1.0 Purpose

The purpose of this procedure is to define the process for validation and verification of analytical test methods. Specific requirements for accredited tests for ISO 17025 are also included in this procedure.

2.0 Scope

2.1 This procedure applies to the validation and verification of analytical test methods intended for use in the QC laboratory at Ion Labs. This procedure is applicable to the validation of non-standard methods and lab-developed methods offered to customers as an accredited test or a part of an accredited test in accordance with ISO 17025:2017. If a published, standard method is utilized beyond its intended scope, then this procedure is applicable as validation of the method is required.

The Test methods listed below will be validated in accordance with ISO 17025:2017 requirements:

Ion Labs ISO 17025:2017 Accredited Test Methods
D-720 Caffeine Determination using HPLC with UV/VIS Detection
D-729 Determination of Tributyrin by GC-FID
D-776 Cannabinoid Determination and Identification by HPLC
D-778 Limit of Citrinin by LC-MS
D-780 Determination of Quercetin by HPLC using UV-VIS Spectroscopy
D-715 Microbial Testing Using 3M Petrifilm
D-715.0 Microbial Limit Test Using Agar

2.2 This procedure is not applicable to discovery activities where tests are being designed.

2.3 This procedure does not apply to heavy metal and mineral validations.

2.4 The parameters listed in this procedure may not apply to the validation of microbial limit tests.

3.0 Responsibility

3.1 It is the responsibility of QC Laboratory analysts and/or AD personnel to execute validation and verification protocols.

3.2 QC Laboratory Management and/or AD personnel are responsible for writing validation and verification protocols, ensuring analysts properly execute protocols, and preparing, reviewing, and approving data summaries and reports.

3.3 It is the responsibility of QC Laboratory Management and/or AD personnel to keep this procedure current with the latest Ion Labs Practices.

4.0 Definitions

4.1 **UV/VIS** – Ultraviolet and Visible Light Spectrums

4.2 **ICP/MS** – Inductively Coupled Plasma / Mass Spectrometry

4.3 **AOAC** – Association of Analytical Communities

4.4 **USP** – United States Pharmacopeia

4.5 **EP** – European Pharmacopeia

4.6 **QC** – Quality Control

4.7 **AD** – Analytical Development

4.8 **R&D** – Research and Development

4.9 **SOP** – Standard Operating Procedure

4.10 **DL** – Detection Limit

4.11 **QL** – Quantitation Limit

4.12 **Customer** – The Ion Labs Inc. Laboratory is employed by Ion Labs and the customer described in this procedure is a designee of Ion Labs Inc

5.0 References

5.1 Analytical Procedures and Methods Validation for Drugs and Biologics, US Department of Health and Human Services, Food and Drug Administration, July 2015

5.2 USP <1225> Validation of Compendial Procedures

5.3 USP <1226> Verification of Compendial Procedures

5.4 ICH Q2(R1) Validation of Analytical Procedures: Text and Methodology

5.5 USP <1092> The Dissolution Procedure: Development and Validation

5.6 D-777, SOP, Elemental Analysis by ICP-MS

5.7 D-715, SOP, Microbial Testing using 3M Petrifilm

5.8 D-715.0, SOP, Microbial Limit Test Using Agar

6.0 Method Development and Documentation

6.1 Test methods which refer to or meet a published/accepted standard will be developed to refer to the most current revision of that standard. The method will identify the revision used. Test methods will be reviewed and updated as the referenced standard changes.

6.1.1 If published/accepted methods do not describe the specific equipment or facilities used or not what is available in the laboratory, or if the method does not contain full details to allow the method to be completed, a supplemental method document including the above-described requirements will be generated to allow Lab personnel to complete the method.

6.1.2 All methods, procedures and supporting documentation, such as instructions, standards, manuals and reference data relevant to the laboratory activities, shall be kept up to date and shall be made readily available to personnel.

6.1.3 The laboratory shall ensure that it uses the latest valid version of a method unless it is not appropriate or possible to do so. When necessary, the application of the method shall be supplemented with additional details to ensure consistent application.

6.2 Deviations from methods for all ISO 17025 accredited laboratory activities are authorized only if the deviation has been documented, technically justified, authorized, and accepted by the customer. Records of the deviation authorization and customer acceptance are retained.

6.3 Every standard ISO 17025 accredited test which is offered to customers will be documented in a test method. The method may refer to other existing documentation and

instructions e.g., test item preparation specifications, sampling procedures, identification, handling and preservation procedures and facility, maintenance, and cleaning procedures, as necessary.

7.0 Procedural Principles and Methodology

7.1 Validation and verification are different processes. Typically, non-standard (e.g., developed in-house) methods, standard methods used outside their intended use, or amplified or modified standard methods are validated while compendial or standard (official) test methods are verified. If non-standard methods are appropriate to a situation within the accredited scope of test for ISO 17025:2017, including lab-developed methods, standard methods used outside of their intended scope and modifications, extensions etc., the non-standard method will be developed and documented in accordance with this procedure and agreed to by the customer. These non-standard methods will also be fully validated before use. The degree of planning, development, documentation, and validation is as extensive as necessary to meet the needs of the given application or field of application.

7.2 Methods intended for use only in investigational testing (not for product or raw material release) do not require validation or verification.

7.3 Validation of an analytical procedure is the process by which it is established, by laboratory studies, that the performance characteristics of the procedure meet the requirements for the intended analytical application. Validation for ISO 17025 accredited tests (listed above in section 2.1) will also take the factors that contribute to the total uncertainty of all measurements into account when developing methods and procedures.

7.4 Verification of a compendial or official test method is the assessment of whether the procedure can be used for its intended purpose, under the actual conditions of use.

7.4.1 A compendial or official test method that falls under the method verification process meets one of the following attributes.

7.4.1.1 The method is listed in the current USP or in another pharmacopeia such as the EP.

7.4.1.2 The method is listed in a document such as the Official Methods of

Analysis of AOAC International or the Food Chemicals Codex.

7.4.1.3 The method is validated and the validation documented (e.g. vendor's test method or published journal article).

7.4.1.4 Only modifications to the stated procedure listed in USP <621> Chromatography (System Suitability section) may be made for verifications. If modifications not listed in USP <621> are made, validation must be performed on the method.

7.4.1.5 Verification is not required for common compendial test procedures that are routinely performed in a laboratory such as:

7.4.1.5.1 Loss on drying.

7.4.1.5.2 Residue on ignition.

7.4.1.5.3 Wet chemical procedures such as acid value.

7.4.1.5.4 Instrumental methods such as pH or Karl Fischer.

7.4.1.6 Verification of compendial or official test methods intended for the analysis of dietary supplements is optional (EXCEPTION: an ISO 17025 accredited test listed above in Section 2.1- all ISO 17025 accredited tests using compendial or official test methods must be verified); however, verification is prudent for highly complex analytical determinations.

7.4.1.6.1 Verification is required for compendial or official test methods that will be incorporated into an SOP.

7.5 This procedure outlines the typical elements that may be used to validate or verify a test method. The parameters and acceptance criteria listed in this document are directly applicable to chromatographic test methods; however, the concepts may be applied to different test methods that may require alternate schemes.

7.6 Method validation and verification are protocol driven activities. The validation or verification protocol should include, at a minimum, the following information:

7.6.1 A brief description of the method including the source, purpose, and general

operating principle.

7.6.2 The specific analytical attributes to be evaluated.

7.6.2.1 Attributes to be evaluated should be chosen based on the guidance given in Sections 8.0 (for validations) and 9.0 (for verifications).

7.6.2.2 More or fewer analytical attributes may be required depending on the intended purpose and complexity of the procedure.

7.6.3 The acceptance criteria to be met.

7.6.3.1 Acceptance criteria should be established based on the guidelines given in Section 10.0.

7.7 The results generated during method validation, method verification, or method development shall not be used for finished product release. To ensure this, the following guidelines will be adhered to:

7.7.1 Sequences generated during method validation, verification or development shall not be stored in the same project folder as those generated for finished product release.

7.7.2 Sequence names and sample names for sequences generated during method validation, verification or development shall not include a batch number. The product name, formula number, or test identifier (e.g. PREC-1, PREC-2, PREC-3) should be used instead.

7.7.3 Finished product samples shall not be used for method validation, method verification, or method development. Samples that are acceptable to use for these activities include:

7.7.3.1 A sample that has been generated in the laboratory according to the product formulation and is not intended for commercial sale.

7.7.3.2 A sample that was obtained from a production batch immediately after blending but before further processing (e.g. encapsulation, compression, packaging).

7.7.3.3 A finished product sample that has been adulterated by the addition of an unknown quantity of excipient. The amount of adulterant added shall not be quantified, but should be limited to no more than ¼ of the total sample amount by visual inspection. Acceptable adulterants

include:

7.7.3.3.1 Microcrystalline cellulose for tablets, capsules, powders, and chewable tablets.

7.7.3.3.2 Sucrose for chewable gels (gummies).

7.7.3.3.3 Glycerin for glycerin based liquids or liquid capsules.

7.7.3.3.4 Olive oil for oil based liquids or liquid capsules.

7.7.3.3.5 Other adulterants may be used with scientific justification.

7.8 Upon successful completion of a validation or verification, a technical report will be generated documenting the results.

7.9 Upon successful completion of a validation or verification, all details of the method as executed should be compiled into a Standard Operating Procedure for routine use.

8.0 Method Validations

8.1 USP <1225> Validation of Compendial Procedures defines the following categories of analytical test methods. These categories may be extended to apply to dietary supplements, functional foods, cosmetics, and companion animal products.

8.1.1 Category I – Analytical procedures for quantitation of major components of bulk drug substances or active ingredients (including preservatives) in finished pharmaceutical products.

8.1.2 Category II – Analytical procedures for determination of impurities in bulk drug substances or degradation compounds in finished pharmaceutical products. These procedures include quantitative and limit tests.

8.1.3 Category III – Analytical procedures for the determination of performance characteristics (e.g. dissolution). Validation or verification of a dissolution procedure is beyond the scope of this document. Refer to USP <1092> The Dissolution Procedure for further guidance on validation of dissolution procedures.

8.1.4 Category IV – Identification tests.

8.2 For each category, different analytical information is needed. The performance characteristics required for each category of analytical procedure are listed in Table 1.

Table 1: Performance Characteristics Required for each Method Category

Analytical Performance Characteristic	Category I	Category II		Category III	Category IV
		Quantitative	Limit Test		
Accuracy	Yes	Yes	*	*	No
Precision	Yes	Yes	No	Yes	No
Specificity	Yes	Yes	Yes	*	Yes
Detection Limit	No	No	Yes	*	No
Quantitation Limit	No	Yes	No	*	No
Linearity	Yes	Yes	No	*	No
Range	Yes	Yes	*	*	No

* May be required, depending on the nature of the specific test

8.3 Robustness is typically evaluated during the method development stage. If the method is found to be susceptible to variation in its parameters, these parameters should be adequately controlled and a precautionary statement included in the method documentation.

8.4 Refer to Section 10.0 for guidance regarding analytical approach and establishing acceptance criteria.

8.5 Analytical methods for dietary supplements, cosmetics, and functional foods are validated or verified for individual analytes as opposed to individual products.

8.6 Analytical methods for drug products are validated for each individual product. For application of a previously validated or verified method to a finished product that has not been analyzed using the method before, Specificity, Accuracy, and Precision should be performed for the new product, a report documenting the results should be generated, and the procedure should be updated to refer to the report, and the scope of the procedure should be updated to include the formula number of the product.

9.0 Method Verification

9.1 Verification requirements should be based on the complexity of the procedure and the material to which the procedure is applied. Although complete revalidation of a compendial method is not required to verify the suitability of a procedure under actual

conditions of use, some of the analytical performance characteristics listed in Table 1 may be used for the verification process. Only those characteristics that are considered appropriate for the verification of the procedure need to be evaluated.

9.2 Assessment of the laboratory personnel, facilities, equipment, instrumentation, and item(s) under test should be performed.

9.2.1 Laboratory personnel should have the appropriate experience, knowledge, and training to understand and perform the compendial procedures.

9.2.2 Facilities, equipment, and instrumentation should be capable of performing the compendial method as intended.

9.2.3 The item(s) under test should be assessed for complexity of the "formulation" as compared to the intended use of the compendial procedure.

9.3 **Robustness** is generally not evaluated during method verification.

9.4 For method verification, the **System Suitability** criteria listed in the compendial method should be used without alteration.

10.0 Acceptance Criteria for Validations and Verifications

10.1 The criteria outlined in this section are general guidelines that are appropriate for the majority of validations and verifications performed at Ion Labs. Alternate criteria may be established if they are scientifically justified, and results meeting the requirements provide confidence that the procedure is suitable for its intended use.

10.2 **Accuracy** expresses the closeness of agreement between the true value and the observed value.

10.2.1 Category I methods are typically evaluated for Accuracy across the range of the analytical procedure. For example, a placebo may be spiked at 80%, 100%, and 120% of the nominal concentration.

10.2.2 Category II methods are typically evaluated for Accuracy at the limit level. The sample may be spiked with target analyte to approximate the limit level.

10.2.3 A number of approaches for evaluating Accuracy are listed below. The most appropriate strategy for the method category and test sample should be chosen.

10.2.3.1 Apply the procedure to synthetic mixtures of the product (placebo) to which known quantities of the target analyte have been added. Calculate the recovery of spiked target analyte.

10.2.3.2 Apply the procedure to a test sample with known quantity of the target analyte (reference substance). Evaluate the difference between the theoretical and observed values.

10.2.3.3 Apply the procedure to a test sample and compare the results to that of a second, well-characterized method.

10.2.3.4 Amend a sample with the target analyte. Apply the procedure to the test sample and the spiked test sample. Calculate the recovery of the spiked analyte.

10.2.3.5 Infer accuracy based on the successful demonstration of Specificity, Precision, and Linearity.

10.2.4 The degree of replication required is dependent upon the sample type.

10.2.4.1 For dietary supplements, functional foods, cosmetics, and companion animal products, a single replicate, or one replicate at each concentration level may be sufficient.

10.2.4.2 For drug products, triplicate sample preparations at each of three concentration levels is appropriate for Category I methods while six replicates at the limit level is appropriate for Category II methods.

10.2.5 Acceptance criteria should be chosen to ensure that the method performs as intended based on the method category and sample type. For strategies using replicate sample preparations, only the mean need be evaluated for acceptance since precision is assessed independently of accuracy.

10.2.5.1 For dietary supplements, functional foods, cosmetics, and companion animal products: within 5% of the expected value is usually appropriate for Category I methods, while within 10% of the expected value is acceptable for Category II methods.

10.2.5.2 For drug products: within 2% of the expected value is usually

appropriate for Category I methods, while within 10% of the expected value is acceptable for Category II methods.

10.3 **Precision** expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions.

10.3.1 Precision is evaluated by analyzing replicate sample preparations and calculating the %RSD of the results.

10.3.2 For Category II methods, the sample may be amended with target analyte to approximate the limit level if sufficient analyte is not already present.

10.3.3 The degree of replication is dependent upon the sample type.

10.3.3.1 For dietary supplements, functional foods, cosmetics, and companion animal products, the sample should be prepared in triplicate. Alternately, for Category I procedures, the sample may be prepared at three concentrations spanning the range of the method.

10.3.3.2 For drug products, six replicate sample preparations is appropriate. For Category I methods, triplicate sample preparations at each of three concentration levels spanning the range of the method is also acceptable.

10.3.4 Acceptance criteria should be chosen to ensure that the method performs as intended based on the method category and sample type.

10.3.4.1 For dietary supplements, functional foods, cosmetics, and companion animal products, less than 5.0% RSD for Category I and III methods, or less than 10.0% RSD for Category II methods is acceptable.

10.3.4.2 For drug products, less than 2.0% RSD for Category I methods, or less than 10.0% RSD for Category II methods is appropriate.

10.4 **Specificity** is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present such as impurities, degradants, or matrix components.

10.4.1 **Specificity** will be determined using retention time, UV/VIS spectral analysis, chromatographic resolution, or a combination thereof.

10.4.1.1 Identity by retention time will be assessed by comparing the retention time of a reference standard to that of the sample. A tolerance of +/- 0.3 minutes is generally acceptable.

10.4.1.2 Peak purity by spectral analysis will be performed by comparing the analyte peak spectra against that of a reference standard across a set wavelength. A spectral match of ≥ 900 is required for Specificity.

10.4.1.3 The USP Resolution between the target analyte and any adjacent peak(s) should be characterized. For Drug Products, a minimum resolution of 1.2 is required for Specificity. For dietary supplements, cosmetics, and functional foods, a minimum resolution of 1.0 is acceptable.

10.4.1.4 For Drug Products, forced degradation studies are required to ensure a stability indicating method. Conditions generally include exposure to acid, base, oxidation, light, and/or heat. In general, conditions should be adjusted to obtain 5% - 20% degradation. In some cases (e.g. light or heat stable analytes), this may not be attainable for all conditions. The above requirements for Identity by Retention Time, Peak Purity, and USP Resolution should be realized in the stressed samples.

10.5 **Detection Limit** is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

10.5.1 It is almost never necessary to determine the actual DL or QL. Rather the limit is shown to be sufficiently low by the analysis of a reference standard or sample with concentration of analyte at the limit or specification level. For procedures that exhibit instrument noise, the signal/noise ratio (S/N) is calculated using the equation below. DL is defined as the concentration that results in a S/N of 3.

S/N ratio = 2H/h

Where H is the height of the peak measured from the peak apex to a baseline extrapolated over a distance ≥ 5 times the peak width at its half-height, and h is the differenece between the largest and smallest noise

values observed over a distance ≥ 5 times the width of the peak at half-height and, if possible, situated equally around the peak of interest.

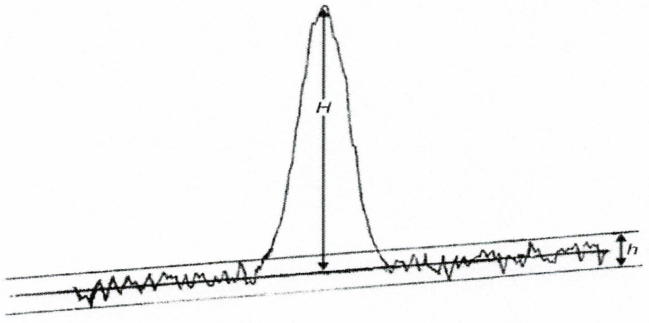

Figure 1: Evaluation of DL/QL

10.5.2 Perform six replicate injections of a reference standard or sample solution prepared at the DL level. The S/N is ≥ 3 for all injections.

10.6 **Quantitation Limit** is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy.

10.6.1 QL is defined as the concentration that results in a S/N of 10.

10.6.2 Perform six replicate injections of a reference standard or sample solution prepared at the QL level. The S/N is ≥ 10 for all injections.

10.7 **Linearity** is the ability, within a given range, of an analytical procedure to obtain measurement results which are directly, or by a well-defined mathematical transformation, proportional to the concentration of analyte in the sample.

10.7.1 **Linearity** requires at least five concentrations spanning the characterized linear range of the assay. The requirement is that $R^2 \geq 0.99$ unless scientific justification is given.

10.8 **Range** is the interval between the upper and lower concentration of analyte for which it has been demonstrated that the procedure has a suitable level of precision, accuracy, and linearity. Range is evaluated concurrently with Accuracy (Section 10.2) or Linearity (Section 10.7).

10.9 **Robustness** is 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.

10.9.1 **Robustness** is characterized during the method development stage. Robustness is typically not performed for methods that have been previously validated at outside laboratories. Acceptance criteria are generally not applied to Robustness, but evaluation helps to identify critical method parameters. The following parameters should be characterized as applicable for Robustness. Additional robustness parameters not listed here may be considered depending on the nature of the analytical method.

10.9.1.1 Flow Rate

10.9.1.2 Column Temperature

10.9.1.3 Injection Volume

10.9.1.4 Mobile Phase Composition

10.9.1.5 Compound Stability

10.9.1.6 Sample Preparation

10.10 **System Suitability**

10.10.1 Criteria for system suitability can include, but are not limited to, retention time matching, spectral matching, %RSD of replicate injections, peak resolution, peak symmetry, and column efficiency (theoretical plates). The chosen system suitability criteria should be incorporated into the respective SOP.

10.10.2 For method validations, acceptance criteria for system suitability should be chosen based on the Accuracy, Precision, and/or Robustness results to ensure that, if system suitability is met, the analytical procedure will perform as intended.

10.10.3 For method verifications, the acceptance criteria for system suitability listed in the compendial or official method should be used without alteration.

10.11 Changes to Validated Methods for use in ISO 17025 accredited tests:

10.11.1 When changes are made to validated methods, the influence of such changes are determined and where it is determined that they affected the original

validation, a new method validation will be performed.

11.0 Facilities and Environmental Conditions

11.1 The laboratory where testing activities will be conducted will be such that the energy sources, lighting, and environmental conditions will facilitate the correct performance of the tests.

11.2 The technical requirements for the accommodation and environmental conditions that can affect the results of the tests will be documented in the associated test methods.

11.3 Environmental conditions requiring control, including storage conditions, will be monitored by calibrated equipment.

12.0 Records

12.1 Method Verification and Validation records including the validation procedure used, the specifications of the requirements, determination of the performance characteristics of the method, results obtained and a statement on the validity of the method, detailing its fitness for the intended use will be maintained per SOP C-502 Record Storage, Retention, and Destruction.

14.0 Revision History

Revision	Date	Description of Changes	CCR #	By
1	03/19/13	New procedure.	13-164	B. Johns
2	12/01/14	Changed SOP number from F-102 to D-103. Updated SOP format. Expanded current validation practices in line with FDA Guidance 2014. Generalized microbial limit testing validation criteria. Updated responsibilities.	14-0966	B. Johns
3	06/20/16	Add use of validated AOAC methods for use without validation. Use of validated methods for investigational purposes. Improve clarity.	16-0548	B. Johns
4	11/17/16	Validation criteria for use in pharmaceuticals and cosmetics	16-1054	B. Johns
5	08/17/20	Update responsibilities. Remove category IIa. Add USP and ICH references. Combine with D-101.0. Add language to allow choice between validation and verification. Remove mass spec from specificity section. Add resolution and forced degradation to specificity section. Change discussion of range to more closely align with USP. Introduce new product verifications. Remove microbial limit testing since this will be addressed in a separate SOP.	CC-20-0577	S. Sassman
6	11/09/21	Added ISO 17025:2017 requirements.	CC-21-0428	J. Maignan
7	10/10/22	Remove new product verifications section and replace with product specific method optimization section.	CC-22-0391	S. Sassman
8	12/20/22	Remove product specific method optimization section since this will go into its own SOP, add language to ensure clear separation of development/validation activities from finished product release.	CC-22-0473	S. Sassman