ANIMAL HEALTH INSTITUTE

Representing manufacturers of animal health products

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Food and Drug Administration, Rm. 1061
5630 Fishers Lane
Rockville, Maryland 20852

RE: Docket No. 01D-0224
Draft Guidance for Industry: Mass Spectrometry for Confirmation of the Identity of Animal Drug Residues; Availability

The ANIMAL HEALTH INSTITUTE ("AHI") submits these comments on the draft guidance for industry (#118) titled "Mass Spectrometry for Confirmation of the Identity of Animal Drug Residues."

AHI is the national trade association representing research-based manufacturers of animal health products – the pharmaceuticals, vaccines and feed additives used in modern food production, and the medicines that keep livestock and pets healthy. Our licensed member companies produce the vast majority of all such products in the United States, as well as the world market.

AHI commends the Center for recognizing the need to update the requirements for confirmation of animal drug residues by mass spectrometry. We endorse the use of new, expanded criteria in response to the development and use of newer mass spectral techniques. With regard to mass spectral matching, we agree with the draft document that confirmation criteria vary depending on the technique for mass spectral data acquisition. However, the draft document has some items that should be clarified and we have both general and specific comments.

General Comments

It is extremely difficult, if not impossible, to define a rigid set of criteria for application to all possible mass spectral techniques. The current draft is still heavily oriented toward the historical three ion criteria developed in 1978 and is based on electron impact ionization. As a number of people have subsequently commented (in many of the references cited in the draft document), the historical three ion rule does not acknowledge the specificity of higher molecular weight ions formed by softer ionization techniques. The softer ionization techniques produce fewer ions that can be problematic in producing the requisite three ions, even though the higher molecular weight ions are generally more specific for identification than lower molecular weight ions. Instead of trying to define criteria to address all mass spectral techniques, the most

01D-0224 C1

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efficient use of resources would be to focus on the most common methodology. As most confirmatory methods recently have been LC/MS/MS methods, and probably will be in the foreseeable future, it is probably best to focus on criteria for these methods. The others could be handled on a case-by-case basis.

The criteria for LC/MS/MS defined under Items III.C.5-8 are generally acceptable, but they do not apply to all situations. They do not address the situation where the precursor ion produces only one significant product ion. For these situations, we propose that the LC retention time criteria be applied and the precursor ion and product ion be measured. The relative abundance ratio for the precursor and the product ion ratio should match the relative abundance ratio of the standard ± 10%.

Section I.E. of the guidance document states "demonstration of non-interference by drugs approved in the same species." We are unclear as to the value added by this requirement within the scope of an LC/MS/MS-based mass spectrometric confirmatory assay and request that it be reconsidered. The generally held scientific opinion supports the position that the combination of analysis of contemporaneous standards, retention time matching, analysis of two to three characteristic ions, and intensity matching of the ions will provide unambiguous identification of analytes with low probability of false positives without the need for non-interference testing. This requirement is more appropriate for a determinative procedure than for a confirmatory procedure. If an interference study performed using a determinative procedure shows no interference by other drugs approved in same species with the analyte of interest, we do not see any reason to demonstrate once again the non-interference by the other drugs by a confirmatory procedure involving MS/MS analysis.

The non-interference requirement also raises the question of how many approved drugs to evaluate. By investigating any compounds in this context, we are implying that there is a possibility of another drug compromising the integrity of the specificity of the mass spectrometric confirmation. This logically progresses to one inquiring as to whether just looking at compounds similar in structure is sufficient, or whether even diverse structures can serendipitously generate an ion of similar m/z to one monitored in the confirmatory assay. The overall effect of this is a negation of recognizing that mass spectrometry is a reliable, unequivocal confirmatory technique, and it has previously been so recognized by CVM Guidance Documents (c.f.: V. GUIDELINE FOR APPROVAL OF A METHOD OF ANALYSIS FOR RESIDUES: B.1; SPECIFICITY).

Specific Comments

Section I.A.1. ...subsamples from one source, but see part 1.F below. Is there a difference in objective between Sect 1.A.1 and Sect 1.F? Which one should be applied?

Section I.A.3. Ten residue-incurred, 5 at each of two levels. In the GUIDELINE FOR APPROVAL OF A METHOD OF ANALYSIS FOR RESIDUES, the criteria are for n=5 incurred tissues for the confirmatory procedure. Why is there a difference with this guidance? If the requirement is

going to be n=5 at two levels, the definition of levels needs to be specified? Do the levels represent different concentrations or different time points?

Section I.C. Demonstration of <10% false negative rate at the tolerance or safe level is recommended (based on fortified and incurred samples). What does the term <10% false negative rate mean? Some explanation would be very useful. For a better understanding of the guidance document, it would be helpful to include the definitions for at least two additional terms, false negative and false positive, in the glossary section.

It appears unnecessary to use incurred samples for this purpose. Unless the surveillance method is a combined determinative/confirmatory procedure, this is more appropriate for the determinative step. Moreover, in most cases, as only the extracts of incurred samples from the determinative procedure will be available for confirmation, the required demonstration can be done using fortified samples.

Section I.D. Demonstration that data can be acquired on more than one day. This helps to ensure data reproducibility. This is actually interassay precision and should be stated as such. Presumably this can be done using the fortified matrix samples. It is necessary to specify the replication and the acceptable parameters of accuracy and precision for the method. (cf. to Part IV: Quality Control)

Section I.F. Demonstration of non-interference by matrix components in control samples from more than one source. Suggest making the wording more flexible (e.g. by adding "if available" at the end) since it is not always possible to obtain control samples from more than one source, unless this just means more than one animal. Conceivably, as stated, this can lead to a major task since interference can vary depending on the strain, gender, age, feeding conditions, etc., and may not be practical. It would be easier to change the experimental conditions (chromatography, cleanup, etc.) to fit the purpose should an interference be detected in a control matrix.

Section II.K. Estimate of concentration limits for confirmation in matrix. This needs some clarification. Does it mean LOQ and a linear dynamic range encompassing the tolerance? Confirmatory procedures are not quantitative in nature, and frequently done in tandem with a determinative procedure using a common sample extract.

Section III.A. Comparison Standard. A definition of Comparison Standard needs to be supplied for clarity

Section III.B.2. A tolerance for retention time matching should be specified in the SOP. The tolerance should not exceed 2% for GC/MS or 5% for LC/MS, relative to the retention time of standard: The guideline should allow the provision of using an internal standard or a retention time marker with the sample and use relative retention time to determine tolerance for retention time.

Section III.C.1. ...strict numerical criteria need not be applied. Clarification needs to be supplied for "numerical criteria". If this is referring to the intensity of the 3 specific structural ions meeting a 20% arithmetic difference, then numerical criteria is insufficient. How should an acceptable minimum relative abundance be determined? Would it be acceptable to CVM if this level is defined in the SOP as any abundance greater than a signal-to-noise ratio of 3:1 (s/n ratio defined in Draft Guidance Section III.B.1.)? If the 3:1 signal-to-noise ratio or some other low abundance is defined as the minimum acceptable level, will the acceptance window be altered? For example, if Selected Ion Monitoring is employed in LC/MS/MS and 3 ions are monitored, the acceptable relative ion abundance window defined by the Draft Guidance would be ±20%. If a monitored ion abundance is 25% and the minimum abundance level specified in the SOP is lower, the Draft Guidance could then be interpreted to allow acceptance of an ion relative abundance of 5%, assuming correspondence with the standard spectra and an acceptable signal to noise ratio.

Section III.C.2. General Comments: If high resolution mass spectrometry is used for confirmation (R=10,000 or higher), exact mass ion ratios of two characteristic ions or two isotope ions (e.g. $^{37}Cl/^{35}Cl$) alone may be sufficient for confirmation provided that the time resolution (LC RT) is used as a complementary criterion.

Section III.C.5.c and III.C.7.a. Use of terminology is inconsistent for precursor and parent. One of these terms should be identified and used consistently.

Section IV. E. Ad Hoc Confirmatory Packages should meet or exceed the following minimal data recommendations: The number of replicates required of the fortified controls (in I and II) used for quality assurance purpose should be specified. AHI recommends three replicates as being adequate.

AHI appreciates the opportunity to comment on this draft guidance document.

Respectfully submitted,

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