

Risk-Based Tiered Approach to Demonstrate Method Practicability

Pilot Program

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Center for Veterinary Medicine

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List of Abbreviations and Terminology

ADUFA	Animal Drug User Fee Act
CVM	Center for Veterinary Medicine
ERL	Early Response Letter
FDA	Food and Drug Administration
FD&C Act	Federal Food, Drug, and Cosmetic Act
FDA or USDA Method	A method currently being used or previously used by a government monitoring agency or a method that CVM previously accepted as a practicable method for a new animal drug approval
Functional Independence	An attribute of an analyst or laboratory when they are operating without prior involvement in or influence from the method development or single-laboratory validation and operating using separate instruments for analysis
GFI	Guidance for Industry
LOD	Limit of Detection
LOQ	Limit of Quantitation
MCM	Method Classification Meeting
MCM-ERL	Method Classification Meeting – Early Response Letter
Method Qualification	A limited validation study demonstrating that a sponsor's laboratory has the capability, equipment, expertise, and quality systems to perform an already-validated method
MOC	Memorandum of Conference
MRS	Method Reproducibility Study
Naïve Analyst	An analyst with no prior involvement in method development or single-laboratory validation who has not previously performed the method and is functionally independent
Naïve Laboratory	A laboratory with no prior involvement in method development or single-laboratory validation that has not previously performed the method and is functionally independent
QC	Quality Control
SLV	Single-Laboratory Validation
SOP	Standard Operating Procedure
SST	System Suitability Test
Study Director	The individual with overall responsibility for the technical conduct of the study, as well as for the interpretation, analysis, documentation and reporting of results, and represents the single point of study control
USDA	United States Department of Agriculture
VICH GL49	Veterinary International Conference on Harmonisation Guideline 49

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Introduction

The FD&C Act requires FDA to determine whether the edible tissues (*i.e.*, meat, milk, eggs and honey) derived from animals treated with new animal drugs are safe for human consumption.¹ As part of this evaluation, sponsors of new animal drugs are required by the FD&C Act and implementing regulations to submit an application that includes,

a description of practicable methods for determining the quantity, if any, of such drug in or on food, and any substance formed in or on food, because of its use.^{2,3}

As part of the reauthorization of ADUFA for the years 2024 through 2028 (*i.e.*, ADUFA V), FDA and stakeholders formed the ADUFA V Residue Method Trial Exploratory Working Group, which was tasked with examining the historical process for demonstrating method practicability described in Guidance for Industry #3 (*i.e.*, the Method Trial). The outcome of the working group was a set of recommendations on how the demonstration of method practicability can be streamlined and modernized.⁴

The working group identified three key attributes of a practicable analytical method:

- Validity
- Reproducibility
- Usability in a government laboratory setting

In addition, the working group developed the concept of a Risk-Based Tiered Approach to demonstrating that an analytical method possesses the attributes of practicability. In general, the Risk-Based Tiered Approach is comprised of four tiers:

- Tier 1: Negligible risk
- Tier 2: Low risk
- Tier 3: Moderate risk
- Tier 4: Elevated risk

The working group recommended that a pilot program be developed for Tiers 1, 2 and 3 and implemented no later than October 2026.

The tier into which a method is placed depends on CVM's confidence in the proposed method's reproducibility and usability in a government laboratory. CVM's confidence in a method's reproducibility and usability is based on the similarity between the proposed method and a method currently being used or previously used by a government monitoring agency or a method that CVM previously accepted as a practicable method for a new animal drug approval (*i.e.*, an FDA or USDA method). As CVM's confidence in a method's reproducibility and usability in a government laboratory increases, the risk decreases along with the requirements needed to

¹ 21 U.S. Code § 360b(d)(1)(A), (B), (D), and (F)

² 21 U.S. Code § 360b(b)(1)(G)

³ 21 Code of Federal Regulations § 514.1(b)(7)

⁴ Report of the Animal Drug User Fee Act (ADUFA) V Residue Method Trial Exploratory Working Group. October 1, 2025.

reach acceptance of the method. The end result of such an approach is approval requirements that are commensurate with the level of risk.

This document describes the pilot program for implementing Tiers 1, 2 and 3 of the Risk-Based Tiered Approach which consists of the following:

- Tier Classification
- Data and Information Requirements for Tiers 1, 2, and 3
- Suggested Submission Strategies for Tiers 1, 2 and 3

The pilot program will take place until a minimum of three new animal drug products have completed the Tier 2 or Tier 3 process or until the end of ADUFA VI, whichever occurs first.

Because this is a pilot program, CVM expects that implementation experience may identify areas where the approach should be clarified, refined, or adjusted. CVM and sponsors may identify process improvements during the pilot, and CVM may update these recommendations as appropriate based on pilot experience.

Tier Classification

The determination of the tier for an analytical method is a key step that establishes expectations for sponsors and CVM during product development. The determination of the tier consists of an optional preliminary classification and then the final classification determination made by CVM.

Preliminary Tier Classification

Although not required, sponsors are encouraged to request a Method Classification Meeting (MCM) with CVM to discuss the preliminary tier classification for their proposed analytical method. At a minimum, the MCM request should contain the relevant tier classification information outlined later in this document for each tier, depending on which tier the sponsor is seeking. However, the MCM request should not include data, and it is not a mechanism to receive a pre-review of the White Paper that is required for Tier 3 methods.

A meeting will be scheduled to take place no later than 60 days after the MCM request is submitted. CVM will review the information and make a preliminary determination of in which tier the sponsor's analytical method likely qualifies. CVM will explain their findings at the meeting, and an MOC will be issued within 45 days of the meeting.

Alternatively, at the time of MCM submission, sponsors can choose the MCM-ERL option. If the MCM-ERL option is selected, sponsors will receive an ERL no later than six days prior to the scheduled meeting. The MCM-ERL will explain CVM's findings and provide the preliminary tier classification. Within three calendar days of receiving the MCM-ERL, the sponsor will notify CVM whether they want to hold the meeting. If the meeting is still held, no further information can be submitted. If the sponsor does not respond within three calendar days of receiving the MCM-ERL, the meeting will be cancelled, and the MCM-ERL will be the final response issued for the MCM submission.

Regardless of the mechanism (MCM or MCM-ERL), CVM will provide a preliminary tier classification commensurate with the information provided. However, CVM's decision will only apply to the information submitted in the MCM request. If changes occur in the development

plan that affect the method or CVM later identifies information that counters the original classification, the preliminary tier classification can be changed.

Final Tier Classification

CVM will make the final determination of which tier applies to the sponsor's method when a P submission containing the method qualification study (Tier 1), SLV study (Tiers 2 and 3), validated method SOP (all Tiers), and White Paper (Tier 3) is submitted to CVM for review. The specific information that needs to be included in the P submission is outlined in this document for each tier. The final classification will be communicated in the letter issued in response to the P submission. Changes made to the method after the P submission might result in CVM altering the final tier classification.

Data and Information Requirements for Tiers 1, 2, and 3

A brief description of each tier and the data and information requirements are provided below. This information is intended to capture most scenarios; however, there may be cases where additional information is needed. In addition, because this is a pilot program, CVM and sponsors have the opportunity to identify process enhancements and refinements that should be incorporated.

Tier 1 (Negligible Risk)

Tier 1 applies to cases where sponsors use an FDA or USDA method. The method already includes the sponsor's analyte at the relevant concentrations in the relevant tissue(s), and no modifications to the method are made. Because the method has previously been determined by FDA or USDA to be suitable for monitoring, the method is considered validated, reproducible, and useable in a government laboratory setting.

There also are cases where a sponsor may have developed both a proprietary long-range method for use in residue depletion studies and a separate short-range method to serve as the practicable method in support of a previous new animal drug approval. In these cases, the sponsor may elect to use their proprietary long-range method for their residue depletion studies and use the short-range method as their practicable method.

MCM Submission or MCM-ERL Submission (optional)

If the sponsor requests an MCM, a Z submission should be submitted containing the following minimum information:

- Identification of the existing FDA or USDA method. This method should be a method currently or previously used by the FDA or the USDA for monitoring or a method that CVM previously accepted as a practicable method for a new animal drug approval.
- Preliminary information to confirm that the method measures the sponsor's analyte at the relevant concentrations (*i.e.*, at least *ca.* 0.3x to 2.5x of the anticipated tolerance).
- Information to confirm that the method includes determinative and confirmatory procedures.

- A statement by the sponsor confirming that no modifications to the existing method SOP will be made and acknowledging that any modifications will result in a different tier classification.

CVM will review the information and communicate the preliminary decision as described previously in the Preliminary Tier Classification section.

Method Qualification Submission

For Tier 1 methods, because the method is an unchanged FDA or USDA method and includes the sponsor's analyte at the relevant concentrations in the relevant tissue(s), sponsors only need to demonstrate that their laboratory is capable of performing the method (*i.e.*, qualify) for use in residue depletion studies (*i.e.*, total residue and metabolism study and marker residue depletion study). The existing, unchanged method SOP will serve as the description of the practicable method required by statute and regulation.

The sponsor will conduct a fit-for-purpose method qualification study, which is a limited validation study designed to demonstrate that the sponsor's laboratory has the capability, equipment, expertise, and quality systems to successfully perform an already-validated method. However, if the laboratory to be used in new residue depletion studies (*i.e.*, total residue and metabolism study and marker residue depletion study) used the method to generate data within the previous 5 years, and CVM found those data acceptable, then a method qualification study is not needed. Alternatively, if 5 years have passed, sponsors may request a meeting to discuss their specific circumstances to determine whether a qualification study is needed.

The requirement to conduct a method qualification study is not intended to question the acceptability of a previously established practicable method. Rather, the purpose of the qualification study is to demonstrate that the analytical laboratory to be used in upcoming depletion studies (*e.g.*, total residue and metabolism study, marker residue depletion study) can perform the method acceptably.

The method qualification study is intended to be fit-for-purpose, such that certain parameters are always included, but others are included on an as-needed basis. The parameters included will be assessed against the acceptance criteria specified in the One Method Approach document, as applicable.

The following method qualification parameters are always included:

- SST
- Calibration Curve and Linearity
- Accuracy and Precision

Depending upon the available information, the following additional parameters may need to be assessed:

- Matrix-interference
 - Necessary when it is not known whether matrix interference was assessed previously.

Contains Nonbinding Recommendations

200 ○ Necessary when the level of interference accepted for the purpose of a
201 practicable method might affect sample analysis during the depletion study
202 (e.g., excessive interference on low concentration samples).

203 • LOD and LOQ

204 ○ Necessary when the method SOP does not state an LOD/LOQ.

205 ○ Necessary when the method SOP LOD/LOQ was not determined according
206 to VICH GL49.

207 • Storage Stability

208 ○ Necessary when the method SOP does not state storage conditions that
209 cover the storage temperature and duration anticipated to be used in the
210 upcoming depletion study.

211 The method qualification study is submitted to CVM as a P submission. CVM will review the
212 study and, if acceptable, the letter will indicate that CVM concludes that Tier 1 is applicable, and
213 that, if no changes to the method SOP are made later in the development phase, the method
214 can serve as the method used in residue depletion studies and the practicable method required
215 by statute and regulation.

216 ***Tier 2 (Low Risk)***

217 Tier 2 applies to cases where sponsors adapt an FDA or USDA method (*i.e.*, the base method),
218 and the only adaptation is the addition of the new analyte in the relevant tissue along with the
219 appropriate standard curve and QC samples. The base method may be a method currently or
220 previously used by FDA or USDA for monitoring or a method that CVM previously accepted as a
221 practicable method for a new animal drug approval. Because the proposed method is only
222 minimally adapted from the base method, there is assurance that the proposed method
223 procedures can be performed in a government laboratory.

224 ***MCM Submission or MCM-ERL Submission (optional)***

225 If the sponsor requests an MCM, a Z submission should be submitted and contain the following
226 minimum information:

227 • Identification of the existing FDA or USDA method that will be minimally adapted. This
228 base method should be a method currently or previously used by FDA or USDA for
229 monitoring or a method that CVM previously accepted as a practicable method for a new
230 animal drug approval.

231 • A statement confirming that the only planned adaptations are the addition of a new
232 analyte, the addition of a new tissue from the same or different species (as applicable),
233 and the addition of a standard curve and QC samples that are appropriate for the
234 intended use.

235 • Information to confirm that the method includes determinative and confirmatory
236 procedures.

237 • A statement by the sponsor acknowledging that an SLV study and MRS will be
238 conducted.

- A statement by the sponsor confirming that adaptations beyond those stated in the MCM request will not be made and acknowledging that adaptations beyond those stated in the MCM may result in a different tier classification.

CVM will review the information and communicate their decision as described previously in the Preliminary Tier Classification section.

Single-Laboratory Validation (SLV) Submission

Methods classified as Tier 2 are minimally adapted from existing FDA or USDA methods. Therefore, Tier 2 methods must undergo full SLV to ensure that the adaptations to the base method are compatible and capable of generating acceptable results. CVM recently developed a One Method Approach that integrates the validation principles and criteria from both VICH GL49 and GFI #3. The SLV study for Tier 2 studies should demonstrate that the minimally adapted method is validated to specifications detailed in the One Method Approach.

The SLV study is submitted to CVM as a P submission. CVM will review the study and, if acceptable, the letter will indicate that CVM concludes that Tier 2 is applicable, and that, if no changes are made later in the development phase, the method can serve as the method used in residue depletion studies and serve as the basis of the practicable method required by statute and regulation following successful completion and acceptance of an MRS.

Method Reproducibility Study (MRS) Submission

Methods classified as Tier 2 are minimally adapted from existing FDA or USDA methods. Therefore, the sponsor must demonstrate that the adapted method is capable of producing reproducible results across laboratories by conducting an MRS. The MRS is a GLP study involving a reference analyst and two independent, naïve analysts.

The three analysts involved in the MRS should be functionally independent. The naïve analysts should be unfamiliar with the method SLV process and have no prior experience performing the method. Moreover, the reference analyst should not provide coaching or guidance to the naïve analysts. Functional independence requires that analysts use separate instruments for analysis during the study.

Certification should be provided affirming that the naïve analysts did not participate in the SLV study and do not have prior experience performing the method. If all analysts are located in the same laboratory, Quality Assurance Unit oversight should be provided to ensure that the analysts remain functionally independent.

The specific data that need to be generated by the MRS are detailed in Appendix 1. Incurred samples are used to generate reproducibility data. The source of the incurred samples is at the discretion of the sponsor and may include samples retained from other studies provided that, throughout the MRS, there is no evidence of analyte degradation. The following three options (detailed in Appendix 2) are provided regarding the source of the incurred samples to be used in the MRS:

- Option A: Incorporate the MRS as a component of the total residue and metabolism study or marker residue depletion study.
- Option B: Transfer incurred samples from another GLP study and conduct a standalone MRS.

- Option C: Conduct a standalone MRS that generates its own incurred samples.

The MRS is submitted to CVM as a P submission. CVM will review the study and, if all parameters described in the One Method Approach and Appendix 1 for the MRS have been addressed acceptably through the SLV study and MRS, the letter will indicate that CVM concludes that, if no changes are made later in the development phase, the method is accepted as the practicable method required by statute and regulation.

Tier 3 (Moderate Risk)

Tier 3 applies to cases where sponsors adapt an existing FDA or USDA method (*i.e.*, the base method). Like Tier 2, the adaptations include the addition of the new analyte in the relevant tissue along with the appropriate standard curve and QC samples. However, for Tier 3 methods, additional adaptations are needed, but CVM determines that the other adaptations won't affect the usability in a government laboratory. The base method may be a method currently or previously used by FDA or USDA for monitoring or a method that CVM previously accepted as a practicable method for a new animal drug approval. Because the proposed method still is based on the base method, there is some level of confidence about the usability in a government laboratory, and this is confirmed through a desk review of a White Paper.

MCM Submission (optional)

If the sponsor requests an MCM, a Z submission should be submitted and contain the following minimum information:

- Identification of the existing FDA or USDA method that will be adapted. This base method should be a method currently or previously used by FDA or USDA for monitoring or a method that CVM previously accepted as a practicable method for a new animal drug approval.
- A description of the planned adaptations and a statement confirming that the only planned adaptations are those stated in the MCM submission.
- Information to confirm that the method includes determinative and confirmatory procedures.
- A statement by the sponsor acknowledging that an SLV study and MRS will be conducted and that a Tier 3 White Paper will be submitted.
- A statement by the sponsor confirming that adaptations beyond those stated in the MCM request will not be made and acknowledging that adaptations beyond those stated in the MCM may result in a different tier classification.

CVM will review the information and communicate their decision as described previously in the Preliminary Tier Classification section.

SLV Submission

The SLV study information and requirements are the same as those previously described for Tier 2 methods.

Contains Nonbinding Recommendations

317 In addition to the SLV study, sponsors may elect to include the Tier 3 White Paper with the Tier 3
318 SLV study submission (see below for information on the Tier 3 White Paper).

319 The SLV study is submitted to CVM as a P submission for review. If the P submission only
320 includes the SLV study (*i.e.*, no Tier 3 White Paper) and if the study is acceptable, the letter will
321 indicate that CVM concludes that the method can serve as the method used in residue depletion
322 studies.

323 If the P submission also includes the Tier 3 White Paper and if CVM agrees with the White
324 Paper and finds the SLV study to be acceptable, the letter will indicate that Tier 3 is applicable
325 and that CVM concludes that if no changes are made later in the development phase, the
326 method can serve as the method used in residue depletion studies and serve as the basis of the
327 practicable method required by statute and regulation following successful completion of an
328 MRS.

329 *MRS Submission*

330 The MRS information and requirements are the same as those previously described for Tier 2
331 methods.

332 The MRS is submitted to CVM as a P submission. In addition to the MRS, sponsors may elect
333 to include the Tier 3 White Paper with the Tier 3 MRS submission (see below for information on
334 the Tier 3 White Paper) if they have not already done so.

335 CVM will review the MRS and, if all parameters described in the One Method Approach and
336 Appendix 1 for the MRS have been addressed acceptably through the SLV study and MRS, and
337 CVM has reviewed and accepted the Sponsor's Tier 3 White Paper, the letter will indicate that
338 CVM concludes that, if no changes are made later in the development phase, the method is
339 accepted as the practicable method required by statute and regulation.

340 If CVM has not reviewed and accepted Tier 3 White Paper at the time of the MRS submission,
341 the letter will only comment on whether the MRS study has demonstrated reproducibility.

342 *White Paper*

343 Tier 3 methods incorporate adaptations to an existing FDA or USDA method beyond the addition
344 of the new analyte in the relevant tissue along with the appropriate standard curve and quality
345 control (QC) samples. Therefore, information is needed to indicate that it is reasonable to
346 expect that a government laboratory can perform the additional procedures.

347 The goal of the White Paper is to leverage the fact that, even though the proposed method
348 contains different procedures from an existing FDA or USDA method, there is evidence that the
349 different procedures can be performed in a government laboratory. For example, the additional
350 procedures (*e.g.*, clean-up procedures, equipment changes, *etc.*) may have been incorporated
351 in other FDA or USDA methods. Or, if the different procedures are not explicitly found in an FDA
352 of USDA method, the White Paper provides sponsors the opportunity to justify that the
353 procedures are common analytical practices, and, therefore, it is reasonable to expect that a
354 government laboratory can perform them. To that end, CVM suggests that the White Paper
355 utilize multiple sources of information to justify that the proposed modifications, while more
356 significant than those in Tier 2, can be routinely performed by a government monitoring

laboratory using standard equipment, reagents, and analyst expertise without significant additional burden.

A P submission is the appropriate mechanism by which sponsors submit the White Paper. Although CVM encourages sponsors to submit the White Paper with the SLV P submission or with the MRS P submission, the timing of the submission is entirely within the remit of the sponsor. CVM will review the White Paper, and, if acceptable, the response letter will indicate that Tier 3 is applicable.

Suggested Submission Strategies

Decisions on when to conduct residue chemistry studies and submit them to CVM for review are entirely at the sponsor's discretion. However, based on the components of the Risk-Based Tiered Approach, CVM does suggest certain strategies that maximize the utility of incurred samples generated during the residue depletion studies. Because the analytical method is an integral part of the Residue Chemistry Component of the Human Food Safety technical section, the suggested strategies below incorporate all residue chemistry segments required for an approval. For ease of presentation, protocol submissions are not included. However, for all studies, CVM strongly encourages sponsors to take advantage of the CVM protocol review process. Sponsors are encouraged to consider these strategies but are not obliged to follow them. The strategies presented below assume that the product being developed is a new chemical entity that requires a full residue chemistry data package. For some supplemental approvals, the components needed may vary.

Tier 1 (Negligible Risk)

Suggested Submission Strategy for the Residue Chemistry Portion of the Human Food Safety Technical Section for Tier 1 Methods

Submission Sequence	Component	Submission Code	Description
1	MCM-ERL	Z	The sponsor requests a meeting to discuss the tier classification and utilizes the ERL option.
2	Total Residue and Metabolism Study + Method Qualification	P	The sponsor conducts the total residue and metabolism study and incorporates method qualification. The study is submitted to CVM.
3	Comparative Metabolism Study	P	The sponsor conducts and submits the comparative metabolism study.
4	Marker Residue Depletion Study	P	The sponsor conducts the marker residue depletion study. The study is submitted to CVM.

382 **Tier 2 (Low Risk)**

383 **Suggested Submission Strategy for the Residue Chemistry Portion of the Human Food**
 384 **Safety Technical Section for Tier 2 Methods**

Submission Sequence	Component	Submission Code	Description
1	MCM-ERL	Z	The sponsor requests a meeting to discuss the tier classification and utilizes the ERL option.
2	SLV Study	P	The sponsor conducts the SLV study prior to conducting pivotal residue studies and submits it to CVM.
3	Total Residue and Metabolism Study + Storage Stability	P	The sponsor conducts the total residue and metabolism study and incorporates the incurred sample storage assessment to leverage the availability of incurred samples. The study is submitted to CVM.
4	Comparative Metabolism Study	P	The sponsor conducts and submits the comparative metabolism study.
5	Marker Residue Depletion Study + MRS	P	The sponsor conducts the marker residue depletion study and incorporates the MRS as a component of the depletion study to leverage the availability of incurred samples. The study is submitted to CVM.

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387 **Tier 3 (Moderate Risk)**

388 **Suggested Submission Strategy for the Residue Chemistry Portion of the Human Food**
389 **Safety Technical Section for Tier 3 Methods**

Submission Sequence	Component	Submission Code	Description
1	MCM-ERL	Z	The sponsor requests a meeting to discuss the tier classification and utilizes the ERL option.
2	SLV Study + White Paper	P	The sponsor conducts the SLV study prior to conducting pivotal residue studies and submits it to CVM. The submission also includes the Tier 3 White Paper.
3	Total Residue and Metabolism Study + Storage Stability	P	The sponsor conducts the total residue and metabolism study and incorporates the incurred sample storage assessment to leverage the availability of incurred samples. The study is submitted to CVM.
4	Comparative Metabolism Study	P	The sponsor conducts and submits the comparative metabolism study.
5	Marker Residue Depletion Study + MRS	P	The sponsor conducts the marker residue depletion study and incorporates the MRS as a component of the depletion study to leverage the availability of incurred samples. The study is submitted to CVM.

390 **Effect on the Product Development Plan**

391 The pilot program eliminates six elements of the preexisting method trial process. The
392 elimination of these five elements is projected to reduce the time to complete the residue
393 chemistry component of the human food safety technical section by approximately 2.25 years
394 for approvals that would undergo the preexisting method trial process. In addition, because the
395 pilot program enables sponsors to demonstrate method reproducibility without the need for
396 government laboratory participation, the MRS study report can be submitted with another
397 residue chemistry study (e.g., marker residue depletion study) as one single P submission with
398 a 180-day review time. This further reduces the time spent on activities required for approval by
399 6 months because, under the preexisting method trial process, the final report from the
400 interlaboratory study was submitted as a separate P submission with its own 180-day review
401 time. Thus, overall, the pilot program reduces the time to complete the residue chemistry
402 component of the human food safety technical section by 2.25 to 2.75 years depending on the
403 tier that applies.

404

405 **Elements of the Preexisting Method Trial Process Eliminated by the Pilot Program**

Element	Sponsor Activity	CVM Activity	Time for Sponsor per Exploratory Group Discussion	Time for CVM per Established Processes	Time Eliminated by Pilot
Short-range method validation	Sponsor conducts and submits the study	CVM reviews the study	180 days	180 days	360 days
3-hour method meeting	Sponsor prepares and submits request	CVM reviews information and schedules the meeting	90 days	60 days	150 days
Incurred sample generation	Sponsor generates incurred samples	None	90 days	0 days	90 days
Method demonstration scheduling	None	CVM schedules the method demonstration	0 days	120 days	120 days
Method demonstration	Sponsor participates in the demonstration	CVM participates in the demonstration	3 days		3 days
Method SOP revision	Sponsor revises the method SOP based on the method trial and submits it to CVM.	CVM files the method SOP	90 days	0 days	90 days
Total days eliminated	-				813
Total years eliminated	-				2.25

406 **Appendix 1: Recommendations for Conducting Tier 2 and Tier 3 MRS**

407 This appendix describes CVM's recommendations for the MRS for Tier 2 and Tier 3 methods.
408 The core objective of the MRS is to demonstrate that the method, as written in the standard
409 operating procedure (SOP), can be independently and reliably executed by naïve analysts and
410 that results are reproducible across analysts. The method SOP used in the MRS should be the
411 same version reviewed and accepted by CVM as part of the SLV submission. If the SOP
412 requires revision, the revised SOP should receive Study Director approval before the MRS
413 begins and these changes should be justified when submitted to CVM for review. The logistical
414 process for conducting the study differs based on how the sponsor chooses to source the
415 incurred samples. Sponsors may choose from three options as described in Appendix 2.

416 The MRS involves the same set of samples being analyzed by one reference analyst and two
417 blinded naïve analysts operating with functional independence. The method SOP is the only
418 instruction the naïve analysts receive, and all analytical decisions made during the MRS should
419 be traceable to the method SOP or documented as deviations. The acceptance criteria for the
420 SST, calibration curve, and fortified QC accuracy and precision should meet the criteria
421 specified in the One Method Approach document.

Contains Nonbinding Recommendations

Before the MRS begins, the Study Director should ensure that each analyst receives the finalized method SOP, analytical reference standards and internal standard with COAs and Safety Data Sheets, control tissue samples, incurred tissue samples, a sample transfer list, and chain of custody documentation for all distributed materials. All reference standards should be characterized and traceable, and purity corrections should be applied in all concentration calculations.

Each analyst should conduct analytical work on a minimum of three separate days. Each analytical run should include a freshly prepared calibration curve, a System Suitability Test (SST), fortified QC samples at low, medium, and high concentration levels, and incurred tissue samples. Each analyst should verify instrument and system performance, demonstrate acceptable accuracy and precision with fortified samples, and analyze incurred samples. All three analysts, including the reference analyst and both naïve analysts, should perform both the determinative and confirmatory procedures on all incurred samples, consistent with the criteria outlined in the One Method Approach document. Any deviations from the method SOP or study protocol should be documented and reported to the Study Director. All analytical days should be completed before the Study Director applies the blinding key.

CVM recommends applying the between-run precision criteria specified in VICH GL49 for evaluation of between analyst precision.

All communications between the Study Director and participating analysts should be documented. Once the incurred sample analysis begins, communication between participating analysts regarding results, sample identities, or method performance is not permitted. Sponsors are encouraged to contact CVM if they believe an issue may affect the method's regulatory acceptability or tier classification.

Individual Analyst Data Package

Upon completion of all analytical work, each analyst submits a complete data package to the Study Director. The table below summarizes what each analyst is expected to submit to the Study Director at the end of the study.

450

Data Element	Details
Stock solution preparation records	Weights, purity corrections, dilution calculations, and stock solution comparability data (between calibration standard stock and QCs stock solutions, reported as peak area ratio difference)
SST results	All SST parameters with measured values and pass or fail determination for each analytical day
Calibration curve data	Regression equation, weighting factor, back-calculated concentrations, percent deviation, and pass or fail for each analytical day
Fortified QC results	Measured concentrations, percent theoretical, and within-run and between-run precision for each QC level across all analytical days
Blinded sample results	Measured concentrations and calculated tissue concentrations for each blinded sample on each analytical day
Confirmatory data	For all positive samples including fortified QC samples and blinded incurred samples, and for control tissue samples to confirm absence of response, consistent with the criteria outlined in the One Method Approach document
Chromatograms	Blank, zero, each QC level, all blinded samples, and calibration standards for each analytical day
Instrument details	Instrument parameters and any adjustments made during the study with justification
Deviations	Any deviations that occurred during the study, including the nature of the deviation and the analyst's assessment of the impact on the data
Raw Data	All raw data files including instrument data files, chromatograms, XML files, and any other electronic records necessary to reconstruct and verify the analytical results, submitted along with the individual analytical report.

451

452 After all analysts have submitted their results, the Study Director applies the blinding key,
 453 compiles the data from all analysts, and performs the between-analyst reproducibility
 454 assessment, and prepares the final study report as described in the following section.

455 **Final Study Report Submission to CVM**

456 The Study Director prepares a final study report for submission to CVM that includes the
 457 individual analytical reports from all participating analysts as appendices. The final study report
 458 includes the following elements: sample preparation and blinding, sample distribution, blinding
 459 key, compiled analytical results, individual analyst performance evaluation, between-analyst
 460 reproducibility assessment, confirmatory analysis summary, deviations, overall conclusions,
 461 GLP compliance statement, study protocol and amendments.

462

Appendix 2: Conducting the MRS: Option-Specific Processes for Incurred Samples

The process for executing the MRS depends on the source of the incurred samples. Sponsors should select the option that best aligns with their drug development timeline and resources.

Option A: Incorporating the MRS into a Pivotal Residue Depletion Study

Under Option A, the MRS is not a separate study. Instead, it is integrated directly into the GLP protocol of the total residue and metabolism study or the marker residue depletion study. This option is highly efficient and minimizes additional animal use.

Protocol Integration: The sponsor should ensure that the GLP study protocol for the pivotal depletion study explicitly addresses the reproducibility components, including the identification of the participating naïve analysts and the specific data reporting requirements for the MRS.

Sample Sourcing and Blinding: Incurred samples are generated during the normal in-life phase of the pivotal study. The Study Director selects a subset of these incurred samples (spanning appropriate low and high concentration ranges) and control samples. The Study Director blinds and randomizes this specific subset and distributes them to the naïve analysts.

Execution: All three analysts (including the reference analyst) execute the MRS by analyzing the distributed incurred samples over the recommended three analytical days. The reference analyst may analyze these samples as part of the routine pivotal study analysis, provided the data are isolated for the reproducibility assessment.

Option B: Standalone MRS Using Transferred Incurred Samples

Under Option B, the MRS is conducted as a separate, standalone GLP study using incurred samples that were previously generated and stored during another GLP study.

Study Protocol: A separate GLP protocol is written specifically for the MRS.

Sample Transfer and Verification: Incurred samples from a prior pivotal study are transferred to the MRS. Because these samples were previously analyzed, their approximate concentrations are known. Incurred samples retained from a stability study may also be used provided that, throughout the MRS, there is no evidence of analyte degradation. The Study Director pools these samples into appropriate low and high concentration groups. If necessary, the Study Director may adjust concentrations by dilution with control tissue or fortification with reference standard.

Blinding and Distribution: Once the target concentration ranges are verified, the Study Director blinds, randomizes, and distributes the samples, along with control tissues and reference standards, to the participating analysts.

Execution: All three analysts (including the reference analyst) execute the MRS by analyzing the distributed incurred samples over the recommended three analytical days.

Option C: Standalone MRS by Generating Incurred Samples

Under Option C, the MRS is conducted as a separate, standalone GLP study that includes its own in-life animal dosing phase solely to generate new incurred samples. This option is typically used when historical incurred samples are unavailable, degraded, or insufficient in volume.

Contains Nonbinding Recommendations

502 *Study Protocol:* A separate GLP protocol is written for the MRS. The protocol should include
503 both the in-life phase (animal dosing and tissue collection) and the analytical phase.

504 *Sample Generation and Verification:* Animals are dosed according to the protocol to generate
505 tissues with residues near the tolerance. The reference laboratory conducts a preliminary
506 analysis of the newly collected tissues to verify that the incurred samples fall within the required
507 low and high concentration target ranges.

508 *Blinding and Distribution:* Following verification, the Study Director pools, blinds, randomizes,
509 and distributes the newly generated incurred samples and control tissues to the participating
510 analysts.

511 *Execution:* All three analysts execute the MRS by the standalone MRS protocol concurrently,
512 analyzing the distributed incurred samples over the recommended three analytical days.

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514

515 **Version History**

516 October 27, 2026 – Original Version