Validation of Quantitative Methods

1. Purpose
    1.1 The purpose of this document is to describe the minimum requirements for validating quantitative methods. The reason for validation is to ensure confidence and reliability in the test results by showing the method is fit for the intended use.
    1.2 Validation is required for the following events but not limited to:
        1.2.1 Development of a new method
        1.2.2 Transfer of current method to a different instrument
        1.2.3 Modification of a current method to improve its performance
    1.3 Transfer or modification of a current method may not require all the experiments to be validated. The required experiments shall be determined based on how the change affects each of the parameters.

2. Validation Plan
    2.1 Before starting a validation, a validation plan shall be written and signed by the laboratory director and quality manager. The plan shall include the following: the reason for the validation, the instrument used for the validation, the parameters and the acceptance criteria for each parameter.
    2.2 The validation plan can be adjusted during validation if needed. The adjustments shall be evaluated and must demonstrate that the method is fit for purpose.

3. Validation Experiments
    3.1 Calibration Model
        3.1.1 The calibration range shall be determined in method development and should span the range of concentrations expected in day to day operations. Once the range is established, the calibration model shall be determined using the signal response (peak area ratio of the analyte and internal standard). The signal response is correlated with the analyte concentration. The calibration model is the mathematical model describing the correlation.
        3.1.2 Procedure
            3.1.2.1 Prepare at least 6 different non-zero concentration levels of calibrator samples over 5 runs.
            3.1.2.2 A minimum of 5 replicates for each concentration shall be used to establish the calibration model. The data points from the five runs shall be plotted together to form the calibration model. The origin shall not be used as a calibration point.
        3.1.3 Data Analysis
            3.1.3.1 All data points shall be used to make calibration curves using $1/x$, $1/x^2$ and no weighting calibration models. This can be done by exporting the data into an excel based software.
            3.1.3.2 The response ratio (area of the analyte divided by the area of the internal standard) of each calibration point shall be used to verify each weighting scheme.
            3.1.3.3 If linear regression does not achieve acceptable correlation, other appropriate regressions can be applied (e.g., quadratic).
        3.1.4 Acceptance Criteria

3.1.4.1 The weighting shall be verified by evaluating the sum of the %RE for unweighted, 1/x and $1/x^2$. The least complex weighting should be used.

$$\%RE = \frac{C_{calculated} - C_{nominal}}{C_{nominal}} X\ 100$$

3.1.4.2 Using residual plots, visual inspection of the combined calibration curves should verify the linearity across the calibration range.

3.1.4.3 Linear regression should meet the following criteria: 1.) 95% confidence interval (CI) of slope should include 1 and 2.) 95% CI of intercept should include 0.

3.1.4.4 Each calibration curve point shall be within 20% of the target in order to validate the calibration.

3.2 Bias and Precision

3.2.1 Procedure

3.2.1.1 Prepare fortified blank matrix samples using three different concentrations (low, medium and high) that span the calibration curve.

3.2.1.2 Analyze each concentration in triplicate over 5 runs.

3.2.1.3 Bias and precision studies can be run concurrently.

3.2.2 Data analysis

3.2.2.1 Evaluate the concentration of the analyte of interest at each concentration using the calibration curve.

3.2.2.2 Calculate bias, within run precision and between run precision for each concentration level.

$$Bias = \frac{Grand\ mean\ of\ calculate\ concentrations - Nominal\ concentration}{Nominal\ concentration} X\ 100$$

$$Within\ Run\ Precision = \frac{Standard\ deviation\ of\ a\ single\ run}{Average\ of\ a\ single\ run} X\ 100$$

$$Between\ Run\ Precision = \frac{Standard\ deviation\ of\ all\ runs}{Average\ of\ all\ runs} X\ 100$$

3.2.3 Acceptance criteria

3.2.3.1 Bias: %bias $\leq$20%

3.2.3.2 Within run precision: %CV $\leq$20%

3.2.3.3 Between run precision: %CV $\leq$20%

3.2.3.4 Blood alcohol analysis should require a lower %bias and %CV ($\leq$10% or better).

3.3 Limit of Detection (LOD)

3.3.1 Procedure

3.3.1.1 Using the lowest non-zero calibrator concentration, analyze a minimum of three samples per run over three runs.

3.3.1.2 The lowest non-zero calibrator in the calibration curve can be used as one of the samples above.

3.3.2 Data analysis

3.3.2.1 Evaluate the chromatograms for retention time, peak shape, ion ratios and other criteria used to identify the analyte of interest.

3.3.2.2 Evaluate signal to noise using the instrument software. Signal to noise is the height of the analyte divided by the height of the background.

3.3.3 Acceptance Criteria

3.3.3.1 The response of the analyte of interest shall be $\geq$ 3.3 times the noise level of the background.

3.3.3.2 The analyte of interest shall meet detection and identification acceptance (e.g., retention time, peak shape, and ion ratios).

3.4 Limit of Quantitation (LOQ)

3.4.1 Procedure

3.4.1.1 Using the lowest non-zero calibrator concentration, analyze a minimum of three samples per run over three runs.

3.4.1.2 The lowest non-zero calibrator in the calibration curve can be used as one of the samples above.

3.4.1.3 LOD and LOQ can be run concurrently.

3.4.2 Data Analysis

3.4.2.1 Evaluate the chromatograms for retention time, peak shape, ion ratios and other criteria used to identify the analyte of interest.

3.4.2.2 Evaluate the concentration of the analyte of interest using the calibration curve.

3.4.2.3 Calculate bias, within run precision, and between run precision.

3.4.3 Acceptance criteria

3.4.3.1 Bias: %bias <20%

3.4.3.2 Within run precision: %CV <20%

3.4.3.3 Between run precision: %CV <20%

3.4.3.4 Blood alcohol analysis should require a lower %bias and %CV (≤10% or better).

3.5 Carryover

3.5.1 Procedure

3.5.1.1 Analyze blank samples immediately after a highly concentrated sample.

3.5.1.2 Perform this analysis three times.

3.5.2 Data analysis

3.5.2.1 Compare the response of the blank sample with the response of the LOD (limit of detection) response for the analyte of interest.

3.5.3 Acceptance criteria

3.5.3.1 No analyte carryover is observed in the blank samples.

3.5.3.2 The response of the blank shall be ≤ 20% (10% for alcohol analysis) of the average responses of the LOD.

3.6 Interference Studies

3.6.1 Procedure

3.6.1.1 Evaluating interference from stable-isotope internal standards – (1) analyze a blank matrix sample fortified with the internal standard with no analyte and (2) analyze a blank matrix sample fortified with the analyte(s) of interest with no internal standard at the upper limit of the calibration range.

3.6.1.2 Evaluating interference from commonly encountered analytes – analyze a blank matrix sample fortified with the analyte of interest at the low control concentration level and the commonly encountered analyte(s) at high therapeutic levels.

3.6.2 Data analysis

3.6.2.1 Interference from stable-isotope internal standards – Evaluate the response of any peak at the retention time of the analyte of interest and at the retention time of the internal standard.

3.6.2.2 Interference from commonly encountered analytes – Evaluate the concentration of the analyte of interest using the calibration curve.

3.6.3 Acceptance criteria

3.6.3.1 Interference from stable-isotope internal standards – Response of the blank matrix shall be <20% of the average response of the LOQ over the validation.

3.6.3.2 Interference from commonly encountered analytes – The concentration of the analyte(s) of interest shall be within ±20% (5% for alcohol analysis) of the average concentration of the low control obtained in the bias and precision studies.

3.7 Dilution Integrity

3.7.1 Procedure

3.7.1.1 Dilute highly concentrated samples with blank matrix to evaluate dilution ratios that may be used in casework. Common dilutions include x2, x5 and x10.

3.7.1.2 Analyze at least three replicates for each dilution over 5 runs.

3.7.2 Data analysis

3.7.2.1 Calculate bias and precision with each concentration pool.

3.7.3 Acceptance criteria

3.7.3.1 The bias, within run precision and between run precision of the diluted samples shall be within the acceptance criteria (typically 10% for alcohol and 20% for drugs) of the bias and within run precision studies.

3.8 Autosampler Stability

3.8.1 Procedure

3.8.1.1 The stability of the analytes in the vials shall be evaluated in case the vials need to be injected at a later time due to an atypical event (e.g., instrument failure or power loss).

3.8.1.2 Determine the maximum amount of time the vials can be maintained before they undergo unacceptable changes prior to being injected (e.g., 72 hours/over the weekend).

3.8.1.3 Autosampler stability of controls: At time zero, prepare two sets of low and high control samples in triplicate. Inject one set of low and high controls and leave the second set unpunctured and maintained in a manner that they would typically be stored during routine analysis (e.g., refrigerated or room temperature on the autosampler). At the predetermined time interval, the remaining vials shall be analyzed by re-injecting the first set analyzed at time zero (punctured) and injecting the second set (unpunctured) with newly prepared calibrators and controls.

3.8.2 Data analysis

3.8.2.1 Evaluate the responses of the stored samples and compare them to the responses of the time 0 (t_0) responses.

3.8.2.2 Evaluate the responses of the re-injected time 0 (t_0) samples compared to the responses of the originally injected time 0 (t_0) responses.

3.8.3 Acceptance criteria

3.8.3.1 The average signal (peak area or ratio of peak area of analyte to internal standard) shall be within 20% of time 0 (t_0) (10% for alcohol analysis).

4.0 References

4.1 ANSI/ASB Standard 036, Standard Practices for Method Validation in Forensic Toxicology, 2019, 1st Ed.

Date	Section	Previous	Changed to	Reason	By whom
11/3/2023	3.1.4.3	Linear regression should meet the following criteria: 1.) 95% confidence interval (CI) of slope should include 1 and 2.) 95% CI of intercept should include 0.	Linear regression should meet the following criteria: 1.) 95% confidence interval (CI) of slope should include 1 and 2.) 95% CI of intercept should include 0.	Removed highlight.	KB/MH
11/7/2023	1.2.1	Development of new method	Development of a new method	Corrected grammatical errors	KB/MH
	3.1.1	The calibration range should be determined in method development and should span the range of concentrations expected in day to day operations.	The calibration range shall be determined in method development and should span the range of concentrations expected in day to day operations.		
	3.6.3.2	Interference from commonly encountered analytes – The concentration of the analyte(s) of interest shall be within +20% of the average concentration of the low control obtained in the bias and precision studies.	Interference from commonly encountered analytes – The concentration of the analyte(s) of interest shall be within +20% (5% for alcohol analysis) of the average concentration of the low control obtained in the bias and precision studies.	Updated to include alcohol criteria	