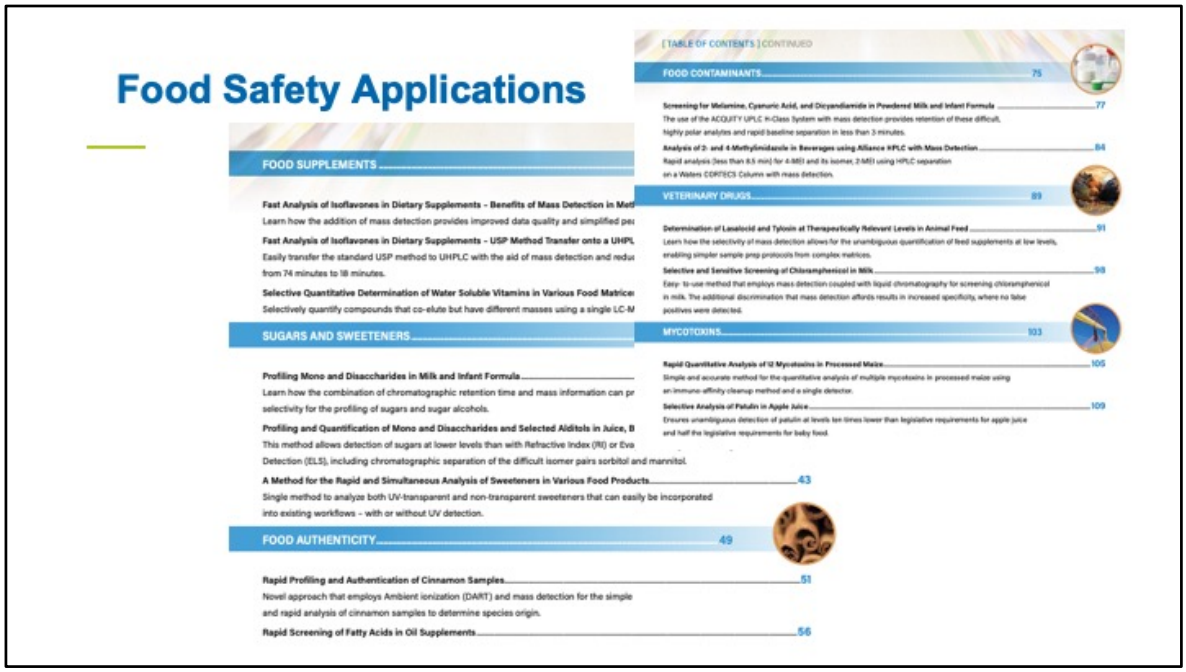
Welcome to module 6 of this online training program on confirmation methods for organic chemical contaminants.

IFSTL International Food Safety Training Laboratory | parera

SECTION 3

Applications of MS in the Measurement of Food Contaminants



In this last section of module 6, we briefly look at popular applications of mass spectrometry in the food safety laboratory. I used a couple of screen captures from Waters' food safety application brochures as an example. Every vendor has a similar catalogue, but I wanted an example to make the point that MS is no longer a tool for niche applications. A lot of regulatory testing is done every day using mass spectrometry. Let's look at some areas and why MS is so popular.

Applications of MS Measurements in Food Safety

Applications of MS Measurements in Food Safety

•Pesticide Residues

Pesticide residues present analytical challenges:

- Thousands of pesticides and products
- Trace levels (ppt → ppm)
- Unknown ID and level ("known unknown")
- Virtually infinite food matrices
- Matrices are extremely complex
- Report results within a day or two of receipt
- Up to 50 samples per day or more in a routine laboratory

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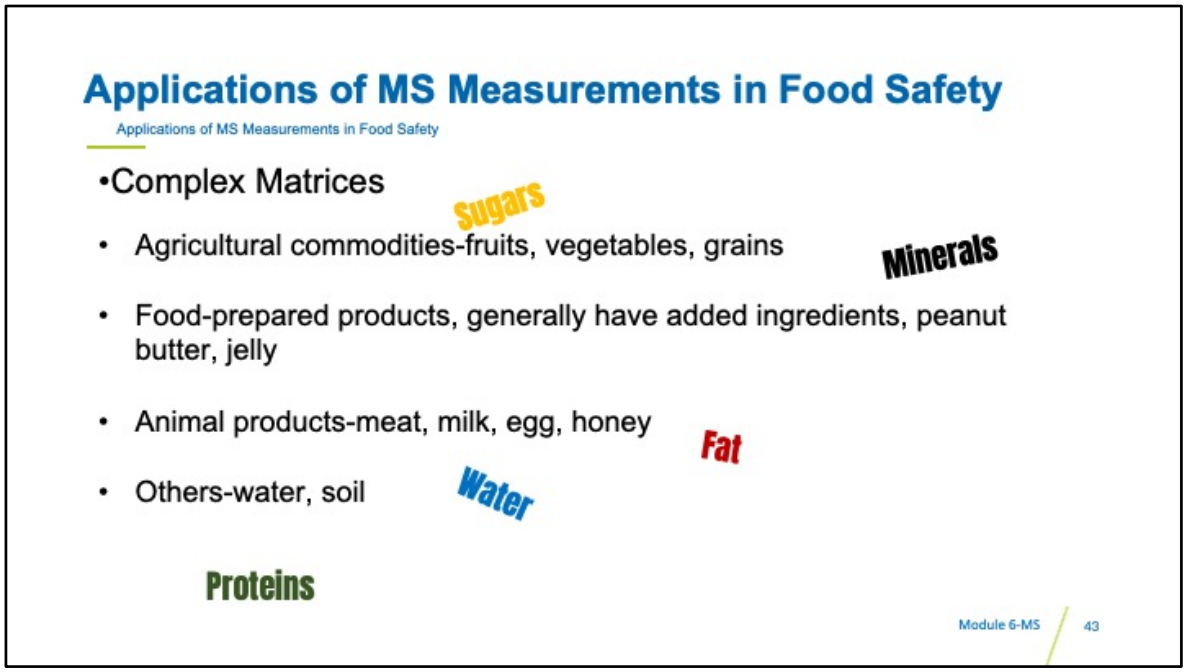
Pesticide residue analysis was the first application broadly deployed for food contaminants. Mass spectrometry provided an answer to many of the challenges presented by pesticide residues. For example, there are over a thousand of pesticides plus their degradation products may also need to be measured. Thanks to good agricultural practices, these compounds are often found only at trace levels.

One advantage here is that most pesticides are synthetic chemicals and they are therefore known. So they are known-unknowns, which means that we have information about which standards to include in a residue method. One of the challenges of the food matrix is its extreme complexity and the potential for some of the matrix components to affect the measurement or the extractability of the residues for chromatography. This is where the additional filtering capability of mass spectrometry comes in handy, by allowing some level of matrix components in the sample.

The short shelf life of fruits and vegetables is another challenge that translates in the requirement for fast analysis as many circumstances require reporting of the results within one or two days of receipt of the samples. In addition, regulatory and private laboratories analyzing for pesticides typically work on a large number of samples, which also requires speed.



It was mentioned before that the food matrix is a complex one. First and foremost, we are dealing with a broad range of agricultural commodities which means fruits vegetables and grains, in addition to the occasional meet and fish sample. Moreover, some laboratories must make the measurements in prepared products that contain a large number of added ingredients. These products can also be in a form that renders them more difficult such as prepared foods containing high amounts of sugar or fat. Finally, the technology is also applied in measuring pesticides and decomposition products in other matrices such as water and soil.

Applications of MS Measurements in Food Safety

Veterinary Drug Residues

- Hundreds of drugs and metabolites
- Trace levels (ppt → ppm)
- Unknown ID and level ("known unknown")
- Matrices are extremely complex
- Report results within a day or two of receipt
- Up to 50 samples per day or more in a routine laboratory

Farm animal entering the food supply may have been treated with veterinary medicines to cure or prevent diseases. These pharmaceutical compounds may be present in the edible portion of the animals along with some of their metabolites. In order to ensure the safety of consumers and the availability of antibiotics for the treatment of diseases in humans, regulations are in place to limit the amounts of residues of these veterinary drugs that may be present in animal meat. Veterinary drugs are typically present in very low concentrations from ppt to ppm, but we generally know what we are testing for; they are therefore known unknowns.

Meat, fish matrix and meat product, such as sausage, are all very complex matrices. In addition, these foods have a relatively short shelf life if they are not frozen and therefore must be analyzed as rapidly as possible. While most regulatory laboratories that analyze a broad range of commodities may not see has many requests for analysis for veterinary drug residues as they see pesticides for example, some specialized laboratories must analyze upwards of a hundred samples per day.

One element that is very different between pesticides and veterinary drugs is that veterinary drugs are almost all heat sensitive and consequently not amenable to analysis by gas chromatography.

Applications of MS Measurements in Food Safety

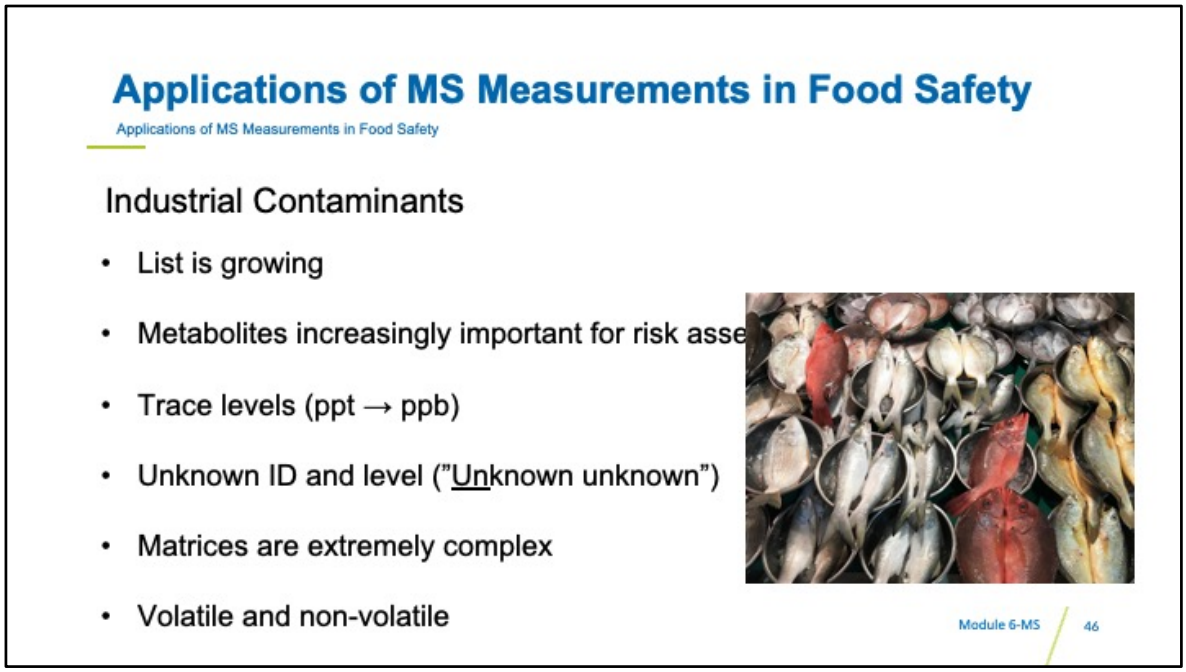
Mycotoxins

- Initially only 4 aflatoxins, but now ~20
- Metabolites increasingly important for risk assessment
- Trace levels (ppt → ppb)
- Unknown ID and level ("known unknown")
- Matrices are complex
- Screening in real-time
- Confirmation within one day (milk) or longer (grains and pulses)

Mass spectrometry is also increasingly used for the determination of mycotoxins. While the focus was placed on aflatoxins only in the past, there is growing interest in analyzing up to 20 different mycotoxins. It is possible to analyze them individually or in small groups using less expensive techniques and less intensive instrumentation. However, the advantage of using mass spectrometry is to develop a single method that applies to a large number of these toxins and commodities. One important disadvantage is that mass spectrometry technology requires of course expensive instrumentation, but also expensive infrastructure because the instruments need to be operated 24 hours a day, 7 days a week in a temperature-controlled laboratory with low humidity and a very stable source of electricity. It also requires a highly specialized workforce.

Nevertheless, mass spectrometry is gaining popularity for mycotoxins especially in the field of risk assessment. In this situation, the quantitation must be performed at ppt levels, which is not compatible with rapid techniques. Mycotoxins are what we call known unknowns and their standards are commercially available. Much like pesticides, mycotoxins must be extracted from complex matrices and in the case of milk for example, results are expected within one day of this admission of the sample. Grains and pulses benefit from longer shelf life and typically can be held for testing for a longer period.

Mycotoxins are compatible with mass spectrometry because they are small molecules that are easily transferred into solution and ionized. They are generally however heat sensitive but soluble in aqueous solutions or weak organic solvents, which makes them compatible with liquid chromatography. While a fluorescence detector attached to an HPLC is sufficient for the determination of aflatoxins due to their natural fluorescence, it is not possible to analyze many of the other mycotoxins in that way because they don't display natural fluorescence. Multiresidue methods using a mass spectrometer coupled to an HPLC or a UHPLC have a broad scope of application in this field.



The list of industrial contaminants of interest is growing. In addition, metabolites are increasingly important for risk assessments. These contaminants are typically measured at trace level and up to hundreds of ppbs. In this case, some measurements qualify as known unknowns while others are unknown unknowns. The first is true in a regulatory laboratory that is measuring to verify compliance with MRLs, while health research, risk assessment, and some pre-regulatory data acquisition programs may not know the breath of contaminants that should be investigated in a particular region or for a particular food. For the unknown unknowns, high resolution mass spectrometry is typically the preferred tool, especially in the research laboratory. The tandem quadrupole instrument operated in multi residue mode is the norm in regulatory monitoring laboratories.

Industrial contaminants are analyzed primarily in foods that are known to concentrate residues. For example, fish may concentrate industrial contaminants along their food chain, while root vegetables may take up contaminants from the soil. Many industrial contaminants are volatile and can be analyzed with gas chromatography, but others are either compatible for both HPLC and GC or better suited for liquid chromatography. Therefore, both chromatographic techniques are used ahead of the mass spectrometer.

Intentional Adulteration

Applications of MS Measurements in Food Safety

- "Unknown unknowns"
  - *E.g.,* Melamine in milk to increase "protein"
- Volatile and non-volatile
- Infinite number of possibilities
  - Artificial colors
  - Preservatives
  - Flavors and aromas
  - Substitutions
  - *Etc.*

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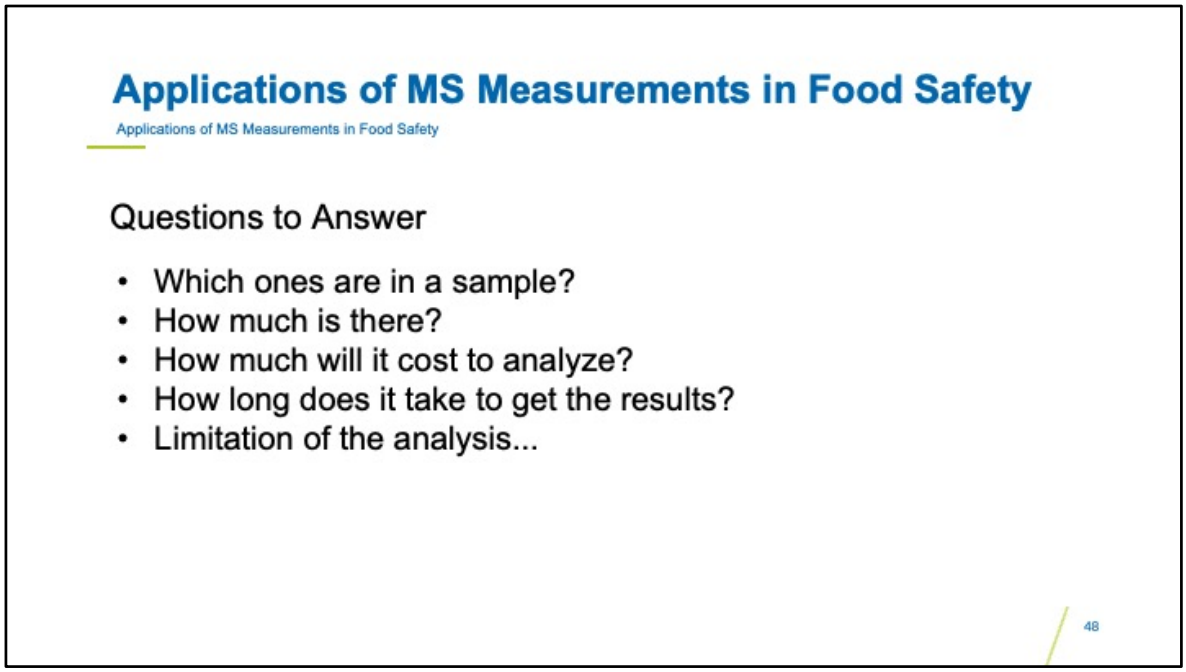
Intentional adulteration is a relatively new concern in the regulatory laboratory. In this case, we don't know what we should be looking for and therefore are definitely looking for unknown-unknowns. The first large scale crisis related to intentional adulteration was the addition of melamine in milk by unscrupulous producers who were just aiming to trick the protein content test. Briefly protein is indirectly tested by measuring the amount of nitrogen present in the milk. Consequently, adding any source of nitrogen will increase the perceived protein content.

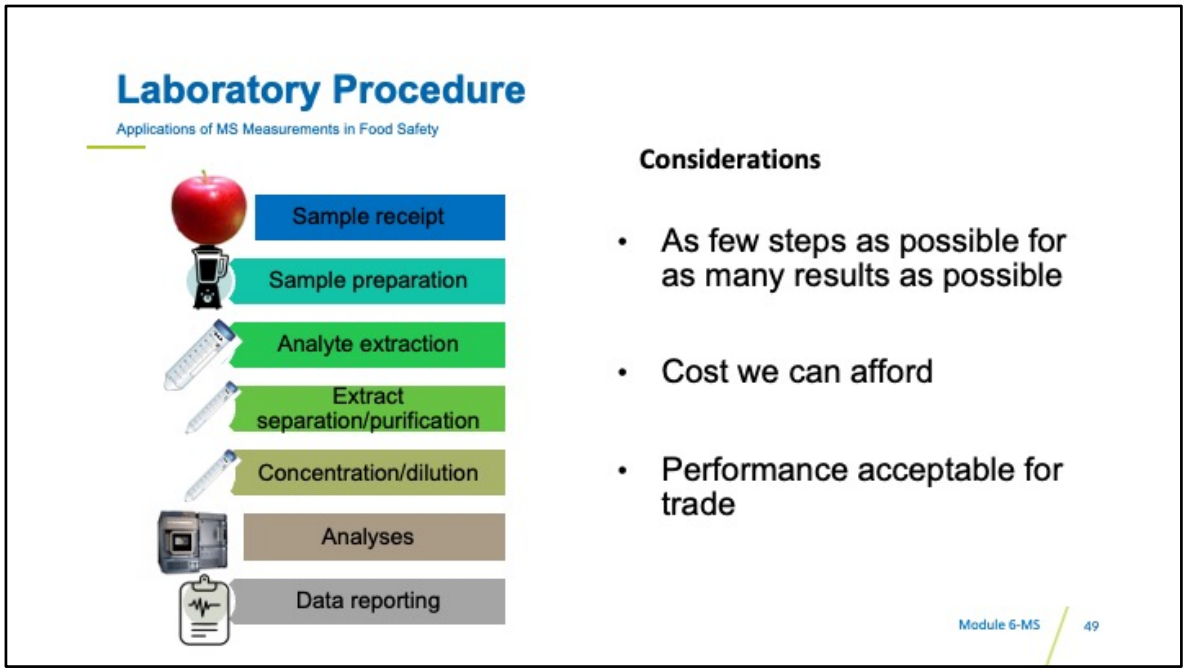
Intentional adulteration can use volatile and non-volatile molecules and there is essentially an infinite number of possibilities. However, the most likely scenarios are the use of an unapproved additive, color, preservative or flavor and sometimes it is due to a simple lack of understanding of what is approved or not. There have been very few situations where adulteration involve the purposeful use of a toxic substance to increase the economic value of a commodity or product. Nevertheless, these would be completely unexpected and consequently are still considered unknown unknowns. Because this is unknown-unknowns, high resolution mass spectrometry would be the tool of choice for these contaminants.



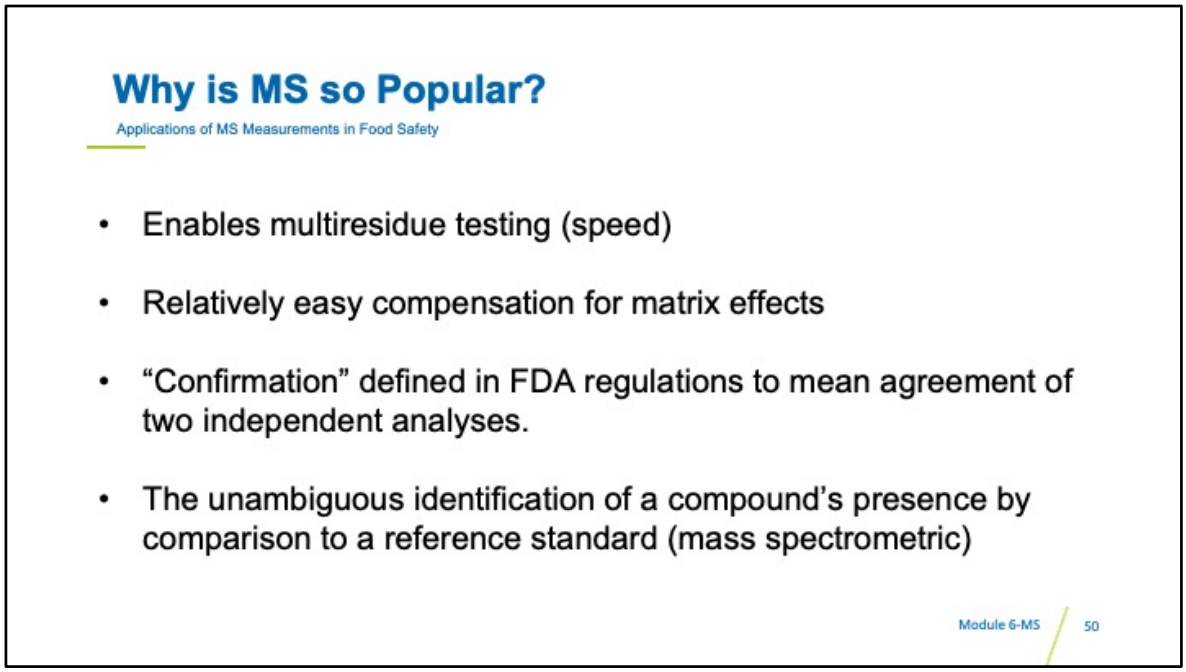
When deciding to apply ms methods in our laboratories, we need to consider a number of questions. Of course, there is the cost and the need for specialized staff, but there are also other questions that help decide which instrumentation is fit for our purpose. We can ask: Which ones are in a sample? –do we know which contaminants... Can we use a low resolution mass spectrometry, or do wee HRMS because we need a large screening library? Another question is how much is there? – The LOQ and LOD of the technique must match the levels we are expecting to measure.
How much will it cost to analyze? Can we afford to perform this test on the number of samples that we expect to be sent to the laboratory?

How long does it take to get the results? – will the commodity still be fresh and have kept its commercial value by the time we are able to deliver the results? That's a very important question.
Finally, we need to decide and then live with the limitation of the analytical method that we chose. If we implement LC-MS/MS, we have access to a really large number of official method. That's why the triple-quad is usually the first choice. Going with high-resolution opens the scope to unknowns, but it may not as sensitive as MS/MS and may not meet the LOQs are LODs that we need for our regulations... HRMS can also be unnecessarily overwhelming for a lab that has a regulatory compliance

purpose and should be testing for a known set of contaminants.

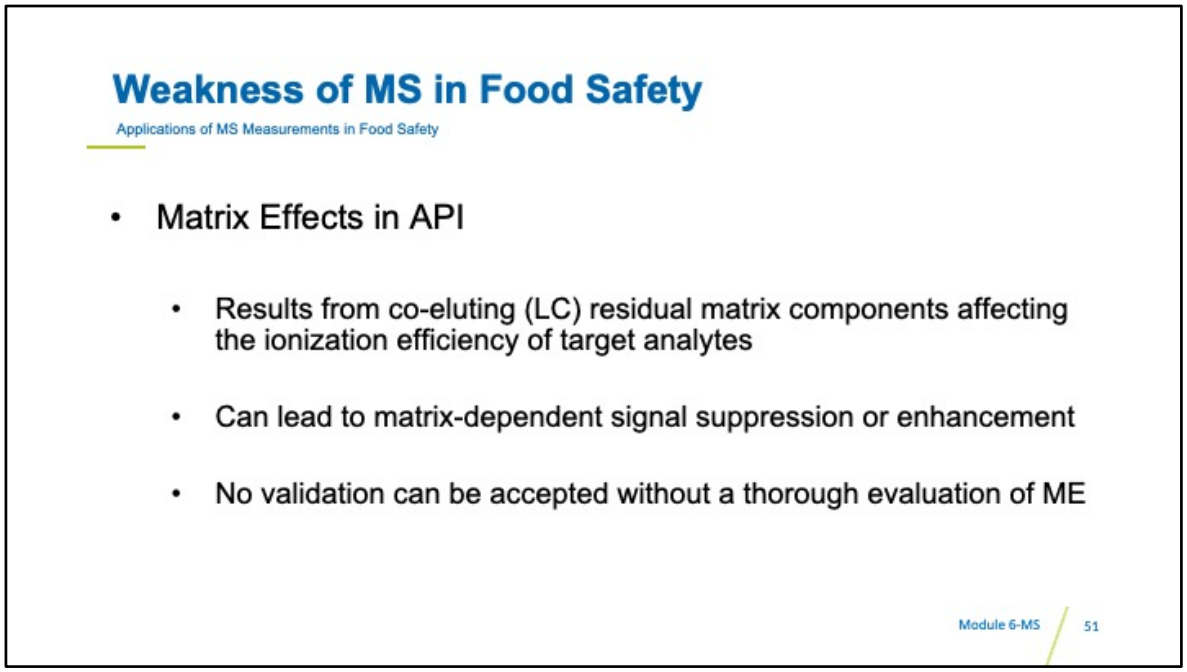


As I mentioned before, one of the great attractions of MS in the food safety laboratory is the possibility to use pretty simple sample preparation, such as steps illustrated here on the left that would be typical of a QuECheRS sample prep. With fewer steps and using less solvent and glassware, we can afford to analyze more samples. The simplified procedures do not produce ncessarily the lowest limits of quantitation possible for example, but the performance is fit for the purpose of trade. The results are reliable and the speed matches the need.



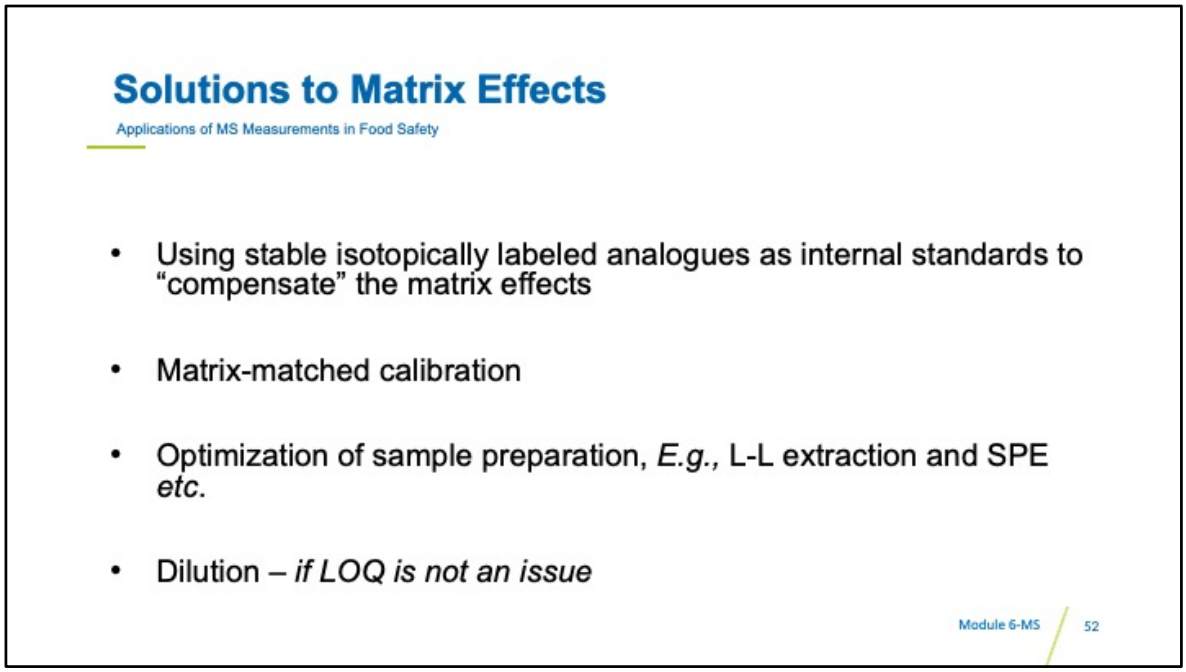
Mass spectrometry has become an important tool in the food safety laboratory in large part due to the ability to perform multi residue testing, which saves a lot of time. The possibility to use the 1st quadrupole as a filter, which in turn allows for simple sample preparation, combined with relative ease to compensate for matrix effects contribute further to this speed of analysis. As a result, it is quite common for a laboratory to analyze a few dozen samples in a day for hundreds of pesticide residues; translated into equivalent single residue analyses, this corresponds to thousands of tests in a single day.

For regulatory testing, it is essential to confirm the identity of the analytes being quantified. Confirmation is typically defined to mean the agreement of two independent analysis. The tandem quadrupole instrument combined with chromatography provides a sufficient number of identification points for confirmation of identity. It bears repeating that comparison of the chromatograms and MS/MS profiles must always be verified with the standard ran on the same instrument and under the same conditions and on the same day.



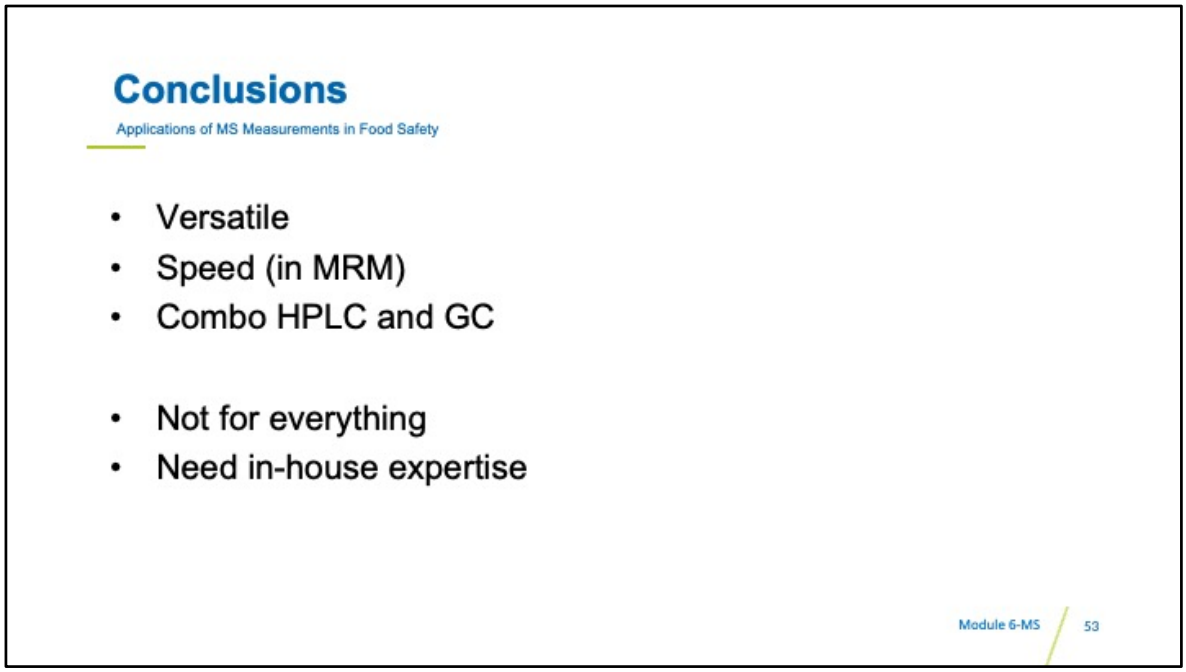
Matrix components are eluted alongside contaminants and their presence can affect the ionization efficiency of the target analytes in the source. This can lead to matrix dependence signal suppression or enhancement.

Matrix effects is a notorious issue in atmospheric pressure ionization (API), and API is the most common source in food safety applications. It is the most common because it is versatile and enables us to analyze a lot of contaminants in the same test, but it can be affected by matrix effects and we always have to check.



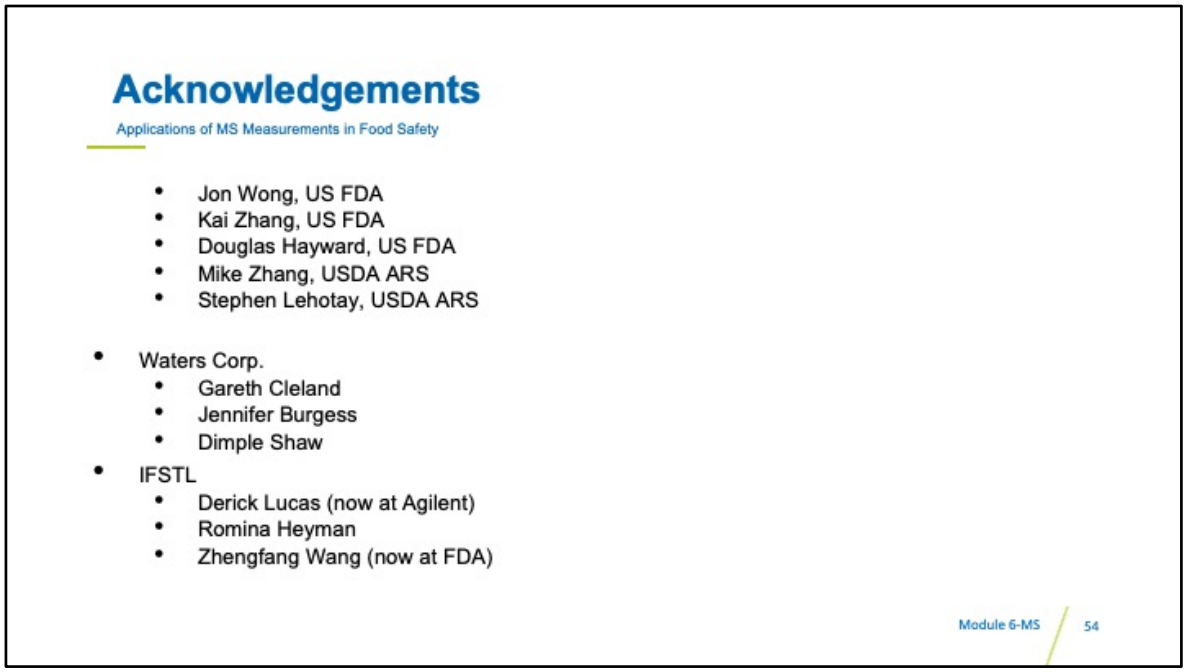
Stable isotope internal standards can be used to calculate the matrix effect, but their optimal use is very expensive because, in theory, each compound should be measured alongside its stable isotope. This is actually rarely applied because these standards are too expensive or are simply not available. Matrix-matched calibrations are often used to compensate for matrix effects because they involve a smaller expense, but there is a price to pay in the amount of time it takes to prepare the matrix-matched calibration and such calibration must be prepared for each matrix or group of matrices that show a similar behavior. So there is an amount of development associated with matrix-matched calibrations.

Another solution to matrix effects is simply to dilute the sample until the effect is no longer observed. Of course, this solution can only be used if the limit of quantitation is not an issue. Finally, optimizing sample preparation by adding steps that focus on the removal of the compounds causing the effect is also a possible option. The disadvantage of increasing the number of steps in the sample preparation which costs time and costs for consumables and the fact that it may limit which matrices are adequate for this new sample preparation. For example, one would need to ensure that the additional cleaning steps do not remove some of the analytes of interest in that particular matrix.



In summary, while mass spectrometry is a versatile analytical technique for the food safety laboratory, it is not the solution for all contaminants in all foods. The speed brought about by parallel analysis of hundreds of pesticides, for example, and the simple sample preparation procedures like QuEChERS that produce sufficiently cleaned samples for MS/MS are the main reasons for the popularity of the technique. Combining the strength of HPLC or GC to separate large numbers of analytes of interest, and the identification and quantification capabilities of mass spectrometry instruments has dramatically changed the expectations in the food safety laboratory.

Very large numbers of samples are expected to be analyzed each day and for an extremely large number of contaminants. In spite of the relative ease of use of chromatography and mass spectrometry-based methods, it is imperative that each laboratory utilizing these tools have the expertise required to understand the potential sources of errors as well as the situations where the instrumentation may not appropriate.



As is the case for each of these modules that are part of the training, a large number of people have been involved in the preparation of training material for the IFSTL over the years, and I would like to recognize them in this slide!. Thank you!

You have reached the end of module 6 focusing on mass spectrometry. Goodbye!