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Workshop/Conference Report

Bioanalytical Method Validation—A Revisit with a Decade of Progress

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PURPOSE OF THE REPORT

This report is a synthesis of (1) the earlier conference on Analytical Methods Validation–Bioavailability, Bioequivalence and Pharmacokinetic Studies (Conference held in Arlington, VA, December 3–5, 1990 and the report published in Pharmaceutical Research, 9: 588-592, 1992) and (2) the workshop on "Bioanalytical Methods Validation—A Revisit with a Decade of Progress," (Workshop held in Arlington, VA, January 12–14, 2000), sponsored by the American Association of Pharmaceutical Scientists and the U. S. Food and Drug Administration. The bioanalytical method validation workshop of January 12–14, 2000 was directed towards small molecules. A separate workshop was held in March 1–3, 2000 to discuss validation principles for macromolecules. The purpose of this report is to represent the progress in analytical methodologies over the last decade and assessment of the major agreements and issues discussed with regard to small molecules at both the conference and the workshop. The report is also intended to provide guiding principles for validation of bioanalytical methods employed in support of bioavailability, bioequivalence, and pharmacokinetic studies in man and in animals.

OBJECTIVES

The objectives of the conference and the workshop were as follows:

- To agree on what should be required in bioanalytical method validation and the procedures to establish validation.
- To determine processes of application of the validation procedures in bioavailability, bioequivalence and pharmacokinetics studies.
- To review the progress, impact and advances made during the last decade of bioanalytical methods validation since the first conference on Bioanalytical Methods Validation, held in 1990.
- To identify, discuss and resolve scientific issues related to the validation of quantitative bioanalytical methodology implemented over the past decade.
- To evaluate and discuss the comments on the FDA Draft Guidance, *Bioanalytical Methods Validation for Human Studies.*
- To develop an updated report on bioanalytical method validation (which may be referred to in developing future formal guidances).

Acceptable practices for documenting and validating bioanalytical methods with regard to processes, parameters, and data treatments were discussed because of their importance in the assessment of pharmacokinetic, bioavailability and bioequivalence studies. The general field of hyphenated mass spectrometric-based assays was discussed in depth, as were ligand-based assays. High throughput systems and support of their reliability were also discussed. Other topics considered essential in the conduct of pharmacokinetic/bioequivalence studies including the measurement and stability of drug metabolites and regulatory submission data packages were deliberated. The need for stereoselective determinations was discussed in the earlier conference.

INTRODUCTION

Analytical methods employed for the quantitative determination of drugs and their metabolites in biological samples are the key determinants in generating reproducible and re-

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liable data which in turn are used in the evaluation and interpretation of bioavailability, bioequivalence, and pharmacokinetic findings. It is essential to employ well-characterized and fully validated analytical methods to yield reliable results which can be satisfactorily interpreted. It is recognized that analytical methods and techniques are constantly undergoing changes and improvements; and in many instances, they are at the cutting edge of the technology. It is also important to emphasize that each analytical technique has its own characteristics, which will vary from analyte to analyte. Moreover, the appropriateness of the technique may also be influenced by the ultimate objective of the study. Specific validation criteria are needed for methods intended for analysis of each analyte (drug and/or metabolite). While validation of each method will stand on its own, there may be situations when comparison of methods will be necessary, e.g., when more than one method has been employed in a long-term study. When sample analysis for a given study is conducted at more than one site, it is necessary to validate the analytical methods(s) at each site and provide appropriate validation information for different sites to establish interlaboratory reliability. Unless a method is used on a regular basis that provides confidence in its continued validity, it is essential to document that the method is still valid prior to analysis of samples in a study.

The original bioanalytical validation conference workshop was the first major meeting dedicated to investigating and harmonizing procedures required in method validations. The original 1990 conference report (Pharmaceutical Research: 9:588–592, 1992) functioned well as an industry outline for practice by bioanalytical laboratories worldwide and as a reference by the regulatory agencies worldwide. The recent advances in technology, a decade of experience and different perspectives in science and compliance warrant an updating of this significant document and reconfirming and updating the principles of bioanalytical method validation.

In the last decade there have been tremendous advancements in the field of mass spectrometry with the development of new interfaces, ionization and detection techniques. These advancements resulted in the rapid emergence and widespread commercial use of hyphenated mass spectrometry (LC-MS-MS) based assays, which have largely replaced conventional HPLC, GC and GC-MS assays. During this time, the use of multi-well plates, automated robotic sample processing and electronic data reporting have become common place. Given the certainty of continued technological advances, the future will very likely bring new, even more powerful bioanalytical approaches as the search for more rapid throughput and increased sensitivity continues. Despite these widespread changes in technology, the need for clearly defined validation criteria for bioanalytical methods intended for analyses of each analyte (drug and/or metabolite(s)) in each separate biological matrix, remains. In the case of hyphenated mass spectrometry techniques, such as LC-MS-MS, there may be unique requirements that demand attention. This current summary of the "Bioanalytical Methods Validation–a Revisit with a Decade of Progress" workshop sponsored by AAPS and FDA expands and updates all aspects of the previous report.

BIOANALYTICAL METHOD VALIDATION

Bioanalytical method validation includes all of the procedures required to demonstrate that a particular bioanalytical method for the quantitative determination of the concentration of an analyte (or series of analytes) in a particular biological matrix is reliable for the intended application. The most widely employed bioanalytical techniques include, but are not limited to, conventional chromatographic based methods (such as GC-ECD and HPLC-UV), mass spectrometry based methods (such as GC-MS and LC-MS), tandem mass spectrometry based methods (such as LC-MS-MS) and ligand-based assays (such as RIA and ELISA). Many of the principles, procedures, and requirements of bioanalytical method validation are common to all types of analytical methodologies.

It is accepted that during the course of a typical drug development program, a defined bioanalytical method will undergo many modifications. These evolutionary changes (e.g. addition of a metabolite, lowering of the lower limit of quantification (LLOQ), etc.) are required to support specific studies and will require different levels of validation to demonstrate continually the validity of an assay's performance. In addition to assay modifications, the required utilization of a defined assay will often change as the growing number of samples leads to the potential necessity of multiple laboratories being involved. This common scenario raises the question of assay comparability at different laboratories and what types of validation data are necessary to "qualify" the different laboratories. Moreover, the possibility of a bioanalytical method not being used on a regular basis will require adequate revalidation data when needed to be used, in order to document and demonstrate that a method is still valid prior to analyses of samples in a study. Throughout this conference report, references will be made to different levels and types of method validations, including "Full Validation, Partial Validation, and Cross Validation". These different types of bioanalytical method validations are defined and characterized as follows:

(1) Full Validation

- Full Validation is necessary when developing and implementing a bioanalytical method for the first time.
- Full Validation is required for a new drug entity.
- If metabolites are added to an existing assay for quantification, then Full Validation of the revised assay will be necessary for all analytes measured.

(2) Partial Validation

Partial Validations are modifications of validated bioanalytical methods that do not necessarily require full revalidations. Partial Validation can range from as little as one intra-assay accuracy and precision determination to a "nearly" Full Validation. Typical bioanalytical method changes which fall into this category include, but are not limited to:

- Bioanalytical method transfers between laboratories or analysts.
- Instrument and/or Software Platform changes.
- Change in species within matrix (e.g. rat plasma to mouse plasma).
- Changes in matrix within a species (e.g., human plasma to human urine).

- Selectivity demonstration of an analyte in the presence of specific metabolites.
- Selectivity demonstration of an analyte in the presence of concomitant medications.
- Change in analytical methodology (e.g., change in detection systems).
- Change in sample processing procedure(s).
- Rare matrices.
- Change in anti-coagulant in harvesting biological fluid.
- Limited volume changes (e.g. planned pediatric study).

(3) Cross Validation

Cross Validation is a comparison of two bioanalytical methods. Cross Validations are necessary when two or more bioanalytical methods are used to generate data within the same study. For example, an original validated bioanalytical method serves as the "reference" and the revised bioanalytical method is the "comparator". The comparisons should be done both ways. Cross validation with spiked matrix and subject samples:

- Should be conducted at each site or laboratory to establish interlaboratory reliability when sample analyses within a single study is conducted at more than one site, or more than one laboratory.
- Should be considered when data generated using different analytical techniques (e.g. LC-MS-MS vs. ELISA) in different studies are included in a regulatory submission.

BIOANALYTICAL METHOD DEVELOPMENT ESTABLISHMENT (CHEMICAL ASSAYS)

The following principles of bioanalytical method validation provide steps for the development of a new method or establishing an existing method in a particular laboratory for the first time. The parameters essential to ensure the acceptability of the performance of a bioanalytical method are accuracy, precision, selectivity, sensitivity, reproducibility and stability. All these parameters need to be defined during Full Validation of a bioanalytical method. Although there are various stages in the development and validation of a bioanalytical procedure, the bioanalytical method validation can be envisaged to consist of two distinct phases: (1) the bioanalytical method development phase in which the assay is defined and validated and (2) application to actual analysis of samples from pharmacokinetic, bioavailability, bioequivalence and drug interaction studies. The following principles of bioanalytical method validation provide steps for the development and Full Validation of a new bioanalytical method or establishing an existing method in a particular laboratory for the first time. Full Validation should be performed to support pharmacokinetic, bioavailability, bioequivalence and drug interaction studies in a new drug application (NDA) or an abbreviated new drug application (ANDA).

Quantification of metabolites, if and when considered necessary, should follow an identical protocol for validation including accuracy, precision, selectivity, sensitivity, reproducibility and stability.

Quantitative determination of stereoisomers in any studies including pharmacokinetic, bioavailability, bioequivalence and drug interaction studies, if and when considered necessary, should follow an identical protocol for validation including accuracy, precision, selectivity, sensitivity, reproducibility and stability of the intended isomers.

Principles of Bioanalytical Method Validation and Establishment

Parameters essential to ensure the acceptability of the performance of a bioanalytical method validation are accuracy, precision, selectivity, sensitivity, reproducibility and stability.

A specific, detailed description of the bioanalytical method should be written. This may be in the form of a protocol, study plan, report and/or Standard Operating Procedure.

Each step in the bioanalytical method should be investigated to determine the extent to which environmental, matrix, material, or procedural variables, from the time of collection of the material up to analysis and including the time of analysis, may affect the estimation of analyte in the matrix. Variability of matrix due to physiological state may need to be considered. In the case of LC-MS-MS based procedures, it is essential that appropriate steps be taken to ensure the lack of a matrix effect(s) throughout the application of the method, especially if the nature of the matrix changes from that used during initial method validation.

A bioanalytical method should be validated for the intended use or application. All experiments used to make claims or draw conclusions about the validity of the method should be presented in a report (method validation report).

Whenever possible, the same biological matrix as that in the intended samples should be used for validation purposes. (For tissues of limited availability, such as bone marrow, physiologically appropriate proxy matrices may suffice.)

The stability of the analyte (drug and/or metabolite(s)) in the matrix during the collection process and the sample storage period should be assessed, preferably prior to sample analysis.

For those compounds with potentially labile metabolites, it is recommended that stability of the analyte in matrix from dosed subjects (or species) be confirmed.

The accuracy, precision, reproducibility, response function, and selectivity of the method with respect to endogenous substances, metabolite(s), and known degradation products should be established with reference to the biological matrix. With regard to selectivity, there should be evidence that the substance being quantified is the intended analyte.

The concentration range over which the analyte will be determined must be defined in the bioanalytical method, based on the evaluation of actual standard samples over the range, including their statistical variation. This defines the *standard curve*.

It is necessary to use a sufficient number of standards to define adequately the relationship between concentration and response. The relationship between response and concentration must be demonstrated to be continuous and reproducible. The number of standards to be used will be a function of the dynamic range and nature of the concentration-response relationship. In many cases, five to eight concentrations (excluding blank values) may define the standard curve. More standard concentrations may be necessary for nonlinear than for linear relationships.

The ability, in terms of accuracy and precision, to dilute samples originally above the upper limit of the standard curve should be demonstrated in the validation.

The accuracy and precision with which known concentrations of an analyte in biological matrix can be determined, must be demonstrated. This can be accomplished by analysis of replicate sets of analyte samples of known concentrations – quality control (QC) samples — from an equivalent biological matrix. At a minimum, three concentrations representing the entire range of the standard curve should be studied: one within 3× of the LLOQ (low QC sample), one near the center (middle QC), and one near the upper boundary of the standard curve (high QC). In consideration of high throughput analyses including but not limited to multiplexing, multicolumn and parallel systems, sufficient QC samples are required to ensure control of the assay. The number of QC samples to ensure proper control of the assay should be determined based on the run size. The placement of QC samples should be judiciously considered in the run.

For a bioanalytical method to be considered valid, specific *a priori* acceptance criteria must be set and achieved for accuracy and precision for the validation of QC samples over the range of the standards.

Repeat analysis of incurred samples is usually not necessary. Any potential issues of degradation should be investigated in a stability experiment utilizing incurred samples.

Specific Recommendations for Bioanalytical Method Validation

The matrix-based standard curve should consist of a minimum of five standard points, excluding blanks, using single or replicate samples. The standard curve should cover the entire range of expected concentrations.

Standard curve fitting is determined by applying the simplest algorithm (model) which best describes the concentration-response relationship using appropriate weighting and statistical tests for "goodness of fit" requirement. This is based on the actual standard points during each run in the analysis. It must be continuous and reproducible, and should be based on minimizing the percent relative error in the back-calculated values.

The lower limit of quantification (LLOQ) is the lowest concentration of the standard curve which can be measured with acceptable accuracy and precision. The LLOQ should be determined using at least five samples independent of standards and determining the coefficient of variation and/or appropriate confidence interval. The LLOQ should serve as the lowest concentration on the standard curve and should not be confused with the limit of detection (LOD; see glossary) and/or the low QC sample. The highest standard will define the Upper Limit of Quantitation (ULOQ) of an analytical method.

For the validation of a bioanalytical method, the accuracy and precision should be determined using a minimum of five (excluding blank sample) determinations per concentration level. The mean value should be within ±15% of the theoretical value except at LLOQ, where it should not deviate by more than ±20%. The precision around the mean value should not exceed 15% coefficient of variation (CV), except for LLOQ, where it should not exceed 20%CV. Other methods of assessing accuracy and precision, which meet these limits may be equally acceptable.

The stability of the analyte in biological matrix at intended storage temperature(s) should be established. In addition, the influence of freeze/thaw cycles (a minimum of three cycles at two concentrations in triplicate) should be studied.

The stability of the analyte in matrix at ambient temperature should be evaluated over a time period that encompasses the duration of typical sample preparation, sample handling and analytical run time.

Reinjection reproducibility should be evaluated to determine if an analytical run could be reanalyzed in case of unexpected delay in the analyses such as instrument failure.

The specificity of the assay methodology should be established using a minimum of six independent sources of the same matrix. This requirement for six independent matrices to be tested for interference may not be necessary for hyphenated mass spectrometry based methods. In the case of LC-MS and LC-MS-MS based procedures, matrix effects should be investigated to ensure that precision, selectivity and sensitivity will not be compromised. Assessment of method selectivity requires evaluation during method development and validation and may continue throughout application of the method in actual study samples.

Recovery should be reproducible at each concentration.

Acceptance/Rejection criteria for spiked, matrix-based calibration standards and validation QC samples should be based on nominal (theoretical) concentration of analyte(s). Specific *a priori* criteria should be set up and achieved for accuracy and precision over the range of the standards, if so desired.

APPLICATION OF A VALIDATED BIOANALYTICAL METHOD TO ROUTINE DRUG ANALYSIS

Many of the above principles under method establishment and validation are relevant to within study validation. This section will emphasize the validation parameters that should be evaluated during routine application of a validated bioanalytical method to a particular study.

Following a successful validation which meets an *a priori* set acceptance criteria for accuracy and precision, analysis of biological samples can be done by single determination without a need for duplicate or replicate analysis. The need for duplicate analysis may arise for special cases. For example, in the case of a difficult procedure for labile analyte(s), when the precision and accuracy tolerances are difficult to achieve, duplicates analyses may be essential. Under these conditions, a rationale and standard operating procedure for duplicate analyses and for reporting results should be developed *a priori.*

A matrix-based standard curve should be generated for each analytical batch for each analyte and should be used for calculating the concentration of analyte in the unknown samples assayed with that run. It is important to use a matrix-based standard curve that will cover the entire range of concentrations in the unknown samples. Estimation of unknowns by extrapolations of standard curve below the LLOQ or above the ULOQ is not recommended. Instead, it is recommended that the standard curve be redetermined or samples be reassayed after dilution with the matrix.

- A matrix-based standard curve should consist of a minimum of five to eight standard points, excluding blanks (either single or replicate), covering the entire range.
- Response Function: Typically, the same curve fitting, weighting and goodness of fit determined during prestudy validation should be utilized for the standard curve within study. Response function is determined by appropriate statistical tests based on the actual standard points during each run in the validation. Changes in response function relationship between prestudy validation and routine run validation are indicative of potential problems.
- The QC samples should be used to accept or reject the run. These QC samples are matrix spiked with analyte.
- System suitability: Based on the analyte and technique, a specific standard operating procedure (or sample) can be identified to assure the optimum operation of the system employed.
- Any required sample dilutions must utilize like matrix (e.g. human to human) obviating the need to incorporate actual within-study dilution matrix QC samples.

Acceptance Criteria for the Batch

Standards and QC samples can be prepared from the same spiking stock solution, provided the solution stability and accuracy have been verified. A single source of matrix may also be used, provided selectivity has been verified.

Standard curve samples can be positioned anywhere in the run. An example of standard curve sample position is at the beginning and end of the run. Blanks, QCs and study samples can be arranged as considered appropriate within the batch.

Matrix-based standard calibration samples. 75%, or a minimum of 6 standards, when back-calculated (including ULOQ) should fall within ±15%of nominal, except for LLOQ when it should be within ±20% of the nominal value. Values falling outside these limits can be discarded provided they do not change the established model algorithm). Acceptance criteria for accuracy and precision as outlined in the section "specific recommendation for method validation" should be provided for both within and between batch experiment.

Quality-Control Samples: Replicate quality-control samples, (at least duplicated), at a minimum of three concentrations [one within 3x of the LLOQ (low QC), one in the midrange (middle QC), and one approaching the high end of the range (high QC)] should be incorporated into each run. The results of the QC samples provide the basis of accepting or rejecting the run. At least 67% (four out of six) of the QC samples must be within 15% of their respective nominal (theoretical) values; 33% of the QC samples (not all replicates at the same concentration) may be outside the ± 15% of the nominal value. A confidence interval approach yielding comparable accuracy and precision is an acceptable alternative.

Incorporated into a run should be a minimum of 5%QCs relative to the number of samples in a run (in multiples of three), or 6 total QCs, whichever is greater.

Repeat Analysis: A standard operating procedure or guideline for repeat analysis and their acceptance criteria must be established *a priori.* This SOP or guideline should define acceptable reasons for repeating sample analysis, such as sample processing errors, equipment failure, poor chromatography, etc. Cautious use of "pharmacokinetic fit" such as double peak may call for repeat analysis of some samples in the study. The rationale for the repeat analysis and the reporting of the repeat analysis should be clearly documented.

The need for occasional reintegration in chromatography should be conducted according to *a priori* criteria.

Samples involving multiple analytes should not be rejected based on the data from one analyte failing the acceptance criteria. The data from rejected runs does not need to be documented, but the fact that a run was rejected should be filed including the reason for failure. The documentation of accepted runs should include outlier standards and QC samples with reasons for decision(s).

BIOANALYTICAL METHODS VALIDATION (MICROBIOLOGICAL AND LIGAND BASED ASSAYS)

The balance of new methodology for the quantification of conventional, low molecular weight drugs in biological fluids has shifted dramatically in favor of mass spectrometry based methods, particularly LC-MS and LC-MS-MS. Nonetheless, there remain situations in which immunoassays or, in some cases, microbiological assays, are the methods of choice. These include assays for analytes that are not amenable to chromatographic analysis and assays for support of Phase III and IV clinical studies, where the high throughput and relatively low per sample cost of immunoassays may be advantageous. Many of the bioanalytical validation parameters and principles discussed above are also applicable to microbiological and ligand based binding assays.

Selectivity Issues

As with chromatographic methods, ligand binding assays must be shown to be selective for the analyte. Two types of selectivity may be considered, as indicated below:

"Specific" Nonselectivity (interference from substances that are physiochemically similar to the analyte)

- Evaluate crossreactivity of metabolites, concomitant medications or endogenous compounds individually and in combination with the analyte of interest.
- When possible, compare the immunoassay with a validated, reference method (such as often LC-MS) using incurred samples and pre-determined criteria for agreement of accuracy of immunoassay and reference method.
- Use study (incurred) samples to assess dilutional linearity to the reference standard.
- For some analytes, selectivity may be improved by incorporation of separation steps prior to immunoassay.

"Nonspecific" Nonselectivity (also termed "matrix effects"; interference from matrix components that are unrelated to the analyte, such as from homolysis, serum proteins, lipemia, etc.)

- Compare standard curves in biological fluids with standards in buffer to detect matrix effects.
- Evaluate parallelism of diluted study samples with diluted standards to detect matrix effects.

Quantification Issues

Immunoassay standard curves are inherently nonlinear and, in general, require more concentration points to define the fit over the standard curve range than do chemical assays. In addition to their nonlinear characteristics, the response-error relationship for immunoassay standard curves is a non-constant function of the mean response (heteroscadisticity). For these reasons, a minimum of six non-zero calibrator concentrations, run in duplicate, is recommended. The concentration-response relationship is most often fitted to a 4- or 5-parameter logistic model, although others may be used with suitable validation. The use of "anchoring points" in the asymptotic high- and low-concentration ends of the standard curve may improve the overall curve fit. Generally, these anchoring points will be at concentrations that are below the established LLOQ and above the established ULOQ. Whenever possible, calibrators should be prepared in the same matrix as the study samples, or in an alternative matrix of equivalent performance (background, etc.).

Both ULOQ and lower LLOQ must be defined by acceptable accuracy, precision, or confidence interval criteria based on the study requirements.

For all assays it is the accuracy of the *reported results* which is the key factor. This accuracy may be improved by the use of replicate samples. In the case where replicate samples need to be measured during the validation to improve accuracy, the same procedure must be followed for unknown samples.

If separation is employed prior to assay for study samples but not for standards, it is necessary to establish recovery and use recovery in determining results. Possible approaches to assess efficiency and reproducibility of recovery are (i) the use of radiolabeled tracer analyte (quantity too small to affect the assay), (ii) prior establishment of reproducible recovery, (iii) the use of an internal standard which is not recognized by the antibody but can be measured by another technique.

Key reagents, such as antibody, tracer, reference standard and matrix should be characterized appropriately and stored under defined conditions.

Assessments of analyte stability should be conducted in true study matrix (e.g. should not employ a matrix stripped to remove endogenous interferences).

Due to the greater inherent imprecision of immunoassays, QC sample acceptance criteria of ±25% for accuracy in routine assay implementation are proposed. Thus, at least 67% of QC samples must be within ±25% of their nominal values, with no two at the same concentration level exceeding ±25% of nominal.

Changes in key reagents require assay re-optimization or validation as follows:

For Labeled analyte (tracer)

- Binding should be re-optimized.
- Performance should be verified with standard curve and QCs,

Antibody

- Key cross-reactivities should be checked.
- Tracer experiments above should be repeated.

Matrix

- Tracer experiments above should be repeated.

Pre-study validation experiments should include a minimum of 6 runs conducted over several days, with at least four concentrations (LLOQ, low, medium and high) analyzed in duplicate in each run.

USE OF COMMERCIAL KITS

The performance of a commercial kit must be validated under the conditions of intended use. Performance characteristics evaluated should include accuracy, precision, stability of key reagents under study conditions and relevant selectivity verification of antiserum and assay, including interferences from compounds included in the study samples to be analyzed.

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GLOSSARY

Accuracy: The degree of closeness of the determined value to the nominal or known true value under prescribed conditions. This is sometimes termed trueness.

Analyte: A specific chemical moiety being measured, which can be intact drug, biomolecule or its derivative, metabolite and/or degradation product in a biologic matrix.

Analytical Batch (or Run): A complete set of analytical and study samples with appropriate number of standards and QCs for their validation. Several runs (batches) may be completed in one day or one run (or batch) may take several days to complete.

Analytical Standard: An analyte of known purity and molecular composition.

Analytical Stock/Standard Solution: A known amount of an analytical standard dissolved in a known volume of solvent which is further diluted to generate working or secondary standard solution(s) used to prepare calibration standards and QC samples.

Biological matrix: A discrete material of biological origin that can be sampled and processed in a reproducible manner. Examples are blood, serum, plasma, urine, feces, saliva, sputum and various discrete tissues.

Calibration Standard: A biological matrix to which a known amount of analyte has been added or spiked. Calibration standards (also referred to as standard curve samples) are used to construct calibration curves (also referred to as standard curve) from which the concentrations of analyte(s) in QCs and in unknown study samples are determined.

Internal Standard: Test compound(s) (e.g. structurally similar analog, stable labeled compound, etc.) added to both calibration standards and samples at known and constant concentration to facilitate quantification of the target analyte(s).

Limit of detection (LOD): The lowest concentration of an analyte that the bioanalytical procedure can reliably differentiate from background noise.

Lower Limit of quantification (LLOQ): The lowest amount of an analyte in a sample that can be quantitatively determined with suitable precision and accuracy.

Linear range: The interval between the upper and lower concentration (amounts) of analyte in the sample (including these concentrations) for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity.

Matrix effect: The direct or indirect alteration or interference in response due to the presence of unintended analytes (for analysis) or other interfering substances in the sample.

Method: A comprehensive description of all procedures used in sample analysis.

Precision: The precision expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogenous sample under the prescribed conditions.

Quantification Range: Range of concentration including ULOQ and LLOQ which can be reliably and reproducibly quantified with acceptable accuracy and precision through the use of a concentration response relationship.

Recovery: Recovery is the extraction efficiency of an analytical process, reported as a percentage of the known amount of an analyte carried through the sample extraction and processing steps of the method.

Reproducibility: Reproducibility is the precision between two laboratories. It also represents precision of the method under same operating conditions over a short period of time.

Sample: A generic term encompassing controls, blanks, unknowns and processed samples, as described below:

Blank: A sample of a biological matrix to which no analyte(s) have been added. It is to assess the specificity of the bioanalytical method.

Quality Control (QC): A sample used to monitor the performance of a bioanalytical method and to assess the integrity and validity of the results of the unknown samples analyzed in an individual batch.

Unknown: A biological sample that is the subject of the analysis.

Processed: The final extract (prior to instrumental analysis) of a sample that has been subjected to various manipulations (extraction, dilutions, concentration, etc.)

Selectivity: Selectivity is the ability of the bioanalytical method to measure unequivocally and differentiate the analyte(s) in the presence of components, which may be expected to be present. Typically these might include metabolites, impurities, degradants, matrix components, etc.

Stability: The chemical stability of an analyte in a given matrix under specific conditions for given time intervals.

Standard Curve: The relationship between the experimental response value and the analytical concentration (also called a calibration curve).

System suitability: A reference standard used to check instrument performance, e.g. sensitivity and chromatographic retention prior to running the analytical batch.

Upper Limit of quantification (ULOQ): The highest amount of an analyte in a sample that can be quantitatively determined with suitable precision and accuracy.

Full Validation: Establishment of all acceptable validation parameters for the bioanalytical method for each analyte in order to apply to sample analysis.

Partial Validation: Modification of validated bioanalytical methods that do not necessarily require full re-validation.

Cross Validation: Comparison of validation parameters of two or more bioanalytical methods.

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