

AOAC INTERNATIONAL

Expert Review Panel

Guidelines for Validation of Botanical Identification Methods

The expert review panel consisted of:

James Harnly, Chair	USDA, ARS
Wendy Applequist	Missouri Botanical Gardens
Steven Caspar	US FDA, CFSAN
Peter Harrington	Ohio University
Norma Hill	Alcohol & Tobacco Tax & Trade Compliance lab
Robert LaBudde	Least Cost Formulations
James Neal-Kababick	Flora Research Laboratories
Danica Harbaugh-Reynaud	AuthenTechnologies, LLC
Mark Roman	Tampa Bay Analytical Laboratories
Shauna Roman	4Life Research
Darryl Sullivan	Covance Labs
Barry Titlow	Compound Solutions
Paul Wehling	General Mills

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1. Scope

The purpose of this document is to provide comprehensive technical guidance for conducting AOAC INTERNATIONAL (AOAC) validation studies for botanical identification methods submitted for *Performance Tested Methods* (PTM) status and/or for *AOAC Official Methods of Analysis* (OMA) status. The requirements for single laboratory validation studies, independent validation studies, and collaborative validation studies for those methods are described.

2. Applicability

These guidelines are intended to be applicable to the validation of all candidate botanical identification methods that are submitted to AOAC for (1) a Collaborative Study or an Alternative Pathway (AP) Study to achieve OMA status or for (2) a PTM certification.

3. Terms and Definitions

- 3.1 Botanical:** Of, or relating to, plants or botany. May also include plant-like algae and fungi. May refer to the whole plant or any part of the plant (e.g. bark, woods, leaves, stems, roots, rhizomes, flowers, fruits, seeds, extracts, etc.). **Paula to send definition.**
- 3.2 Botanical Identification Method (BIM):** A method that establishes identity specifications for a botanical material and allows determination, within a specified statistical limit, that a test material is a true example of the target botanical material and meets the identity specifications. Thus, a method answers the question, “*Is the test material the same as the target material?*” not “*What is this material?*”. In most cases, the method will achieve this goal by comparison of the test material with botanical material(s) from the inclusivity panel and will return a yes/no (or, in some cases, a consistent/non-consistent) answer.
- 3.3 Candidate method:** The method to be validated or submitted for validation.
- 3.4 Exclusivity:** Ability of a BIM to correctly reject non-target botanical materials.
- 3.5 Exclusivity sample frame:** A list of practically obtainable non-target botanical materials that have similar taxonomic, physical, or chemical composition characteristics that are expected to give a negative result when tested by the BIM.
- 3.6 Exclusivity panel:** A subset of the exclusivity sample frame that is selected for the validation study. These materials should be authenticated by an appropriate method.
- 3.7 Identity specification (IS):** The morphological, genetic, chemical or other characteristics that define a target botanical material. Specifications may include, but are not limited to, data from macroscopic, microscopic, genetic (e.g., DNA sequencing), chromatographic

fingerprinting (e.g., CE, GC, LC, TLC), and spectral fingerprinting (e.g., IR, NIR, NMR, MS, UV-vis) methods.

- 3.8 Inclusivity:** Ability of a BIM to correctly identify variants of the target material that meet the identity specification.
- 3.9 Inclusivity sample frame:** A list of practically obtainable botanical materials that are expected to give a positive result when tested by the BIM. The inclusivity frame should be sufficiently large that the botanical variation is adequately represented. Sources of variation may include, but are not limited to, species, sub-species, cultivar, growing location, growing conditions, growing season, and post-harvest processing.
- 3.10 Inclusivity panel:** A subset of the inclusivity sample frame that is selected for the validation study. These materials should be authenticated by an appropriate method.
- 3.11 Laboratory Sample:** Sample as prepared for sending to the laboratory intended for inspection or testing.
- 3.12 Non-target botanical material:** Any botanical material that does not meet the identity specification.
- 3.13 Physical form:** Botanical materials exist in a number of physical forms. The form(s) will be specified by the SMPRs.
- 3.14 Probability of Identification (POI):** The expected or observed fraction of test portions at a given concentration that give a positive result when tested by the BIM.
- 3.15 Sample:** A small portion or quantity, taken from a population or lot that is ideally a representative selection of the whole.
- 3.16 Specified inferior test material (SITM):** A botanical material mixture that has the maximum concentration of target material that is considered unacceptable, as specified by the SMPRs. The BIM must reject this material with a specified minimum level of $(1 - \text{POI})$ with 95% confidence. The ideal BIM would reject the SITM 100% of the time (i.e., identify 0% of the time). The SITM will typically be high quality target material mixed with the worst-case (for identification) non-target material.
- 3.17 Specified superior test material (SSTM):** A botanical material mixture that has the minimum acceptable concentration of the target material, as specified by the SMPR. The BIM must identify this material with a specified minimum level of POI with 95% confidence. The ideal BIM would identify the SSTM 100% of the time. The SSTM will typically be high quality target material mixed with a small amount of worst-case (for identification) non-target material.
- 3.18 Standard Method Performance Requirements (SMPRs):** AOAC documents that are developed by the stakeholder panel to include fitness for purpose, and relevant method

performance requirements, such as panels used to determine sensitivity, specificity, and selectivity; and minimum criteria for key validation parameters. SMPRs are codified and published.

3.19 Target botanical material: The botanical material of interest as described in the identity specification.

3.20 Test portion: The portion of the laboratory sample that is subjected to analysis by the method.

4 Validation Study Guidelines

A validated BIM requires SMPRs and a method validation study. The guidelines presented here are intended to be applied to any qualitative BIM that returns a binary result. The guidelines provide technical guidance in validating the method.

4.1 Standard Method Performance Requirements

The SMPRs will be prepared by the appropriate AOAC body as per AOAC policy. The SMPRs will specify (a) the target botanical material, (b) the physical form of the material, (c) a list of botanical materials for the inclusivity/exclusivity frames, (d) composition of the SSTM and SITM, (e) maximum POI for the SITM and minimum POI for the SSTM, and (f) any other relevant criteria for the target material.

4.2 Single Laboratory Validation Study

4.2.1 Scope

An SLV study is intended to determine the performance of a candidate method. For validation purposes, the candidate botanical identification method may be regarded as a black box providing a yes/no test result. The study is designed to evaluate performance parameters for the candidate method including (1) inclusivity/exclusivity, (2) POI for the specific superior test material (SSTM) and the specific inferior test material (SITM), and (3) POI as a function of the concentration of the target material (analytical response curve).

4.2.2 Inclusivity/Exclusivity Study

The purpose of this study is to confirm the ability of the candidate method to provide positive results for botanical materials on the inclusivity panel and negative results for materials on the exclusivity panel.

4.2.2.1 Inclusivity/Exclusivity Panel Selection

Botanical materials selected from the inclusivity/exclusivity sample frames will comprise the inclusivity/exclusivity panels. If the Inclusivity/exclusivity sample frames are sufficiently large, a representative sub-group will be selected for the panels. A primary requirement for the panel materials is their availability. These materials should be

authenticated by an appropriate method. All test portions should be as uniform and homogeneous as possible. The level of replication of the inclusivity/exclusivity panels will be specified in the SMPRs.

4.2.2.2 Study Design

Prepare the test samples in a form appropriate for the candidate method. All test samples will be blind coded and randomized so that the analyst(s) cannot know the identity of the samples. Analyze the test samples following the instructions of the candidate method.

4.2.2.3 Data Analysis and Reporting

The data will be analyzed for positive and negative responses. Unexpected results will be investigated, evaluated, and resolved prior to continuing the validation. The data is reported as a number of inclusivity/exclusivity materials detected relative to the total tested. For example, “Of the 30 specific inclusivity botanical materials tested, 28 were detected and 2 were not detected. Those materials not detected were the following:...” or “Of the 30 specific exclusivity botanical materials tested, 27 were not detected and 3 were detected. Those detected were the following:...” The study report should include a table titled “Inclusivity/Exclusivity Panel Results,” which lists all materials tested, their source, origin, and essential characteristics and testing outcome.

4.2.3 SSTM/SITM Study

The purpose of this study is to demonstrate method performance at two concentrations, the SSTM and the SITM.

4.2.3.1 Test Samples

The appropriate amount of a target material is selected from the inclusivity panel and is mixed with an appropriate amount of a non-target material from the exclusivity panel to produce the SSTM and SITM as specified by the SMPRs. The test materials may be prepared using a mixture of botanical materials from the inclusivity list as the target material and a mixture of materials from the exclusivity list as the non-target material.

All test portions should be as uniform and homogeneous as possible. The level of replication of the SSTM and SITM will be specified in the SMPR.

4.2.3.2 Study Design

Prepare the test samples in a form appropriate for the candidate method. All test samples will be blind coded and randomized so that the analyst(s) cannot know the identity of the samples. Analyze the test samples following the instructions of the candidate method.

4.2.3.3 Data Analysis and Reporting

The data will be analyzed for positive and negative responses. For the SSTM and the SITM, report the POI results with 95% confidence intervals, and the total number tested and the total number of positive responses.

4.2.4 Analytical Response Curve

This study will characterize the POI curve for mixtures of SSTM and SITM.

4.2.4.1 Test Samples

The appropriate amount of a target material is selected from the inclusivity panel and is mixed with an appropriate amount of a non-target material from the exclusivity panel to produce the mixtures with concentrations between the SSTM and SITM. The test materials shall be prepared using the same target and non-target botanical materials used in the SSTM and SITM study.

4.2.4.1 Study Design

Prepare the test samples in a form appropriate for the candidate method. All test samples will be blind coded and randomized so that the analyst(s) cannot know the identity of the samples. Analyze the test samples following the instructions of the candidate method.

4.2.4.2 Data Analysis and Reporting

The data will be analyzed for positive and negative responses. For each mixture, report the POI results with 95% confidence intervals, and the total number tested and the total number of positive responses. Plot the POI curve and confidence intervals. **Add Appendix**

4.3 Independent Validation Study

The independent validation study is a portion of the method evaluation required by the AOAC Performance Tested Methods program and is conducted by an AOAC Research Institute approved laboratory. The independent laboratory attempts to reproduce certain results and verify selected performance parameters as listed in **Section 4.2**. AOAC, not the method developer, coordinates this study.

4.4 Collaborative Study

The collaborative study is a route to an OMA method. The purpose of the Collaborative Study is to estimate the reproducibility and determine the performance of the candidate method among collaborators.

4.4.1 Number of Collaborators

A minimum of 10 laboratories reporting valid data are required. The study director should plan on including additional laboratories due to potential invalid data sets.

4.4.2 Number of Tests

Each collaborator receives 12 replicates of each material to be studied. At a minimum these materials will include the SSTM and SITM. Prepare the test samples in a form appropriate for the candidate method. All test samples will be blind coded and randomized so that the analyst(s) cannot know the identity of the samples. Analyze the test samples following the instructions of the candidate method.

4.4.3 Data Analysis and Reporting

The data will be analyzed by laboratory for positive and negative responses. For the SSTM and the SITM, report the POI results with confidence intervals for each laboratory, and for the combined results. Estimate reproducibility as in Appendix XX and evaluate compared to the SMPR.

5 References – To be added

Appendix A - Method Developer Study Pre-validation Study

A.1 Scope

The method developer will produce a candidate method that will meet the SMPRs. (suggest different language for this first line – Candidate methods to be considered for validation must use appropriate measurement methods that will meet pre-determined SMPRs). The method submitted may be based on new principles or modifications of an existing method. The identity specifications will be based on morphological, genetic, and/or chemical characteristics, or any other defining feature of the botanical material. The candidate method may use visual inspection, DNA sequencing, instrumental analysis, or other appropriate measurement. The measured characteristics will collectively provide a single analytical parameter that will be used to determine the final yes or no result. The analytical parameter may be based on the similarities or differences of the test sample and the reference material.

A.2 Inclusivity/Exclusivity Panel Selection

The method developer will select representative botanical materials from the inclusivity and exclusivity sample frames for use as target and non-target botanical materials, respectively, in development of the method. These materials should be authenticated by an appropriate method.

A.3 Study Design

The method developer will prepare all the botanical samples in a form appropriate for the candidate method. The developer will analyze the target and non-target botanical materials using the candidate method and develop an analytical parameter that is suitable for distinguishing between the two sets of materials.

Target materials will be mixed with systematically increasing amounts of non-target materials to produce a series of target materials whose concentrations range from 100% to a concentration below the minimum acceptable concentration specified by the SMPRs. The developer will analyze the target and diluted target materials using the candidate method and determine the analytical parameter for each concentration.

A.4 Data Analysis and Reporting

Based on the analytical parameters measured for the diluted target materials, a threshold value will be established that will permit positive identification of the minimum acceptable concentration of the target material with the specified confidence (e.g. 95%). The developer will use the threshold to determine a probability of identification (POI) for each

concentration (Appendix B). The POIs measured for each concentration will be used to construct the POI curve.

The method developer will document the candidate method and the POI results.

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Appendix B: Understanding the POI Model Insert POI paper ??

Appendix C: Number of Test Portions

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Sample Size Required for Proportion

HIGH

Version:

0.0

- ASSUME:
1. Binary outcome (occur / not occur).
 2. Constant probability rho of event occurring.
 3. Independent trials (e.g., simple random sample).
 4. Fixed number of trials N.

INFERENCE: 95% confidence interval lies entirely at or ABOVE specified minimum rho.

DESIRED: Sample size N needed.

- NOTES:
1. Based on modified Wilson score 1-sided confidence interval.
 2. AOQL = Average Outgoing Quality Level

Minimum Probability rho	Sample Size N	Minimum Number Events x	Maximum Number Non-events y	1-sided Lower Confidence Limit on rho	Expected Lower Confidence Limit on rho	Expected Upper Confidence Limit on rho	Effective AOQL rho
50%	3	3	0	52.6%	43.8%	100.0%	71.9%
50%	10	8	2	54.1%	49.0%	94.3%	71.7%
50%	20	14	6	51.6%	48.1%	85.5%	66.8%
50%	40	26	14	52.0%	49.5%	77.9%	63.7%
50%	80	48	32	50.8%	49.0%	70.0%	59.5%
55%	4	4	0	59.7%	51.0%	100.0%	75.5%
55%	10	9	1	65.2%	59.6%	100.0%	79.8%
55%	20	15	5	56.8%	53.1%	88.8%	71.0%
55%	40	28	12	57.1%	54.6%	81.9%	68.2%
55%	80	52	28	55.9%	54.1%	74.5%	64.3%
60%	5	5	0	64.9%	56.5%	100.0%	78.3%
60%	10	9	1	65.2%	59.6%	100.0%	79.8%
60%	20	16	4	62.2%	58.4%	91.9%	75.2%
60%	40	30	10	62.4%	59.8%	85.8%	72.8%
60%	80	56	24	61.0%	59.2%	78.9%	69.1%
65%	6	6	0	68.9%	61.0%	100.0%	80.5%
65%	10	9	1	65.2%	59.6%	100.0%	79.8%
65%	20	17	3	67.8%	64.0%	94.8%	79.4%
65%	40	31	9	65.1%	62.5%	87.7%	75.1%
65%	80	59	21	65.0%	63.2%	82.1%	72.7%
70%	7	7	0	72.1%	64.6%	100.0%	82.3%
70%	10	10	0	78.7%	72.2%	100.0%	86.1%
70%	20	18	2	73.8%	69.9%	97.2%	83.6%
70%	40	33	7	70.7%	68.0%	91.3%	79.7%
70%	80	63	17	70.4%	68.6%	86.3%	77.4%
75%	9	9	0	76.9%	70.1%	100.0%	85.0%
75%	10	10	0	78.7%	72.2%	100.0%	86.1%
75%	20	19	1	80.4%	76.4%	100.0%	88.2%
75%	40	35	5	76.5%	73.9%	94.5%	84.2%
75%	80	67	13	75.9%	74.2%	90.3%	82.2%
80%	11	11	0	80.3%	74.1%	100.0%	87.1%
80%	20	19	1	80.4%	76.4%	100.0%	88.2%
80%	40	37	3	82.7%	80.1%	97.4%	88.8%
80%	80	70	10	80.2%	78.5%	93.1%	85.8%
85%	20	20	0	88.1%	83.9%	100.0%	91.9%
85%	40	38	2	86.0%	83.5%	98.6%	91.1%
85%	80	74	6	86.1%	84.6%	96.5%	90.6%
90%	40	40	0	93.7%	91.2%	100.0%	95.6%
90%	60	58	2	90.4%	88.6%	99.1%	93.9%
90%	80	77	3	91.0%	89.5%	98.7%	94.1%
95%	60	60	0	95.7%	94.0%	100.0%	97.0%
95%	80	80	0	96.7%	95.4%	100.0%	97.7%
95%	90	89	1	95.2%	94.0%	100.0%	97.0%
95%	96	95	1	95.5%	94.3%	100.0%	97.2%
98%	130	130	0	98.0%	97.1%	100.0%	98.6%
98%	240	239	1	98.2%	97.7%	100.0%	98.8%
99%	280	280	0	99.0%	98.6%	100.0%	99.3%
99%	480	479	1	99.1%	98.8%	100.0%	99.4%