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A Guide to Hemp Method Validations

Paper One — Five Part Series

Executive Summary

Accurate analysis of hemp products is crucial for cultivators, whether to confirm a legal level of THC or to establish the quality of the product. This means the method itself must be proven trustworthy. To make an informed decision about which method to use, a user must first understand the language surrounding method validation. This paper highlights key differences in similar-sounding terms and provides important definitions that will allow a user to understand method validation and verification processes. This paper is the first in a series of five papers explaining method validations to help each user choose the strongest fit for their needs.

Unexpected Results

Hemp cultivators recognize the significance of having accurate analysis methods, as a difference in a tenth of a percentage for THC can render their product illegal. Other required analyses, such as those for cannabinoid profiles, microbial contaminants, or pesticides, can also impact the product's value or require it to be destroyed. When receiving an unexpected result, what action should cultivators take? To answer this question, a cultivator should first trust that the result is accurate, and to do that, an understanding of the method, including the validation of the method, is crucial to making informed decisions when unexpected results are obtained.

Method Validation 101

Method validation is a complex process that often takes months or years to complete. Internal validation studies are performed by the method developer (or subcontracted to specialized expert laboratories) to ensure the method's fitness before being released to the industry. Following the internal validation, method developers will often seek an external certification or independent evaluation of the method. However, not all internal or external validations are the same as variations in study design and complexity exist based on the type of method (chemistry or microbiology), state requirements, and commodity-specific regulations (e.g., inhalables, concentrates, or edibles). Before selecting a method for analyzing your product, it is critical to be informed on specifics about the method's validation process. Some key questions to ask are:

- Was the method validated internally or through an approved third-party organization? (E.g. AOAC INTERNATIONAL, Health Canada, AFNOR, MicroVal, other).
- Did the validation study contain an independent laboratory study?
- What are the validated matrices and categories?
- Are you willing to share the validation data with my team?
- If the method developer can't or won't provide answers to these questions, it's time to start looking for a new method!

Validation Terminology

To prepare for this discussion, we first need to gain familiarity with the terminology used in the validation landscape.

The two most common terms, validation and verification, while often used synonymously, have distinctly different meanings. Knowing the differences between these two terms helps ensure the method is both robust and fit for purpose.

- Validation — The establishment of the performance characteristics of a method and provision of objective evidence that the performance requirements for a specified intended use are fulfilled.[1]
- Verification — The demonstration that a validated method functions in the user's hands according to the method's specifications determined in the validation study and is fit for purpose.

Simply, validation ensures that a method is robust enough to be used in the marketplace, whereas verification ensures that it works in the user's hands.

- Scope of a validation — The analytes, matrices, and concentrations for which a validated method of analysis can be used satisfactorily.
  - The analytes are the targets that a method detects or enumerates. Methods are traditionally separated into two distinct categories based on the analytes they detect: microbiology or chemistry.[2]
    - Microbiology assays are designed to detect pathogens (*Salmonella, Aspergillus*) or to quantify a group of microorganisms (coliforms, yeast, and mold).
    - Chemistry assays are designed to detect or quantify compounds of interest (cannabinoids, residual solvents, heavy metals).
- The matrices are the specific items that were tested in the validation study. Examples of matrices are flower (hemp, cannabis with > 0.3% THC), concentrates (solvent-based, oils, tinctures), infused edibles (beverages, chocolates, gummies), or infused non-edibles (therapeutic patches, lotions, balms).
- The concentrations provide information on the method's ability to detect or enumerate at low, medium, or high contamination levels. See the next section for additional details.

The scope of a validation tells us the exact analytes (cannabinoids, pesticides, microorganism), matrices (hemp, high THC cannabis flower, concentrates, etc.), and detection levels of the method. This information is vital to making informed decisions! More details will be provided in the second paper in this series, highlighting the specific requirements for a method to be truly validated.

Qualitative vs Quantitative

Methods are typically separated into two categories: qualitative (presence/absence) or quantitative (a specified amount of a target). Each of these types of methods has specific terms associated with their validation studies.

Qualitative

- Limit of detection (LOD) — Measured analyte concentration obtained by a given measurement procedure. An example of LOD is the LOD95 of a method is the level of detection for which 95% of tests give a positive result.
  Note: For quantitative methods, the LOD is defined as the lowest concentration at which the analyte can be reliably distinguished from the absence of that analyte.
- Specificity — The ability of a method to detect target analytes and distinguish them from non-targets.

When selecting a qualitative method, users should look for data that indicates the method has a LOD_{100} at or below actionable limits and a highly specific method to the targets it claims to detect. Qualitative methods that are not specific may lead to false positive results (indicating an analyte is present when it is not) or false negative results (indicating an analyte is not present when it actually is). The method developer should have data to show whether closely related compounds are detected on the method in review.

Quantitative

- Limit of quantification (LOQ) — The lowest analyte concentration that can be reliably quantitated with an acceptable level of precision and trueness.
  - Precision — The closeness of agreement between measured values obtained by replicate.

Measurements

- Trueness — The closeness of agreement between measured values and a reference value.

When choosing a quantitative method, users should ask for validation data that shows the method is repeatable (precision) and can accurately quantify levels from a known standard (trueness).

Conclusions

Many test methods are validated, but that does not necessarily make them fit for every user's needs. Understanding the scope of a method's validation is the first step in deciding whether the method is suitable; a method must be shown to work in the matrices that the user will be working with and target the analytes that the user is interested in. It must be able to detect its targets at useful levels, whether it is a qualitative method to screen for certain levels or a quantitative method that must accurately and reliably produce more exact measurements. Knowledge is power, and knowledge of terms and definitions gives the user power to make informed choices about analytical methods.

1. *All definitions attributable to ISO 16140-1:2016 Microbiology of the food chain – Method validation – Part 1: Vocabulary;*
2. *Details on differences between chemistry and microbiology validation studies can be found in the third paper in this series: Differences in requirements for microbiology, chemistry and allergen validation studies;*