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White Paper

Nonclinical Dose Formulation: Out of Specification Investigations

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Abstract. Nonclinical safety studies are required to follow applicable Good Laboratory Practice (GLP) regulations. Nonclinical dose formulations are required to be analyzed to confirm the analyte concentration, homogeneity, and stability. Analytical samples that fall outside of the acceptance criteria are considered out of specification (OOS), and an investigation should be conducted. The US FDA has issued a guidance document for GMP studies on conducting OOS investigations. However, no regulatory guidance has been issued regarding nonclinical safety study (GLP) OOS investigations, which often vary in regard to content, assessment, and impact statements. There is opportunity to improve the quality of OOS investigations by defining expectations and providing guidance in several areas including root cause assessment, impact statements, and acceptable paths forward. This paper will provide recommendations of best practices for nonclinical dose formulation OOS investigations.

KEY WORDS: guidance document; nonclinical dose formulation; out of specification investigation; root cause; standard operating procedures.

INTRODUCTION

Nonclinical studies intended to support applications for research or marketing permits are required to follow applicable federal regulations as specified in 21 CFR, Part 58 (Good Laboratory Practice (GLP) for Nonclinical Laboratory Studies) (1), the OECD "Principles of Good Laboratory Practice" (2), or Japanese MHLW no. 21 (3). The primary purpose of nonclinical studies is to establish safety margins that can then be extrapolated to clinical studies. Therefore, nonclinical dose formulation analysis (NCDFA) is required in all nonclinical regulated studies to verify the documented test article concentrations in formulations used to determine these safety margins (1–3). These analytical methods are used to assess the concentration of the test article in nonclinical formulations, formulation homogeneity, and formulation stability in support of regulated nonclinical studies (for example, safety, toxicokinetic, and pharmacokinetic studies). Recommended specifications according to the AAPS white paper for "Nonclinical dose formulation analysis method validation and sample analysis" are 100±10 % recovery with ≤10 % relative standard deviation (RSD) for solutions, 100± 15 % recovery with ≤10 % RSD for suspensions, and 100±20 % recovery with ≤20 % RSD for solids (4). Analytical samples that fall outside of this range are considered out of specification (OOS). When samples are OOS, an investigation should be conducted to verify if the result is OOS, to assess the root cause of the OOS, to determine the impact to the study, and to determine the path forward.

Presently, no regulatory authority has a guidance document for nonclinical dose formulation analysis out of specification (NCDFA-OOS) investigations. The U.S. Food and Drug Administration (FDA) has issued a guidance document for Good Manufacturing Practices (GMP) studies (U.S. Department of Health and Human Services, FDA, CDER, October 2006) on conducting OOS investigations (5). This document provides guidance on how to conduct an OOS investigation as it relates to GMP test results for pharmaceutical production,

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DEFINITIONS: API, Active pharmaceutical ingredient; CAPA, Corrective and preventive action; CFR, Code of Federal Regulations; CRO, Contract research organization; CoA, Certificate of analysis; FDA, Food and Drug Administration; GLP, Good Laboratory Practices; GMP, Good manufacturing practices; MHLW, Ministry of Health Labor and Welfare; NCDFA, Nonclinical dose formulation analysis; OECD, Organisation for Economic Co-operation and Development; OOS, Out of specification; OOS Result, Results that do not meet preestablished specification/acceptance criteria; PC, Performance check; QC, Quality control; Reanalysis, Repeat analysis of the existing analytical sample; Re-sampling, Collection of a new representative sample from the formulation preparation; Re-preparation, To prepare another formulation preparation; SOP, Standard operating procedure; SST, System suitability test.

including the responsibility of the analyst and the laboratory supervisor, as well as the investigation of OOS test results, including review of production, additional laboratory testing, reporting testing results, and concluding the investigation. This GMP guidance is sometimes used as a basis for performing NCDFA-OOS investigations; however, it is not specific to nonclinical dose formulation sample test results. Its applicability is limited primarily to the analytical portion of the investigation and adopting it for NCDFA-OOS investigations, in the opinion of the authors, is not adequate. Nonclinical studies are typically short term (days to months) in nature where dose formulations are in early development stages and samples are limited. OOS investigations detailed in the GMP guidance would be time consuming, expensive, and would not necessarily provide any additional benefits since any impact of NCDFA-OOS results would be determined by the Study Director and Toxicologist.

Across the industry (contract research organizations, pharmaceutical companies, and biotechnology companies), dose formulation OOS investigations for nonclinical studies vary in regard to content, assessment, and impact statements. The amount of resources required for the investigation may vary depending upon the individual study and the study type. The opportunity exists to improve the consistency of these investigations by defining expectations of the NCDFA-OOS investigation and providing guidance in the areas of root cause assessment, impact to the study statements, and acceptable paths forward.

GOALS AND OBJECTIVES

A standard process for conducting an NCDFA-OOS investigation for nonclinical studies is proposed. This includes the following steps: review of data and methods (analytical and formulation), assessment of OOS root cause, assessment of the impact to the study, and determination of the path forward. The path forward may include reanalysis, re-testing, re-sampling, and/or re-preparation of the formulation (6).

ROLES AND RESPONSIBILITIES

The roles and responsibilities for conducting the OOS investigation need to be clearly defined. This may vary among companies in terms of specific responsibilities as defined by Standard Operating Procedures (SOPs); however, the process should be similar among companies. This should include the analytical lab, the formulation lab, quality assurance, and the Study Director (7). The analytical and formulation investigations should be conducted by the respective laboratories, and the results reviewed by management. Both Quality Assurance and the Study Director need to be involved in the investigation. The analytical and formulation laboratories are responsible for keeping the Study Director informed throughout the entire investigation. The Study Director is responsible for approving the conclusions of the investigation.

INVESTIGATION PROCEDURE

The primary objective of the investigation is to assess whether there is a specific identifiable reason for the OOS result (i.e., a root cause). The investigational procedure is divided into three sections: the "General Section" which covers items common to both analytical and dose formulation preparation, and the "Formulation Analysis" and "Dose Formulation Section". The latter two sections include topics designed to address the unique aspects of analytical and formulation dose preparation investigations. The investigation for these sections may be conducted in parallel or sequentially. It is important to note that although these sections attempt to include the most probable areas for investigation/review, it is not possible to have an all-inclusive check list for every OOS investigational scenario.

If errors are found in any of the areas discussed, one must first determine whether these errors are indeed the root cause of the OOS or simply a deviation or contributing factor. It is necessary to evaluate the error in the context of the data. If, for example, a weight-check verification was not performed for a specific day, this would be a deviation; however, it may not be the root cause of the OOS result. As is the case with all laboratory investigations, sound science must be the top priority.

General Review

The "General Review" section of this White Paper deals with areas of the investigation that are common to both the analytical and formulation preparation laboratories. These points should be common to all GLP laboratories. Table I summarizes the OOS investigational components that have overlap between the two major branches of the investigation.

While these points are identified as overlaps between the "Analytical" and "Dose Formulations" sections, it is important to remember that other areas of overlap might also exist and should be considered, regardless of the stage of the investigation.

Formulation Analysis

When a dose formulation sample analysis result does not meet the stated acceptance criteria, the typical first reaction is to assume an error was made by the analyzing laboratory. This is not necessarily a correct assumption; however, review of the analytical procedure is a logical starting point of the investigation.

It is common for the analytical laboratory to be a separate test site from the test facility/study director, whether it is the pharmaceutical/biotech company's in-house laboratory or a third-party CRO. Therefore, it is essential that the analytical laboratory have its own SOPs for conducting and documenting the investigation as well as for communicating the investigation results to the Study Director.

There are various areas where errors may occur during the sample analysis procedure and for ease of discussion, investigation; they have been divided into three main categories: analytical data, analytical equipment, and supporting information. If the root cause is identified, no further investigation is required.

It should be noted that the NCDFA OOS investigation procedures outlined within are not required for instances where System Suitability Test (SST) samples fail to meet the acceptance criteria if the standards and study samples have not yet been analyzed, and should be handled according to internal SOPs.

Analytical Data

The investigation should include a thorough review of all analytical results, sample preparation, and analysis items

Table I. General Review

Instrument/equipment history	Instrument/equipment within calibration date Instrument/equipment maintenance up-to-date—history of maintenance issues Previous use reviewed for incompatibility (i.e., apparatus cleaned and flushed adequately before changing methods) Instrument system error logs—system or acquisition errors recorded in software log
Glassware/containers	Cleaned appropriately Free of possible analyte contamination Verification of container material (i.e., glass, plastic) Verification of container closure material
Facility environmental conditions	Verification of facility conditions (i.e., temperature, relative humidity, subdued lighting [if applicable]) Differences between analytical and dose preparation laboratory environmental conditions
Laboratory facilities	Appropriate for activities Free of possible cross contamination issues
SOP review	Appropriate SOPs in place SOPs followed as written
Training and records	Laboratory personnel trained in methodology Laboratory personnel have all necessary training Review records—certificate(s) of analysis (CoA), protocol, method, standard, and reagent expiration dates

associated with the OOS sample result. This review should attempt to determine if the dose formulation sample analysis was performed correctly by answering the following questions: Was the method followed and performed as written? Was the sample prepared as per the method? Did the instrument(s) perform as expected? Were there any calculation or dilution errors? Were any issues noted for the dose formulation sample itself? Items to check during the analytical data review are listed in Table II.

Analytical Equipment

The analytical equipment review should evaluate whether the equipment used was operating properly and in accordance with the method and any associated SOPs. Dose formulation analysis is commonly performed using high-pressure liquid chromatography with ultraviolet (HPLC-UV) for separation and detection. The analytical equipment review has been written for HPLC-UV instrument methods, but the review procedure can be adapted to other methodologies, such as liquid chromatography–mass spectrometry (LC–MS), LC–MS/MS, gas chromatography (GC)-FID, GC-MS, and GC-MS/MS. Items to check during the analytical equipment review are listed in Table III.

Analytical Supporting Information

The analytical supporting information review should include verification of all associated documentation that accompanies dose formulation analysis. This includes the review of protocols, SOPs, test methods, method validations, certificate of analysis (CoA), material safety data sheets, training records, and any other associated documents that may have an impact. Items to check during the analytical supporting information review are listed in Table IV.

If no assignable cause of the OOS result is identified after performing the analytical section investigation, the investigation should be expanded to the dose formulation preparation.

Dose Formulation Section

The "Dose Formulation Section" of the OOS investigation may be conducted in parallel to or sequentially after the analytical investigation. In many organizations, the dose formulation group may be notified while the analytical OOS investigation is being conducted, and the dose formulation investigation may be started. This portion of the OOS investigation should be conducted and approved by the dose formulation group. It is essential that the formulation laboratory have its own procedures or SOPs for conducting and documenting the investigation as well as communicating the investigation results to the Study Director.

In the dose formulation section of the OOS investigation for GLP studies, the two main sections are the "Formulation Equipment Review" and the "Formulation Preparation Review". In the "Formulation Equipment Review" section, the equipment used in the preparation is reviewed and verified. In the "Formulation Preparation Review" section, supporting data/information and the formulation preparation procedure are reviewed and verified. If the root cause is identified, no further investigation is required.

Formulation Equipment Review

The formulation equipment review should systematically review the equipment used and assess whether there is a root cause for the OOS result. This review should attempt to determine if the formulation equipment used was appropriate by answering the following questions: Was all equipment used calibrated and correctly maintained? Were equipment verifications performed? Was the appropriate equipment used for the specific purpose? Suggested items to check during the formulation equipment review which is not all-inclusive are included in Table V.

Table II. Analytical Data Review

Dose formulation sample	Physical appearance—color/clarity/viscosity as expected Suspensions—no large particles visible in the formulation Sample stored as specified in method (i.e., container, temperature, light sensitivity, etc.) Sample analyzed within its established stability time interval
Sample preparation	Correct sample analyzed Sample diluted per validated method Dilution factor calculated correctly Proper reagents used Proper equipment used (pipette and/or balance within calibration, volumetric flask of acceptable grade, heating, or mixing apparatus acceptable) Handling issues noted during preparation (e.g., spillages, transfer difficulties)
Calibration standards/performance check standards (PC)/quality control (QC) samples	Prepared per validated method Reference standard material within expiration date Correction factor calculated correctly and applied, if applicable Proper reagents used Proper equipment used (pipette and/or balance within calibration, volumetric flask acceptable grade, heating, or mixing apparatus acceptable) Calibration standards, performance check, and/or QC samples met acceptance criteria Proper containers and closures used Dilution factor calculated correctly Handling issues noted during preparation (e.g., spillages, transfer difficulties)
Blanks	Presence of analyte peak could indicate carryover, contamination, or impurity/interference from the vehicle
Run setup	Vials placed in autosampler in accordance with the analytical run sequence Dilution factors entered correctly in software Concentrations (standards, PC, QC, and samples) entered correctly in software Customized software calculations entered correctly
System suitability	System suitability results acceptable (e.g., injection precision [retention time, peak area], theoretical plates, tailing factor, capacity factor, resolution)
Autosampler	Autosampler vial septa were pierced Autosampler vial cap tight to prevent evaporation Sufficient sample volume in vial
Chromatography	No interfering peaks at analyte retention time No analyte peak shape anomalies Baseline acceptable Retention time consistent
Integration	Proper and consistent integration of the analyte peak
Calibration	Standard calibration regression equation as per the validated method (i.e., slope and intercept) Peak response is consistent with validation (peak height *vs*. peak area) Regression coefficient acceptable
Sample result	Sample result calculation correct Acceptance criteria correct If replicate results, precision is within acceptance criteria

PC performance check, *QC* quality control

Formulation Preparation Review

This section systematically reviews the supporting data/information and the formulation preparation process to assess if there is a root cause for the OOS result. Questions asked here include: Were all study personnel appropriately trained, as documented in their training records? Was the correct factor applied to account for purity/activity as defined on the CoA or the study protocol? Did the grade of all materials used, including the active pharmaceutical ingredient (API), match that used for development and exploratory studies? Was the formulation in question prepared and stored as outlined in the protocol? Did the preparation instructions match the protocol and preliminary development methodology?

Table III. Analytical Equipment Review

HPLC-UV instruments	
Method setup	Method parameters entered into software correctly Method parameters agree with validation parameters
Instrument setup	Proper mobile phase, needle washes, and liquid chromatography column installed Detector turned on and stabilized before starting (check hours on deuterium lamp) LC pressure acceptable Column heater used and acceptable (or check room temperature if ambient temperature is used) Autosampler temperature set accurately if required by method
System suitability	Meets the acceptance criteria as per the validated analytical method
Performance check (PC) standards	PC standard results within acceptance criteria

LC liquid chromatography

During the course of the investigation in this section, the cause may not be identified; however, it may be recognized that it might be necessary to further evaluate the formulation. This might be due to different lots of API or vehicle components or different formulation scale. In many instances, development work may be necessary during the nonclinical study to optimize the formulation preparation instructions. Suggested topics for review of the formulation preparation section of an OOS investigation are included in Table VI and are not an all-inclusive list.

The grade of the materials used must be evaluated to determine their appropriateness. The API lots used for formulation development and exploratory studies may be different lots and could have differences in particle size, morphology, and impurity levels between the lots. Some of these changes may influence suspendability and kinetic dissolution of the formulation. Therefore, even though the API may be the same chemically, there may be physical differences between lots, which could affect the formulation preparation. The excipients and vehicles used for the formulation preparation must also be evaluated to determine if the correct grade and viscosity (if applicable) for the study have been used. The expiry and storage conditions of the excipients should also be explored, as these might also contribute to an OOS result. If the wrong grade of excipient or vehicle has been used, they should be assessed to determine if this is the root cause of the OOS result. The recommended grade may not have been used; however, this may not be the root cause of the OOS result.

Formulations developed prior to nonclinical studies and/or for formulation stability studies are typically developed on a very small scale. The quantity of formulation prepared for formulation development purposes is "fit-for-purpose" and may not mimic the scale at which formulations are prepared for nonclinical studies. An assessment must be made to determine if the differences in scale (milliliter to liter) may influence whether the formulations are within specification. Route of administration and physical state of the specific formulation must be taken into account. If there was a change in scale, it is important that the investigator evaluate whether the change in scale may have caused the OOS results. If a batch size has been changed by greater than 20 %, additional homogeneity analysis should be performed.

Compare the actual formulation preparation instructions to the protocol formulation instructions, and verify that the instructions match the proposed formulation instructions. The proposed formulation instructions may

Table IV. Analytical Supporting Information Review

Study protocol	Protocol procedures followed as written Protocol procedures correct (i.e., concentrations, dosages, calculations)
Method	Validated method followed as written Method calculations correct Method history—previous issues
Method validation	Validation experiments exhibit any trends All validation results met acceptance criteria Dose formulation preparations consistent with study dose formulation preparations
Certificate of analysis (CoA)	Standard reference material within expiration date and appropriate purity assigned Standard reference material correction factor calculated and applied correctly, if applicable Standard reference material stored in accordance with CoA Vehicle and reagents within expiration dates and of appropriate quality
Sample receipt documentation	Concentrations correct Vehicle correct Were any issues recorded during the shipment procedure?

Table V. Formulation Equipment Review

Formulation equipment	Appropriate equipment for each function was used; specifically, balances, temperature probes, stir plates, overhead stirrers, bath sonicators, probe sonicators, homogenizers, mixers, blenders Appropriate equipment was used based on the size of the batch (i.e., small-scale batch may use magnetic stirrers and large-scale batches may need overhead or paddle stirrers) Equipment calibration and performance verifications were performed and met specifications Appropriate container size used during preparation and storage (general rule is that the containers should be filled at least 50 %)
Storage Containers	Storage container material and type appropriate for active pharmaceutical ingredient (API) and vehicle Closure type compatible with formulation Opacity/color of storage container consistent with formulation stability requirements
Cleaning	Review cleaning process for equipment and containers All glassware used has been appropriately cleaned to ensure it is free of possible contaminants

not match the actual formulation instructions. For example, a change in scale may cause the preparation conditions to require a change. Review the formulation records in the protocol, and assess if the formulations were prepared according to protocol instructions. Then compare these instructions to the formulation development work. This assessment will determine if the changes made to the procedure produced an equivalent formulation. Be sure to check not only that all reagents were added in the correct ratio, but also that they were added in the same order as originally planned. Variation in either of these could be a major contributor to the root cause.

Verify the procedure for sampling of the formulation for testing purposes. If data are available, verify the density of the sample taken against the density of the bulk formulation or the anticipated density of the sample. Make an assessment of sampling technique, for example taking an aliquot of the formulation to determine the technique, could influence the OOS result. If trituration is used with a mortar and pestle, the size of the mortar and pestle and the trituration time may affect content uniformity and particle size of the suspension.

If no assignable cause is identified, it is necessary to evaluate the data in context. Determine whether the OOS result is systematic or random. Are all of the values higher or lower than expected? The investigator must assess if it is an error with the formulation preparation method, the scale of the formulation, or the method for taking the analytical sample.

Table VI. Formulations Preparation Review

Active Pharmaceutical Ingredient (API)	CoA reviewed for purity and purity/activity factor calculation (correct and same as used by analysis group?) Stored appropriately and within expiration, if applicable Differences in exploratory and development lots, specifically particle size, morphology, and impurities, and impurity levels
Vehicle/excipients	Correct grade and viscosity, if applicable Source evaluated (equivalent grades between sources) Stored appropriately and within expiration, if applicable
Dose formulation preparation	Concentration and correction factor calculations verified Dose formulation preparation compared to the study protocol instructions and formulation development work (Determine if changes are necessary due to scale or batch size) Dose formulation stored according to the validated formulation stability conditions
Scale-up	Difference in scale between current formulation and previous formulations or developmental work (mL *versus* L preparations) If differences in scale exist, assess potential impact to the formulation preparation (i.e., appropriate equipment used, homogeneity) Consistent pH of the formulation over the concentration range (changes in pH may affect solubility and formulation stability)
Sampling	Sampling procedure appropriate for the formulation type Accurate sampling procedure (i.e., density measurements in agreement with anticipated dose formulation density)

In addition to evaluating the data in context, it is sometimes necessary to observe the preparation of the formulation in order to assess the root cause of the OOS result. This allows the investigator to visually assess if there may be a potential cause for the OOS result.

Summary of Investigation

After the investigation is conducted, an out of specification report should be issued. It should include a summary of the analytical and/or the formulation investigations. The investigation summary should include an assessment of the root cause, the study impact, the path forward, and signatures from the appropriate parties. The root cause must be evaluated in both the analytical and formulation sections of the investigation summary. Although it would be expected to be an unusual occurrence, if after a thorough investigation a root cause is not identified, it should be noted that a root cause could not be determined and the impact on the study would be assessed by the study director. The study director will consult others as needed. When a root cause is identified, it must be taken into account when determining the path forward. A CAPA plan should be generated, when appropriate. If separate investigations were conducted for the analytical and formulation sections, reports should be issued by their respective groups. Reports will include a signed document for each of the individual investigations.

Within the investigation summary, study impact is assessed by the study director. The study director will rely upon the analytical data provided and utilize the expertise of the analytical and formulation investigation groups to determine what, if any, impact the OOS has on the study as a whole. The study director may try to correlate the dose formulation data to available pharmacokinetic data to determine if there were any changes to the expected exposure during the study.

As part of the investigation, the path forward must be identified. Possible paths include, but are not limited to, analyzing the sample duplicates (back-ups), analyzing additional samples (re-sampling), generating new dose formulations (re-preparation), reanalyzing the initial sample, and adjusting the reported dose level. The Study Director must be informed of and involved in the decisions made throughout this process. SOPs will typically determine how the process proceeds. Once an OOS investigation is concluded, the results of the OOS investigation may determine if new or additional investigative or study work will need to be completed.

CONCLUSIONS

This document provides an outline for performing NCDFA-OOS investigations of nonclinical studies in an attempt to overcome the current lack of regulatory guidance. A guidance document should be created to include review of data and methods (analytical and formulations), assessment of OOS root cause, assessment of the impact to the study, and the path forward (actions identified). This would benefit organizations that conduct regulated nonclinical studies.

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