Application guide

Biacore™ SPR system assay validation



cytiva

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01

Purpose

This guide provides practical, regulation-aligned recommendations for developing, qualifying, and validating Biacore™ SPR assays for use in analytical development and GxP-regulated quality control environments. The goal is to help users design robust assays, establish system suitability, and perform validation according to ICH Q2(R2), ICH Q14, and regional regulatory expectations (1).

02

Introduction

Validation is the process of demonstrating that an analytical assay consistently delivers results that are accurate, reliable, and suitable for its intended purpose. A well validated assay provides confidence that the data generated are reproducible over time and robust enough to support scientific, development, and quality related decisions. For many applications, particularly in regulated environments, assay validation is not only best practice, but a formal requirement before the assay can be used for development activities, product characterization, and decision making.

In biomolecular interaction analysis, validation is especially important due to the high sensitivity and complexity of the underlying surface plasmon resonance (SPR) technology. Small changes such as assay conditions, surface chemistry, and reagent quality can influence results if they are not well controlled. SPR is widely used in quality control and product characterization because it provides direct, label free measurements of binding activity that reflect the native behavior of biomolecules. SPR data has been included in regulatory submissions for many licensed biologics, underscoring regulatory confidence in SPR technology when assays are properly developed and validated.

Biacore SPR system, together with Biacore Insight Software, support traceable data handling, automated workflows, and analytical consistency required for late stage development, quality control, and release testing (Fig 1). This application guide provides practical recommendations for validating SPR based assays for potency, concentration, kinetics, affinity, and comparability assessments. The guidance is aligned with current ICH recommendations and is intended to support the development of robust, well controlled analytical methods in regulated environments.

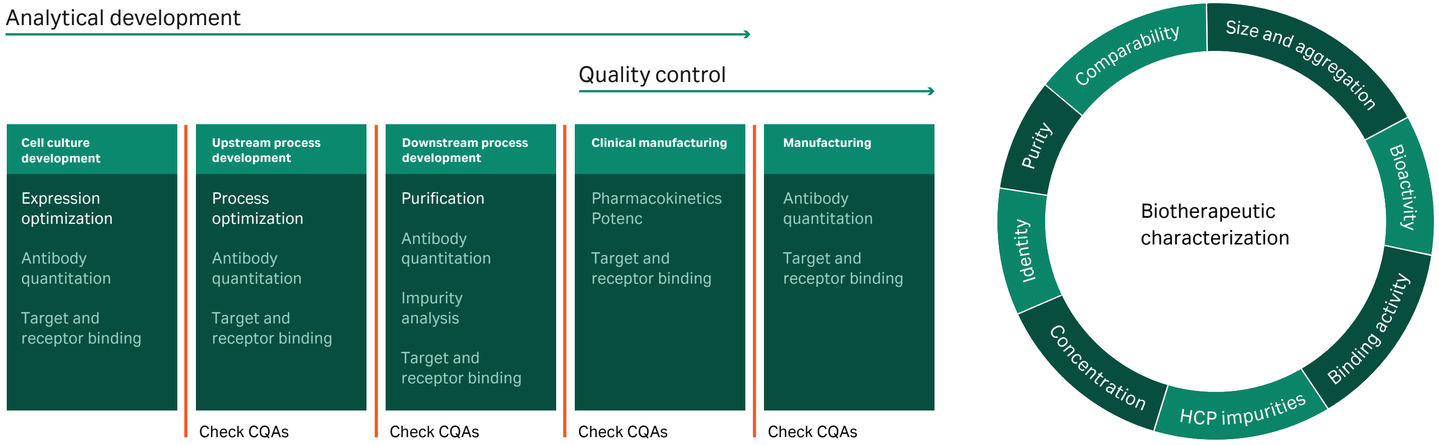

Fig 1. Biacore SPR system supports the entire drug development process from basic research, through the analytical development phase, to late-stage development with quality control and release testing.

03
Terminology

Term	Meaning
Assay validation	The task that ensures accuracy, precision, reliability, quality, and overall integrity of an analytical procedure.
GxP (Good x practice)	A broad term for quality guidelines and regulations in pharma and life sciences ensuring products are safe and effective, and data is reliable. The “x” is a variable and can be replaced with M for manufacturing (GMP), C for clinical (GCP), or L for laboratory (GLP), each covering specific operational areas.
Drug substance (DS)	The active pharmaceutical ingredient (API) in a drug responsible for the therapeutic effect
Drug product (DP)	The final formulated form of a drug (e.g., tablet, capsule, injection) containing the API plus excipients.
Pharmacopeia	An officially recognized (and often legally enforceable) reference book that contains standards, quality specifications, and analytical methods for medicines, drug substances, and pharmaceutical preparations, aimed at ensuring the quality of medicines and thereby supporting their safety and efficacy. Examples include the European Pharmacopoeia (Ph. Eur.) and the United States Pharmacopeia (USP) (5, 6). Pharmacopoeias typically provide detailed and sometimes prescriptive methods (with allowance for equivalent alternatives), whereas ICH guidelines offer broader, harmonized regulatory principles.
21 CFR part 11 compliance	A definition of criteria under which electronic records and signatures are considered trustworthy, reliable, and equivalent to paper records. It requires industries like pharmaceuticals, biotech, and medical devices to implement secure, time-stamped audit trails, system validation (IQ/OQ/PQ), and restricted access controls to ensure data integrity. 21 CFR compliance is mandated by FDA.
Installation qualification (IQ)	A documented, mandatory verification process in regulated industries (e.g., pharma, medical devices) that confirms equipment, systems, or software are installed, assembled, and configured according to manufacturer specifications and design requirements.
Operational qualification (OQ)	A critical validation step in regulated industries (pharma, medical devices) that verifies equipment, software, or systems function according to intended operational specifications, typically following Installation Qualification (IQ).
Performance qualification (PQ)	A documented, evidence-based verification that equipment, systems, or processes consistently operate according to approved specifications under routine conditions.
Initial performance qualification (IPQ)	A test that verifies that the system functions according to its operational specifications under conditions like those utilized by the user. IPQ enables regular assessment and verification of the system’s performance with an independent test kit developed specifically to meet qualification standards.
Totality of evidence	A regulatory and scientific framework, primarily used by FDA for approval of biosimilars. This approach requires a comprehensive evaluation of all available data rather than relying on a single study.
Comparative efficacy studies (CES)	Studies designed to confirm that a proposed biosimilar has no clinically meaningful differences in efficacy, safety, and immunogenicity compared to its reference product.
Comparative analytical assessment (CAA)	Comparison of a proposed biosimilar to its reference product, ensuring they are highly similar in structure, function, purity, and potency through the assessment of critical quality attributes. The purpose of CAA is to minimize the need for extensive clinical trials.
Limit of detection (LOD)	The lowest concentration of a substance that can be reliably distinguished from background noise but not necessarily quantified with precision.
Limit of quantitation (LOQ)	The lowest concentration of a substance that can be measured with acceptable accuracy and precision.
International Council for Harmonization (ICH)	An international body that develops harmonized guidelines for the pharmaceutical industry to ensure the quality, safety, and efficacy of medicinal products across different regions (e.g., EU, US, Japan).

04
Regulatory guidelines and expectations

Analytical methods used in drug development and manufacturing are governed by official industry guidelines that define the requirements for product release. These guidelines aim to ensure patient safety, product quality, and consistent performance by setting expectations for facilities, equipment, analytical procedures, data integrity, and documentation. Assays used for release testing, stability studies, and comparability assessments must be demonstrated to be suitable for their intended purpose through structured development and validation.

For SPR based assays, these regulatory expectations translate into the need for controlled assay design, qualified systems, defined system suitability criteria, and documented validation of assay performance over time (Fig 2).

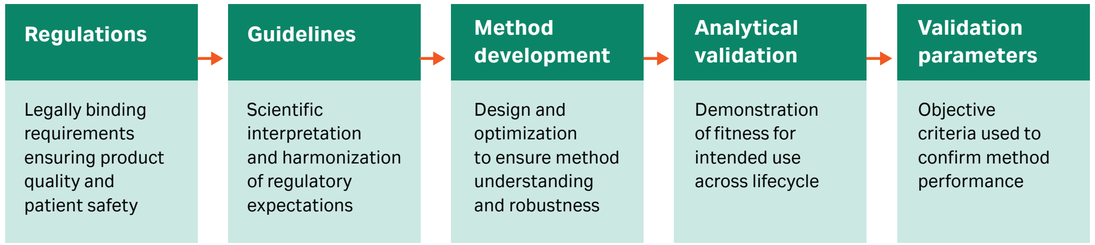

Fig 2. Regulatory-aligned analytical validation workflow.

4.1 ICH guidelines: Regulatory expectations

The International Council for Harmonization (ICH) develops globally harmonized scientific and technical guidelines that are adopted by regulatory agencies worldwide. Although ICH itself is not a regulatory authority, its guidelines form the foundation for regulatory expectations in regions such as the US, Europe, and Asia. Relevant ICH guidelines are organized into Quality (Q), Safety (S), Efficacy (E), and Multidisciplinary (M) categories.

For analytical methods, the most relevant guidelines are ICH Q2(R2), ICH Q14, and ICH M10. Together, they define how analytical procedures should be developed, validated, and maintained throughout their lifecycle.

What this means for Biacore SPR assays

- SPR assay development and validation should follow globally harmonized principles to reduce the need for region specific assay redesign.
- A risk based approach can be applied to SPR methods, with the level of validation matched to assay purpose (e.g., QC release vs. characterization).
- SPR methods for potency, concentration, kinetics, affinity, or comparability can be positioned clearly within an ICH aligned validation framework.

4.2 ICH Q2(R2): Validation of analytical procedures

ICH Q2(R2) defines the validation characteristics required for analytical procedures included in regulatory submissions. It applies to assays used for release and stability testing of drug substances and drug products, both chemical and biological. The guideline describes required validation parameters such as specificity, accuracy, precision, linearity, range, robustness, and, where relevant, LOD and LOQ.

What this means for Biacore SPR assays

- SPR assays must have clearly defined validation parameters based on their intended use (e.g., potency vs. kinetics).
- Validation studies should demonstrate that the assay produces reliable and reproducible results under routine operating conditions.
- Acceptance criteria for parameters such as accuracy, precision, and linearity should be predefined, justified, and documented.
- System suitability testing forms a critical part of demonstrating continued suitability of the SPR method.

4.3 ICH Q14: Analytical procedure development

ICH Q14 describes science and risk based approaches to analytical procedure development and lifecycle management. It emphasizes understanding of method variables, control strategies, and robustness to ensure that analytical procedures remain suitable throughout product development and manufacturing.

ICH Q14 complements ICH Q2(R2) by focusing on how methods are developed before validation.

What this means for Biacore SPR assays

- SPR assay development should generate process understanding, such as the impact of ligand density, contact time, regeneration conditions, and matrix effects.
- Development data can be used to justify validation design and acceptance criteria.
- Robustness testing during development reduces the risk of validation failure and simplifies any changes of the method in later phases.
- SPR specific parameters (e.g., surface stability, mass transport effects, RI effects) should be addressed early.

4.4 ICH M10: Bioanalytical method validation

ICH M10 harmonizes global requirements for bioanalytical method validation, particularly for ligand binding and functional assays supporting pharmacokinetic, pharmacodynamic, and immunogenicity assessments. The guideline focuses on data reliability, reproducibility, and consistency across laboratories and regions.

What this means for Biacore SPR assays

- SPR assays used in bioanalytical or functional contexts should demonstrate consistent performance across analysts, runs, and instruments.
- Validation parameters such as precision, accuracy, and specificity should reflect biological variability where appropriate.
- Standardized workflows and well controlled system performance are particularly important when SPR data supports clinical or biosimilar programs.

4.5 Regional regulatory guidelines

Regional agencies such as the FDA, EMA, NMPA, and PMDA adopt and implement ICH guidelines within their local regulatory frameworks (Table 1). While regional requirements may differ in emphasis or documentation detail, they are largely built on the ICH foundation to ensure global consistency and avoid redundant testing.

Table 1. Examples of regulatory bodies in various regions of the world

Name	Acronym	Used in
The Food and Drug Administration (2)	FDA	USA
The European Medicines Agency (3)	EMA	Europe
The National Medical Products Administration	NMPA	China
Pharmaceutical and Medical Devices Agency	PMDA	Japan
Health Canada	-	Canada
Central Drugs Standard Control Organization	CDSCO	India
Therapeutic Goods Administration	TGA	Australia

What this means for Biacore SPR assays

- SPR assays validated according to ICH principles are generally acceptable across multiple regions.
- Regional expectations may influence documentation depth, data presentation, or ongoing performance monitoring.
- Using standardized Biacore SPR system, qualified software, and harmonized validation practices support global assay deployment and technology transfer.

05
Integrating SPR into GxP-regulated workflows

Integrating SPR into GxP regulated environments is essential to ensure that data generated across the product lifecycle is accurate, traceable, and compliant. GxP frameworks require strong control of data integrity, method consistency, and system performance. SPR platforms used in these environments must therefore demonstrate reliable operation, controlled workflows, and documented validation status.

In regulated settings, SPR assays are commonly applied to characterize critical quality attributes (CQAs), support comparability and biosimilarity studies, and monitor batch to batch consistency of biologics. When properly qualified and validated, SPR assays enable confident decision making while meeting regulatory expectations for data integrity, method validation, and lifecycle control. The sections below describe how key GxP principles apply specifically to SPR workflows, focusing on reagent qualification, system and software qualification, and ongoing performance verification.

5.1 Qualification of reagents

In GxP regulated SPR assays, reagents should be classified as critical or non critical based on their potential impact on assay performance and data integrity.

- Non critical reagents typically include running buffers, coupling reagents, and regeneration solutions.
- Critical reagents include the ligand and analyte in direct binding assays. In capture based formats, the capture molecule is also considered critical. Likewise, inhibitors used in competition or inhibition assays should be treated as critical reagents.

All critical reagents must be qualified and requalified throughout the assay lifecycle to ensure consistent performance.

Reagent qualification typically includes:

- Sourcing from qualified or well established suppliers.
- Retaining and reviewing certificates of analysis.
- Verifying identity, activity, and suitability through functional testing.
- Performing parallel testing of new reagent lots against an established reference lot.

Note: In Biacore terminology, the ligand is the interactant coupled to the sensor surface, and the analyte is the interactant injected in solution and passed over the ligand. The term sample typically refers to the injected analyte. However, in certain applications, such as capture-based antibody screening, the capture injection can also be referred to as the sample. In these assays the capture solution is varied, whereas the analyte remains constant.

5.2 Qualification of SPR equipment and software for use in GxP applications

SPR assays used in GxP-regulated workflows must be conducted in a controlled environment and supported by features such as audit trails, electronic signatures, controlled documentation, and validated system functionality. These measures safeguard product quality, patient safety, and regulatory compliance throughout the lifecycle of the regulated products, for confident decision making in clinical or commercial applications.

All system components, including the software that operates the instrument, must meet the requirements of 21 CFR Part 11. Achieving compliance typically involves completing installation qualification (IQ), operational qualification (OQ), and performance qualification (PQ) before introducing the system into any regulated process.

To maintain accurate biomolecular interaction measurements, routine performance checks (such as verifying optical detector function with a sucrose solution) should be supplemented with assay relevant tests. Cytiva's Initial Performance Qualification (IPQ) kit is one option, designed to confirm that the instrument performs as expected for its intended application.

Instrument performance and data integrity must be reassessed and documented at regular intervals throughout the system's operational life. Although the frequency depends on usage, requalification should generally take place at least every six months. Monitoring results over time helps identify trends or performance drift that may signal emerging instrument issues.

Software validation is also essential for analytical instruments used in regulated environments. Application software should be developed and verified according to a documented development model, and vendors are expected to provide transparency into their processes, including audit support and evidence that both hardware and software comply with established development and quality standards.

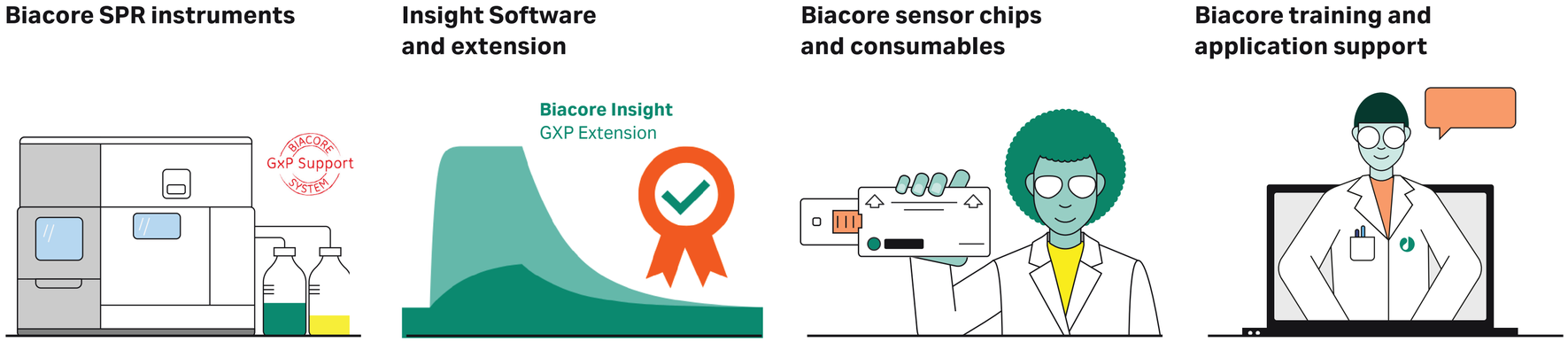

Fig 3. Biacore GxP support tools include SPR instruments, software, and assay reagents, backed up with training and application support.

Table 2. Key components of Biacore GxP package.

Regulatory compliance and documentation	Validated tools and software	GxP services and support
Instrument and software compliant with regulatory expectations	Design-validated Biacore Insight Software with full 21 CFR part 11 compliance	IQ/OQ/IPQ services performed by GxP-trained personnel
Certificate of hardware or software conformance	Extensions and tools that simplify SPR assay setup and ensure reliability	Comprehensive guidelines for GxP users
System assessment report for qualification	Stringent production methods and quality control for sensor chips and other consumables	Online validation training courses and free support materials
Change control protocols for hardware, software, and consumables		Application support and training to ensure smooth implementation

06
Assay validation framework

Assay validation defines and demonstrates that the SPR method is fit for its intended use. For regulated analytical workflows, validation is not a single event, but a lifecycle activity that begins during assay development and continues throughout routine use, maintenance, and ultimately system retirement.

A structured validation framework ensures that SPR assays generate reliable, reproducible, and decision ready data across different analysts, instruments, and time points. A robust validation strategy typically includes the following elements (Fig 4):

- **Defining the validation scope and intended use**
  Clearly describe the purpose of the SPR assay, such as QC release testing (kinetics, affinity, concentration), stability testing, or comparability assessment, and identify the relevant critical quality attributes (CQAs).
- **Establishing controlled documentation**
  Prepare and approve validation protocols, predefined acceptance criteria, and validation reports to ensure traceability and regulatory compliance.
- **Executing appropriate validation experiments**
  Perform experiments and data analysis that demonstrate assay performance for parameters such as specificity, accuracy, precision, linearity, range, robustness, and system suitability, as applicable
- **Aligning with regulatory expectations**
  Ensure that the validation approach is consistent with applicable guidelines, such as ICH Q2(R2) for analytical procedure validation, and supported by principles outlined in ICH Q14 and ICH M10, where relevant.

Lifecycle and phase appropriate validation

SPR assay validation should follow a phase appropriate and risk based approach. The validation effort evolves as the assay moves from early development to routine QC use.

- **Early development phase:**
  Focus on assay understanding, robustness screening, and feasibility to support method selection and development.
- **Late stage development and quality control:**
  Apply full validation with defined acceptance criteria, formal documentation, and system suitability testing to support regulated use.

The most critical decisions are made early, during assay planning and development, when method design choices determine validation success. Over investing too early may limit flexibility, while under investing increases the risk of late stage rework. A balanced, phase oriented strategy ensures efficient development while maintaining regulatory readiness.

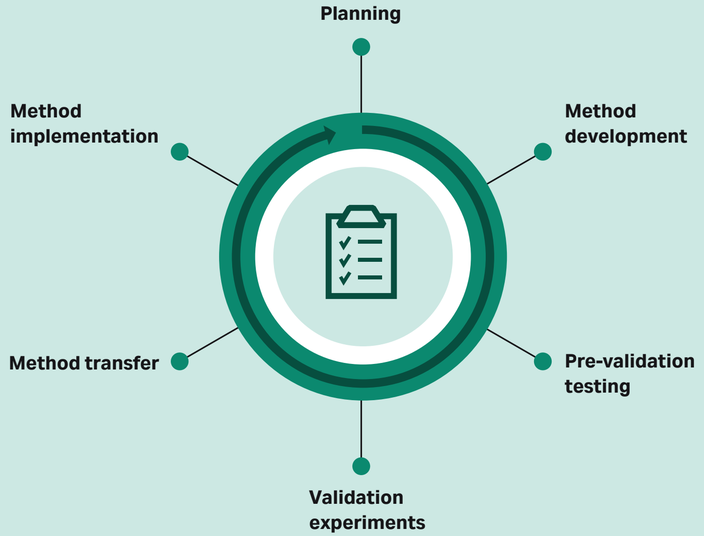

Fig 4. Workflow for Biacore method development and validation.

07
Developing an SPR assay ready for validation

A successful assay validation starts with a well developed and well understood SPR assay. In regulated environments, assay development is not separate from validation. It is the foundation that defines how validation can be designed, justified, and successfully executed. Development work should demonstrate that the assay is stable, controlled, and fit for its intended purpose before formal validation begins.

Key development steps that prepare an SPR assay for downstream validation are outlined to the right.

7.1 Define ligand or capture level

Selecting an appropriate ligand or capture level for the application is a critical early step, as it directly impacts sensitivity, precision, and data quality (Fig 5).

- For kinetics assays, low ligand densities are essential to reduce the risk of mass transport limitation and rebinding. A practical target is a theoretical R_{max} of 10–30 RU for the analyte. For calculation of suitable ligand level to obtain theoretical R_{max} of 10–30 RU see Figure 6.
- For calculation of suitable ligand level to obtain a theoretical Rmax of 10–30 RU see Figure 6.
- For affinity measurements using steady state analysis, ligand, or capture levels do not need to be kept low, as steady state responses are not influenced by mass transport limitation. Higher levels may be used to ensure measurable responses at low analyte concentrations.
- Small molecule (LMW) assays typically require higher ligand levels due to their size and inherent low signals.
- For specificity, potency and concentration assays, ligand density is less critical but should be sufficiently high to reliably measure the lowest required analyte concentrations.



Fig 5. Appropriate ligand levels for various Biacore applications.

$$R_{ligand} = \frac{R_{max}}{n} \times \frac{MW_{ligand}}{MW_{analyte}}$$

Term	
R_{ligand}	Ligand attachment level (RU)
R_{max}	Theoretical maximum analyte binding capacity (RU)
MW	Molecular weight
n	Interaction stoichiometry (analyte/ligand)

Fig 6. Calculation of suitable ligand level to obtain a theoretical R_{max} of 10-30 RU

7.2 Establish baseline stability

A stable baseline is essential for reliable SPR measurements and reproducible validation results.

- The ideal baseline shows minimal noise and no drift.
- A slight downward drift is typically more acceptable than an upward drift, which may indicate surface instability or buffer or regeneration issues.

7.3 Optimize analyte contact times

Analyte contact times should be matched to type and purpose of the assay.

- For kinetics experiments, the association phase should be long enough to show curvature, with the highest analyte concentrations ideally approaching saturation.
- Dissociation time should preferably allow at least a 5–10% decrease in binding response to support reliable dissociation rate constant determination.
- For steady state affinity analysis, it is critical that injections reach steady state during the association phase. Using non steady state data will underestimate affinity.
- For potency and concentration assays, contact times are generally less critical but should be long enough to achieve reproducible response levels.

7.4 Define analyte concentration range

The concentration range must be appropriate to the assay objective.

- For kinetics and affinity assays, analyte concentrations should ideally span 0.1 to 10× the expected K_D of the interaction.
- For concentration based assays, the calibration curve should cover the full expected analyte range, including the lower quantitation limit.

7.5 Define sample injection strategy

Sample run order can influence assay results if not controlled.

- For most assay types, inject samples in random order to avoid systemic bias.
- For kinetics and affinity assays, inject analyte concentrations from lowest to highest to minimize carryover and surface artifacts.

7.6 Define fitting model for kinetics and affinity assays

Choice of fitting model and the quality of the fit strongly influence the reliability of the results.

- Ensure the selected fitting model is appropriate for the interaction to be studied.
- Assess the quality of the fit by using QC parameters supplied by the software, such as Chi^2, U-value, and T-values.

7.7 Minimize bulk refractive index effects and assess specificity

Differences in refractive index (RI) between samples and running buffer can introduce bulk shifts and bias results.

- Whenever possible, dilute samples and standards in running buffer.
- If matrix matching is not feasible, assess and monitor RI mismatches and their effect on assay performance.
- Confirm that binding is specific by evaluating responses on the reference surface.
- Assess non specific binding to the ligand by injecting blank samples (e.g., sample matrix without analyte).

7.8 Establish effective regeneration conditions

Regeneration conditions must remove bound analyte completely while preserving ligand activity.

- Evaluate regeneration by performing repeated analyte injections at relevant concentrations.
- Monitor both binding responses and baseline recovery to confirm regeneration efficiency and surface stability.
- The same principles should be applied to capture-based assays.

7.9 Assess biological plausibility and assay design

Ensure that kinetic and affinity parameters are realistic and biologically meaningful.

- Compare derived rate constants with expected interaction behavior.
- Use simulation tools, such as Biacore Simul8 (6), to evaluate experimental design and identify potential artifacts before validation.

An SPR assay is ready for validation when its critical parameters—ligand level, baseline stability, contact times, concentration range, regeneration, and specificity are understood, controlled, and documented. Development data should directly inform validation design, acceptance criteria, and system suitability testing for a smooth transition from assay optimization to validation.

7.10 System suitability testing

System suitability testing (SST) is a mandatory pre-analysis check in pharmaceutical and analytical labs to ensure that the entire system (instrument, reagents, operator, etc.) is functioning properly to produce reliable and accurate results. System suitability criteria should be established so results obtained for the test sample fall within the operating parameters. The amount of testing required will depend on the purpose of the assay. In practice, each assay submitted for validation should include an appropriate number of system suitability tests defining the necessary characteristics of that system.

System suitability testing should be carefully conducted and evaluated for SPR-based assays. To establish criteria for an SPR assay multiple batches of ligand, analyte, coupling reagents, and sensor surfaces should be used. It is recommended to track assay results for both reference standard and QC samples over time. For example, trending analyses could be performed to make sure that the assay remains in control. To enable tracking, it is recommended to include the reference standard in all assays runs. Examples of system suitability parameters are listed in Table 3.

Table 3. Parameters for system suitability testing for SPR assays

Parameter	Testing
Ligand or capture levels	Monitor ligand or capture levels. Variation between runs should typically not be larger than 20-30%.
R_{max}	The experimental R_{max} should be within a certain percentage of the theoretical R_{max}, which reflects a functional and active surface.
Sensor surface stability	Monitor sensor surface stability by running known control samples at regular intervals throughout the experiment to detect any deterioration in system performance over time.
Baseline stability	Monitor noise and drift in the baseline before injection. An unstable baseline may indicate air bubbles, temperature fluctuations, poorly filtered buffer, or assay drift.
Response on reference surface	The reference surface should show minimal binding. A high or shifting response indicates non-specific binding.
Reference material	Check life length and precision of reference material.
Precision	Check precision of system by performing replicate injections of a standard solution with a known concentration.
Regeneration efficiency	Confirm that the regeneration solution restores the baseline to its original level without damaging the coupled ligand.
Visual evaluation of sensorgrams	Inspect the shape of the binding curves to identify deviations that may indicate aggregation or instrument issues.

08
Validation parameters and acceptance criteria

This chapter provides an overview of the key characteristics of analytical validation parameters as defined by the ICH guidelines. It outlines the purpose and application of each parameter to establish a clear foundation for understanding how they contribute to the reliability, accuracy, and overall quality of analytical methods.

8.1 Specificity and selectivity

Confirm that the assay specifically measures the analyte of interest, even in the presence of impurities, degradants, or complex matrices (e.g., serum). Also verify that the analyte itself does not interfere with other components in the process.

Demonstrate that there is no non-specific binding to the sensor surface.

For in process control, assess specificity and selectivity by testing samples spiked with relevant process contaminants (e.g., HCP, Protein A). Include an appropriate specificity control, for example an alternative antibody or an antibody lacking the binding domain for antigen binding assays. For Fc receptor binding assays, use a commercially available Fab drug or an appropriate isotype control. Stressed samples (e.g., heat-, pH-, or oxidation stressed) can also be used to demonstrate assay specificity and selectivity.

8.2 Linearity and range

Working sample concentrations and samples tested for accuracy should be in the linear range of the assay. The regression coefficient R2 is typically expected to be > 0.95. The linearity range to be examined depends on the purpose of the test method. The correlation coefficient, y-intercept, slope of the regression line as well as residual sum of squares are all information that normally should be submitted to health authorities. For classical potency assays relative binding range is usually 70-130%.

8.3 Accuracy and precision

Accuracy and precision are both crucial parameters to assess during an assay validation. Accuracy measures how close results are to a true or accepted value, while precision measures how close measurements are to each other (Fig 7). They are independent parameters and, thus, you can have high precision but poor accuracy and vice versa.

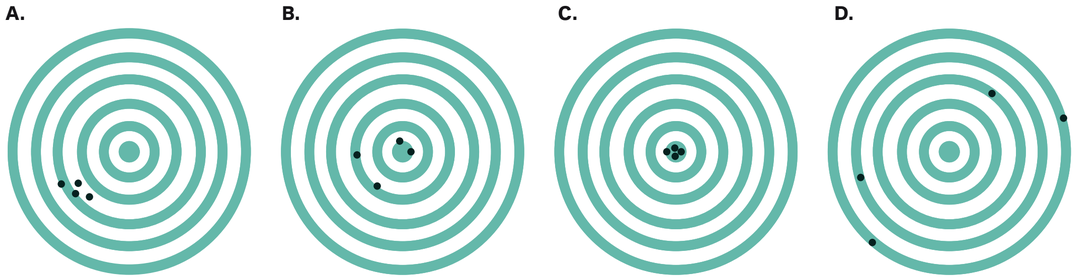

Fig 7. The difference between precision and accuracy. a) precise but inaccurate measurements, b) measurements with high accuracy but low precision, c) accurate and precise measurements, d) measurements with low accuracy and low precision.

Accuracy studies for drug substance and drug product are recommended to be performed from 70 to 130% of label claim. Evaluation of accuracy for the drug product is often performed by adding known amounts of drug (weight or volume) to the buffer/sample matrix within the linear range of analyte detection. Replicate samples should be run and evaluated at each concentration. The %CV of each concentration is calculated, and each run is then repeated on more than one occasion. The mean of %CV of each run, expressed as % label claim, indicates how accurate the test method is. A typical value for accuracy is +/- 20% of the expected value.

Precision can be determined at three different levels: repeatability, intermediate precision, and reproducibility.

Repeatability

Repeatability, or intra-assay precision, expresses the precision under the same operating conditions over a short interval of time, typically within a single run. Repeatability can be further divided into two components:

Injection repeatability

Injection repeatability refers to the ability of a system to deliver consistent, identical results over multiple consecutive cycles under the same conditions by delivering consistent volume of sample. This is typically part of the system suitability testing, for example using Cytiva's IPQ kit. As part of the assay validation, a minimum of six injections (two samples in triplicate) is recommended with a targeted %CV of <15.

Analysis repeatability

Analysis repeatability refers to the whole system and is assessed through multiple measurements of a sample by the same analyst under the same analytical conditions and is expressed in %CV. For practical purposes, it is often combined with accuracy and carried out as a single run. Recommendations for analysis repeatability are to have at least three samples of 100% and use a fourth as a QC sample and run each sample in groups of six.

Intermediate precision

Intermediate precision, or inter-assay precision, is a measure of the precision of the method in a different environment from that used during method development. The objective of this test is to ensure that the method will provide the same results once the method development phase is over. Depending on time and resources, intermediate precision can be evaluated on multiple days, analysts, and instruments. Intermediate precision in the test method can be partly assured by good system suitability specifications. Thus, it is important to set tight, but realistic, system suitability specifications. Recommendations for evaluating intermediate precision is to evaluate accuracy at least on three separate occasions. It is strongly recommended to indicate the intermediate precision of the test method in an application.

Reproducibility

As defined by ICH, reproducibility expresses the precision between laboratories as in collaborative studies. Multiple laboratories are desirable, but not always attainable. Evaluation of reproducibility is not normally expected.

8.4 Limit of detection (LOD) and limit of quantitation (LOQ)

LOD is the lowest concentration of analyte in a sample that can be detected, but not necessarily quantitated, under the stated experimental conditions. LOQ is the lowest concentration of analyte in a sample that can be determined with acceptable precision and accuracy under the stated experimental conditions. LOD and LOQ should be accurately evaluated for concentration-based assays but are not required for affinity, kinetics, or potency assays. It is only essential for calibrated assays used for detection of, for example, impurities in the drug product.

Several blank samples, typically 20, are run and assessed in relation to the calibration curve. Make sure the blank samples are representative of the sample matrix (e.g., buffer with serum, cell lysate) to account for background noise. Also prepare and run low concentration samples to identify the lowest detectable concentration. Calculate the standard deviation (SD) of the blank samples and then calculate LOD and LOQ according to the following formula:

LOD: $3 \times SD_{blank}$

LOQ: $10 \times SD_{blank}$

Assess the lowest analyte concentration that can be reliably distinguished from LOQ and quantified with acceptable precision.

8.5 Robustness

Robustness is a measure of a method's capability to remain unaffected by small, deliberate variations in method parameters, such as different ligand and/or capture levels, sensor chip lots, protein lots, and buffer and reagent lots (amine coupling and/or capture kits). Data obtained from studies for robustness, though usually not submitted, are recommended to be included as part of the final assay validation.

09
Validation by assay type

9.1 Potency-based assays

Validation of potency-based SPR assays involves demonstrating that the method is accurate, precise, specific, and robust for measuring the functional activity (relative potency) of a biopharmaceutical compared to a reference standard (Fig 8). The process generally follows ICH Q2(R2) guidelines, requiring validation of key parameters to support GMP lot release and stability testing. Potency-based assays are the most common assay type submitted for release testing.

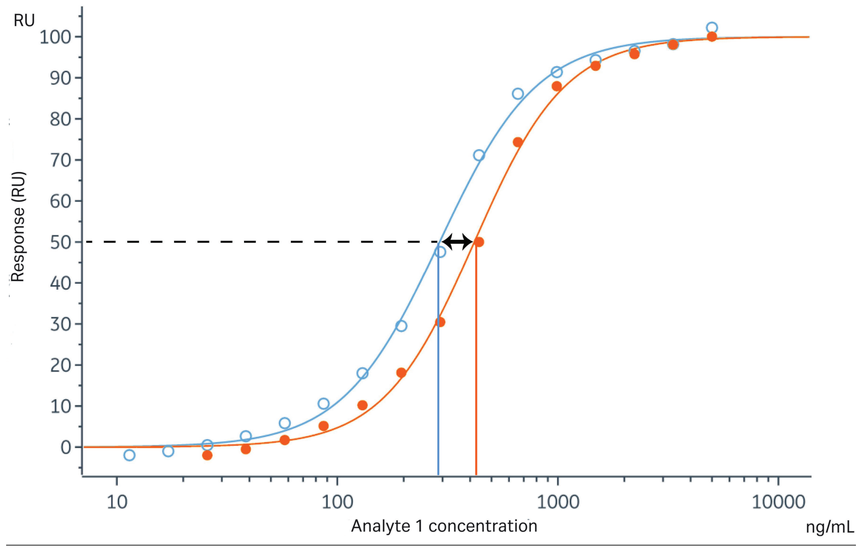

Fig 8. Example of a potency assay. Normalized dose-response curves are fitted to a 4-parameter equation. The EC50 value is the x-value at the inflection point of the fitted curve. Relative potency is then calculated as the ratio of EC50 values for reference and test samples, expressed as a percentage.

When conducting validation studies linearity, range, accuracy, and precision should be evaluated. These characteristics should be assessed using results from at least three independent runs using triplicate replicate samples by different operators on different days to produce datasets for assessment of validation characteristics. % nominal concentrations of the true standard concentration are often used. For potency and stability samples, it is normally recommended to use five different concentrations in a 50-150% range. At each level, replicate samples are evaluated.

Besides submitting the actual results for this assay type, it is also recommended to include relevant sensorgrams as the binding curves represent the true raw data and enable a direct qualitative assessment of data quality. In addition, there might be special cases when curves are needed to be submitted to health authorities, such as in trouble-shooting reports, etc.

Accuracy is assessed by testing samples with known relative potencies, typically 50%, 100%, and 150% prepared from the reference standard. Acceptance criteria for accuracy are commonly defined such that the measured values do not deviate by more than ±20% from the average value (7).

The assay must show a linear relationship between the concentration (dose) and the response (in RU) across a range that covers the expected specifications (e.g., 50 to 150% of the target potency). At least five different concentrations should be used for solutions prepared independently in at least triplicate. It should also be assessed whether the test sample and reference standard show similar dose-response behavior.

Precision should be determined from preferably triplicate measurements of reference material for drug substance (DS) and/or drug product (DP). For repeatability an acceptance criteria of <15 %CV is recommended, and for intermediate precision, where the assay is run on three independent occasions, a typical acceptable result would be %CV < 20.

Specificity should be included in the validation study. The common approach is to use a negative control, a non-specific but closely related product, and test under the exact same conditions as the reference material. It is recommended to overlay sensorgrams of negative control and reference material for visual inspection and qualitative assessment of binding profiles. Data and profiles should be assessed for deviations from acceptance criteria.

9.2 Concentration-based assays

Validating concentration-based SPR assays ensures the method accurately measures the amount of active analyte.

The validation should align with ICH Q2(R2) guidelines for quantitative assays, specifically assessing accuracy, precision, linearity, range, specificity, LOD, LOQ, and robustness, often with special attention to surface stability. These characteristics should be assessed using results from at least three independent runs using triplicate replicate samples by different operators on different days to produce datasets for assessment of validation characteristics. It is also recommended to include representative sensorgrams for concentration-based assays to enable a qualitative evaluation of data quality.

Acceptance criteria for accuracy in a concentration-based assay are typically defined such that results do not deviate by more than ±20% from the average value.

Precision should be determined from preferably triplicate measurements of reference material for drug substance (DS) and/or drug product (DP). For repeatability an acceptance criteria of <15 %CV is acceptable, and for intermediate precision, where the assay is run on three independent occasions, an acceptable result would be < 20 %CV.

Establish dose-dependency (response level in RU vs concentration) for the calibration curve. Verify that the calibration curve maintains a linear response, often using a 4-parameter logistic (4PL) or linear regression. Define limits within which the assay is precise and accurate. Acceptance criteria for linearity should be a regression coefficient R2 > 0.95. For the establishment of linearity, at least five different concentrations should be used for solutions prepared independently in at least triplicate.

For specificity use a negative control, a non-specific but closely related product, and test under the exact same conditions as the reference material. It is recommended to overlay sensorgrams of negative control and reference material for visual inspection and qualitative assessment of binding profiles. Data and profiles should be assessed for deviations from acceptance criteria.

Assess the lowest analyte concentration that can be reliably distinguished from LOQ and quantified with acceptable precision. For impurity assays the recommendations for determining LOD and LOQ are:

- Analysis repeatability and injection repeatability (optional) should be evaluated at the quantitation limit.
- The use of an additional reference standard at the quantitation limit level in the test method is typically required.

9.3 Kinetics and affinity assays

Validation of SPR kinetics and affinity assays ensures that the measured rate constants are accurate and precise. Validation typically adheres to ICH Q2(R2) guidelines for analytical procedures.Key parameters to assess include precision, specificity, robustness, and range.

For accuracy, evaluate the closeness of the measured affinity or kinetics to a known reference standard. Accuracy should not deviate by more than ±20% from the average value.

The consistency of k_a, k_d and K_D values across multiple runs, different days, and different operators should be assessed. Within a run, multiple injections of the same analyte concentrations should yield similar R_{max} and rate constants. Rate constants should also be reproducible across different days, operators, and chip batches.

The level of precision in a kinetics assay varies with the type and the speed of the interaction. Normally, a higher variation is expected for rapid association rates compared to slow association rates. Correspondingly, high precision is normally obtained for medium dissociation rates while slow and rapid dissociation rates tend to have a higher degree of variation (Fig 9). In general, it can be better to set acceptance criteria based on a range rather than using standard deviations. This makes it easier to judge whether a result falls within the acceptance criteria.

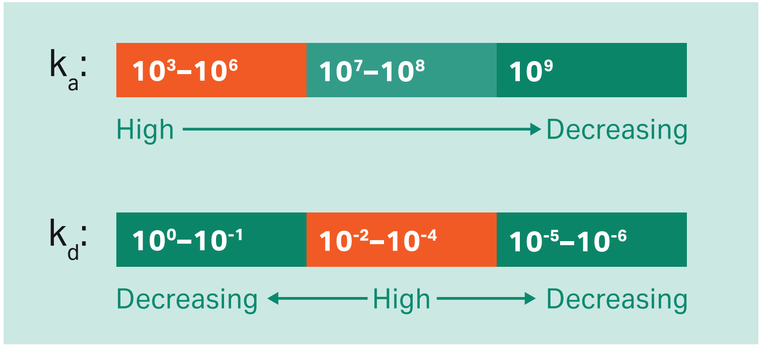

Fig 9. The level of robustness of kinetic rate constants varies with the speed and stability of the interaction. A rapid association rate typically has a higher variation than a slow association rate. Slow and rapid dissociation rates tend to have a higher degree of variation compared to medium dissociation rates.

Specificity should be assessed during validation by evaluating matrix interference and the impact of potential product-related impurities. Parameters to evaluate include k_a, k_d, K_D, and R_{max}. Evaluate the quality of fit by assessing quality parameters provided in the software, such as Chi^2, U-value, T-values. Perform a visual examination of overlaid sensorgrams of negative control and reference material.

Robustness is generally assessed during assay development but should be revisited, verified, and documented also at a later stage to ensure a fully validated assay. This is preferably done when more experience of the assay has been gained.

9.4 *Sensorgram comparison* assays

SPR binding assays are increasingly used in biosimilar development to support demonstration of similarity as part of a totality of evidence approach. SPR based functional assays provide high sensitivity, label free binding data that reflect structure–function relationships. Recent regulatory guidance, including the FDA draft guidance on demonstrating biosimilarity (8), highlights a shift away from comparative efficacy studies (CES) toward comparative analytical assessment (CAA) supported by advanced analytical technologies, reducing reliance on routine comparative efficacy studies when appropriate. Within this framework, Biacore ***Sensorgram comparison*** plays a central role in biosimilarity evaluation and QC decision making (Fig 10).

Sensorgram comparison quantifies similarity between a test sample and a reference standard by directly comparing the shape of binding curves, without requiring kinetic model fitting (9). A similarity score is generated based on how closely the sample sensorgram matches the reference sensorgram, enabling objective and quantitative classification of samples as similar or dissimilar using the similarity score. This approach is well suited for rapid and robust quality control of biosimilars.

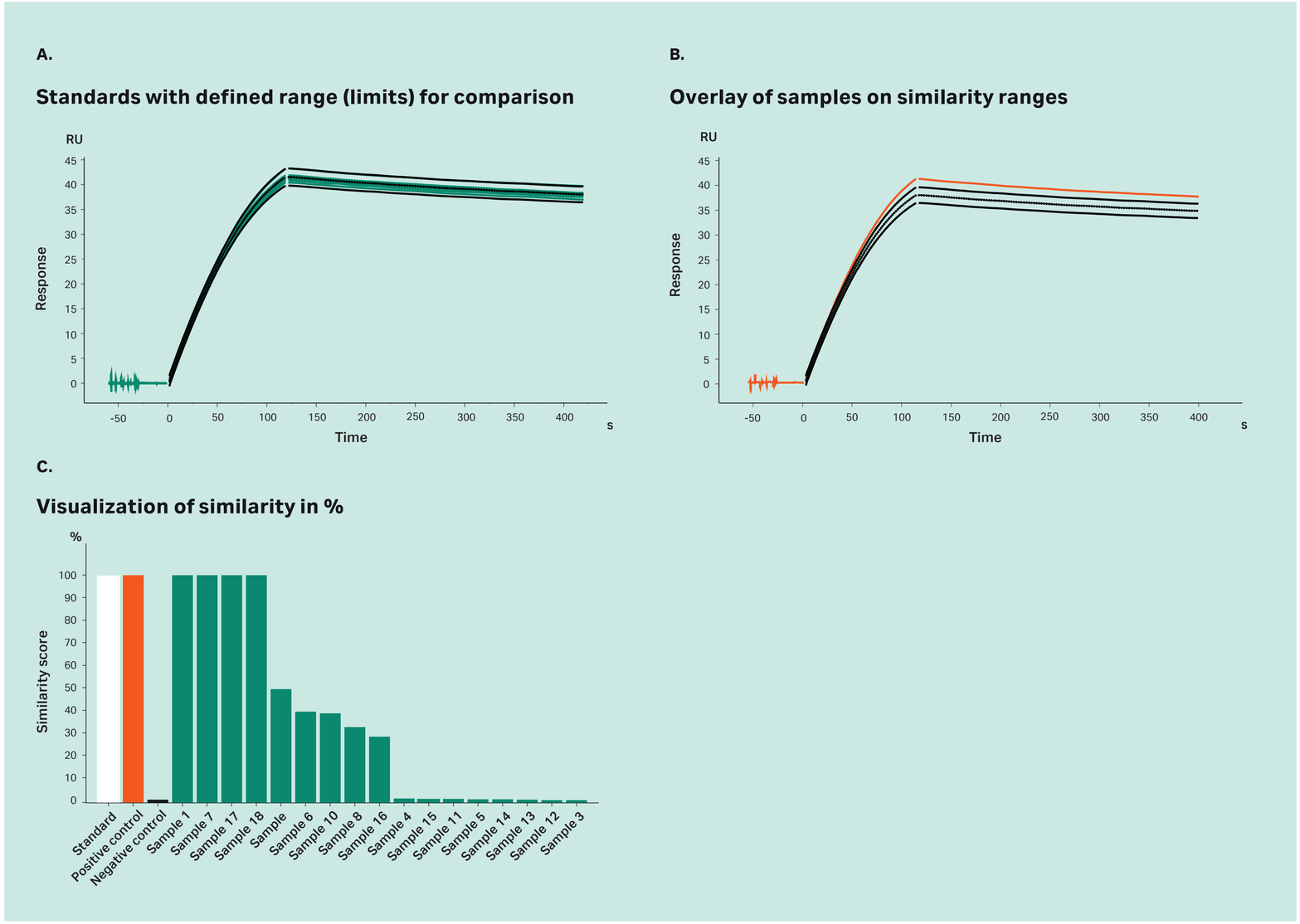

Fig 10. ***Biacore Sensorgram*** comparison workflow illustrating how samples are compared with standards to assess similarity. (a) Standards with defined range limits for comparison. (b) Overlay of samples on similarity range. (c) Visualization of similarity in %.

Validation considerations for *Sensorgram comparison* assays

Validation of SPR assays for ***sensorgram comparison*** focuses on three key elements:

- Establishing representative reference standards
- Defining appropriate controls
- Selecting a fit for purpose comparison approach

Sensorgram comparison assays should be validated according to ICH Q2(R2) principles, with assay specific justification of the selected comparison metrics.

Reference standard validation

A well characterized reference standard that reflects the expected binding behavior of the analyte is essential. During validation, the standard should be tested to capture normal assay variability, including differences in reagent lots, sample preparations, operators, instruments, run instances, and ligand or capture levels. In ***Sensorgram comparison*** approach, the standards data are used to define statistically meaningful similarity limits (upper and lower comparison curves) for the sample's comparability assessment.

Controls validation

The use of both positive and negative controls is highly recommended to strengthen confidence in assay performance and specificity. It is also recommended to consider defined quality checks as part of method validation, including visual inspection of sensorgrams for expected shape and absence of artifacts, confirmation of baseline stability, verification of ligand or capture levels where applicable, and review of reference surface responses to exclude non specific binding.

During validation, a comparison approach should be selected to define acceptance limits for standard behavior and support reliable assessment of samples. The choice must be data driven, justified, and aligned with the intended assay use:

- **Standard deviation (SD) approach:** Defines symmetric upper and lower limits around the average standard curve based on statistical variability. This is the preferred approach when standards behave consistently and variability is well controlled. It is suitable for most validated assays, performance monitoring, assessment of reagents, and routine QC of production batches.
- **Min/max approach:** Defines acceptance limits based on the minimum and maximum responses, with limits established around the median curve. This approach can be applied when data show asymmetric variability or non normal behavior. It is most appropriate for heterogeneous interactions, and for screening assays where predefined cut offs are required.
- **Percentage deviation approach:** Applies fixed percentage limits around the average curve and is suitable during early development when limited amount of standard data is available.

After completion of validation, the selected reference standards and comparison approach, together with defined acceptance limits (similarity limits), should be applied consistently to test samples to support comparability assessments and confident decision making throughout the assay lifecycle.

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Tips for validation of Biacore SPR assays

- Make sure your assay is stable before moving into the validation phase. A well optimized assay leads to a stronger validation outcome.
- Do not underestimate the importance of system suitability testing. Well executed system suitability testing will save time in the end.
- Select ligand levels according to the assay's purpose: use low ligand densities for kinetic assays and higher levels for potency or concentration assays.
- Monitor matrix effects carefully, including any non specific binding to the ligand or the sensor surface.
- Leverage the information in the sensorgram—its visual detail often reveals insights that data alone cannot.
- Avoid setting acceptance criteria that are unnecessarily strict.
- As always, keep your Biacore SPR instrument clean and properly maintained.

11

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