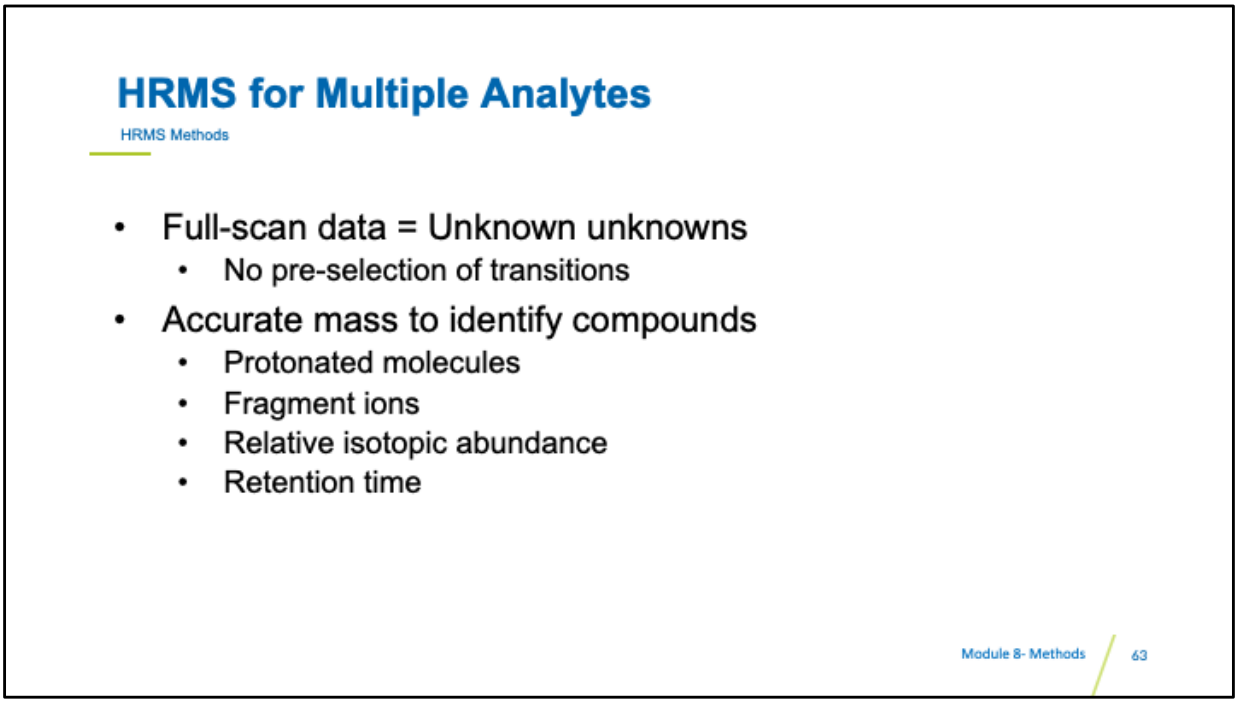
SECTION 4
Introduction to HRMS Method

Welcome to section 4 where we introduce high resolution mass spectrometry methods.

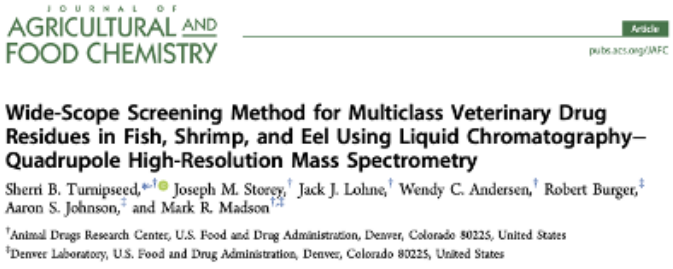


Using high-resolution mass spectrometry (HRMS) instruments, a virtually unlimited number of compounds can be simultaneously analyzed because the full-scan data are collected rather than preselected ion transitions corresponding to specific compounds. Selectivity is achieved by taking advantage of the instrument's ability to provide accurate mass measurements. Residue identification can be based on the calculated exact masses of protonated molecules and fragment ions, relative isotopic abundances, and retention times. This can lead to the development of methods that can monitor for a wide scope of residues and contaminants

Considerations

HRMS Method

- Need high-enough mass resolution to measure exact mass in complex matrix
- Sensitive enough for trace levels (10 ppb)



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Article

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Wide-Scope Screening Method for Multiclass Veterinary Drug Residues in Fish, Shrimp, and Eel Using Liquid Chromatography–Quadrupole High-Resolution Mass Spectrometry

Sherri B. Turnipseed,*[†] Joseph M. Storey,[†] Jack J. Lohne,[†] Wendy C. Andersen,[†] Robert Burger,[‡] Aaron S. Johnson,[‡] and Mark R. Madson[†,‡]

[†]Animal Drugs Research Center, U.S. Food and Drug Administration, Denver, Colorado 80225, United States
[‡]Denver Laboratory, U.S. Food and Drug Administration, Denver, Colorado 80225, United States

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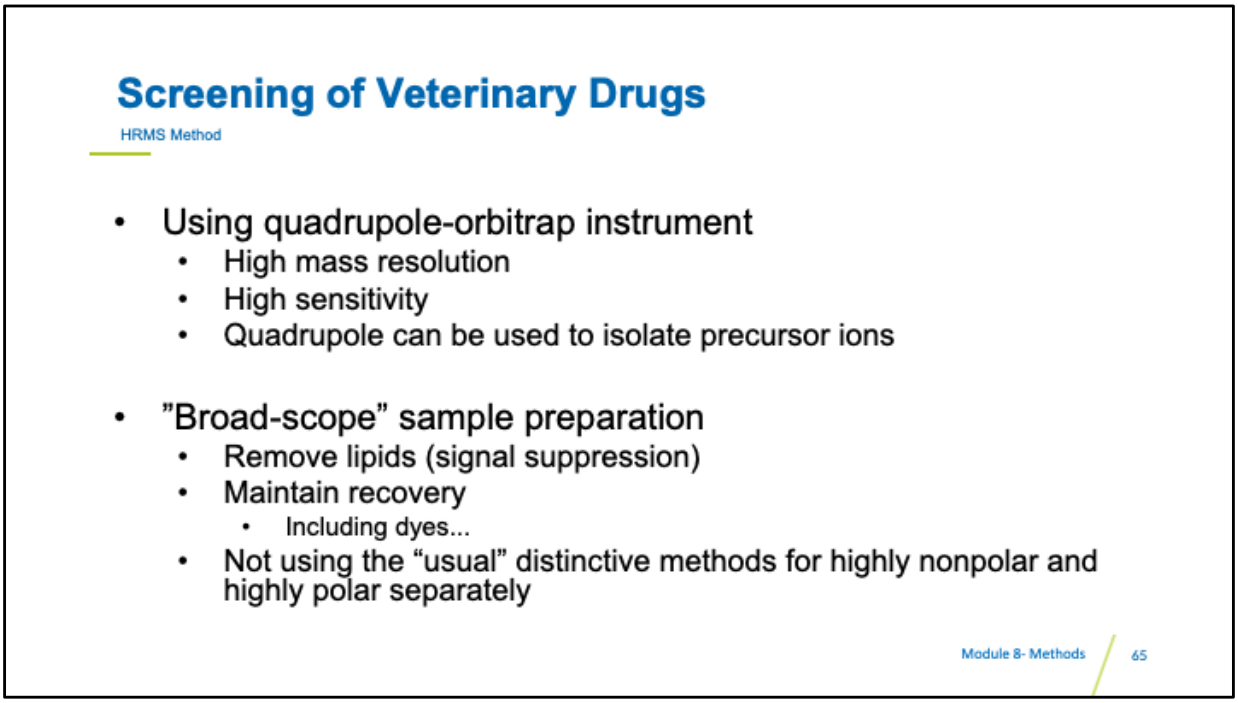
In this section, we are looking at the example of a method used at the US FDA's Office of Regulatory Affairs, the enforcement arm of the FDA. They use it for veterinary drug residues in fish and seafood.

For those who are familiar with drug residues in these commodities, the difficulty is that some drugs are very polar while others are very nonpolar. So we typically split the sample preparation into two streams. One of the objectives here was a good compromise –single stream of sample preparation, and then seeing if the recovery and identification criteria could be met.



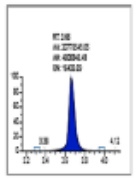
The method uses a quadrupole-orbitrap instrument, which means that it has the ability to isolate precursor ions in addition to measuring exact mass.

The broad scope sample preparation, what I called the compromise before, includes steps to remove lipids because they cause ion suppression in mass spectrometry, and then a very generic extraction and clean-up. We will discuss these further in the laboratory. For now, we focus on the instrumentation.

Criteria

HRMS Method

- Screening “presumptive positives”
  - Precursor ions must be present (signal-to-noise > 3) and match theoretical exact mass within a 5-ppm mass tolerance
  - Retention time match to a standard injected the same day was typically observed (±0.1 min)
  - At least one fragment ion with 500 count minimum intensity threshold within a 10-ppm maximum mass deviation window
  - A sample would be considered presumptive positive for a test compound if the qualitative criteria were met and the signal was ≥50% as compared to the matrix-extracted standard fortified at the TTL.



Lincomycin
Quan Peak: 407.22103 m/z
RT: 3.66 min
Amount: 11 ng/g

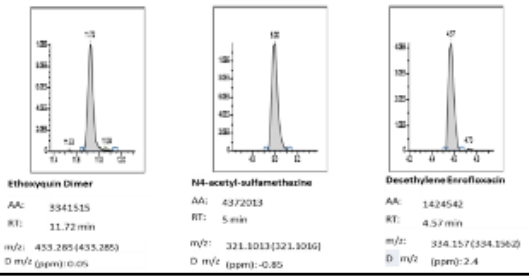
The criteria used used in this method to accept a presumptive positive were: the precursor ions must be present (with a signal-to-noise > 3) and match theoretical exact mass within a 5 ppm mass tolerance. The retention time match to a standard injected on the same day was typically observed (±0.1 min), at least one fragment ion with 500 count minimum intensity threshold within a 10 ppm maximum mass deviation window must be present.

A sample would be considered presumptive positive for a test compound if the qualitative criteria were met and the signal was ≥50% as compared to the matrix-extracted standard fortified at the target test level. The peak copied from the publication illustrates the excellent signa to noise obtained.

Criteria

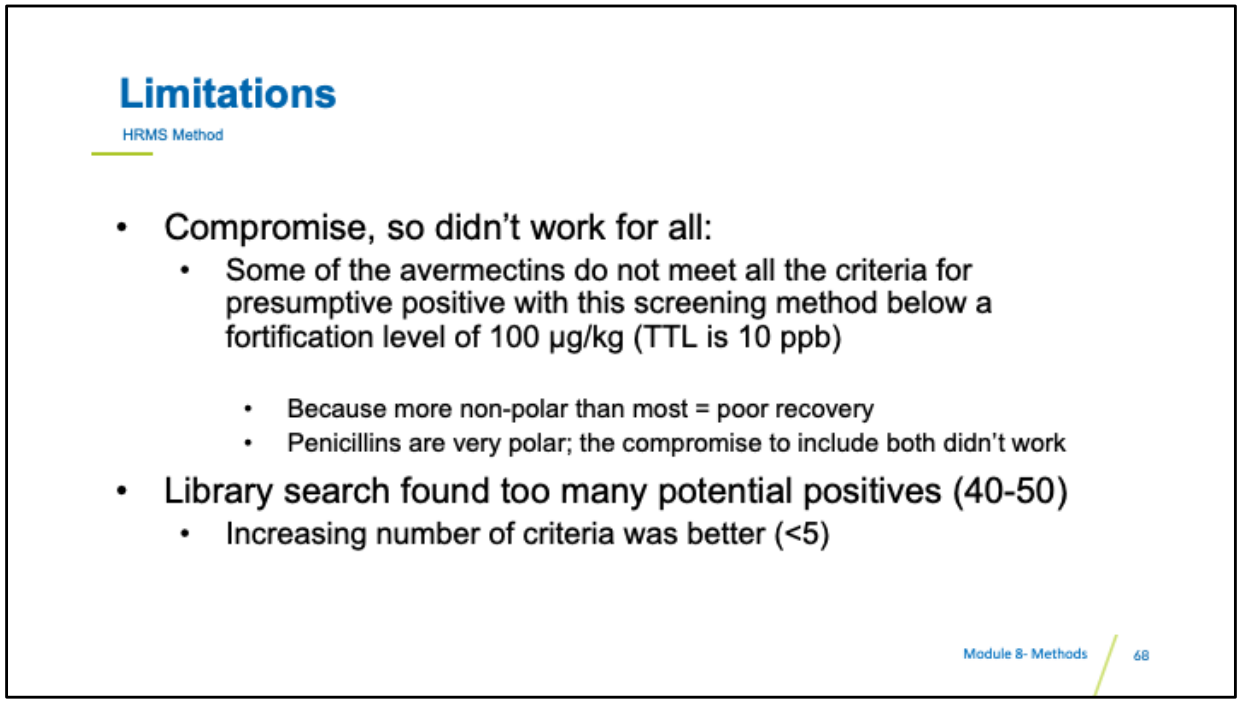
HRMS Method

- Unknown unknowns
  - A Thermo TraceFinder "Screening Method" was used to search for additional residues beyond the test compounds.
    - Data collected using AIF were compared to a compound database containing >330 potential veterinary drug residues, including metabolites and minor components.



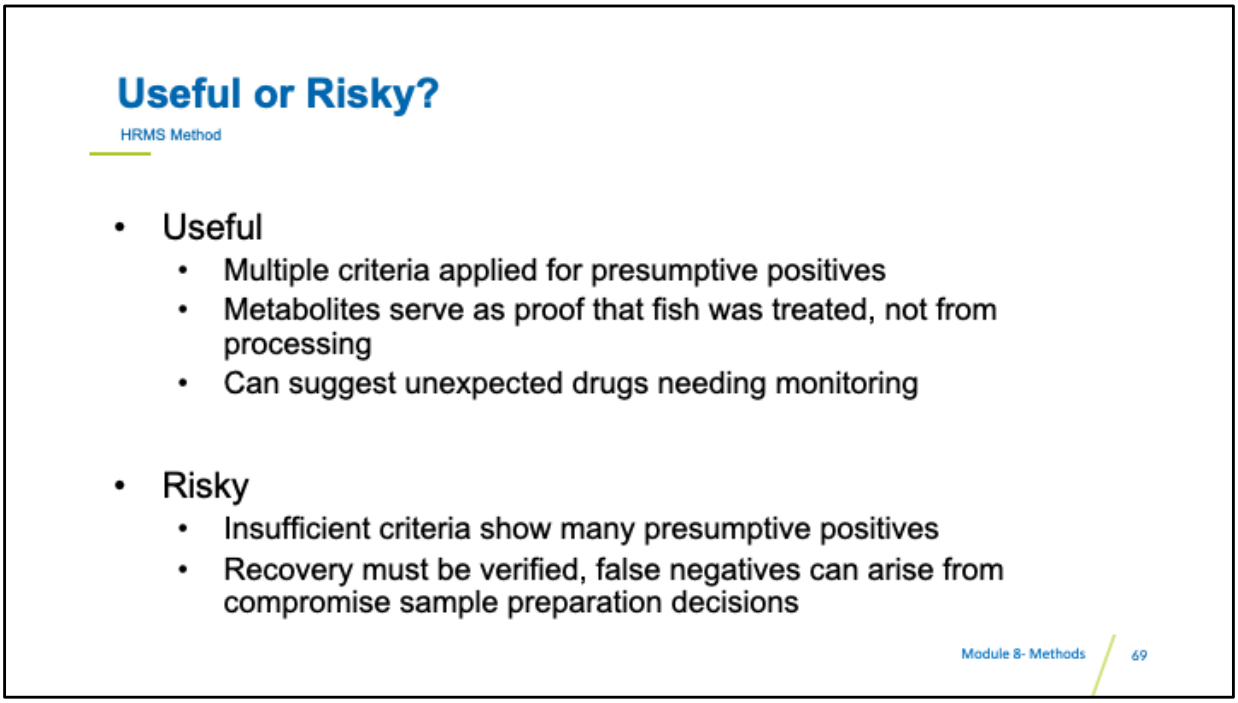
The previous criteria were for known-unknowns since they included a specific verification of precursor ion. For unknown-unknowns, a library search was used with the software TraceFinder from Thermo. The "all ion fragmentation" mode, AIF, was used for this. AIF is a nontargeted method in which a full scan MS is followed by an MS^2 scan where all precursors are allowed into the high collision dissociation cell to form product ions simultaneously. The library contained over 330 potential veterinary drug residues, including metabolites and minor components

The peaks at the bottom part of the slide once again show excellent S/N, and in this case the difference in m/z for these presumptive compounds in calculated. We can see that it ranges between 0.05 and 2.4 ppms in this cases.



This method was a compromise, and it ended up not working for all the analytes of interest. Some of the avermectins do not meet all the criteria for presumptive positive below a fortification level of 100 µg/kg. The target test level is 10 ppb, so this is not good enough. It is probably due to their being more nonpolar than most, which led to poor recovery. Penicillins are also very polar and the compromise to include both highly polar and non-polar didn't work.

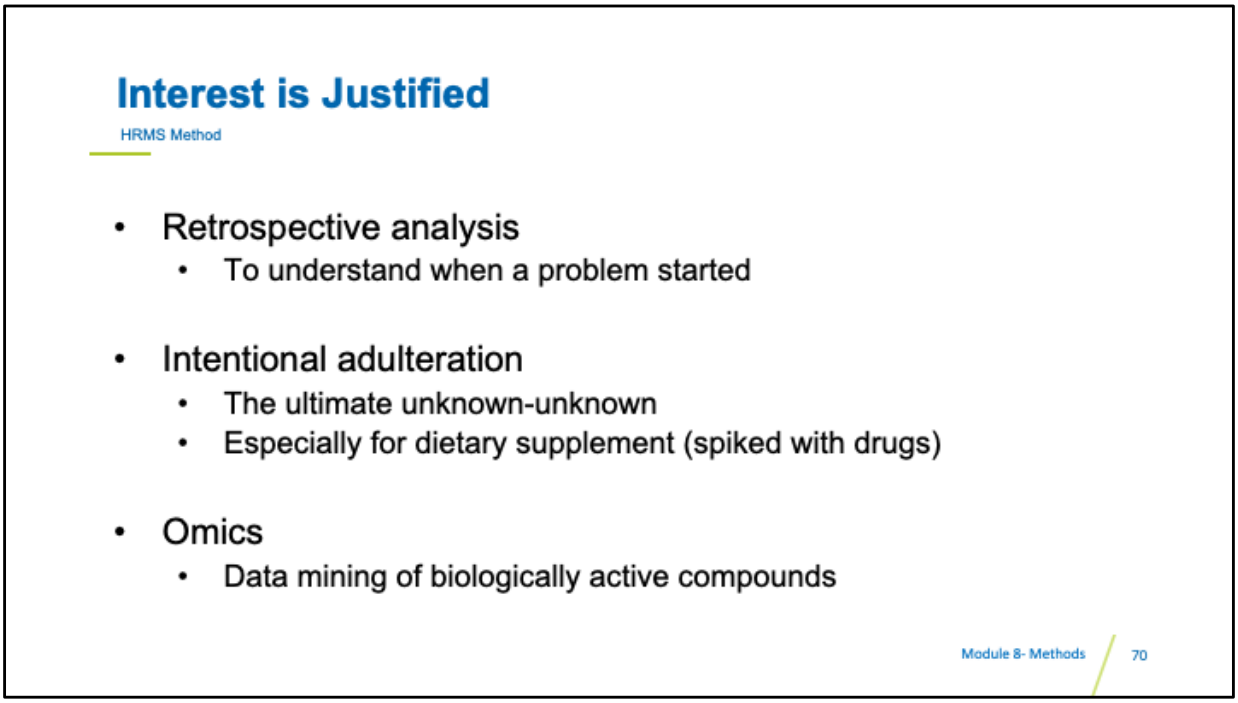
The library search used for the non-targeted identification of unknowns found too many potential positives to be useful at 40-50. Increasing the number of criteria led to a more realistic and useful set of less than 5 potential positives.



The important question is whether the method useful or too risky to deploy? First and foremost, it is useful when multiple criteria were applied to identify a presumptive positive, which gives confidence in the results. In addition, metabolites were included in the library, which serves to prove that the fish was treated and metabolized the drug, as opposed to a contact with the drug during storage or processing. The most useful outcome of the method for a regulatory agency like the FDA that must always be on the lookout for the next problem is that it can suggest unexpected drugs needing monitoring. This information would be very difficult to obtain from an MS/MS measurement.

But the method is also risky. It led to too many presumptive positives when insufficient criteria were used. This was spotted by the research team because they are very knowledgeable about the drugs used in aquaculture and could see that some of the hits simply did not make sense. So they were able to adjust the number of criteria. A less knowledgeable application team in a regulatory laboratory may feel that they need to confirm all 40 to 50 presumptive positives, an amount of work that is far beyond the capacity of any laboratory. In the worst-case scenario, a regulatory agency could take action on these results if they did not understand the risks associated with a library search without confirmation with standards.

Finally, there is always a risk of jeopardizing recovery for some analytes when compromising to include a very large number of analytes or commodities, which could lead to false negative results.



HRMS is a promising technique in the food safety laboratory. Some of the areas receiving the most interest at this time include retrospective analysis of samples that were not tested for certain new contaminants. They can help understand when the new contaminant started appearing in commodities.
Intentional adulteration is a relatively uncommon issue, but it can be extremely risky for public health if toxic compounds are used. This is the ultimate unknown-unknown as anything can be selected simply because of its color or texture. In the field of dietary supplements, we are especially concerned with the addition of synthetic drugs in the presumably natural remedies.

Finally, the field of omics, such as proteomics, metabolomics and now foodomics rely on data mining of very large databases, so HRMS is perfectly aligned with this.

You have reached the end of module 8. Module 9 focuses on quality assurance.