# Step-by-Step Analytical Methods Validation and Protocol in the Quality System Compliance Industry

BY GHULAM A. SHABIR

## Introduction

*Methods Validation: Establishing documented evidence that provides a high degree of assurance that a specific method, and the ancillary instruments included in the method, will consistently yield results that accurately reflect the quality characteristics of the product tested.*

Method validation is an important requirement for any package of information submitted to international regulatory agencies in support of new product marketing or clinical trials applications. Analytical methods should be validated, including methods published in the relevant pharmacopoeia or other recognized standard references. The suitability of all test methods used should always be verified under the actual conditions of use and should be well documented.

Methods should be validated to include consideration of characteristics included in the International Conference on Harmonization (ICH) guidlines[1, 2] addressing the validation of analytical methods. Analytical methods outside the scope of the ICH guidance should always be validated.

ICH is concerned with harmonization of technical requirements for the registration of products among the three major geographical markets of the European Community (EC), Japan, and the United States (U.S.) of America. The recent U.S. Food and Drug Administration (FDA) methods validation guidance document,[3-5] as well as the United States Pharmacopoeia (USP),[6] both refer to ICH guidelines.

The most widely applied typical validation characteristics for various types of tests are accuracy, precision (repeatability and intermediate precision), specificity, detection limit, quantitation limit, linearity, range, and robustness *(Figure 1)*. In addition, methods validation information should also include stability of analytical solutions and system suitability.[7]

Health Canada (HC) has also issued guidance on methods validation entitled *Acceptable Methods Guidance*.[8] HC has been an observer of ICH, and has adopted ICH guidelines subsequent to its reaching Step Four of the ICH process. An acceptable method predates ICH, and HC plans to revise this guidance to reflect current ICH terminology.

*Figure 2* shows the data required for different types of analysis for method validation. Where areas of the *Acceptable Methods Guidance* are superseded by ICH Guidelines Q2A[1] and Q2B,[2] HC accepts the requirements of either the ICH or *Acceptable Methods Guidance*; however, for method validation, ICH acceptance criteria are preferred. HC's *Acceptable Methods Guidance* provides useful guidance on methods not covered by the ICH guidelines (e.g., dissolution, biological methods), and provides acceptance criteria for validation parameters and system suitability tests for all methods.

HC has also issued templates recommended as an approach for summarizing analytical methods and validation data ICH terminology was used when developing these templates.

This paper suggests one technique of validating methods. There are numerous other ways to validate methods, all

***Figure 1***

**ICH, USP, and FDA Methods Validation Characteristics Requirements for Various Types of Tests**

| Validation Characteristics | Assay | Testing for Impurities | | Identification |
|---|---|---|---|---|
| | | Quantitative | Limit | |
| Accuracy | Yes | Yes | No | No |
| Precision - Repeatability | Yes | Yes | No | No |
| Precision - Intermediate Precision | Yes[1] | Yes* | No | 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 | No |
| Robustness | Yes | Yes | No | No |

* In cases where reproducibility has been performed, intermediate precision is not needed.[7]

***Figure 2***

**Health Canada Methods Validation Parameter Requirements for Various Types of Tests**

| Validation Parameters | Identity Tests | Active Ingredients | | Impurities / Degradation Products | | Physico-Chemical Tests |
|---|---|---|---|---|---|---|
| | | Drug Substance | Drug Product | Quantitative | Limit Tests | |
| Precision (of the system) | No | Yes | Yes | Yes | 1 | Yes |
| Precision (of the method) | No | 1 | Yes | Yes | 1 | Yes |
| Linearity | No | Yes | Yes | Yes | No | Yes |
| Accuracy | No | Yes | Yes | Yes | 1 | Yes |
| Range | No | 1 | Yes | Yes | No | Yes |
| Specificity | Yes | 1 | Yes | Yes | Yes | * |
| Detection Limit | 1 | No | No | Yes | Yes | * |
| Quantitation Limit | No | No | No | Yes | No | * |
| Ruggedness | 1 | Yes | Yes | Yes | Yes | Yes |

* May be required depending upon the nature of the test.

equally acceptable when scientifically justified.

## Prepare a Protocol

The first step in method validation is to prepare a protocol, preferably written, with the instructions in a clear step-by-step format, and approved prior to their initiation. This approach is discussed in this paper. The suggested acceptance criteria may be modified depending on method used, required accuracy, and required sensitivity. (Note: Most of the acceptance criteria come from the characterization study.) Furthermore, some tests may be omitted, and the number of replicates may be reduced or increased based on scientifically sound judgment.

A test method is considered validated when it meets the acceptance criteria of a validation protocol. This paper is a step-by-step practical guide for preparing protocols and per-

forming test methods validation with reference to High Performance Liquid Chromatography (HPLC) (use similar criteria for all other instrumental test method validation) in the quality system compliance industry.

## Analytical Methods Validation Protocol Approval Cover Page

Methods validation must have a written and approved protocol prior to its initiation. A project controller will select a validation Cross-Functional Team (CFT) from various related departments and functional areas. The project controller assigns responsibilities. The following tables illustrate one suggested way of documenting and preserving a record of the approvals granted at the various phases

### Summary Information

| Summary Information | |
|---|---|
| Organization name | |
| Site location | |
| Department performing validation | |
| Protocol title | |
| Validation number | |
| Equipment | |
| Revision number | |

### Project Controller

| Project Controller | Name | Signature | Date |
|---|---|---|---|
| | | | |

### Document Approval

| Document Approval | | | |
|---|---|---|---|
| **Department / Functional Area** | **Name** | **Signature** | **Date** |
| Technical Reviewer | | | |
| End Lab Management | | | |
| Health & Safety | | | |
| Quality Assurance | | | |
| Documentation Control (reviewed and archived by) | | | |

### Revision History

| Revision History | | | |
|---|---|---|---|
| **Revision No.** | **Date** | **Description of change** | **Author** |
| | | | |

of the validation:

## Writing a Test Method Validation Protocol

Analytical method validations should contain the following information in detail:

**Purpose:** This section provides a short description of what is to be accomplished by the study.

Project scope: Identify the test methods and which products are within the scope of the validation.

**Overview:** This section contains the following: a general description of the test method, a summary of the characterization studies, identification of method type and validation approach, test method applications and validation protocol, the intended use of each test method application, and the analytical performance characteristics for each test method application.

**Resources:** This section identifies the following: end user laboratory where the method validation is to be performed; equipment to be used in the method validation; software to be used in the method validation; materials to be used in the method validation; special instructions on handling, stability, and storage for each material.

**Appendices:** This section contains references, signature, and a review worksheet for all personnel, their specific tasks, and the documentation of their training. Listings of all equipment and software necessary to perform the method validation should be found here along with document and materials worksheets used in the method validation and in the test method procedure(s).

### 1. Analytical Performance Characteristics Procedure

Before undertaking the task of methods validation, it is necessary that the analytical system itself be adequately designed, maintained, calibrated, and validated. All personnel who will perform the validation testing must be properly trained. Method validation protocol must be agreed upon by the CFT and approved before execution. For each of the previously stated validation characteristics *(Figure 1)*, this document defines the test procedure, documentation, and acceptance criteria. Specific values are taken from the ICH, U.S. FDA, USP, HC, and pertinent literature as references. (See the References section at the end of this article for further definitions and explanations.)

### 1.1. Specificity

*1.1.1. Test procedure*

The specificity of the assay method will be investigated by injecting of the extracted placebo to demonstrate the absence of interference with the elution of analyte.

*1.1.2. Documentation*

Print chromatograms.

*1.1.3. Acceptance criteria*

The excipient compounds must not interfere with the analysis of the targeted analyte.

### 1.2. Linearity

*1.2.1. Test procedure*

Standard solutions will be prepared at six concentrations, typically 25, 50, 75, 100, 150, and 200% of target concentration. Three individually prepared replicates at each concentration will be analyzed. The method of standard preparation and the number of injections will be same as used in the final procedure.

*1.2.2. Documentation*

Record results on a datasheet. Calculate the mean, standard deviation, and Relative Standard Deviation (RSD) for each concentration. Plot concentration (x-axis) versus mean response (y-axis) for each concentration. Calculate the regression equation and coefficient of determination (r2). Record these calculations on the datasheet.

*1.2.3. Acceptance criteria*

The correlation coefficient for six concentration levels will be ≥ 0.999 for the range of 80 to 120% of the target concentration. The y-intercept must ≤ 2% of the target concentration response. A plot of response factor versus concentration must show all values within 2.5% of the target level response factor, for concentrations between 80 and 120% of the target concentration.[9,10] HC states that the coefficient of determination for active ingredients should be ≥ 0.997, for impurities 0.98 and for biologics 0.95.[8]

### 1.3. Range

*1.3.1. Test procedure*

The data obtained during the linearity and accuracy studies will be used to assess the range of the method.

| Linearity - Data Sheet | | Electronic file name: | |
|---|---|---|---|
| **Concentration (mg/ml)** | **Concentration as % of Analyte Target** | **Peak Area (mean of three Injections)** | **Peak Area RSD (%)** |
| 5 (e.g.) | 25 | | |
| 10 | 50 | | |
| 15 | 75 | | |
| 20 | 100 | | |
| 30 | 150 | | |
| 40 | 200 | | |
| Equation for regression line = | | Correlation coefficient ($r^2$) = | |

| Range - Data Sheet | Electronic file name: |
|---|---|
| Record range: | |

| Accuracy - Data Sheet | | Electronic file name: | | |
|---|---|---|---|---|
| **Sample** | **Percent of Nominal (mean of three injections)** | **Amount of Standard (mg)** | | **Recovery (%)** |
| | | **Spiked** | **Found** | |
| 1 | 75 (e.g.) | | | |
| 2 | 100 | | | |
| 3 | 150 | | | |
| Mean | | | | |
| SD | | | | |
| RSD% | | | | |

| Repeatability - Data Sheet | | Electronic file name: | |
|---|---|---|---|
| **Injection No.** | **Retention Time (min)** | **Peak Area** | **Peak Height** |
| Replicate 1 | | | |
| Replicate 2 | | | |
| Replicate 3 | | | |
| Replicate 4 | | | |
| Replicate 5 | | | |
| Replicate 6 | | | |
| Replicate 7 | | | |
| Replicate 8 | | | |
| Replicate 9 | | | |
| Replicate 10 | | | |
| Mean | | | |
| SD | | | |
| RSD% | | | |

The precision data used for this assessment is the precision of the three replicate samples analyzed at each level in the accuracy studies.

*1.3.2. Documentation*

Record the range on the datasheet.

*1.3.3. Acceptance criteria*

The acceptable range will be defined as the concentration interval over which linearity and accuracy are obtained per the above criteria, and in addition, that yields a precision of ≤ 3% RSD.[9]

## 1.4. Accuracy

*1.4.1. Test procedure*

Spiked samples will be prepared at three concentrations over the range of 50 to 150% of the target concentration. Three individually prepared replicates at each concentration will be analyzed. When it is impossible or difficult to prepare known placebos, use a low concentration of a known standard.

*1.4.2. Documentation*

For each sample, report the theoretical value, assay value, and percent recovery. Calculate the mean, standard deviation, RSD, and percent recovery for all samples. Record results on the datasheet.

*1.4.3. Acceptance criteria*

The mean recovery will be within 90 to 110% of the theoretical value for non-regulated products. For the U.S. pharmaceutical industry, 100 ± 2% is typical for an assay of an active ingredient in a drug product over the range of 80 to 120% of the target concentration.[9] Lower percent recoveries may be acceptable based on the needs of the methods. HC states that the required accuracy is a bias of ≤ 2% for dosage forms and ≤ 1% for drug substance.[8]

## 1.5. Precision - Repeatability

*1.5.1. Test procedure*

One sample solution containing the target level of analyte will be prepared. Ten replicates will be made from this sample solution according to the final method procedure.

*1.5.2. Documentation*

Record the retention time, peak area, and peak height on the datasheet. Calculate the mean, standard deviation, and RSD.

*1.5.3. Acceptance criteria*

The FDA states that the typical RSD should be 1% for drug substances and drug products, ± 2% for bulk drugs and finished products. HC states that the RSD should be 1% for drug substances and 2% for drug products. For minor components, it should be ± 5% but may reach 10% at the limit of quantitation.[8]

## 1.6. Intermediate Precision

*1.6.1. Test procedure*

Intermediate precision (within-laboratory variation) will be demonstrated by two analysts, using two HPLC systems on different days and evaluating the relative percent purity data across the two HPLC systems at three concentration levels (50%, 100%, 150%) that cover the analyte assay method range 80 to 120%.

*1.6.2. Documentation*

Record the relative % purity (% area) of each concentration on the datasheet.

Calculate the mean, standard deviation, and RSD for the operators and instruments.

*1.6.3. Acceptance criteria*

The assay results obtained by two operators using two instruments on different days should have a statistical RSD ≤ 2%.[9, 10]

## 1.7. Limit of Detection

*1.7.1. Test procedure*

The lowest concentration of the standard solution will be determined by sequentially diluting the sample. Six replicates will be made from this sample solution.

*1.7.2. Documentation*

Print the chromatogram and record the lowest detectable concentration and RSD on the datasheet.

*1.7.3. Acceptance criteria*

The ICH references a signal-to-noise ratio of 3:1.[2] HC recommends a signal-to-noise ratio of 3:1. Some analysts calculate the standard deviation of the signal (or response)

| Intermediate Precision - Datasheet | Electronic file name: | | | | | |
|---|---|---|---|---|---|---|
| | Relative % Purity (% area) | | | | | |
| | Instrument 1 | | | Instrument 2 | | |
| Sample | S1 (50%) | S2 (100%) | S3 (150%) | S1 (50%) | S2 (100%) | S3 (150%) |
| Operator 1, day 1 | | | | | | |
| Operator 1, day 2 | | | | | | |
| Operator 2, day 1 | | | | | | |
| Operator 2, day 2 | | | | | | |
| Mean (Instrument) | | | | | | |
| Mean (Operators) | | | | | | |
| RSD% | S1 + S1 | S2 + S2 | S3 + S3 | | | |
| Instruments | | | | | | |
| Operators | | | | | | |

| Limit of Detection - Data Sheet | Electronic file name: |
|---|---|
| Record sample data results: (e.g., concentration, S/N ratio, RSD%) | |

| Limit of Quantitation - Data Sheet | Electronic file name: |
|---|---|
| Record sample data results: (e.g., concentration, S/N ratio, RSD%) | |

of a number of blank samples and then multiply this number by two to estimate the signal at the limit of detection.

### 1.8. Limit of Quantitation

*1.8.1. Test procedure*

Establish the lowest concentration at which an analyte in the sample matrix can be determined with the accuracy and precision required for the method in question. This value may be the lowest concentration in the standard curve. Make six replicates from this solution.

*1.8.2. Documentation*

Print the chromatogram and record the lowest quantified concentration and RSD on the datasheet. Provide data that demonstrates the accuracy and precision required in the acceptance criteria.

*1.8.3. Acceptance criteria*

The limit of quantitation for chromatographic methods has been described as the concentration that gives a signal-to-noise ratio (a peak with height at least ten times as high as the baseline noise level) of 10:1.[2] HC states that the quantitation limit is the best estimate of a low concentration that gives an RSD of approximately 10% for a minimum of six replicate determinations.[8]

### 1.9. System Suitability

*1.9.1. Test procedure*

System suitability tests will be performed on both HPLC systems to determine the accuracy and precision of the system by injecting six injections of a solution containing analyte at 100% of test concentration. The following parameters will be determined: plate count, tailing factors, resolution, and reproducibility (percent RSD of retention time, peak area, and height for six injections).

*1.9.2. Documentation*

Print the chromatogram and record the data on the datasheet

*1.9.3. Acceptance criteria*

Retention factor (k): the peak of interest should be well resolved from other peaks and the void volume; generally k should be ≥2.0. Resolution (Rs): Rs should be ≥2 between the peak of interest and the closest eluted peak,

| System Suitability – Data Sheet | | Electronic file name: | | |
|---|---|---|---|---|
| System Suitability Parameter | Acceptance Criteria | Results | | Criteria Met/ Not Met |
| | | HPLC 1 | HPLC 2 | |
| Injection precision for retention time (min) | RSD ≤ 1% | | | |
| Injection precision for peak area (n = 6) | RSD ≤ 1% | | | |
| Injection precision for peak height | RSD ≤ 1% | | | |
| Resolution ($R_s$) | Rs = ≥ 2.0 | | | |
| USP tailing factor (T) | T = ≤ 2.0 | | | |
| Capacity factor (k) | K = ≥ 2.0 | | | |
| Theoretical plates (N) | N = ≥ 2000 | | | |

| Robustness - Data Sheet | Electronic file name: |
|---|---|
| Explain / record sample data: | |

which is potentially interfering (impurity, excipient, and degradation product). Reproducibility: RSD for peak area, height, and retention time will be 1% for six injections. Tailing factor (T): T should be 2. Theoretical plates (N): ≥2000.[3]

### 1.10. Robustness

As defined by the USP, robustness measures the capacity of an analytical method to remain unaffected by small but deliberate variations in method parameters. Robustness provides some indication of the reliability of an analytical method during normal usage.

Parameters, which will be investigated, are percent organic content in the mobile phase or gradient ramp, pH of the mobile phase, buffer concentration, temperature, and injection volume. These parameters may be evaluated one factor at a time or simultaneously as part of a factorial experiment.

The chromatography obtained for a sample containing representative impurities, when using modified parameter(s), will be compared to the chromatography obtained using the target parameters. The effects of the following changes in chromatographic conditions will be determined: methanol content in mobile phase adjusted by ± 2%, mobile phase pH adjusted by ± 0.1 pH units, column temperature adjusted by ± 5°C. If these changes are within the limits that produce acceptable chromatography, they will be incorporated in the method procedure.[9, 10]

### 2. Appendices

List all appendices associated with this protocol. Each appendix needs to be labeled and paginated separately

**Article Acronym Listing**

| | |
|---|---|
| CFT: | Cross-Functional Team |
| EC: | European Community |
| FDA: | Food and Drug Administration |
| HC: | Health Canada |
| HPLC: | High Performance Liquid Chromatography |
| ICH: | International Conference on Harmonization |
| RSD: | Relative Standard Deviation |
| U.S.: | United States |
| USP: | United States Pharmacopoeia |

## *List of Appendices*

| Appendix No. | Document Title | Total Pages |
|---|---|---|
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |

## *Appendix 1*

**Method Validation Personnel Signature and Review Worksheet**

| Analyst Name | Dept. | Validation Activity | Protocol Reference | Analyst Signature | Date |
|---|---|---|---|---|---|
| | | | | | |
| | | | | | |
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| | | | | | |
| | | | | | |

**Comments:**

| Completed By: | Signature: | Date: |
|---|---|---|
| | | |

## *Appendix 2*

**Equipment and Software Used in Method Validation Worksheet**

| Equipment Name/Module # | Last Calibration Date | Next Calibration Date | Software Name and Version | Validation Reference |
|---|---|---|---|---|
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |

**Comments:**

| Completed By: | Signature: | Date: |
|---|---|---|
| | | |

## *Appendix 3*

**Document and Materials Used in Method Validation Worksheet**

| Complete Pre-protocol Execution | | | | |
|---|---|---|---|---|
| **Document Name/Ref. No.** | **Edition/Version Number** | **Material Name** | **Supplier/ Lot Number** | **Expiration Date** |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| **Comments:** | | | | |
| **Completed By:** | **Signature:** | | **Date:** | |

## *Appendix 4*

**Analytical Test Method Procedure**

This procedure should include the entire testing method and all procedures associated with it. This appendix can appear in any format, but it should always be included in the documentation

from the body of the document. The following information must be found on every page of each appendix: validation protocol number; validation protocol title; appendix number (e.g., 1, 2, 3, … or A, B, C, …); and page X of Y. ❑


## Acknowledgements

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