DCN: 16-001438 v. 3.1

BIOSPECTRA

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Effective Date:	22-Jan-2020	21-Jan-2023	: Date of Next Review
Initiated By:	Gathagan, Danielle E	3.0	: Supersedes
Reason for Print:	MasterControl		

Approval:

Approvers

Date	Time	Group	Name
21-Jan-2020	04:19:20 PM	DOC SPEC	Gathagan, Danielle E
21-Jan-2020	04:50:24 PM	EDITOR	Kuchmas, Hannah T
21-Jan-2020	05:31:31 PM	SNR MGMT	Meissner, Dora
22-Jan-2020	08:44:38 AM	QUALITY	Miller, Jenna M

ANALYTICAL METHODS VALIDATION MASTER PLAN

Printed On:	22-Nov-2021 09:18:26 AM	Gathagan, Danielle E	: Printed By
Print Expiration:	This document expires on the Date of Next Review listed in the header of this document or upon next revision.		
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1.0 INTRODUCTION

"Test procedures for assessment of the quality levels of pharmaceutical articles are subject to various requirements. According to Section 501 of the Federal Food, Drug, and Cosmetic Act, assays and specifications in monographs of the United States Pharmacopeia and the National Formulary constitute legal standards. The Current Good Manufacturing Practice regulations [21 CFR 211.194(a)] require that test methods, which are used for assessing compliance of pharmaceutical articles with established specifications, must meet proper standards of accuracy and reliability. Also, according to these regulations [21 CFR 211.194(a)(2)], users of analytical methods described in *USP–NF* are not required to validate the accuracy and reliability of these methods, but merely verify their suitability under actual conditions of use. Recognizing the legal status of *USP* and *NF* standards, it is essential, therefore, that proposals for adoption of new or revised compendial analytical procedures be supported by sufficient laboratory data to document their validity". (*USP 1225)*

The purpose of the Analytical Method Validation Master Plan (AMVMP) is to define sections required in the individual Method Validation Protocols and Reports. The AMVMP will also outline the performance parameters required for specific categories of analytical methods. The AMVMP will establish guidelines for acceptable limits on specifications and individual performance parameters.

2.0 RESPONSIBILITIES

Quality Control Manager: The Quality Control Manager (QC Manager), or qualified designee, is responsible for creating the Analytical Method Validation Protocol. The QC Manager is responsible for ensuring that analysts working on the analytical method validation are properly trained and that the training is documented correctly in the employee's training binder. The QC Manager is responsible for ensuring that the Analytical Method Validation Protocol is approved, in a timely manner, prior to carrying out the validation. The QC Manager is responsible for assigning the validation to qualified analysts. The QC Manager, or qualified designee, is responsible for creating the Analytical Method Validation Report.

Quality Assurance Manager: The Quality Assurance Manager (QA Manager) is responsible for supporting the QC Manager in the development of the method or the Analytical Method Validation Protocol or Report. The QA Manager or qualified designee is responsible for review and approval of the Analytical Method Validation Protocol and Report at the Bangor, PA or Stroudsburg, PA Facility.

Qualified Analyst: A qualified analyst has completed their Introductory Period at BioSpectra. This step may be circumvented at the discretion of the QC Manager based on previous or current

experience with the method or instrument. If the Introductory Period qualification is circumvented, the QC Manager must write a statement of why this occurred and attach it to the Analytical Method Validation Report and place a copy in the analyst's training binder. The analysts must be trained to perform the procedure and this training must be documented in the analyst's training binder. If the method or instrument is new to BioSpectra, the analyst to complete the IQ/OQ/PQ (Installation Qualification / Operational Qualification / Performance Qualification) or the bulk of the validation testing will be designated as the Subject Matter Expert (SME). It is then the responsibility of the SME to train the other analysts in the laboratory.

3.0 DEFINITIONS

Analytical Method: For the purpose of the AMVMP, an Analytical Method is defined as any analysis that is performed in the QC Laboratory at BioSpectra.

Subject Matter Expert: The Subject Matter Expert (SME) is defined as the qualified analyst that has developed the method and procedure for a specific instrument or analysis.

Specificity: Specificity is the ability to detect the desired analyte in the presence of other compounds that can be expected, such as impurities, degradants and intermediate products.

Accuracy: The accuracy of an analytical method expresses the closeness of agreement between the value which is accepted as true or an accepted reference value and the value obtained.

Precision: The precision of an analytical method expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision may be considered at three levels: repeatability, intermediate precision and reproducibility.

Repeatability: Repeatability expresses the precision of a series of measurements performed under the same conditions over a short period of time. This may also be referred to as intra-assay precision.

Intermediate Precision: Intermediate precision expresses the precision between inter-laboratory variations. This may include different analysts, different days or different equipment.

Reproducibility: Reproducibility expresses the precision between different laboratories.

Detection Limit: The detection limit expresses the lowest level of analyte that can be detected but may not be quantified at an exact value.

Quantitation Limit: The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and

accuracy. The quantitation limit is a parameter of quantitative assays for low levels of compounds in sample matrices, and is used particularly for the determination of impurities and/or degradation products.

Linearity: Linearity is the analytical methods ability to obtain results that are directly proportional to the concentration of analyte present in the sample.

Range: The range is defined as the interval between the upper and lower concentrations of analyte in the sample that have demonstrated suitable precision, accuracy, and linearity.

Robustness: The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

4.0 PRE-QUALIFICATION REQUIREMENTS

For all method validations performed at BioSpectra the following Pre-Qualifications Requirements are necessary. There may be additional Pre-Qualification Requirements based on the analysis or instrument being validated, but the list included in this AMVMP is required to be addressed as part of the Analytical Method Validation Protocol.

Equipment: A list of all equipment that is used will be created as part of the report. This list will include any serial numbers, date of last calibration and the calibration due date for each piece of equipment.

Personnel: All qualified analysts assigned to complete the Validation Protocol must be adequately trained to complete the Protocol. This training must be documented appropriately in the analyst's training binder.

Supplies: Supplies required to carry out the Validation must be listed and identified with the Supplier and description.

Reagents: The reagents used for the Validation must be identified and deemed suitable for use by the QC Manager, or qualified designee, prior to performing the Validation. A list of the name of the reagent, lot number, manufacturer, date of opening, date of expiration and part number should be provided in the Validation Report.

Reference Standards: There are three types of industry accepted standards: USP, commercially supplied, and materials of a known purity as determined by the QC Laboratory at BioSpectra. A list of the name of the reference standard, lot number, manufacture, date of opening, date of expiration and part number must be provided in the validation report and recorded during validation testing.

5.0 MATERIALS AND EQUIPMENT

A list of all equipment utilized must be provided in the Analytical Method Validation Report. This should include any instrumentation used. The instrumentation should be listed including the following information: Instrument Name, Model Number, Manufacturer, Serial Number, Calibration Information and Validation Information. Other equipment used must be listed as well including, but not limited to: pipettes, pipette tips, stir beads, stir plates, glassware, parafilm, hot plates, spatulas or scoops, weigh boats and weigh paper.

Any specifications on materials or equipment must be listed here as well. For example, if a titration is being performed on a water sensitive material, it may be necessary to use previously dried glassware. If a specific level of accuracy is required for any of the instruments, this must be listed here as well. This list must be started prior to the start of the validation. However, this does not mean that additional needs for materials will not arise during the validation. These may be added to this list as used. In cases where various pieces of equipment are used but fall into the same category, i.e. different volume pipettes, all must be listed separately.

6.0 PROCEDURE

The Analytical Method Validation Procedure should be separated into distinct sections that have a procedure for all performance parameters required for the method being validated. Compendial test requirements vary from highly exacting analytical determinations to subjective evaluation of attributes. Considering this broad variety, it is only logical that different test procedures require different validation schemes. The most common categories of tests for which validation data should be required (as per USP) are as follows:

Category I: Analytical procedures for quantitation of major components of bulk drug substances or active ingredients (including preservatives) in finished pharmaceutical products.

Category II: Analytical procedures for determination of impurities in bulk drug substances or degradation compounds in finished pharmaceutical products. These procedures include quantitative assays and limit tests.

Category III: Analytical procedures for determination of performance characteristics (e.g., dissolution, drug release, etc.)

Category IV: Identification tests.

The Table below will outline the performance parameters required for each category of method. This will provide a guideline for method validations of new analysis.

Analytical Performance Characteristics	Category I	Category II		Category III	Category IV
		Quantitative	Limit Tests		
Accuracy	Yes	Yes	[1]	[1]	No
Precision	Yes	Yes	No	Yes	No
Specificity	Yes	Yes	Yes	[1]	Yes
Detection Limit	No	No	Yes	[1]	No
Quantitation Limit	No	Yes	No	[1]	No
Linearity	Yes	Yes	No	[1]	No
Range	Yes	Yes	[1]	[1]	No

[1] May be required, depending on the nature of the specific test.

There are seven main performance parameters that may be evaluated for each method. Guidelines of the analysis required for these parameters are listed below.

Linearity: A linear relationship should be evaluated across the range of the analytical procedure. It may be demonstrated directly on the drug substance (by dilution of a standard stock solution) and/or separate weighing of synthetic mixtures of the drug product components, using the proposed procedure. The latter aspect can be studied during investigation of the range. Linearity should be evaluated by visual inspection of a plot of signals as a function of analyte concentration or content. If there is a linear relationship, test results should be evaluated by appropriate statistical methods, for example, by calculation of a regression line by the method of least squares. In some cases, to obtain linearity between assays and sample concentrations, the test data may need to be subjected to a mathematical transformation prior to the regression analysis. Data from the regression line itself may be helpful to provide mathematical estimates of the degree of linearity. The correlation coefficient, y-intercept, slope of the regression line and residual sum of squares should be submitted. A plot of the data should be included. In addition, an analysis of the deviation of the actual data points from the regression line may also be helpful for evaluating linearity. Some analytical procedures, such as immunoassays, do not demonstrate linearity after any transformation. In this case, the analytical response should be described by an appropriate

function of the concentration (amount) of an analyte in a sample. For the establishment of linearity, a minimum of 5 concentrations is recommended. Other approaches should be justified.

Range: The specified range is normally derived from linearity studies and depends on the intended application of the procedure. It is established by confirming that the analytical procedure provides an acceptable degree of linearity, accuracy and precision when applied to samples containing amounts of analyte within or at the extremes of the specified range of the analytical procedure.
The following minimum specified ranges should be considered:

- For the assay of a drug substance or a finished (drug) product: normally from 80 to 120 percent of the test concentration;
- For content uniformity, covering a minimum of 70 to 130 percent of the test concentration, unless a wider more appropriate range, based on the nature of the dosage form is justified;
- For dissolution testing: +/-20 % over the specified range;
- For the determination of an impurity: from the reporting level (ICH) or 50% (USP) of the impurity to 120% of the specification.
- If assay and purity are performed together as one test and only a 100% standard is used, linearity should cover the range from the reporting level of the impurities1 to 120% of the assay specification.

Accuracy: Accuracy should be assessed using a minimum of 9 determinations over a minimum of 3 concentration levels covering the specified range (e.g., 3 concentrations /3 replicates each of the total analytical procedure). Accuracy should be reported as percent recovery by the assay of known added amount of analyte in the sample or as the difference between the mean and the accepted true value together with the confidence intervals.

The preparation of the spiked samples will be detailed in the procedure portion of the Analytical Method Validation Protocol. The data will be assessed by calculating the percent recovery for each concentration. The acceptance criterion will depend on the assay and its variability and on the product. Setting an acceptance criterion based on the lack of statistical significance of the test of the null hypothesis that the slope is 1.0 is not an acceptable approach. Accuracy of physical property methods may be assessed through the analysis of standard reference materials, or alternatively, the suitability of the above approaches may be considered on a case-by-case basis. The average recovery, standard deviation, and relative standard deviation should be calculated after all samples are analyzed in order to determine the overall accuracy of the method.

Precision: The precision of an analytical procedure is determined by assaying a sufficient number of aliquots of a homogeneous sample to be able to calculate statistically valid estimates of standard deviation or relative standard deviation (coefficient of variation). Assays in this context are independent analyses of samples that have been carried through the complete analytical procedure from sample preparation to final test result.

The ICH documents recommend that 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.

Ruggedness (intermediate precision): The extent to which intermediate precision should be established depends on the circumstances under which the procedure is intended to be used. The applicant should establish the effects of random events on the precision of the analytical procedure. Typical variations to be studied include days, analysts, equipment, etc. It is not considered necessary to study these effects individually. The use of an experimental design (matrix) is encouraged.

Specificity: In the case of qualitative analyses (identification tests), the ability to select between compounds of closely related structure that are likely to be present should be demonstrated. This should be confirmed by obtaining positive results (perhaps by comparison to a known reference material) from samples containing the analyte, coupled with negative results from samples that do not contain the analyte and by confirming that a positive response is not obtained from materials structurally similar to or closely related to the analyte.

In the case of analytical procedures for impurities, specificity may be established by spiking the drug substance or product with appropriate levels of impurities and demonstrating that these impurities are determined with appropriate accuracy and precision.

In the case of the assay, demonstration of specificity requires that it can be shown that the procedure is unaffected by the presence of impurities or excipients. In practice, this can be done by spiking the drug substance or product with appropriate levels of impurities or excipients and demonstrating that the assay result is unaffected by the presence of these extraneous materials.

If impurity or degradation product standards are unavailable, specificity may be demonstrated by comparing the test results of samples containing impurities or degradation products to a second well-characterized procedure (e.g., a Pharmacopeial or other validated procedure). These comparisons should include samples stored under relevant stress conditions (e.g., light, heat, humidity, acid/base hydrolysis,

and oxidation). In the case of the assay, the results should be compared; in the case of chromatographic impurity tests, the impurity profiles should be compared.

The ICH documents state that when chromatographic procedures are used, representative chromatograms should be presented to demonstrate the degree of selectivity, and peaks should be appropriately labeled. Peak purity tests (e.g., using diode array or mass spectrometry) may be useful to show that the analyte chromatographic peak is not attributable to more than one component.

For validation of specificity for qualitative and quantitative determinations by spectroscopic methods, chapters related to topics such as near-infrared spectrophotometry, raman spectroscopy, and X-ray powder diffraction should be consulted.

Detection Limit: Several approaches for determining the detection limit are possible, depending on whether the procedure is a non-instrumental or instrumental. Approaches other than those listed below may be acceptable.

- Based on Visual Evaluation: Visual evaluation may be used for non-instrumental methods but may also be used with instrumental methods. The detection limit is determined by the analysis of samples with known concentrations of analyte and by establishing the minimum level at which the analyte can be reliably detected.
- Based on Signal-to-Noise: This approach can only be applied to analytical procedures which exhibit baseline noise. Determination of the signal-to-noise ratio is performed by comparing measured signals from samples with known low concentrations of analyte with those of blank samples and establishing the minimum concentration at which the analyte can be reliably detected. A signal-to-noise ratio between 3 or 2:1 is generally considered acceptable for estimating the detection limit.
- Based on the Standard Deviation of the Response and the Slope The detection limit (DL) may be expressed as: $DL = (3.3\ \sigma)/ S$ where σ = the standard deviation of the response, and S = the slope of the calibration curve. The slope S may be estimated from the calibration curve of the analyte. The estimate of σ may be carried out in a variety of ways, for example:
    - Based on the Standard Deviation of the Blank Measurement of the magnitude of analytical background response is performed by analyzing an appropriate number of blank samples and calculating the standard deviation of these responses.
    - Based on the Calibration Curve A specific calibration curve should be studied using samples containing an analyte in the range of DL. The residual standard deviation of a regression line

or the standard deviation of y-intercepts of regression lines may be used as the standard deviation.

The detection limit and the method used for determining the detection limit should be presented. If DL is determined based on visual evaluation or based on signal to noise ratio, the presentation of the relevant chromatograms is considered acceptable for justification.

Quantitation limit: Several approaches for determining the quantitation limit are possible, depending on whether the procedure is a non-instrumental or instrumental. Approaches other than those listed below may be acceptable.

- Based on Visual evaluation: Visual evaluation may be used for non-instrumental methods but may also be used with instrumental methods. The quantitation limit is generally determined by the analysis of samples with known concentrations of analyte and by establishing the minimum level at which the analyte can be quantified with acceptable accuracy and precision.
- Based on Signal-to-Noise Approach: This approach can only be applied to analytical procedures that exhibit baseline noise. Determination of the signal-to-noise ratio is performed by comparing measured signals from samples with known low concentrations of analyte with those of blank samples and by establishing the minimum concentration at which the analyte can be reliably quantified. A typical signal-to-noise ratio is 10:1.
- Based on the Standard Deviation of the Response and the Slope: The quantitation limit (QL) may be expressed as QL = (10σ)/S, where σ = the standard deviation of the response, and S = the slope of the calibration curve. The slope S may be estimated from the calibration curve of the analyte. The estimate of σ may be carried out in a variety of ways:
  - Based on Standard Deviation of the Blank: Measurement of the magnitude of analytical background response is performed by analyzing an appropriate number of blank samples and calculating the standard deviation of these responses.
  - Based on the Calibration Curve: A specific calibration curve should be studied using samples, containing an analyte in the range of QL. The residual standard deviation of a regression line or the standard deviation of y-intercepts of regression lines may be used as the standard deviation.

The quantitation limit and the method used for determining the quantitation limit should be presented. The limit should be subsequently validated by the analysis of a suitable number of samples known to be near or prepared at the quantitation limit.

Robustness: The robustness of an analytical procedure is a measure of capacity to remain unaffected by small but deliberate variations in the procedural parameters listed in the procedure documentation and provides an indication of its suitability during normal usage. The need to analyze the robustness of an analytical method, as well as the specifications will be developed on an individual method basis. This performance characteristic is typically analyzed during the development of an analytical method and is not included in the Analytical Method Validation Protocol

System Suitability: If measurements are susceptible to variations in analytical conditions, these should be suitably controlled, or a precautionary statement should be included in the procedure. One consequence of the evaluation of robustness and ruggedness should be that a series of system suitability parameters is established to ensure that the validity of the analytical procedure is maintained whenever used. Typical variations are the stability of analytical solutions, different equipment, and different analysts. In the case of liquid chromatography, typical variations are the pH of the mobile phase, the mobile phase composition, different lots or suppliers of columns, the temperature, and the flow rate. In the case of gas chromatography, typical variations are different lots or suppliers of columns, the temperature, and the flow rate.

System suitability tests are based on the concept that the equipment, electronics, analytical operations, and samples to be analyzed constitute an integral system that can be evaluated as such. System suitability test parameters to be established for a particular procedure depend on the type of procedure being evaluated.

The definitions and determinations of the performance parameters described in this section are referenced from ICHQ2(R1) and USP <1225>.

7.0 TEST REPORT AND CONCLUSIONS

The Analytical Method Validation Report should include a brief introduction that explains what analysis is being validated and why. The next section of the report should include all of the Pre-Validation Requirements. This includes but is not limited to all resources defined in the Pre-Validation Section 4.0 of this AMVMP. The Report will then detail the procedures carried out for all of the separate performance parameters. This should include all reagents, equipment, instrumentation, reference standards, and materials where used in the analysis. The results section will detail all results of the Analytical Method Validation. The conclusion will detail any lab investigations or discrepancies that may have occurred that directly affected the Analytical Method Validation. It will also explain any deviations from expected results in the analysis. A final conclusive statement will be made at the end of the conclusion to state the

following items: Method Validation status, date effective, location of validation, and any exceptions to the validation.

Any changes made to a Method Validation Protocol may be approved by the validation approval team. Appropriate justification is required in the Method Validation Report.

8.0 EQUIVALENCY

In the absence of a Revalidation, an equivalency study may be performed. An equivalency study will act as an abbreviated Analytical Method Validation. The Report or Protocol (when applicable) and requirements will be created by the QC Manager or designee. Equivalency is proven for an outside testing lab or the Stroudsburg Laboratory by obtaining analytical results of three lots that are within the error of the method when compared to the original validation results. The following are additional instances where an Equivalency study may be acceptable: new model of the same equipment, equivalent methods or instrument, change in key reagents and minor differences in compendia methods.

9.0 REVALIDATION

The need for Revalidation of Analytical Method will be based on method or instrument changes. However, as a general guideline, the following instances will result in a Revalidation: Repeated failure to meet validity criteria, significant change in sample preparation, or change in reagent formulation.