

**#264**

# **Standardized Medicated Feed Assay Limits**

## **Guidance for Industry**

### **Draft Guidance**

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**U.S. Department of Health and Human Services  
Food and Drug Administration  
Center for Veterinary Medicine (CVM)  
February 2020**

## **Standardized Medicated Feed Assay Limits**

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#### **I. Introduction**

CVM establishes assay limits for drugs in medicated feeds (i.e., Type B and Type C medicated feeds) as part of the approval process for Type A medicated articles. The term “assay limits” refers to how much the measured amount of drug can deviate from the labeled amount of drug. The assay limits for Type B and Type C medicated feeds are specified as a range and published in 21 CFR §558.4(d). When a medicated feed assay value falls within this range using a validated method accepted by CVM, it indicates that the measured drug level in medicated feed is consistent with the labeled drug level. The assay limits are not intended to permit a feed manufacturer to add more or less than the labeled amount of drug to the medicated feed. Assay limits are used pre-approval to ensure that medicated feeds in Target Animal Safety (TAS), Effectiveness (EFF), Chemistry, Manufacturing, and Controls (CMC), Bioequivalence (BE), and Human Food Safety (HFS) residue chemistry studies contain the appropriate amount of drug, and post-approval for compliance and customer service purposes.

This guidance recommends a standardized set of assay limits for medicated feeds. Standardized medicated feed assay limits allow predictability in the review process as sponsors can determine early in the drug development process what assay limits they should expect to meet for medicated feeds used in TAS, EFF, CMC, and HFS residue chemistry studies. The implementation of standardized medicated feed assay limits does not lower or otherwise change the current standards for safety and effectiveness and does not change the expectation regarding medicated feed assay methods, medicated feed assay method validation, and method transfer studies (see Guidance for Industry (GFI) #135, “Validation of Analytical Procedures for Type C Medicated Feeds,”<sup>1</sup> GFI #136, “Protocols for the Conduct of Method Transfer Studies for Type C Medicated Feed Assay Methods,”<sup>2</sup> and GFI #137, “Analytical Methods Description for Type C Medicated Feeds”<sup>3</sup>). CVM will not require any additional medicated feed studies beyond those already required in the CMC technical section due to the implementation of standardized

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<sup>1</sup> <https://www.fda.gov/regulatory-information/search-fda-guidance-documents/cvm-gfi-135-validation-analytical-procedures-type-c-medicated-feeds>

<sup>2</sup> <https://www.fda.gov/regulatory-information/search-fda-guidance-documents/cvm-gfi-136-protocols-conduct-method-transfer-studies-type-c-medicated-feed-assay-methods>

<sup>3</sup> <https://www.fda.gov/regulatory-information/search-fda-guidance-documents/cvm-gfi-137-analytical-methods-description-type-c-medicated-feeds>

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medicated feed assay limits. CVM does not intend to tighten any previously approved medicated feed assay limits but would be willing to consider widening previously approved medicated feed assay limits to match the assay limits in this guidance at the request of the pioneer Type A medicated article sponsor. Sponsors should request a meeting with CVM if they wish to discuss widening previously established medicated feed assay limits. Sponsors of generic Type A medicated articles may not initiate a change to widen the medicated feed assay limits and should follow the assay limits listed in 21 CFR §558.4(d). CVM would also be willing to discuss alternate medicated feed assay limits with sponsors on a case-by-case basis (e.g., for medicated feeds with drug inclusion rates below 5 ppm<sup>4</sup>), but this type of discussion should occur early in the drug development process, based on the sponsor's knowledge of their drug, assay methodology, and proposed indications.

In general, FDA's guidance documents do not establish legally enforceable responsibilities. Instead, guidances describe the Agency's current thinking on a topic and should be viewed only as recommendations, unless specific regulatory or statutory requirements are cited. The use of the word *should* in Agency guidances means that something is suggested or recommended, but not required.

## **II. Background**

Medicated feed assay limits are based on Permissible Analytical Variability (PAV). Previously, CVM used an equation for PAV that quantified the variability due to the analytical method and adjusted for the other variations that have not been quantified.<sup>5</sup>

$$\text{PAV} = (2 \times \%CV) + 5\%$$

In this equation,  $\%CV = (\text{standard deviation}/\text{mean}) \times 100\%$  and was derived from the feed assay method transfer study results for fortified feed samples according to GFI #136; 5% was added to account for the sources of variability that are not quantified. The following equation was used to calculate the assay limits:

$$\text{Assay Limits} = \% \text{ Recovery} \pm \text{PAV},$$

where % Recovery was obtained from the feed assay method transfer study results for fortified feed samples (see GFI #136). PAV values typically ranged from 15% to 20%; however, CVM has required tighter assay limits and accepted wider assay limits for some medicated feeds on a case-specific basis. A major limitation to this approach was that medicated feed assay limits were not finalized until the CMC technical section was complete. In many cases, significant work on safety and effectiveness had already been conducted prior to establishing the medicated feed assay limits, and that work had to be reassessed if medicated feeds used in those studies assayed outside of the finalized medicated feed assay limits.

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<sup>4</sup> 5 ppm is equivalent to 4.5 g/ton.

<sup>5</sup> Association of American Feed Control Officials (AAFCO) Official Publication 1985, pp 88 – 90.

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### III. Standardized Assay Limits for Medicated Feeds

This guidance recommends a standardized set of PAVs for medicated feeds, based on feed type and drug inclusion rate. For the purpose of determining PAVs, medicated feeds are categorized, based on similarity, into two main types: 1) swine, poultry, and minor avian; and 2) cattle and minor ruminant. In general, swine, poultry, and minor avian feeds are similar with respect to their flow and handling characteristics, including particle size, particle size distribution, moisture content, and composition; thus, it is reasonable to assign the same standardized PAVs to the medicated feeds for these species.<sup>6</sup> Likewise for cattle and minor ruminant feeds. Due to the use of unique manufacturing processes and lack of historical data, the PAV for other species (e.g., fish, exotic animals) will be determined on a case-by-case basis. PAVs for each feed type will vary, as shown below, depending on the drug inclusion rate.

PAVs for Medicated Feeds<sup>7</sup>

Species	PAV < 25 ppm	PAV 25 - 500 ppm	PAV > 500 ppm
Swine, poultry, minor avian	20%	15%	15%
Cattle, minor ruminant	25%	20%	15%

Standardized medicated feed assay limits are calculated by adding and subtracting the standard PAV from 100%, unless the sponsor has data to show that the typical recovery is not 100%, in which case the sponsor could propose that the assay limits be centered on the average percent recovery. If a Type A medicated article is intended for use in multiple species or is used across medicated feed inclusion rate ranges that cross 25 or 500 ppm, there could be more than one set of approved medicated feed assay limits for that product.

For free-choice medicated feeds,<sup>8</sup> CVM suggests using the same PAVs listed in the table above; however, sponsors may meet with CVM on a case-by-case basis to discuss alternate PAVs for these types of products.

The details of how CVM calculated the standardized PAVs for medicated feeds are provided as an appendix to this guidance.

### IV. Appendix: CVM's Calculation of Standardized PAVs for Medicated Feeds

The standardized PAVs are obtained from two contributing factors: analytical method variability and mixing/manufacturing variability,

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<sup>6</sup> Chiba, L. *Animal Nutrition Handbook*, Section 12: Poultry Nutrition and Feeding, 2014.

<sup>7</sup> 25 and 500 ppm are equivalent to 22.7 and 453.6 g/ton, respectively.

<sup>8</sup> Free-choice medicated feeds contain one or more animal drugs and are placed in feeding and grazing areas but are not intended to be fully consumed at a single feeding. These include medicated blocks, mineral mixes, and liquid feeds (see 21 CFR §510.455).

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PAV = Analytical Method Variability + Mixing/Manufacturing Variability.

Analytical method variability, as a percent, is calculated using the Horwitz equation for predicted interlaboratory variability ( $\sigma_H$ )<sup>9</sup> as follows:

$$\sigma_H = 2C^{-0.15},$$

where C is the concentration of the drug in the medicated feed expressed as a dimensionless fraction (e.g., 1  $\mu\text{g/g}$ ,  $C=10^{-6}$  g/g). In the Horwitz equation, the predicted interlaboratory variability is the overall measure of analytical variability, including the intralaboratory component.

The mixing/manufacturing variability represents the variability in the uniformity of a batch of medicated feed. The uniformity of a batch of medicated feed is generally assessed by assaying samples across the batch and calculating the variability of the assay results. Therefore, the uniformity variability includes the analytical variability associated with the method used to assay the samples (i.e., the intralaboratory analytical variability). Because the amount of total analytical variability, both inter- and intra-laboratory variability, is accounted for using the Horwitz equation, the intralaboratory analytical variability is subtracted from the uniformity variability to obtain the mixing/manufacturing variability, as shown in the equation below.

Mixing/Manufacturing Variability = Uniformity Variability – Intralaboratory Analytical Variability.

Historically, an intralaboratory analytical method variability, %CV, of 5% was applied to drug inclusion levels of greater than 10 ppm and a %CV of 7.5% was applied to drug inclusion levels of 10 ppm or less. These %CV values and the 10 ppm drug inclusion cut-off for analytical variability were established using medicated feed assay data from sponsors of New Animal Drug Applications (NADAs) for Type A medicated articles and the values published in GFI #135. More recently, in an effort to reevaluate the drug inclusion cut-off for analytical variability, CVM analyzed blinded medicated feed assay data provided by NADA sponsors for cattle, poultry, and swine medicated feeds and determined that for this guidance, the drug inclusion cut-off will be set at 25 ppm.<sup>10</sup> For ease of use, an intralaboratory analytical method variability value of 8% is applied to medicated feed concentrations less than 25 ppm and a value of 5% is applied to medicated feeds with concentrations of 25 ppm or greater. It is noted that the assigned intralaboratory analytical method variability is in line with Horwitz study results, which found that intralaboratory variability is usually one-half to two-thirds the predicted interlaboratory

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<sup>9</sup> Vander Heyden, Y., Smeyers-Verbeke, J., Massart, D., Benchmarking for Analytical Methods: The Horwitz Curve, LCGC Europe, Vol. 18, Issue 10, pp 528 – 531, October 2005.

<sup>10</sup> CVM evaluated the data by applying the standardized medicated feed assay limits in this guidance at drug inclusion cut-off values of 10, 25, 50, and 100 ppm. The proportion of data that fell within the assay limits did not meaningfully increase as the drug inclusion cut-off values increased from 25 – 100 ppm. Therefore, 25 ppm was selected as the drug inclusion cut-off.

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variability ( $\sigma_H$ ).<sup>11, 12</sup> At low concentrations, such as 5 ppm, the intralaboratory analytical method variability can be calculated as  $\frac{2}{3} \sigma_H = 8\%$ , and at the drug inclusion cut-off of 25 ppm the intralaboratory analytical method variability can be calculated as  $\frac{1}{2} \sigma_H = 5\%$ .

For uniformity variability, it is generally accepted that a %CV for uniformity across the batch of 10 - 15% is considered “good,” while a %CV of 15 - 20% is regarded as “fair.”<sup>13</sup> A uniformity variability of 15% is applied to swine, poultry, and minor avian medicated feeds less than 25 ppm and 10% uniformity variability is applied to swine, poultry, and minor avian medicated feeds greater than or equal to 25 ppm. It is known that mixing drugs in cattle feed homogeneously is difficult due to the nature of the feed (e.g., particle size, feed ingredients). Therefore, a uniformity variability of 20%, 15%, and 10% is applied to medicated cattle and minor ruminant feeds at concentrations of less than 25 ppm, 25 - 500 ppm, and greater than 500 ppm, respectively, where 500 ppm could be either a concentrated Type C or Type B medicated feed.

Standardized PAVs are calculated as follows:

$$\text{PAV} = 2C^{-0.15} + (\text{Uniformity Variability} - \text{Intralaboratory Analytical Variability}),$$

where,

C = medicated feed concentration as a dimensionless fraction (e.g., 1  $\mu\text{g/g}$ ,  $C=10^{-6}$  g/g)

Uniformity Variability =

Species	Medicated Feed Concentration < 25 ppm	Medicated Feed Concentration 25 - 500 ppm	Medicated Feed Concentration > 500 ppm
Swine, poultry, minor avian	15%	10%	10%
Cattle, minor ruminant	20%	15%	10%

Intralaboratory Analytical Variability =

Medicated Feed Concentration	Intralaboratory variability
< 25 ppm	8%
$\geq$ 25 ppm	5%

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<sup>11</sup> See footnote 9.

<sup>12</sup> Horwitz, W. Evaluation of Analytical Methods Used for Regulation of Foods and Drugs. Analytical Chemistry, Vol. 54, No. 1, pp 67 – 76, January 1982.

<sup>13</sup> Herman, T., Behnke, K. Testing Mixer Performance. MF1172. Kansas State University Agricultural Experiment Station and Cooperative Extension Service Bulletin, Manhattan, KS: Kansas State University, October 1994.

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The recommended PAVs are derived as shown below, where 5 ppm is considered a low drug inclusion concentration, 25 ppm is the drug inclusion cut-off, and 500 ppm could be either a concentrated Type C or Type B medicated feed.

For swine, poultry, minor avian:

$$\begin{aligned} 5 \text{ ppm: PAV} &= 2(0.000005)^{-0.15} + (15\% - 8\%) = 12\% + 7\% = 19\% \\ 25 \text{ ppm: PAV} &= 2(0.000025)^{-0.15} + (10\% - 5\%) = 10\% + 5\% = 15\% \\ 500 \text{ ppm: PAV} &= 2(0.0005)^{-0.15} + (10\% - 5\%) = 6\% + 5\% = 11\% \end{aligned}$$

For cattle, minor ruminant:

$$\begin{aligned} 5 \text{ ppm: PAV} &= 2(0.000005)^{-0.15} + (20\% - 8\%) = 12\% + 12\% = 24\% \\ 25 \text{ ppm: PAV} &= 2(0.000025)^{-0.15} + (15\% - 5\%) = 10\% + 10\% = 20\% \\ 500 \text{ ppm: PAV} &= 2(0.0005)^{-0.15} + (10\% - 5\%) = 6\% + 5\% = 11\% \end{aligned}$$

For ease of use, 19%, 24%, and 11% are rounded up to the nearest 5%. The rounded PAVs are shown in section [III. Standardized Assay Limits for Medicated Feeds](#).