Validation of Qualitative Identification Methods for Botanicals

-Update on AOAC Working Group-

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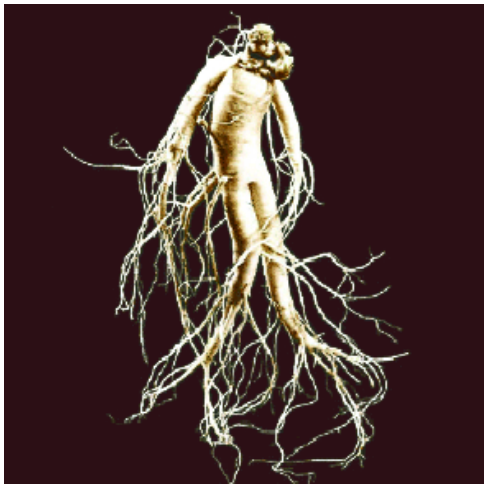
GMP Requirements

- CGMP requires that a person who manufactures a dietary supplement conduct at least one appropriate test or examination to verify the identity of each dietary ingredient that will be used in the manufacture of the dietary supplement.
- Quality means that the dietary supplement consistently meets the established specifications for identity, purity, strength, and composition, and limits on contaminants…
- You must establish an identity specification;

Identity Testing in a GMP Environment

- Many “identity” methods used for botanicals are actually qualitative methods to meet standards of identity.
  - TLC, microscopy, NIR, etc.

- Many of the traditional identity methods (macro/ microscopic examination, TLC) were developed for identifying whole plants or part of plant (e.g. leaf, root, flower, etc.).
  - Using one or more tests, one can usually definitively state from what plant an individual leaf, root, flower, etc., came.



Identity Testing in a GMP Environment

- “Identity” and “Purity” are two separate specifications.
  - “Purity” implies some type of quantitative method.
  - Purity tests look for specific impurities
  - An identity test says nothing about purity, strength, or composition.
- An extract of a botanical is not the plant – it is an extract of the plant
  - Different extraction techniques can lead to different chemical profiles.
  - Carriers can play an important role in the manufacturing process, as well as physical and chemical stability of finished products.

The Problem

- How do we ensure that the qualitative test method we are using to verify the identity of the botanical is reliable?
  - The series of experiments performed to demonstrate the reliability of a method is known as “Validation.”
  - AOAC OMA requires a statistically valid model to demonstrate that a method is reproducible between laboratories.

Validation

- Quantitative Methods
  - Numerical Answers
  - Statistical Analysis
  - Estimate uncertainty and establish confidence intervals
- Qualitative Methods
  - Binary results
    - Positive/Negative
    - Yes/No
    - Present/Absent

From a GMP perspective, a material either meets the identity specification or it does not.

Validation of Quantitative Methods

- Many well-established guidelines for validation of quantitative methods.
  - AOAC
  - USP
  - ICH
- Reliability of quantitative methods measured by:
  - Accuracy (% recovery)
  - Precision (% RSD)
  - Linearity/Range
  - LOD/LOQ
  - Selectivity
  - Ruggedness

Validation of Qualitative Methods

- Botanicals are never 100% pure
  - Dirt/filth
  - “Weeds”
  - Insects
  - Pesticides/herbicides/heavy metals
  - Other related species
- “Identity” and “Purity” are two separate specifications.
  - “Purity” is a test for specific contaminants/adulterants
- An extract of a botanical is not the plant – it is an extract of the plant

Validation of Qualitative Methods

- How to we validate (i.e. determine the reliability) a qualitative method?
- Three levels of validation:
  - Method developer study (single laboratory validation)
  - Independent laboratory study (peer verification)
  - Collaborative study

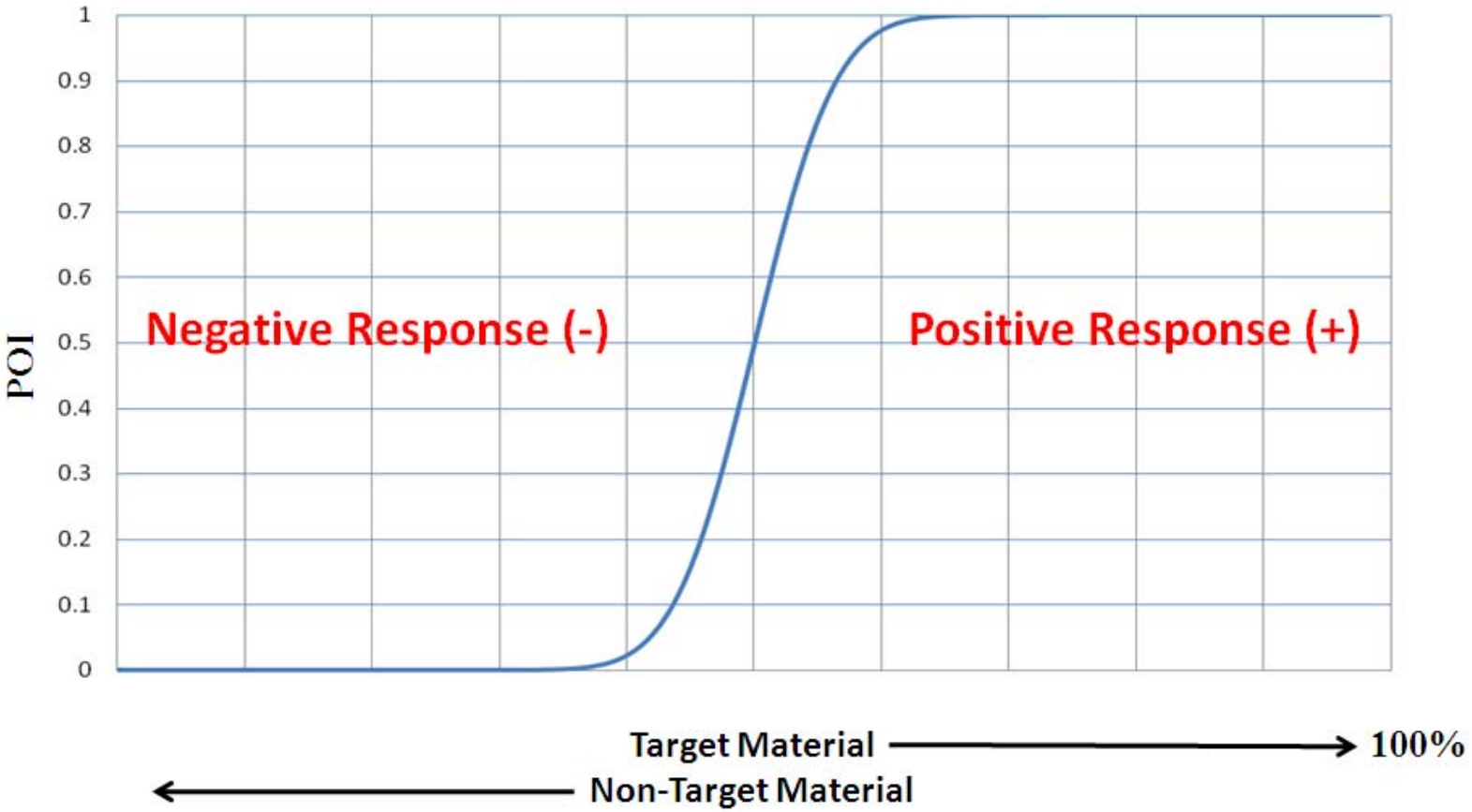
Single Lab Validation

- Performance Characteristics
  - Detection response
  - Specificity
  - Inclusivity
  - Repeatability
  - Environmental factors (Ruggedness)
- Concepts borrowed from microbiological guidelines

Single Lab Validation

- Detection Response (Sensitivity)
  - The ability of the method to detect the target analyte in the matrix.
    - Probability of Identification (POI) = probability of a positive response at a given concentration.
    - In microbiology, it is the Probability of Detection (POD).
  - Characterization of the response curve of POI by concentration is a primary goal of method validation for qualitative assays.
  - The ability of the method to detect the target botanical in the presence of other “stuff.”
  - What happens when target botanical gets diluted with other “stuff?”

Validation of Qualitative Methods



Validation of Qualitative Methods

- POI model correlates to and harmonizes with quantitative statistical models.
  - Allows use of existing statistics, e.g. collaborative study spreadsheet.
    - Positive Result = 1
    - Negative Result = 0
  - Allows reporting of:
    - POI (mean result)
    - Repeatability
    - Reproducibility (for collaborative study)

Validation of Qualitative Methods

- Selectivity
  - The ability of the method to differentiate target species from related species and/or potential adulterants.
    - The probability of the method giving a (-) response when the sample is truly without target species
    - Can be estimated as 1-POI(0)
  - Exclusivity study
    - Non-target botanicals, or related compounds that must not detected by the method.

Validation of Qualitative Methods

- Repeatability
  - Method Developer Study and Independent Laboratory Study to determine the repeatability of the method and the transferability of the method to a second site.
  - The number of test portions determines the confidence interval around the repeatability results.

Validation of Qualitative Methods

Number of replicates (X)	Maximum No. of Incorrect Results	Confidence
30	0	90%
99	0	95%
30	1	75%
96 (96)[a]	1 (0)	95%

a - sequential test plan of 96 initial test portions and an additional 96 test portions if 1 failure in the initial 96 analyses.

Validation of Qualitative Methods

- Collaborative Study - Determine reproducibility of the method at a minimum of 10 sites.
- Select 12 to 15 collaborators. Number of collaborators = *C*
- Prepare:
  - 5**C* test portions of a similar botanical that contains no target botanical
  - 5**C* test portions of 10% target botanical (w/w)
  - 5*C test portions of 50% (w/w) botanical
  - 5*C test portions of 90% (w/w) target botanical
  - 5*C test portions of 100% (w/w) target botanical
- Code test portions.
- All collaborating laboratories receive 5 test portions of each of the 4 concentrations plus 5 test portions containing a non-target botanical. Record qualitative results for all test portions.
- Analyze qualitative results using POI analysis

Validation of Qualitative Methods

- Requirements to determine performance characteristics:
  - Authenticated botanicals
    - botanical of interest
    - likely contaminants/adulterants
  - Samples must include definite positives, definite negatives, and a range of potential adulterants.
    - What are known contaminants/adulterants?
    - What are likely contaminants/adulterants?

Validation of Qualitative Methods

- Expert Review Panels would be required to:
  - Establish specifications for identity
    - What is minimum concentration required to obtain positive result?
    - What confidence level is required?
  - Identify likely contaminants/adulterants
    - Economic
    - Mis-identified species
  - Select candidate methods

Validation of Qualitative Methods

- POI Model allows unified model between quantitative and qualitative methods

	Qualitative Methods	Quantitative Methods
Chemical Methods	POI $s_R^2 = s_r^2 + s_L^2$	mean $s_R^2 = s_r^2 + s_L^2$
Microbiological Methods	POI $s_R^2 = s_r^2 + s_L^2$	mean $s_R^2 = s_r^2 + s_L^2$

Validation of Qualitative Methods

- Validation model will establish confidence level of result.
  - Use of poorly defined terms such as “consistent with,” “matches,” not needed any longer.
- ERPs will be critical in establishing specifications of identity for each botanical.
- POI manuscript written by J. Harnley and R. LaBudde has been accepted for publication by JAOAC.

Urban Legends Surrounding AOAC Working Group

- Working group is specifying which methods/techniques to use for particular botanicals.
  - Working group is only recommending guidelines for validation of ID methods.
- Labs will have to follow these guidelines to validate their ID guidelines.
  - GMPs do not require validation of methods.
  - Guidelines are specific to AOAC Official Methods Program.
- The working group has been working in secret on these guidelines.
  - Progress updates have been given at last 2 AOAC meetings, as well as trade shows.
  - Updates have been published in 2 issues of ILM.
  - I have given approximately 2 bazillion talks on validation of ID methods over last 4 years.

Conclusions

- There currently are no official accepted guidelines for the validation of qualitative methods for the identification of botanicals.
- Identity methods are qualitative, and say nothing about the purity, strength, or composition.
  - Separate tests needed for these characteristics
- POI model allows harmonization between quantitative method validation and qualitative method validation.