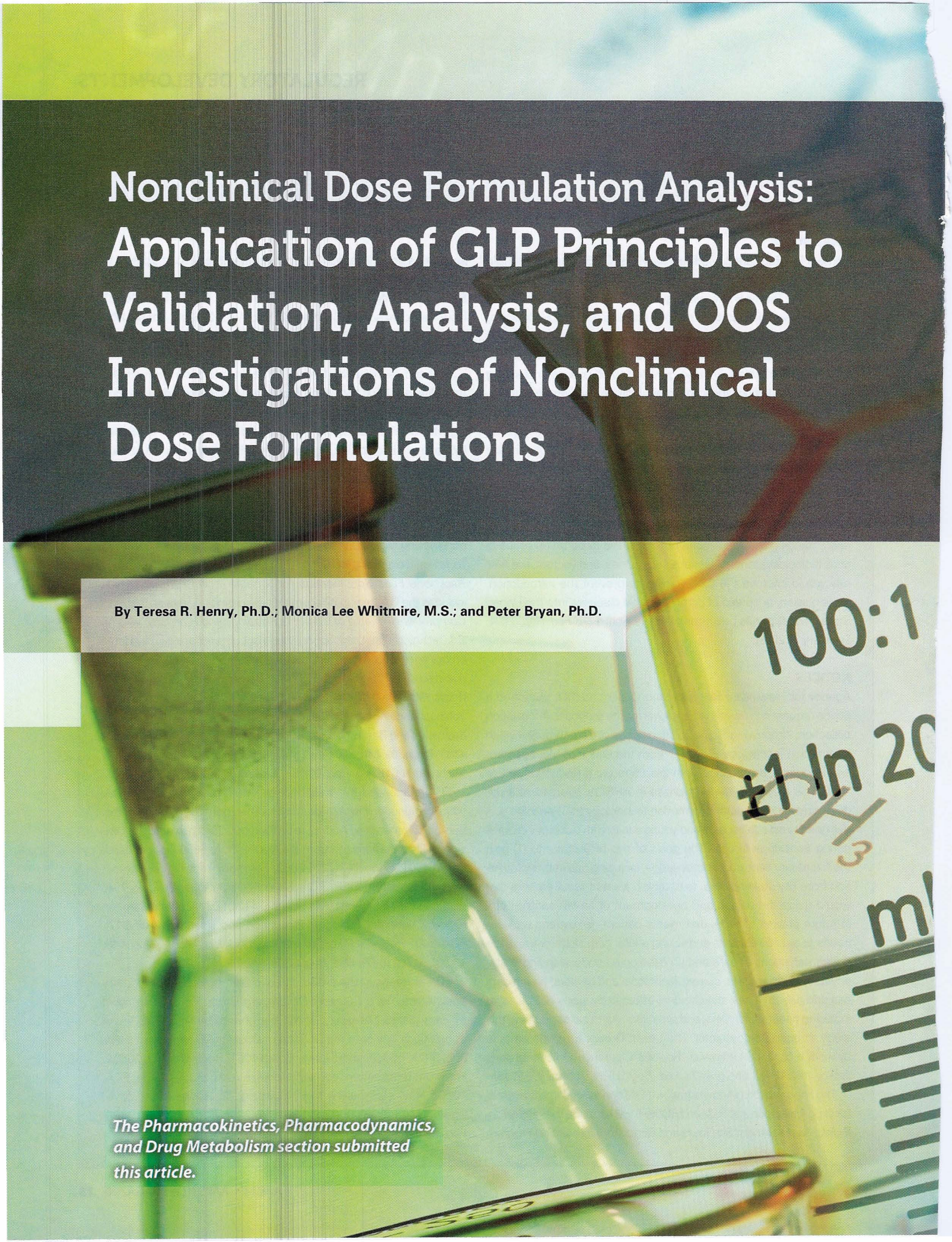
Nonclinical Dose Formulation Analysis: Application of GLP Principles to Validation, Analysis, and OOS Investigations of Nonclinical Dose Formulations

By Teresa R. Henry, Ph.D.; Monica Lee Whitmire, M.S.; and Peter Bryan, Ph.D.



The Pharmacokinetics, Pharmacodynamics, and Drug Metabolism section submitted this article.

Robust analytical results for nonclinical dose formulation testing are possible, and application of best practices in good laboratory studies can standardize processes across the industry.

Nonclinical pharmacokinetic (PK) and toxicokinetic (TK) safety studies are performed using good laboratory practice (GLP) regulations to ensure the availability of safe medicines. International GLP regulations uniformly require that dose concentration, homogeneity/uniformity, and stability be established prior to administration.[1–3]

However, the Food and Drug Administration (FDA) and the Organisation for Economic Cooperation and Development (OECD) both proclaim that GLPs do not apply to validation of analytical methods used to determine the concentration of GLP test article in drug dosage forms.[1,2] Yet, the outcome of nonclinical toxicology safety studies is fundamentally dependent upon accurate and precise dose formulations. Therefore, formulation method validation and sample analysis for supporting nonclinical toxicology studies should be consistently conducted around the world under the framework of GLP principles.

GLP studies are planned, performed, monitored, recorded, reported, and archived according to approved protocols, study plans, or standard operating procedures (SOPs). All applicable experimental parameters and associated acceptance criteria are predefined.

Guidance regarding the validation of formulation analysis methods and subsequent use for supporting GLP toxicology study sample analysis is warranted at this time to ensure such studies are conducted consistently. Adherence to standard principles for method validation, sample analysis, and out-of-specification (OOS) investigations would inherently improve the quality of nonclinical safety studies. Furthermore, the recently published white papers *Nonclinical dose formulation analysis method validation and sample analysis*[4] and *Nonclinical Dose Formulation: Out of Specification Investigations*[5] should be the keystones of this effort.

Before analysis of GLP study samples can be attempted, a method must be developed and validated. Although similar to bioanalytical (BA) methods, where validation is clearly defined by regulatory guidance, nonclinical dosage forms analytical method validation has not been fully described by any regulatory bodies. These methods are often high-performance liquid chromatography (HPLC) based, and similar to their BA counterparts, they must be validated for accuracy, precision, selectivity, and sensitivity.

Unlike plasma or other BA samples, the formulation samples in GLP studies are typically of known concentrations and are of substantially higher concentration than typical bioanalytical samples. This means that the methods designed for their analysis generally utilize ultraviolet-visible spectroscopy detection and may not require the complicated calibration curves of BA methods. Regardless of their simplicity, GLP formulation samples should be assessed for stability and homogeneity to ensure the integrity and reproducibility of the study results.

THE VALIDATION PROCESS

The validation process for nonclinical dosage forms analysis involves the evaluation of several key parameters: system suitability, performance checks, standard preparation checks, linearity, recovery/accuracy, specificity, sensitivity, and stability. It is essential that all of these are confirmed prior to use of a new method for GLP testing to ensure that the resultant data are both reliable and reproducible.

System suitability testing is used to scientifically qualify that the instrumentation to be used in the analytical method are operating as designed. These checks should be done as part of all validation and routine analysis to ensure the equipment is functioning properly at the time of analysis. Typical system suitability tests include injection precision, tailing factor, resolution, etc.

Calibration curves are used to correlate the area or height of a chromatographic response to the actual concentration of the sample. Often a single point calibration is acceptable for analysis where the sample can be diluted to the same theoretical concentration as the standard.

Regardless of whether a single- or multipoint curve is utilized for the correlation, the linear range of the assay must be established as part of the validation. Only samples whose results fall within the validated linear range can be quantifiably reported.

It is therefore important to agree on a planned dose range prior to method validation to avoid additional work. Typical acceptance criteria for linearity as well as many other validation parameters are included in Table 1.

Standards are a vital part of any analytical method. To ensure that the stock solutions used to prepare the working standards are prepared properly, a standard check should be conducted where two separate preparations of stock solutions are compared with one another (see Table 1). Errors in standard preparation or storage can lead to OOS results.

Performance check standards, also known as quality control samples, are standards prepared in a formulation vehicle that are injected over the course of a validation or analytical run to confirm consistent performance of the method (see Table 1). If a performance check standard result does not meet the acceptance criteria, this is a good indication of system instability or lack of method robustness.

Recovery, also known as accuracy,

Table 1. Typical Acceptance Criteria for Validation Studies[4]

TEST	ACCEPTANCE CRITERIA
Stock Standard Comparison	Not more than (NMT) 5% difference between two preparations
Performance Check	Within 100 ± 10% recovery for solutions Within 100 ± 15% recovery for suspensions Within 100 ± 20% recovery for solids
Linearity	Coefficient of determination not less than 0.99 with a y-intercept near 0
Accuracy	Within 100 ± 10% recovery for solutions Within 100 ± 15% recovery for suspensions Within 100 ± 20% recovery for solids
Specificity	NMT 1% target concentration for single point calibration NMT 20% of the limit of quantitation (typically the lowest concentration examined for linearity) for multipoint calibration
Stability	Within 100 ± 10% recovery for solutions Within 100 ± 15% recovery for suspensions Within 100 ± 20% recovery for solids

can be determined in a few different ways. One option is to create small-scale preparations of analyte in the vehicle that can be used to examine the entire expected range of sample concentrations. The second approach involves the use of spike preparations to create samples to study the entire proposed analytical range. Regardless of the method chosen for sample preparation, the acceptance criterion remains the same (see Table 1).

Intra- and interrun accuracy should be determined as part of each validation. This helps to determine that the method will provide reproducible results from the beginning to the end of a single analytical run and when comparing one run to the next. Inability to reproducibly analyze dose formulations will result in studies where correlation of dose to exposure is difficult if not impossible.

Specificity is evaluated during validation to ensure that components of the vehicle or diluent will not yield an interfering response. Blank sample analysis is the most common approach to ensuring specificity. It is also important to ensure that carryover from one injection to the next is minimized to prevent interference from one sample or standard injection to subsequent injections.

Sensitivity of the method or assay is usually based on the validated linear range rather than an analytically determined limit of quantitation. As such, both the upper and lower limits of quantitation can be adjusted by repeating a portion of the validation (a partial validation).

Stability of the materials involved in a validation should also be established under the planned storage and use conditions. This includes pre- and post-processed stability, freeze/thaw, storage, and in-use stability, as well as stock solution stability. Stability can be tested as part of the validation protocol or as a separate study but must be established prior to sample analysis.

SAMPLE ANALYSIS

GLPs require analysis of all dose formulations to assure accuracy of concentration, dose homogeneity, and storage stability. There are three primary types of dose formulation study samples: concentration, homogeneity, and stability.

Regardless of the sample type, the analytical sequence used for testing is essentially the same. Once a validated method is available, it can be used for routine sample analysis by trained analysts.

Analytical runs associated with sample analysis typically involve the following components: system suitability, calibration curve, sample analysis, and performance check. A stock standard comparison may also be carried out within a sample analysis run if a new standard has been prepared.

As was the case in validation, the acceptance criteria for sample analysis may vary based on the sample type. Typical specifications are similar to the sample stability criteria noted in Table 1.

Table 2. Common Formulation Analysis Investigation Topics[5]

Sample	Appearance Storage Stability
Analytical Sample Prep	Correct sample analyzed Dilution factor Preparation/dilution Reagents and equipment
Standards	Preparation/dilution Stability Correction factor Reagents and equipment Performance check standards meet acceptance criteria of method
Blanks	No interference with the analyte
Instrument Setup	Entry errors Mobile phase All components on and correctly set
System Suitability	Any indication of problems
Autosampler	Sample septum pierced Sufficient sample available
Chromatography	Interference Abnormalities
Integration	Consistent
Calibration	Consistent with method
Results	Calculations Reproducibility between injections
Supporting Information	Protocol Method Method validation Certificates of Analysis (COAs) Sample receipt documentation

Table 3. Common Formulation Preparation Investigation Topics[5]

Active Pharmaceutical Ingredient	Purity correction factor matches certificate of analysis and analytical calculations Storage Correct grade or lot used
Vehicle/excipients	Correct grade Approved source Storage
Preparation	Concentrations and correction factor verified Preparation/dilution Storage
Scale-up	Compare scale to previous batches and validation batch Confirm pH
Sampling	Appropriate for formulation type Accurate procedure

OOS INVESTIGATIONS

No matter how well designed a method is or how well trained a formulator may be, OOS results do occur. When such issues arise, it is important to have and follow an established OOS investigation procedure.[6–8] Such an investigation should cover three key areas: formulation analysis, dose formulation, and general.

The formulation analysis investigation should carefully review all of the relevant analytical data, equipment, and supporting information to determine if any portion of these may have contributed to the unexpected result (see Table 2). The dose formulation section should explore whether any issues occurred relating to equipment or preparation of the dose (test article) that may result in an OOS result (see Table 3).

Finally, the general section of review should explore all areas that may be common to both formulation preparation and analysis. These include but are not limited to instrument/equipment history, cleanliness of glassware/containers, environmental factors, standard operating procedures, and training. Once the OOS investigation is complete it must be summarized and provided to the appropriate parties for review.

Following the aforementioned suggestions and the two recently published white papers will lead to robust analytical results for nonclinical dose formulation testing. The application of these best practices in GLP studies are encouraged to standardize processes across the industry.

DISCUSSION POINT

We want to know your opinion! Please discuss the following question with your colleagues via the *AAPS Blog*. Go to the *AAPS Newsmagazine* digital edition to link directly to the blog entry associated with this article.

What regulatory gaps do you see related to the application of GLP principles, and how would you rectify them?

Learn more about the AAPS Pharmacokinetics, Pharmacodynamics, and Drug Metabolism section; visit the section's Web page via the *AAPS Newsmagazine* digital edition.

REFERENCES

1. U.S. Department of Health and Human Services, Food and Drug Administration, 21 CFR Part 58, Good Laboratory Practice Regulations; Final Rule. September 4, 1987. Available at: www.fda.gov/downloads/ICECI/EnforcementActions/BioresearchMonitoring/ucm133730.pdf. Accessed on December 22, 2011.
2. Organisation for Economic Co-operation and Development, Environment Directorate, Chemicals Group and Management Committee. January 26, 1998. OECD Principles of Good Laboratory Practice. ENV/MC/CHEM(98)17. Available at: www.oecd.org/officialdocuments/displaydocumentpdf?cote=env/mc/chem(98)17&doclanguage=en. Accessed December 22, 2011.
3. Japan, Ministry of Health, Labor and Welfare. Ordinance No. 21. March 26, 1997.
4. Whitmire ML, Bryan P, Henry TR, et al. NonClinical Dose Formulation Analysis Method Validation and Sample Analysis. *AAPS J.* 2010;12(4):628-34. doi:10.1208/s12248-010-9226-9.
5. Appleton T, Bryan P, Contos D, et al. Nonclinical Dose Formulation: Out of Specification Investigations. *AAPS J.* 2012;14(3):523-529. doi:10.1208/s12248-012-9347-4.
6. U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research. Guidance for Industry: Investigating Out of Specification (OOS) Test Results for Pharmaceutical Production. October 2006. Available at: www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM070287.pdf. Accessed December 22, 2011.
7. Appleton TA. Out of Specification (OOS) Investigations for Good Laboratory Practice (GLP) Studies. Poster presented at: International Pharmaceutical Federation Pharmaceutical Sciences World Congress in association with the American Association of Pharmaceutical Scientists Annual Meeting and Exposition; November 14-18, 2010, New Orleans, LA.
8. Carvalho VM. Investigating for Failures. *Lab Manager Magazine.* 2011:6(6):80-83.