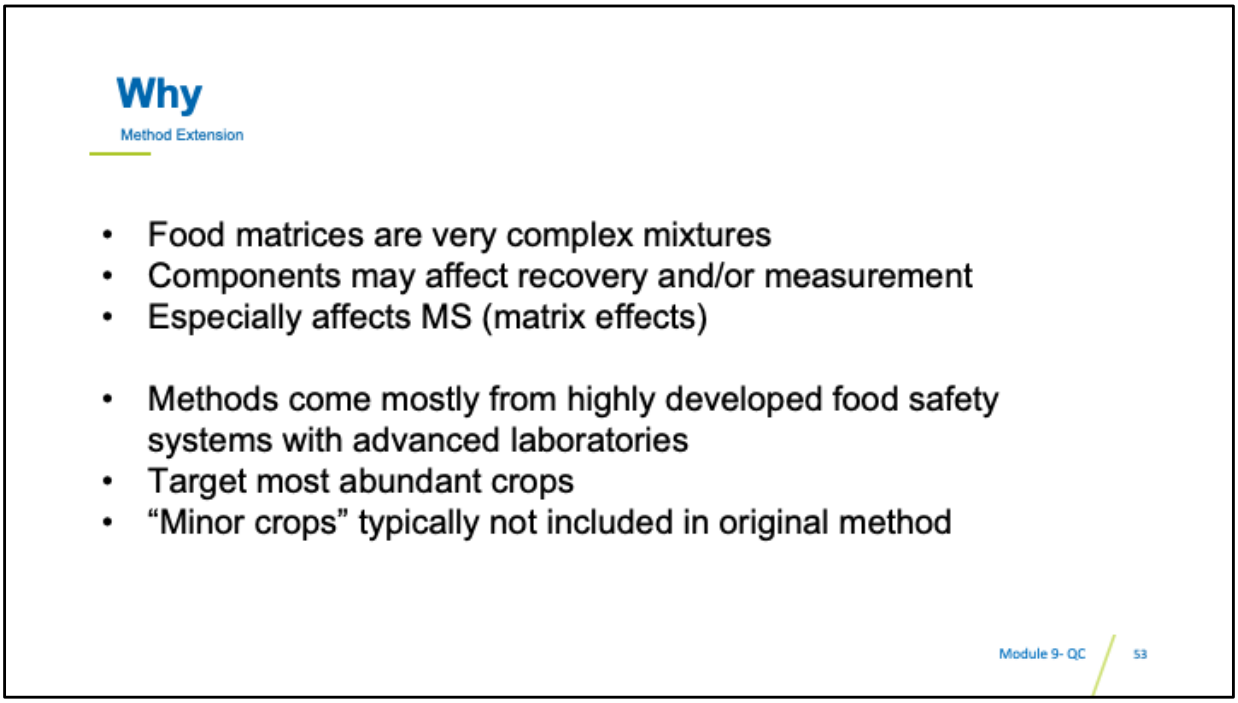
Welcome to section 3 of module 9, where we discuss validation of matrix extension.



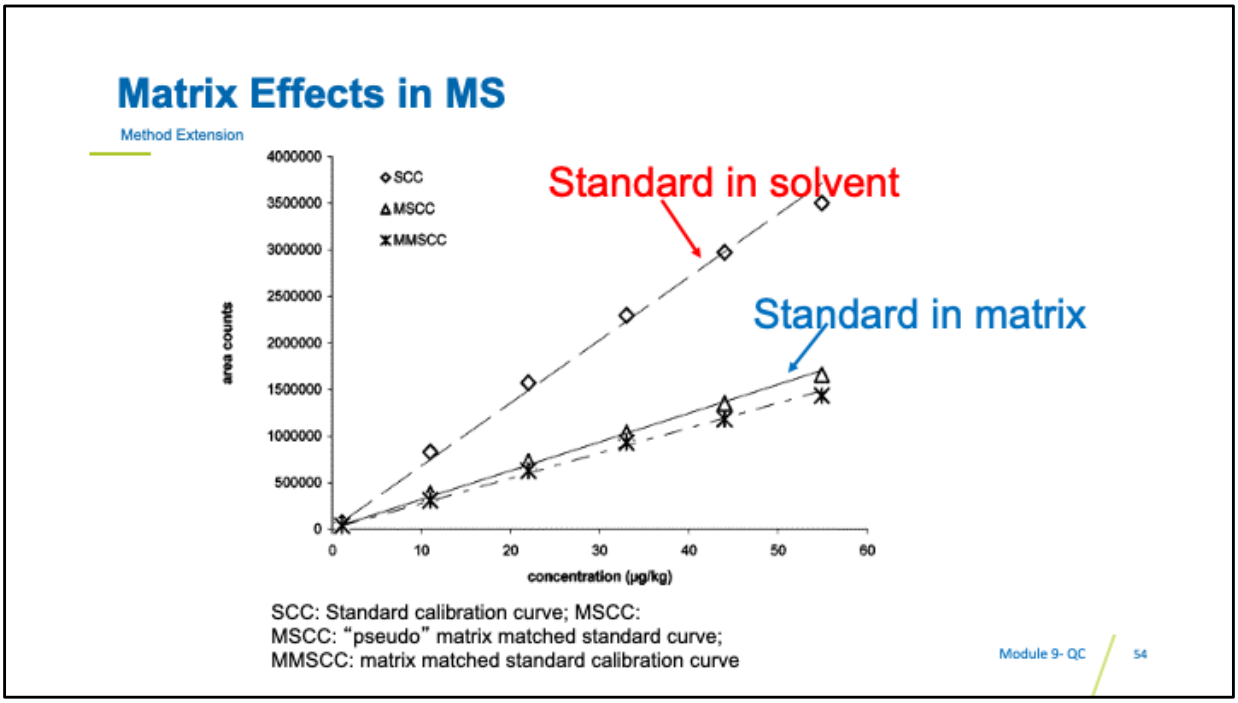
The concern with the matrix is that its composition may affect the ability of the procedures incorporated in the method to extract the analytes of interest with the same efficacy as observed in the matrix originally included in the scope of the method.

The performance of some measurement technologies is more affected than others. Matrix extension became a very important consideration when mass spectrometry became commonplace in the food safety laboratory because of the matrix effects of suppression and enhancement that are common.

Laboratory methods are typically developed in advanced laboratories associated with highly sophisticated food safety systems. These laboratories, at least originally, were located in the United States, Europe and Japan, which meant that their scope was limited by these countries' diversities of crops. Indeed, methods were mostly developed to test the most abundant crops and as such, they did not include many of the fruits found in tropical countries for example. While matrix extension is seen in most commodity groups and for different contaminants, the biggest challenge is in the analysis of pesticide residues.

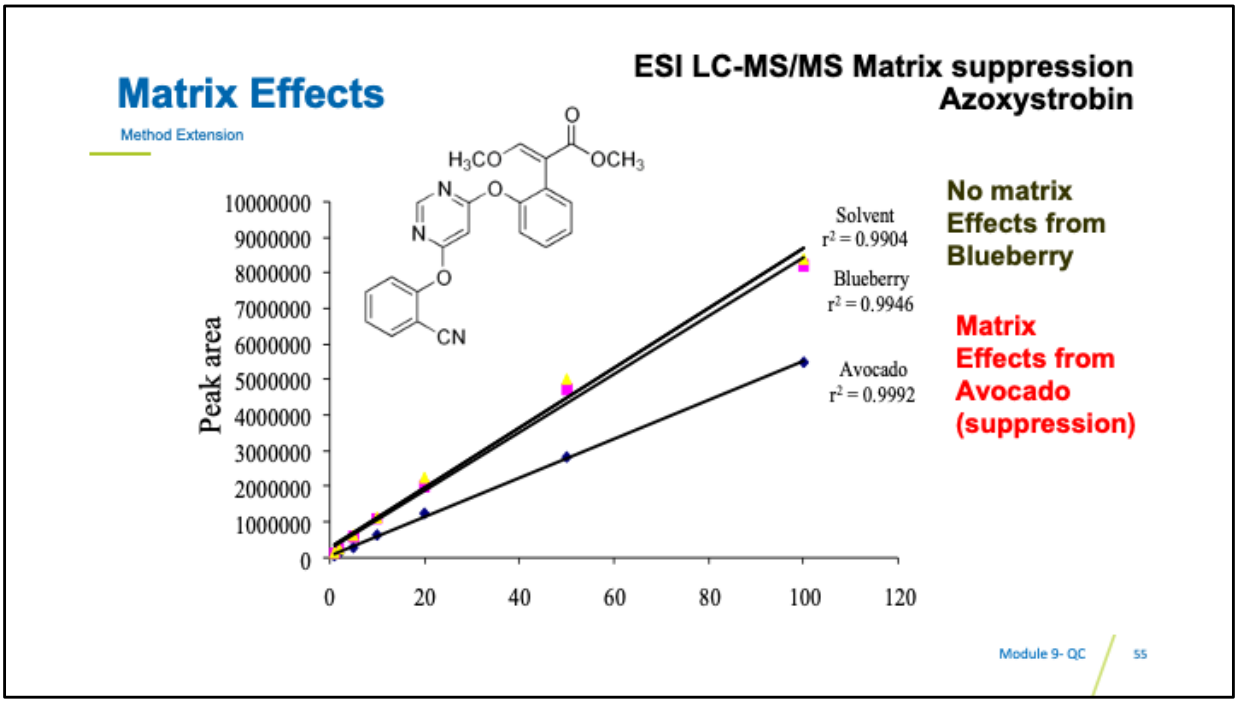
With more countries supporting their regulatory system with advanced

laboratories, we are seeing a rapid growth in the commodity scope of methods, but most of the expansion is done through matrix extension to avoid reinventing the wheel!



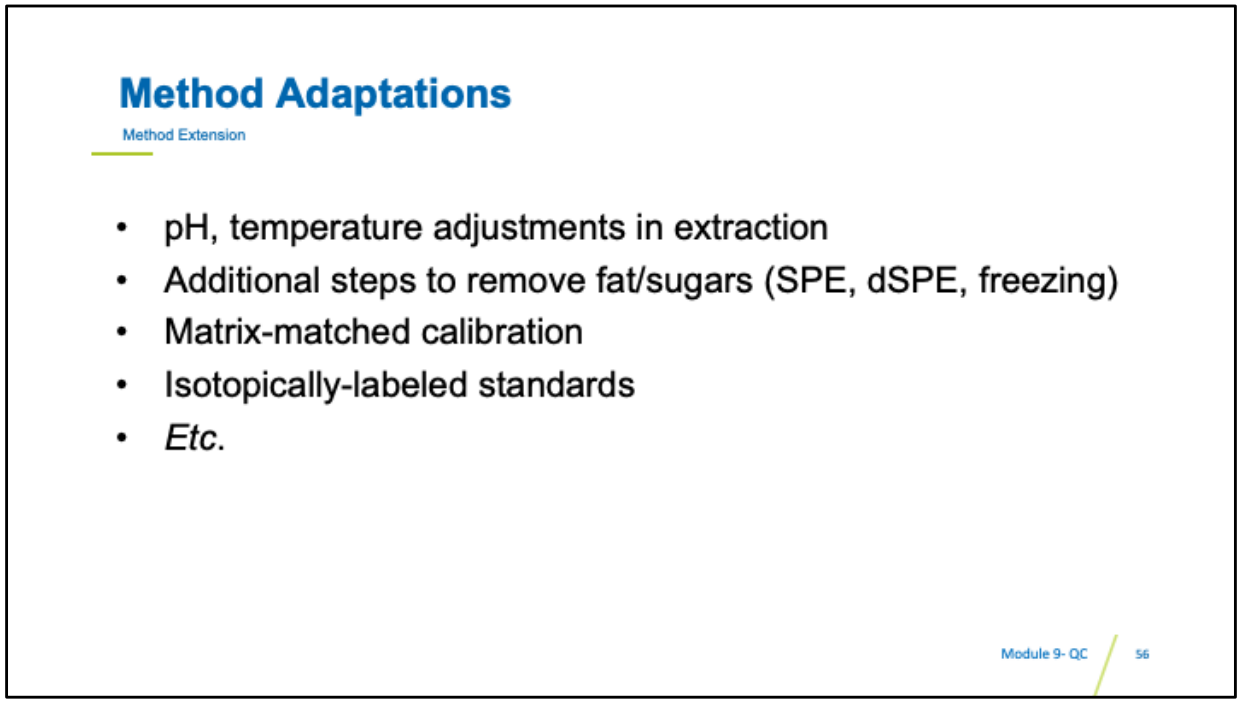
Mass spectrometry is especially sensitive to matrix effects because the specificity of the detector has led us to minimize the burden of sample preparation. When using quick and easy sample preparation methods, we leave quite a large number of matrix components in the sample that we inject in the chromatograph. When the sample reaches the ionization source of the mass spectrometer, these matrix components can affect the ionization process, resulting in either suppression or enhancement of the signal.

This graphic shows an example of suppression. The top line is the calibration using standards in solvent. The bottom two lines are standard in matrix. We can see that for the same concentration, the area under the curve is a lot smaller in the matrix. So, if we used the calibration curve made with standards in solvent, we would be underestimating the concentrations, or producing false negatives.



This slide is a specific example of the same pesticide analyzed in blueberry and in avocado. The solvent and blueberry matrix lines are equivalent, which means that there is no matrix effect. However, the avocado matrix calibration curve is suppressed.

The effect can be opposite, where the signal can be enhanced in the matrix. This happens when the matrix promotes better ionization that the standard in solvent.



Methods can be adjusted to become compatible with additional matrices. Sometimes, adjusting the pH of the sample may be enough. In other cases, like with honey, performing the extraction at higher temperature helps. We have options to add salts in our QuEChERS procedures to remove more fat, sugar or colors. We can also remove fat by freezing it out. All these would need to be tested to ensure that the recovery of all analytes is not reduced beyond the acceptable limits.

Matrix-matched calibrations are very popular to deal with matrix effects and are much more economical than the next option of using isotopically labeled standard. But the most expensive approach is easier to implement... There is always a price to pay, in time, in money, in effort or in performance...

Representative Matrices

Method Extension

- Purpose
  - Identify matrices that behave similarly in the method
  - When a representative matrix is validated, there is no need to validate the other matrices in the group
    - Reduce validation burden
  - Extends the geographical relevance of methods

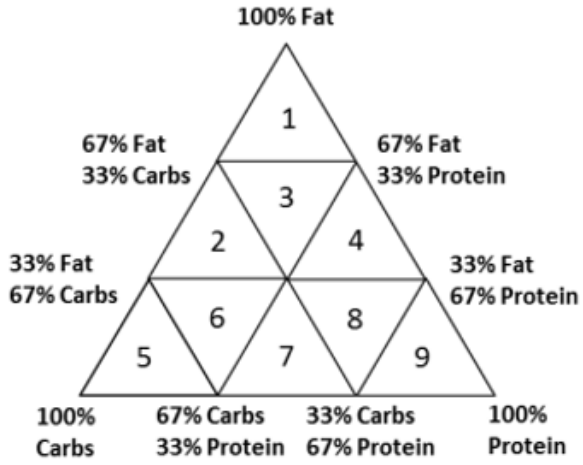
As introduced before, one approach that is facilitating this process is the acceptance of representative matrices. By grouping commodities that behave similarly in the method, we can validate the method for all the members of a group. This is especially useful for pesticide residue analysis where we deal with commodities that grow in very different regions of the world.

AOAC Food Matrix Triangle

Method Extension

Possible Criteria

- Lipids/proteins/sugars
- Acidity
- Water content



https://www.fda.gov/media/81810/download

AOAC proposes a food matrix triangle that is useful to understand how to group commodities. Essentially, we use the lipid, protein and sugar ratios to create groups of commodities that behave similarly in common methods.

Further, we can refine the grouping with information such as acidity. Usually, high water commodities are a group of their own.

Commodity Representatives

Method Extension

Commodity groups	Typical commodity categories	Typical representative commodities
1. High water content	Pome fruit	Apples, pears
	Stone fruit	Apricots, cherries, peaches
	Other fruit	Bananas
	Alliums	Onions, leeks
	Fruiting vegetables/cucurbits	Tomatoes, peppers, cucumber, melon
	Brassica vegetables	Cauliflower, Brussels sprouts, cabbage, broccoli
	Leafy vegetables and fresh herbs	Lettuce, spinach, basil
	Stem and stalk vegetables	Celery, asparagus
	Fresh legume vegetables	Fresh peas with pods, peas, mange tout, broad beans, runner beans, French beans
	Fresh Fungi	Champignons, canterelles
	Root and tuber vegetables or feed	Sugar beet and fodder beet roots, carrots, potatoes, sweet potatoes

https://www.fda.gov/media/81810/download

This table, reproduced in part in this slide and the next, is the matrix grouping used by the US FDA for pesticide residue testing. The entire table can be found at the link indicated here.

What this table says is that if the method has been validated for apples for example, then it should work for pears as well and any other pome fruit. If it works with potatoes, it probably works for carrots too. Etc.

Commodity Representatives

Method Extension

2. High acid content and high water content	Citrus fruit	Lemons, mandarins, tangerines, oranges
	Small fruit and berries	Strawberry, blueberry, raspberry, black currant, red currant, white currant, grapes
3. High sugar and low water content	Honey, dried fruit	Honey, raisins, dried apricots, dried plums, fruit jams
4a. High oil content and very low water content	Tree nuts	Walnuts, hazelnuts, chestnuts
	Oil seeds	Oilseed rape, sunflower, cotton-seed, soybeans, peanuts, sesame, etc.
	Pastes of tree nuts and oil seeds	Peanut butter, tahini, hazelnut paste
4b. High oil content and intermediate water content	Oily fruits and products	Olives, avocados and pastes thereof
5. High starch and/or protein content and low water and fat content	Dry legume vegetables/pulses	Field beans, dried broad beans, dried haricot beans (yellow, white/navy, brown, speckled), lentils
	Cereal grain and products thereof	Wheat, rye, barley and oat grain; maize, rice, whole meal bread, white bread, crackers, breakfast cereals, pasta, flour.
6. "Difficult or unique commodities"		Hops, cocoa beans and products thereof, Coffee, tea, spices

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This is another section of that same table. The next few rows would have milk and meats... Once again, you can access the table in the document available at this link.

I wanted to include this portion because of the category of difficult commodities. These always need special attention.

FDA Guidance for MS Methods

Method Extension

Guidelines for the Validation of Chemical Methods for the FDA FVM Program, 3rd Ed.

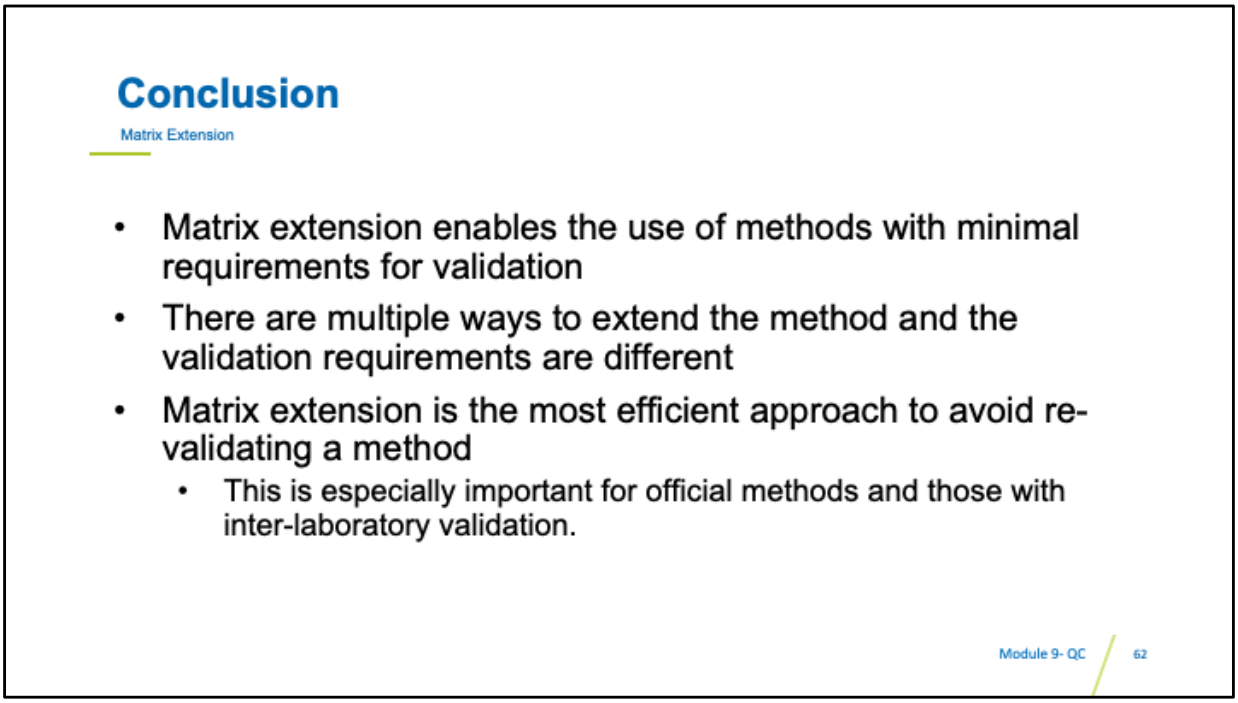
Technique used in the Original Method	Minimum Requirements for Matrix Extensions	Notes
Methods using isotopically labeled internal standards or matrix matched calibration curves	Spike run in duplicate, along with a matrix blank (if available).	Can be run prior to or concurrent with regulatory samples
All other methods	Two matrix spike levels, run in duplicate, along with a matrix blank (if available)	Can be run prior to or concurrent with regulatory samples

Table 2: Guidance for Matrix Extensions

https://www.fda.gov/media/81810/download

FDA also includes its own guidance for demonstrating the validity of a method extended to a new matrix. When using a matrix-matched calibration or isotopically-labeled standards, only a blank and a spike are needed. When using other approaches, then 4 spikes and a blank are needed.

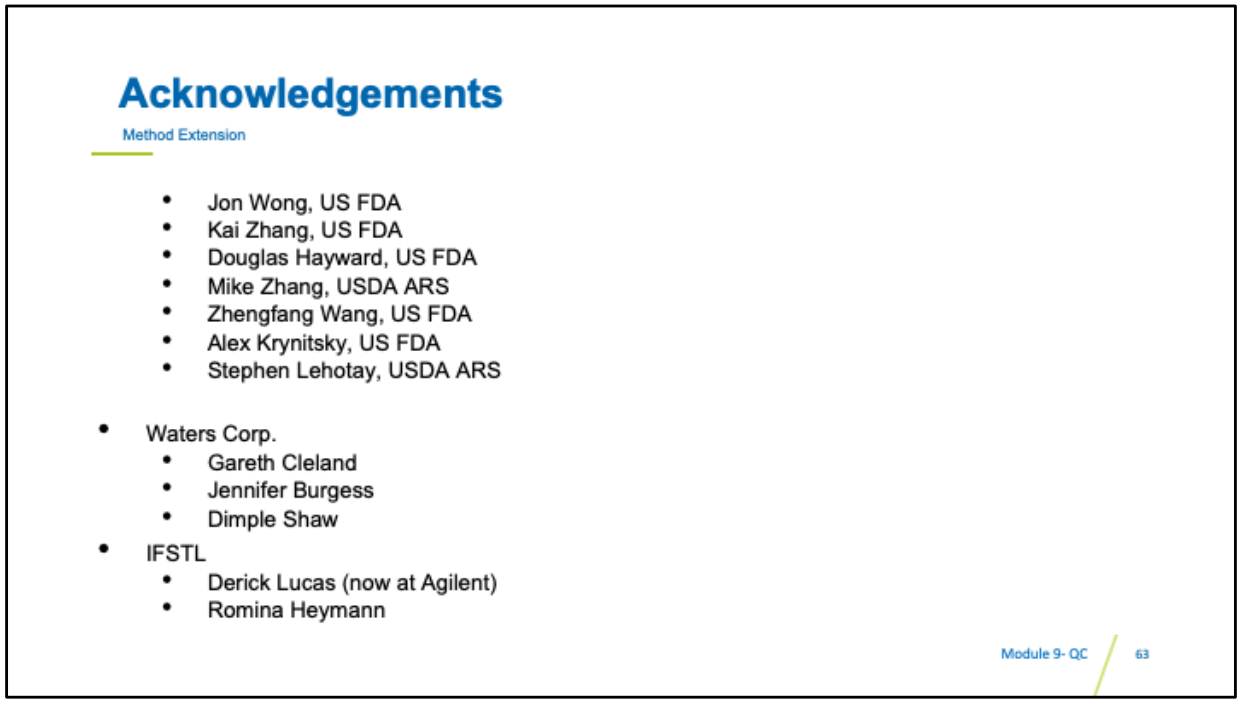
We will perform a matrix-matched calibration and an isotopically-labeled standards correction during the practical session of this training, so we will go through these topics in more details then.



In conclusion, matrix extension enable us to use of methods with minimal requirements for validation

There are multiple ways to extend the method and the validation requirements are different.

Matrix extension is the most efficient approach to avoid re-validating a method. This is especially important for official methods and others that have undergone inter-laboratory validation.



Once again, many people have contributed to the slides used in this module and I want to recognize them here. Special thanks go to Alex Krynitsky of the FDA who provided the slides on the effect of matrices on calibration curves.

You have reached the end of Module 9 on quality assurance. Module 9 was the last module of this online training. We hope to see you soon for the in-laboratory hands-on portion of this training program. Goodbye!