

Technical Report No. 42
Process Validation of
Protein Manufacturing

**PDA Journal of
Pharmaceutical
Science and
Technology**

September/October 2005

Supplement

Volume 59

No. S-4



PDA Technical Report No. 42

Process Validation of Protein Manufacturing

Process Validation of Protein Manufacturing Task Force Members/Authors

Chris Bussineau, Ph.D., Chiron Corporation, Co-Chair
Robert Seely, Ph.D., Amgen, Inc., Co-Chair
Genevieve Lovitt, G.I. Lovitt & Associates, Project Manager/Technical Editor
James Fernandez, Fernandez and Associates, PDA Liaison
Greg Blank, Ph.D., Genentech, Inc.
E.J. Brandreth, Favril, Inc.
Chris Bussineau, Ph.D., Chiron Corporation
Doris Conrad, Consultant, GlaxoSmithKline Biologicals
Rebecca A. Devine, Ph.D., Independent Regulatory Consultant
Robert J. Ferris, Ph.D., Chiron Corporation
Koen de Heyder, GlaxoSmithKline Biologicals
Robert W. Juffras, M.S., Wyeth BioPharma
Peter Levy, Millennium Pharmaceuticals, Inc.
Wolfgang List, Ph.D., Aventis Behring
Morten Munk, CMC Biopharmaceuticals A/S
Brian C. Neely, Don Hill and Associates, Inc.
Stephen Notarnicola, Ph.D., Biogen Idec
Rhona O'Leary, Ph.D., Genentech, Inc.
Harold van Deinse, Baxter Healthcare Corporation

Process Validation of Protein Manufacturing

Technical Report No. 42
Supplement
Vol. 59, No. S-4
September/October 2005

© 2005 by PDA



PDA TECHNICAL REPORT NO. 42
PROCESS VALIDATION OF PROTEIN MANUFACTURING

Table of Contents

1. INTRODUCTION	1	6.3 Validation Reports	22
1.1 Purpose	1	6.4 Compliance Program	22
1.2 Scope	1	7. APPENDICES	22
2. DEFINITIONS	2	7.1 Idealized Process Validation Workflow	22
3. PROCESS VALIDATION PREREQUISITES ...	4	7.2 Process Description Example	22
3.1 Introduction	4	7.2.1 Cell Culture Process Description	23
3.2 Planning	5	7.2.2 Downstream Purification Process Flow ..	24
3.3 Process Development	5		
3.3.1 Sequence of Activities Leading to Formal Process Validation	5		
3.3.2 Raw Materials	7		
3.3.3 Process Description	7		
3.3.4 Analytical Methods	7		
3.3.5 Risk Assessment	8		
3.3.6 Process Characterization	8		
3.3.7 Cell Line and Expression Construct Char- acterization	10		
3.3.8 Extractables from Product-contact Surfaces	11		
3.3.9 Development Documentation	11		
3.4 Equipment and Facilities	11		
3.5 Training	12		
4. PROCESS VALIDATION			
4.1 Introduction	12		
4.2 Process Validation Options	12		
4.3 Validation Approaches	12		
4.3.1 Unit Operation Validation	12		
4.3.2 Family and Matrix Validation	13		
4.4 Scale of Studies	13		
4.5 Process Validation Studies	14		
4.5.1 Viral Clearance	14		
4.5.2 Impurity Clearance	15		
4.5.3 Process Consistency	16		
4.5.4 Process Intermediate Stability	16		
4.5.5 Process Solution Stability	17		
4.5.6 Drug Substance Fill/Freeze/Thaw/ Storage	17		
4.5.7 Mixing Studies	18		
4.5.8 Chromatography Resin and Reusable Membrane Lifetime Validation	18		
5. POST-VALIDATION ACTIVITIES	19		
5.1 Change Control	19		
5.2 Ongoing Process Monitoring	20		
5.3 Process Re-validation	20		
6. DOCUMENTATION	20		
6.1 Introduction	20		
6.2 Validation Protocols	21		

1 Introduction

Significant advancement in the design and implementation of effective validation programs has been made since the 1987 Food and Drug Administration (FDA) Guideline on General Principles of Process Validation. (1) Open, candid discussions at conferences, many of which were dedicated specifically to process validation, and at other open forums have resulted in common practices.

The guidelines presented here are intended to assist in the design and execution of process validation studies to ensure the reproducibility and robustness of the process. Points to consider are provided to facilitate collection of data to support a regulatory filing for the approval of a drug substance intended to be used in a pharmaceutical product. The information provided in these guidelines is one technical approach to process validation activities and documentation based on the experiences and practices of a PDA Task Force that represents a cross-section of industry professionals.

The following guidelines represent what the authors believe to be best practices that are science-based and value-added and are believed to make good business sense as well as meet current regulatory expectations. The document does not contain isolated responses to individual inspections or reviews. Such responses are often driven by case-by-case requirements particular to specific organizational needs.

1.1 Purpose

This technical report provides practical guidance for compliance with current Good Manufacturing Practices (cGMP) and International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) guidances for the validation of biopharmaceutical processes to the drug substance stage.

1.2 Scope

The focus of this technical report is validation of biopharmaceutical processes used to manufacture drug substances.

proteins and polypeptides. These proteins and polypeptides are produced from recombinant or non-recombinant cell-culture expression systems and can be characterized using an appropriate set of analytical procedures. Selected principles outlined in this document may also apply to other product types, such as proteins and polypeptides isolated from tissues and body fluids. The intent is to provide clear technical guidance for the development and design of a process validation plan and provide a comprehensive overview of strategies that may be used to validate a manufacturing process or unit operation.

The strategies contained in this document primarily focus on the validation of non-sterile, low bioburden, protein-based drug substances. The guidelines do not cover validation as it relates to reprocessing, reworking, aseptic processing of drug products, Process Analytical Technologies (PAT), facilities, design qualification, stability, cleaning, or shipping. These topics are either covered in depth elsewhere or are in the early stages of definition.

The guidelines are not intended to establish mandatory standards for process validation; they are intended to be a single-source overview that complements existing guidance documents listed in the reference section. For greater detail on various topics throughout this technical report, references to published documents are provided. It is always advisable to consult with the appropriate regulatory authority for agreement on the strategies employed for process validation studies.

2 Definitions

Current FDA, ICH, and other regulatory definitions are used except when more clarity is added by the Task Force. In some instances two definitions used in current guidances are provided where both are considered applicable. Regulatory guidelines offer other definitions that may be considered.

Variations in the usage of some terms may differ from company to company, and some may be subject to change in the future. However, the terms used in a validation program must be clearly defined and well understood within the company and clearly defined in internal Standard Operating Procedures (SOPs), standards, and in regulatory filings. For the purposes of this technical report, the following definitions are used.

Action Limit: An internal (in-house) value used to assess the consistency of the process. The cause of the excursion should be investigated and documented and corrective action is generally required. Action limits are not specifications. (Synonym: action level)

Acceptance Criteria: Numerical limits, ranges, or other suitable measures for acceptance of test results. (2) Exceeding the acceptable range for a critical parameter during subsequent validation studies may result in questionable product quality that would require initiation of an investigation. Exceeding the operating range should be documented and explained in the validation report and evaluated for validation study impact.

Active Pharmaceutical Ingredient (API): See Drug Substance.

Characterization Study: A late-stage study that evaluates the process to increase process knowledge and examines proposed operational ranges and their individual and/or combined impact on target protein quality. (Synonyms: evaluation studies, process justification studies, engineering studies, development studies, robustness studies)

Conformance Lots: A pre-determined number of production lots, typically three, that represent the licensed process and are evaluated to demonstrate consistency. (Synonyms: validation, consistency, demonstration, or qualification lots).

Contaminant: Any adventitiously or externally introduced material(s) (e.g., chemical, biochemical, or microbial species) not intended to be part of the process. (3)

An undesired impurity of a chemical or microbiological nature that is introduced into a raw material, intermediate, or API [drug substance] during manufacture. (4)

Documentation:

Development Reports: Documentation and description of work done during the early phases of development. The goal is to document information about the way the process works and to document why key choices were made in selecting the specifics of the process (e.g., flow rate or temperature). These documents can serve as a reference during investigations of discrepancies and during the design of specific validation and characterization studies.

Process Characterization Report: A report that includes results from a study characterizing the performance of a unit operation and/or operations conducted in a process characterization study. The report describes process characteristics, the operational parameters (e.g., critical, key, and non-key) and their acceptable ranges (limits), and acceptance criteria for validation protocols.

Process Validation Master Plan: A document that defines the process validation scope and activities and

contains the list of process validation studies to be performed.

Process Validation Protocol: A written plan pre-approved by the quality unit that specifies critical steps, controls, and measurements. The Process Validation Protocol states how validation will be conducted, identifying sampling, assays, specific acceptance criteria, production equipment, and operating ranges. Results obtained for each study described in the protocol should be evaluated in an associated process validation report.

Process Validation Report: A report approved by the quality unit that summarizes specific tests performed, compares the test results with the protocol acceptance criteria, and addresses deviations encountered during the study.

Drug Product: A pharmaceutical product that contains a drug substance, generally in association with excipients. (5)

The dosage form in its final immediate packaging intended for marketing. (6) (Synonyms: dosage form; finished product, medicinal product)

Drug Substance: The active ingredient that is subsequently formulated with excipients to produce the drug product. It can be composed of the desired product, product-related substances, and product- and process-related impurities. It may also contain excipients, including other components such as buffers. (7) (Synonyms: bulk drug substance, bulk material, active pharmaceutical ingredient (API))

Impurity: Any component present in the drug substance or drug product that is not the desired product, a product-related substance, or excipient. Impurities are to be distinguished from contaminants, which are defined above.

In-process Control: Checks performed during production to monitor and, if appropriate, to adjust the process and/or to ensure that the intermediate or API [drug substance] conforms to its specifications and/or other defined quality criteria (e.g. limits for bioburden and endotoxin). (8) (Synonym: process control)

Information-only Tests: Tests that provide data that are collected without pre-established acceptance criteria to further evaluate the process.

Installation Qualification (IQ): Documented verification that the equipment or systems, as installed or modified, comply with the approved design, the manufacturer's recommendations, and/or user requirements. (9)

Intermediate: A material produced during steps of the processing of a drug substance that undergoes further molecular change or purification before it becomes a drug substance. (10)

Operational Qualification (OQ): Documented verification that the equipment or systems, as installed or modified, perform as intended throughout the anticipated operating ranges. (11)

Parameters:

Operational Parameter: An input variable or condition of the manufacturing process that can be directly controlled in the process. Typically, these parameters are physical or chemical (e.g., temperature, process time, column flow rate, column wash volume, reagent concentration, or buffer pH). (Synonym: process parameter)

Critical Operational Parameter: An input process parameter that should be controlled within a meaningful, narrow operating range to ensure that drug substance quality attributes meet their specifications. Although, parameters with wide operating ranges may also impact product quality, they are generally easily controlled and not as likely to result in excursions that impact quality and are therefore low risk. [For further discussion see Sub-section 3.3.6] (Synonym: critical process parameter (CPP))

Non-critical Operational Parameter: All input process parameters that fall outside the definition for critical operational parameter are non-critical. Non-critical operational parameters are divided into key and non-key operational parameters. [For further explanation, see Sub-section 3.3.6]

Key Operational Parameter: An input process parameter that should be carefully controlled within a narrow range and is essential for process performance. A key operational parameter does not affect critical product quality attributes. If the acceptable range is exceeded it may affect the process (e.g., yield, duration) but not product quality. [For further explanation, see Sub-section 3.3.6]

Non-key Operational Parameter: An input process parameter that has been demonstrated to be easily controlled or has a wide acceptable limit. Non-key operational parameters may have an impact on drug substance quality or process performance if acceptable limits are exceeded. [For further explanation, see Sub-section 3.3.6]

Performance Parameter: An output variable or outcome that cannot be directly controlled but can be measured.

that the process performed as expected. (Synonym: performance attribute)

Performance Qualification (PQ): Documented verification that the equipment and ancillary systems, as connected together, can perform effectively and reproducibly based on the approved process method and specifications. (12)

Process Validation: The documented evidence that the process, operated within established parameters, can perform effectively and reproducibly to produce an intermediate or API [drug substance] meeting its predetermined specifications and quality attributes. (13)

Concurrent Process Validation: Validation that occurs during manufacturing of drug substance for batches that can be released and used in a final drug product for commercial distribution based on thorough monitoring and heightened testing of the drug substance batches. (14)

Prospective Process Validation: Validation conducted prior to the distribution of either a new product or a product made under a revised manufacturing process where the revisions may affect the product's characteristics. (15)

Retrospective Process Validation: Validation of an existing manufacturing process that occurs by reviewing data from relevant historical and test production records.

Re-validation: Repeating partial or full validation of a process after a process change is implemented. Re-validation is change-based, not time-based.

Qualified Assay: An assay that is not fully validated but is documented to be suitable for its intended use, including sample collection and handling procedures. Such an assay should be demonstrated to be accurate, precise, linear within the range of use, and show no interference from process stream components (i.e., spike recovery). (16)

Specification: A list of tests, references to analytical procedures, and appropriate acceptance criteria that are numerical limits, ranges, or other criteria for the test described. It establishes the set of criteria to which a material should conform to be considered acceptable for its intended use. (17)

Set Point: The target for an operational parameter. The range around the set point is commonly stated in the manufacturing procedures or batch records.

Unit Operation: A discrete step or manipulation in a manufacturing process where process and operating pa-

rameters are defined to achieve a specific process objective. (Synonym: process step)

Validation: A documented program that provides a high degree of assurance that a specific process, method, or system will consistently produce a result meeting predetermined acceptance criteria. (18)

3 Process Validation Prerequisites

3.1 Introduction

There are several aspects of cGMP and development that should be completed prior to validation of the manufacturing process. Process validation requires that validated assays, calibrated instruments, and qualified production support systems are already established with documentation completed prior to execution of process validation studies. Furthermore, the process should be fully developed, characterized and documented in a development report. (19) These and other specific supporting activities are briefly described in the following sub-sections. (20)

Fundamental to process validation is process knowledge and full understanding of the equipment and technology to be used in the process. The process must show consistency and be well defined, controlled, and reproducible. Operational (input) parameters and performance (output) parameters should be identified, with appropriate operating limits or acceptance criteria defined for both parameters. Process characterization studies performed during process development and scale-up establish this process knowledge database. Final written reports should include discussion, justification or rationale, details of the study, resulting data, recommendations, and cautions. These reports should be internally approved and controlled. (21) Regulators may review development documentation containing the justification for operational parameter ranges. For full-scale conformance lots, process validation usually focuses on process performance at the set-point of each operating parameter range. Justification of the operating range for each operation may not be demonstrated during full-scale process validation; therefore, the ranges should be justified during the development phase using appropriate process characterization studies, which for practical reasons are usually done at lab-scale.

Process validation studies that are performed at lab-scale should be done with pre-approved protocols that contain predetermined acceptance criteria and final validation reports. For example, spiking studies may demonstrate impurity removal that requires a scale-down model study because they cannot be conducted at full, commercial scale.

scaled-down model used in process characterization or validation should be justified as being representative of the full-scale unit operation; this may include demonstrating that the resultant intermediate or drug substance is comparable. Some differences in performance are unavoidable; these are acceptable as long as they are understood and considered when interpreting the results. (22)

Central to defining the process is a documented change control program, which should be in place throughout clinical product development. (23) Change control ensures that proposed changes are justified, examined, and documented so that the process is not altered without a thorough review and proper documentation. (24) Information about changes during development may affect the product application review process, as some clinical trials may have been conducted with material made prior to a change that took place during development. Change control should also be in place for equipment.

3.2 Planning

A successful validation program is one that is initiated early in the product life cycle and is completed only when the process or product reaches the end of its life cycle. The foundation of a successful validation program begins with implementation of a comprehensive corporate policy that defines the organization's expectations and commitment to process validation principles. This policy should define the corporation's quality management philosophy, components of validation, periodic review or re-qualification timeframes, documentation requirements (including a validation master plan), (25) validation protocols and reports, and responsibilities of key stakeholders within the organization.

Successful process validation begins early in the development phase of a product with acquisition of preliminary process data and knowledge. Although formal process validation is not generally required in Phase 1, some exceptions are made for product safety issues (e.g., viral clearance validation for mammalian cell products). After Phase 1 and the decision to continue product development, efforts to develop a commercial process must ensure that the process meets all validation requirements. During the commercial development phase, more detailed process knowledge should be collected that describes critical and key operational parameters; normal, maximum, and minimum operating ranges; performance parameters with acceptance criteria where appropriate; and product impurity profiles as a function of each individual unit operation and the process as a whole. This information, together with the history of the process,

should be captured in a series of development reports and/or process characterization reports. These documents will serve as the repository of key development information; they are critical to assembling subsequent process validation plans and protocols, as well as providing ancillary process support during pre-approval inspections.

A multi-function process validation team should be assembled prior to manufacture of conformance lots. The team should minimally consist of members from validation, process development, quality assurance, regulatory affairs, manufacturing, and technical operations and/or engineering departments. The team's first assignment is to design and assemble the validation master plan. This high-level document is a blueprint for process validation activities and describes the required content of the individual process validation protocols (e.g., the need for test requirements and acceptance criteria and the responsibilities of various groups for protocol implementation). The validation master plan also includes requirements for implementation of the individual process validation protocols, including starting material requirements, execution of consecutive conformance lots, status of analytical methods (validated versus qualified), and procedures for handling deviations and revisions. Criteria for determining acceptance or failure of the validation program should also be defined.

Following assembly of the validation master plan, process validation protocols should be written describing in detail the procedures to be followed to produce documented evidence that the process has been validated. The protocols must specify critical and key operational parameters and their respective ranges, acceptance criteria, procedures required to perform the validation (including the sampling plan), and responsibilities of various functional team members participating in the validation.

3.3 Process Development

Process validation is an evolving process, where process understanding is gradually built up during the development phase and continues throughout the commercial phase of the product's life-cycle. **Appendix 7.1** represents an idealized process validation workflow and illustrates the essential elements and activities leading up to formal process validation, as well as validation and post-validation activities.

3.3.1 Sequence of Activities Leading Up to Formal Process Validation

Process validation is the culmination of a long series of process development activities. While the validation process

process development may not be to develop a fully validated process, many of the activities integral to developing a robust, reliable process necessitate an awareness that process validation may be an end goal. Some of the activities that occur during development are listed below. Many of these may remain constant throughout development; however, others will change throughout the various stages of scale up and process optimization. With each incremental change, an overall awareness of the ability to validate the process is of primary importance.

These activities include:

- Molecule selection
- Host cell system/cell line selection
- Cell culture/fermentation process development
- Harvest process development

- Purification process development
- Identification and development of all filtration systems
- Formulation development and product presentation
- Analytical method development
- Pharmacokinetic/toxicology studies
- Process scale up
- Process characterization

Those activities that have a primary impact on process validation are discussed below.

The following figure, **Figure 3.3.1-1: Common Sequence of Process Validation Activities**, depicts typical

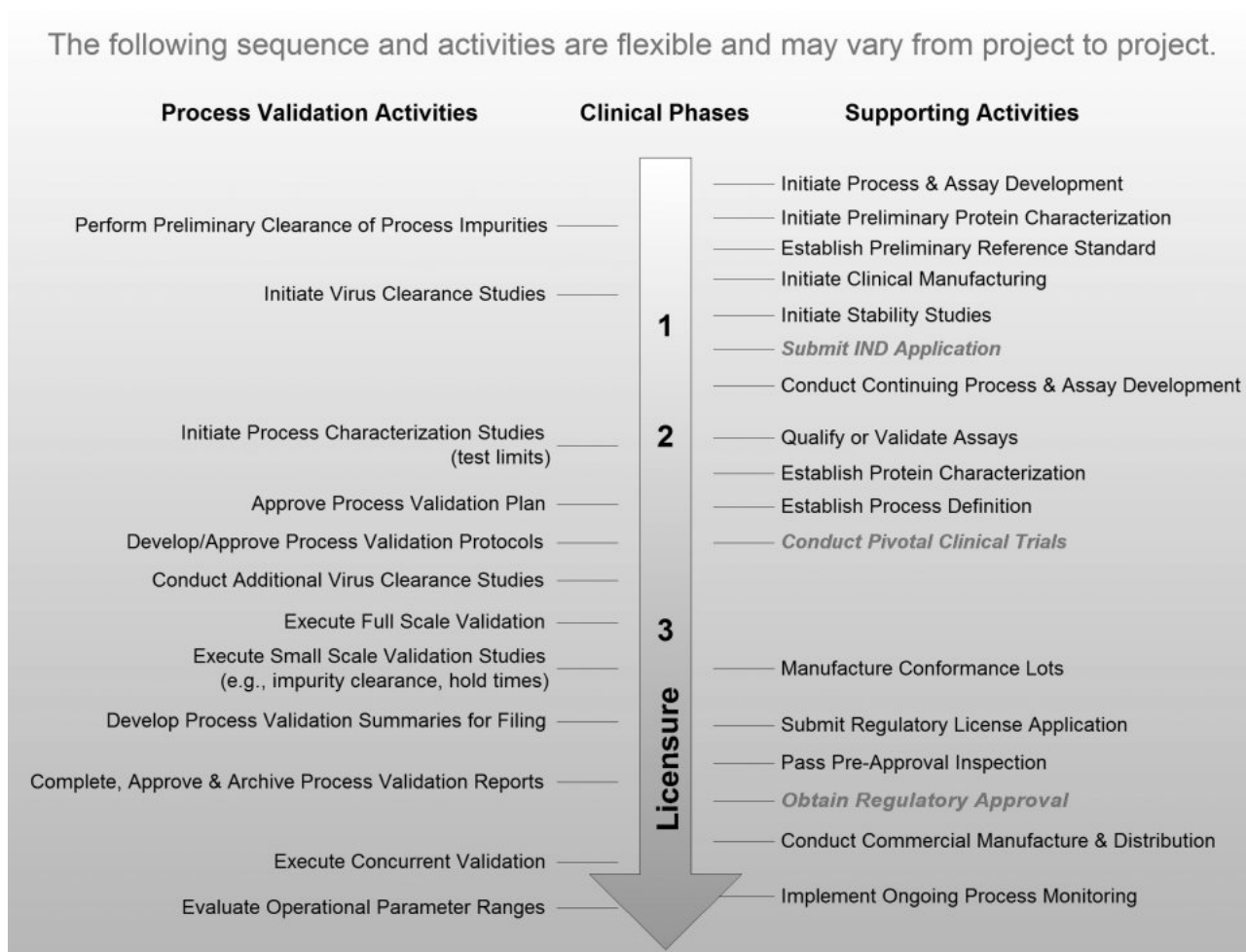


Figure 3.3.1-1
Common Sequence of Process Validation Activities

process validation activities and their relationship to other development activities.

3.3.2 Raw Materials

The beginning of a material qualification program should be in place for raw materials that will be used for the initial manufacturing. At a minimum, vendors should be selected and specifications should be established, especially for non-compendial-grade materials. As the manufacturing evolves through the clinical trials, a more complete program should be defined, including systems to qualify new materials or alternate vendors, defined responses to material discrepancies, material stability, vendor audit requirements, and supplier sustainability. (26) A raw materials control program should be well established prior to proceeding with process validation.

3.3.3 Process Description

The process description is part of a master document such as the validation master plan and describes the manufacturing process. It details each process step and specifies the process operational and performance parameters associated with each step. The purpose of each step (e.g., inducing expression, DNA reduction, or buffer exchange) should be clearly stated so performance of that step can be meaningfully evaluated. Flowcharts and summary tables are helpful tools in this description to supplement the text. Summary table examples are provided in **Appendix 7.2: Process Description Example**.

3.3.4 Analytical Methods

Analyses of product and process stream characteristics are an integral part of the process validation exercise. Demonstrating an effective and reproducible process relies on the availability of appropriate assays. The level of assay qualification and validation increases as the product proceeds through the clinical trials.

Process validation studies are designed to explore process characteristics in more depth and detail than the studies applied during routine drug substance manufacture. Assays, additional to those used for normal in-process monitoring and lot release testing, are often used during the execution of process validation studies. Assays should be chosen to demonstrate that each process step (unit operation) is performing as designed.

In-process and lot release analytical methods that are used during routine manufacturing of commercial product should be validated prior to initiation of process validation studies. Methods may need to be qualified for the sample matrix being tested, as process validation usually entails non-routine sample matrices. These assays will always be performed in a cGMP laboratory according to approved SOPs. Some examples of these assays are provided in **Table 3.3.4-1** (Note: this table is not meant to be all-inclusive and does not cover product-specific assays). Detailed guidance for the validation of analytical methods is contained in the ICH Harmonized Tripartite Guideline “Validation of Analytical Procedures: Methodology”. (27)

TABLE 3.3.4-1
Examples of In-process Test and Lot Release Assays Typically Requiring Validation

Assay	Application
SDS PAGE*	Identity, purity
Iso-electric focusing	Identity
Carbohydrate analysis	Identity
Peptide mapping	Identity
Protein concentration (A280 absorbance, HPLC**)	Quantity (drug substance, drug product, and in-process streams)
Biological activity assay (or potency assay)	Activity
Size exclusion chromatography	Purity (aggregated species)
Endotoxin	Safety
Bioburden or sterility	Safety
Mycoplasma	Safety
<i>In vitro</i> adventitious virus testing	Safety

* SDS PAGE: sodium dodecyl sulfate polyacrylamide gel electrophoresis

** HPLC: high performance liquid chromatography