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Guidance for Industry
Validation of Analytical Procedures for Type C Medicated Feeds
Draft Guidance

This draft guidance document is being distributed for comment purposes only.

This draft guidance discusses characteristics that may be considered during the validation of non-microbiological analytical procedures for the analysis of drugs in Type C medicated feeds included as part of original and supplemental new animal drug applications (NADAs) and abbreviated new animal drug applications (ANADAs) for Type A Medicated Articles submitted to the Food and Drug Administration (FDA). It provides guidance and recommendations on considering various validation characteristics for each analytical procedure used in medicated feed assays and indicates the data that may be included in the applications.

Comments and suggestions regarding this draft guidance should be sent to the Division of Dockets Management (HFA-305), Food and Drug Administration, 5630 Fishers Lane, Room 1061, Rockville, MD 20852. All comments should be identified with the Docket No. 2004D-0146. Comments may also be submitted electronically on the Internet at http://www.fda.gov/dockets/ecomments.

For questions regarding the draft guidance document, contact Mary G. Leadbetter, Center for Veterinary Medicine (HFV-141), Food and Drug Administration, 7500 Standish Place, Rockville, MD 20855, 301-827-6964, E-mail: mleadbet@cvm.fda.gov .

Additional copies of this draft guidance document may be requested from the Communications Staff (HFV-12), Center for Veterinary Medicine, Food and Drug Administration, 7519 Standish Place, Rockville, MD 20855, and may be viewed on the Internet at http://www.fda.gov/cvm.

According to the Paperwork Reduction Act of 1995, a collection of information must display a valid OMB control number. The existing valid OMB control numbers for this information collection are 0910-0032 and 0910-0154. This draft guidance contains no new collections of information.

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Veterinary Medicine
April 26, 2004

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Validation of Analytical Procedures for Type C Medicated Feeds[1]

This draft guidance, when finalized, will represent the agency's current thinking on this topic. It does not create or confer any rights for or on any person and does not operate to bind FDA or the public. You can use an alternative approach if the approach satisfies the requirements of the applicable statute(s) and/or regulation(s). If you want to discuss an alternative approach, contact the FDA staff responsible for implementing this guidance. If you cannot identify the appropriate FDA staff, call the appropriate number listed on the title page of this guidance.

INTRODUCTION

The purpose of this draft guidance is to provide recommendations on how to consider the various validation characteristics for each analytical procedure used in medicated feed assays. This guidance is written primarily for chromatographic methods; however, the guidance does not limit the analytical technique to chromatographic procedures, as other techniques may be appropriate. In some cases (for example, demonstration of specificity), the overall capabilities of a number of analytical procedures in combination may be investigated in order to ensure the quality of the medicated feed.

Section 512(b) of the Federal Food, Drug, and Cosmetic Act (21 U.S.C. § 360b) establishes the requirements for new animal drug approval. 21 C.F.R. § 514.1 specifies the information required to be submitted as part of the application and the proper form for the submission. Section 514.1(b)(5)(vii) requires an applicant to describe analytical procedures that should be capable of determining the active component(s) within a reasonable degree of accuracy and of assuring the identity of such components. Section 514.1(b)(5)(vii)(a) states that a description of practicable methods of analysis of adequate

[1] This draft guidance has been prepared by the Office of New Animal Drug Evaluation in the Center for Veterinary Medicine at the Food and Drug Administration.

sensitivity to determine the amount of the new animal drug in the final dosage form should be included.

FDA's guidance documents, including this guidance, do not establish legally enforceable responsibilities. Instead, guidances describe the Agency's current thinking on a topic and should be viewed only as recommendations, unless specific regulatory or statutory requirements are cited. The use of the word "should" in Agency guidances means that something is suggested or recommended, but not required.

DISCUSSION

The objective of the analytical procedure should be clearly understood since this will govern the validation characteristics that are evaluated. Typical validation characteristics that may be considered are listed below:

- Specificity
- Linearity
- Range
- Accuracy
- Precision
- Limit of Detection
- Limit of Quantitation
- Robustness

Each of these validation characteristics is defined in the attached Glossary.

Approaches other than those set forth in this guidance may be acceptable. It is the responsibility of the applicant to choose the validation procedure and protocol most suitable for the product. However, it is important to remember that the main objective of validation of an analytical procedure is to demonstrate that the procedure is suitable for its intended purpose.

It is recommended that a well-characterized reference standard, with documented purity, be used throughout the validation study. The degree of purity necessary depends on the intended use.

For the sake of clarity, this document considers the various validation characteristics in distinct sections. The arrangement of these sections reflects the process by which an analytical procedure may be developed and evaluated.

In practice, it is recommended to design the experimental work such that the appropriate validation characteristics can be considered simultaneously to provide a sound, overall

knowledge of the capabilities of the analytical procedure. Appropriate validation characteristics may include: specificity, linearity, range, accuracy, and precision.

1. SPECIFICITY

It is recommended that an investigation of specificity be conducted during the validation of the medicated feed assay. The procedures used to demonstrate specificity will depend on the intended objective of the analytical procedure.

Identification of the analyte may be made by means of retention time of the standard.

For chromatographic procedures, it is recommended that representative chromatograms be used to demonstrate specificity, and individual feed components and drug products be appropriately labeled. The chromatographic profile using peak shape and tailing criteria may be used to indicate either co-eluting peaks or sample matrix effects. The peak parameters should be in agreement between the standard and analyte peaks. In addition, to ensure that the peaks are single components, a diode array detector may be used to obtain peak purity information for the analyte peaks in a variety of feed matrices. Similar considerations may be given to other separation techniques.

For the assay, it is recommended that there be a demonstration of a lack of interference by feed ingredients or other drug products that may be in the feed. This may be done by demonstrating that the responses of a blank placebo made from the feed ingredients and/or drug products, either separately or in combination, are either different from the absorbance (for UV methods) or retention time (for GC and HPLC methods) of the analyte of interest or not significant (i.e., that the signal measured as a percent concentration is not greater than 10%). It is recommended that additional information be provided showing that common feed ingredients do not interfere with the detection system. If potential interference is observed, it is recommended that the ingredient be evaluated by the complete method. Some examples of interfering ingredients are clay agents (for flowability) and pellet binding, molasses, grass meals (e.g., alfalfa), high mineral content, corn cob meal, cottonseed by product meal, meat and bone meal, and fish meal. Many methods developed to give good recovery in a simple corn-soy feed do not work well when the analyte is added to high mineral feeds, and are not recommended.

Typically, 2-3 feed mixtures, based on the species that will be medicated, geographical location where the feed may be prepared, and life cycle of the species (e.g., starter, finisher), should be tested. For drug products, it is recommended that applicants consider the most common products that may typically be present within the feed.

Applicable literature references demonstrating non-interference may be supplied in lieu of actual testing.

2. LINEARITY

It is recommended that a linear relationship be evaluated across the range (see section 3) of the analytical procedure. It may be demonstrated directly on the drug substance by separate weighings (two separate weighings preferred) and/or dilution of a standard stock solution, using the proposed procedure.

It is recommended that linearity be evaluated by visual inspection of a plot of signals as a function of analyte concentration or content. If there is a linear relationship, it is recommended that test results be evaluated by appropriate statistical methods, for example, by calculation of a regression line by the method of least squares. Data from the regression line itself may be helpful to provide mathematical estimates of the degree of linearity. It is recommended that the correlation coefficient (R) be at least 0.995. The regression line intercept should not differ from zero if a single point calibration technique is used. This may be demonstrated if the confidence limits of the intercept include zero or if the intercept value is a small percentage of the target level. If the intercept is significantly different from zero, then a single point calibration technique is not recommended.

For the establishment of linearity, a minimum of 5 concentrations, covering the intended dosing range with one concentration 50% of the lowest dose, is recommended. It is recommended that the sponsor contact CVM if other approaches are used.

3. RANGE

The specified range should be derived from linearity studies and depends on the intended application of the procedure. It may be 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.

For the assay of a drug in a medicated feed, the range should be from 50 to 150 percent of the labeled concentration.

4. ACCURACY

It is recommended that accuracy be established across the specified range of the analytical procedure used for medicated feed assays.

It is recommended that two (2) typical feed matrices with known quantities of the drug added be analyzed.

It is recommended that accuracy be assessed using a minimum of 15 - 20 determinations over the concentration levels covering the specified range for each feed matrix tested (e.g. 3 - 4 concentrations (depending on the dose range) / 5 replicates each of the total analytical procedure). Recovery from fortified blank matrix samples should be between 80 - 110%.

5. PRECISION

Validation of tests for assay of medicated feeds should include an investigation of precision.

5.1. Repeatability

For fortified medicated feed samples, it is recommended that repeatability be assessed using a minimum of 15 determinations covering the specified range for the procedure (e.g., 3-4 concentrations / 5 replicates each).

For drugs incorporated into medicated feeds at greater than 10 ppm, the within-laboratory variation coefficient should be less than 5.0%. For drugs incorporated into medicated feeds at less than 10 ppm, the within-laboratory variation coefficient should be less than 7.5%.

5.2. Intermediate Precision

The extent to which intermediate precision should be established depends on the circumstances under which the procedure is intended to be used. It is recommended that the applicant establish the effects of random events on the precision of the analytical procedure. It is recommended that variations to be studied include days, analysts, equipment, etc. It is not recommended to study these effects individually. Instead, the use of a statistical experimental design (matrix) is encouraged (see Statistical Manual of the AOAC by W.J. Youden and E.H. Steiner, 1975, page 33 for more information on statistical design of experiments). The performance of the method by a second independent laboratory is encouraged.

5.3. Reproducibility

It is recommended that reproducibility be assessed by means of an inter-laboratory trial. It is recommended that reproducibility be considered in the case of standardization of an analytical procedure.

5.4. Proof of Performance

It is recommended that proof of performance of the assay be demonstrated by testing two (2) batches of the proposed medicated feed manufactured in mixing equipment of the appropriate size and under conditions representative of typical commercial processing. When feasible, batches should be manufactured using different configurations of mixers.

It is recommended that a minimum of 10 determinations covering the specified range for the procedure be made (e.g., 2 concentrations (high and low) / 5 replicates each). If the feed is pelletized, it is recommended that the mash and the pelletized feed be tested separately. Results should be reported in both concentration and percent label claim.

6. LIMIT OF DETECTION

There are several approaches for determining the limit of detection (LOD), depending on whether the procedure is non-instrumental or instrumental. Approaches other than those listed below may be used.

6.1. Based on Visual Evaluation

Visual evaluation may be used for non-instrumental or instrumental methods.

The detection limit may be 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.

6.2. Based on Signal-to-Noise

It is recommended that this approach be applied only to analytical procedures that exhibit baseline noise.

Determination of the signal-to-noise ratio may be 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 of between 3 or 2:1 is generally recommended for estimating the detection limit.

6.3. Based on the Standard Deviation of the Response and the Slope

It is recommended that the LOD be expressed as:

$$LOD = 3.3 \, \delta / S$$

where δ = the standard deviation of the responses and S = the slope of the calibration curve. The slope S may be estimated from the calibration curve of the analyte. The estimate of δ may be carried out in a variety of ways, for example:

6.3.1. Based on the Standard Deviation of the Blank

It is recommended that the measurement of the magnitude of analytical background response be performed by analyzing an appropriate number of blank samples and calculating the standard deviation of these responses.

6.3.2. Based on the Calibration Curve

It is recommended that a specific calibration curve be studied using samples containing an analyte in the range of the LOD. 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.

7. LIMIT OF QUANTITATION

Several approaches for determining the limit of quantitation (LOQ) are possible, depending on whether the procedure is non-instrumental or instrumental. Approaches other than those listed below may be used.

7.1. Based on Visual Evaluation

Visual evaluation may be used for non-instrumental or instrumental methods.

The LOQ may be 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.

7.2. Based on Signal-to-Noise Approach

It is recommended that this approach be applied only to analytical procedures that exhibit baseline noise. Determination of the signal-to-noise ratio may be 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 quantified. A signal-to-noise ratio of 10:1 is recommended.

7.3. Based on the Standard Deviation of the Response and the Slope

The LOQ may be expressed as:

$$LOQ = 10\ \delta / S$$

where δ = the standard deviation of the responses and S = the slope of the calibration curve. The slope S may be estimated from the calibration curve of the analyte. The estimate of δ may be carried out in a variety of ways, for example:

7.3.1. Based on the Standard Deviation of the Blank

Measurement of the magnitude of analytical background response may be performed by analyzing an appropriate number of blank samples and calculating the standard deviation of these responses.

7.3.2. Based on the Calibration Curve

It is recommended that a specific calibration curve be studied using samples containing an analyte in the range of the LOQ. 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.

8. ROBUSTNESS / RUGGEDNESS

It is recommended that the evaluation of robustness be considered during the development phase and demonstrated during the analytical validation phase. Robustness depends on the type of procedure under study. It should show the reliability of an analysis with respect to deliberate variations in method parameters.

If measurements are susceptible to variations in analytical conditions, it is recommended that the analytical conditions be suitably controlled or a precautionary statement be included in the procedure. One consequence of the evaluation of robustness should be

that a series of system suitability parameters (e.g., resolution test) are established to ensure that the validity of the analytical procedure is maintained whenever used.

Examples of typical variations are:

-stability of analytical solutions and feed extracts; and
-extraction time.

(Note: it is recommended that results of the stability studies of the analytical solutions and feed extracts be included in the procedure)

In the case of high performance liquid chromatography (HPLC), examples of typical variations are:

-influence of variations of pH in a mobile phase;
-influence of variations in mobile phase composition;
-different columns (different lots and/or suppliers); and
-temperature-flow rate.

In the case of gas chromatography (GC), examples of typical variations are:

-different columns (different lots and/or suppliers); and
-temperature-flow rate.

9. SYSTEM SUITABILITY TESTING

System suitability testing is an integral part of many analytical procedures. The 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 validated and may include, for example, data acceptability testing for feed controls. It is recommended that system suitability tests and criteria for the HPLC or GC detection system be evaluated. Performance specifications for critical reagents and steps, such as solid phase extraction, should be included when appropriate. If specific tests and criteria are used, then the recommended actions taken if performance does not meet the criteria should be determined. Additional information is available in Pharmacopoeias..

10. RECOMMENDED DATA

It is recommended that data collected during validation and formulae used for calculating validation characteristics be submitted for each feed type and discussed as outlined below:

Specificity:

It is recommended that representative sample sets of chromatograms be provided so that recalculation can be performed, including:

- Baseline / mobile phase
- Extraction solvent
- Feed ingredient placebo that cause interference
- Other drug product placebo that causes interference
- Standards
- Samples (high and low concentration, different feed mixtures)

Retention times and a comparison of relative retention times should be provided.

Tabular listing of feed mixture ingredients and other drug products tested should be provided.

Linearity & Range:

It is recommended that the correlation coefficient, y-intercept, slope of the regression line, and residual sum of squares 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.

Accuracy:

It is recommended that accuracy 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.

Tabular listing of feed mixture ingredients used should be provided.

For each feed matrix studied, it is recommended that the complete set of data including weighings, sample and standard preparation, chromatography, calculations, and results be provided. A representative set of chromatograms should be provided and, for the concentration(s) in between, a table of relevant parameters should be provided. All individual area or height measurements for controls, standards, and samples and all other information such as sample weights, standard concentrations, and dilutions should also be provided.

Precision:

It is recommended that the standard deviation, relative standard deviation (coefficient of variation), and confidence interval be reported for each type of precision investigated.

It is recommended that the complete set of data including weighings, sample and standard preparation, chromatography, calculations, and results be provided. A representative set of data should be provided and, for the concentration(s) in between, a table of relevant parameters should be provided for each type of precision investigated.

Limit of Detection:

It is recommended that the limit of detection and the method used for determining the detection limit be presented. If the LOD is determined based on visual evaluation or based on signal-to-noise ratio, the presentation of the relevant chromatograms may be considered acceptable for justification.

In cases where an estimated value for the LOD is obtained by calculation or extrapolation, this estimate may subsequently be validated by the independent analysis of a suitable number of samples known to be near, or prepared at, the LOD.

Limit of Quantitation:

It is recommended that the limit of quantitation and the method used for determining the LOQ be presented. The limit should be subsequently confirmed by the analysis of a suitable number of samples known to be near, or prepared at, the LOQ.

Robustness/Ruggedness:

It is recommended that tabular representation including conditions tested, retention times, tailing factors, effects on resolution, and potency be presented.

GLOSSARY

1. ANALYTICAL PROCEDURE

The analytical procedure refers to the way an analysis is performed. It describes in detail the steps that should be followed to perform each analytical test. This may include, but is not limited to, the sample, the reference standard and the reagents preparations, use of the apparatus, generation of the calibration curve, and use of the formulae for the calculation.

2. SPECIFICITY

Specificity is the ability to assess unequivocally the analyte in the presence of components that may be expected to be present. Typically, these might include impurities, degradation products, matrix, other approved drugs, etc.

Lack of specificity of an individual analytical procedure may be compensated for by other supporting analytical procedure(s).

This definition includes the following:

Identification: to ensure the identity of an analyte.
Assay (content or potency): to provide an exact result which allows an accurate statement on the content or potency of the analyte in a sample.

3. LINEARITY

The linearity of an analytical procedure is its ability (within a given range) to obtain test results that are directly proportional to the concentration (amount) of analyte in the sample.

4. RANGE

The range of an analytical procedure is the interval between the upper and lower concentration (amounts) of analyte in the sample (including these concentrations) for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy, and linearity.

5. ACCURACY

The accuracy of an analytical procedure refers to the closeness of agreement between the value that is accepted either as a conventional true value or an accepted reference value, and the value found.

This is sometimes termed trueness.

6. PRECISION

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

Precision is investigated using homogenous, authentic samples. However, if it is not possible to obtain a homogenous sample, it may be investigated using artificially prepared samples or a sample solution (although extraction variability will not be measured).

The precision of an analytical procedure is usually expressed as the variance, standard deviation, or coefficient of variation of a series of measurements.

6.1. Repeatability: Repeatability expresses the precision under the same operating conditions over a short interval of time. Repeatability is also termed intra-assay precision.

6.2. Intermediate precision: Intermediate precision expresses within-laboratories variations: different days, different analysts, different equipment, etc.

6.3. Reproducibility: Reproducibility expresses the precision between laboratories (collaborative or transfer studies, usually applied to standardization of methodology).

7. LIMIT OF DETECTION

The limit of detection of an individual analytical procedure is the lowest amount of analyte in a sample that can be detected, but not necessarily quantitated as an exact value.

8. LIMIT OF QUANTITATION

The limit of quantitation of an individual analytical procedure is the lowest amount of analyte in a sample that 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.

9. ROBUSTNESS / RUGGEDNESS

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

10. SYSTEM SUITABILITY

A procedure run prior to the individual analytical analysis to demonstrate that the instrument, column, mobile phase, etc., parameters are within defined criteria. Adequate system suitability is demonstrated before proceeding with the analysis.