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## 1. Purpose

This procedure provides basic requirements to Office of Regulatory Science (ORS) laboratories for development, validation, and verification of method performance specifications for new methods, modified methods or procedures previously validated externally.

## 2. Scope

ORS laboratories verify standard method performance and validate new or modified methods introduced into the laboratory.

Refer to ORA-LAB.5.9 Assuring the Quality of Test Results for continuing verification of acceptable performance for each analytical batch (EAB) through a quality control program.

## 3. Responsibility

- A. ORS Office of Research Coordination, Evaluation and Training (ORCET)
  - 1. Directs, monitors, and coordinates ORS method development and validation activities designed to address regulatory gaps.
  - 2. Issues call for proposals, assigns reviewers, approves proposals and maintains approved and completed project list.
  - 3. Tracks research progress, achievement of deliverables and evaluation of impact.
- B. Laboratory Management:
  - 1. Provides resources and ensures implementation of method verification and validation procedures.
  - 2. Develops and executes approved method development and validation program.
- C. Analysts:
  - 1. Adhere to written protocol for method performance verification, validation or modification.

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2. Submit method development and validation needs through local management in response to ORCET initiatives and/or programmatic needs.
3. Develop and submit proposals in accordance with approved procedures.
4. Accomplish approved projects in timely manner.
5. Adhere to the highest standards of intellectual honesty and ethical standards in formulating, conducting and presenting method development and validation work.
6. Follow Agency level and ORCET research and publication requirements and guidelines.
7. Submit new validation proposals into the Component Automated Research Tracking System (CARTS) to include projected timelines and milestones.

D. Quality System Manager:

1. Ensures all procedures are implemented to ensure traceability and defensibility of data provided for method development and method validation in accordance with maintaining accreditation.
2. Ensures an SOP exists or is written when use of the original Method does not provide guidance required in testing processes, for instance where the original method has been modified.
3. Provides concurrence for approval with the Laboratory Management for requests and ensures that completed analytical studies have met all quality validation and/or verification criteria.
4. Maintains records of completed verified/validated studies.
5. Monitors progress/status of open method validation, verification, and modification.

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#### 4. Background

As a regulatory agency, it is imperative that the analytical methods ORS laboratories employ for surveillance, compliance and outbreak investigations continue to meet the highest standard of review and performance.

When a standard analytical method is verified, the laboratory is required to demonstrate with objective evidence that it can achieve specified performance characteristics/ parameters proven during the initial validation study.

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The validation study must establish pertinent performance characteristics (e.g., accuracy, precision, sensitivity, limit of detection, etc.) that demonstrate or confirm a method is suitable for its intended purpose.

Any significant modification to existing procedures involving a major change in part of the original process that will affect the instruments and/or samples used to produce data must be validated.

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## 5. References

- A. Code of Federal Regulations. Title 40, Part 136, Appendix B, Definition and Procedure for the Determination of the Method Detection Limit
- B. The Analytical Laboratory Accreditation Criteria Committee (ALACC) How to Meet ISO 17025 Requirements for Method Verification, AOAC International, 2007
- C. ISO/IEC 17025:2017 General requirements for the competence of testing and calibration laboratories.
- D. AOAC International Guidelines for Laboratories Performing Microbiological and Chemical Analysis of Food, Dietary Supplements, and Pharmaceuticals – An Aid to Interpretation of ISO/IEC 17025:2017; August 2018.
- E. Guidelines for the Validation of Analytical Methods for the Detection of Microbial Pathogens in Foods and Feeds, 3<sup>rd</sup> Edition
- F. Guidelines for the Validation of Chemical Methods for the FDA FVM Program, 3<sup>rd</sup> Edition
- G. USFDA Center for Veterinary Medicine, ‘Mass Spectrometry for Confirmation of the Identity of Animal Drug Residues’, Guidance for Industry # 118, 2003
- H. Food and Drug Administration, “Acceptance Criteria for Confirmation of Identity of Chemical Residues using Exact Mass Data within the Office of Foods and Veterinary Medicine”
- I. USP <621> (Chromatography)
- J. SANCO/12495/2011 ‘Method Validation and Quality Control Procedures for Pesticide Residue Analysis in Food and Feed’

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## 6. Procedure

### 6.1. Method Selection

- A. An integral part of the laboratory quality system is the use of standard methods. Standard methods are used, whenever possible, unless otherwise specified by the Compliance Program or the customer.
- B. Methods of analysis that are specified in law or regulations must be followed in accordance with those requirements.
- C. 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 validated.
- D. If a standard test method is not available for the requested analysis, a new method may be developed and validated. See ORA Laboratory Manual, Volume III Section 6, ORA Method Development and Validation Program.

### 6.2. Method Evaluation & Record

- A. Any method selected for use must be appropriate to the requirements of the regulatory function and must be within the capabilities of the laboratory. Analytical methods are to be evaluated based on attributes such as accuracy/trueness, precision, specificity/selectivity, sensitivity, ruggedness/robustness, and practicality.
- B. There shall be a record of evaluation and approval for use in the laboratory by the designated official(s) regardless of whether or not a validation is deemed necessary. The method of evaluation, explanations, and justifications shall be described in the record.

### 6.3. Standard Method Verification

- A. Standard or FDA official methods need verification to ensure that the laboratory is capable of meeting the test method performance specifications. Verification of a test method demonstrates that the laboratory has met the test method's performance specifications and must be completed before the method is used for routine testing.
- B. Verification ensures that the laboratory can obtain comparable results on the same matrix, using equivalent equipment and circumstances, as the standard method; in other words, the method is suitable under actual conditions of use in a laboratory.

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### 6.3.1. Chemistry

The requirements below outline general principles for quantitative and qualitative chemistry methods.

For additional special considerations and requirements for drug chemistry work, please refer to Attachment C. Compendial drug methods should follow the compendial requirements.

For additional special considerations and requirements for food chemistry work, please refer to the Guidelines for the Validation of Chemical Methods for the FDA FVM Program, 3<sup>rd</sup> Edition.

#### 6.3.1.1. Quantitative Chemistry

##### A. Minimum Requirements

1. Meets System suitability requirements, if applicable
2. Accuracy,
3. Precision,
4. Working Range/Linearity
5. Method Detection Level/Limit of Detection
6. Limit of Quantitation

B. Critical requirements for quantitative analysis are the accuracy and the precision (also known as repeatability and reproducibility) obtained from actual lab data which are reflected in the measurement uncertainty.

C. Analyze reference materials, standard, or spike samples at  $\geq 2$  concentration levels in triplicate. Consider any relevant regulatory limits/action levels when selecting spike ranges (e.g. spiking at 0.5x any applicable limit). Run a matrix blank when available and a method blank with each spike level.

D. Determine working range or target level. As guidance for a curve, the mid-point is set at the target level with the lowest calibrator at one-half this concentration and the highest calibrator at twice this concentration. For a one-point calibration, use the target level.

E. The  $R^2$  (coefficient of determination) for a calibration curve must be equal to or better than documented in the method. If the  $R^2$  is not specified in the method:

1. Single analyte / single matrix methods  $R^2$  should be  $\geq 0.995$

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2. Multi residue, multi class mass spectrometer analysis may have a R<sup>2</sup> value of  $\geq 0.990$  with a written justification included in the study package narrative(s).

F. Method Detection Limit (MDL)/Limit of Detection (LOD)

1. If the MDL is stated in the method, verify by running the method stated concentration. The result should be within  $\pm 30\%$  of the known value.
2. If the MDL is not provided in the method, determine detection limit by either method detection limit (MDL) according to 40 CFR, Part 136, Appendix B or limit of detection (LOD). LOD may be determined by analyzing sample blanks, calculating the standard deviation, and expressed as the mean plus 3 standard deviations.

G. Limit of Quantitation (LOQ)

LOQ (the minimum amount/concentration that can be quantified with acceptable precision)

H. If available, prepare and analyze a Quality Control Sample/Certified Reference Material (CRM).

1. The concentration must be within the range specified within the Certificate of Analysis for CRM or 80% - 120% of stated or known value for other control material used.
2. This check is intended as an independent check of technique, methodology and standards.

I. For a standard curve, analyze calibration standards containing known amounts of analyte.

J. Calculate percent recovery. The result should be within  $\pm 20\%$  of the known value for acceptable method performance.

**6.3.1.2. Qualitative Chemistry Methods**

A. Meet system suitability requirements, if applicable.

B. Establish a detection limit (LOD). The detection limit is the lowest level of analyte that gives a positive response that can be reliably distinguished from zero (term is usually restricted to the response of the detection system. When applied to the complete analytical method it is referred to as the MDL)

C. Linearity

D. Analyze  $\geq 3$  matrices where available

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- E. Analyze a blank. The result must be negative.
- F. Analyze a quality control sample or reference material, if available. The results must be positive.
- G. Analyze  $\geq 3$  matrix spikes for each matrix tested. The matrix or sample should be spiked at the relevant regulatory limits/action levels. The result must be positive.

### 6.3.2. Microbiology

- A. Meet method system suitability requirements, if applicable.
- B. Prepare and analyze a positive culture control to assess and demonstrate accuracy.
  - 1. Accuracy must be  $> 95\%$ .
  - 2. A positive culture control must exhibit positive growth or exhibit expected characteristics to assure the system is working. For example, turbidity in a tube filled with enrichment broth showing growth or a physical (phenotypic) change to the bacterial culture showing a positive test result.
- C. Include all other controls as required by ORA-LAB.001 Microbiological Controls for Food and Feed Sample Analysis and SOP-000288 Microbiological Controls for Medical Product Sample Analysis.
- D. Sample duplicates are not required when precision is calculated by the number of false negatives or false positives. Precision must be  $< 5\%$ .

### 6.3.3. Other Scientific Disciplines

Laboratories will address, at their local level, method verification of scientific disciplines not discussed in this procedure. At a minimum, scientific disciplines must meet the requirements to demonstrate lack of contamination, accuracy, precision, detection limits, quantitation limits, and linearity as applicable.

### 6.4. Validation of Method Performance

- A. Non-standard and laboratory-developed methods must be validated. 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.
- B. Typical validation characteristics which should be considered are:
  - 1. accuracy,

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2. precision,
3. specificity,
4. detection limit,
5. limit of quantitation,
6. linearity,
7. range, and
8. ruggedness and robustness.

(See Section 7 for definitions of these characteristics)

#### **6.5. Validation of Method Modifications:**

- A. If there is any difference between the current samples and those for which the method was originally validated, the extent of the difference and its impact must be assessed. See Attachment A & B for general chemistry guidelines for allowable modifications to a method before a revalidation protocol is needed.
- B. For protocol requirements for modification to Food and Feed chemistry methods, refer to Guidelines for the Validation of Chemical Methods for the FDA FVM Program, 3<sup>rd</sup> Edition.
- C. For protocol requirements for modification to microbiology methods, refer to the Guidelines for the Validation of Analytical Methods for the Detection of Microbial Pathogens in Foods and Feeds 3<sup>rd</sup> Edition.
- D. Modifications of methods that alter the fundamental nature of the method shall be validated to demonstrate that equivalent results are obtained; do not adversely affect the precision and accuracy of the data obtained; and that the method is suitable for its intended use.
- E. Each major modification is verified against the original method.
- F. Additional statistics that may be employed to validate method modifications are:
  1. The t test for significance of difference between the two data set means. If your t-stat value is less than or equal to the t-critical value (0.5 would provide 95% confidence level), there is no statistically significant difference between your methods.
  2. The f test provides a ratio of the calculated data from the validated method and the modified method. A high F-statistic would indicate a significant difference.

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## 6.6. Validation Tools

The following tools can be used to substantiate a method's ability to meet satisfactory specifications of performance:

- A. Blanks (matrix, reagent, system): Use of various types of blanks enables assessment of how much is attributable to the analyte and how much is attributable to other causes.
- B. 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.
- C. Fortified (spiked) materials and solutions: Recovery determinations can be made from fortification or spiking with a known amount of analyte.
- D. Incurred materials: These are materials in which the analyte of interest has been introduced to the bulk at some point prior to the material being sampled.
- E. Measurement standards: These are substances or stable artifacts 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.
- F. Replication: Replicate analysis provides a means of checking for changes in precision in an analytical process which could adversely affect the results.
- G. Statistics: Statistical techniques are employed to evaluate accuracy, precision, linear range, limits of detection and quantification, and measurement uncertainty.

## 6.7. Validation Protocol Guidance

### 6.7.1. General

- A. **Note:** Refer to program validation guidance documents such as *Guidelines for the Validation of Analytical Methods for the Detection of Microbial Pathogens in Foods and Feeds* and *Guidelines for the Validation of Chemical Methods for the FDA FVM Program* for specific requirements pertaining to the program.
- B. Laboratories record their validation protocol, the performance characteristics measured, and acceptance limits for the validation of non-standard and laboratory developed methods. The validation record also includes a statement indicating the method is fit for the intended use.

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- C. 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.
- D. Guidelines for determining performance characteristics are outlined below.

### 6.7.2. Chemistry

- A. Perform system suitability requirements: i.e. 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 may be determined by analyzing sample blanks, calculating the standard deviation, and expressed as the mean plus 3 standard deviations.
- C. For qualitative measurements, determine the concentration threshold below which specificity becomes unreliable.
- D. Quantitative measurements: Determine 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.
- E. 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 be assessed with these samples. Accuracy (percent recovery) and precision (relative standard deviation or relative percent recovery) are calculated from the results.
- F. Analyze blanks (reagent, solvent and matrix).
- G. Evaluate interferences. i.e. spectral, physical, chemical or memory by analyzing a sample containing various suspected interferences in the presence of the measure:
  1. Spectral interference may be observed when an overlap of a spectral line from another element or background contribution occurs.
  2. Physical interference may occur from effects associated with sample transport processes on instruments.
  3. Chemical interferences can be characterized by compound formation, ionization or vaporization effects.

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4. Memory interference occurs from contribution of signal from previous sample to sample being tested.

### 6.7.3. 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.

### 6.7.4. Other Scientific Disciplines

Laboratories will address, at their local level, method validation of scientific disciplines not discussed in this procedure.

## 6.8. Documentation

### 6.8.1. Method Verification

- A. Verification studies are planned and approved with local management and the Quality System Manager.
- B. Objective evidence of standard method verification can be in method verification reports, analyst worksheets, memos of analyses, and method quality control records.
- C. Method verification reports include at a minimum:

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1. A summary (e.g. standard method used, method criteria, how sample was prepared and analyzed, equipment used, measurement uncertainty, analysts demonstrating competence).
2. Data
3. Verification statistical evaluation (e.g. accuracy, precision, LOQ, MDL, linearity, etc.).

### **6.8.2. Method Validation**

Validation studies are initially planned and approved with local management and the Quality System Manager and then submitted in CARTS; refer to ORA Laboratory Manual, Volume III Section 6.

- A. Submit method validation plans, results and documentation to Laboratory Management and the QSM for review and approval.
- B. The validation information contains all relevant data to confirm that method performance specifications, as defined by the established acceptance criteria, are met.
- C. The method validation package includes:
  1. A study summary (how sample was prepared and analyzed, equipment used, robustness, measurement uncertainty, analysts demonstrating competence)
  2. Data
  3. Validation study statistical evaluation and acceptance criteria (accuracy, precision, LOQ, MDL, linearity, etc.)
  4. Laboratory Method Procedure. Note: infrequently used or non-routine methods do not need a Laboratory Procedure until they become routine.
  5. A record of the person authorizing adoption of the method and the date authorization was granted.
  6. A statement on the validity of the method, detailing its fitness for the intended use.
- D. Management and the Quality System Manager review and approve validated methods for routine use. The validation package is retained by the Quality System Manager.

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## 7. Glossary/ Definitions

- A. **Accuracy** – Accuracy is the nearness of a result or the mean of a set of measurements to the true value.
- B. **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.
- C. **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.
- D. **Limit of quantitation (LOQ)** – This is the level above which quantitative results may be determined with acceptable accuracy and precision. Limit of quantitation (or quantification) is variously defined but must be a value greater than the Method Detection Limit (MDL) and should apply to the complete analytical method.
- E. **Linearity** – Linearity is the ability of the method to elicit results that are directly proportional to analyte concentration within a given range.
- F. **Method Detection Limit (MDL)** - The minimum amount or concentration of analyte in the test sample that can be reliably distinguished from zero. MDL is dependent on sensitivity, instrumental noise, blank variability, sample matrix variability, and dilution factor.
- G. **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.
- H. **FDA "official" methods** - This refers to methods found in compendia specified in the FD&C Act and prescribed in the CFR and methods in applications and petitions that have official status are included. These methods include those in the United States Pharmacopeia, National Formulary, Homeopathic Pharmacopeia of the United States, Official Methods of Analysis of the Association of Official Analytical Chemists (AOAC) International or any supplement of any of them, American Public Health Association (APHA) Compendium of Methods for the

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Microbiological Examination of Foods, FDA compliance programs, the Pesticide Analytical Manual (PAM), the Food Additives Analytical Manual, the Food Chemicals Codex, FDA Bacteriological Analytical Manual (BAM), FDA Macroanalytical Procedures Manual (MPM), and ORA. Laboratory Information Bulletins (LIBs) that are included in compliance programs and special assignments. Standard methods are preferred for use and are verified for use in the laboratory. A standard method may be supplemented with additional details in the form of a laboratory procedure to ensure consistent application. Those methods specified by the manufacturer of the equipment are considered as standard methods. Standard methods are verified according to the procedures described above.

- I. **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. The precision is described by statistical methods such as a standard deviation or confidence limit of test results. Intermediate precision expresses within-laboratory variations, such as different days, different analysts, and different equipment. Reproducibility expresses the precision between laboratories.
- J. **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.
- K. **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.
- L. **Selectivity** – The extent to which a method can determine particular analyte(s) in a mixture(s) or matrix(ces) without interferences from other components of similar behavior. Selectivity is generally preferred in analytical chemistry over the term *Specificity*.
- M. **Sensitivity** – The change in instrument response which corresponds to a change in the measured quantity (e.g., analyte concentration). Sensitivity is commonly defined as the gradient of the response curve or slope of the calibration curve at a level near the LOQ.
- N. **Specificity** – In quantitative analysis, specificity is the ability of a method to measure analyte in the presence of components which may

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be expected to be present. The term *Selectivity* is generally preferred over Specificity.

- O. **Standard method** – Standard methods are those published by international, regional or national standards-writing bodies; by reputable technical organizations; in legal references; and FDA published methods. FDA “official” methods are considered to be standard methods.
- P. **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.
- Q. **Verification** – A verification is the confirmation by examination and provision of objective evidence that specified requirements have been fulfilled.

## 8. Records

- A. Validation and verification documentation and reports

## 9. Supporting Documents

- A. ORA-LAB.001 Microbiological Controls for Food and Feed Sample Analysis
- B. SOP-000288 Microbiological Controls for Medical Product Sample Analysis International Conference on Harmonization (ICH) Topic Q2A
- C. SOP-000107 Method Verification
- D. ORA Laboratory Manual, Volume III Section 6, ORA Method Development and Validation Program.

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## 10. Document History

Revision #	Status* (D, I, R)	Date	Author Name and Title	Approving Official Name and Title
1.1	I	10/03/2003	LMEB	LMEB
1.2	R	09/09/2005	LMEB	LMEB
1.3	R	11/15/2007	LMEB	LMEB
1.4	R	02/25/2009	LMEB	LMEB
1.5	R	04/01/2009	LMEB	LMEB
1.6	R	02/03/2012	LMEB	LMEB
1.7	R	09/08/2014	LMEB	LMEB
02	R	06/30/2020	LMEB	LMEB

\* - D: Draft, I: Initial, R: Revision

## 11. Change History

Revision #	Change
02	<ul style="list-style-type: none"> <li>• Revisions made as needed to align this procedure with new ISO/IEC 17025 and AOAC requirements.</li> <li>• Revision to formatting and other changes were made in all the document sections to provide policy clarification and enhance areas like documentation requirements, method modification requirements, etc.</li> <li>• Addition of requirement to submit validation requests in CARTS</li> <li>• Significant revision was also made to the documents in the Attachments section.</li> </ul>

## 12. Attachments

### List of Attachments

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## Attachment A - Food and Feed Chemistry Modification Criteria

If adjustments to method conditions are needed, the following documents may be consulted for specifications to sample preparation or operation parameter conditions: USP <621> (Chromatography), SANCO/12495/2011, or an FDA LIB that qualifies as a standard method for the set of target analytes in a given matrix.

### A. Sample Inlet

#### 1. LC

- a. **Mobile phase pH:** the pH of the aqueous buffer used in the preparation of the mobile phase can be adjusted to within  $\pm 0.2$  pH units of the value or range specified. Applies to both gradient and isocratic separations.
- b. **Concentration of salts in buffer:** The concentration of the salts used in the preparation of the aqueous buffer employed in the mobile phase can be adjusted to within  $\pm 10\%$ , provided the permitted pH variation is met. Applies to both gradient and isocratic separations.
- c. **Mobile Phase Composition:** The following adjustment limits apply to minor components of the mobile phase (specified at  $\leq 50\%$ ). The amount(s) of these components can be adjusted by  $\pm 30\%$  relative. However, the change in any component cannot exceed  $\pm 10\%$  absolute, nor can the final concentration of any component be reduced to zero. Examples of adjustments for binary and ternary mixtures are given below:
  - i. **Binary Mixtures:** Specified Ratio of 50:50 – Thirty percent of 50 is 15% absolute, but this exceeds the maximum permitted change of  $\pm 10\%$  absolute in either component. Therefore, the mobile phase ratio may be adjusted only within the range 40:60 to 60:40.  
Specified Ratio of 95:5 – Thirty percent of 5 is 1.5% absolute. However, because adjustments of up to  $\pm 2\%$  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 to 97.4.

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- ii. ***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  $\pm 10\%$  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  $\pm 2\%$  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%.
  - d. ***Flow rate***: May be adjusted by as much as  $\pm 50\%$ . Consult the column configuration table in USP <621> (Table 2) when particle size is changed, because smaller particle columns will require higher linear velocities for the same performance (as measured by reduced plate height).
  - e. ***Injection volume***: The injection volume can be adjusted as far as it is consistent with accepted precision, linearity, and detection limits. Note that excessive injection volume can lead to unacceptable band broadening, causing a reduction in the number of theoretical plates (N, which is a measure of column efficiency) and resolution, which applies to both gradient and isocratic separations.
2. GC
- i. ***Carrier gas***. Hydrogen for FID use, Helium for MS use. Hydrogen can be used with MS detection if sensitivity does not vary more than 10% in the working range for every target analyte in the sample.
  - ii. ***Flow rate***: The flow rate can be adjusted by as much as  $\pm 50\%$ , provided that the carrier gas system can be maintained under control at the desired set points.
  - iii. ***Injection volume***: The injection volume and split volume (which is controlled by the split ratio) can be varied as much as possible, as long as the detection and repeatability are satisfactory for the entire range concentrations of the calibration curve.

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## B. Sample Analysis

### 1. LC

- a. **Stationary phase type:** The column stationary phase type can be changed according to the USP L-rating number (consult the USP LC column equivalence chart).
- b. **Column dimensions:** For isocratic separations, the particle size and/or length of the column may be modified provided that the ratio of the column length (L) to the particle size (dp) remains constant or into the range between -25% and 50% of the prescribed L/dp ratio. Alternatively (as for the application of particle-size adjustment to superficially porous particles), other combinations of L and dp can be used provided that N is within -25% to 50%, relative to the prescribed column. For gradient elution separations, changes in length, column inner diameter, and particle size are not recommended. If UHPLC is used, then the columns dimensions should be chosen such that the resolution of all target analytes is conserved.
- c. **Column temperature:** The column temperature can be adjusted by as much as  $\pm 10^{\circ}\text{C}$ . Column thermostating is recommended to improve control and reproducibility of retention time, which applies to both gradient and isocratic separations.

### 2. GC

- a. **Stationary phase type:** The column stationary phase type can be changed according to the USP G-rating number (consult the USP GC column equivalence chart). Stationary film thickness can be adjusted by as much as -50% to 100%. A capillary column can be used in substitution of a packed column if performance (resolution of target analytes and analysis time) is demonstrated to be equivalent or superior.
- b. **Column dimensions:** Column length can be adjusted by as much as  $\pm 70\%$ . Column inner diameter can be adjusted by as much as  $\pm 50\%$ .
- c. **Temperature program:** Oven temperature can be to any rate that the instrument can reproducibly deliver, and up to a temperature that will not produce excessive column bleed (typically  $20^{\circ}\text{C}$  below the stationary phase's prescribed maximum allowable operating temperature). The retention index of target analytes should not vary by more than 10 index units in the

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modified method, and peak shapes (peak asymmetry) should be consistent (or better) than in the method that is being modified.

C. Sample Detection

1. LC

a. **Non-MS Detectors:** Reference samples should provide adequate S/N ratios (>10) over the working range.

b. **MS Detectors**

Table 1 MS				
MS detector/Characteristics		Acquisition	Requirements for Identification	
Resolution	Typical Systems (Examples)		Minimum number of ions	Other
Unit Mass Resolution	Single MS Quadrupole Ion trap Time of Flight (TOF)	Full scan, Limited m/z range Selected ion monitoring (SIM)	3 ions	S/N ≥ 3 Analyte peaks from both product ions in the extracted ion chromatogram must overlap
	MS/MS Triple Quad Ion trap Q-trap Q-TOF Q-Orbitrap	Selected or multiple reaction monitoring (SRM, MRM) Mass resolution for precursor-ion Isolation equal to or better than unit mass resolution	2 product ions	Ion ratio from sample extracts should be within ± 30% of average of calibration standards from same sequence. Please consult CVM118 reference for additional information.
Accurate Mass Measurement	High Resolution MS Q-TOF Q-Orbitrap FT-ICR-MS Magnetic Sector MS	Full scan Limited m/z range SIM Fragmentation with or without precursor-ion selection	2 ions with mass accuracy ≤ 5 ppm	S/N ≥ 3 Analyte peaks from precursor and/or product ion(s) in the extracted ion chromatograms must fully overlap Please consult CVM 118 for additional information.

2. GC

a. **Non-MS Detectors:** Reference samples should provide adequate S/N ratios (>10) over the working range.

b. **MS-MS and HRMS:** refer to Table 1 MS.

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### Attachment B - Drug Chemistry Modification Criteria

If adjustments to operating conditions are needed, each of the following is the maximum specification (USP General Chapter <621> Chromatography) 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  $\pm 0.2$  pH units of the value or range specified. Applies to both gradient and isocratic separations.

**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  $\pm 10\%$ , provided the permitted pH variation is met. Applies to both gradient and isocratic separations.

**Ratio of Components in Mobile Phase (HPLC):** The following adjustment limits apply to minor components of the mobile phase (specified at  $\leq 50\%$ ). The amount(s) of these component(s) can be adjusted by  $\pm 30\%$  relative. However, the change in any component cannot exceed  $\pm 10\%$  absolute (i.e., in relation to the total binary phase), Adjustment can be made to one minor component in a ternary mixture. 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  $\pm 10\%$  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 2:98 – Thirty percent of 2 is 0.6% absolute. 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  $\pm 10\%$  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. In all cases, a sufficient quantity of the first component is used to give a total of 100%. Therefore, mixture ranges of 50:45:5–70:25:5 or 58.5:35:6.5-61.5:35:3.5 would meet the requirement.

**Wavelength of UV-Visible Detector (HPLC):** Deviations from the wavelengths specified in the method are not permitted. The procedure

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specified by the detector manufacturer, or another validated procedure, is to be used to verify that error in the detector wavelength is, at most,  $\pm 3$  nm.

**Column Length (GC):** May be adjusted by as much as  $\pm 70\%$ .

**Column Length (HPLC):** See Particle Size (HPLC) below

**Column Inner Diameter (HPLC):** Can be adjusted if the linear velocity is kept constant. See Flow Rate (HPLC) below

**Column Inner Diameter (GC):** Can be adjusted by as much as  $\pm 50\%$ .

**Film Thickness (Capillary GC):** May be adjusted by as much as  $-50$  to  $+100\%$ .

**Particle size (HPLC):** For isocratic separations, the particle size and/or the length of the column may be modified provided that the ratio of the column length ( $L$ ) to the particle size ( $dp$ ) remains constant or into the range between  $-25\%$  and  $50\%$  of the prescribed  $L/dp$  ratio. Alternatively (as for the application of particle-size adjustment to superficially porous particles), other combinations of  $L$  and  $dp$  can be used provided that the number of theoretical plates ( $N$ ) is within  $-25\%$  to  $50\%$ , relative to the prescribed column. Caution should be used when the adjustment results in a higher number of theoretical plates that generate smaller peak volumes, which may require adjustments to minimize extra-column band broadening by factors such as instrument plumbing, detector cell volume and sampling rate, and injection volume. **For gradient separations, changes in length, column inner diameter, and particle size are not allowed.**

**Particle size (GC):** Changing from a larger to a smaller or from a smaller to a larger particle size GC mesh support is acceptable if the chromatography meets the requirements of system suitability and the same particle size range ratio is maintained. The particle size range ratio is defined as the diameter of the largest particle divided by the diameter of the smallest particle

**Flow Rate (GC):** The flow rate can be adjusted by as much as  $\pm 50\%$ . [Note—When the monograph specifies a linear velocity parameter, the allowed velocity adjustment is between  $+50\%$  and  $-25\%$ , provided the carrier gas system can be maintained under control at the desired set points.]

**Flow Rate (HPLC):** When the particle size is changed, the flow rate may require adjustment, because smaller-particle columns will require higher linear velocities for the same performance (as measured by reduced plate height). Flow rate changes for both a change in column diameter and particle size can be made by:

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$$F2 = F1 \times [(dc2^2 \times dp1)/(dc1^2 \times dp2)]$$

where  $F1$  and  $F2$  are the flow rates for the original and modified conditions, respectively,  $dc1$  and  $dc2$  are the respective column diameters, and  $dp1$  and  $dp2$  are the particle sizes.

When a change is made from  $\geq 3\text{-}\mu\text{m}$  to  $< 3\text{-}\mu\text{m}$  particles in isocratic separations, an additional increase in linear velocity (by adjusting flow rate) may be justified, provided that the column efficiency does not drop by  $> 20\%$ . Similarly, a change from  $< 3\text{-}\mu\text{m}$  to  $\geq 3\text{-}\mu\text{m}$  particles may require additional reduction of linear velocity (flow rate) to avoid reduction in column efficiency by  $> 20\%$ . Changes in  $F$ ,  $dc$ , and  $dp$  are not allowed for gradient separations.

Additionally, the flow rate can be adjusted by  $\pm 50\%$  (isocratic only).

Examples:

Adjustments in column length, internal diameter, particle size, and flow rate can be used in combination to give equivalent conditions (same  $N$ ), but with differences in pressure and run time. [Table 2](#) lists some of the more popular column configurations to give equivalent efficiency ( $N$ ), by adjusting these variables.

Table 2. Column Configurations

Length ( $L$ , mm)	Column Diameter ( $dc$ , mm)	Particle Size ( $dp$ , $\mu\text{m}$ )	Relative Values				
			$L/dp$	$F$	$N$	Pressure	Run Time
250	4.6	10	25,000	0.5	0.8	0.2	3.3
150	4.6	5	30,000	1.0	1.0	1.0	1.0
150	2.1	5	30,000	0.2	1.0	1.0	1.0
100	4.6	3.5	28,600	1.4	1.0	1.9	0.5
100	2.1	3.5	28,600	0.3	1.0	1.9	0.5
75	4.6	2.5	30,000	2.0	1.0	4.0	0.3
75	2.1	2.5	30,000	0.4	1.0	4.0	0.3
50	4.6	1.7	29,400	2.9	1.0	8.5	0.1
50	2.1	1.7	29,400	0.6	1.0	8.5	0.1

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For example, if a monograph specifies a 150-mm × 4.6-mm; 5- $\mu$ m column operated at 1.5 mL/min, the same separation may be expected with a 75-mm × 2.1-mm; 2.5- $\mu$ m column operated at 1.5 mL/min × 0.4 = 0.6 mL/min, along with a pressure increase of about four times and a reduction in run time to about 30% of the original.

**Injection Volume (HPLC):** The injection volume can be adjusted as far as it is consistent with accepted precision, linearity, and detection limits. Note that excessive injection volume can lead to unacceptable band broadening, causing a reduction in *N* and resolution, which applies to both gradient and isocratic separations.

**Injection volume and split volume (GC):**

The injection volume and split volume may be adjusted if detection and repeatability are satisfactory.

**Column Temperature (HPLC):** May be adjusted by as much as  $\pm 10^\circ$ . Applies to both gradient and isocratic separations.

**Oven Temperature (GC):** May be adjusted by as much as  $\pm 10\%$ .

**Oven Temperature (GC):** May be adjusted by as much as  $\pm 10\%$ .

**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  $\pm 20\%$  is permitted.

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**Attachment C - ORA Validation (non-compendial) and Verification  
Guidance for Human Drug Analytical Methods**

- A. **Purpose:** Products that are non-compendial, Over-the-counter (OTC), or pharmacy-compounded do not require a New Drug Application (NDA) or Abbreviated New Drug Application (ANDA) to be marketed. Nevertheless, ORS 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 attachment provides uniform guidance to ORS laboratories on minimum requirements for validation and verification 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 the same or equivalent equipment, as the originator of the method; in other words, that the method is suitable under actual conditions of use in a particular laboratory.

In general, presented here are the *minimum* requirements for validation and verification of drug methods within ORS. 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

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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, suspensions, 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. below).

**B. References:**

1. International Conference on Harmonisation (ICH) Harmonised Tripartite Guideline: Validation of Analytical Procedures: Text and Methodology Q2(R1)
2. United States Pharmacopeia (USP) section <1225>: Validation of Compendial Procedures
3. United States Pharmacopeia (USP) section <1226>: Verification of Compendial Procedures.

**C. 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:** Should be assessed by using a minimum 9 determinations over a minimum of three concentration levels, covering the specified range (i.e., three concentrations and three replicates of each concentration). Prepare 3 sample 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.) Based on the

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nature of the dosage form (e.g. metered dosed inhalers) a wider range maybe used with appropriate justification.

- 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. below)

- 2. **Precision (repeatability):** Repeatability should be assessed using a minimum of nine determinations covering the specified range for the procedure (i.e., three concentrations and three replicates of each concentration) or using a minimum of six determinations at 100% of the test concentration (e.g. prepare 6 sample solutions at 100% of the test concentration and inject each), unless otherwise specified.

Acceptance Criteria: Drug products RSD less than or equal to 3.0%, unless otherwise specified; Drug active pharmaceutical ingredients - RSD less than or equal to 2.0%,

- 3. **Linearity:** Prepare and analyze a set with a minimum of 5 concentrations of analyte standard, across a minimum range as recommended by ICH based on the type of analysis described below: defined for Content Uniformity solutions under “accuracy,” above.
  - a. Assay of a drug substance (or a finished product): From 80% to 120% of the test concentration
  - b. For content uniformity: A minimum of 70%–130% of the test concentration, unless a wider or more appropriate range based on the nature of the dosage form (e.g., metered-dose inhalers) is justified
  - c. For dissolution testing:  $\pm 20\%$  over the specified range (e.g., if the acceptance criteria for a controlled-release product cover a region from 30% after 1 h, and up to 90% after 24 h, the validated range would be 10%–110% of the label claim).
  - d. Determination of an impurity: From 50% to 120% of the acceptance criterion

Perform the determination and generate a standard curve. The correlation coefficient, y-intercept, slope of the regression line, and residual sum of squares should be submitted. Acceptance Criteria:

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Linear Regression Coefficient of Determination  $r^2$  (2) greater than or equal to 0.995 (see note a. and e. below)

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 excipients, known 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. **Detection Limit (DL)/Limit of Detection (LOD):** For chromatographic or spectrophotometric methods, determine the minimum level at which a compound can be detected, using analyte solutions of decreasing concentration. LOD generally defined as 3 times the noise level (signal to noise ratio  $S/N \geq 3$ ). Some applications may depend on standard deviation or slope of the calibration curve. 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. **Quantitation Limit (QL)/Limit of Quantitation (LOQ):** For chromatographic or spectrophotometric methods, determine the minimum level at which a compound can be quantitated, using analyte solutions of decreasing concentration. LOQ is generally defined as 10 times the noise level ( $S/N \geq 10$ ). The standard deviation should also be considered along with the  $S/N$  ratio. 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.

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**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. 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 parameter “Robustness” is frequently discussed. When the method being validated is for single-occurrence use or internal use, this determination 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”, robustness should be assessed through variations in the analytical procedure (e.g. change in flow rate, use of differing equipment, different column lots, etc.) Consult with the lab manager for specifics on test requirements.
- 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, ISO17025:2017 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, e.g. Atomic Emission Spectroscopy, a non-linear standard curve may be expected, and can be used. Linear Regression analysis would not apply to such situations.

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#### D. 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: Performance Tests Components:** (e.g. dissolution, drug release, particle size). Required parameter: Precision, all other parameters are considered based on nature of the specified test)
5. **Category IV: Identification tests:** Required parameter: Specificity

E. **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, at 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 five (RSD requirement  $\leq 2.0\%$ ) or six (RSD requirement  $> 2.0\%$ ) replicate injections or as specified in the method; 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:

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97.0%103.0% recovery for APIs, 95.0%-105.0% for finished dosage forms; see note a. above).

**F. Ad Hoc Sample Analyses (example Consumer Complaint Samples)**

For ad hoc samples, such as consumer complaint samples, the analysis is intended for a particular sample without consideration for wider application. In addition, the amount of sample is usually limited, and the analytical results need to be expedited. In such cases, it may not be practical to perform full method validation or verification. However, efforts should be made to demonstrate accuracy, precision and linearity. Precision can be assessed from the RPD (relative percent difference) of duplicate sample analyses. Accuracy may be assessed from a single or duplicate spike recovery. Method assessment results are documented in a memo of analysis that is included with the analytical report