variations in experimental conditions such as changes in sample preparation, chromatographic conditions, or equipment.

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Sample Analysis for Anti-Drug Antibody Analysis

Immunogenicity analysis involves the detection and characterization of ADAs in patient samples using bioassays. Bioassays are functional assays that measure the biological activity of a bio therapeutic product and can be used to determine the potency and stability of the product. Immunogenicity analysis typically consists of three main steps: sample collection, ADA screening, and ADA confirmation.

Sample Collection - Sample collection determines the quality and integrity of the data obtained from bioassays. Patient samples, such as serum or plasma, are collected at various time points during the clinical trial or post-approval period. Samples should be collected using standardized procedures and stored under appropriate conditions to maintain their stability until analysis.

ADA Screening - ADA screening is the initial step in immunogenicity analysis and involves the detection of ADAs in patient samples using a screening assay. Screening assays are typically designed to be highly sensitive and capable of detecting low levels of ADAs. Positive samples identified in the screening assay are further confirmed using a confirmation assay.

ADA Confirmation - ADA confirmation assays are used to confirm the presence of ADAs in samples that tested positive in the screening assay. They are designed to be highly specific and capable of distinguishing between ADAs and other cross-reacting antibodies. They provide additional evidence of ADA presence and can help determine the titer, or level, of ADAs in patient samples.

ADA Titer - ADA titer refers to the level or concentration of anti-drug antibodies (ADAs) in patient samples, which can be quantified using validated bioassays. It provides information on the magnitude and persistence of the immune response against the drug and the data can be used to evaluate the impact of ADAs on the safety, efficacy, and pharmacokinetics of the bio therapeutic

ADA Neutralization - Neutralization refers to the ability of ADAs to neutralize the biological activity of the bio therapeutic product, which can result in reduced efficacy or increased safety concerns. They are designed to measure the functional impact of ADAs on the bio therapeutic product, and they are important in assessing the clinical relevance of ADA presence.

Method Validation for Anti-Drug Antibody Analysis

Method validation is an essential step in immunogenicity analysis to ensure that the bioassays used for ADA detection are accurate, reliable, and fit for their intended purpose. Some key considerations for method validation in immunogenicity analysis include:

Plate Analysis → Cut Point Calculations → Validity Negative Control → Sensitivity → LPC Calculation → Between Run Precision → System Suitability Controls → Selectivity → Selectivity (Drug Tolerance) → Hook Effect → Stability → Other Assessments

The table below shows some of the relevant experiments for method validation for ADA

Sensitivity and Specificity

- Sensitivity refers to the ability of the assay to detect low levels of ADAs.
- Specificity refers to the ability of the assay to accurately identify ADAs and distinguish them from other antibodies.
- Includes experiments to determine the LOD and the LLOQ of the assay, as well as specificity evaluations.

Precision and Accuracy

- Precision refers to the variability of results obtained from repeated measurements of the same sample.
- Accuracy denotes the proximity of the measured values to the true values.
- Perform intra-assay and interassay evaluations using spiked samples with known ADA levels or reference samples with established ADA status.

Selectivity (Drug Tolerance)

- It is expected that samples containing drug will exhibit interference due to competition for drug-specific antibodies.
- It is necessary to mix antibody samples with varying concentrations of study drug to assess how much drug is required to eliminate or reduce detection in the assay.

Hook Effect

- A high dose hook effect refers to falsely low responses or even false negative results caused by very high levels of the analyte in the sample.

Robustness and Stability

- Evaluations are important to assess the robustness of the bioassay to minor changes in assay conditions.

  Stability evaluations assess the stability of reagents, samples, and the assay performance over the intended duration of sample analysis.

A Solution for the **"Lab of Tomorrow"**

Conclusion

A sophisticated analytics tool that offers options for all the above analyses and integrates easily with LIMS (both reading sample data directly from LIMS and writing back the analysis outcome into LIMS for the analyst to take further action) is of a huge advantage to lab analysts, boosting productivity and reducing the risk of manual errors.

Labvantage Analytics (LVA) is an analytics module that comes integrated with Labvantage LIMS and offers a complete library of analytics capabilities along with the ability to apply assay specifications and options for visualizations and scenario creation.

- Seamless integration with LIMS, with the ability to read data directly from LIMS and write back the analysis outcome into LIMS for the analyst to take further action.
- Access to multiple curve fitting models (linear and non-linear) and options for residual weights, to generate the standards curve.
- Generation of run acceptance parameters and relevant reports.
- Support for defined user interactions for scenario creations.
- Calculation of pass/fail criteria applied at individual sample / well level as well as plate level.
- Covers analysis screening assays, confirmatory assays, titer assays, and neutralizing assays.
- Supports both parametric & non-parametric analysis for immunogenicity cut-point computations.
- Ability to validate methods by supporting the analysis for the relevant validation experiments with acceptance criteria.

The use of the integrated approach has been yielding tremendous benefits for organizations –

Upto **50%**	Reduction in cost and time for analysis and validation for a leading bio-tech company
Upto **25%**	Expected cost savings from manpower efficiency and reduced cost of rework for a leading bio-tech company
Upto **20%**	Expected cost savings from manpower efficiency and reduced cost of rework for a big pharma

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