TG 41-03

RECOMMENDED GUIDELINES FOR THE VERIFICATION AND VALIDATION OF METHODS IN FORENSIC CHEMISTRY

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1. Purpose and Scope

This guideline is supplemental to TG01 and ISO/IEC 17025:2017 and is applicable to all methods in Forensic Chemistry, specifically those in the fields of Controlled substances, Toxicology and Trace Evidence. It aims to define the concepts and processes of method verification and validation, and to provide practical guidelines in order to facilitate a standardised approach.

2. Introduction

It is a requirement of SANAS TG01 "Criteria for Laboratory Accreditation in the Field of Forensics" and ISO/IEC 17025:2017 that "all technical procedures used by a forensic science laboratory must be fully validated before being used on casework". It is preferable to use this guideline, but if an individual laboratory uses a different approach, it is their responsibility to proof the validity of their approach, with the necessary literature references and/or historical data.

2.1 **Validation** of an analytical method is the process by which it is established, by laboratory studies, that the performance characteristics of the method meet the requirements for the intended analytical applications. The process also establishes the limitations of the method, and the influences, which may change these characteristics, and to what extent.[6.1]

a) A method published in the scientific literature, with no validation characteristics given, should be fully validated.[6.6]
b) A method developed in-house, should be fully validated.[6.6]
c) When significantly modifying a fully validated method, the modification should be appropriately validated.

2.2 **Verification** refers to a process that provides evidence that the laboratory can achieve the performance characteristics given in a specific analytical method, especially accuracy and precision, and demonstrating that the method is suitable for the intended use. The extent and nature of such verification work depends on the needs of the customer, and the intended use.[6.6]

a) A method that has been fully validated (studied in a collaborative trial), should be proven by the laboratory to be able to fulfill the requirements of the analytical task, or that the laboratory is capable of achieving the performance characteristics of the method.[6.6]
b) In a method that has been fully validated, but where a new matrix or new instrument is used, trueness, precision, and possibly the detection limit should be verified.[6.6]
c) In a well-established, but not collaboratively studied method, verification should be supplemented with limited validation, for example with regard to reproducibility.[6.6]
d) A method published in the scientific literature, with validation characteristics given, a verification should be supplemented by a limited validation of the method's repeatability and reproducibility.[6.6]

3. Definitions

Accuracy - The accuracy of an analytical method, is the closeness of test results obtained by that method, to the true value,[6.1] and is expressed as the percent recovery of the analyte of interest.

Bias (Trueness) - Measurement trueness describes the closeness of agreement between the average of replicate measured values and a reference quantity value. Recovery tests are used to assess bias. Bias is measured by the detection of a known amount of an analytical parameter added to a specimen and included throughout the method of analysis. A blank should also be analysed. A significant departure from the accepted reference value is manifested in a high level of bias.

Certified reference material (CRM) - This refers to material of which the identity and/or purity have been certified by one or more external certification institution. Such material will have certificates of identity and/or purity supplied with it, and such certificates will be on record.

Limit of detection (LOD) - It is the lowest amount of analyte in a sample that can be detected, but not necessarily quantified, under the stated experimental conditions. The detection limit is usually expressed as the concentration of analyte in the sample.[6.1] Alternatively, the laboratory may determine the LOD by determining the mean value for the blank, and adding three standard deviations (SD) to this value (LOD = Xm + 3 SD)[6.8, 6.9] LOD in chromatography - The smallest concentration of analyte in a sample, needed to give a peak height three times the noise level of the background signal of a blank sample.[6.8]

Limit of quantitation (LOQ) - It is the lowest amount of analyte in a sample that can be determined with acceptable precision and accuracy under the stated experimental conditions.[6.1] The LOQ may be derived by adding ten standard deviations to the true value of the blank. However, it is preferable to determine the LOQ experimentally as the lowest concentration for which an acceptable coefficient of variation can be routinely achieved.[6.8]

Linearity refers to the ability (within a given range) of a method to obtain test results which are directly proportional to the concentration of analyte in the sample, or to its ability to elicit test results that are directly, or by a well-defined mathematical transformation, proportional to the concentration of analyte in samples within a given range.[6.1]

Precision - The precision of an analytical method refers to the closeness of results to each other and is usually expressed as the standard deviation (coefficient of variation) of a series of measurements, and may be a measure of either the degree of reproducibility or repeatability.[6.1]

Primary standard (P) - This refers to material that is designated or widely acknowledged as having the highest metrological qualities. It is accepted without reference to any other standard.

Range - The range of an analytical method is the interval between the upper and lower levels of analyte, including these levels that have been demonstrated to be determined with a suitable level of precision, accuracy, and linearity, using the method as written. The range is normally expressed in the same units as the test results obtained by the analytical method.[6.1]

Recovery - Recovery of the analyte of interest from a given matrix can be used as a measure of the accuracy or the bias of the method. The same range of concentrations as employed in the linearity studies is used. The linearity experiment is repeated in the presence of matrix constituents.

Relative Standard Deviation (RSD) - Standard deviation of the mean, also known as the coefficient of variation (CV),[6.7]

Reproducibility - This refers to replicate analysis by different operators, using different equipment, over a set time period/the use of the analytical method, using different operators and/or different instrumentation and/or different laboratories. (Between-run precision[6.7])

Specificity refers to the ability of a method to respond to the particular analyte measured.

Selectivity refers to the ability of a method to respond to the particular analyte of interest in the presence of possible interferences.

Standard - This refers to material of a known identity, which can be used as a reference to determine the identity of unknown material.

Standard Deviation (SD) - Measure of spread, see formula in 4.1.5 below.[6.7]

4. General List of Requirements

All assessments are done in accordance with the relevant ISO/IEC Standards and SANAS requirements. SANAS documents are available from SANAS and are made available on receipt of the application for Accreditation. Additional copies of SANAS documentation may be purchased from the office.

4.1 Examination Procedures (Section 7.2)

4.1.1 Confirmatory qualitative and quantitative analytical methods

a) Verification of a qualitative analytical method[6.4, 6.5]

Characteristics	Procedures to be followed	Acceptance criteria
Limit of detection	Analysis of blanks and a range of standards (use P's or CRM's where possible), in duplicate, with samples spiked within working range of the analytical method/instrumentation/tool	LOD = Xm + 3 SD Xm = mean value of the blank SD = standard deviation, or S/N=3 from blank studies or calibration[6.10]
Matrix effects	Analysis of matrix blanks, matrix spiked with standards, and standards (use P's or CRM's where possible), in duplicate in each sample matrix type	None, or a constant matrix effect.
Selectivity	Analysis of reagent blanks, matrix blanks, matrix spiked with standards representative of the analyte and possible interferences, and standards (use P's or CRM's where possible), one of each, analysed once	Selective response to the analyte of interest in the presence of possible interferences.
Bias	Analysis of reagent blanks, matrix blanks, matrix spiked with standards representative of the analyte (replicate samples).	Determine the significance of the departure from the true value.
Precision (repeatability)	Replicate analysis of samples. Looking at linearity range, select three different concentrations representing the upper, middle and lower end of the linear range. At least 5 analyses at each concentration in each sample matrix type, and calculate the RSD, using the formula given in 4.1.	Instrument repeatability, 10 measurements, with RSD-acceptable for given method.
Precision (reproducibility)	Replicate analysis by different operators, over a set time period. (using different equipment and doing inter-laboratory studies if possible)	Influenced by random errors. Random errors cause the individual results to fall on both sides of the average value.6.7 It should thus be such that it is acceptable for the individual laboratory and technique, as supported by literature or historical data.
Accuracy (can be expressed as the percent of analyte recovered)	Analysis of matrix spiked with standards within the working range (use P's or CRM's where possible). At least 5 replicate analyses of each CRM. (For formula, see 4.1.5)	A comparison of each mixture's true value versus the measured result for samples of known concentration, should have less than the acceptable % error for the particular method and application.[6.11]

b) Verification of a quantitative analytical method[6.4, 6.5, 6.8]

Characteristics	Procedures to be followed	Acceptance criteria
Limit of detection and quantification	Analysis of blanks and a range of standards (use P's or CRM's where possible), in duplicate, with samples spiked within the working range of the analytical method/instrumentation/tool.	LOD = Xm + 3 SD Xm = mean value of the blank SD = standard deviation, or S/N=3 from blank studies or calibration[6.10] LOQ = Xm + 10 SD, or LOQ=the concentration which gives a defined RSD, or S/N=10[6.10]
Matrix effects	Analysis of matrix blanks, matrix spiked with standards, and standards (use P's or CRM's where possible), in duplicate in each sample matrix type.	None, or a constant determinable matrix effect.
Selectivity	Analysis of reagent blanks, matrix blanks, matrix spiked with standards representative of the analyte and possible interferences, and standards (use P's or CRM's where possible), one of each, analysed once.	Selective response to the analyte of interest in the presence of possible interferences.
Accuracy (can be expressed as the percent of analyte recovered)	Analysis of matrix spiked with standards within the working range (use P's or CRM's where possible). At least 5 replicate analyses of each CRM. (For formula, see 4.1.5)	A comparison of each mixture's true value versus the measured result for samples of known concentration, should have less than the acceptable % error for the particular method and application.[6.11]
Linearity range	Analysis of a range of standards (use P's or CRM's where possible) At least 7 at each of 7 concentrations over working range.	Judge linearity by: I) visual inspection of the response/concentration curve, and the correlation coefficient, r, (for r, see 4.1.5), with r □0,998[6.11], and ii) performing linear regression on each of the original area or height data points, by using the calculated best fit line, Y= mX+ b. (least squares method)[6.2, 6.3] *b*=*y*-intercept of best line fit *m*=the slope of best line fit. (For formulas of m and b, see 4.1.5)
Bias	Analysis of reagent blanks, matrix blanks, matrix spiked with standards representative of the analyte (replicate samples).	Determine the significance of the departure from the true value.
Precision (repeatability)	Replicate analysis of samples. Looking at linearity range, select three different concentrations representing the upper, middle and lower end of the linear range. At least 5 analyses at each concentration in each sample matrix type, and calculate the RSD, using the formula given in 4.1.	Instrument repeatability, 10 measurements, with RSD-acceptable for given method.
Precision (reproducibility)	Replicate analysis by different operators, over a set time period. (using different equipment and doing inter-laboratory studies if possible)	Influenced by random errors. Random errors cause the individual results to fall on both sides of the average value.6.7 It should thus be such that it is acceptable for the individual laboratory and technique, as supported by literature or historical data.

c) Validation of a qualitative analytical method [6.4, 6.5]

Characteristics	Procedures to be followed	Acceptance criteria
Limit of detection	Analysis of blanks and a range of standards (use P's or CRM's where possible). At least 7 determinations of at least 7 concentrations covering the anticipated range.	LOD = Xm + 3 SD Xm = mean value of the blank SD = standard deviation
Matrix effects	Analysis of matrix blanks, matrix spiked with standards, and standards (use P's or CRM's where possible), once at each of 3 concentrations in each sample matrix type.	None, or a constant matrix effect.
Selectivity	Analysis of reagent blanks, matrix blanks, matrix spiked with standards representative of the analyte and possible interferences, and standards (use P's or CRM's where possible), one of each, analysed once.	Selective response to the analyte of interest in the presence of possible interferences.
Bias	Analysis of reagent blanks, matrix blanks, matrix spiked with standards representative of the analyte (replicate samples).	Determine the significance of the departure from the true value.
Precision (repeatability)	Replicate analysis of samples. Looking at linearity range, select three different concentrations representing the upper, middle and lower end of the linear range. At least 5 analyses at each concentration in each sample matrix type, and calculate the RSD, using the formula given in 4.1.	Instrument repeatability, 10 measurements, with RSD-acceptable for given method.
Precision (reproducibility)	Replicate analysis by different operators, over a set time period. (using different equipment and doing inter-laboratory studies if possible)	Influenced by random errors. Random errors cause the individual results to fall on both sides of the average value.[6.7] It should thus be such that it is acceptable for the individual laboratory and technique, as supported by literature or historical data.
Accuracy (can be expressed as the percent of analyte recovered)	Analysis of matrix spiked with standards within the working range (use P's or CRM's where possible). At least 5 replicate analyses of each CRM. (For formula, see 4.1.5)	A comparison of each mixture's true value versus the measured result for samples of known concentration, should have less than the acceptable % error for the particular method and application.[6.11]

d) Validation of a quantitative analytical method [6.4, 6.5, 6.8]

Characteristics	Procedures to be followed	Acceptance criteria
Limit of detection and quantification	Analysis of blanks and a range of standards (use P's or CRM's where possible). At least 7 determinations of at least 7 concentrations covering the anticipated range.	LOD = Xm + 3 SD Xm = mean value of the blank SD = standard deviation or S/N=3 from blank studies or calibration[6.10] LOQ=the concentration which gives a defined RSD, or S/N=10[6.10]
Selectivity	Analysis of reagent blanks, matrix blanks, matrix spiked with standards (in working range) representative of the analyte and possible interferences, and standards (use P's or CRM's where possible), one of each, analysed once.	Selective response to the analyte of interest in the presence of possible interferences.
Linearity range	Analysis of a range of standards (use P's or CRM's where possible). At least 7 at each of 7 concentrations over working range (representing approximately 10-200% of the target concentration).	Judge linearity by: I) visual inspection of the response/concentration curve, and the correlation coefficient, r, (for r, see 4.1.5), with r □0,998[6.11], and ii) performing linear regression on each of the original area or height data points, by using the calculated best fit line, Y= mX+ b. (least squares method)[6.2, 6.3] *b*=*y*-intercept of best line fit, and *m*=the slope of best line fit. (For formulas of m and b, see 4.1.5)
Precision (repeatability)	Replicate analysis of samples. Looking at linearity range, select three different concentrations representing the upper, middle and lower end of the linear range. At least 5 analyses at each concentration in each sample matrix type, and calculate the RSD, using the formula given in 4.1.	Instrument repeatability, 10 measurements, with RSD-acceptable for given method.
Accuracy (can be expressed as the percent of analyte recovered)	Analysis of matrix spiked with standards within the working range (use P's or CRM's where possible). At least 5 replicate analyses of each CRM. (For formula, see 4.1.5)	A comparison of each mixture's true value versus the measured result for samples of known concentration, should have less than the acceptable % error for the particular method and application.[6.11]
Recovery	Analysis of samples spiked with standards within the working range (use P's or CRM's where possible). At least 5 analysis at each of three concentrations in each sample matrix type..	Recovery of standards (P's or CRM's). ±2% at concentrations of 80% to 120% of the target concentration.[6.10]
Reproducibility	Replicate analysis by different operators, over a set time period. (using different equipment and doing inter-laboratory studies if possible)	Influenced by random errors. Random errors cause the individual results to fall on both sides of the average value.[6.7] It should thus be such that it is acceptable for the individual laboratory and technique, as supported by literature or historical data.
Bias	Analysis of reagent blanks, matrix	Determine the significance of the departure from

Characteristics	Procedures to be followed	Acceptance criteria
	blanks, matrix spiked with standards representative of the analyte (replicate samples).	the true value.

4.1.2 Formulas

a) Correlation coefficient, r, where r= $\frac{\sum_{i=1}^{n} X_i Y_i}{\sqrt{(\sum_i^n X_i^2)(\sum_i^n Y_i^2)}}$ with X= analyte concentration, and Y= analyte response

b) Slope of the best line fit, m, where $m = \frac{n\sum x_i y_i - \sum x_i \sum y_i}{n\sum x_i^2 - (\sum x_i)^2}$

c) *y-intercept of the best line fit, b, where* $b = \frac{\sum x_i^2 \sum y_i - \sum x_i \sum x_i y_i}{n\sum x_i^2 - (\sum x_i)^2}$

d) Relative Standard Deviation, RSD, where %RSD= $s100/\bar{x}$, s=standard deviation of the mean observed value, where s= $\sqrt{\sum_{i=1}^{n}(x_i - \bar{x})^2 / n-1}$, and $\bar{x}$ is the arithmetic mean $\bar{x} = \frac{\sum x}{n}$, i.e. the sum of all the measurements, divided by the number of measurements.

e) Accuracy=μ/$\bar{x}$ x 100%, $\bar{x}$ = mean observed value, μ=true or nominal value at which sample has been spiked.

f) $w_{LOD}=w_B+3s_B$; $w_{LOQ}=w_B+10\ s_B$; w_{LOD}=analyte concentration giving a signal equal to the blank signal(w_B), w_B=blank signal, s_B=standard deviation of blank signal.

4.1.3 Presumptive test methods

Presumptive test methods need not be validated. The minimum requirement for the use of a specific presumptive test method, which is not validated, is that it should be documented in a recognized scientific publication and verified within the laboratory. The origin of all reagents used, will be fully documented. The establishment of the proper function of the method through the analysis of standards will be fully documented. This standard could be an analyte identified by using a fully validated method, a certified reference material or a primary standard.

4.2 Control of Records (Section 8.4)

Records of all printouts and calculations generated during validation procedures should be kept with a designated person, for a specified time period. Validated methods Should be officially designated in writing by the laboratory Director, or a suitable designated person. Each laboratory seeking accreditation Should have in place a policy and relevant procedures regarding method validation and verification.

ADDENDUM 1: Amendment Record

Proposed By:	Section	Change
STC	Whole document	Aligned document with the new ISO/IEC 17025:2017. Changed all “shall” to “should”