TABLE OF
CONTENTS

ANALYTICAL METHODS VALIDATION

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 Where areas of the *Acceptable Methods Guidance* are superseded by ICH Guidelines Q2A[1] and Q2B,[2] HC accepts the requirements of either the ICH or *Acceptable Methods Guidance*; however, for method validation, ICH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 $\leq 2\%$ 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 ≤ 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

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

I thank Abbott Laboratories and MediSense for permission to publish this article. I also thank Dr. Alison Ingham (Health Canada) for his comments on the text.

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, 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.

References

1. International Conference on Harmonization (ICH), Q2A: Text on Validation of Analytical Procedures, March 1995.
2. International Conference on Harmonization (ICH), Q2B: Validation of Analytical Procedures: Methodology, May 1997.
3. U.S. Center for Drug Evaluation and Research, Reviewer Guidance: Validation of Chromatographic Methods, November 1994.

4. U.S. FDA, Guidance for Submitting Samples and Analytical Data for Methods Validation, Rockville, Md., USA, Center for Drugs and Biologics, Department of Health and Human Services, February 1987.
5. U.S. FDA DHHS, 21 CFR Parts 210 and 211, Current Good Manufacturing Practice of Certain Requirements for Finished Pharmaceuticals, Proposed Rule, May 1996.
6. Validation of Compendial Methods, <1225>, U.S. Pharmacopoeia 26-National Formulary 21, United States Pharmacopeial Convention, Rockville MD, 2003.
7. U.S. FDA, Guidance for Industry: Analytical Procedures and Methods Validation: Chemistry, Manufacturing and Controls Documentation, August 2000.
8. Drugs Directorate Guidelines, Acceptable Methods, National Health and Welfare, Health Protection Branch, Canada, July 1994. (This guidance is available from HC as a print copy, but is soon to be released on the website http://www.hc-sc.gc.ca/hpfb-dgpsa/tpd-dpt/).
9. M.J. Green, Anal. Chem., Vol. 68, 1996. p. 305A.
10. G.A. Shabir, J. Chromatogra. A, Vol. 987, 2003. p. 57.

Validation of Analytical Methods Used in Cleaning Validation

BY HERBERT J. KAISER, PH.D. & BRUCE RITTS, M.S.

The information used to establish a positive cleaning validation is based on the result of validated analytical measurements. There must be a high degree of confidence in these results, as human safety depends on the lack of residues remaining on equipment. This article will describe various aspects regarding the validation of analytical methods used in cleaning validations. The validation elements are explored from both a theoretical point of view and through examples. References are provided to guide the reader to more in-depth information.

An analytical method is one of the deciding factors in establishing the cleanliness of pharmaceutical manufacturing equipment. It is, therefore, important that there be a high level of confidence in the results obtained using the method. This high level of confidence is established by testing and defining the usefulness of the analytical method. A properly developed cleaning validation strategy includes the analytical method validation, which defines the method parameters necessary in providing a high level of confidence in the cleaning results. The analytical method validation study demonstrates to scientific staffs, manufacturing personnel, and regulatory agencies, that the method performs as required, and that the results are reliable. There are many articles available that address analytical method validation within and outside of the pharmaceutical industry, both domestically and worldwide.[1,2,3,4,5,6,7,8]

Personnel other than analytical chemists may not understand the need for analytical method validation, let alone the extent to which these methods need to be evaluated. They may not understand that analytical method validation, as well as cleaning validation, has an important impact on everyday pharmaceutical manufacturing.[9] As is the case with cleaning validation, analytical methods may need to be revalidated.[10] This revalidation may arise from changes in instrumentation, analytes or manufacturing methods, or cleaning processes that affect the ability of the analytical method to determine the correct analyte level.

Measuring cleanliness is a difficult task. Essentially, trace residues on surfaces are the target analytes. The residue must first be extracted from a surface, recovered from the extraction medium, and then suitably quantitated. Residue analysis is quite different from analyzing bulk or formulated drug actives, as obtainable precisions and accuracies may be larger than the analyst is accustomed. Sensitivity levels of the techniques employed need to be considered for linearity, precision, and accuracy. The first decision to be made is the decision as to which residue will be measured. This residue could be the active drug, formulation excipients, or a component of the cleaner.[11] In most cases, the residue being analyzed has the potential to be a combination of all of these. The next step is to decide on the allowed residue limit,[12,13] followed by the choice of whether to use a specific or nonspecific technique. It is only after these decisions have been made, that the analytical technique can be selected.

Analytical method validation is the analysis of reproducibility of the method developed. There should not be any surprises in a validation study. All of the parameters required in a validation study need to be preliminarily evaluated during method development. Method development is the process by which the analytical chemist obtains the initial information to establish the limits and goals that are listed in a validation protocol, e.g., precision of 5% between analysts, linearity, accuracy, repeatability, etc. Understanding the required parameters during method development is a requirement for successful analytical method validation.

Regulations

The requirement for analytical method validation is identified in the Good Manufacturing Practice (GMP) regulations (21 CFR 211). The United States Pharmacopoeia (USP) provides a widely used standard for analytical method validation, and is probably the most often used reference regarding the subject.[14] The Food and Drug Administration (FDA) submitted guidelines for analytical method validation[15] in 1995 that correlate with the recommendations of the International Conference on Harmonization (ICH).[16] The ICH then issued a document describing different approaches that can be used in analytical method validation.[17] The FDA has also issued a guide for the validation of cleaning processes that state the need for validated analytical methods[18] in cleaning validations.

The ICH documents, along with the USP document, describe validation guidelines for methods used in different applications. The USP describes four different categories of methods. Category I methods involve the quantitation of major components of bulk drug substances or active ingredients in finished pharmaceutical products. Category II methods involve the determination of impurities in bulk drug substances, or degradation compounds in finished pharmaceutical products. These include quantitative and limit tests. Category III methods are used for the evaluation of performance characteristics, and Category IV methods are identification tests. The ICH guidelines define the same categories as the USP, except for USP Category III. *Table 1* lists the ICH categories and required parameters.

What parameters apply to analytical methods used in cleaning validations? The residues being determined are potential impurities. Therefore, the parameters that should be evaluated are most closely associated with Category II requirements (quantitative analysis of residues). It could be argued that the most important parameters are the limits of quantitation and detection, because these are the measures of sensitivity of the analytical method.

Chromatographic versus non-Chromatographic Methods

While various chromatographic methods, specifically High Performance Liquid Chromatography (HPLC), may be the more common methods used in analytical laboratories, there are certainly other applicable methods.[19,20] Some examples are Total Organic Carbon (TOC),[21] capillary electrophoresis[22], Atomic Absorption (AA), Inductively Coupled Plasma (ICP), titrations, ultraviolet spectroscopy, near infrared,[23] enzymatic,[24] etc. These "other" methods also require

Table 1

ICH method parameters.

Parameter	Identity	Impurities		Assay
		Quantitative	Limit	
Accuracy	-	+	-	+
Precision				
Repeatability	-	+	-	+
Intermediate	-	+	-	+
Specificity	+	+	+	+
LOD	-	-*	+	-
LOQ	-	+	-	-
Linearity	-	+	-	+
Range	-	+	-	+

* May be needed for some applications

validation, and while the USP and ICH parameters may appear more suitable for chromatographic methods, they are certainly applicable to non-chromatographic methods.[25] A good understanding of how to adapt and measure the required parameters using the specified analytical technique, is all that is required. In fact, various methods can be used to validate each other,[26] i.e., a mass spectroscopic technique could be used to determine the specificity of another technique.

Specificity

Specificity is the ability of a method to measure the analyte in the presence of components, which may be expected to be present. For cleaning validation methods, the potential presence of drug actives, formulation excipients, impurities, known degradation products, and cleaner components (if any)[27] should be anticipated. Experiments must be conducted that demonstrate the absence of interferences when the analyte is in a typical matrix.

Co-elution of components in chromatographic methods is typically the primary concern here. If HPLC with diode array capabilities is utilized, peak purity can be evaluated by examining the spectra across the peak. Most HPLC software programs will automatically calculate peak purity. Poor peak purity may be an indication of the presence of excipients, degradants, or cleaning components within the peak of interest.

Cleaning validation methods should be carefully evaluated for interferences. Studies should be conducted involving the analyte in the presence of the cleaning agent. If the cleaning agent is being quantitated, the effect of the drug active and formulation components should be evaluated. If the drug active or a formulation component is being analyzed, it must be shown that the cleaning process does not affect the analyte. This means that the effect of the cleaning process does not change the analyte in such a manner that it is no longer analyzable using the method being validated. One approach is to perform a recovery study of the analyte, by exposing it to the cleaning agent at use concentrations, time, and temperature. If suitable, recovery can be obtained, then the method can be used. If not, the method must be modified, or a new method developed.

Table 2

Data used for linearity evaluations

[Active X], ppm	Response	Standard Deviation	% RSD
0.10	613	72.5	11.80
0.40	900	100.0	11.00
0.70	998	33.3	3.33
1.1	1472	51.1	3.50
1.7	2398	53.0	2.21
2.5	3398	55.3	1.63
5.0	6800	100.0	1.50
10.0	13467	153.0	1.10
20.0	27133	208.0	0.77
30.0	40417	284.0	0.70
40.0	49500	500.0	1.00

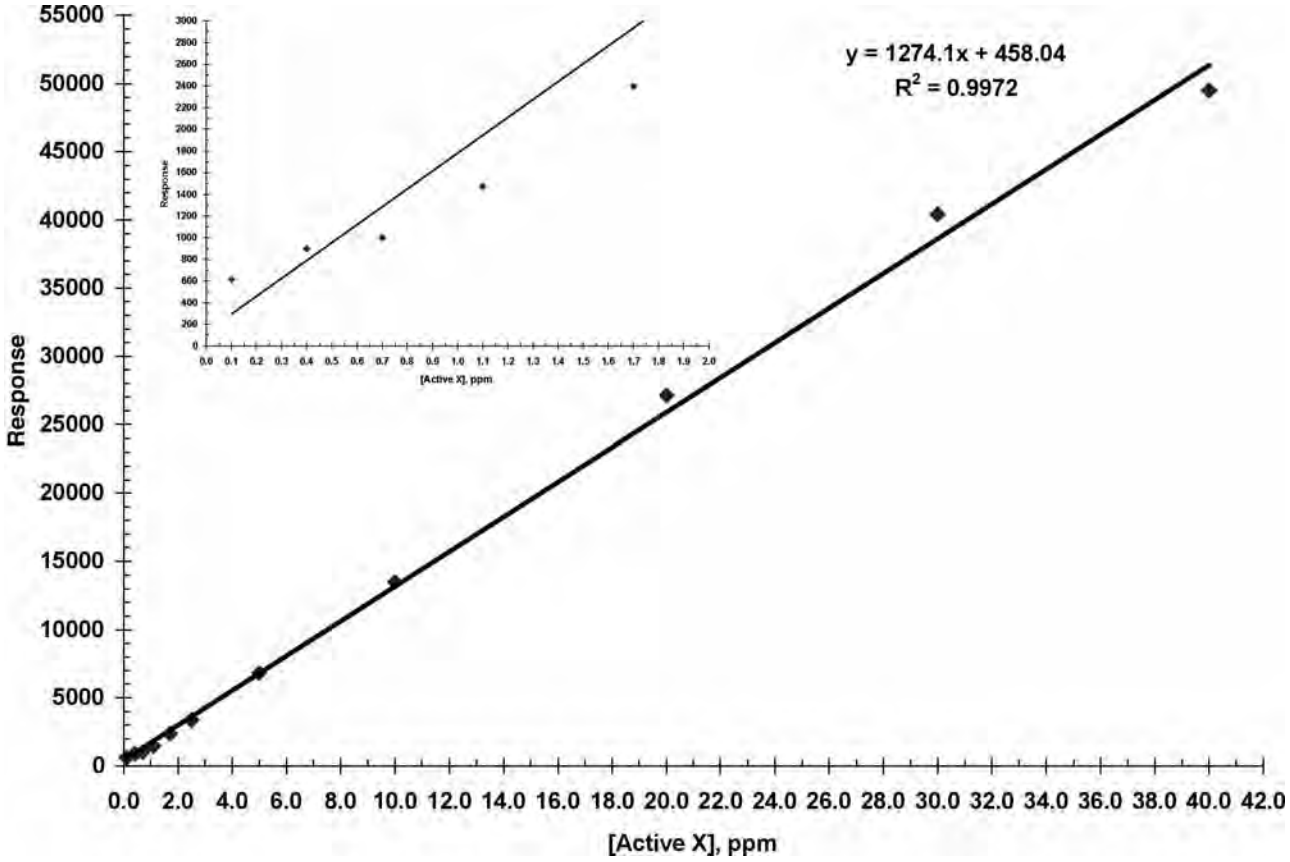
Linearity and Range

The linearity of a method is the ability of an assay to elicit a direct and proportional response to changes in analyte concentration. There are some detectors that produce, or have the ability to produce, non-linear responses (e.g., gas chromatography with flame photometric detectors and others, such as evaporative light scattering or mass spectrometers may have limited linear ranges when compared to flame ionization or UV-visible detectors). However, specific ranges could be found within the non-linear response that approach near linearity. If a non-linear curve must be used, a suitable number of points need to be utilized that will accurately describe the curve.

The range of a method is the interval between the upper and lower concentrations of analyte for which the method has been shown to have suitable precision, linearity, and accuracy. ICH recommends a range of 80 to 120% of the test concentration for finished drug products, 70 to 130% of the test concentration for content uniformity, and up to 120% of the reporting level for impurities, ensuring that the detection and quantitation limits are lower than the controlled level. For cleaning validation analysis, the range will potentially be much greater than what ICH recommends for drug actives and impurities. Generally, the range will extend from the limit of quantitation to perhaps 200% or greater for the amount of allowable residue in the sample. The wide range is important

Figure 1

Linear evaluation of all data points



in order to allow monitoring of the residue. If the low end of the range was equal to the allowable residue limit, process monitoring would not allow for early warning of potential problems. Again, the key requirement for the range is that it is suitable with regard to precision, linearity, and accuracy.

There are several methods described in industry literature to determine the linearity and the range of a method.[28, 29, 30] These methods range from simple observation to comprehensive statistical treatments. Statistical methods should be used to verify observed results. The method of choice will normally be dictated by a pharmaceutical company's policies. The specifics should be described in the validation, protocol if not in Standard Operating Procedures (SOPs).

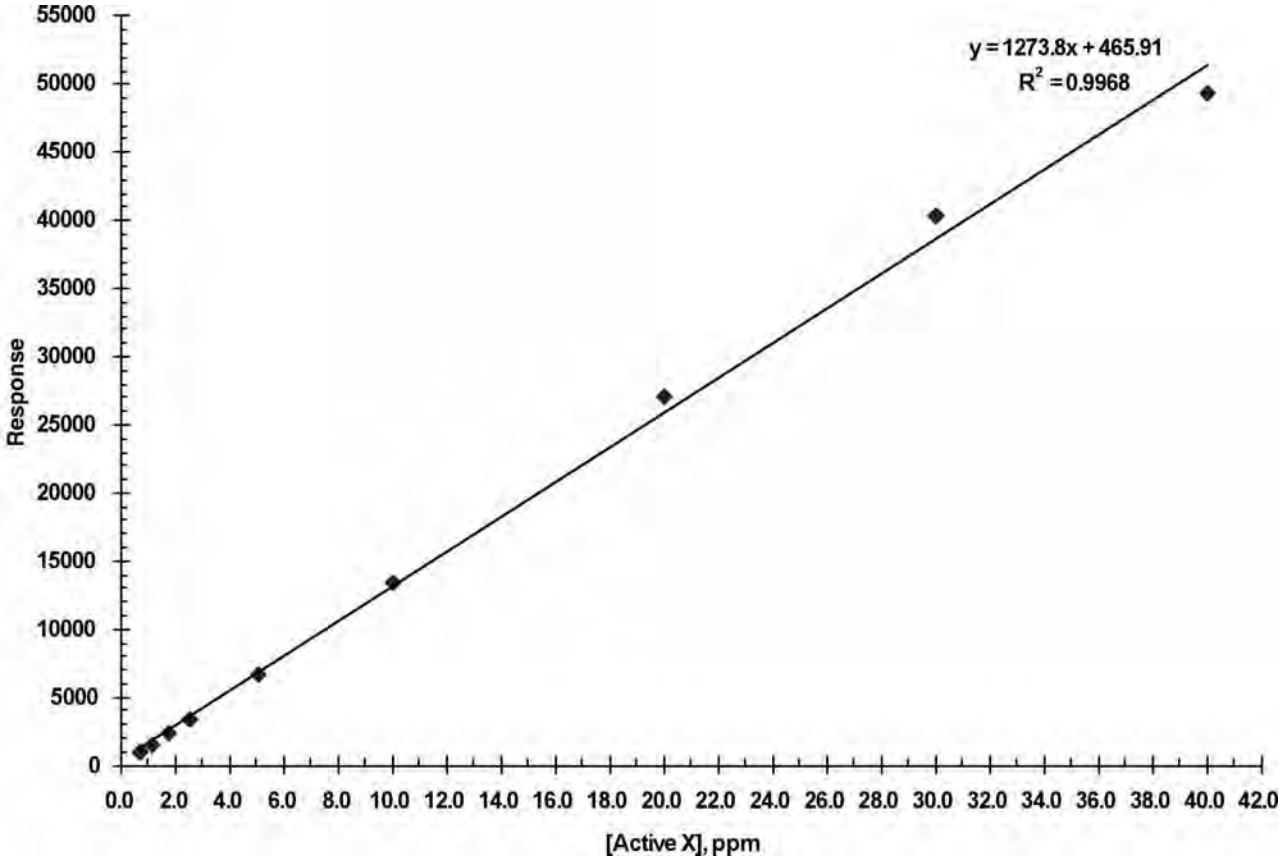
The linearity criteria are to be set prior to the validation. For example, for an HPLC analysis, the criteria for linearity may be that injections at each level must have an RSD < 3%, and the regression analysis yield an R^2 value of greater than 0.999 over the defined range. It should be noted that these requirements will vary greatly by method type and instrumentation in use. Acceptable %RSD values will also vary greatly, depending on the level of analyte. This will be covered in detail later.

The linearity experiment should include a minimum of five concentration levels. Each concentration level should be analyzed minimally in duplicate. Three analysis for each point are generally utilized, but more are preferred. The %RSD is then calculated for each injected level. This data is the base information set that will be utilized in establishing the linearity and range of the method.

Two simple methods for determining linearity will be described here. Both methods involve preparing a series of solutions containing known concentrations of the analyte of interest. This series encompasses the range of results expected from the analysis of actual samples. Usually, these solutions are free of expected matrix components. For the following examples, the pre-established (and somewhat arbitrary) criteria that was applied, is that all points must have a %RSD < 5 and $R^2 > 0.999$. These solutions would then be analyzed by

Figure 2

Linear evaluation less the first two data points



the method of choice. *Table 2, (see prior page)* presents the data that will be used for both examples. These data are only presented by way of example. A graph of the response versus the concentration shown earlier in *Figure 1*.

The first step would be to examine the %RSD values for each point. The very low concentrations of 0.10 and 0.40 ppm produce a %RSD of 12 and 11, respectively. Since these %RSDs are greater than the 5% RSD required, these data would be eliminated from the curve. The rest of the data meet the criteria. It should be noted that data cannot be arbitrarily eliminated. For example, if the 0.10 ppm data point produced an acceptable %RSD, but the 0.40 ppm point did not, the 0.40 ppm alone could not be eliminated. All data below 0.40 ppm would have to be eliminated. There may also be a need to investigate the discrepancy in RSD values.

Figure 2 shows the resulting graph after the lowest concentration levels have been eliminated. The R^2 value has decreased, due to the shortening of the range. Since the lower concentration levels have been eliminated, it is not surprising that the y-intercept has also increased. The y-intercept value is a good indication of bias. If the y-intercept is 0, no bias exists. Bias exists if the y-intercept deviates from 0. Statistical significance testing of the regression can provide evidence of bias. Examination of the y-intercept can be used to justify single point calibration criteria. If there is no statistical evidence of bias, or the bias is judged to be small, then single point calibration can be successfully used. In making this evaluation of the statistical evidence, it is the P-value and the upper and lower 95% confidence intervals for the y-intercept that are important to examine. These are typically values obtained from automated regression analysis as is shown in *Figure 3 (see next page)*. If the P-value is large and 0 is included in the confidence interval, there is no evidence that the y-intercept is anything other than 0.

Close examination of this calibration curve reveals that the highest concentration point (40 ppm) may be deviating from linearity. This concentration level is eliminated from the data set as a result of the fact that the inclusion of this data

Figure 3

Typical output from a regression

SUMMARY OUTPUT

Regression Statistics	
Multiple R	0.999930643
R Square	0.999861291
Adjusted R Square	0.999843952
Standard Error	339.9654599
Observations	10

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	6664892272	6664892272	57666.49335	1.01229E-16
Residual	8	924612.1115	115576.5139		
Total	9	6665816884			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	324.5939254	133.9655524	2.422965603	0.041656901	15.66860789	633.519243
X Variable 1	2684.578938	11.17929476	240.1384879	1.01229E-16	2658.799422	2710.358455

Figure 4

Linear evaluation less first two and the last data point. This produced quite acceptable results.