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# Evaluation of criteria for the acceptance of bioanalytical data*

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**Abstract**: Results from bioanalytical analyses for registration of a new drug entity are used to define its pharmacokinetics and bioavailability/bioequivalence. Whilst analytical data may be derived from the application of a validated method, it is essential to apply mathematical criteria to its acceptance, in order that the analyst can be assured that the assay is performing within defined limits and to its validated specification.

Parameters evaluated for acceptability are the batch calibration curve, the minimum quantifiable concentration and the quality control (QC) sample acceptability. Specifically, six QC samples per analytical batch are used, two samples at each of three concentrations. The rationale for the definition of these criteria is evaluated together with a consideration of their applications and limitations. The relevance and use of Shewhart and Cusum plots to monitor assay performance is illustrated.

**Keywords**: *Acceptance criteria; calibration curves; minimum quantifiable concentration; QC samples; control charts.*

## Introduction

Bioanalytical data which are used for the determination of pharmacokinetic parameters and bioequivalence comparisons should be the subject of rigorous control in order to ensure the quality of derived parameters. Although data may be derived from applications of a validated analytical method, it is essential to apply mathematical criteria to its acceptance so that the analyst, "client" or reviewer is assured that the method is performing within defined limits and to its validated specification. This will minimize requests for repeat assays by statisticians and pharmacokineticists who may request repeat analysis of samples on the basis of lack of fit with preconceived models. Although this discussion is limited to chromatographic assays, with minimal modification it is possible to apply these criteria to immunoassays.

Those specifications which are generally accepted for validation include precision, accuracy, sensitivity, selectivity, stability, linearity and recovery. Providing the conditions under which the method is run follow a standard operating procedure (SOP) or similar method report, selectivity, stability and recovery can usually be regarded as definitive characteristics of the method. Changes to the methodology together with inherent operator and transient system variations may be monitored by observing changes in precision, accuracy, sensitivity and detector linearity.

## A Scheme of Evaluation for Analytical Batches

Since biological samples for analysis are usually analysed in batches consisting of 40–120 samples, it is good practice to monitor the assay performance of individual batches and collate the results to assess overall performance. A cost effective way to do this is to provide acceptance criteria for calibration curves, quality control (QC) samples and the minimum quantifiable concentration (MQC). Graphical methods such as the Shewhart and Cusum charts [1] can then be used to assess the assay performance with time.

## Qualitative Acceptance of Chromatography

Before invoking these criteria, however, it is essential that the analyst is satisfied with the "quality" of the chromatography and the integrity of the data management system. A qualitative assessment of the chromatograms must include attention to the peak shape,

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resolution and detector response for the analyte and internal standard from control and extracted samples. The chromatograms must also be inspected to confirm that baseline settings and integration parameters are appropriate for the analysis. The authors would recommend that a system suitability test such as that described by Szepesi [2] be used to this end. Only after such evaluation is it then possible to apply quantitative criteria to determine the acceptability of analytical batches.

*Batch acceptance criteria*

Evaluation of batch acceptability criteria is based on the performance of the following elements: (1) the calibration curve; (2) MQC; and (3) the QC samples.

*Acceptability of the calibration curve.* The same calibration function should be used for each batch in the entire study. A minimum of four or five calibration points is needed to define a linear function. However, there might be cause for concern if the number of rejected standards exceeds one or two. The range of the calibration should be set to extend from the MQC to beyond the anticipated maximum concentrations $C_{max}$. The correlation coefficient ($r$) should be at least 0.9900 and "blank" matrix extracts should show interference of <20% of the MQC.

What criteria should be used for rejection? Generally a rejected standard should be one which by its inclusion significantly changes the calibration slope or intercept. Once the calibration curve has been shown to be acceptable, it will then be possible to evaluate the MQC and the quality control samples.

*Evaluation of the MQC.* The MQC must be distinguished from the limit of detection which has no associated confidence limit. A reliable MQC is essential for the evaluation of definitive pharmacokinetics.

Empirically a judgement must be made on a single concentration. The authors follow the levels described by Aarons *et al.* [3], defining the MQC as the level having a predefined relative standard deviation of 20%. The MQC validated in this way should have a back-calculated concentration value within 20% of nominal. Any interfering peak should have a height or area 20% or less than that of the MQC. If the MQC is rejected on this basis, reference to the next appropriate calibration standard should be made. For this reason it is important to ensure that there are sufficient standards around the MQC. This will ensure that the assay may be used over the widest concentration range practicable.

*Acceptability of QC samples.* The use of three concentrations for QC samples is becoming widely accepted; usually two samples at each concentration are included in a batch. By setting limits of ±1 standard deviation (SD), the limits of the low, medium and high QC samples may be set at ±20, 15 and 10% of nominal, respectively. These values may be modified depending on assay characteristics and the objectives of the analysis.

For normal distributions, approximately 66% of data will fall within ±1 SD. On this basis, for six QC samples the expected number of acceptable values would be four. In order to minimize bias, at least one QC sample at each concentration should be acceptable.

When setting the concentration of the QC samples it is important that specific guidelines are followed to enable objective comparison of data between different studies and laboratories. The authors base these concentrations on the calibration range of an assay. Thus for the medium and high concentration QC samples the values are set at 40–60 and 70–90% of the highest calibration value, and for the low concentration QC sample, 3–5 times the MQC. In addition, it is essential that the calibration range is accurately defined with respect to the expected range of concentrations in the samples.

In preparing QC samples and calibration standards, it is essential to ensure that a consistent source of drug-free control biological matrix is used in order to minimize bias. Similarly, QC and calibration standards should be prepared by different analysts using different stocks of material. Careful consideration should be given to the location of QC and calibration standards within a batch. Standards or QC samples, either in duplicate or single, may be randomly or evenly distributed throughout the batch, or placed at the beginning and/or end of the batch. As random distribution may result in standards and QC samples aggregating, the authors have a preference for selecting the position of a QC sample or standard location within a batch, but to randomize the order to such samples assigned to these positions.

**Table 1**
Control parameters useful in monitoring assay performance with Shewhart and Cusum plots

| |
|---|
| QC sample concentration |
| Variance of QC samples |
| Mean of QC samples |
| Peak asymmetry |
| Theoretical plates |
| Internal standard replication |
| Slope of calibration function |

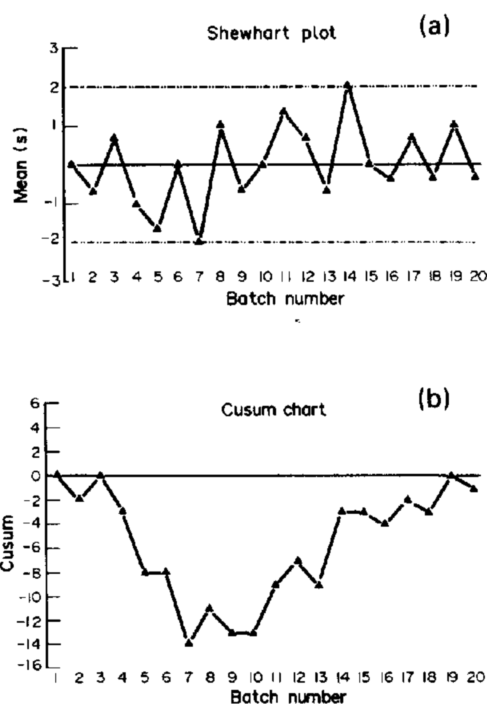


**Figure 1**
Use of Shewhart (a) and Cusum (b) plots in monitoring assay performance.

## The Use of Control Charts to Monitor Assay Performance

Control charts such as the Shewhart and cumulative sum or Cusum plots are ideal methods with which to monitor the performance of an assay over a number of batches. In its simplest form the Shewhart plot can be constructed from the mean and SD of a control parameter. Table 1 lists a range of control parameters which the authors have used in such analyses. The control parameter is plotted on the ordinate axis against the batch number (or date of analysis). If a normal distribution for the random error is assumed, 66% of the data should fall within ±1 SD, and 95% of the data within ±2 SD. Setting "warning" levels at ±2 SD will alert the analyst to diagnostic trends in control parameters (Fig. 1a). Action limits set at ±3 SD indicate severe assay problems requiring attention.

Whilst the Shewhart plot is a good way of monitoring the imprecision of assays it is difficult to detect incremental changes in accuracy (for example a consistent assay drift). For this reason a Cusum plot is useful as drifts from the data line are easier to identify at an early stage. Changes in the mean are identified by plotting the cumulative total of deviations from a reference value [4]. A comparison of both plots is shown in Fig. 1. The Shewhart plot (Fig. 1a) indicates a slight trend in the data, although the data appear to be satisfactory as the variation is within the acceptance limits. When the same data are represented as a Cusum plot (Fig. 1b) it is easier to identify assay drift, which would alert the analyst to potential problems. The Cusum plot highlights such assay drift *from* batch 10 by a *sustained* change in the slope of the line.

Often the original reference mean for Cusum plots differs from the ultimate mean value. This causes the plot to drift from the horizontal. This is not significant as the plot is used to monitor changes in slope; the greater the severity of a sustained change in slope the greater is the bias. For a fuller description of the use of Cusum charts the reader is referred to the work of Wetherill [5] and Johnson [4].

## Conclusion

The aim of batch acceptance criteria is to provide assurance to the analyst that during routine batch analysis the methodology has been performing within its originally validated specification. A minimum number of quality control samples must be included to provide the necessary data on which to make a judgement. To meet these goals it is essential that acceptance criteria are tailored to the purpose for which the data are to be used. The "quality" of the data required is therefore based on consideration of the investigator's objectives, the technical feasibility of the work and the logistical considerations of time and cost. These factors are often given a high weighting in deciding whether to pass or fail batches, the ultimate responsibility resting with the judgement of the analyst.

Assessment of these factors is made by reference to the database collated during the initial validation of the assay. It is essential therefore that some form of pilot study resembling as closely as possible the anticipated batch size and routine constraints be included in the validation. This will allow the pattern of random error to be assessed in the context of the overall study aims.

The constant dilemma in applying acceptance criteria is the difficulty of providing guidelines which can be applied to ensure that satisfactory data are produced, whilst ensuring there is sufficient latitude for the application of good scientific judgement.

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