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Analytical Survey

A comprehensive method validation strategy for bioanalytical applications in the pharmaceutical industry — 1. Experimental considerations

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Abstract: The method validation strategy described consists of four components which are the prevalidation, validation proper, study proper and statistical analyses. These components constitute the platform upon which to evaluate the reliability and reproducibility of a bioanalytical method. Consideration has been given to emulate the study proper conditions to understand the method's limitations and performance expectations. The validation strategy will be presented in two papers. This first paper will describe the overall validation strategy, and the second paper will discuss the statistical analyses and data interpretation.

Keywords: *Method validation; bioanalytical methods; comprehensive strategy; FDA regulations; prevalidation; validation proper; study proper; statistical analyses; confidence limits; control charts; calibration standards; quality control samples.*

Introduction

Method validation is an important component in determining the reliability and reproducibility of a bioanalytical method, and is a requirement of any regulatory submission [1]. The policy of Food and Drug Administration (FDA) states that for each analytical method used to quantitate drug concentrations from biological fluids, specific analytical parameters must be determined with respect to accuracy, linearity, precision, sensitivity, specificity and recovery [2].

After the bioavailability–bioequivalence regulations were introduced in 1977, the quality of FDA submissions has significantly improved. However, inadequate analytical documentation describing validation data has remained a major cause of deficient biopharmaceutic submissions [3]. Therefore, in designing a validation strategy, it is important that it be defensible and satisfy regulatory requirements.

Method-validation publications have generally defined analytical terms identified as important to a regulatory submission [4–9]. There does not appear to be a published strategy for determining the validity of a bioanalytical procedure. In designing the present, proposed validation strategy, the approach taken was for it to satisfy the following criteria. The method should be defensible with respect to regulatory requirements and reliable by incorporating statistical analyses to evaluate its performance. The method should also incorporate continuous validation to monitor the reproducibility of the method over time and be a timely procedure to generate adequate validation data.

A detailed description of the validation strategy will be presented in two papers. This first paper will describe the overall validation strategy and the second paper will discuss the statistical analyses and data interpretation [10].

Validation Strategy

The overall validation strategy consists of four components which are the prevalidation, validation proper, study proper, and statistical analyses. These components constitute the

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platform upon which to evaluate the reliability and ruggedness of a bioanalytical method. This paper will focus on the first three components and describe each in detail. The validation flowchart summarizes the strategy and sequence of events (Fig. 1).

Prevalidation

Prior to initiating the validation proper, a prevalidation is performed by the primary analyst. This component provides the analyst an opportunity to obtain some practical experience with the method and helps to identify the optimum chromatographic conditions. It is recommended that the following studies be conducted prior to initiating the validation proper. The appropriate peak response to use in quantitating drug concentrations should be selected first. This involves comparing internal and external methods to evaluate the reproducibility and ruggedness. The optimum standard curve range and the number of calibrators should be established. The appropriate regression model which best fits the data is then selected.

The extraction scheme and its recovery should be optimized to give insight into the limit of quantitation and to help determine if the extraction procedure is reproducible. If possible, potential compounds that could interfere with the chromatography should be evaluated. The remaining system suitability parameters are then investigated by optimizing the mobile phase composition, selecting an appropriate chromatographic column, and determining the temperature effects on the chromatographic separation.

Validation Proper

The validation proper consists of four analytical runs generated on separate days involving two analysts. Each analyst has specific responsibilities that help determine the performance and reproducibility of the method. Each validation run emulates the analytical conditions of the study proper and its expected run time. This strategy helps the primary analyst develop a daily routine to use during the study proper and anticipate potential assay problems.

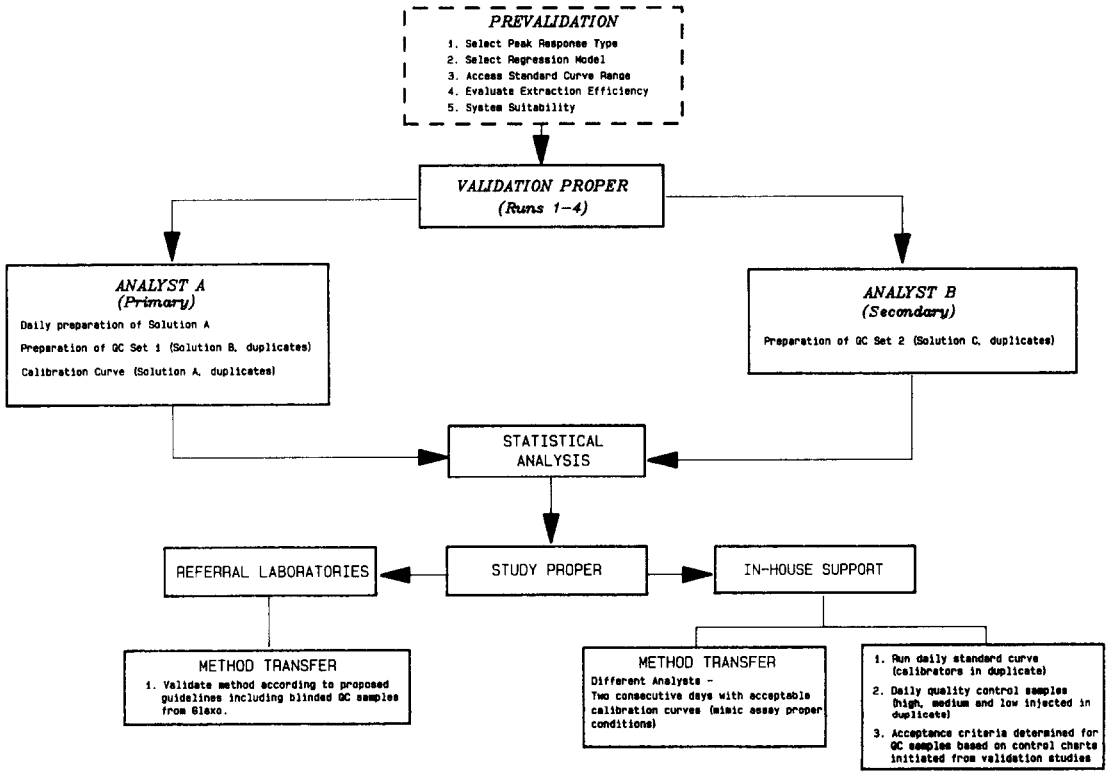

Figure 1
Validation strategy.

Suggestions for additional samples to be analysed during each validation run include samples fortified with metabolites and potentially interfering compounds to identify chromatographic behaviour and ensure assay specificity; spiked biological samples to determine extraction characteristics and recovery; stability samples to begin generating information pertinent to sample processing and storage; additional biological matrices to identify and reserve for use in future clinical studies; and predose study samples to determine if there are coeluting contaminants in the samples to help prevent analytical difficulties during the sample analysis.

From the data generated, specific analytical parameters are reported, including linearity, accuracy, precision, sensitivity and recovery. From each standard curve, the slope, intercept, correlation coefficient, and variance are monitored. Statistical analyses are used to determine within- and between-run variances and to demonstrate how the method can be expected to perform on a daily basis. Based on the initial quality control data concentration results, acceptance criteria are established. Subsequent analytical runs are monitored during the study proper using these acceptance criteria to determine if the data generated are valid.

Preparation of stock solutions

During the method validation, two analysts are involved in the preparation of stock solutions. The primary analyst, Analyst A, is responsible for preparing stock Solutions A and B from different weighings. During the validation, Solution A will be made daily to use in preparing the calibration standards. This will serve to incorporate realistic variability into the validation runs and to demonstrate the ruggedness and reproducibility of the assay. The secondary analyst, Analyst B, will prepare stock Solution C from a separate weighing. From Solutions B and C, separate quality control sample sets are prepared by the two analysts. From these stock solutions, each analyst will prepare either calibration standards or quality control samples as described in the following sections.

Preparation of calibration standards

The primary analyst is responsible for preparing daily calibration standards during the method validation. Calibrators comprising the standard curve will be prepared by making serial dilutions from Solution A and spiking them into the appropriate biological matrix. It is recommended at least five calibrators, evenly spaced, be in the standard curve. If the standard curve range is wide, additional calibrators can be included.

Preparation of quality control samples

Quality control samples are prepared by Analysts A and B. The primary analyst prepares quality control samples from Solution B and the secondary analyst prepares them from Solution C. The quality control samples prepared by the second analyst will verify the controls prepared by the primary analyst. Solutions B and C may be used repeatedly during the validation if additional quality controls are required. Three different quality control concentrations (low, medium and high) are required and prepared from these two stock solutions. Before preparing the quality control samples, the biological matrix should be screened for endogenous components that might interfere with the chromatography. When acceptable lots of the biological matrix have been identified, adequate volumes should be reserved and used during the method validation and study proper.

Sufficient quantities of each quality control concentration are prepared by Analysts A and B for approximately 25 analytical runs. These control batches are separated into aliquots, frozen in appropriate containers, and used in the method validation and subsequent study proper analyses. When additional quality control samples are required for a study proper, they are prepared before the original quality control sample sets are depleted. Both the original and freshly prepared quality control samples are analysed concurrently to determine if they are statistically equivalent.

Method specificity

The method must be specific with no endogenous components interfering with the separation and quantitation of the principal analyte. The method should be capable of resolving co-administered drugs and metabolites from the parent drug. Retention times will be identified for all compounds and included in the validation report.

Recovery

Recovery of the analyte from the biological

matrix must be determined to ensure adequate and consistent recoveries. The recovery will be documented throughout the standard curve range. The recovery will be calculated by comparing the interpolated (extracted) from the theoretical (unextracted) concentration.

Stability

For new chemical entities, stability data are generated in biological fluids, sample containers, freeze–thaw studies, and under the appropriate chromatographic conditions. Quality control samples (low, medium and high) prepared by the primary analyst can be used to initiate the stability study. Quality control charts may be used to monitor the drug's stability characteristics under the above-mentioned conditions.

Method transfer

If the method has been validated and is to be performed by another analyst using the same chromatographic conditions, the analyst must generate at least two consecutive analytical runs with acceptable quality control results. Both runs must emulate the validation run conditions.

If a referral laboratory is contracted to perform the sample analysis and has not used the procedure previously, a complete method validation is performed by that laboratory using the validation procedure. During the validation exercise, blinded quality control samples are analysed to verify the assay results. Blinded quality control results are analysed in duplicate in two analytical runs and are subject to the validation-acceptance criteria.

Method cross-validation

If a referral laboratory intends to use an alternative analytical procedure that has not been validated, the following cross-validation guideline outlines the necessary procedure to verify that the method is reliable and reproducible.

Prior to beginning the method cross-validation, the referral laboratory will provide a copy of the methodology and their validation results. After approving the procedure and validation results, the blinded quality control samples (low, medium and high) are analysed in two different runs in at least duplicate. The acceptance criteria for the quality control samples are based on the established control chart limits determined for that method. The acceptance of the standard curve and study proper results is subject to the criteria described in the statistical-analysis paper [10].

From each standard curve, the following cross-validation data are reported.

Standard curve statistics:
- correlation coefficients;
- slopes;
- intercepts;
- interpolated standard concentrations;
- summary standard deviation for each calibrator concentration;
- summary relative standard deviation for each calibrator concentration.

Quality control results:
- individual quality control data;
- blinded quality control results;
- summary standard deviation for each calibrator concentration;
- summary relative standard deviation for each calibrator concentration.

Chromatograms:
- complete standard curve;
- quality control samples;
- blank.

Study Proper

Daily standard curves are generated to determine the sample concentrations. All calibrators and quality control samples are analysed in duplicate. Sample concentrations are based on a single determination. To ensure the assay and chromatographic system are working properly, calibrators are placed at the beginning and end of the analytical run. Quality control samples are analysed in duplicate and evenly interspersed among the clinical samples. The quality control sample sequence is carefully monitored for systematic errors. For each standard curve, the slope, intercept, variance, correlation coefficient, and the interpolated calibrator concentrations are reported.

Acceptance of the assay results are determined by monitoring the quality control results. If the interpolated concentrations are within the control charts' confidence limits, established during the method validation, the data are considered valid. Upon completing a study proper and accepting the analytical runs, the quality control results are incorporated into

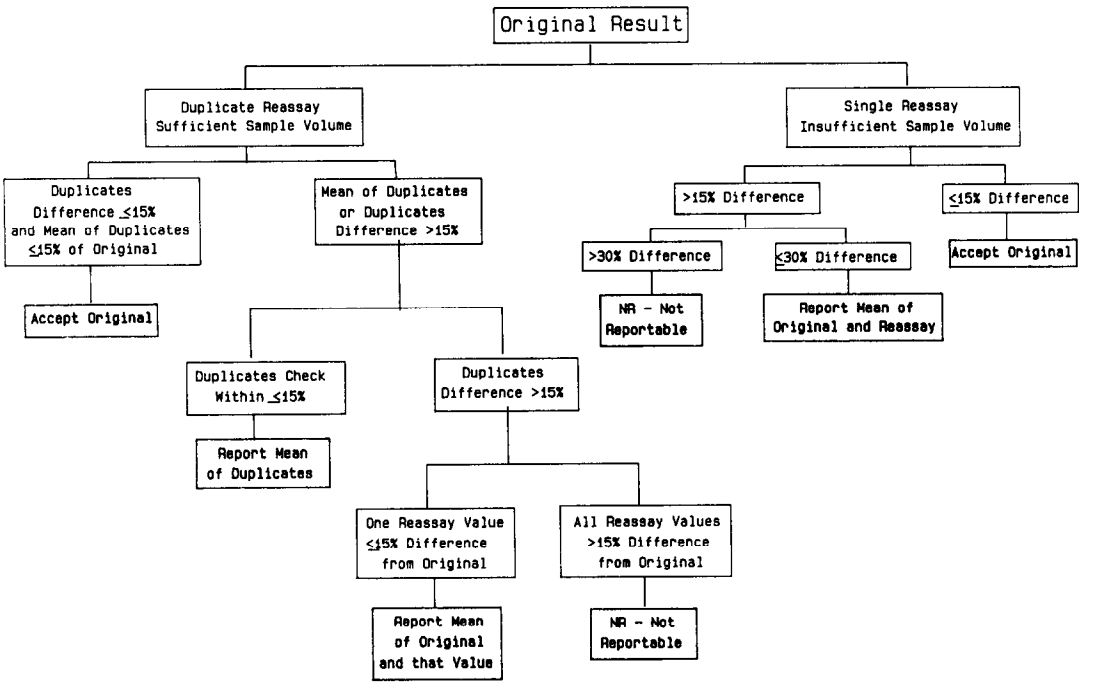

Figure 2
Reanalysis flowchart.

their respective databases to update their confidence limits.

When subject samples are reanalysed to verify the drug concentration, final data are reported using the described flowchart strategy. When possible samples are reanalysed in duplicate, and based on these results, a final concentration is reported (Fig. 2).

Conclusions

Bioanalytical method validation papers have described basic terminology but do not suggest a validation strategy to evaluate the performance of a method. The approach described in this paper provides a validation strategy that utilizes statistical analyses.

The validation strategy describes a procedure that can be used to validate any bioanalytical method as a continuous process. Taking a more statistical view of the validation results will enable clinical and bioanalytical laboratories to understand the limitations and performance expectations of a method. The second paper [10] in this two-part series will address the statistical analyses and data interpretation using the validation results.

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