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## White Paper

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# NonClinical Dose Formulation Analysis Method Validation and Sample Analysis

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Received 30 April 2010; accepted 6 July 2010; published online 14 August 2010

**Abstract.** Nonclinical dose formulation analysis methods are used to confirm test article concentration and homogeneity in formulations and determine formulation stability in support of regulated nonclinical studies. There is currently no regulatory guidance for nonclinical dose formulation analysis method validation or sample analysis. Regulatory guidance for the validation of analytical procedures has been developed for drug product/formulation testing; however, verification of the formulation concentrations falls under the framework of GLP regulations (not GMP). The only current related regulatory guidance is the bioanalytical guidance for method validation. The fundamental parameters for bioanalysis and formulation analysis validations that overlap include: recovery, accuracy, precision, specificity, selectivity, carryover, sensitivity, and stability. Divergence in bioanalytical and drug product validations typically center around the acceptance criteria used. As the dose formulation samples are not true “unknowns”, the concept of quality control samples that cover the entire range of the standard curve serving as the indication for the confidence in the data generated from the “unknown” study samples may not always be necessary. Also, the standard bioanalytical acceptance criteria may not be directly applicable, especially when the determined concentration does not match the target concentration. This paper attempts to reconcile the different practices being performed in the community and to provide recommendations of best practices and proposed acceptance criteria for nonclinical dose formulation method validation and sample analysis.

**KEY WORDS:** acceptance criteria; formulation method validation; formulation sample analysis; nonclinical dose formulation analysis; test article concentration and homogeneity.

## INTRODUCTION

Nonclinical dose formulation analysis (NC DFA) is required in all regulated studies used to assess the safety of drugs during the development process. The primary purpose of nonclinical studies is to establish safety margins which can then be extrapolated to clinical studies. Therefore, NC DFA is required in all regulated studies to verify the documented test article concentrations in formulations used to calculate these safety margins (1,2). These analytical methods are used to assess the concentration of test article in nonclinical formulation, formulation homogeneity and formulation stability in support of regulated nonclinical studies (for example: safety, toxicokinetic, and pharmacokinetic studies).

The in-life phase and therefore the dose formulation analysis phase of regulated nonclinical studies are typically conducted in compliance with one or more of the following:

(1) the Food and Drug Administration (FDA) Good Laboratory Practice Regulations (GLP) as set forth in Title 21 of the US Code of Federal Regulations, Part 58 (1,2); (2) the Organisation for Economic Co-operation and Development (OECD) Principles of Good Laboratory Practice, [ENV/MC/CHEM(98)17] (3); (3) the Japanese Ministry of Health, Labor and Welfare (MHLW) No. 21, March 26, 1997 (4); and (4) the FDA Guidance for Industry: Bioanalytical Method Validation, May 2001 (5). It should be mentioned that the final guidance listed above, as the name suggests, is specifically for bioanalytical method validation, not dose formulation analysis validation. The fundamental parameters for bioanalytical and NC DFA validations that overlap include: recovery, accuracy, precision, selectivity (specificity), carryover, sensitivity and stability (6–9). Divergence in bioanalytical and NC DFA validations typically center around the acceptance criteria used. As the dose formulation samples are not true “unknowns”, the bioanalytical concept of quality control samples that cover the entire range of the standard curve serving as the indication for the confidence of the data generated from the “unknown” study samples may not always be necessary. Also, the standard bioanalytical acceptance criteria may not be directly applicable, especially when the determined concentration does not match the target concentration.

Since none of the aforementioned guidance or the numerous guidelines that have been written for the validation

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of analytical procedures (10–16) specifically address the analysis of nonclinical dose formulations, the members of the NonClinical Dose Formulation Analysis Focus Group of the American Association of Pharmaceutical Scientists (AAPS) met to discuss issues associated with NC DFA of small molecules, in June and November of 2009. The attendees represented a cross-section of the focus group from contract research organizations, pharmaceutical companies and biotech companies with analytical, bioanalytical, toxicology, and formulation experience represented. The intent of these meetings was to gain consensus on nonclinical dose formulation analysis criteria for validation and sample analysis. The resulting consensus is the framework of this white paper.

## GOALS AND OBJECTIVES

The purpose of this AAPS NC DFA Focus Group-sponsored white paper is to outline the general requirements for validating analytical methods and performing routine sample analysis in support of nonclinical drug formulation assessments. This paper focuses on method validation and sample analysis; however, the intent is not to minimize the importance of performing good science when developing an analytical method.

The analytical method should be suitable for the intended use/purpose and should generate reliable results that are free from significant bias. These analytical methods will be utilized to provide routine GLP support of confirmation of analyte (test article) dosage concentration, homogeneity assessment and stability testing. These analytical methods are not primarily intended to provide quantification of minor impurities for use in stability-indicating methods.

### Analyte and Vehicle

*Analyte.* An active pharmaceutical ingredient (API) is known as a test article when dosed and as an analyte during method validation and sample analysis. The analyte should be characterized with established purity, storage conditions, and retest or expiration date ideally documented in a certificate of analysis (or purity statement).

*Vehicle.* A vehicle (also known as excipients) is the material(s) used to deliver the test article to a biological system. Examples of vehicles include 0.5% methylcellulose, saline, water, feed, *etc.* Documentation of the vehicle components of the test article system is necessary during method validation.

## PROPOSED METHOD VALIDATION APPROACH

The general approach and criteria for validating methods for dose formulation analysis are instrument-independent. Currently, the majority of formulation analysis methods utilize high-performance liquid chromatography-ultraviolet (HPLC-UV), LC-mass spectrometry (MS)/MS, or GC-FID instrumentation. The suggested criteria provided in the remainder of this document are specific to HPLC-UV methodologies, but the underlying principles may be applied to other suitable analytical techniques. NC DFA validation

experiments should encompass the anticipated dosage concentration range and include accuracy, precision, specificity, standard curve linearity and analytical solution stability (10–19). In addition, formulation stability and homogeneity should be established for the conditions a sample is expected to encounter during sample collection, storage, analysis, and dosing. Each validation experiment is discussed in detail. Before initiating a method validation, a validation protocol, validation plan or appropriate SOP(s) to support the method validation experimentation should be written and approved. The validation protocol should include the acceptance criteria used to pass/fail individual runs and assess other validation experimental results. The method is considered valid if these acceptance criteria are achieved.

An analytical method is often used to support many different studies after it is validated. Therefore the method development phase must be rigorous enough to ensure the final method will be suitable for its intended purpose. The analytical method should cover the entire dose concentration range for all studies that it will be used to support. If a method development study exhibits highly variable results (*e.g.*, high%RSD) or lower than desired percentage recovery values, various parameters should be assessed prior to proceeding to validation including but not limited to the following: (1) container composition; (2) protection from light; (3) temperature; (4) filter bias; (5) dosing apparatus, and (6) stability. Assessment of mini-dose formulations or spiked additions of the analyte and vehicle components should be conducted in the method development phase. A typical item that is often overlooked is that low-dose concentrations are made using high amounts of vehicle components and exactly the opposite for high dose concentrations. Therefore, pH and specificity for the entire dosing range need to be considered during method development. Solubility is also a critical attribute and depends on the formulation vehicle properties. Most laboratories rely on visual observations for solubility measurements; however, instrumentation exists for more accurate evaluations. Although calculation of correction factors may vary from one compound to the next, correction factors used during validation should be representative of those planned for use in the preparation of study samples. Further definition of critical method development parameters are outside of the scope of this paper.

### Types of Validations

For all analytical method validations, acceptance criteria must be defined prior to the initiation of the validation (10–19). The analytical methods discussed herein are not intended to provide quantification of minor impurities for use in stability-indicating methods. The analytical method should be suitable for the intended purpose. It is conceivable to have a rapid analytical method for test article only, which would not be suitable as a stability indicating assay where degradants are separated from the test article or a more elaborate method which may have the additional advantage of identifying degradants. Both types of assays are acceptable and would require the appropriate level of validation for their intended purposes.

*Full Validation.* A full validation is conducted for assay methods used for chronic toxicity studies (>3 months in

duration) and encompasses all elements of the validation set forth in this paper. A full validation includes multiple sets of accuracy and precision data.

**Early Phase Validation.** An early phase validation is conducted for assay methods for acute toxicity studies ( $\leq 3$  months). Early phase validation testing may include a single validation run due to time constraints and limited availability of API. An early phase validation will assess system suitability, linearity and range, accuracy, specificity, selectivity, and carryover in one analytical run. Precision data obtained may be limited as replicates and multiple runs may not be performed.

**Partial Validation.** A partial validation should be conducted for a validated method when there is a significant change in the method. These changes may include but are not limited to: vehicle composition, dose formulation concentration range, analytical concentration range, chromatographic conditions, detector type, sample-processing procedure, or software. Partial validations are sometimes known as method qualifications and require a minimum of one set of accuracy and precision data.

**Transfer Validation.** A transfer validation should be conducted when transferring a fully validated method from one laboratory to another which utilizes the same method, same vehicle, same validation range, and same predefined acceptance criteria. Transfer validations require a minimum of one set of accuracy and precision data.

### System Suitability Test

Scientifically qualified and properly maintained instruments should be used for implementation of analytical methods in routine dose formulation analysis. Performance of system suitability ensures that the system is operating properly at the time of analysis. System suitability tests are more appropriately used for chromatographic methods to ensure that the system is sufficiently sensitive, specific and reproducible for the current analytical run. Examples of typical system suitability test (SST) factors are injection precision (retention time and peak area), theoretical plates (N), tailing factor (T), capacity factor ( $k'$ ), and resolution.

### Stock Standard Comparison

The accuracy of standard preparation should be demonstrated by comparing the response of two separately weighed stock solutions which have been diluted to a single concentration within the linear range of the method. Only stocks which compare within 5% difference should be used to prepare subsequent standards or quality control samples.

### Performance Check Standards

The system suitability check samples or quality control samples prepared in vehicle should be injected periodically throughout every chromatographic run to assess consistent analytical performance and serve as a performance check.

The typical performance measure for performance check standards is accuracy.

### Calibration Curves

A calibration curve is assessed as a function of the detector response at known concentrations of analyte: the assay's linear range (using a particular regression formula) is the lowest to the highest diluted concentration. Standards are usually prepared as simple solutions of analyte in diluent. If significant bias exists, standards should be prepared in vehicle.

### Linearity and Range

The formulation range is the concentration of the API in the on-study formulation. The analytical range is the validated linear range of the analytical method within which the dose formulation samples will be diluted. Linearity should be demonstrated over the entire analytical range. The linearity data may be used to support a single-point or multi-point calibration curve provided the appropriate criteria are met.

Single-point or multi-point calibration curves may be used if acceptable linearity (3 or more concentrations) is demonstrated during validation with acceptable coefficient of determination values ( $>0.99$ ). If the y-intercept is significantly different from zero, then the standard curve should be prepared with the vehicle.

### Recovery/Accuracy/Precision

**Accuracy (recovery):** Accuracy is the closeness of agreement between the average of replicate test results and the nominal value, measured in terms of percentage of recovery, relative error, or deviation from theoretical.

One approach to validate recovery or accuracy is based on small scale preparations of analyte in vehicle which mimic formulation preparations. These preparations should be made in vehicle at a minimum of the low and high concentrations with respect to the anticipated dosing range. The preparations should then be diluted to a target concentration within the anticipated analytical range. Multiple preparations or dilutions from a stock may be used to assess precision.

Another approach to validate recovery and accuracy is based on spike preparations of analyte and vehicle in the diluent. This approach is helpful when validating the low end of the formulation range where weighing requirements cannot be met or when homogeneity of mimic formulations is not likely.

### Intra-run and Inter-run Accuracy

Intra-run accuracy should be established during validation at a minimum. Inter-run accuracy should be performed in situations where there is a complex vehicle (*e.g.*, suspensions, solids and feeds). Inter-run accuracy should be measured using a minimum of three determinations per concentration. The analytical method should result in accuracy values of  $100 \pm 10\%$  recovery for solutions,  $100 \pm 15\%$  recovery for suspensions, and  $100 \pm 20\%$  recovery for solids where recovery is measured by dividing the found concentration by the nominal value. Values outside of this range may be acceptable if recovery is consistent across the concentration range.

### Intra-run and Inter-run Precision

Intra-run precision should be established during validation at a minimum. Precision is the closeness of agreement (degree of scatter) between replicate independent test results, measured in terms of relative standard deviation (%RSD) or coefficient of variation (%CV). Precision should be measured using a minimum of three preparations per concentration. The analytical method should result in precision values of  $\leq 5\%$  RSD for solutions,  $\leq 10\%$  RSD for suspensions, and  $\leq 20\%$  RSD for solids

### Specificity/Selectivity/Carryover

Specificity or selectivity is the degree to which a method can quantify the analyte accurately in the presence of inter-ferents (e.g., vehicle components, impurities and degradants). For chromatographic procedures, representative chromatograms should be used to demonstrate specificity (e.g., diluent blank, vehicle blank, analyte in diluent, and analyte in vehicle).

A method should be shown to be specific and selective in that it should be able to quantify the analyte accurately in the presence of potential interferences from degradants, the vehicle and diluent. There should be no significant peaks at the retention time of the analyte in diluent or vehicle blanks. Any carryover should be minimized in order to increase the ability to detect analyte in the vehicle control samples. Methods utilizing single-point calibration curves should have carryover no greater than 1% of the target standard concentration. Methods involving multi-point calibrations should have a carryover response of no more than 20% of the limit of quantification (LOQ).

### Sensitivity

The LOQ of an assay should be defined as the lowest concentration at which an assay is validated. All values that are below the LOQ should be reported as  $<LOQ$  and not 0 to allow the correct interpretation of the results. If samples are diluted as part of the analysis, the dilution should be incorporated into the reporting of the LOQ. The LOQ should have a signal to noise ratio of  $\geq 10$ .

### Stability Recommendations: Preprocessed, Postprocessed, Storage, Stock Solution

Stability of the analyte during storage of the test article prior to use, during use and throughout sample preparation and analysis should be demonstrated. Additionally, stock solution stability should be assessed when solutions are stored at room temperature, refrigerated or frozen for a relevant period of time. Stability should be compared with the nominal concentrations of freshly prepared standards. Acceptance criteria are typically based on the formulation vehicle composition: for example, solutions =  $100 \pm 10\%$  recovery with  $\leq 10\%$  RSD, suspensions =  $100 \pm 15\%$  recovery with  $\leq 10\%$  RSD and solids =  $100 \pm 20\%$  recovery with  $\leq 15\%$  RSD.

*Preprocessed Stability.* Stability should be generated so as to cover all likely temperatures and times that the samples will be exposed as part of the in-life administration or sample

analysis portions of a study. Generally 1 to 48 h of room temperature or refrigerated storage is sufficient.

*Postprocessed Stability.* Stability of the processed samples should be established to confirm the integrity of the samples after storage (for example, within the autosampler) in the case where a reinjection is required due to instrument malfunctions or power outages. Typically ambient and/or refrigerated conditions are investigated for the time it takes to perform the second chromatographic run (2 to 3 days).

*Storage Stability.* Stability used to determine formulation storage conditions and expiration dates. Storage stability is used to determine how long formulations may be used for dosing as well as to support storage prior to sample analysis. Stability must be established to cover the time from sample preparation to sample analysis.

*Freeze/Thaw Stability.* Based on the anticipated storage conditions and sample analysis procedure, it may be necessary to evaluate freeze/thaw stability. If evaluated, stability should be generated so as to cover the number of times/conditions under which a sample is likely to be frozen and thawed during shipment and analysis. Although most samples only experience one freeze/thaw cycle, reanalysis, or inadvertent thawing during transport may occur. In such cases, stability should be checked over multiple cycles of freezing and thawing at the relevant conditions.

*Stock Solution Stability.* The stability of stock solutions of the analyte should be evaluated at room temperature for at least 6 h. If the stock solutions are refrigerated or frozen for a relevant period, the stability should be evaluated. After completion of the desired storage time, the stability should be assessed by comparing the instrument response of the test solution with that of a freshly prepared solution. Only stocks which compare within 5% difference should be used to prepare subsequent standards or quality control samples.

### Effective and Efficient Documentation—Validation Summary Report Minimal Contents

All method validation data should be summarized in a validation summary or report. The validated method should be followed as written during sample analysis.

### FORMULATION SAMPLE ANALYSIS REQUIREMENTS

GLPs require testing of the formulations to assure accurate concentration, uniform homogeneity and stability at the intended storage conditions for the duration of use and storage. All formulation samples should be assessed within the validated storage time frame following the analytical method as written. Acceptance criteria are typically based on the formulation vehicle composition: for example, solutions =  $100 \pm 10\%$  recovery with  $\leq 10\%$  RSD, suspensions =  $100 \pm 15\%$  recovery with  $\leq 10\%$  RSD and solids =  $100 \pm 20\%$  recovery with  $\leq 15\%$  RSD.

## Sample Analysis Procedures

The following components should be included as part of a study sample analysis run:

### *System Suitability Test*

The “system suitability” should be demonstrated before initiating a study sample batch analysis. The system suitability tests performance and criteria should have been demonstrated during validation and incorporated into the analytical method. Typically, standards or quality control samples are used to confirm acceptable sensitivity and reproducible response while blanks are used to confirm there is no interference. Examples of typical SST factors are injection precision (retention time and peak area), theoretical plates (N), tailing factor (T), capacity factor ( $k'$ ), and resolution.

### *Calibration Curve*

A single-point calibration curve may be used if all formulations are diluted to the same concentration as the single-point calibrator and the curve has been shown to be linear. A multi-point calibration curve should have multiple concentration levels and show suitable response over the analytical range, demonstrating an acceptable coefficient of determination value.

### *Stock Standard Comparison*

The accuracy of weighing should be demonstrated by comparing the response of two separately weighed stock solutions which have been diluted to a single concentration within the linear range of the method. Only stocks which compare within 5% difference should be used to prepare subsequent standards or quality control samples.

### *Performance Check Standards*

The performance of the method should be assessed over the course of each analytical run. For this purpose, a single dilution of a stock solution or of a quality control solution in vehicle may be made to the target concentration and injected multiple times during the run. The measured concentration should be within  $\pm 5\%$  of the target concentration. Any samples not bracketed by acceptable performance check standards or calibration curve standards should be considered nonreportable and reanalyzed.

## Types of Dose Formulation Study Samples

### *Concentration Analysis*

Concentration assessments should be performed for every dosage concentration including control/vehicle samples, at a minimum for the first and last test batches. More frequent assessments during the course of the study may be required by the SOP/protocol. Long-term (3 months or longer) studies typically assess concentration throughout the course of the study at predefined intervals (for example, once a month or beginning, middle, and end). If

replicate samples are analyzed, the individual values and the average% recovery value are reported as well as the % RSD. If fewer than three samples are analyzed, then a % RSD will not be reported.

### *Homogeneity Analysis*

Homogeneity assessments are required for all formulations at study initiation with the exception of “true solutions.” Homogeneity assessments are usually performed for the first test batch low and high dosage form concentrations and whenever the batch size changes significantly (for example, homogeneity is usually repeated when there is a 20–50% change in batch size). Homogeneity assessment may also be conducted as part of a validation to confirm whether a “true solution” or suspension has been prepared. Homogeneity is usually performed by assessing replicate samples from the top, middle and bottom strata of the dosage form preparation vessel. In addition to the measured concentration of each sample, the average and %RSD of all aliquots analyzed from a single preparation should be reported.

Resuspension homogeneity should be performed when a formulation is prepared, stored, and used on a daily basis over a period of time. Over time, a formulation may settle without mixing or precipitate if stored at a temperature less than the conditions for preparation. Resuspension homogeneity is usually performed by assessing replicate samples from the top, middle and bottom strata of the dosage form storage vessel after a defined period of time and storage conditions.

### *Stability Analysis*

Stability assessments are usually performed during the assay validation or during the toxicology studies by collecting samples from at least the lowest and highest dosage concentrations. Replicate samples are stored at the conditions to be used during the in-life phase of the study to cover the time from preparation to the time of final dose analysis.

## STUDY SAMPLE COLLECTION

Dose formulation study samples may be solutions, suspensions (for example, microemulsions) or solids. The method of collection of study samples can be critical to accurate analysis. For example, an analyte in a microemulsion may fall out of suspension before analysis is performed, but is still chemically stable in the vehicle. In such a situation, taking a sub-aliquot of the study sample may not provide an accurate result. For these types of formulations, special collection procedures should be provided within the study protocol. Exact aliquots where the entire sample is analyzed should be used for suspensions. It is also recommended that replicate study samples be collected to ensure sufficient sample volume for out of specification investigations and repeat analyses for failed runs. If the samples are to be shipped, it is recommended that the back-up samples be shipped separately.

## OUT OF ACCEPTANCE/SPECIFICATION INVESTIGATIONS

All study sample results that do not meet acceptance criteria/specification criteria should be investigated. The procedures for an out of acceptance investigation should be clearly defined within a SOP, study protocol or plan. The FDA guidance for Industry “Investigating Out-of-Specification (OOS) Test Results for Pharmaceutical Production” (20) has been commonly used as guidance for NCDFA OOS.

## SUMMARY

NCDFA is an important part of all preclinical studies. Methods used for sample analysis in GLP studies should be validated for accuracy, precision, selectivity and sensitivity in compliance with existing FDA guidelines. Formulations should be assessed for stability and homogeneity before or during GLP studies to ensure sample integrity and reproducibility of results. Close adherence to the recommendations of this paper should be used to insure that robust, reliable and appropriate methods are used for formulation analysis.

## ACKNOWLEDGEMENTS

The authors would like to thank the following: John Buckholz, B.S., Brian Burbach Ph.D., Binh Do, Dannette Doolittle, Ph.D., Rodrigo Laureano, M.S., Josef Ludwig, B.S., MBA, Emily Mainstone, B.S., Stacy Marden, Ph.D., Tony Nakhla, M.S., Shaundel Percey, B.S., MT (ASCP), Yuan-Shek Chen, Ph.D., Phillip J. Wang, Ph.D, MBA, and Laixin Wang, Ph.D.

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## REFERENCES

1. FDA Nonclinical Laboratory Studies Good Laboratory Practice Regulations. Available at: [http://www.fda.gov/ora/compliance\\_ref/bimo/glp/78fr-glpfinalrule.pdf](http://www.fda.gov/ora/compliance_ref/bimo/glp/78fr-glpfinalrule.pdf)
2. FDA 21 CFR Part 58 Good Laboratory Practice Regulations Final Rule. Available at: [http://www.fda.gov/ora/compliance\\_ref/bimo/glp/87fr-glpamendment.pdf](http://www.fda.gov/ora/compliance_ref/bimo/glp/87fr-glpamendment.pdf)
3. Organisation for Economic Co-operation and Development (OECD) Principles of Good Laboratory Practice, [ENV/MC/CHEM(98)17], 26 Jan 1998.
4. Japanese Ministry of Health, Labor and Welfare (MHLW) No. 21, March 26, 1997
5. FDA. Guidance for Industry: Bioanalytical Method Validation. In: U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research, May 2001.
6. Shah V, Midha K, Dighe S, McGilveray I, Skelly J, Yacobi A, *et al.* Analytical methods validation: bioavailability, bioequivalence and pharmacokinetic studies. Conference report. Eur J Drug Metab Pharmacokinet. 1991;16(4):249–55.
7. Shah V, Midha K, Findlay J, Hill H, Hulse J, McGilveray I, *et al.* Bioanalytical method validation—a revisit with a decade of progress. Pharm Res. 2000;17(12):1551–7.

8. Viswanathan C, Bansal S, Booth B, DeStefano A, Rose M, Sailstad J, *et al.* Workshop/conference report—quantitative bioanalytical methods validation and implementation: best practices for chromatographic and ligand binding assays. Pharm Res. 2007;24(10):1962–73. Epub 2007 Apr 26.
9. Shah V. The history of bioanalytical method validation and regulation: evolution of a guidance document on bioanalytical methods validation. The AAPS Journal. 2007;9(1):E43–7. Article 5.
10. USP 32-NF 27 (United States Pharmacopeial Convention, Rockville, MD, 2009), pp. 3988–91.
11. FDA, “Analytical Procedures and Methods Validation: Chemistry, Manufacturing and Controls.” Federal Register (Notices) 65(160), 52,776-52,777 (August 2000).
12. Q2A “Text on validation of analytical procedures.” International Conference on Harmonization. Fed Regist. 1995; 60: 11260
13. Q2B “validation of analytical procedures: methodology.” International Conference on Harmonization. Federal Register. 1996; 60: 11260.
14. Q2 (R1) “Validation of analytical procedures: text and methodology.” International Conference on Harmonization. Federal Register. 1997; 62: 27463–27467.
15. “International Conference on Harmonization; Draft Guidance on Specifications: Test Procedures and Acceptance Criteria for New Drug Substances and Products: Chemical Substances.” Federal Register (Notices) 2000; 65(251): 83041–63.
16. “Harmonized guidelines for single-laboratory validation of methods of analysis.” International Union of Pure and Applied Chemistry (IUPAC) Technical Report. Pure Appl Chem. 2002; 74(5): 835–55.
17. FDA CDER Guidance for Industry Analytical Procedures and Methods Validation. Available at: <http://www.fda.gov/cder/guidance/2396dft.pdf>
18. FDA Guidance for Industry Good Laboratory Practices Questions and Answers revised July 2007. Available at: [http://www.fda.gov/ora/compliance\\_ref/bimo/glp/81GLP-qanda.pdf](http://www.fda.gov/ora/compliance_ref/bimo/glp/81GLP-qanda.pdf)
19. FDA CDER Reviewer Guidance Validation of Chromatographic Methods. Available at: [http://www.fda.gov/cder/guidance/cmc3\\_rev.pdf](http://www.fda.gov/cder/guidance/cmc3_rev.pdf)
20. FDA. Guidance for Industry: Investigating Out-of-Specification (OOS) Test Results for Pharmaceutical Production. In: U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research, October 2006.

## Definitions

Accuracy Calculations:

$$\% \text{Recovery} = (\text{found concentration}) / (\text{nominal concentration}) \times 100$$

$$\% \text{Relative Error} (\% \text{Deviation}) = [(\text{found concentration}) - (\text{nominal concentration})] / (\text{nominal concentration}) \times 100$$

Feed: Feeds or chows are blended from various raw materials and additives and are formulated according to the specific requirements of the target animal. They may be meals, pellets or crumbles.

Intra-run: within one day or analytical run or analytical sequence

Inter-run: between days or analytical runs or analytical sequences

Nominal concentration: The theoretical concentration, corrected for salt form and purity (as applicable), of a formulation based on the amount of analyte weighed per total volume of analyte plus vehicle.

Precision Calculations:

$$\% \text{Relative Standard Deviation} (\% \text{Coefficient of Variation}) = \text{SD} \times 100 / \text{Mean}$$

Note: % Relative Standard Deviation is appropriate only for data sets containing 3 or more points.

Quality Control Sample: A solution, suspension or solid containing test article in formulation vehicle designed to mimic actual dosage formulations.

Solution: A solution is a homogeneous mixture composed of

two or more substances dissolved in a solvent.

**Solid:** A solid object does not flow to take on the shape of its container, nor does it expand to fill the entire volume available. Examples are powders, powders in capsules and tablets.

**Suspension:** A suspension is a heterogeneous mixture in which solute-like particles may settle out of solvent-like phase some time after their introduction.

**True Solution:** A “true solution” is a solution in which the analyte is completely dissolved in the liquid phase. If a true solution is filtered, then the filtrate and the retentate generate the same concentration value. If a true solution is centrifuged, then no particles are observed. If a true solution is analyzed utilizing a solubility scanner, then the particle size maintains a horizontal axis across the solubility range.