

One Method Approach

*Recommendations to Enable One Analytical Method to be Used
in Residue Depletion Studies and Serve as the Basis of the
Practicable Method*



Center for Veterinary Medicine

Table of Contents

1		
2	Introduction	2
3	Purpose and Scope of the One Method Approach	2
4	FDA Documents Referenced	2
5	One Method Validation	3
6	Validation Parameter Criteria	3
7	System Suitability Test (SST).....	3
8	Linearity and Range	3
9	Limit of Detection (LOD) and Limit of Quantitation (LOQ)	4
10	Accuracy and Precision	4
11	Interference (Selectivity, Specificity, and Carryover).....	4
12	Dilution Integrity (Parallelism)	5
13	Robustness and Ruggedness	5
14	Stability in Matrix.....	5
15	Stability of Stock Standard and Working Standards Solutions	6
16	Long-range Calibration Curve	6
17	Weighting Factors	6
18	Variance and Residual Plots	7
19	Sum of the Absolute Values of Relative Errors Expressed as a Percentage ($\sum$ %RE)	8
20	Weighting Factor Decision Making	9
21	LOD/LOQ Considerations.....	9
22	Confirmatory Procedure	10
23	Timing of Method Validation Submission	11
24	Version History	11
25		
26		

Introduction

Generally, there are two methods developed as part of the new animal drug approval process for food-producing animals. First, there is a validated method that is used in the sponsor's residue depletion studies. This method usually has a long-range calibration curve and, therefore, typically is referred to as the long-range method. Second, there is the proposed practicable method, which is typically the same as, or very similar to, the long-range method. The practicable method has a truncated calibration curve range that encompasses the tolerance (determinative procedure), and a procedure is included to confirm the presence of the targeted residue (confirmatory procedure). This approach to residue methods typically results in sponsors conducting and submitting at least two separate method validation studies, one for the long-range method and one for the short-range, practicable method.

This document outlines recommendations to enable a single analytical method to be used in residue depletion studies and serve as the basis of the practicable method. This is referred to as the One Method Approach. The end goal of the One Method Approach is to enable sponsors to potentially conduct a single method validation study, instead of two.

Purpose and Scope of the One Method Approach

The One Method Approach integrates the validation principles and criteria from both VICH GL49 and GFI #3 to create a streamlined pathway for sponsors. It is important to clarify that CVM is not replacing either guidance document. Rather, this document provides an integrated approach that enables sponsors to conduct one comprehensive method validation that enables a single analytical method to be used in residue depletion studies and serve as the basis of the practicable method. This document is an intermediate working document developed to support the ADUFA V Residue Method Trial Exploratory Working Group recommendations. This document represents CVM's current thinking on this topic. In cases where these criteria cannot be met, CVM highly encourages sponsors to discuss a path forward with CVM.

Demonstration of practicability of the method and usability in a government laboratory is outside the scope of this document and will be addressed in CVM's Risk-Based Tiered Approach to Demonstrate Method Practicability.

FDA Documents Referenced

- CVM GFI #3 General Principles for Evaluating the Human Food Safety of New Animal Drugs Used in Food-Producing Animals
- CVM GFI #208 VICH GL49(R) Studies to Evaluate the Metabolism and Residue Kinetics of Veterinary Drugs in Food-Producing Animals: Validation of Analytical Methods Used in Residue Depletion Studies
- CVM GFI #118 Mass Spectrometry for Confirmation of the Identity of Animal Drug Residues

One Method Validation

Validation Parameter Criteria

FDA has two guidance documents that describe validation criteria for residue methods, depending on whether the method is to be used in residue depletion studies (VICH GL49) or as the practicable method (GFI #3). Discussion of each parameter is provided below.

System Suitability Test (SST)

A System Suitability Test is a quality control measure in real-time analysis to verify that the analysis system can generate reliable and accurate data before sample analysis begins. VICH GL49 does not specify any requirements for a System Suitability Test. GFI #3 recommends including an SST and that the %CV ratios for the analyte to the internal standard peak areas be less than 6%.

One Method Approach Recommendation: CVM recommends validating an analytical method per the SST criteria described in GFI#3.

Linearity and Range

VICH GL49 allows for calibration curves to be created in three formats: solvent, fortified in matrix, or fortified in matrix and taken through the extraction procedure. Linearity can be described by a linear, polynomial, or other appropriate regression plot of known concentration vs. response using a minimum of 5 different concentrations. Acceptability of weighting factors is determined by an evaluation of the residuals across three separate runs. Solvent and matrix-based calibration curves have a repeatability acceptable limit of $\leq 15\%$ at all concentrations except at or below the LOQ where it can be $\leq 20\%$. Calibration curves generated through extraction are subject to the same acceptance criteria as samples.

GFI#3 recommends the use of solvent-based standards but allows for matrix-based standards that include an isotopically labeled internal standard and acceptable justification. Additionally, the guidance recommends calibration curves range from 30-250% of the tolerance. If a weighting factor is used, a justification should be provided.

One Method Approach Recommendation: CVM recommends a single calibration curve using solvent-based standards that covers at least 30 to 250% of the anticipated tolerance and covers the anticipated range of residue concentrations in the residue depletion study. A minimum of five calibration standards is needed. Where solvent-based standards are not appropriate due to matrix effects or other method-specific considerations, matrix or matrix-matched standards may be used when scientifically justified. The following acceptance criteria should be met: calibration curves have a repeatability acceptable limit of $\leq 15\%$ at all concentrations except at the lowest calibration standard where it can be $\leq 20\%$. For methods with a long-range calibration curve, a weighting factor may be needed to address heteroscedasticity and ensure accurate quantification across the broader concentration range. Weighting factor acceptability is discussed below in the *Long-range Calibration Curve* section. To ensure that the method can be used in residue depletion studies and serve as the basis of the practicable method, sponsors should include an adequate number of QCs such that the span of the calibration curve is

represented and such that QCs approximating 0.5x, 1x, and 2x of the anticipated tolerance are included. If the anticipated tolerance is not known at the time of initial single lab validation, the accuracy and precision at 0.5x, 1.0x, and 2x tolerance may be demonstrated as part of the study to demonstrate reproducibility that will be described in CVM's Risk-Based Tiered Approach to Demonstrate Method Practicability. In these cases, the method SOP should be updated to reflect the additional QCs approximating 0.5x, 1x, and 2x of the confirmed tolerance.

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

VICH GL49 describes different ways to determine LOD and LOQ. GFI #3 refers to VICH GL49 Annex 2 for calculation of the LOD and LOQ. For clarification, LOQ is statistically derived and is not the same as the lowest calibration standard.

One Method Approach Recommendation: CVM recommends use of VICH GL49 Annex 2 for LOD and LOQ calculations using the standard error of the intercept (SEI), calculated from at least three calibration curves, instead of root mean square error (RMSE). CVM prefers the use of SEI over RMSE because SEI specifically quantifies the uncertainty in the y-intercept of the calibration curve, which is the point where the regression line crosses the y-axis at zero concentration. Because LOD and LOQ calculations are fundamentally concerned with the ability to detect and quantify analytes at very low concentrations (near zero), the SEI provides a more direct and relevant measure of uncertainty at this critical region of the calibration curve. In contrast, RMSE represents the overall variability of the entire calibration curve across all concentration levels and does not specifically focus on the region where instrument signal corresponding to the LOD and LOQ is located (is estimated). This distinction is particularly important for long-range calibration curves where the concentration span may be very wide.

Additional discussion regarding LOD and LOQ in relation to the use of long-range calibration curves is discussed below in the *Long-range Calibration Curve* section.

Accuracy and Precision

VICH GL49 relates accuracy and precision as dependent upon the analyte concentration, with Tables 1 and 2 in the guidance document defining the acceptable limits for accuracy and precision, respectively.

GFI #3 lists that accuracy of recovery should be 80-110% when the tolerance is ≥ 0.1 ppm, or 60-110% when the tolerance is less than 0.1 ppm. Additionally, acceptable within-laboratory precision is defined as $\leq 10\%$ CV where the tolerance is greater than or equal to 0.1 ppm, or $\leq 20\%$ CV for a tolerance below 0.1 ppm.

One Method Approach Recommendation: CVM recommends use of VICH GL49 for accuracy acceptance criteria. CVM recommends use of VICH GL49 within-run precision acceptance criteria for all precision acceptance criteria.

Interference (Selectivity, Specificity, and Carryover)

VICH GL49 provides that selectivity is acceptable if the response in control tissue at the expected retention time of the analyte is no greater than 20% of the response at the LOQ.

Contains Nonbinding Recommendations

GFI#3 recommends the method should demonstrate the absence of interference from other animal drugs, no interference from control tissue greater than 10% of the tolerance, and no interference from carryover peak greater than 10% of the tolerance.

One Method Approach Recommendation:

- Matrix Interference: The response should be no more than 20% of the response at the LOQ.
- Interference from other new animal drugs: Interference from other new animal drugs is only applicable to addressing whether the method can serve as the basis of the practicable method. To this end, interference from other compounds of similar chemical structure approved for use in the same target species should not exceed 10% of the tolerance.
- Carryover: In general, the response from carryover should be no more than 20% of the response at the LOQ.

Dilution Integrity (Parallelism)

Dilution integrity is not described in VICH GL49. GFI #3 describes parallelism as the ability to dilute samples with analyte concentrations above the highest calibration standard. The sponsor should provide data to demonstrate that using control matrix extract to dilute samples above the highest calibration standard does not compromise the accuracy or precision of the method when the sample is analyzed post-dilution.

One Method Approach Recommendation: Although not required for method acceptance, CVM strongly recommends validating a method for dilution integrity and applying the VICH GL49 criteria for accuracy and precision as described above in the Accuracy and Precision section. The choice of diluent should be based on what yields acceptable accuracy and precision for the specific method.

Robustness and Ruggedness

Robustness evaluation in VICH GL49 relates to reagent lots, incubation temperatures, extraction solvent composition and volume, extraction time and number of extractions, solid phase extraction (SPE) cartridge brand and lots, analytical column brand and lots and HPLC elution solvent composition and other parameters that may affect the overall performance of the method.

GFI #3 robustness evaluation is the same as VICH GL49. GFI #3 states that ruggedness is a measure of the transferability of the method between operators, laboratories, instrumental platforms, or long intervals of time.

One Method Approach Recommendation: For robustness, CVM recommends use of VICH GL49 because it provides more detail. Ruggedness will be addressed in CVM's Risked-Based Tiered Approach to Demonstrate Method Practicability.

Stability in Matrix

VICH GL49 describes evaluating stability in matrix and processed sample stability. The guidance additionally specifies different temperatures appropriate for use of the method should

be studied (frozen, benchtop, autosampler) as well as a freeze/thaw stability study. The stability of samples after extraction is recommended, as extracted samples may be stored for a period of time at varying temperatures and exhibit differing stability behavior. VICH GL49 outlines different concentrations to evaluate and refers to the accuracy and precision criteria specified elsewhere in the guidance.

GFI#3 states that data for stability in matrix and stability of the analyte in extracts should be provided.

One Method Approach Recommendation: CVM recommends use of the procedures described in VICH GL49 for stability evaluations and applying the criteria outlined in the Accuracy and Precision section of this document. The sponsor should select time points and temperatures that are appropriate for future intended use of the method. CVM recommends including an assessment of fortified sample stability in the One Method validation study. An assessment of incurred sample stability should also be conducted at some point and may be included as part of the One Method validation study, as a standalone stability study, as part of a residue depletion study, or as part of the study to demonstrate reproducibility that will be described in CVM's Risk-Based Tiered Approach to Demonstrate Method Practicability. Sponsors should consider their goals and needs when determining when to assess stability of incurred samples.

Stability of Stock Standard and Working Standards Solutions

GFI #3 specifies that the stability of standard stock solutions should be evaluated. VICH GL49 does not address this topic.

One Method Approach Recommendation: CVM recommends that data be provided on the stability of standard stock solutions and working standard solutions under the storage conditions used during method validation and routine analysis.

Long-range Calibration Curve

Weighting Factors

Historically, sponsors were generally expected to use unweighted calibration curves for the practicable method. VICH GL49 allows for weighting to be applied for methods used in residue depletion studies and contains the following excerpt:

“Acceptability of weighting factors should be determined by evaluation of the residuals across three runs to determine if the residuals are randomly distributed.”

For the practicable method, GFI #3 states,

“If a weighting factor is used for the calibration curve, justification for weighting should be provided.”

Additionally, the Bioanalytical Method Validation Guidance for Industry from FDA (May 2018) states:

“The sponsor should use the simplest model that adequately describes the concentration-response relationship, as well as an appropriate weighting scheme and regression equation.”

Guidance does not necessitate the use of any specific weighting scheme, only that an appropriate one is used. The default assumption should be that the most appropriate weighting scheme be applied (*i.e.*, unweighted or weighted) based on an evaluation of the data.

Tissue residue analytical methods quantify analytes by use of calibration curves and linear regression. Linear regression assumes that the variance of the response variable y is homogenous among the levels of the prediction variable x . In other words, each data point is assumed to contribute equally to the total error estimate in the model.¹ However, when there is a wide range of predictor variables in the data set (*e.g.*, long-range calibration curve), it is common for the variance to increase as the predictor variable increases.² This is known as proportional error, and the data are said to be heteroscedastic. When this occurs, it is no longer appropriate to assume that every observation should be treated equally.¹ Also, the calculations in least squares linear regression favor numbers of larger value compared to smaller value numbers, which can result in regression model predictions being more accurate at the higher end and less accurate at the lower end.³ Weighted least squares linear regression is a statistical technique that overcomes the issues of proportional error and greater influence of larger value numbers. Weighted linear regression attempts to provide each data point the appropriate amount of influence by applying a weighting factor.¹ For calibration curves, common weights used are $1/x$ and $1/x^2$. The weights are applied to the sums of squares during the regression calculations⁴ and generally result in improved prediction accuracy (*i.e.*, less model error).²

For evaluating the presence of proportional error and heteroscedasticity, CVM recommends plotting the regression residuals and instrument response variance from the calibration curve. In addition, comparing the sum of Relative Error expressed as a percentage (%RE) can be used to determine which weighting factor should be used. Utilizing these three tools will guide sponsors and CVM when determining which weighting scheme is most appropriate.

Variance and Residual Plots

Simple plots of instrument response variance and regression residuals can be generated from calibration curve data to help determine whether the data exhibit proportional error and heteroscedasticity. Briefly, for a variance plot, the variance of the instrument response (y -value) is plotted against the concentration of the calibration standards (x -value). For a residual plot, the residuals (predicted vs. observed concentration) from the calibration curve unweighted regression model (y -value) are plotted against the concentration of the calibration standards (x -values). The plots are visually inspected for increases in variance and increases in the magnitude of the residuals as the calibration standard concentration increases. Increasing variance values and increasing magnitude of residuals are evidence that the data exhibit

¹ Weighted Least Squares Regression. <https://www.itl.nist.gov/div898/handbook/pmd/section1/pmd143.htm>

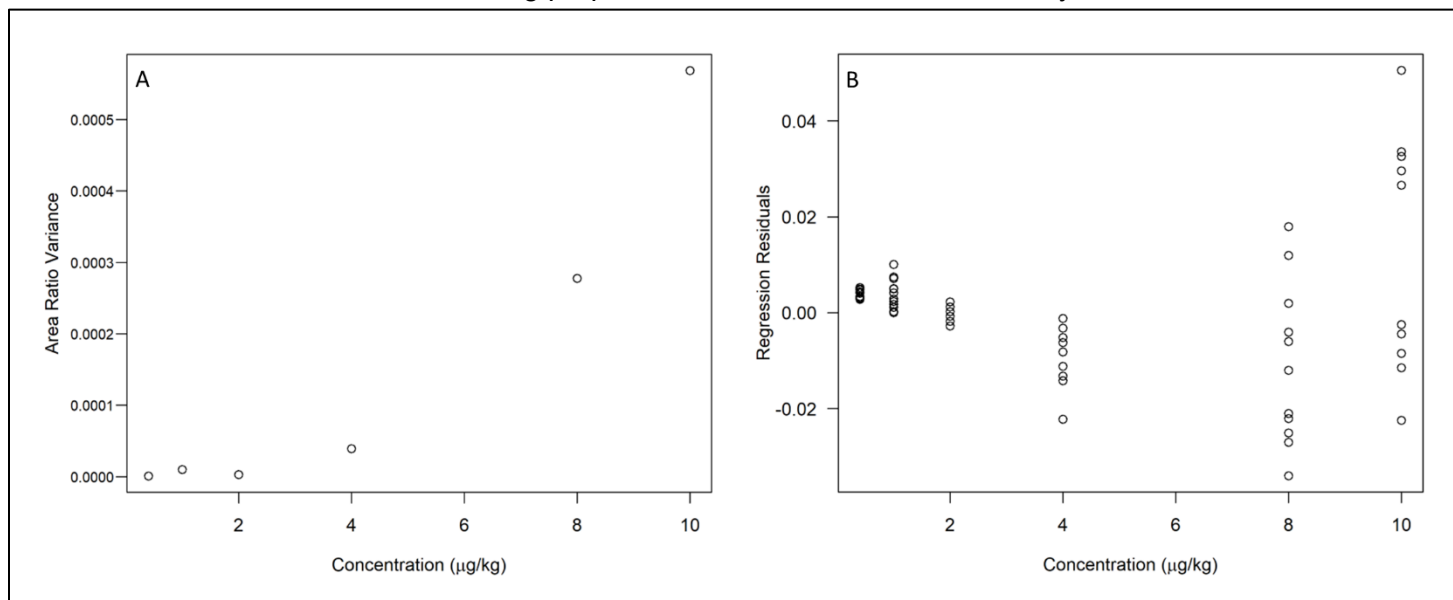
² <https://www.chromatographyonline.com/view/calibration-curves-part-v-curve-weighting>

³ Environmental Protection Agency Method 8000C.

⁴ <https://ms.mcmaster.ca/canty/teaching/stat3a03/Lectures7.pdf>

proportional error and heteroscedasticity and indicate that the use of weighted regression should be pursued. Below are two example plots exhibiting these characteristics (Figure 1).

Figure 1. Example plots of instrument response variance (A) and regression residuals (B) exhibiting proportional error and heteroscedasticity.



In addition to plotting, sponsors and CVM may use formal statistical tests (e.g., F-test comparing variances at lowest and highest calibrator concentrations, Breusch-Pagan test, White test, Bartlett's test, or Levene's test) to determine whether the calibration curve data exhibit heteroscedasticity.⁵

Sum of the Absolute Values of Relative Errors Expressed as a Percentage ($\sum$ %RE)

Comparing the $\sum$ %RE for the calibration curve data for a given weighting scheme has been described as a simple, straight-forward approach to determine which weighting factor should be used after it has been determined that weighted linear regression is needed.⁶ To do this, the back calculated concentrations of the calibration standards are expressed as percent recovered relative to their nominal concentration. Then, 100% is subtracted from the percent recovery value for each standard to yield relative error (%RE) value for each standard. The absolute value of relative error values for all calibration standards are added together to yield a sum of %RE ($\sum$ %RE). The $\sum$ %RE values from different weighting schemes are then compared against each other to quickly determine which weighting scheme is most appropriate. Generally, the weighting factor with the lowest $\sum$ %RE value can be taken as being the most appropriate.

⁵ Cheng, W. L.; Markus, C.; Lim, C. Y.; Tan, R. Z.; Sethi, S. K.; Loh, T. P.; for the IFCC Working Group on Method Evaluation Protocols. Calibration Practices in Clinical Mass Spectrometry: Review and Recommendations. *Ann. Lab. Med.* **2023**, 43 (1), 5–18. DOI: 10.3343/alm.2023.43.1.5

⁶ Kiser, M.M. and Dolan, J.W. 2004. Selecting the Best Curve Fit. *LC•GC Europe*. 17(3).138-143.

Weighting Factor Decision Making

Weighting should be based on a total evaluation of the validation data and in consideration of the above tools. No one test can give a complete picture of the data, and the decision should be used in concert with the totality of method validation data.

Weighting decisions should not be made solely to achieve a higher R^2 value, to lower the LOQ/LOD values, or to compensate for consistent issues with the analytical method. Choosing to weight without careful consideration of the broader method performance may obscure deeper issues with the method that are better resolved through changing method parameters.

Generally, when determining whether weighting is needed, the following steps, as described previously, are recommended:

1. Plot the variance of the instrument response for each concentration of calibration standards (y-value) against the concentration of the calibration standards (x-value).
2. Plot the residuals (predicted vs. observed concentration) from the calibration curve unweighted regression model (y-value) against the concentration of the calibration standards (x-values).
3. Determine whether the variance and the magnitude of the residuals increase as the calibration standard concentration increases. If necessary, a formal statistical test can be performed to assess whether the data are heteroscedastic.

If these evaluation techniques indicate that weighted linear regression is needed, calculate $\sum \%RE$ as described previously. Use the results of the $\sum \%RE$ in conjunction with the totality of the validation data to select the most appropriate weighting factor.

LOD/LOQ Considerations

CVM historically has calculated LOD and LOQ values through VICH GL49 Annex 2. Step 1 in Annex 2 describes how to estimate the LOD and LOQ values based on the calibration curve data. Step 2 is used to verify that the method recoveries at the LOQ/LOD values estimated in Step 1 are acceptable and provides a better estimate of the LOQ. In recent times, CVM has not asked sponsors to conduct Step 2.

However, increased use of weighted linear regression under the One Method Approach will yield lower LOQ and LOD values compared to values determined by unweighted linear regression. This may result in calculated LOQ/LOD values that are below the ability of the method to accurately and precisely measure. To address concerns about whether calculated LOQ values accurately reflect the method's true quantitative capability, CVM recommends that sponsors follow the complete VICH GL49 Annex 2 procedure, including both Step 1 (using SEI as discussed previously) and Step 2, for estimating and verifying the LOD and LOQ. This two-step approach ensures that the reported LOQ represents a concentration at which the method can actually quantify the analyte with acceptable performance, not merely a mathematical calculation that may fall below the method's true capability.

If the accuracy and precision of samples fortified at the estimated LOQ (from Step 1) do not meet the criteria specified in VICH GL49 during the Step 2 verification process, the LOQ should

be adjusted. Specifically, the sponsor should evaluate additional concentrations above the calculated LOQ in a systematic and scientifically justified manner until acceptable accuracy and precision are achieved. The final reported LOQ should be the lowest concentration at which the method reliably meets VICH GL49 accuracy and precision criteria which ensures that the LOQ represents the method's true quantitative capability.

Confirmatory Procedure

The Analytical Methods for Residues section of GFI #3 contains the following:

“A method for analyzing the marker residue in the target tissue consists of two procedures: the determinative (quantitative) and confirmatory procedures. The determinative and confirmatory procedures may be combined into a single procedure as instrumentation allows.”

GFI #3 then references GFI #118 as describing the criteria for the confirmatory procedures. GFI #3 Section VI.G.3.a (Specific Data Needed - Sponsor's Submission) specifies the minimum samples to be analyzed using the determinative procedure: 5 control tissues, 5 control tissues fortified at 0.5× tolerance, 5 control tissues fortified at 1× tolerance, 5 control tissues fortified at 2× tolerance, and 5 tissues containing biologically incurred marker residue at approximately the tolerance. For the confirmatory procedure, GFI #3 specifies: 5 control tissues, 5 control tissues fortified at 1× tolerance, and 5 tissues containing biologically incurred marker residue at approximately the tolerance.

Data generated from the determinative procedure applied to 5 control tissues, 5 control tissues fortified at 0.5× tolerance, 5 control tissues fortified at 1× tolerance, 5 control tissues fortified at 2× tolerance can be included as part of the One Method validation study. Because the tolerance likely will not be known at this time, sponsors are encouraged to assay multiple fortified sample concentrations.

Data generated from the determinative procedure applied to 5 tissues containing biologically incurred marker residue at approximately the tolerance can be included as part of the One Method validation study. Alternatively, these incurred data may be generated at another time more suitable to the sponsor's development plan when biologically incurred samples are available.

Data generated from the confirmatory procedure applied to 5 control tissues and 5 control tissues fortified at 1× the anticipated tolerance can be included as part of the One Method validation study. Because the tolerance likely will not be known at this time, sponsors are encouraged to assay multiple fortified sample concentrations.

Data generated from the confirmatory procedure applied to 5 tissues containing biologically incurred marker residue at approximately the tolerance can be included as part of the One Method validation study. Alternatively, these incurred data may be generated at another time more suitable to the sponsor's development plan when biologically incurred samples are available.

349 **Timing of Method Validation Submission**

350 CVM recommends submitting the One Method validation study prior to conducting any residue
351 depletion studies. This will ensure that any unforeseeable issues can be addressed prior to
352 conducting pivotal *in vivo* studies. Another option is to submit the One Method validation study
353 when sponsors submit their pivotal total residue and metabolism study.

354 Version History

355 October 27, 2026 – Original Version

356