Section 3: Advantages and challenges of multi-residue methods

What Made MS so popular?

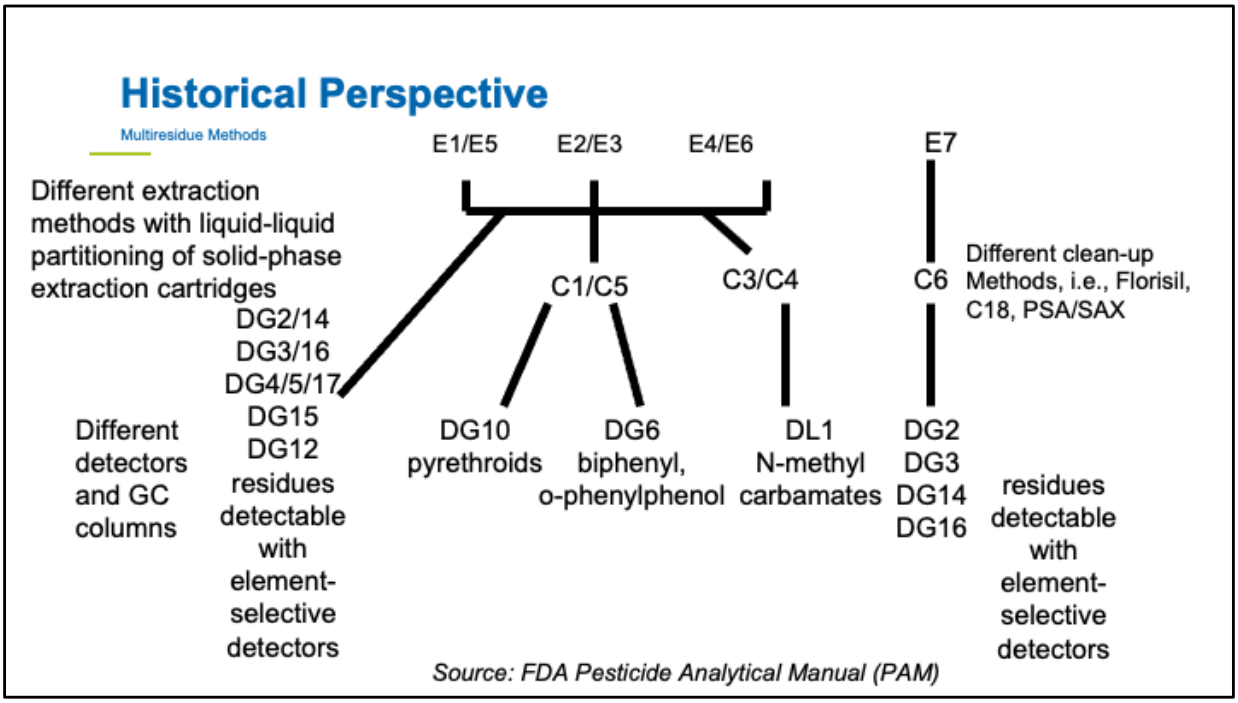
Multiresidue Methods

- Multiresidue methods
- With HPLC – simple sample preparation
- Relatively easy compensation for matrix effects

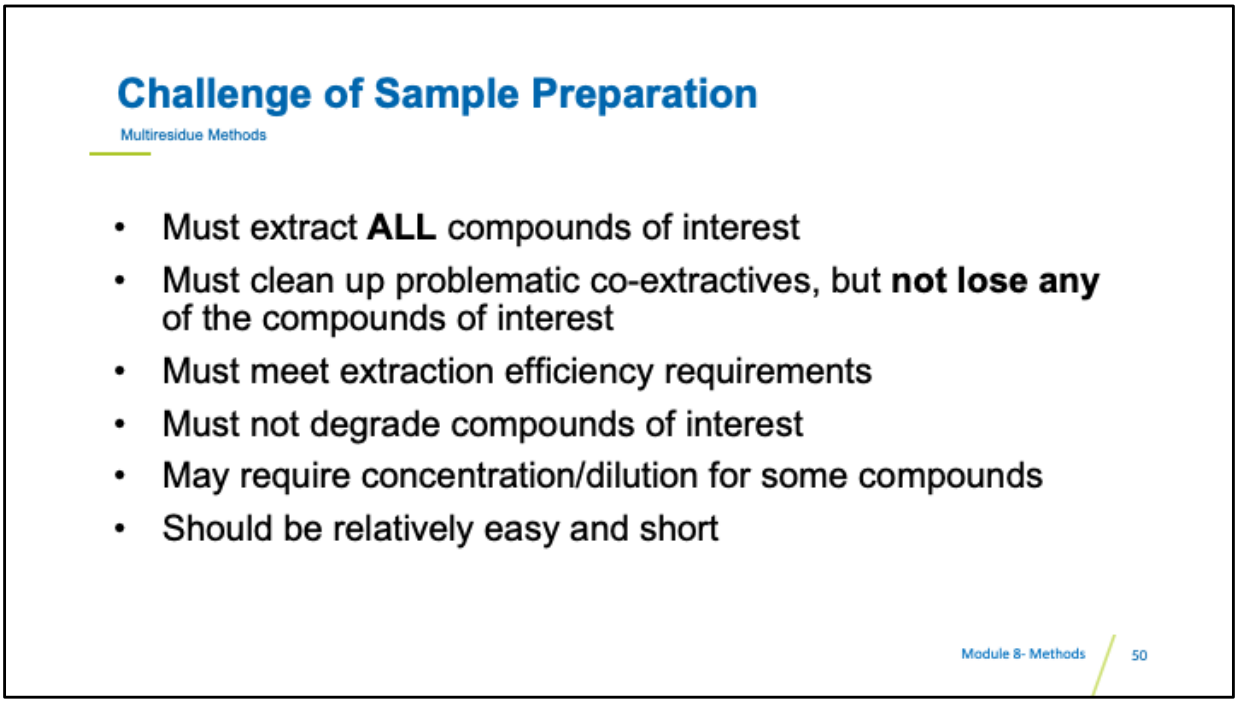
Let's start with a summary of why MS has become so popular. First and foremost, it enables us to do multi-residue analyses, which means that we get sometimes 200 or even 300 answers with one test. Sample preparation can be easy, especially with the QuEChERS techniques.

It can be relatively easy, or cheap to deal with matrix effects. I say easy or cheap, usually not both! Easy is to used isotopically labelled standards, and cheap is more work, with matrix-matched calibration. But then you have to make a calibration for every matrix, or test if you can mix matrices together to make a more general matrix-matched calibration. We will talk about this further in the hands-on session.

Finally, the fact that it accumulates enough points to obtain a confirmation of identity is also quite practical.

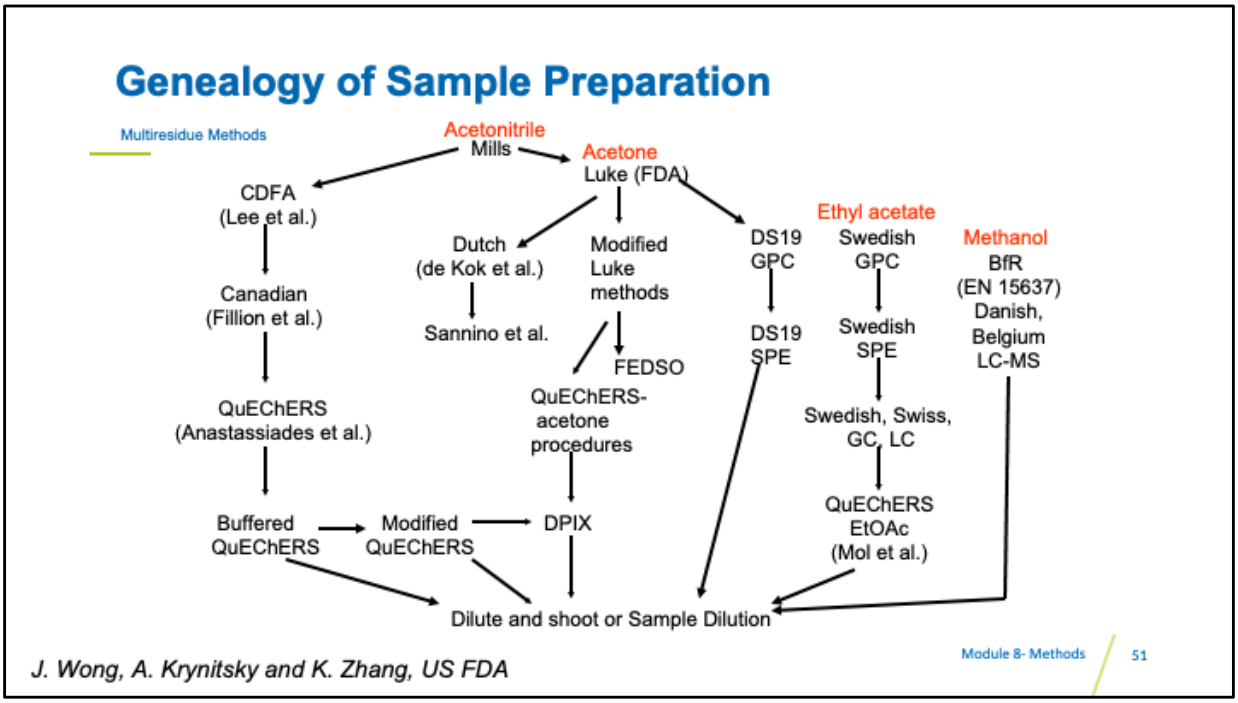


Historically, there were completely different extraction techniques and combinations of detectors and columns on GC.



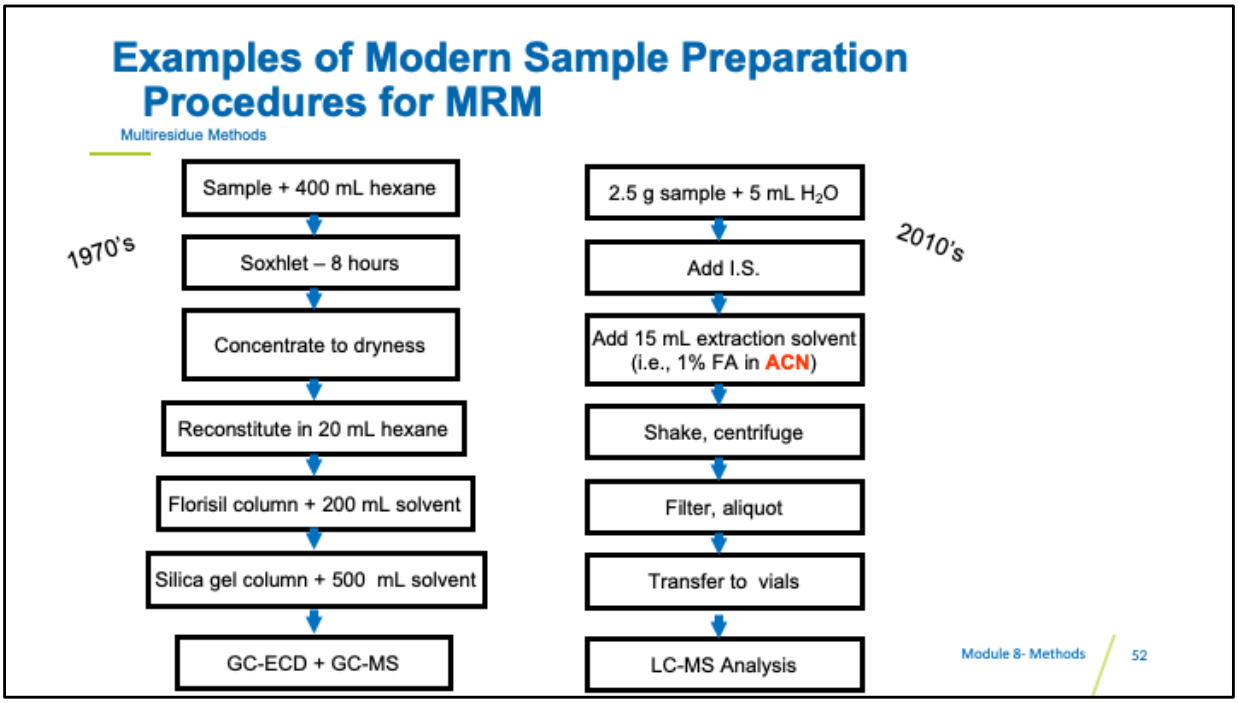
Multiresidue methods pose a number of challenges. For example, the sample preparation method must extract all compounds of interest. The cleanup steps must remove problematic co-extractives but do so without losing any of the compounds of interest. Using a tandem quadrupole instrument reduces the level of clean up needed in a wide variety of samples, but there is still a need to remove any analytes that could negatively impact the ionization in the source, especially if it increases the limits of quantitation and the limit of detection to above the levels needed for the purpose of the method.

The sample preparation with suitably broad retention must lead to an extraction efficiency meeting regulatory requirements, as discussed in lesson 2. It must not cause any degradation of any of the compounds of interest. When contaminants are present in vastly different concentrations, it may be necessary to include steps of concentration or dilution in order for the signal to fall within the linear range of the detector. Finally, it has become expected that multi residue methods will involve relatively easy and fast sample preparation. It is overall a tall order!



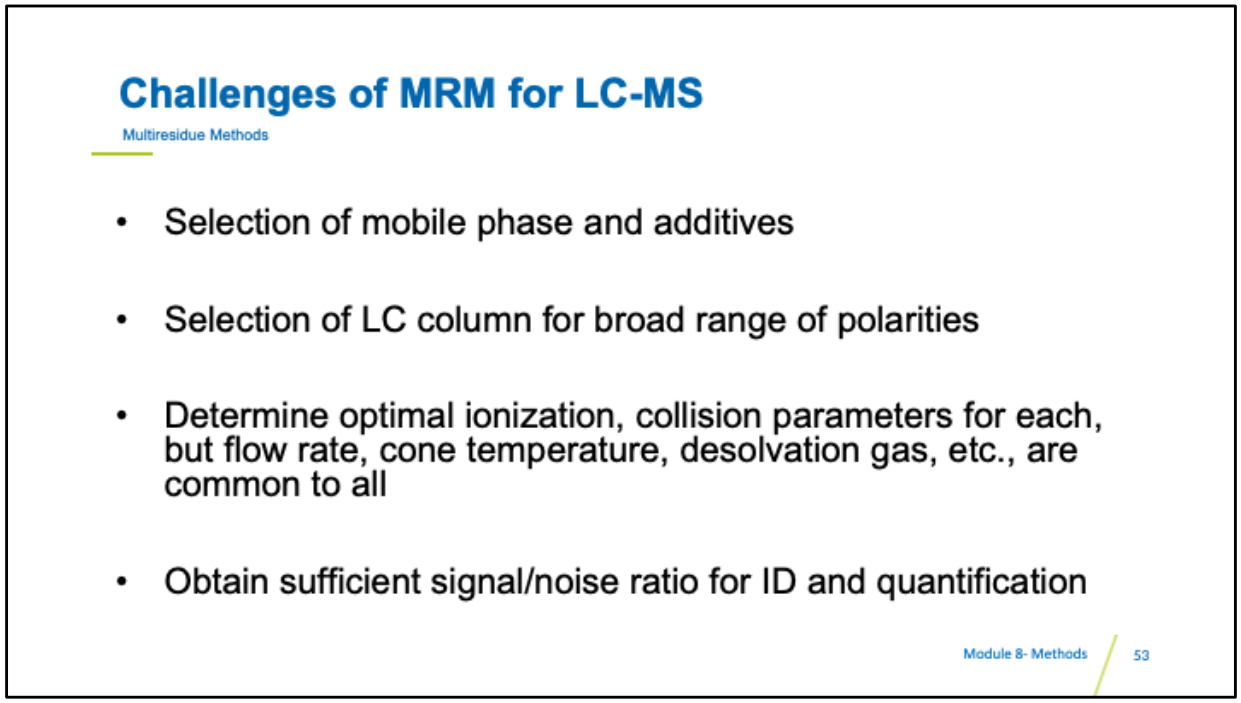
Similarly to the GC sample preparation tree, there are many different versions of sometimes relatively similar sample preparation procedures for liquid chromatography.

The graphical representations of the genealogy of sample preparation methods for pesticides was prepared by colleagues at the US FDA: Drs. Jon Wong, Alex Krynitsky and Kai Zhang).



Let's look at an example of how sample preparation procedures have changed in time for MRMs. Sample preparation procedures used in the 1970s for the determination of halogenated pesticide residues required 400 mL of hexane and using a Soxhlet for 8 hours. The extract was then concentrated down to dryness and reconstituted in 20 mL of hexane. The next step was to run the sample through a florisil column to separate fats, using 200 mL of solvent, and a silica gel column to separate pesticides from PCB's, using another 500 mL of solvent. The quantitation was performed using gas chromatography with an ECD detector and confirmation was done by GC-MS.

This method used over 1 L of organic solvent (hexane) per sample for the measurement of a few halogenated pesticides; in comparison, the QuEChERS methods described in the figure on the right uses 15 mL of acetonitrile for LC and an additional 1.5 mL of toluene for GC per sample for the measurement of 20 pesticides. Modern variations on the QuEChERS method use the same volume of solvent for the measurement of upwards of 200 and even 300 pesticides. Besides being easier to perform, faster and less costly, these methods are better for the environment.



The LC-MS/MS multiresidue method needs to be set up in order to separate the analytes sufficiently to enable MS analysis and produce peaks that are a compromise between sensitivity (*i.e*. narrow) and wide enough to allow enough time for the mass spectrometer to go through all the components of interest that elute in the same retention window. The effect of the selection of mobile phase on all analytes of the method must be understood. We discussed examples of the effect of using a gradient and different mixtures for isocratic runs in Module 4. The effect of pH is also important to produce symmetrical peaks and favor ionization. The column also has to accommodate all analytes; luckily, food contaminants analysis has grown globally and now represents an interesting market for column manufacturers who now market columns with optimal performance for these applications.

Effect of Mobile Phase

Multiresidue Methods

Simultaneous Determination of Deoxynivalenol, Its Modified Forms, Nivalenol and Fusarenone-X in Feedstuffs by the Liquid Chromatography-Tandem Mass Spectrometry Method

June 2020

Authors:

Lukasz Panasiuk
ul 4.51 · Państwowy Instytut Weterynaryjny

Piotr Jedziniak

Katarzyna Pietruszka
ul 10.53 · Państwowy Instytut Weterynaryj...

Andrzej Posyniak
ul 36.59 · Państwowy Instytut Weterynaryj...

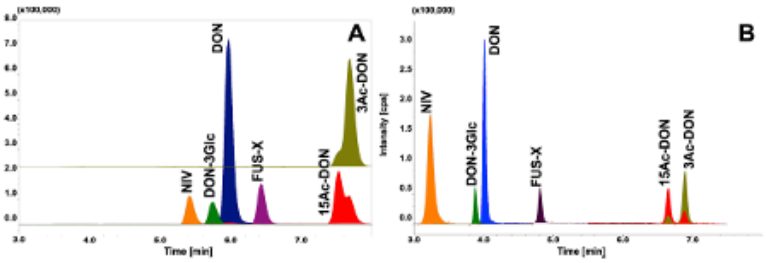


Figure 2. Chromatographic separation of tested compounds on the same column (Phenomenex Luna ® Omega C18 100 × 2.1, 1.6 µm) with different organic mobile phase: (**A**)–MeOH; (**B**)–ACN.

This example illustrates the effect of the mobile phase on the measurement of mycotoxins. Chromatograms in A were acquired using methanol, while acetonitrile was used for B. The separation is cleaner in B.

Effect of LC Column

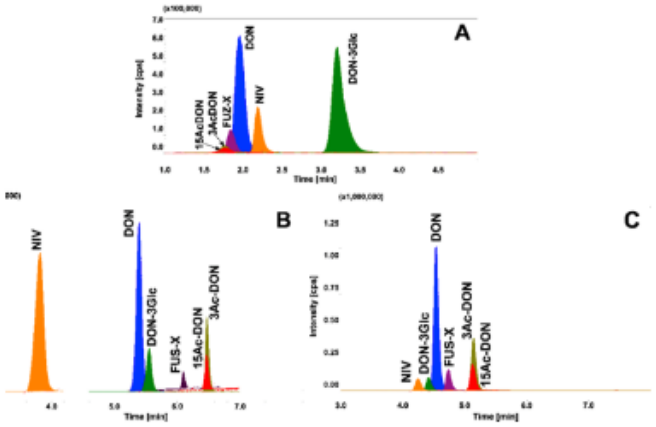
Multiresidue Methods

Simultaneous Determination of Deoxynivalenol, Its Modified Forms, Nivalenol and Fusarenone-X in Feedstuffs by the Liquid Chromatography-Tandem Mass Spectrometry Method

June 2020

Authors:

Lukasz Panasiuk
Piotr Jedziniak
Katarzyna Pietruszka
Andrzej Posyniak

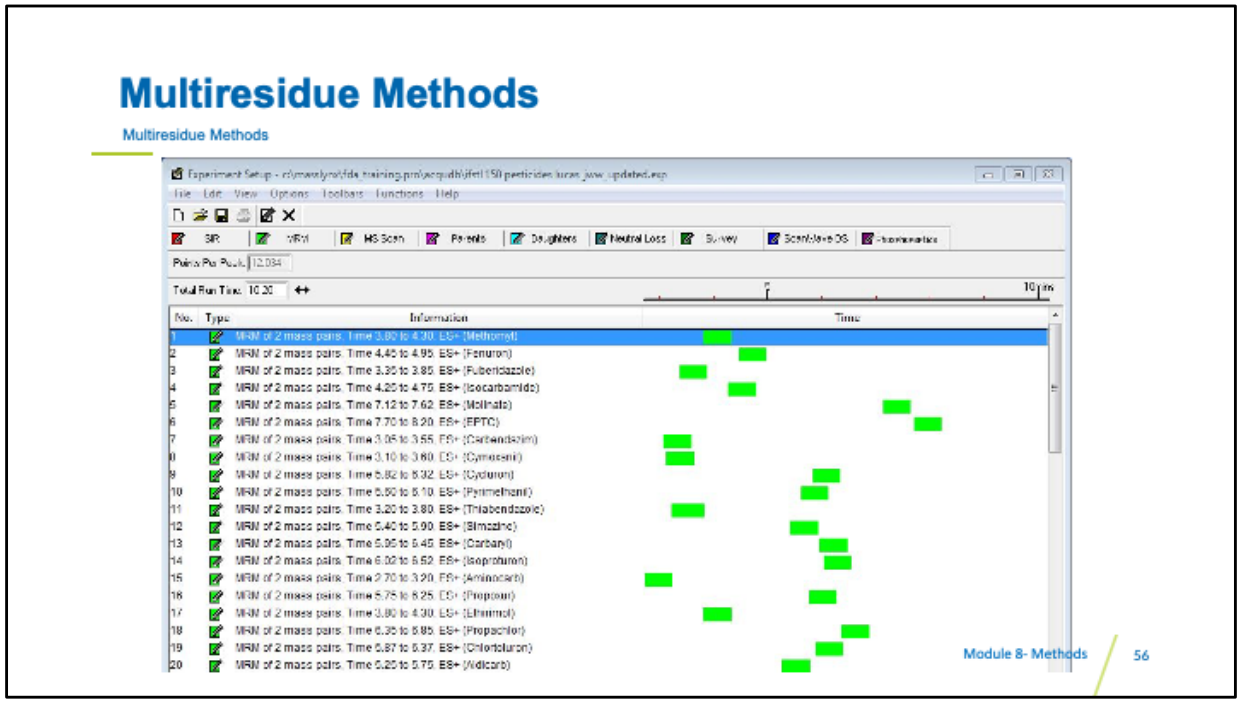


LC-MS/MS chromatograms of analysed toxins, tested in the same mobile phase but at different chromatographic columns: (A)-Phenomenex Luna HILIC (B)-Phenomenex Kinetex Biphenyl 100 × 2.1mm, 1.7 µm; (C)-Phenomenex Kinetex C18 100 × 2.6 mm, 2.1 µm.

https://www.researchgate.net/publication/342067694_Simultaneous_Determination_of_Deoxynivalenol_Its_Modified_Forms_Nivalenol_and_Fusarenone-X_in_Feedstuffs_by_the_Liquid_Chromatography-Tandem_Mass_Spectrometry_Method

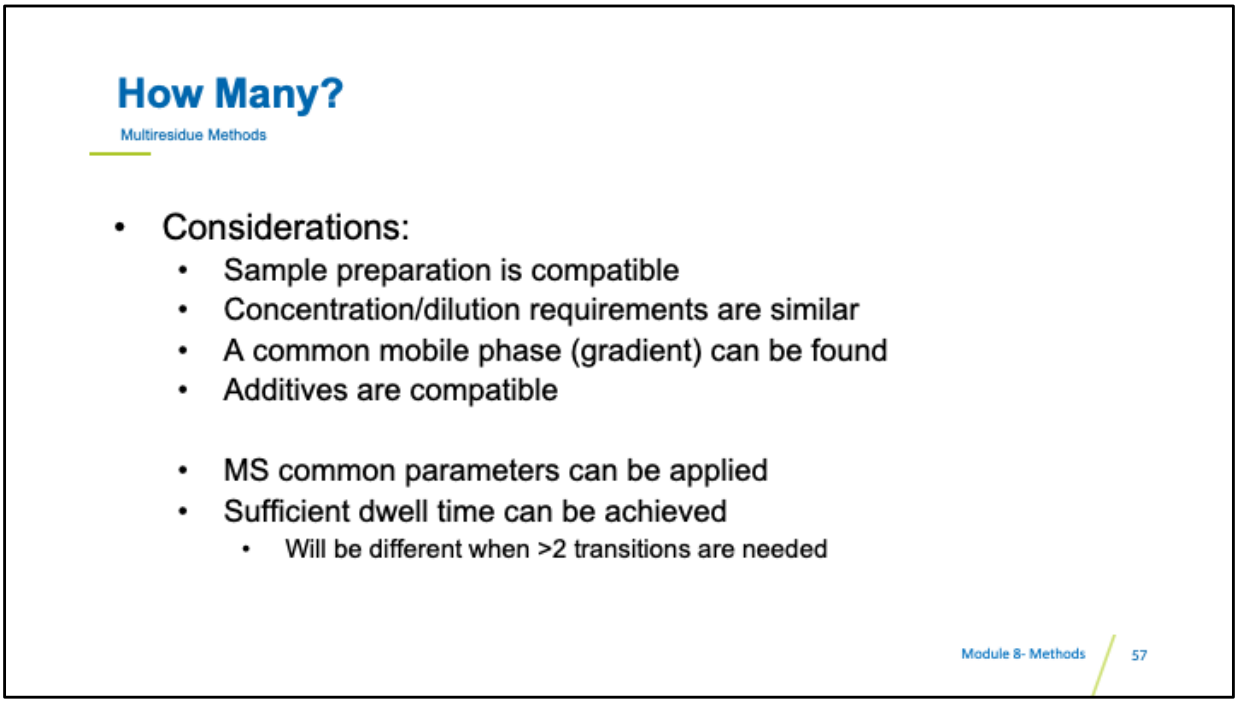
From the same article, we can look at the figure showing the effect of the column on the separation. There is rarely a perfect answer, especially because we are constantly adding analytes to our methods. in this case, the three original mycotoxins of interest, namely DON, fumonisin and nivelanol are perfectly separated in B, but some of the other peaks are not. None of these 3 columns used in the conditions shown produce a perfect separation. Luckily, the mass spectrometer can resolve two or more overlapping peaks.

This publication is available for free from its authors on ResearchGate.



The real “new” topic in this section is the concept of running more than one analyte at the same time in the mass spectrometer. It is not strictly what happens since only one m/z is allowed through MS1 in the MRM mode, but it describes the need for sharing of a time frame by two or more analytes.

This slide shows a typical software window for a multi-reaction monitoring experiment in MS/MS. The green boxes span the portion of the time axis located at the top; data is to be acquired for each of the pesticides listed on the left (and including 2 product ions, identified here as mass pairs) during this time window. In this experiment, the period between, for example, 5.5 and 6.5 minutes shows 8 analytes. Consequently, MS1 will need to allow 8 different precursor ions through, the collision chamber will need to be energized at the best collision energy for each of them to produce 2 product ions. Finally, MS 2 will need to filter 16 product ions onto the detector. This particular method includes over 150 pesticides, so there may be more than 16 overall.



Some of the MS parameters are fixed during a run, such as the ionization flow rate at injection, desolvation gas and source temperature. Other parameters can be tuned for each analyte, such as ionization current (positive/negative) and the collision energy for fragmentation in the collision cell. Now comes the concept of dwell time. Dwell time is the amount of time that the mass spectrometer spends accumulating data. We usually report dwell time and resulting points per peak, where a minimum of 10 to 15 points per peak is preferred. During the period of accumulation of the information (i.e. the dwell time), the signal is averaged, but so is the noise. This results in an increased S/N since the noise is random and will add/subtract, while the signal should always be about the same. Of course, a greater S/N translates in lower LOD and LOQ. In summary, we are working with very low concentrations in food safety, so we need to compromise in a way that provides a good-enough S/N ratio to meet the LOQs required by the regulations.

Dwell Time

Multiresidue Methods

- How long you allow the instrument to measure one m/z or transition.

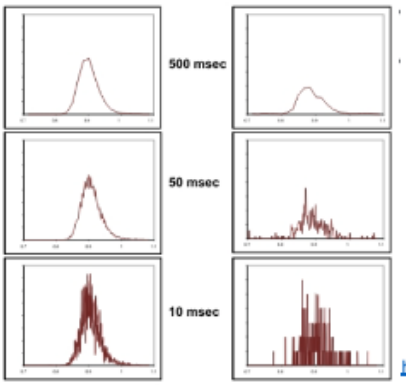
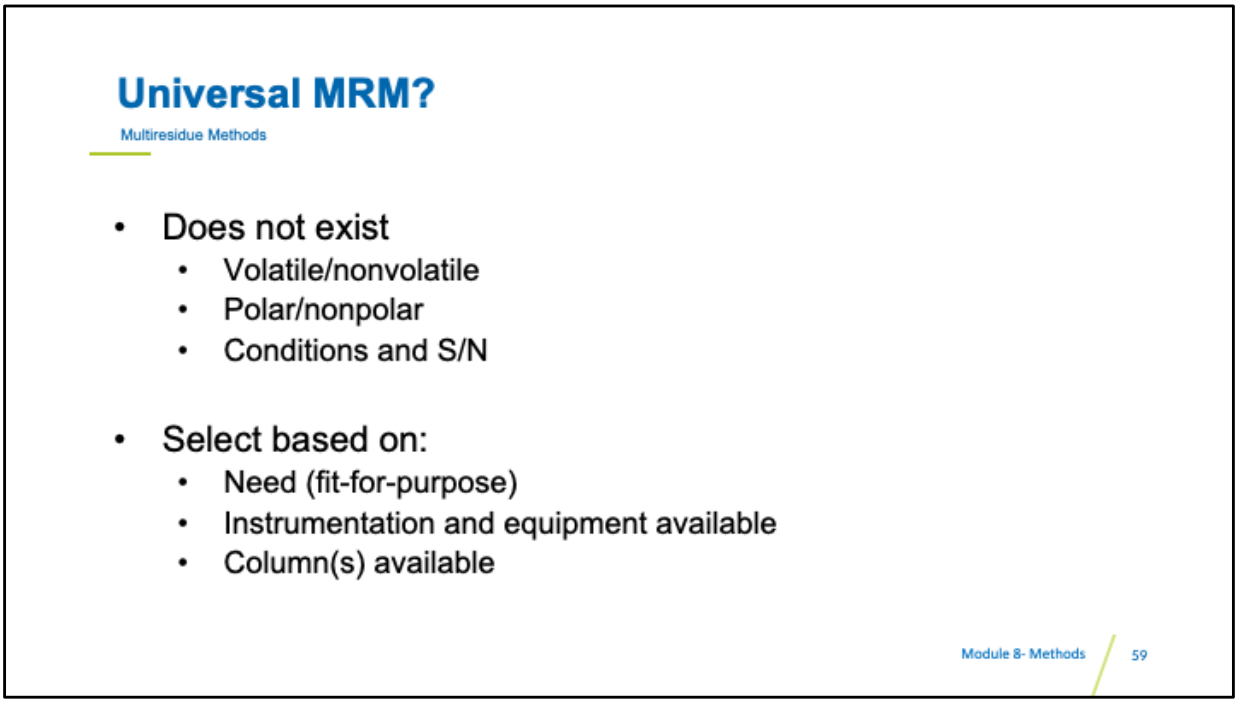


Figure 1. Effect of dwell time on peak shape for a 40 ng/mL (left) and 200 pg/mL (right) injection

Signal/noise ratio

https://www.basinc.com/library/presentations/biochem/alexander_01

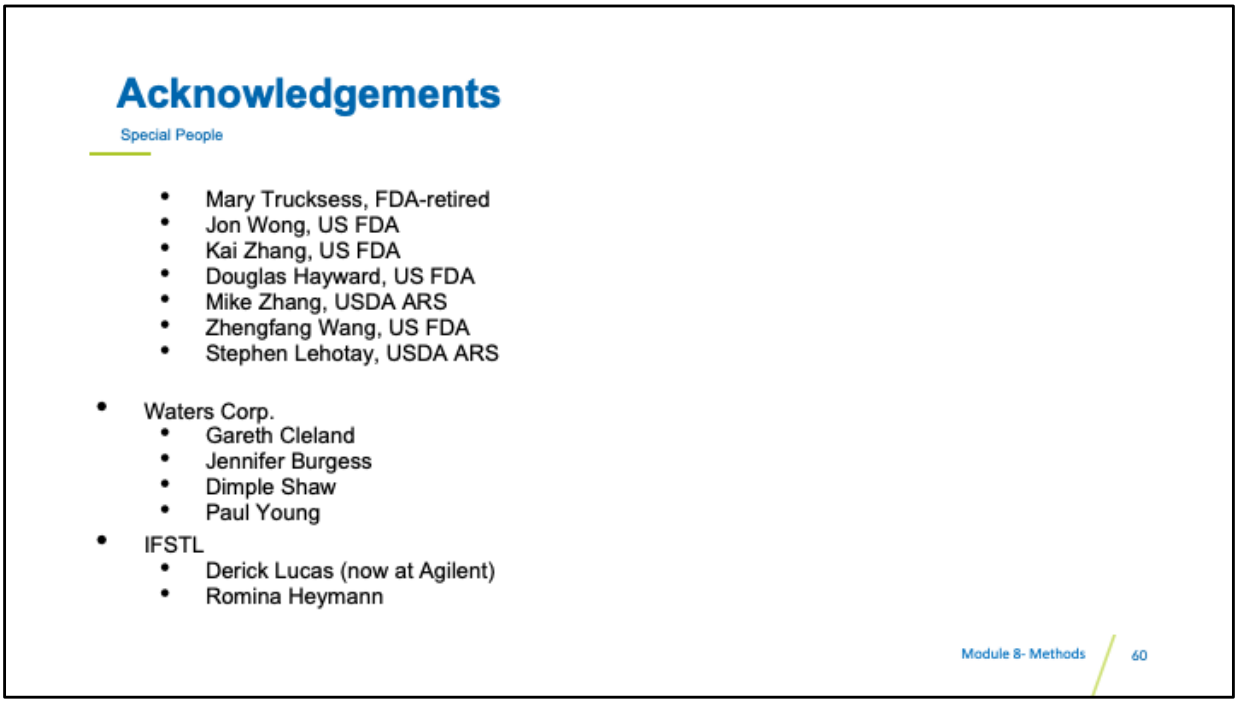
Circling back to the dwell time, this is a value that can vary between instruments, but generally not by an order of magnitude. Using round numbers for illustrative purposes, an analyte may produce a S/N of 3:1 with 10 msec dwell time. This would be the absolute minimum dwell time to use to make a screening measurement (which needs S/N of 3 or more). In theory, the S/N improves according to the square root of the additional time. So, moving to a 100 msec dwell time should improve the S/N by 3.16X. Considering the margin of error, this is approximately the dwell time that would be needed to obtain a peak with a S/N of 10, the minimum requirement for a quantitative measurement.



Putting all these considerations together clearly emphasizes why there is a limit to the number of analytes that can be included in a multiresidue method. It could be possible to increase the number of analytes included in the method by lengthening the experiment in a manner that would cause better separation of the analytes. A sufficiently long dwell time could then be allocated for each analyte in a very large method. At this time, methods including upwards of 300 pesticides are some of the largest methods for regulatory testing. As mentioned before, regulatory testing requires a high degree of certainty because it will trigger regulatory action that is very costly. Screening methods comprising of around 1,000 pesticides are advertised, but they are generally not very popular because the preparation of the calibration curve for such a large method is both arduous and expensive.

In conclusion, there is no such a thing as a universal MRM. Analyte characteristics such as whether they are volatile or not, polar or not, or sensitive to degradation at high temperature fundamentally make the universal method impossible. More practically, there is generally not a need for exceedingly large methods because many laboratories have a restricted mandate that can limit the scope of commodities analyzed, the list of pesticides of interest, or both. When the concern is with completely unexpected analytes, high

resolution mass spectrometry has a better fit for the purpose.



I wish to acknowledge contributions from many people to the material presented in this section.

You have reached the end of Section 3. Section 4 discusses methods using high resolution mass spectrometry.