Deviation from Documented Procedures for Signatures Acknowledging Revisions to the Toxicology Section Operations Guide (TOX)

Dates of Deviation: TBD

Type of Deviation: Signature Requirements for TOX-03-00 (Validation of Quantitative Methods)

Describe the Deviation:
The Crime Laboratory normally uses a digital document management system (PowerDMS) to acknowledge the reinstatement/revision of the Toxicology Section Operations Guide (TOX) by Laboratory staff. Due to Laboratory Director not having access to this system, this deviation will be used to record the signatures of the Laboratory staff. The Quality Manager is on leave and cannot acknowledge the reinstatement/revision to the Toxicology Section Operations Guide (TOX). The revision to TOX-03-00 will go into effect once all staff have signed this deviation to exclude the Quality Manager. The Quality Manager shall acknowledge the revision on their return from leave. Once all signatures have been recorded, this deviation shall be digitized and included within the controlled document files.

LOG-17-04 Document Management
"4.6 All laboratory personnel shall be responsible for:
4.6.1 Reading and acknowledging the existence of new/revised policies and procedures"

BCCL Quality Manual 8.3.2 Document Issuance and Maintenance
"8.3.2.4 CHANGES TO ELECTRONICALLY STORED DOCUMENTS… Staff shall be notified when revised and updated documents become available…. Personnel shall be responsible for verifying that they are using and following current policies…."

Reason for Deviation: Due to leave on the part of the Quality Manager, access to the digital document management system has been disrupted until their return.

Laboratory Number(s) (if applicable): Not applicable.

Summer Swango — Evidence Technician — 10.19.23 — Date

Seized Drug — Analyst — 10/17/23 — Date

Kayla M. Bayless — Analyst — 10/17/2023 — Date

Melina Henry — Analyst — 10/17/2023 — Date

Laboratory Director — 17 Oct 2023 — Date

Quality Assurance Manager — 20 Nov. 2023 — Date

Approval Date: November 30, 2021
Effective Date: November 30, 2021

Issuing Authority: Upper Management
Authorized for Distribution by Laboratory Director

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 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 will 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 will 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 should be determined in method development and should span the range on concentrations expected in day to day operations. Once the range is established, the calibration model will 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.
       - 3.1.2.2 A minimum of 5 data points for each concentration shall be used to establish the calibration model. The data points shall be plotted together to form the calibration model. The origin should 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.

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% CI of slope should include 1 and 2.) 95% CI of intercept should include 0.

3.1.4.4 Each calibration curve point must 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.

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 may 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 must have 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 may 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 $\leq$ 20% (10% for alcohol analysis) of the response 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 and (2) analyze a blank matrix sample fortified with analyte of interest 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 on interest at the low control concentration level and the commonly encountered analyte 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 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 LOD over the validation.

3.6.3.2 3.4.2.2 Interference from commonly encountered analytes – The concentration of the analyte of interest shall be within ±20% 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 and within run precision of the diluted samples shall be within the acceptance criteria (usually 20%) 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 there is a possibility that 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 may need to set before being injected (e.g., 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. Re-inject the first set and inject the second set at the predetermined time interval with calibrators.

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 responses.

3.8.2.2 Evaluate the responses of the re-injected time 0 samples compared to the responses of the originally injected time 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 (10% for alcohol analysis).

4.0 References

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