Sections Included in this Document and Document History

1. Purpose

This document establishes the guidelines for method selection and the procedures for verification of standard method performance, as well as the validation of non-standard methods.

2. Scope

ORA laboratories verify standard method performance and validate non-standard methods introduced into the laboratory.

3. Responsibilities

A. Directors:

- ensures implementation of method verification and validation procedures.

B. Supervisors:

- implements method verification and validation procedures in respective division.

C. Staff:

- adheres to written protocol for method performance verification, validation or modification.

4. Background

None

5. References

A Laboratory Guide to Method Validation, (Eurachem).

6. Procedure

6.1 Method Selection

A. Selection of Methods

An integral part of the laboratory quality system is the use of standard methods. Standard methods are used, whenever possible, or unless otherwise specified by the Compliance Program or the customer. Non-standard methods are used in cases where a standard method does not exist and the customer has agreed to its use. A clear expression of quality objectives and testing parameters or criteria are made when a non-standard method is employed.

6.2 Standard Method Verification

A. Standard or FDA *official* methods need verification to ensure that the laboratory is capable of performing the analysis. The laboratory's quality control program as described in ORA-LAB.5.9 addresses this need. Verification of an analytical procedure is the demonstration that a laboratory is capable of replicating with an acceptable level of performance a standard method. Verification under conditions of use is demonstrated by meeting system suitability specifications established for the method, as well as a demonstration of accuracy and precision or other method parameters for the type of method. Method performance is accomplished by using performance characteristics such as:

- blanks in chemistry, or un-inoculated media in microbiology, to assess contamination;
- laboratory control samples - spiked samples for chemistry or positive culture controls for microbiology, to assess accuracy;
- precision based on the analysis of duplicates;
- calibration check standards analyzed periodically in the analytical batch for quantitative analyses; and
- monitoring quality control samples, usually through the use of control charts.

6.3 Method Validation

A. Validation of Method Performance

1. Non-standard and laboratory-developed methods need method validation. This activity is planned and assigned to qualified personnel. The method's performance characteristics are based on the intended

use of the method. For example, if the method will be used for qualitative analysis, there is no need to test and validate the method's linearity over the full dynamic range of the equipment.

2. Typical validation characteristics which should be considered are:

   - accuracy,
   - precision,
   - specificity,
   - detection limit,
   - limit of quantitation,
   - linearity,
   - range, and
   - ruggedness and robustness.

   See Section 7 for definitions of these characteristics.

B. Validation Tools

The following tools can be used to demonstrate the ability to meet method specifications of performance:

1. *Blanks:* Use of various types of blanks enables assessment of how much is attributable to the analyte and how much is attributable to other causes.

2. *Reference materials and certified reference materials:* Use of known materials can be used to assess the accuracy of the method, as well as obtaining information on interferences.

3. *Fortified (spiked) materials and solutions:* Recovery determinations can be made from fortification or spiking with a known amount of analyte.

4. *Incurred materials:* These are materials in which the analyte of interest may be essentially alien, but has been introduced to the bulk at some point prior to the material being samples.

5. *Measurement standards:* These are substances used for calibration or identification purposes. When placed periodically in an analytical batch, checks can be made that the response of the analytical process to the analyte is stable.

6. *Replication:* Replicate analysis provides a means of checking for changes in precision in an analytical process which could adversely affect the results.

7. *Statistics:* Statistical techniques are employed to evaluate accuracy, precision, linear range, limits of detection and quantification, and measurement uncertainty.

C. Validation Protocol Guidance

Laboratories document their protocol, the performance characteristics measured, and acceptance limits for the validation of non-standard and laboratory developed methods. The extent of validation will depend on constraints imposed such as time, cost, amount of sample or standard, future use of method, or type of information (quantitative, qualitative, screening). Due to these constraints, not all characteristics may be applicable. The following gives guidelines for determination of performance characteristics:

1. Chemistry

   a. *Perform system suitability requirements:* injection repeatability, peak resolution, relative retention for liquid chromatography analyses.

   b. *Quantitative measurements:* Determine detection limit, either method detection limit (MDL) according to 40 CFR, Part 136, Appendix B or limit of detection (LOD). LOD is determined by analyzing sample blanks, calculating the standard deviation, and expressed as the mean plus 3 standard deviations. For qualitative measurements, determine the concentration threshold below which specificity becomes unreliable.

   c. *Quantitative measurements:* Determine linear calibration range if a standard curve is to be used or determine the target calibration standard and linearity if only a one calibration point is to be used.

   d. *Quantitative measurements:* Prepare and analyze spiked blanks, solvent or matrix samples of known concentration utilizing at least three different concentration levels: low, middle, and high. These samples are carried through the complete sample preparation

procedure. Matrix effects can also be assessed with these samples. Accuracy (percent recovery) and precision (relative standard deviation or relative percent recovery) are calculated from the results.

e. *Analyze blanks (reagent, solvent and matrix*).

f. *Evaluate interferences:* spectral, physical, chemical or memory by analyzing a sample containing various suspected interferences in the presence of the measure:

- Spectral interference may be observed when an overlap of a spectral line from another element or background contribution occurs.
- Physical interference may occur from effects associated with sample transport processes on instruments.
- Chemical interferences are characterized by compound formation, ionization or vaporization effects.
- Memory interference occurs from contribution of signal from previous sample to sample being tested.

g. *Prepare the laboratory procedure (LP).* Infrequently used or non-routine methods do not need an LP until they become routine.

2. Microbiology

a. *Meet method system suitability requirements*, if applicable. The suitability of the method is checked and confirmed by comparing with requirements typical for the intended use of the method. For example, a filtration method for a non-filterable food, a five day test where three days are needed, a 1 gram test where 100 grams are needed, surface tests for Colony Forming Units (CFU)/square area where CFU/gram is needed.

b. *Include un-inoculated medium control* to assess contamination from the laboratory. This control is considered a blank and is to exhibit no growth.

c. *Prepare and analyze positive and negative culture controls.* A

negative control is atypical, negative or no growth and the positive control is positive or shows microbial growth.

d. *A spike positive culture control is prepared and analyzed.* Unless otherwise specified, it is recommended that a 25 gram sample be spiked with an inoculum of 30 cells or less. This assesses the matrix effects as well as the sensitivity of the method.

e. *Evaluate interferences.* This assesses the selectivity and specificity of the method.

f. *Prepare the laboratory procedure (LP).* Infrequently used or non-routine methods do not need a LP until they become routine.

D. Validation of Method Modifications

1. In cases where the testing procedure is modified from the standard or existing testing procedure and protocol, it is demonstrated that the modifications do not adversely affect the precision and accuracy of the data obtained.

2. See Attachment A for general guidelines for allowable modifications to a method before a modification protocol is needed.

3. In order to implement the modification, the standard or existing method is first performed. Each major modification is then verified against the original method.

4. Additional statistics employed to verify performance specifications are:

   a. The *t* test for significance of difference between the two sample means to determine degree of accuracy. The *t*-Stat value is to be less than or equal to the *t*-critical value.

   b. The F test for significance of difference between the two sample variances to determine degree of precision. The F value is to be less than or equal to the F-critical value.

E. Documentation

The results from the validation study are submitted for approval as designated by the laboratory. Statistical techniques are employed to

evaluate the method performance and determine its use. See Attachment B for an example of a form to record validation results.

7. Definitions

Accuracy **–** Accuracy is the nearness of a result or the mean of a set of measurements to the true value.

Analytical batch – An analytical batch consists of samples which are analyzed together with the same method sequence and the same lots of reagents and with the manipulations common to each sample within the same time period or in continuous sequential time periods.

Detection limit – A detection limit is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value. It is often called the *limit of detection (LOD)* which is the lowest concentration level that can be determined statistically different from a blank at a specified level of confidence. It is determined from the analysis of sample blanks. *Method detection limit (MDL)* is the minimum concentration of a substance than can be measured and reported with 99% confidence that the analyte concentration is greater than zero. It is determined from analysis of a sample in a given matrix containing the analyte.

Limit of quantitation – This is the level above which quantitative results may be determined with acceptable accuracy and precision.

Linearity – Linearity is the ability of the method to elicit results that are directly proportional to analyte concentration within a given range.

Non-standard method – This refers to a method that is not taken from authoritative and validated sources. This includes methods from scientific journals and unpublished laboratory-developed methods.

Precision – Precision is the agreement between a set of replicate measurements without assumption of knowledge of the true value. *Repeatability* expresses the precision under the same operating conditions over a short period of time. *Intermediate precision* expresses within-laboratory variations, such as different days, different analysts, and different equipment. *Reproducibility* expresses the precision between laboratories.

Range – A range is the interval between the upper and lower concentration of analyte in sample for which it has been demonstrated that the analytical procedure has an acceptable level of accuracy, precision, and linearity.

Ruggedness or robustness – Ruggedness is a measure of an analytical procedure's capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

Specificity **–** Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present.

Standard method –This is a method that is traceable to a recognized, validated method. FDA "official" methods are those in compendia specified in the Food Drug & Cosmetic Act and prescribed in the Code of Federal Regulations. Additionally, methods in Applications and Petitions that have *official* status are included under the umbrella of standard methods. *Official* methods include those in the United States Pharmacopeia, National Formulary, Homeopathic Pharmacopeia of the United States, Official Methods of Analysis of the Assocaition of Official Analytical Chemists (AOAC) International or any supplement of any of them, American Public Health Association (APHA) Compendium of Methods for the Microbiological Examination of Foods. *Official* methods also include methods that are found in a FDA Compliance Program, the Pesticide Analytical Manual (PAM), the Food Additives Analytical Manual, the Food Chemicals Codex, FDA Bacteriological Analytical Manual, FDA Macroanalytical Procedures Manual (MPM), and ORA Laboratory Information Bulletins (LIBs). In addition, methods in approved Abbreviated New Drug Applications (ANDA), New Drug Applications (NDA), New Animal Drug Applications (NADA), Food Additive Petitions (FAP) and Pesticide Petitions (PP) are considered standard methods.

Validation, method – A method validation is the process of establishing the performance characteristics and limitations of a method and the identification of the influences which may change these characteristics and to what extent. (Eurachem Guide)

Verification – A verification is the confirmation by examination and provision of objective evidence that specified requirements have been fulfilled. (ISO 8402:1994)

8. Records

Validation data and statistics

9. Supporting Documents

ORA-LAB1 Microbiological Controls for Sample Analysis
International Conference on Harmonization (ICH) Topic Q2A
Code of Federal Regulations. Title 40, Part 136, Appendix B

10. Attachments

Attachment A: Modification Criteria
Attachment B: Example Validation Form

11. Appendix

Appendix 1: ORA Validation and Verification Guidance for Human Drug Analytical Methods

Document History					
Version No.	**Status** (I, R, C)	**Date Approved**	**Change History**	**Name & Title**	
				Author	**Approving Official**
1.1	I	10-03-03	NA	LMEB	DFS
1.2	R	09/09/05	Appendix 1 added as section 11 and inserted	DFS	DFS/LMEB

Approving Official's signature: _____ Date:_____

If adjustments to operating conditions are needed, each of the following is the maximum specification that can be considered. All adjustments falling outside the maximum specifications will be considered as method modifications and will be subject to the method modification protocol.

pH of Mobile Phase (HPLC): The pH of the aqueous buffer used in the preparation of the mobile phase can be adjusted to within ±0.2 pH units of the value or range specified.

Concentration of Salts in Buffer (HPLC): The concentration of the salts used in the preparation of the aqueous buffer used in the mobile phase can be adjusted to within ±10%, provided the permitted pH variation is met.

Ratio of Components in Mobile Phase (HPLC): The following adjustment limits apply to minor components of the mobile phase (specified at 50% or less). The amount(s) of these component(s) can be adjusted by ±30% or ±2% absolute (i.e., in relation to the total mobile phase), whichever is larger. However, the change in any component cannot exceed ±10% absolute, nor can the final concentration of any component be reduced to zero. Examples of adjustments for binary and ternary mixtures are given below.

Binary Mixtures: Specified Ratio of 50:50 – Thirty percent of 50 is 15% absolute, but this exceeds the maximum permitted change of ±10% absolute in either component. Therefore the mobile phase ratio may be adjusted only within the range of 40:60 to 60:40. Specified Ratio of 95:5 – Thirty percent of 5 is 1.5% absolute. However, because adjustments up to ±2% absolute are allowed, the ratio may be adjusted within the range of 93:7 to 97:3. Specified Ratio of 2:98 – Thirty percent of 2 is 0.6% absolute. In this case an absolute adjustment of –2% is not allowed because it would reduce the amount of the first component to zero. Therefore, the maximum allowed adjustment is within the range of 1.4:98.6 to 2.6:97.4.

Ternary Mixtures: Specified Ratio of 60:35:5 – For the second component, thirty percent of 35 is 10.5% absolute, which exceeds the maximum permitted change of ±10% absolute in any component. Therefore, the second component may be adjusted only within the range of 25 to 45% absolute. For the third component, thirty percent of 5 is 1.5% absolute. Since ±2% absolute is permitted and provides more flexibility, the third component may be adjusted within the range of 3 to 7% absolute. In all cases, a sufficient quantity of the first component is used to give a total of 100%.

Wavelength of UV-Visible Detector (HPLC): Deviations from the wavelengths specified in the method are not permitted. The procedure specified by the detector manufacturer, or another validated procedure, is to be used to verify that error in the detector wavelength is, at most, ±3 nm.

Column Length (GC, HPLC): May be adjusted by as much as ±70%.
Column Inner Diameter (GC, HPLC): may be adjusted by as much as ±50%.

Flow Rate (GC, HPLC): May be adjusted by as much as ±50%.
Injection Volume (GC, HPLC): May be reduced as far as is consistent with accepted precision and detection limits. It may be increased to as much as twice the volume specified, provided there are no adverse effects on factors such as baseline, peak shapes, resolution, linearity and retention times.

Particle Size (HPLC): May be reduced by as much as 50%.
Column Temperature (HPLC): May be adjusted by as much as ±20%.

Film Thickness (Capillary GC): May be adjusted by as much as –50 to +100%.
Column Temperature (GC): May be adjusted by as much as ±2%, in terms of absolute temperature.
Oven Temperature Program (GC): Adjustment of temperatures is permitted as stated above. For the times specified for the temperature to be held or for the temperature to be changed from one to another, an adjustment of up to ±20% is permitted.

REPORT FOR: _____ REQUEST #: ___

ANALYST(S) NAME AND DATE: _____
_____

METHOD NAME: _____
_____

RESULTS:

ACCURACY: ____PASS ____FAIL ____N/A (Criteria – Define)
PRECISION: ____PASS ____FAIL ____N/A (Criteria – Define)

DETECTION LEVEL:____PASS____FAIL ____N/A (Criteria-Define)

INTERFERENCE(S): ____PASS ____FAIL ____N/A (Criteria – Pass=interferences resolved; Fail=interferences not resolved)

t TEST: ____PASS ____FAIL ____N/A F TEST: ____PASS ____FAIL ____N/A
(Criteria = < t Critical; < F Critical)
ADDITIONAL PERFORMANCE MEASUREMENTS (i.e. linearity, corr.coef..)
_____

____PASS ____FAIL ____N/A

COMMENTS: _____
_____

ANALYST(S) SIGNATURE AND DATE: _____
_____

CONCURRANCES:
SUPERVISOR: ____YES____NO
SIGNATURE AND DATE: _____
QMS MANAGER: ____YES____NO
SIGNATURE AND DATE: _____
LABORATORY DIRECTOR: ____YES____NO
SIGNATURE AND DATE: _____

A. Purpose: Products that are non-compendial, OTC, or pharmacy-compounded do not require an NDA or ANDA to be marketed. Nevertheless, ORA labs can be called upon to test these products. Instances also exist where "Standard Methods" (Compendial or NDA/ANDA methods) are not applicable to a certain product, require equipment not available in the laboratory, are outdated or not readily available, or are not the most efficient use of a laboratory's resources. This document provides uniform guidance to ORA laboratories on minimum requirements for validation of drug analytical methods developed for this purpose.

Validation, in simplest terms, is defined as the demonstration that an analytical method is suitable for its intended purpose. It is important to recognize that, especially in drug analysis, different types of methods exist for different purposes. These methods can be grouped into categories, each category requiring a different set of validation parameters. Categories of methods are discussed below.

The ORA Laboratory Manual directs that validation is required when a new method is developed, when an existing validated method is significantly modified, or when an existing validated method is applied to a sample matrix significantly different from that for which the method was developed.

Verification (sometimes also referred to as "method transfer") is defined as an assurance that a laboratory other than the originator of a Standard Method or other previously-validated method can obtain comparable results, using its equipment and personnel, as the originator of the method; in other words, that the method is suitable under actual conditions of use in a particular laboratory.

Presented here are the ***minimum*** requirements for validation of drug methods within ORA. This is primarily designed to address methods for single-occurrence or internal use: for a single sample or a small group of similar samples. Validation of methods intended for use by multiple labs, for publication in a scientific journal, or for establishment as a future "Standard Method" require additional validation; this is addressed in Notes (c) and (d), below. In any case, labs may in certain circumstances justifiably find the need to perform additional validation steps. However, the value of additional information gained by such work must be weighed against the resources expended in the process.

Also presented are acceptance criteria for each validation parameter. These must be considered carefully. These acceptance criteria apply to "conventional" dosage forms (tablets, capsules, solutions, aqueous injections, etc.) where matrix interference is usually minimal. For more complex matrices (creams, suppositories, etc.), meeting these criteria may be impossible. Other considerations, such as reduction of spiking levels due to limited standard availability, may also cause difficulties in meeting the criteria. Such situations must be evaluated, approved, and documented on a case-by-case basis (see note a).

References:

International Conference on Harmonisation (ICH) Harmonised Tripartite Guideline: Text on Validation of Analytical Procedures (Q2A)

International Conference on Harmonisation (ICH) Harmonised Tripartite Guideline: Validation of Analytical Procedures: Methodology (Q2B)

United States Pharmacopeia (USP) section <1225>: Validation of Compendial Methods

B. Validation Parameters: The following validation parameters (referred to as "Analytical Performance Characteristics" in USP and ICH documents), are to be applied based on the category of method being validated, according to the chart below. Definitions are as are commonly accepted by the scientific community, and expressed in the USP and ICH documents referenced above.

1. **Accuracy**: Prepare 3 preparations of composited sample, containing a known quantity of added analyte ("matrix spike"), so that the expected concentrations are as follows:
   a. Assay: range at least 80%-120% of expected content
   b. Content Uniformity: range at least 70%-130% of expected content (note: if Assay and Content Uniformity methods are the same, accuracy determination ranging 70%-130% of expected content will satisfy requirements for both methods.)
   c. Dissolution/release rate determinative step: range at least 20% less than lower dissolution limit to 20% greater than higher dissolution limit

   Acceptance Criteria: 97.0% - 103.0% recovery for each spike level for APIs; 95.0% - 105.0% for finished dosage forms. (see note a.)
2. **Precision (repeatability):** Perform 5 replicate injections of standard solution of analyte at 100% of expected concentration, unless otherwise specified.
   Acceptance Criteria: RSD less than or equal to 2.0%, unless otherwise specified
3. **Linearity:** Prepare a set of a minimum of 5 concentrations of analyte standard, with minimum range as defined for Content Uniformity solutions under "Accuracy," above. Perform the determination, and generate a standard curve.
   Acceptance Criteria: Linear Regression Coefficient of Determination r(2) greater than or equal to .995 (see note e.).
4. **Specificity:** Assessment of specificity depends on the technique being used. Certain techniques (i.e. titrations) are non-specific by nature; a combination of two or more analytical procedures is necessary to achieve the required level of discrimination. Techniques such as HPLC-UV or UV spectrophotometry are somewhat more specific in nature: visual comparison of standard and sample spectra or chromatograms should be performed; no interferences should be apparent. Peak-purity technology should be used when possible to assist in this evaluation. Techniques such as IR spectrophotometry or mass spectrometry are highly specific: sample and standard maxima or bands should occur at the same wavelengths or masses.
   When impurity or degradant standards are available, specificity can be additionally assessed by addition of these compounds to the primary analyte, to assure that interferences do not occur.
5. **Limit of Detection:** For chromatographic or spectrophotometric methods, determine the minimum level at which a compound can be detected, using analyte solutions of decreasing concentration. L.O.D. is generally defined as 3 times the noise level. Other scientifically-sound approaches may also be used.
   For other types of methods, estimate through visual evaluation the minimum level at which a compound can be detected, using analyte solutions of decreasing concentration.
6. **Limit of Quantitation:** For chromatographic or spectrophotometric methods, determine the minimum level at which a compound can be quantitated, using analyte solutions of decreasing concentration. L.O.Q. is generally defined as 10 times the noise level. Other scientifically-sound approaches may also be used.
   For other types of methods, estimate through visual evaluation the minimum level at which a compound can be quantitated, using analyte solutions of decreasing concentration.

Notes:

a. If acceptance criteria are not met, due to situations described in this paragraph, the occurrence should be evaluated in the form of a discussion between analyst(s), lab managers, and QA managers, with the purpose of the analysis and the requirements of the customer being taken into account. The specified acceptance criteria can then be modified, if sufficiently justified.

b. the validation parameter "Range" is often discussed separately. For most purposes, the quantitative or qualitative range of a method will be appropriately established through determination of linearity, accuracy, and LOD/LOQ, as described above.

c. the validation parameters "Ruggedness" and "Robustness" are also frequently discussed. When the method being validated is for single-occurrence use or internal use, these determinations may not be necessary. For a more complete validation, as in cases where the method is intended for publication or establishment as a future "Standard Method", ruggedness and robustness should be assessed through use of differing equipment, by a different analyst or laboratory, over several time intervals, or a combination of the above.

d. ICH and other guidelines recommend, for Accuracy determination, an assessment using a minimum of 3 replicates at each of 3 concentrations, thereby equating to a minimum of 9 determinations. This should be done when the method is intended for publication or establishment as a future "Standard Method." For routine regulatory analytical purposes, 17025 requirements will be considered met if each of the three single preparations evaluated under "Accuracy" meet the Acceptance Criteria. If one or more preparations fails to meet these criteria, the laboratory should conduct a failure investigation, to include an examination of possible causes for this failure

e. For certain types of methods, i.e. Atomic Emission Spectroscopy, a non-linear standard curve may be expected, and can be used. Linear Regression analysis would not apply to such situations.

C. Categories of Methods; Validation Parameter Requirements

1. **Category I: Quantitative Assessment of Major Components:** (i.e. Assay, Content Uniformity, determinative step for Dissolution/Release Rate). Required parameters: Accuracy, Precision, Linearity, Specificity
2. **Category IIa: Quantitative Assessment of Minor Components:** (i.e. Impurity and Degradant quantitative determinations). Required parameters: Accuracy, Precision, Linearity, Specificity, Limit of Quantitation
3. **Category IIb: Qualitative Assessment of Minor Components:** (i.e. Impurity and Degradant Limit Tests). Required parameters: Specificity, Limit of Detection
4. **Category III: Qualitative Assessment of Major Components:** (i.e. Identification). Required parameter: Specificity

D. Verification of Methods: As is mentioned above, a laboratory must verify that any validated method (including USP or other "Standard Methods") can be performed acceptably under actual conditions of use. Method Verification should be performed upon the first use of a method by a particular analyst on a particular instrument to document that the **method performance criteria** can be met. After this, **instrument performance criteria** (for example, system suitability parameters, criteria specified in the method, etc.) should be met as directed by the method or per batch of similar samples.
Verification should include, as a minimum:

1. Full system suitability testing, as defined in the compendial method, with acceptance criteria as defined in the compendium. If this is not applicable, system precision for chromatographic procedures should be assessed using six replicate injections (RSD $\leq$ 2.0); specificity should be assessed using either a chromatographic resolution factor (>1.3) or a visual examination of chromatograms or spectra for freedom from interference.
2. Accuracy determination through analysis of a matrix spike (acceptance criteria: 97.0%-103.0% recovery for APIs, 95.0%-105.0% for finished dosage forms; see note a. above).