

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) guidelines^{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 (re-

peatability 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 Where areas of the *Acceptable Methods Guidance* are superseded by ICH Guidelines Q2A¹ and Q2B,² HC accepts the requirements of either the ICH or *Acceptable Methods Guidance*; however, for method validation, ICH acceptance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 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 (r^2). 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 $\leq 2\%$ 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 $\leq 3\%$ 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, $100 \pm 2\%$ 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 $\leq 2\%$ for dosage forms and $\leq 1\%$ 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, $\pm 2\%$ 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 $\pm 5\%$ 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 $\leq 2\%$.^{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.

concentration that gives an RSD of approximately 10% for a minimum of six replicate determinations.⁸

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 con-

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)	R _s = ≥ 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

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. □

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quality assurance, in-process control, R&D, and validation in the pharmaceutical industry. Ghulam has received many technical excellence industrial awards as well as academic awards for authoring 'best scientific papers.' Ghulam's work has appeared in many publications. He has given several presentations at international conferences as well as having organized and moderated at symposiums. He holds a Master's Degree in Chemistry and Pharmaceutical Sciences. He can be reached by phone at 44-1993-863099, by fax at 44-1235-467737, or by e-mail at ghulam.shabir@abbott.com.

About the Author

Ghulam Shabir is a Principal Scientist at Abbott Laboratories, MediSense UK. His group is responsible for materials characterization, analytical methods development, and validation and equipment qualification. Ghulam is a Fellow of the Institute of Quality Assurance and a Companion of the Institute of Manufacturing with 17 years of broad-based experience in the areas of production, quality control,

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