Guidance for Industry

Mass Spectrometry for Confirmation of the Identity of Animal Drug Residues

FINAL GUIDANCE

This final guidance document is intended to provide specific guidance for the development, evaluation, and application of mass spectrometric methods for confirming the identity of animal drug residues. It elaborates the description of method specificity in CVM Guidance Document 3, General Principles for Evaluating the Safety of Compounds Used in Food-producing Animals, Part V, Guideline for Approval of a Method of Analysis for Residues, section B.1.

Comments and suggestions regarding the document should be submitted to Division of Dockets Management (HFA-305), Food and Drug Administration, 5630 Fishers Lane, Rm. 1061, Rockville, MD 20852. Submit electronic comments to http://www.regulations.gov. All comments should be identified with the Docket No.O1D-0224.

For questions regarding this final document, contact David N. Heller, Center for Veterinary Medicine (HFV-510), Food and Drug Administration, 8401 Muirkirk Rd., Laurel, MD 20708, 301-210-4579, e-mail: david.heller@fda.hhs.gov.

Additional copies of this final guidance document may be requested from the Communications Staff (HFV-12), Center for Veterinary Medicine, Food and Drug Administration, 7519 Standish Place, Rockville, MD 20855 and may be viewed on the Internet at
http://www.fda.gov/AnimalVeterinary/GuidanceComplianceEnforcement/GuidanceforIndustry/default.htm

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Veterinary Medicine
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FINAL GUIDANCE FOR INDUSTRY:

MASS SPECTROMETRY FOR CONFIRMATION OF THE IDENTITY OF ANIMAL DRUG RESIDUES[1]

This guidance represents the Food and Drug Administration's (FDA's) current thinking on this topic. It does not create or confer any rights for or on any person and does not operate to bind FDA or the public. You can use an alternative approach if the approach satisfies the requirements of the applicable statutes and regulations. If you want to discuss an alternative approach, contact the FDA staff responsible for implementing the guidance. If you cannot identify the appropriate FDA staff, call the appropriate number listed on the title page of this guidance.

I. INTRODUCTION

CVM develops, evaluates, and applies qualitative mass spectrometric methods for confirming the identity of animal drug residues. Methods developed in support of a New Animal Drug Application (NADA methods) are designed for residues of an approved new animal drug used in the approved manner. Methods may also be developed for unapproved new animal drugs or approved new animal drugs used in an unapproved manner (non-NADA methods). This final guidance document describes the basic principles recommended by CVM for developing, evaluating and applying these methods.

The purpose of this document is to facilitate and expedite coordination between CVM and its stakeholders so these activities may be carried out in a consistent and timely manner. This final document does not commit CVM to accepting a specific method or data package prior to reviewing the relevant data. This final document is intended for technical professionals familiar with mass spectrometry. Please contact CVM for further information on this document or any technical explanations that may be necessary. For a historical perspective, please see the Bibliography. For definitions of terms used in this document, please see the Glossary.

This guidance document is applicable in the following areas:

1. Consultations on confirmatory methodology

2. Desk reviews of confirmatory procedures

3. Method trials or second-laboratory evaluations of confirmatory procedures

4. Development of confirmatory procedures

[1] This guidance has been prepared by the Division of Residue Chemistry, Office of Research, Center for Veterinary Medicine (CVM) at the Food and Drug Administration.

5. Desk reviews of data generated with confirmatory procedures

It is CVM's view that methods should fit the purpose. This document applies to work done for CVM's purposes, and does not necessarily apply to or invalidate work done for other purposes. This guidance applies only if a reference standard is available.

This guidance document should be used to help in the development of new methods, the review of methods submitted to CVM, and in the laboratory trial of methods submitted to CVM. The document should also help in making decisions about appropriate methodology in various regulatory situations and ensuring consistency in work done for CVM's purposes. This document sets guidance standards and performance specifications as targets. CVM recommends that methods meet or exceed these standards.

FDA's guidance documents, including this guidance, 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. GUIDANCE

Where CVM can predict that use of a new animal drug in food animals will likely result in the presence of drug residues in edible tissue of the treated animal, a full CVM Confirmatory Procedure should be developed and validated. For cases when a full procedure is unavailable and time does not permit a procedure to be fully validated, an Ad Hoc Confirmatory Package may be assembled. (See Glossary for these terms.) The following sections list the specific elements that should be addressed in each case.

CVM Confirmatory Procedures

CVM Confirmatory Procedures are developed and validated in advance of their application. These methods should address each of the following points:

I. Validation package from originating laboratory

   A. Replicate samples

      1. Five Controls (may be subsamples from one source, but see part 1.F. below.)

      2. Five Fortified Controls at tolerance/safe level

      3. Residue-incurred:

         a. Internal (within-laboratory) validation: Five per analyte (may be subsamples from one source)

         b. Interlaboratory study (e.g., trial of NADA method): Ten total (five at each of two levels)

B. Demonstration of zero false positive rate.

C. Demonstration of ≤ 10% false negative rate at or above the tolerance or safe level is recommended (based on fortified and incurred samples). If this criterion cannot be met during method development, contact CVM.

D. Demonstration that suitable data can be acquired on more than one day. This helps to ensure that the method is rugged and under control.

E. Demonstration of non-interference by other animal drugs. It is CVM's view that interference testing should be conducted in proving the specificity of a confirmatory procedure. The selection of drugs for interference testing should be reasonable as opposed to exhaustive. For example, a reasonable selection includes drugs approved in the same species, and may include drugs from the same class of compounds, drugs of similar molecular weight, or known metabolites and degradants. Some compounds may be reasonably excluded based on obvious chemical differences. For clarification, please contact CVM.

F. Demonstration of non-interference by matrix components in control samples from more than one source (i.e., an individual animal). Note: although data to address Part I.A.1. may be acquired with subsamples from one source, more than one source should be tested.

II. Method Description (Standard Operating Procedure, SOP)

A. Scope of applicability

B. Method principles, including technique for mass spectral data acquisition.

C. Stepwise, unambiguous description of all reagents, apparatus, and steps.

D. Structure and full spectrum of marker residue.

E. Spectral data based on at least three structurally-specific ions that completely define the parent molecule (may or may not include the parent ion), or more if non-specific ions are included. Use of water loss and isotopic ions are discouraged, but will be evaluated on a case-by-case basis.

F. Proposed fragment ion structures, consistent with fragmentation pattern.

G. Justification for specificity of selected ions or scan range.

H. System Suitability parameters.

I. Confirmation criteria specified in advance (see Section III of this guidance).

J. Operational criteria for repeat injection of same sample.

K. Estimate of concentration limits for confirmation in matrix.

L. Quality Control section (see Section IV of this guidance).

III. Confirmation criteria

These criteria are an expansion and updating of criteria that CVM has applied in the past. The new, expanded criteria are in response to the use of newer mass spectral techniques for regulatory confirmation. In CVM's judgment, all methods approved prior to issuing this document meet these criteria. Criteria should be specified in the SOP in advance.

A. Comparison standard.

Comparison standard(s) should be analyzed contemporaneously. Preparation and analysis sequence of the comparison standard(s) should be fully described. Examples: first single injection prior to or after samples; average of all standards injected the day of analysis; average of two closest bracketing standards. If a matrix effect alters the spectrum or chromatography of a pure standard so that normal confirmation criteria cannot be met, a control extract containing standard may be substituted for pure standard. Confirmatory procedures that call for spiked control extracts for comparison should be justified. The tissue used as a control should be analyzed without a standard added to demonstrate the absence of interferences.

B. Chromatography/Mass Spectrometry.

Any of the following chromatograms may be used: total ion chromatogram (TIC); reconstructed ion chromatogram (RIC); all single ion chromatograms (from scan, Selected Ion Monitoring (SIM) or Selected Reaction Monitoring (SRM)). Flow injection analysis is discouraged, but will be evaluated on a case-by-case basis.

1. The chromatographic peak(s) should exceed a signal-to-noise (s/n) threshold of 3:1. The technique used for estimating s/n should be described.

2. An acceptability range for retention time matching should be specified in the SOP. The acceptability range should not exceed 2% for GC/MS or 5% for LC/MS, relative to the retention time of standard.

C. Mass spectral matching.

Refer to Section II.E. structurally-specific ions. Confirmation criteria vary depending on the technique used for mass spectral data acquisition.

1. MS^1 full scan

The mass spectrum should include at least three structurally-specific ions. The spectrum obtained from a suspect compound should visually match the spectrum obtained from a contemporaneous standard. Since full scan data may include hundreds of significant data points for comparison, strict numerical criteria need not be applied. [An acceptability range of ± 20% on relative abundance of major ions is a useful rule of thumb, but is not required. See Part III.C.2.a.] Library-

search algorithms should not be used to confirm identity. The following elements should apply when MS^1 full scan data are used:

a. All structurally-specific ions identified in Section II.E. are present above a specified level. The method developer should describe an appropriate minimum level based either on relative abundance or signal-to-noise.

b. There is general correspondence between relative abundances or ranked abundances obtained for sample and standard.

c. Prominent ions other than from the target analyte can be explained (e.g. present in controls, blanks, etc.).

d. If background subtraction is used, this should be specified in the SOP. The range used as background should always be indicated on the chromatogram.

2. MS^1 Selected Ion Monitoring (SIM).

a. Relative abundances for three structurally-specific ions should match the comparison standard within ±10%. This acceptability range is calculated by addition and subtraction. For example, at 50% relative abundance, the acceptability range would be 40-60%, not 45-55%.

b. Relative abundances for four or more unique, structurally-specific ions should match the comparison standard within ±15%.

c. Relative abundances for more than three ions, which include ions due to isotopes or loss of water, should match the comparison standard within ± 10%.

3. MS^1 scan acquisition, SIM treatment.

If scan data is acquired, the data may be treated as for SIM acquisition (Section III.C.2.).

4. MS^1 partial scan

Criteria are the same as for full scan (Section III.C.1. above). All structurally-specific ions should appear in the scan range.

5. MS^n full scan

The spectrum obtained from a suspect compound should visually match the spectrum obtained from a contemporaneous standard. Since full scan data may include hundreds of significant data points for comparison, strict numerical criteria need not be applied.

a. All structurally-specific ions identified in Section II.E. should be present above a relative abundance specified in the SOP.

b. There should be general correspondence between relative abundances or ranked abundances obtained for sample and standard.

c. If a structurally-specific precursor ion completely dissociates to product ions after MS^n, the appearance of at least two additional structurally-specific product ions in the MS^{n+1} spectrum should be sufficient.

d. Prominent ions other than from the target analyte can be explained (e.g. present in controls, blanks, etc.).

e. If background subtraction is used, the range used as background should be specified.

6. MS^n partial scan

Criteria are the same as for full scan (Section III.C.5. above). All structurally-specific ions should appear in the scan range.

7. MS^n Selected Reaction Monitoring (SRM)

a. If a precursor ion selected by MS^n is completely dissociated, and only two structurally-specific product ions are monitored in MS^{n+1}, the relative abundance ratio should match the comparison standard within ± 10%.

b. If three or more structurally-specific ions are monitored, the relative abundance ratios should match the comparison standard within ± 20%.

8. MS^n scan acquisition, SRM treatment

If MS^n scan data is acquired, the data may be treated as for SRM acquisition (Section III.C.7.).

D. New technology.

New Technology. The availability of new instrumentation has led to new options as listed above, but also to new concerns in confirmatory analysis. If the number of ions observed, their structural significance, or their mass measurement accuracy are issues, please contact CVM for further information. Such issues will be handled on a case-by-case basis.

IV. Quality Control

A. System suitability should be established before valid data can be obtained.

B. At least one control and one fortified control sample should be run each day. The fortified control should meet criteria and the control should fail criteria for the day's analyses to be valid.

C. Sufficient blanks or controls should be analyzed after standards or fortified control samples to ensure that carryover does not cause a false positive outcome.

D. Operational criteria for repeat analysis of same sample: If a sample is analyzed but it can be shown that system suitability was not adequate during that analysis, the sample may be reanalyzed after taking steps to improve system performance and reestablish suitability.

E. This document provides options for method developers, so that methods may be fit for their purpose. However, once a procedure is developed and the SOP prepared, a single set of confirmation criteria should be specified and used. Analysts should not substitute other criteria after analyses have been carried out.

***Ad Hoc* Confirmatory Packages**

Ad Hoc Confirmatory Packages should meet or exceed the following minimal data recommendations:

Ad hoc data packages arise when new procedures are applied in response to unanticipated situations, when full confirmatory procedures are unavailable, and when time does not permit a procedure to be fully validated. CVM's confidence in ad hoc data packages is based on good quality assurance, good training, and high expertise in the laboratory. The following analyses are the minimum recommended for an ad hoc data package, although additional supporting data is strongly encouraged (see above). All recommendations for structural specificity (Section II.B-F.), confirmation criteria and recommendations for treatment of data (Section III.), and quality control (Section IV.) still apply.

I. At least two control samples should be analyzed. No control sample should meet criteria (i.e., give a false positive outcome). A surrogate control is from a similar matrix known to be free of the suspect compound, but is used to simulate the same matrix. A survey control is from the same type of matrix, but is of unknown origin, and has been analyzed repetitively and found to fail confirmation in every case.

II. Control samples fortified with the suspect compound should meet confirmation criteria. At least two control samples should be fortified at the suspect compound's tolerance or safe level. If the suspect compound has no tolerance or safe level, at least four fortified control samples should be prepared: two above and two near the suspect compound's estimated level.

III. Sufficient replicate injections of the standard should be made to establish system suitability.

IV. A blank or control sample should be analyzed after a fortified sample or standard to demonstrate that carryover does not cause a false positive outcome. Otherwise, blanks should be analyzed after each sample until the blank analysis appears free of the suspect compound.

Exact Mass Measurements

The following recommendations apply to the use of exact mass measurements in confirmatory analyses. Until specific standards for exact mass measurements in animal drug residue analysis are generally accepted, their use will be evaluated on a case-by-case basis. Multiple structurally-specific ions should be used to confirm the presence of a compound of a specified structure.

* The mass spectrometer design and operating conditions used to assign an exact mass (e.g., analyzer geometry, resolution, lock masses, mass range) should be described.

* Mass resolution and peak purity should be demonstrated to be sufficient to provide only one predominant component per mass peak in the range of the peak of interest.

* Suitability should be demonstrated by applying the method to a standard of known composition to show that mass accuracy is acceptable. Accuracy should be reported as parts-per-million (ppm) = [measured – calculated mass] x 10^6 / calculated mass.

* The possibility of interferences within the mass measurement accuracy of the instrument should be evaluated. Candidate compositions should be calculated using a range of reasonable elements, including but not limited to C, H, N, and O. The candidate compositions should be shown, along with the ranges of each element used to generate them. At low mass (well below m/z 500) < 5 ppm difference may be sufficient to confirm a unique elemental composition. However, as mass increases, the chance of interference increases dramatically. At roughly m/z 500 and above, many elemental compositions can occur within a 5-10 ppm window around the theoretical mass.

* If multiple candidates occur within the mass measurement accuracy of the instrument, the alternatives should be evaluated for their reasonableness. Parameters to consider could include the number of heteroatoms, the isotopic distribution pattern, or results of other chemical analyses.

* If all alternative compositions cannot be excluded by virtue of the instrument's mass accuracy or the unreasonableness of the alternatives, then exact mass measurements do not improve the analysis relative to nominal mass measurements.

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GLOSSARY

***Ad Hoc* Confirmatory Package** - A data package accompanied by a conclusion that is supported by the data. *Ad hoc* data packages may be acceptable when a CVM Confirmatory Procedure is unavailable, and time does not permit a procedure to be fully validated. Examples of the need for such procedures include unanticipated misuse of an approved drug; unanticipated use of an unapproved drug, suspected presence of drug in unexpected tissue matrix, or sabotage of food products.

Comparison Standard - The reference standard which is analyzed contemporaneously with unknown samples. The mass spectrum and retention time from the sample are evaluated against the corresponding data from the standard.

Confirmation - Unambiguous identification of a compound's presence by comparison to a reference standard (mass spectrometric).

Control Sample - A control sample is the same tissue type as the target tissue, but is known to be free of target analyte. A fortified control sample is a control sample to which target analyte has been added.

CVM - FDA Center for Veterinary Medicine

CVM Confirmatory Procedure - A procedure that CVM considers valid for regulatory analyses. This procedure should include a stepwise description of the method for evaluation by CVM. Such procedures can be developed in advance of their application because their need can be anticipated. Examples of the need for such procedures include the approval process for new animal drugs and preparation for surveys of suspected drug misuse. Such procedures are reviewed by CVM prior to a laboratory evaluation of the procedure at CVM. The evaluation consists of a sample set corresponding to Section I.A-D (see above).

Exact Mass Measurement - A mass assignment to more than one decimal place.

False Negative Rate - The percent of samples known to contain a marker residue which do not meet the confirmation criteria.

False Positive Rate - The percent of samples known not to contain a marker residue which do meet the confirmation criteria.

Full Spectrum - A mass range encompassing all diagnostic data or the full width of instrumental capability. For example, full spectrum may include both the molecular ion and low molecular weight fragment ions.

Limit of Confirmation (lower) - The method's limit of confirmation may be defined as the concentration where the weakest diagnostic ion no longer appears at an acceptable signal-to-noise level or where the false negative rate becomes excessive. The estimate of limit of confirmation is recommended for two reasons: to show that the method is sufficiently sensitive for its purpose, and to predict transferability of the method to other laboratories.

Marker Residue - The residue selected for assay whose concentration is in a known relationship to the concentration of the residue of concern in the last tissue to deplete to its permitted concentration.

MS^n - Two or more stages of mass separation conducted sequentially.

Residue - Any compound present in edible tissues of the target animal which results from the use of the compound, including the compound, its metabolites, and any other substances in or on food because of the compounds' use.

Safe Level – A conservative estimate of a drug residue level in edible animal tissue derived from food safety data or other scientific information. Concentrations of residue in tissue below the safe level will not raise human food safety concerns. A safe level is not a safe concentration or a tolerance and does not indicate that an approval exists for the drug in that species or category of animal from which the food is derived.

Standard Operating Procedure (SOP) - A stepwise written procedure for carrying out an analytical method.

Structurally-Specific - Characterizing a compound's molecular weight and/or unique substructure. For a molecule to be completely defined, the spectral data should be unique to that compound and none other.

Suitable Data - Data acquired when system suitability has been met.

System Suitability - The fitness of analytical instruments for the purpose at hand, based on manufacturer specifications, instrumental Standard Operating Procedure, or specific requirements of the analytical method. Suitability may be established through verification of relevant instrumental parameters such as calibration, pressure, flows, temperature, multiplier gain, etc., or through verification of method-specific parameters such as signal-to-noise level for a known amount injected, peak shape, test spectra, etc.

Target Analyte - The chemical entity that a particular method is designed to detect.

Target Tissue - The edible tissue selected to monitor for residues in the target animals, including where appropriate, milk or eggs.

Tolerance (Rm) - The concentration of the marker residue in the target tissue when the residue of concern is equal to the permitted concentration in the last tissue to deplete to its permitted concentration.

Validation - Demonstration that a method performs as claimed through a defined experimental protocol and data evaluation. A claim that a mass spectrometric method is valid for confirmatory analyses should be supported with experimental results.