



Memorandum

Date: September 24, 2015

From: FDA Foods and Veterinary Medicine Science and Research Steering Committee

Subject: Acceptance Criteria for Confirmation of Identity of Chemical Residues using Exact Mass Data within the Office of Foods and Veterinary Medicine

To: FVM Executive Council

The FDA Foods and Veterinary Medicine (FVM) Science and Research Steering Committee (SRSC), made up of representatives from the Office of Foods and Veterinary Medicine, the Center for Food Safety and Applied Nutrition, the Center for Veterinary Medicine, the Office of Regulatory Affairs, the National Center for Toxicological Research, the Office of International Programs, and the Office of the Chief Scientist, is charged with the task of prioritizing, coordinating and integrating food- and feed-related science and research activities across the operating units of FDA's FVM Program.

As a regulatory agency tasked with ensuring the safety of the nation's food supply, it is imperative that the laboratory methods needed to support regulatory compliance, investigations and enforcement actions meet the highest analytical performance standards appropriate for their intended purposes. The attached document, now formally adopted by the SRSC, establishes acceptance criteria for confirmation of identity of chemical residues using high resolution mass spectrometry (HRMS) within the FDA Office of Foods and Veterinary Medicine (OFVM) Program. In 2002 FDA Center for Veterinary Medicine (CVM) published Guidance for Industry (#118) titled "Mass Spectrometry for Confirmation of the Identity of Animal Drug residues". Details of criteria for various types of unit-resolution MS were given, but specific guidelines were not provided for HRMS. With recent technical advances in HRMS and its increased use in the analysis of foods and veterinary medicines, it is imperative to supplement the existing guidance so that users of HRMS are consistent in evaluating and comparing results for regulatory use. This new guidance will harmonize the interpretation of the HRMS data across all of OFVM. In addition, as multi-class, multi-residue methods are commonly used within OFVM, this supplemental guidance will cover more than just veterinary drugs, e.g., pesticides, chemical contaminants, and natural toxins. In the near future, we plan to post this document on FDA's website and additional venues for publication and dissemination of these guidelines are being explored and will be announced when they become available.

Thank you,

Palmer Orlandi, Jr., Ph.D., Chair
FDA FVM Science and Research Steering Committee
Acting OFVM Chief Science Officer/Research Director

**Acceptance Criteria for Confirmation of Identity of
Chemical Residues using Exact Mass Data for the
FDA Foods and Veterinary Medicine Program**

**US Food & Drug Administration
Office of Foods and Veterinary Medicine**

September 2015

Acceptance Criteria for Confirmation of Identity of Chemical Residues using Exact Mass Data for the FDA FVM Program

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Center for Food Safety and Applied Nutrition (CFSAN)

Office of Regulatory Science
Office of Food Safety
Office of Applied Research and Safety Assessment

Center for Veterinary Medicine (CVM)

Office of Research
Office of New Animal Drug Evaluation

Office of Regulatory Affairs (ORA)

Office of Regulatory Science
ORA Laboratories

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APPROVAL PAGE

This document is approved by the FVM SRSC. The FVM SRSC Project Manager is responsible for updating the document as change requirements are met, and disseminating updates to the SRSC and other stakeholders, as required.

APPROVED BY:

OFVM Chief Science Officer/Research Director

CVM, Deputy Director for Science Policy

OFVM Senior Science Advisor

CVM, Director, Office of Research

CFSAN Senior Science Advisor

ORA, Director Office of Regulatory Science

CFSAN, Director, Office of Regulatory Science

ORA, Director, Food and Feed Scientific Staff

CFSAN, Director Office of Applied Research &
Safety Assessment

ORA, Member of the ORA Scientific
Advisory Council

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**US Food & Drug Administration
Office of Foods and Veterinary Medicine**

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Acceptance Criteria for Confirmation of Identity of Chemical Residues using Exact Mass Data for the FDA FVM Program

1.0 INTRODUCTION

In 2002 the FDA Center for Veterinary Medicine (CVM) published Guidance for Industry (#118) titled “Mass Spectrometry for Confirmation of the Identity of Animal Drug residues”. Details of criteria for various types of unit-resolution mass spectrometry (MS) were given, but specific guidelines were not provided for high resolution mass spectrometry (HRMS). With recent technical advances in HRMS and its increased use in the analysis of foods and veterinary medicines, it is imperative to supplement the existing guidance so that users of HRMS are consistent in evaluating and comparing results for regulatory use. This new guidance will harmonize the interpretation of the HRMS data across all of OFVM. In addition, as multi-class, multi-residue methods are commonly used within OFVM, this supplemental guidance will cover more than just veterinary drugs, e.g., pesticides, chemical contaminants, and natural toxins.

HRMS can significantly expand the scope of analytical methods used to monitor for chemical residues. Rather than targeting a select number of expected residues, HRMS analysis with a wide scan range permits the simultaneous detection of a large number of unexpected contaminants. Full spectrum data generated by HRMS allows retrospective analysis. Potentially, queries can be conducted to find information about novel residues in addition to the target residue in previously acquired raw data without reanalysis of samples. Also, the exact mass(es) obtained by HRMS allows for the elucidation of molecular formulas and for library searching in non-targeted screening. Newer generation HRMS instruments are capable of operating at a resolution that can resolve co-eluting isobaric compounds often encountered in complex matrices.

There are only a few published guidances containing information on confirmation of identity of drug residues by HRMS. See Appendix 1 for a detailed description of these guidances. However, none of these documents provide sufficient information to adequately meet the needs of OFVM for using HRMS in residue analysis.

For the purpose of this document, a high resolution mass spectrometer will be an instrument which is consistently measuring at a resolving power greater than 10,000 at FWHM at the peak (m/z) of interest. The most frequently used HRMS instruments in FDA laboratories are based on time-of-flight (TOF) and Orbitrap technology. See Appendix 2 for a detailed description of the mass analyzers.

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2.0 SCOPE

This document is applicable for the confirmation of identity of chemical residues using HRMS for the FDA FVM Program, including but not limited to the following applications:

- The confirmation and identification of small molecules with a molecular weight range typically less than 1000 Daltons at residual levels. Such chemicals include veterinary drugs, pesticides, dyes, food or feed additives, and other natural or synthetic contaminants.
- The applicable matrices include foods of animal and plant origin, animal and pet feeds, ingredients used in the preparation of foods and feeds, dietary supplements, cosmetics, and other FDA regulated commodities that fall within the purview of OFVM.
- The primary focus is the use of HRMS for targeted analysis when the comparison standard is available, although aspects of non-targeted analysis are discussed.
- For the purposes of this document, the definition of “confirmation of identity” is consistent with CVM guidance 118 and is defined as the unambiguous identification of a compound’s presence by comparison to a reference standard (mass spectrometric). It is understood that “confirmation” is also defined in other regulatory documents to mean agreement of two independent analyses. However, the purpose of this document is to describe the specific criteria used to evaluate HRMS data for a residue in a regulatory sample by comparing to a reference standard to confirm its identity.
- Other uses of HRMS in support of regulatory actions within the OFVM.

This guidance is based on accumulated experience in using commercially available HRMS instruments for the analysis of contaminants in food. It cannot anticipate every use of the technology and therefore is not applicable in all cases. Also, the capability of commercially available HRMS instruments is improving over time. However, it is essential that when good science requires one to deviate from this guidance, the reasons for the deviation are explicitly given. This guidance is not applicable to the HRMS methods established by FDA for persistent organic pollutants, including dioxins, due to highly specialized instrumentation and procedures used and well established standard procedures.

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3.0 CONFIRMATION CRITERIA

The confirmation criteria documented in this section expand and update the criteria documented in CVM Guidance for Industry #118. The new criteria stated below are in response to the use of exact mass measurements generated by the use of HRMS instrumentation.

Mass Extraction Window (MEW):

In full scan mass spectrometric measurements using HRMS, the selectivity for a particular analyte is determined by the narrowness of the mass extraction window (MEW) that is used to obtain the extracted ion chromatogram (EIC) of the target analyte. However, selection of an overly narrow MEW can result in a distorted (corrupt) peak resulting in a false negative finding for the worst cases. Yet a too wide MEW may lead to a high rate of false positive findings. Either situation can occur when there are co-eluting ions of nearly the same exact mass. The likelihood of such an event will increase when attempting to detect low levels of an analyte in complex matrices. Therefore, the MEW has to be selected by careful consideration of the resolution of the instrument, drift of the mass axis and the complexity of the matrix. It is recommended that the optimum MEW be experimentally determined from analysis of standards in matrix using the same chromatographic and sample preparation procedures used in the method.

Signal requirement:

EIC generated with narrow MEW from HRMS may produce baselines free of any background noise. Calculation of S/N ratio is not feasible under such conditions. If there is noise, a S/N threshold ≥ 3 is recommended. When there is no noise, relative signal intensity acquired from the test sample vs. a comparison standard can be used to set up the threshold to recognize an EIC peak.

Retention time:

The retention time must match comparison standard within one of the following limits: (1) ≤ 0.2 min; or (2) within $\pm 2.5\%$, not to exceed 0.5 min; or (3) within experimental error (multiples of standard deviation) established in the validation method, not to exceed 0.5 min. The EIC of all ions derived from an analyte using the same MEW must co-elute. Matrices may shift analyte retention times in which case matrix matched standards or standard additions might be necessary.

Mass accuracy:

For confirmation of identity, the measured exact mass of at least two ions (preferably a structurally significant fragment or product ion in addition to precursor ion) should have a mass accuracy of ≤ 5 ppm in the MS¹ mode and ≤ 10 ppm in the MS/MS mode, as is calculated by:

$$\text{Mass accuracy (ppm)} = \frac{\text{Measured mass} - \text{Calculated mass}}{\text{Calculated mass}} \times 10^6$$

Ion Ratio:

If the measured exact mass from two or more ions match the mass accuracy criterion, it is not necessary to calculate and report ion abundance ratios. If, however, the measured mass error is greater than the mass accuracy criterion, the ion ratio criteria for nominal mass data as described in CVM guidance 118 or ORA-LAB010 shall apply.

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Table 1. Summarized Requirements for Confirmation of Identity

MS mode	MS ¹	MS/MS	MS ¹ and MS/MS
EIC: signal requirement (absolute)	A criterion to be set by one of the following methods: (1) a S/N threshold ≥ 3 ; (2) an intensity ratio relative to the comparison standard equal or above a preset threshold		
EIC: retention time, relative to comparison standard	A criterion to be set by one of the following methods: (1) ≤ 0.2 min, or (2) within $\pm 2.5\%$, not to exceed 0.5 min, or (3) within an established error range, not to exceed 0.5 min		
MS: number of structurally significant ions	Minimum 2	Minimum 2	Minimum 2 combined
MS: mass accuracy	≤ 5 ppm	≤ 10 ppm	MS ¹ : ≤ 5 ppm; MS/MS: ≤ 10 ppm

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4.0 NON-TARGETED ANALYSIS

Identification of compounds detected in a sample that were not set *a priori*, i.e., unexpected in the sample, is defined as non-targeted analysis. HRMS provides one of the most promising tools in non-targeted residue analysis. See Appendix 3 for details.

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5.0 GLOSSARY

EIC – Extracted ion chromatogram, created by plotting the intensity of the signal observed at a chosen m/z value or set of values in a series of *mass spectra* recorded as a function of retention time.

TIC – Total ion chromatogram, created by plotting the total ion intensity (count) in a series of mass spectra recorded as a function of retention time.

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 (comparison standard).

Calculated exact mass – Calculated from a molecular formula using known masses of specific (usually the most abundant) isotopes to at least four decimal places.

Measured exact mass (exact mass measurement; accurate mass) – An experimentally determined exact mass.

Mass extraction (tolerance) window – When processing raw MS data to generate EIC, the parameter that sets the range around the target m/z so that all ions within this range are counted as belonging to this m/z . The size of the extraction window typically depends on the mass accuracy and mass resolution of the instrument.

FWHM – Full width, half maximum of an ion peak in a full-scan mass spectrum. See Figure 1.

HRMS (high resolution mass spectrometry) – In this document, it refers to a MS instrument that can give at least 10,000 nominal mass resolving power at FWHM for the compound of interest.

Residue – Any compound present in the sample, in the form of the compound itself, metabolites, chemical derivative, degradant, etc.

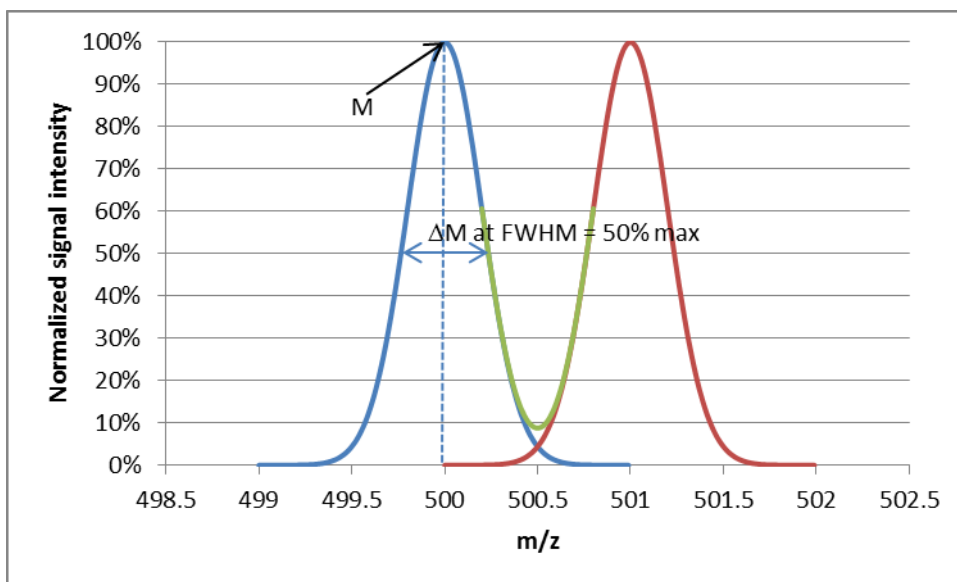
Relative ion abundance – For a particular m/z , the number of detected ions or signal intensity in a mass spectrum relative to that of the most abundant ion.

Resolving power – The ability of a mass spectrometer to separate ions of two different m/z values above a certain valley threshold. Mathematically it is expressed as $M/\Delta M$ (at FWHM; See Figure 1). Resolving power can be a function of m/z depending on type of instrument.

Mass resolution Is the inverse of resolving power ($=\Delta M/M$). Note that there are alternative ways to calculate resolving power, which are not adopted in this guidance document.

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Figure 1. Graphical illustration of FWHM



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APPENDIX 1 - Published Guidances Containing Confirmation Criteria for HRMS

- 1) EU directive 2002/657/EC: This document includes HRMS and employs a point system for the interpretation of confirmatory results. An ion acquired by HRMS is given more points than by unit resolution MS. In this directive, HRMS is defined as MS at a mass resolution of 10,000 according to the 10% valley definition, roughly equivalent to 20,000 FWHM. However, no criteria for mass accuracy have been set in 2002/657/EC.
- 2) SANCO/12571/2013: "Method validation and quality control procedures for pesticide residue analysis in food and feed": A comprehensive list of identification requirements for both low (unit) and high resolution MS data including requirements for mass accuracy are tabulated. This document does not utilize an identification point system as in 2002/657/EC.
- 3) ORA-LAB.010 (published in 2009): "Guidance for the Analysis and Documentation to support Regulatory Action on Pesticide residues": a point system (including use of HRMS) very similar to 2002/657/EU was employed for identification and confirmation.

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APPENDIX 2 – Description of Different Mass Analyzers

TOF mass analyzers: Ions are accelerated in an electric field prior to entering a field-free drift tube. Ions with the same charge enter the drift tube with the same kinetic energy. This results in ions with smaller mass-to-charge needing less time to travel through the drift tube while ions with larger mass-to-charge require more time. More specifically, the drift time is proportional to the square root of the mass-to-charge of the ion of interest.

Orbital trap mass analyzers: The mass analyzer consists of two outer electrodes surrounding what has been best described as a “spindle-like” central electrode. Ions are isolated both radially and axially around the center electrode using an electric field generated using the outer and central electrodes. The axial oscillations are then measured using the outer electrodes as the receiver plates. The frequency of the oscillation is then used to determine the mass-to-charge of the ion.

Table 2 lists various mass analyzer platforms and compares the resolving power (FWHM), mass accuracy (ppm), mass range (Da), linear dynamic ranges and sensitivity of each. The values listed are for comparison purposes only, and may represent the best or specific case scenario under a certain set of conditions, since hardware is vendor dependent and factors such as chemical size, structure and property, matrix effects, and instrumental conditions (scan speed, scan range, etc.) can affect these values. The purpose of the table is to reveal the differences between typical unit resolution instruments (triple quadrupole and quadrupole ion trap) and high resolution, full-scan instruments based on present instrument capabilities. Recent developments and advances in high resolution technologies will eventually lead to improvement in resolving power capabilities and other parameters in future instruments.

Table 2. Comparison of resolving power (FWHM), mass accuracy (ppm), mass range (Da), linear dynamic ranges and sensitivity of various mass analyzer platforms

Mass spectrometer type	Resolving power	Mass accuracy (ppm)	Mass range (Da)	Linear dynamic range	On-column sensitivity (acquisition mode)
Magnetic sector (double focusing)	~100,000	< 1	5 – 15,000	10 ⁹	fg (full scan)
Triple quadrupole	~7,500	100	5 – 2,000	10 ⁶	fg – pg (SRM)
Quadrupole ion trap	>10,000	100	5 – 2,000	10 ⁶	fg - pg (SRM)
Time of flight	>40,000	< 1	5 – 40,000	10 ⁶	pg (full scan)
Orbitrap	>140,000	< 1	50 – 6,000	10 ⁴	fg - pg (full scan)
Fourier transform ion cyclotron resonance	>1,000,000	< 1	100 – 10,000	10 ⁵	pg (full scan)

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APPENDIX 3 – Non-targeted Analysis

This section describes the Agency's current thinking on non-targeted analysis, and it should be viewed only as recommendations. In this document, non-targeted analysis is defined as LC/HRMS analysis of compounds outside of a target list, i.e., any compounds that are unexpected in a sample. A number of software platforms exist for non-targeted workflows. The basic workflow for non-targeted screening consists of:

1. Assessment of a chromatographic peak
2. Interpretation of the detected ions
3. Formula generation
4. Database searching

Chromatography. Chromatographic resolution is often overlooked in non-targeted analysis, and scientists should try to obtain, within reason, optimum chromatography to retain and resolve as many compounds as possible. For complex samples where there is little chromatographic resolution, identification of individual compound by HRMS alone can be challenging. Studies have shown that in mass spectrum generated by Orbitrap MS, when there is a lack of chromatography, ion peak coalescence can occur between isobaric compounds, which translated to up to 12 ppm mass error in some cases¹. In addition, when there is a lack of chromatography, ion suppression can skew the mass spectra.

It is also important to determine the MEW used in the post-acquisition data processing to generate EIC, leading to the overall selectivity of the HRMS process. Currently there is no agreement about a proper value of MEW in non-targeted analysis for generation of EIC. In practice, the MEW can be set at < 20 ppm for a TOF operating at ~20,000 resolving power. High resolving power (> 50,000) is needed when using narrower mass extraction windows (< 5 ppm) for low analyte concentrations (e.g., ppb level) in complex matrices (e.g., animal feed). To avoid missing potential hits when using exact mass data for screening purposes, the MEW could be set wider. Any presumptive positives found with a wider MEW would then need to be further evaluated using additional product or isotope ions as well as retention time matching to a contemporary standard when available.

Interpretation of the detected ions. In typical non-targeted analysis, deconvolution or other appropriate algorithm for peak picking and removal of background noise can be used as the first step of data analysis. Just as the isotopic distribution can be an indication of halogenation, the isotopic ratio of A, A+1 and A+2 can provide information for the elemental composition. The S/N of these ions must be significant in order to differentiate the analyte from the background and the isotopic ratio distribution should meet a pre-determined criteria. It is equally important to look at the mass spectral peak shape. Any shouldering could indicate that there is more than one component contributing to the signal.

Formula generation. Molecular formulas are assigned to the peaks of interest based on exact mass measurement and other information such as isotope pattern. Software is available from different vendors for different stages of this process. To elucidate the correct formula of a completely resolved compound with an 80-99% probability, the mass accuracy should be within 3 ppm and a maximum of 5% absolute isotope ratio deviation should be observed².

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Database searching. The generated formulas are then searched against databases such as an in-house library, Metlin, PubChem, ChemSpider, etc., for the identification of the unknowns. However, one has to be aware that the libraries contain only a limited number of compounds and are not error free.

The most critical and challenging step in the non-targeted screening process is confirmation of detected unknowns. Multiple hits obtained through a library search can be refined by evaluating fragmentation patterns obtained through MS/MS experiments. Final confirmation should be carried out by comparison to a standard using the criteria included in the targeted section of this document. In cases where a comparison standard is not available, orthogonal methods such as NMR may be used and are typically needed for unambiguous identification.

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