



A Guide to Hemp Method Validations

Paper Five – Five Part Series

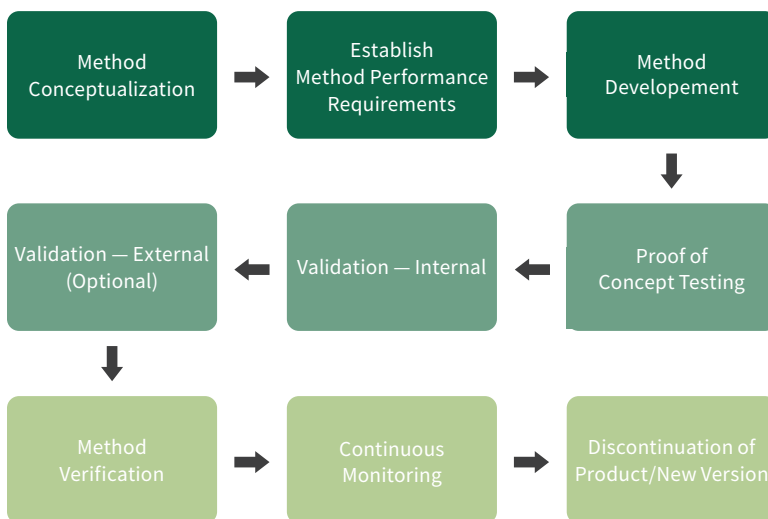
Executive Summary

Once the end user has reviewed validation reports and chosen a method, the final step in their process is verification. While validation of a method establishes known performance requirements, verification ensures the method works as intended for the desired matrices and facilities. Verification provides confidence to the end user that the method will perform accurately and that the results obtained can be trusted. Ongoing method performance monitoring is also recommended.

Life Cycle of a Method: From Conceptualization to Validation and Verification

This series of papers has summarized key requirements for method validation to ensure that end users can make informed decisions on the methodology they utilize. While method validation is essential, it is just one step that occurs in the middle of the life cycle of a method. For method developers, the process begins with an idea and ends with a validation study (internal and external validation¹). However, for end users, this is not the last step in the method's life cycle. As the method begins to be utilized by the industry, method verification will need to be performed to ensure that the method works correctly at the end user facility.

Figure 1: Life Cycle of Methods



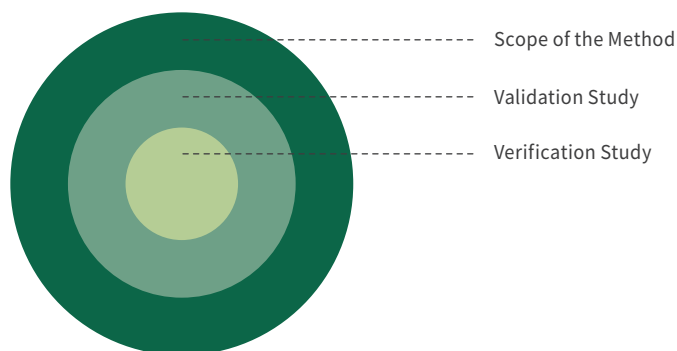
Validation and Verification Relationship

Method verification and method validation are often used interchangeably, but informed readers know from the first paper in this series that these two processes are distinctly different. Method validation establishes the performance characteristics of a method for a specific intended use, while verification is used to demonstrate that a validated method functions as it is supposed to in the hands of an end user².

Understanding the relationship between validation and verification is key to ensuring that your testing facility complies with state regulatory requirements and ISO accreditation. While a method may be suitable for many matrices, the method is only validated for a small subset of those matrices due to cost and timing during the validation study. As end users implement the method, testing facilities must verify that the method is suitable for the specific products tested at that site (figure 2 and table 1).



Figure 2: Validation and Verification Relationship



For example, a method may be validated for potency in CBD-infused chocolate. However, is the method also applicable to infused gummies? A verification study could be performed on the gummies to demonstrate that the method works for that matrix. This is a common nuance that is often overlooked in nascent industries. We will discuss the topic in more detail below.

Table 1: Matrix Relationship for Validation and Verification

Analytical Method for Determining CBD Concentration		
Method Scope	Method Validation	Method Verification
Hemp Flower	Hemp Flower	Hemp Flower
Infused Edibles	CBD Infused Chocolate	CBD Infused Gummies
Concentrates	None	N/A

Verification 101

As outlined throughout this series of papers, testing requirements for validation studies are extensive and traditionally are performed by the method developer or expert laboratories. Verification testing, however, is performed by the end user (site verification) or the method developer (food item verification).

- Site verification is specific to the test matrices analyzed at that facility. It’s performed to demonstrate the method’s fitness for purpose for the end user.
- Food item verification is a process to demonstrate that the method is appropriate for use in more matrices than those studied during the validation study. It can be performed by the method developer or end user.

Similar to validation, there is not a single approach that is required for verification testing. Multiple approaches exist, allowing an end user or method developer flexibility to conduct testing within their technical capabilities but will still ensure that their method is fit for purpose.

Where Do I Start?

The verification process can seem overwhelming for a facility that’s just beginning to verify the methods it uses. This section will outline where to start, or more exactly, which questions need to be asked and answered before beginning verification studies.

1. Which matrices are my method validated to test?
 - a. The first step in the verification process is determining the validated scope of applicability of the method. If the method has been validated internally, ask the method developer for the validation report. If the method has been validated externally as part of a third-party certification process, review the publicly available certificate to determine which matrices were validated.
 - b. If the scope of applicability contains the matrices that your facility tests, a small implementation verification study can be performed to ensure the method works with your team. If the specific matrices your facility tests are not included in the validation, then proceed to question 2. If the answer to question 1 is yes, proceed to question 4.





2. The method was not validated for matrices that my facility tests, but they are similar. What should I do next?
 - a. Review the list of validated matrices and categories to determine the closeness of those products to the ones tested in your facility. Matrices from the same validated category or similar intrinsic properties can often be included in a verification study.
 - b. Additionally, a risk-based approach can be utilized (Matrix Evaluation Level Assessment Tool) to determine if the matrix of interest is likely to contain the analyte and if verification testing is appropriate. If the matrices are not similar, proceed to question 3. If the matrices are determined to be similar enough or the risk is low, proceed to question 4.
3. None of the matrices our facility tests are similar to those studied in the validation. Can we still use the method?
 - a. Additional validation studies may be required if the matrices validated are different from those tested at your facility. Contact the method developer of the assay to determine if they have additional internal matrix data or for assistance with further validation of the method.
4. How should I perform the testing required for a verification study?
 - a. Guidance documents on conducting verification testing can be found from several regulatory bodies (FDA, Health Canada) or within published standards (ISO, USP). Based on the category (chemistry or microbiology) and type (qualitative or quantitative) of the test, determine which approach aligns with the technical capabilities of your facility.

Chemistry

Verification studies for chemistry methods are often designed similar to validation studies, with a smaller subset of testing being performed. These studies often require users to:

- Meet system suitability specifications established for the method.
 - System suitability testing ensures the method's system (equipment, electronics, analytical operations, and controls) is functioning correctly prior to or during use.
 - Testing is performed by analyzing known standards, blanks, or control samples and determining if the values obtained meet specified parameters established during the method's validation.
- Evaluate a subset of matrices tested at the facility.
 - Matrix testing is performed with fortified samples by analyzing multiple replicates from several concentrations across the range of the method.
 - It allows the lab to determine both the accuracy and precision of the method
 - Determine the percent recovery of the analyte in the fortified material for each concentration.
 - Matrix testing should include analysis of blank samples to demonstrate a matrix effect in the result.
- Evaluate the working range of the method in a linearity study.
 - Perform testing on pure analyte at multiple concentrations with multiple replicates.
- Evaluate fortified samples at the LOD or LOQ of the method.

Microbiology

Verification testing in microbiology does require some technical skill, as the use of live/viable cultures is recommended for these evaluations. While this may seem daunting, manufacturers of bacterial/fungal reference materials³ have simplified the process by preparing cultures in ready-to-use formats with known concentrations (Microbiologics, NSI). Recommendations for testing vary between the guidance documents. However, the following items are traditionally included in a verification study:

- Verification testing should be performed on a subset of the matrices analyzed by the facility. If the facility analyzes products from multiple categories, it's recommended to include a matrix from each category.
 - Matrix testing is performed using multiple replicates.
 - Single or multiple contamination levels studies can be performed. If a single contamination level is conducted, more replicates should be analyzed than a multiple contamination study.
 - Blank or control samples should be included with the study to ensure that the method works correctly on non-contaminated samples.



- Contamination levels evaluated are traditionally higher than those used for the validation study.
 - Acceptable contamination levels are often a multiple of the LOD of the validated method (2x, 5x, 9x) or within a recommended threshold (e.g., 30–100 CFU).
- Verification studies may also incorporate aspects of factorial design studies by including multiple:
 - Operators analyzing the same sample
 - Multiple brands or lots of culture media
 - Laboratory equipment

Method Performance Monitoring

While verification studies will ensure that a method is fit for purpose for your facility, one final step is required in evaluating the method. This step, however, is never-ending. A method's performance should be monitored as long as a facility is using it. Method monitoring is much less involved than validation or verification and is something both the testing facility and method developer will conduct.

External Monitoring

Each testing facility should ensure that a method is performing accurately each time it is performed. Routine monitoring is easily accomplished by including standardized controls within the testing protocol. For chemistry methods, this may include using specific standards analyzed at intervals throughout the testing day. For microbiology methods, using positive and negative organism controls and sterile media controls will provide assurance the method is performing correctly.

Long-term monitoring by testing facilities is traditionally accomplished by analyzing proficiency test samples or internally prepared (fortified or inoculated) samples. This type of monitoring is traditionally performed at set intervals (e.g., annually, biannually).

Internal Monitoring

In addition to testing facilities, method developers will monitor the performance of their methods over the life cycle of the method. Method developers will actively seek feedback from end users on the performance of their assays to identify potential issues with certain matrices and determine aspects of the testing protocol that may require adjustments. This information is used to optimize the method for specific industries or matrices and help develop future generations.

Method developers will also monitor the production of new lots through quality control testing with known standards and controls. This process ensures consistency between lots being used for analysis.

Conclusion

Reviewing a method developer's internal or external validation report is the first step an end user will take in choosing a method for their facility. The next step is verification, which ensures the method works as intended in the end user's hands. Site verification is performed by the end user and, when possible, uses a matrix or food item tested in the validation study. Food item verification is performed by the end user, an independent laboratory, or method developer to verify additional food items or matrices that are in scope but were not included in the original validation. After microbiological or chemical verification studies are completed, method monitoring through control analysis and participation in proficiency testing programs ensures the method's ongoing performance.

1 See Paper 4: *Method Validation: Internal vs. External*

2 See Paper 1: *Validation overview, terminology, and definitions*

3 See Paper 3: *Differences in Validation Requirements for Microbiology, Chemistry or Allergen Methods for more details on reference materials.*