Title: Method Validation and Verification

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References:
USP <1225> Validation of Compendial Methods
USP <1226> Verification of Compendial Methods
FDA, 60 FR 11260-2, Mar 1, 1995, "ICH Guidelines....".
Dadgar, D., et al., "Application Issues in Bioanalytical Method Validation, Sample Analysis and Data Reporting", J. Pharm. Biomed. Anal. 13(2), 89-97 (1995).
FDA, "Guideline for Submitting Samples and Analytical Data for Methods Validation", Center for Drugs and Biologics, Rockville, MD.
Hokanson, G.C., "A Life Cycle Approach to the Validation of Analytical Methods during Pharmaceutical Product Development, Part I: The Initial Method Validation Process", Pharm. Tech., Sept. 1994, 18(9), p 118.
ICH Q2(R1), Validation of Analytical Procedures: Text and Methodology, 2005.

Rev. No.	Effective Date	Revision Summary
1.	04-19-95	Original Version
2.	02-22-99	Converted to LWP format
3.	01-07-03	Sec. 1.0: Included method transfer.
4.	07-21-09	Sec. 1.0: Revised to align with ICH Q2(R1), protocol required, 2.2: added, 2.3 Accuracy expanded, 2.7: Peak purity added, 2.13: Stability expanded, 2.14: Stability indicating method added.
5.	MAY 05 2011	Sec. 1.4 -1.7: added to include method verification per USP <1226>.

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Prepared by	Date	Technical Review	Date
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QA Approval/Date: [signature] 05/05/11

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1.0 SCOPE AND APPLICATION

1.1 This SOP provides a guideline for validating, verifying, or transferring analytical methods. While it summarizes requirements for the pharmaceutical industry, the concepts are applicable for most methods. The goals for the study will need to be discussed at length with the client prior to method development and validation work. Method transfer and verification requires demonstration of system suitability and may require partial validation as required by the client.

1.2 A signed protocol for method validation with approvals from both this laboratory as well as the sponsor should be in place prior to the study. The protocol should contain sufficient detail to describe the tests to be performed and the criteria for acceptance.

1.3 There are four common types of analytical procedures with various requirements for validation. Methods are validated separately for drug products and drug substances (Active Pharmaceutical ingredients or excipients):

- Identification tests to ensure the identity of an analyte
- Quantitative tests for impurities
- Limit tests for impurities
- Quantitative tests or assays for components that are supposed to be present in the drug product

Validation requirements for each of these tests is summarized in the following table:

	Identification	Quantitative for Impurity	Limit Test for Impurity	Assay
Accuracy	No	Yes	No	Yes
Precision				
Repeatability	No	Yes	No	Yes
Inter. Prec.	No	Yes	No	Yes
Specificity	Yes	Yes	Yes	Yes
LOD	No	Yes	Yes	No
LOQ	No	Yes	No	No
Range & Linearity	No	Yes	No	Yes

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1.4 Per USP <1226>, compendial procedures that are being performed for the first time must be evaluated to demonstrate that they yield acceptable results utilizing the personnel, equipment, and reagents available. This chapter is not intended for retroactive application to already successfully established laboratory procedures. Due to the long history of acceptable performance, including (in many cases) blinded proficiency testing, the following analytical methods have been assessed and are considered basic procedures for this laboratory, for which additional verification or validation activities are unnecessary for compendial materials:

1.4.1 Description of the appearance of a material or solution of a material, including comparison with colored, turbid, or opalescent reference standards.

1.4.2 Basic gravimetric tests, including: Specific Gravity, Soluble/Insoluble Substances, Residue on Ignition, Residue After Evaporation, Loss on Ignition, Loss on Drying, Alkalies and Alkaline Earths, and equivalent.

1.4.3 Identification or spectral conformance by FTIR or NMR.

1.4.4 Titration of compendial materials with standard volumetric solutions and indicators.

1.4.5 Standard identification tests per USP <191>, or equivalent.

1.4.6 Nitrogen content by USP <461>

1.4.7 Limit of arsenic by USP <211> and EP (2.4.2)

1.4.8 Limit of lead by USP <251>

1.4.9 Limit of iron by USP <241>

1.4.10 Limit of chloride or sulfate by USP <221>, including compendial limit tests for common anions.

1.4.11 Acidity or Alkalinity by titration

1.4.12 Viscosity by USP <911> or EP (2.2.9)

1.4.13 Residual solvents by USP <467>

1.4.14 Basic instrumentation tests, where calibration and qualification of the instrument demonstrates acceptability of the analytical system, including: pH, specific rotation, UV-Vis, fluorometry, turbidity, microscopy, and conductivity.

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1.5 Routine quality control practices, as described in the Quality Manual, ensure that the suitability of methods used are demonstrated with each analytical batch. These include:

1.5.1 Demonstration of acceptable detection limit for limit tests through the analysis of a spiked sample, sample with impurities, or a standard (as appropriate) at or below the required detection limit for the method.

1.5.2 Demonstration of precision for quantitative tests through the analysis of a duplicate sample or duplicate matrix spike sample. In addition, system suitability for chromatographic assay tests includes the analysis of replicate standards with acceptance criteria for precision, as defined by USP <621>.

1.5.3 Demonstration of accuracy for quantitative tests through the analysis of a matrix spike sample.

1.5.4 Range and linearity for quantitative impurity tests is demonstrated through the analysis of standards across a minimum of 50%, 100%, and 150% of the range of the specification.

1.6 The laboratory training program, as defined in SOP 140, ensures that analysts have the appropriate experience, knowledge, and training to understand and be able to perform compendial procedures as written.

1.7 Some USP/EP compendial tests have been found to not pass verification criteria for some matrices.

1.7.1 The USP liaison should be contacted for assistance in resolving the problem.

1.7.2 If the procedure is not suitable for use with the article being tested, it may be necessary to develop and validate an alternate procedure upon contract with the client.

1.7.3 The OOS and NCR systems, and their associated trending activities, further identify errors and non-conformances related to the performance of compendial procedures, which gives additional assurance that problematic methods are identified and corrective actions implemented.

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2.0 DEFINITIONS

These definitions are taken for the most part from USP <1225>, submission requirements for compendial methods, as well as other references and guidances. The definitions below are not in alphabetical order, but in order of logical flow from a discussion of the subject.

2.1 Method Validation - the process by which it is determined that the performance of an analytical method meets the requirements for an intended application. Performance characteristics may include:

Accuracy	Limit of Quantitation
Precision	Linearity & Range
Specificity	Stability of Solutions
Limit of Detection	Robustness
Ruggedness (Intermediate Precision)	

Validation occurs after method feasibility and development activities. Performance expectations should be set prior to method development. While the method validation process begins during the development of a new drug, changes in drug product or analytical technology may make this a dynamic process throughout the lifetime of the drug product, where the method is revalidated several times.

2.2 Method Transfer - Appropriate validation activities are required when a method is transferred to another laboratory, in order to demonstrate that acceptable performance can be documented by the new lab.

2.3 Reference Sample - a well characterized sample with a known, well established value, preferably a primary standard from a government agency (EPA, USP, NIST, etc.) or a certified reference material directly traceable to a primary standard.

2.4 Accuracy - closeness to the true value, measured by % recovery of sample spikes or % error in the analysis of a reference standard. For a drug substance or drug product assay, accuracy may be inferred from precision, linearity, and specificity. For a drug product, accuracy of an assay may be demonstrated by recovery of spikes into a placebo formulation of the drug product at 80, 100, and 120% of the specification. For impurities, accuracy should be demonstrated by recovery of spikes at 50, 100, and 150% of the maximum limit. Usually spikes are prepared with N=6 replicates at the specification or maximum limit with N=3 replicates at other levels. The specification for accuracy and precision will be stated in the approved protocol prior to execution.

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2.5 Precision - the degree of agreement between replicate analyses of an homogenous sample, usually measured as the relative percent difference (RPD) between duplicates or the relative standard deviation (RSD) of a set of replicates. Normally the precision required for an assay of the active ingredients is on the order of 2%, which may require an internal standard method. Precision for trace impurities, where the instrument may be working closer to the detection limit, is often higher (15-20%). The ICH Guideline defines three precision measurements: (a) repeatability or short term precision as defined above, (b) intermediate precision which is essentially the same as intra-lab ruggedness, and (c) reproducibility which is essentially the same as inter-lab ruggedness. Replicate sample preparations should be analyzed as described above for accuracy.

Note that the homogeneity of a sample will depend on sample size, particularly for solids. Adequate sample size should be used to insure the sample is representative of the test article, as demonstrated by the reproducibility between aliquots.

2.6 Internal Standard (IS) - a compound of similar chemistry and structure to the analyte which is used to correct the response for variations in injection amount and other instrument and chromatographic variables. In LC methods, the IS compound should elute at a similar retention time, preferably after the drug to avoid potential interference from faster eluting, more polar metabolites.

2.7 Specificity (Selectivity) - the degree of bias (or lack thereof) caused by expected sample components and common interferences, determined by measuring the analyte with and without anticipated interferences. For example, it has been recommended that when developing an analysis for a drug in blood or plasma, that at least six independent sources of blank matrix be tested for interferences. A known reference material may be spiked with known impurities and product excipients (i.e. placebo). Degradation and metabolite products of the matrix and drug may be obtained from stress tests. See also 2.14 Stability Indicating Methods. Peak purity should be addressed, commonly through the use of a second detector.

2.8 Detection Limit (LOD or DL) - the lowest concentration which can be detected with confidence (usually at the 99% confidence level), estimated as three times the standard deviation (SD) of the background signal of low level sample spikes (3-5X the estimated DL). An instrument detection limit (IDL) may be determined from low level standards or method blanks as opposed to a sample LOD determined from low level sample spikes. The 3 X SD gives a concentration which is statistically known as the 99% confidence level at which the experimental value is known to be above the background concentration (zero). When using standards or spikes, the concentration must be reduced to a level which challenges the analysis, i.e. a level at which the precision of the determination increases over the errors normally experienced at higher concentrations. For example, if precision at 100 ppm is generally demonstrated by an RSD of 10%, the concentration should be reduced until the precision is significantly greater than 10% (perhaps 15-50% RSD).

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2.9 Limit of Quantitation (LOQ) - the concentration level above which the concentration can be determined with acceptable precision (typically RSD < 10 - 25%) and accuracy (typically 80-120% recovery), usually estimated as ten times the SD of the background or low level sample spikes. A standard near the LOQ should be included in the calibration curve for method used for the quantitation of impurities and degradation products.

2.10 Range and Linearity - the variance in the response with concentration, measured as the RSD of response factors or the correlation coefficient (R^2) from a linear regression fit. Note that a normal, unweighted regression fit normally weights the high concentration and understates errors at low concentration. For this reason a response factor graph is preferable. For an assay method, six determinations should be made in the range of 25-125% of anticipated or specified levels. For quantitative determination of impurities and degradation products, the lower level should be extended to the LOQ or background level. Calibration curves are not forced through zero.

2.11 Robustness - the capacity of a method to remain unaffected by small, deliberate variations in the method parameters that one might experience during normal usage, determined by varying reagent and eluent composition (typically 2% variation) and pH, columns (at least two from different lots of the same part number), column temperature, flow rates, extraction times, etc. Robustness may be proposed as part of a separate study after other validation parameters are demonstrated.

2.12 Ruggedness (intermediate precision) - the variance in the analysis of homogenous samples between analysts, instruments, columns, and on different days. May include inter-laboratory performance.

2.13 Stability - Replicates of a homogenous sample should be stored for "worst case" holding times (1-2 weeks) before analysis under typical storage conditions and compared to results from analysis performed immediately after the samples were prepared. Stability of the sample and standard solutions, dilutions, or digests should be addressed.

2.14 Stability Indicating Method (SIM) - a validated analytical procedure that accurately and precisely measures active ingredients (drug substance or drug product) free from potential interferences like degradation products, process impurities, excipients, or other potential impurities. The FDA recommends that all assay procedures for stability studies be stability indicating. This involves demonstrating that interferences are not produced during degradation of the sample. This requires degraded samples to be tested during the development or validation phase of the study. The goal of the SIM is to obtain baseline resolution of all the resulting products (the API and all the degradation products) with no coelutions. Forced degradation of samples by acid and base hydrolysis, oxidation, heat, and light is beyond the scope of this SOP, as it is typically performed by our clients and based on their knowledge about degradation pathways and products that could form during storage. Degradation of at least 10-30% of the assay peak is recommended.

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3.0 PROCEDURE

3.1 Analysts will be assigned by the Study Director, Technical Director, or Group Leader for conducting the validation work. They must have documentation of training on the associated instrumentation and/or technique. If ruggedness is an included parameter, a second analyst will be assigned.

3.2 Prior to method validation, when method development is first contracted, clear goals or guidelines should be identified for performance characteristics.

3.3 Create a draft protocol that addresses the elements of method validation pertinent to the analysis (sec. 1.3). If any elements are declined by the client, a scientifically valid justification must be included in the protocol. A signed copy of the protocol must be filed in the Job Envelope before the analysis of samples begins. Validation protocols should include the following items:

3.3.1 Description of the analyte of interest and the matrix (e.g. chemical name, structure, etc.).

3.3.2 List of standards, placebos, and samples (including identification numbers, when available) to be used.

3.3.3 References relevant to the protocol (e.g. method development reports, methods or previous validations).

3.3.4 Acceptance criteria for validation.

3.3.5 The protocol must be approved by the client; the technical reviewer (the Study Director, Technical Director, or Group Leader), and Quality Assurance.

3.3.6 Attachments: the draft SOP to be validated, the specification, or other relevant product information.

3.4 Assemble any required equipment and reagents. Ascertain that equipment has been qualified and reagents are within expiry. Perform any suitability tests required by the method.

3.5 Conduct the tests as outlined in the signed, approved protocol.

3.6 Any deviations must be documented in the data package and corrected or justified. Any observed deficiencies in the method will be noted in the report. Risk assessment and toxicological evaluation of method deficiencies are the responsibility of the client.

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3.7 The analyst, as assigned by the Group Leader, will prepare the draft method validation report.

3.8 Submit the data package and draft report for review by a Senior Chemist (or above), Group Leader, or Technical Director.

3.9 Submit the data package and draft report for QA review.

3.10 Following QA review, the draft report is submitted to the client and the final report prepared. The final report will include a copy of the raw data.

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