STA

Methods of Analysis for Organic Contaminants

Quality Assurance

Module 9

Janie Dubois, Ph.D.

Food Safety Specialist

parera
Food Risk Analysis and Regulatory Excellence Platform

INAF
INSTITUTE OF NUTRITION AND FUNCTIONAL FOODS

UNIVERSITÉ LAVAL
Faculty of Agriculture and Food Sciences

Table of Contents

Introduction

Analytical laboratories, especially those laboratories performing regulatory testing, must maintain a strong quality management system in order to ensure that they provide a reliable test results for every sample. Quality assurance is the overarching discipline that includes the documentation, standard operating procedures, quality control samples, and external quality assessment schemes. Quality control refers to the procedures that are used to ensure that a method is run as intended and produces valid results. External quality assessment schemes are those assays, some of which are formal proficiency testing, that evaluate the accuracy of the entire testing program from receiving the sample to reporting results. In this module we review some of the aspects of laboratory management that impact the reliability of results. While a short discussion of laboratory accreditation is included, a full description of ISO 17025 is beyond the scope of this training.

Learning Objectives

- Understand the principles of quality assurance systems, the role of ISO 17025 and the basic principles of method validation
- Understand the rationale and execution of single laboratory validation experiments
- Understand the rationale and execution of the validation of a method extension

Lesson 1: Quality Assurance and Method Validation

Quality assurance system are put in place in laboratories to ensure that there is a clear structure in the organization which promotes confidence in the results. While documentation is often perceived as the objective of the quality assurance system, it is rather the actions that ensure quality that are the focus. For example, the organizational structure, or the organigram, of a laboratory organization should illustrate how the skills and experience, as well as the checks and balances that it represents, will ensure reliable results. Similarly, the quality assurance system requires the documentation of employees' skills and experience; it is not having the CV itself on file that matters, it is the fact that it shows that the employee is qualified for their job. Training is also documented because it supports this demonstration that employees are qualified and also the commitment of management to sustainable high-quality results even as technology or best practices evolve. Standard operating procedures are an obvious part of the quality assurance system because they provide employees with procedures to follow that have a demonstrated outcome of producing reliable results.

The quality assurance system for food testing laboratories involved in trade increasingly follows the guidelines from ISO/IEC 17025. The ISO accreditation system for laboratories started in 1999 but was not broadly implemented in food safety laboratories until the late 2000s. Rigorous internal quality assurance systems were considered sufficient to assure the reliability of the results. However, with the dramatic increase in international trade, deferring to international standards provides the advantage of building trust in each other's laboratories without a need to inspect them ourselves. It's important to remember that we, as governments involved in trade, used to inspect the laboratories of our trading partners, a

burden that we have now passed on to accreditation bodies. Compliance with ISO accreditation requirements includes participation in appropriate proficiency testing schemes. It also emphasizes the use of validated methods and internal quality controls that ensure that the application of the validated method produces a reliable result.

It is important to understand that ISO 17025 accreditation is about laboratory management. It does not in itself define what a laboratory must do. What it does is require documentation for what a laboratory decides to do. For example, a number of different methods can be used to measure contaminants, and the laboratory is free to select the method that is best suited for its instrumentation, environmental controls, staff training and experience, and sample throughput. Once this decision is made, the laboratory must document that analysts obtain the right result reliably when they use this method. This is what we call a single laboratory validation, or verification, for the use of an official method. In the same spirit, regulatory agencies typically do not require laboratories providing them with results to use predefined methods, but rather select methods that meet certain performance criteria.

One common misunderstanding relating to laboratory accreditation is to think that an accredited laboratory has received this certification for everything that it does. Actually, laboratories receive accreditation for specific methods that they use to produce results; this is known as the scope of the accreditation. In more rare circumstances, a laboratory may seek accreditation for a broad scope, but this process is both expensive and burdensome, which limits its accessibility to well funded and well-staffed laboratories. As mentioned before, we will not go through the requirements for ISO 17025 in this training; the reader is encouraged to review UNIDO's practical guidebook on complying with ISO 17025[1].

[1] Complying with ISO 17025 A practical guidebook. UNIDO. Available at: https://www.unido.org/sites/default/files/2010-08/Complying_with_ISO_17025_A_practical_guidebook_0.pdf (accessed 11/12/20)

As stated in the UNIDO document referenced above, the documentation of a quality system is meant to demonstrate that the work was done by a properly qualified person who had been trained in the relevant technical operations and had access to all the information necessary for the proper execution of their work. It also should demonstrate that the method used was technically sound and appropriate for the sample and the requirements of the client. As a side note, the term “client” represents different people or entities depending on the laboratory. For example, a private laboratory has clients who pay for tests. These clients can be food producers, or they can be regulatory agencies outsourcing their testing for example. A regulatory laboratory typically produces results for the regulatory agency it belongs to. The quality assurance system should also document that the equipment used for analysis was properly maintained and calibrated and that quality control checks were in place and produced results within the specifications.

In summary a trained analyst uses a validated method that has been demonstrated to work in their own laboratory and runs it on reliable instrumentation to obtain a result whose reliability is confirmed by quality control checks. Internal and external proficiency testing schemes are used to document all the aspects of the previous sentence. While an internal proficiency test typically aims to build confidence in the results obtained by an analyst (*i.e.* training and verification of the effectiveness of the training), formal external proficiency testing is required for accreditation. Proficiency testing (PT) is simply the comparison of results from a number of different laboratories (or analysts in the case of internal schemes) to determine testing performance. Put simply, individual laboratories receive a sample originating from the same source and report their results for statistical analysis. Ideally, all laboratories and analysts involved in a proficiency testing round obtain results that are close to each other and also to the value obtained by the organizers (*i.e.* the assigned value). PT establishes and confirms the accuracy and precision of the laboratory result. The statistical analysis of all participants results is expressed as a z score. The z score

combines an estimate of the error of a result with a standard deviation according to the following equation:

$$z = (x - x_{a)/\sigma_p}$$

Where: x is the result reported by the participant; $x_a$is the assigned value and σ_p is the standard deviation for proficiency.

The z scores are reported for each laboratory identified by a code to keep its identity confidential. A z score of 0 indicates that the laboratory has obtained the value closest to the assigned value. While increasingly high z-scores, both in the positive and negative directions, indicate greater deviations from the assigned value, a z-score of up to plus or minus 2 indicates a satisfactory result whereas a z score greater than +2 or lower than -2 is considered unsatisfactory.

What is Method Validation

We emphasized earlier that laboratories must use validated methods. A validation aims to determine the reliability of a method. It includes parameters such as specificity, accuracy, precision, sensitivity, applicability and ruggedness. The highest level of method validation is performed through a collaborative study that includes laboratories from different countries, using different instruments, having different staff perform the analysis, but all from a common sample source.

Definitions

There is unfortunately not a single definition adopted by all for many of the terms used in method validation. While they aim to express the same outcome, the definitions vary and contain a level of detail proportional to their field of application. For example, we can compare definitions proposed by three different documents with extremely broad, broad and narrow applicability such as FAO in a document focusing on food composition data

(Food composition data -Chapter 6 [2]), by the US Food and Drug administration in a document dedicated to the validation of chemical methods (in general) for food feed cosmetics and veterinary products (Guidelines for the validation of chemical methods in food feed cosmetics and veterinary products[3]), and the one included in the European Union SANTE document entitled Analytical Quality control and method validation procedures for pesticide residues analysis in food and feed (2007)[4].

Specificity (FAO): Specificity is the ability of a method to respond exclusively to the substance for which the method is being used.

Specificity (U.S. FDA): The ability of a method to measure an analyte in the presence of components which may be expected to be present. It should be noted that the US Food and Drug administration prefers to use the term selectivity over specificity. It defines selectivity as the extent to which a method can determine particular analytes in a mixture or matrix without interferences from other components of similar behavior.

Specificity (SANTE): The ability of the detector (supported by the selectivity of the extraction, clean-up, derivatization or separation, if necessary) to provide signals that effectively identify the analyte. GC-MS with EI is a fairly non-selective determination system capable of high specificity. High resolution MS and MSn can be both highly selective and highly specific. SANTE defines selectivity as the ability of the extraction, the clean-up, the

---

[2] Food Composition Data, published by FAO (2003). Available at: http://www.fao.org/3/y4705e/y4705E00.htm#Contents (accessed 11/12/20).

[3] Guidelines for the validation of chemical methods in food, feed, cosmetics, and veterinary products. 3rd edition (2019). US Food and Drug Administration. Available at: https://www.fda.gov/media/81810/download (accessed 11/12/20)

[4] Analytical quality control an method validation procedures for pesticide residues analysis in food and feed. SANTE /2017/11813. Available at: https://ec.europa.eu/food/sites/food/files/plant/docs/pesticides_mrl_guidelines_wrkdoc_2019-12682.pdf (accessed 11/12/20)

derivatization, the separation system and (especially) the detector to discriminate between the analyte and other compounds. For example, GC-ECD is a selective determination system providing no specificity.

Nevertheless, an agreement was reached in the Codex Committee on Methods of Analysis and Sampling on analytical terminology for Codex Alimentarius and government use and published in the Guidelines on Analytical Terminology[5]. Here are some definition adapted for the purpose of this training. The definitions and equations presented in the Codex document will be discussed further in the in-person portion of this training

Accuracy: The closeness of agreement between a test result or measurement result and a reference value.

- Notes: The term "accuracy," when applied to a set of test results or measurement results, involves a combination of random components and a common systematic error or bias component. When applied to a test method, the term accuracy refers to a combination of trueness and precision.

Applicability: The analytes, matrices, and concentrations for which a method of analysis may be used satisfactorily.

- Note: In addition to a statement of the range of capability of satisfactory performance for each factor, the statement of applicability (scope) may also include warnings as to known interference by other analytes, or inapplicability to certain matrices and situations.

Bias: The difference between the expectation of the test result or measurement result and the true value. Bias is the total systematic error as contrasted to random error.

Error: Measured quantity value minus a reference quantity value

[5] Guidelines on analytical terminology, Codex CAC/GL 72-2009). Available at: http://www.fao.org/input/download/standards/11357/cxg_072e.pdf (accessed 11/12/20)

Limit of Detection (LOD): The true net concentration or amount of the analyte in the material to be analyzed which will lead, with a defined probability, to the conclusion that the concentration or amount of the analyte in the analyzed material is larger than that in the blank material. In chromatography, LOD is estimated to be the concentration of analyte that produces a S/N of 3:1.

Limit of Quantification (LOQ): The smallest quantity of an analyte that can be measured with a specified level of confidence. The Codex definition includes the important considerations of standard deviation and matrix. In chromatography, the LOQ is the concentration that produces a S/N of 10:1.

Linearity: The ability of a method of analysis, within a certain range, to provide an instrumental response or results proportional to the quantity of analyte to be determined in the laboratory sample. This proportionality is expressed by an *a priori* defined mathematical expression. The linearity limits are the experimental limits of concentrations between which a linear calibration model can be applied with an acceptable uncertainty.

Precision: The closeness of agreement between independent test/measurement results obtained under stipulated conditions.

- Notes: Precision depends only on the distribution of random errors and does not relate to the true value or to the specified value. The measure of precision is usually expressed in terms of imprecision and computed as a standard deviation of the test results. Less precision is reflected by a larger standard deviation. Quantitative measures of precision depend critically on the stipulated conditions.

Recovery/recovery factors: Proportion of the amount of analyte, present in, added to or present in and added to the analytical portion of the test material, which is presented for measurement.

Repeatability (Reproducibility): Precision under repeatability (reproducibility) conditions.

Repeatability conditions: Observation conditions where independent test/measurement results are obtained with the same method on identical test/measurement items in the same test or measuring facility by the same operator using the same equipment within short intervals of time.

- Note: Repeatability conditions include: the same measurement procedure or test procedure; the same operator; the same measuring or test equipment used under the same conditions; the same location and repetition over a short period of time.

Reproducibility conditions: Observation conditions where independent test/measurement results are obtained with the same method on identical test/measurement items in different test or measurement facilities with different operators using different equipment.

Robustness (ruggedness): A measure of the capacity of an analytical procedure to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

Selectivity: Selectivity is the extent to which a method can determine particular analyte(s) in a mixture(s) or matrice(s) without interferences from other components of similar behavior.

- Note: Selectivity is the recommended term in analytical chemistry to express the extent to which a particular method can determine analyte(s) in the presence other components. Selectivity can be graded. The use of the term specificity for the same concept is to be discouraged as this often leads to confusion.

Sensitivity: Quotient of the change in the indication of a measuring system and the corresponding change in the value of the quantity being measured.

- Notes: The sensitivity can depend on the value of the quantity being measured The change considered in the value of the quantity being measured must be large compared with the resolution of the measurement system.

Trueness: The closeness of agreement between the average of an infinite number of replicate measured quantity values and a reference quantity value.

- Note 1: Measurement trueness is not a quantity and thus cannot be expressed numerically, but measures for closeness of agreement are given in ISO 5725.
- Note 2: Measurement trueness is inversely related to systematic measurement error, but is not related to random measurement error.
- Note 3: Measurement accuracy should not be used for 'measurement trueness' and vice versa.

Validation: Verification, where the specified requirements are adequate for an intended use.

- Verification: Provision of objective evidence that a given item fulfils specified requirements.

Quality Control

As mentioned previously internal quality control procedures in place in the laboratory normally require the use of validated methods. The validation of an official method is performed through an international collaborative study. At the onset of application of a new method in a laboratory, a single laboratory validation should be performed. A single laboratory method validation is essentially a verification that the validated method performs as expected in one's laboratory. It typically involves a single instrument and single analyst but predefined number of different matrices and repetitions of measurements over a certain number of days. The objective is to document that the environmental conditions of the

laboratory, the instrument selected, its maintenance status, and all the equipment and consumables used in the performance of the method, as well as the skills of the analyst, all combine to produce the expected value reliably. This topic will be discussed further in Lesson 2.

Once the method performance has been verified in the laboratory, the quality control processes should confirm that each run delivers a reliable result. For this purpose, it is commonplace to run blanks, standards, spiked samples or naturally contaminated samples, duplicate analysis for at least 10% of the samples, and one type of a reference material. Reference materials come with different levels of confidence; a standard reference material has the highest level of confidence while a reference material is also a substance usually purchased from a reliable organization who guarantees a high level of certainty with regards to the purity and concentration of the material. Laboratory control materials provide a good option to lower the cost by using a sample that is available in large quantity in the laboratory and for which the concentration of the analyte of interest has been confirmed rigorously. Often, laboratories will keep a contaminated sample submitted for analysis when it contains a relevant concentration of an analyte of interest for this purpose. A naturally contaminated sample is called “incurred”. Relevant concentration is obviously a relative term, but it most often refers to the regulatory limit set for the contaminant. The sample is homogenized and preserved, usually in the freezer, to ensure that all sub portions contain the same concentration of analyte and that the control material does not degrade over time.

The laboratory control material is often known as the QA sample. Sources of error associated with the QA sample are insufficient homogeneity typically caused by the presence of large particles, contamination of the QA sample from the use of dirty equipment, poor subsampling for the verification of the concentration, and a number of usual sources of error in a laboratory such as inaccurate weighing, pipetting, and cross contamination.

The quality assurance sample is used to populate a quality control chart. This chart is a graphical representation of the results of an analysis centered around the expected value for the quality assurance sample. The acceptable range of values is usually defined as twice the standard deviation and random distribution of the reported values between these two limits indicates that a method is under good control. In addition, the control chart serves to identify systematic changes in the measurements that may originate from various sources such as deterioration of the signal in the instrument, a change of supplier for some of the reagents and solvents, or at bias in results obtained from one analyst. The graphical representations of problems of dispersion, trend, and shift were reviewed in the video portion of this lesson.

The purpose of the quality control chart is to spot any changes and implement the corrective actions necessary to continue to produce reliable results, ideally before the measured values have exceeded the acceptance limits. The interpretation of the control chart is not as straightforward as everyone would like because there are many sources of error that can cause a change in the measured value in the same direction. For example, a shift in the reported values associated with specific work periods or “work shifts” maybe due to the operator on duty, or to a change in the environmental conditions of the laboratory. For example, a laboratory running mass spectrometry during the night must ensure that the air conditioning stays on. For laboratories that have multiple instruments running in the same methods, the instrument itself, its column and any one of its components can be the source of error. A batch of solvent or reagents could cause a sudden trend. The same is true for a new batch of standard, whether they come from a new supplier or the usual supplier of the laboratory.

Sample management is also an important component of the quality assurance system. Traceability of the sample, all the way back to sample collection, is insured through proper

documentation of the chain of custody. The sample label must always contain sufficient information for traceability before the sample was received in the laboratory, and typically also contains a description of the sample detailed enough to ensure that the label undoubtedly belongs to the sample. Samples must be stored in appropriate conditions throughout the chain of custody, and this includes the preservation of a portion of the sample for re-analysis in the case of disputed results. The sample identity must be tracked through the apportioning process.

Acceptability of Method

Analysts are involved in the documentation for the quality assurance system to varying degrees depending on the size of the laboratory and its management structure. There is however one area where the responsibility falls solely on the analysts and that is method selection. When selecting a method, analyst must determine if its purpose or its goal and scope of applicability fit the purpose of the analysis being requested. This includes considering if the matrix is included in the scope of the method, if the sample preparation steps can be accomplished in the laboratory with the equipment and consumables available or affordable, if the dynamic range and limit of detection of the method aligns with the expected concentrations and with the target concentration (MRL), ease of use by the analysts in the laboratory considering their experience and training, and the cost.

In addition, if results are to be submitted to a regulatory authority, they must meet the acceptance criteria established by the organization. In GC and LC, all retention times must be compared to those of standards ran on the same instrument and under the same conditions. The acceptance criteria may vary depending on the analytical technique, the concentration of the target analyte, and the matrix. They can be an issue when, for example, an aging instrument is used that is unstable. If the repeatability and reproducibility of the retention time cannot be achieved, or if we can’t afford the standards required for the analysis, for example matrix-matched calibrations can require a lot of standard solutions

that increase the cost of the method, then we need to select a method that fits our purpose, including our financial means.

Conclusions

The reliability of results produced by any given laboratory depends on its commitment to assuring quality. Quality assurance systems don't have to be overly burdensome to be effective. When a laboratory seeks international recognition through accreditation according to ISO 17025, the requirements of the standards include the quality assurance system and a number of other parameters that must be documented in a prescribed manner.

Lesson 2: Single Laboratory Validation

Validation is the process to establish the performance characteristics and limitations of an analytical method, including which analytes can be measured, in what kind of matrix, and in the presence of which interferences. Much like many of the definitions in quality control, validation itself is defined differently by various organizations. The table below showcases some examples of definitions for the word “validation” as adopted by Codex, ISO and number of regulatory agencies.

Table 1: Definitions of validation published by different organizations.

Reference	Validation Definition
Codex CAC/GL 74	Process to establish the **performance characteristics** and limitations of an analytical method: which analytes, in what kind of matrices, in the presence of which interference. Result = **precision and trueness values** of a certain analytical method under the examined conditions.[1] [pg 2]
ISO 16140-1	Establishment of the **performance characteristics** of a method and provision of **objective evidence** that the performance requirements for a specified intended use are fulfilled.[2] [pg 2]
USDA FSIS	Process to measure **performance characteristics** of a particular test, with the goal of determining whether the test is equivalent to the reference test for the intended conditions of use. “Equivalent” = the performance characteristics are **statistically indistinguishable**.[3] [pg 2]
US FDA	Demonstration that **adequate confidence** is provided when the results obtained by the alternative method i.e. the commercially available kit, are **comparable to or exceed** those obtained using the reference method using the **statistical criteria** contained in the approved validation protocol.[4] [pg 16]

Health Canada	Evaluation of the **performance parameters** of a new method in comparison to an accepted reference method using paired or unpaired samples. In the context of relative validation, the results of the reference method are assumed to reflect the true microbiological status of the samples and the performance parameters of the alternative method are **calculated in relation** to this. (Source: Part 4)
ISO 17025:2017	Provision of objective evidence that a given item fulfills specified requirements, while **Validation** is **defined** as '**verification**, where the specified requirements are adequate for an intended use.

For the purpose of this document, we will review the requirements for different levels of validation as applied by the US Food and Drug administration for its food program. The FDA defines four levels of validation. Level 1 is a single laboratory validation limited to emergency use. This type of method is only acceptable when no method that has undergone interlaboratory validation is available for an emerging contaminant or in an unexpected matrix. Level 2 validation is more comprehensive than Level 1 and corresponds to the AOAC's single laboratory validation guidelines. This level of validation is enough for long time use within the agency's laboratory. A Level 3 method undergoes multi laboratory validation, which makes it peer verified, a more extensive process that is designed to ensure confidence for widespread long-term use and international trade. Finally, a level 4 method has undergone collaborative validation studies typically under the auspices of the AOAC, where more than eight laboratories participated. A level 4 method has international acceptance.

For multi residue methods and methods that are applicable for multiple matrices, tables of minimum requirements are published. Some of these tables were reviewed in the video portion of this training. In summary, the minimum requirements for a Level 1 validation for FDA, which means for a method to be used in a case of emergency, a single matrix from

two sources should be used, and one blank and three samples spiked at half, one time, and twice the target level are sufficient.

The Level 2 method, traditionally known as the single laboratory validation for routine use, requires the repetition of analysis from three sources of a matrix, over three different days for the same concentrations as Level 1. If a certified reference material is available, it is recommended to include it in the replicate analysis. Levels 3 and 4 differ in that they require measurements to be performed in more than one laboratory, a minimum of two laboratories for Level 3 and a minimum of eight laboratories for Level 4. The number of sources of matrix goes up to three, but the spike levels remain the same, and each matrix source should be replicated three times for a Level 3 and twice for a Level 4.

The acceptance criteria for methods differ between qualitative and quantitative methods. Moreover, the two different types of qualitative methods have different acceptance criteria. A screening method, typically characterized by a pass or fail or yes/no answer, aims to determine the presence or absence of an analyte. The lateral flow tests discussed in Module 2 are examples of screening methods. Some LC/MS (and /MS) and GC/MS methods are also implemented for screening purposes. Semi-quantitative methods, such as ELISA, yield both a determination of presence and absence and a quantity, typically with a higher level of uncertainty.

The validation procedure for a binary test requires only the analysis of a blank matrix and one concentration, typically the target concentration or MRL. The false positive rate is calculated as the number of blank samples testing positive with a 95% confidence interval. The false negative rate is the proportion of test results for spiked samples at the target concentration that don't detect the analyte. Generally, a false negative rate of less than 5% at target level is acceptable. This number can differ depending on the actual target level because the standard deviation and uncertainty is higher for lower concentrations. A 10 to

15% false positive rate at target test level is usually acceptable. A higher false positive rate is accepted because positive samples are verified using a confirmation method, while only a small proportion of negative samples are confirmed. From a health perspective, a false negative means that a food containing a contaminant recognized for posing a health risk is present at a concentration higher than the maximum residue limit allowed on the market.

A method validation necessarily involves the comparison of results with a reference method and a statistical evaluation of the equivalence of the results. Original methods and methods deviating significantly enough from existing methods to be considered new, significant modifications to validated methods, extension of method scope to include additional analytes, highly differing matrices, or changing the intended use of the method, changes involving new technology or automation, and significant changes to important parameters such as critical reagents or steps all result in a requirement for validation. Matrix extensions may require a lower level of verification limited to testing for unintended differences if the new matrices are not significantly different from the matrices included in the original method. Groupings of matrices have been developed and published to assist in the determination of whether a new matrix needs any validation at all. Methods are increasingly validated for “representative matrices” and all other commodities belonging to the same group would not need to be validated because they were considered included in the original method. In these situations, we aim to verify the specificity of the method or in other words, quantitatively indicate the extent to which the method can distinguish between the analyte of interest and interfering substances under the experimental condition, including the new matrix. Random interferences should also be measured through the analysis of a set of representative blank samples, ideally from more than one source. We also need to verify the accuracy of the method, or the closeness of agreement between the true value of the analyte concentration and the mean result that is obtained by applying the experimental procedure many times to a set of homogeneous samples.

As mentioned previously, the accuracy requirements of methods will vary depending upon the planned use of the results. For example, we often allow less accuracy at very low concentrations, especially when these low concentrations do not have an impact on a regulatory action, or in other words, on public health. However, a method providing low accuracy at very low concentration would not fit a purpose of analysis for toxicology or risk assessment for example. Significant differences in matrix could include adding high fat matrices such as avocados or peanuts in a method targeting only high-water matrices like apples and celery. Similarly, matrices with a more extreme pH, like citrus, would warrant a full single laboratory validation.

Significant Modifications

We introduced the concept of significant modifications to a method, which may require more extensive validation. In chromatography and mass spectrometry methods, the multi laboratory validation is expected to ensure that the method will perform well when all the steps of the procedure are followed. There are however some modifications that may need to be applied and, once again, we refer to the FDA's recommendation as an example.

Some modifications are considered minor and would not need a method to be validated, while others are considered major and require a documentation of the validity of the modified method.

Chromatography column: Changes in the column chemistry, particle size, particle type and pore size identified in the multi laboratory validated method are considered major modifications. However, column length or diameter, but not both, can be modified as long as the flow rate is adjusted to achieve the same separation reported in the validated method. FDA allows a relative retention time in chromatography of ±20%.

Mobile phase: The composition of the mobile phase should not be changed, but there is latitude on the mobile phase modifiers. Small concentration differences of up to 10% of salts or a pH difference of less than 0.2 units are acceptable as they should fall within the ruggedness of the method. The same criteria described for the column apply, *i.e.* relative retention time of ±20%.

Injection volume: There may be a need to change the injection volume as a result of a change to the column dimensions and flow rate of the mobile phase. Any change should not impact peak symmetry, resolution, and method sensitivity. If it does affect any of these parameters, it must be validated.

HPCL-UHPLC: When implementing a method developed for HPLC on a UHPLC instrument, the column chemistry must be preserved, but the method can be used on the UHPLC system with the appropriate conversions of injection volume and flow rate. The retention time of the analytes is not part of the procedures, but rather a result of the method. Therefore, moving from HPLC to UHPLC will produce a different retention time, but the acceptance criteria for retention times remain the same (*i.e.* agreement with the retention time of the standard).

Source conditions in MS: The source conditions in mass spectrometry need to be adapted for different instruments, for different models and for different manufacturers. Consequently, these source parameters can and must be changed to meet the performance specifications of the method for its entire calibration range.

MS ion monitoring window: The retention time window assigned for the monitoring of ions in mass spectrometry can and should be changed in response to changes in the elution profile brought about by any of the above-mentioned modifications. The selection of ions and number of transitions monitored should not be changed.

Number of analytes: A reduction of the number of analytes measured by a method does not require any validation. An increase of the number of analytes, or the extension of the method to new analytes, does require a level of validation.

In some cases, the modifications are significant enough for a method to be considered new. FDA provides a list of nine circumstances where a method would be considered new:

- Column characteristics (e.g., separation mode [reverse phase to normal phase], particle type)
- Column temperature
- Data collection mode (e.g., Full Scan, MRM, DDA):
- Ion pair reagent
- Ionization polarity
- Ion selection of precursor and product ions: For confirmatory analysis the use of additional structurally significant products ions is allowable, provided they are compared to a standard analyzed at the time of use and do not reduce the dwell times of the quantifying and qualifying ions listed in the method.
- Ionization source (e.g., ESI, APCI)
- Mass resolution
- Mobile phase (composition and gradient)

Conclusions

A single laboratory validation, also known as a verification, is sufficient when using an official method and only aims to verify that it works as anticipated in one's laboratory. In a nutshell, the specificity of the method reassures us that negative samples are negative. The

accuracy tells us how well it recovers the analyzed from a spiked sample. The precision expresses the standard deviation of replicates and all of these factors are influenced not only by the procedures of the method but also by the instrument, analyst, laboratory conditions and fit for purpose.

A method must be validated before it is used for official purposes. The level of validation that is acceptable is related to the circumstances of the use of the method. Official methods only need single laboratory validation, while emergency methods can be used with even less. The level of validation should be increased with larger or more impactful modifications to a method.

Lesson 3: Validation of Method Extension

The two most common types of validation needed in a routine laboratory are the verification of an official method that will start to be used in the laboratory, and matrix extensions. The concern with the new 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 matrices originally included in the scope of the method, or may affect the measurement. The performance of some measurement technologies is more affected than others. Matrix extension became a very important consideration when mass spectrometry was deployed broadly in the food safety laboratory because of the matrix effects of suppression and enhancement.

Representative Matrix

The Food and Drug Administration published a list of representative commodities relevant for pesticide residue analysis in Appendix 4 of its document "Guidelines for the validation of chemical methods in food, feed, cosmetics, and veterinary products"[6]. For example, the very large group of commodities termed "high water content" includes pome fruit, stone fruit, fruiting vegetables, brassica vegetables, leafy vegetables, fresh legume vegetables, fresh fungi, and root and tuber vegetables. All pome fruits can be considered likely to work well in the method if apples or pears are included. Similarly, all roots and tuber vegetables can be considered likely to make no difference in the method if sugar beet roots, carrots, potatoes, or sweet potatoes are included in the method. It's important to note that each method, and maybe more specifically each type of extraction and analytical technique, may be influenced differently by small differences between matrices. The best approach is to research the perspective of analysts with expertise in the field who can provide guidance about the necessity to extensively examine the influence of a new matrix on the validity of the results. It is normal for methods to have different performance parameters for

[6] FDA, Guidelines for the Validation of Chemical Methods for the FDA FVM Program, 3rd Ed (2019) Available at: https://www.fda.gov/media/81810/download (accessed 11/30/20)

different matrices, and the only concern is when a new matrix drives these performance parameters outside of the acceptability range

The FDA essentially divided all food commodities into 10 different commodity groups. High water content, high acid and high-water content, high sugar and low water content, high oil content, high starch and/or protein content with low water and fat, difficult or unique commodities is the 6th category and includes hops, cocoa beans, coffee, tea and spices. Meat and seafood, milk and milk products, eggs, and fat from animal origin make up the remaining four categories. Figure 1 is a reproduction of the graphical representation of the partitioning of commodities used by the US FDA (but published by AOAC). It clearly shows how the three main factors that affect extraction and measurements, *i.e.* fat, carbohydrates and proteins, are considered in the partitioning.

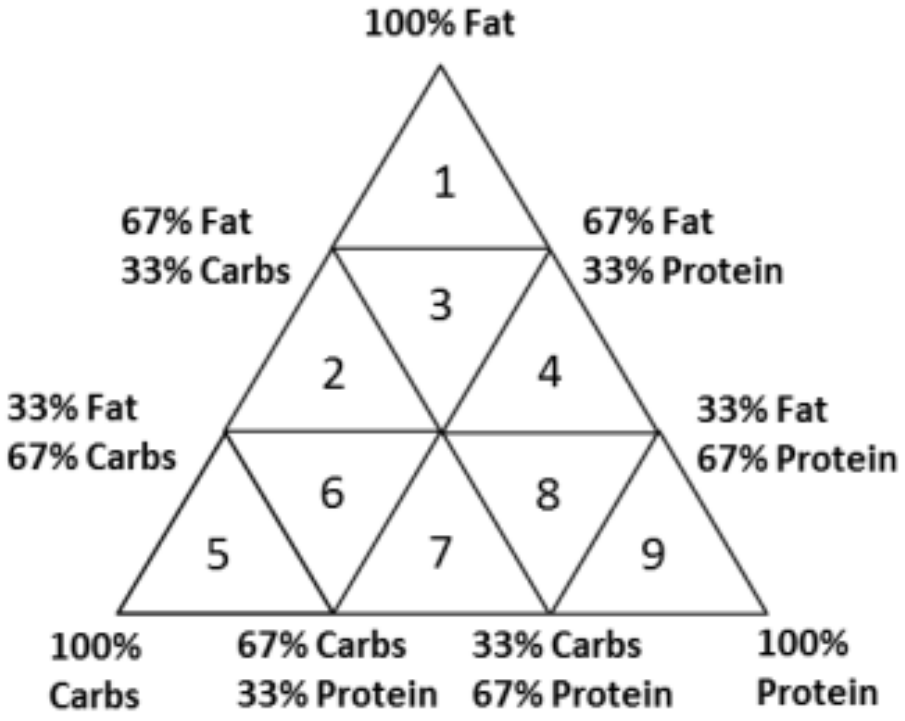

Figure1: AOAC Food Matrix Triangle. Reproduced from FDA (2019).

As discussed before in this text and more extensively in the in-person portion of this training, some mass spectrometry methods incorporate the use of isotopically labeled internal standards for each analyte included in the scope of the method, while most laboratories rely on a matrix matched calibration; in both cases, it is generally accepted that a spike ran in duplicate and a matrix blank analyzed in the same run as samples from matrices covered by the scope of the method are sufficient to verify the performance. FDA requires its own laboratories to use spikes at two concentrations, each analyzed in duplicate, and a matrix blank for other methods. In all cases, the recovery should be within the range of those reported for the matrices in the original validation.

Conclusions

Methods are generally developed to test the commodities traded in higher volumes. In addition, each market establishes a list of commodities that receive more attention based on their consumption patterns. Staple foods are particularly important because they represent a high proportion of the diet of a population and the presence of contaminants at levels of concern could have a much greater impact then a contamination of a niche product. However, with the growth of international trade and the population's appetite for exotic foods, methods need to be adapted for the analysis of different commodities. Luckily, an appreciable number of commodities can be traced back to a group with similar characteristics for which the presence of one representative commodity is indicative of a great likelihood of applicability to the other commodities of the same group. However, each commodity should be evaluated individually; as seen in this lesson, the burden is minimal to determine that there is no difference in method performance. When a difference is seen, it is important to perform the necessary work to ensure that the results obtained, possibly with the requirement of applying small modifications to the method, are accurate and reliable.