Technical Report No. 14 Revised 2008 Validation of Column-Based Chromatography Processes for the Purification of Proteins

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Validation of Column-Based Chromatography Processes for the Purification of Proteins Task Force

E.J. Brandreth, formerly with Favrille, Inc.

Chris Bussineau, PhD, BioVascular, Inc.

Michelle Butler, Genentech, Inc.

Tony Christensen, Scantago ApS

Doris Conrad, Conrad Associates

Becky Devine, Independent Regulatory Consultant

Ranga Godavarti, PhD, Wyeth BioPharma

Eric Grund, PhD, GE Healthcare

Norbert Hentschel, Boehringer Ingelheim Pharma GmbH & Co KG

Philip Lester, Genentech, Inc.

Jennifer McNay, Regeneron Pharmaceuticals Inc.

Michael Muegge, Bayer Healthcare Corporation

Stephen Notarnicola, PhD, Biogen Idec, Inc.

Rahul Patel, Immunomedics, Inc.

Anurag Rathore, PhD, Amgen, Inc.

Laurie Scaggs, formerly with Abbott Laboratories

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Table of Contents

1.0 Introduction

Since publication of the original Technical Report No. 14 in 1992, the biotech industry has grown rapidly. Technological development has resulted in improved pharmaceutical processing equipment and advances in separation science. There has also been an increase in regulatory scrutiny with respect to validation of protein purification processes warranting an update on this important topic.

Chromatographic purification steps are used in most protein purification processes. The practices developed for validation of chromatography processes today are the result of better understanding of the design requirements for biopharmaceutical manufacturing processes and the experience gained from developing novel products requiring new applications. This technical report revision reflects current practice in column-based chromatography validation and follows a lifecycle validation approach using risk-based tools. Chromatography validation should provide a high degree of confidence that the process performs consistently, removing process and product-related impurities and viruses when executed as designed.

Chromatography validation prerequisites are addressed first in this report and cover development activities that includes process characterization, analytical methods selection, and a comprehensive risk assessment program. Because small-scale studies can occur before, during, and after production-scale conformance batch studies, the task force chose to first address conformance batch validation, followed by a discussion of small-scale studies. Supporting activities (e.g., raw material selection, equipment qualification, column packing, and testing) to chromatography validation and areas necessitating greater detail (e.g., types of chromatography and experimental design) are addressed in the appendices.

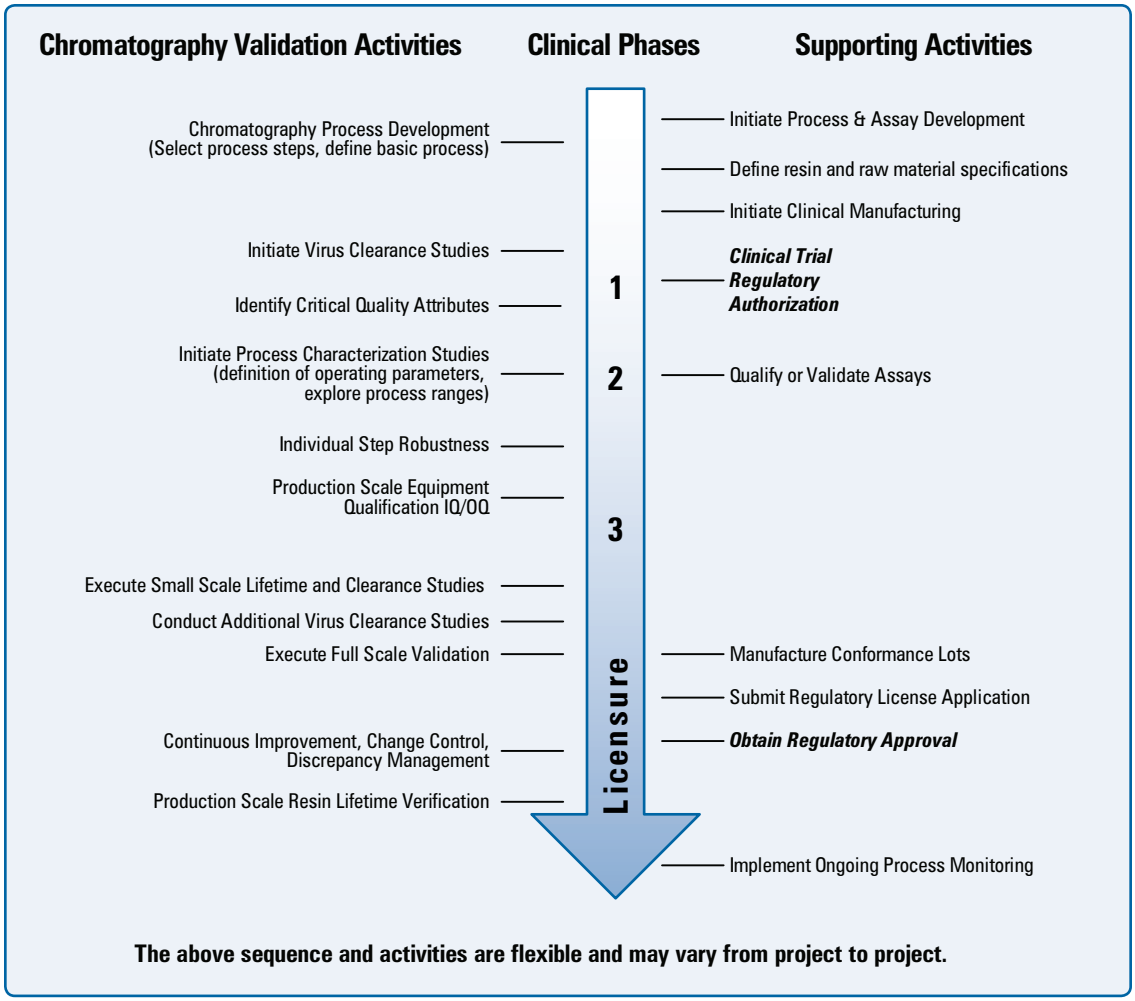
1.1 Purpose/Scope

The purpose of this technical report is to provide a current industry perspective and scientific guidance on the validation of column-based separation processes for the purification of biopharmaceutical proteins.

The structure of Technical Report No. 14 follows the lifecycle of the validation process. As illustrated in **Figure 1.1-1**, the chromatography process lifecycle begins in early development and impurity clearance studies with scale-down models, and culminates in manufacturing-scale process validation and post-validation monitoring. The intent of this report is to provide guidelines with supporting rationale on developing a chromatography process, rather than a step-by-step guide on how to perform validation. Chromatography processes commonly used in the industry are the focus of this report. Other unique processes like expanded bed adsorption (EBA), simulated moving bed (SMB), and membrane chromatography are not addressed.

In development and characterization studies, process operating parameters are investigated and categorized identifying critical, key, and non-key parameters. Product and process knowledge achieved during development are the foundation for the full-scale validation of chromatography processes which typically occur during the production of conformance batches. After validation, process monitoring and change control programs should be in place to maintain the validated state. Depending on the type and extent of changes, revalidation may be required.

Figure 1.1-1 Idealized Chromatography Validation Sequence of Activities



The following topics are discussed at a high level and are not given a comprehensive review: process analytical technologies (PAT), quality by design (QbD), computer hardware and software, facilities, equipment, cleaning, and sanitization. Although the task force recognizes the importance of these topics, the scope of this technical report will remain focused on current standard practices specific to chromatography validation.

This technical report is intended to complement PDA Technical Report No. 42, "Process Validation of Protein Manufacturing" *(1)* and to provide more detailed guidance on validation of the chromatography aspect of the protein purification process. It is not intended to establish any mandatory or implied standards.

2.0 Glossary of Terms

Current US Food and Drug Administration (FDA), International Conference on Harmonisation (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.

Acceptance Criteria
Numerical limits, ranges, or other suitable measures for acceptance of test results. ***(2)***

Active Pharmaceutical Ingredient (API)
See Drug Substance.

Characterization Study
A series of tests designed to increase process knowledge by examining proposed operational ranges and their individual and/or combined impact on the chromatography process. [Synonyms: evaluation studies, process justification studies, engineering studies, development studies, robustness studies]

Chromatogram
Data recorded during performance of a chromatography unit operation typically includes UV absorption (280 nm), pH, and conductivity, as well as other data (e.g., flow rates or pressure). [Synonym: chromatography profile]

Chromatography
The passage of a solute (mobile phase) through resin (stationary phase) to achieve purification of substances based on the chemical, physical, and biological properties of the molecules involved.

Chromatography Resin
Material used to interact with the process stream in order to purify the target protein. Chromatography resin usually consists of porous particles within a defined particle size range that are insoluble in the process stream (e.g., ceramic beads, agarose). [Synonyms: chromatographic medium, media, stationary phase]

Column Load
The solute that is passed through the column for separation. [Synonyms: feed, feedstock]

Column Packing
Preparation of a column that includes the addition of resin slurry into a column to create a bed suitable for its intended use. Characteristics of a packed column bed include bed height and diameter, backpressure, and number of theoretical plates.

Conformance Batches
A pre-determined number of production lots, typically three, that represent the process and are evaluated to demonstrate consistency. [Synonyms: validation, consistency, demonstration lots, 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. ***(2)***

Critical Quality Attribute (CQA)
A physical, chemical, biological, or microbiological property or characteristic that should be within an appropriate limit, range, or distribution to ensure the desired product quality.

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 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. *(3)*

The dosage form in its final immediate packaging intended for marketing. *(2,4)* [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 buffers and other components. *(3)* [Synonyms: bulk drug substance, bulk material, active pharmaceutical ingredient (API)]

Effluent
The liquid flowing out of a column.

Eluate
Solution (effluent) that flows out of the chromatographic column containing the drug substance.[Synonym:collectedproductfractions]

Elution
Desorption of a drug substance from a chromatographic column.

Equilibration
Column washing with a solution or buffer(s) in preparation for the column load.

Extractable
A chemical component that is removed from a material by application of an artificial or exaggerated force (e.g., solvent, temperature, time). The term extractable is often erroneously used to describe a leachable.

Flow-through
Effluent that may contain the product that is not retained by chromatography resin during column loading.

Frit
A porous sieve or screen installed at the top and bottom of a column used to retain chromatography resin particles and allow passage of the process stream. [Synonym: sinter]

HETP (Height Equivalent to the Theoretical Plate)
A measurement of column packing efficiency or integrity, calculated from the column height divided by the number of theoretical plates. *(5)*

Impurity
Any component present in the drug substance or drug product that is not the desired product, a product-related variant, 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). *(2)* [Synonym: process control]

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. *(2)*

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. *(2)*

Leachable
A chemical component that migrates from a contact surface into a drug product or process fluid during storage or normal use conditions. The term *leachable* is often erroneously used to describe an extractable.

Ligand
A functional molecule (small molecule or protein) coupled to the chromatography resin that selectively interacts with the target protein, impurities, or other molecules from the process stream.

Load Density
The amount of target molecules per volume of resin (e.g., gram protein per milliliter resin).

Operational Qualification (OQ)
Documented verification that the equipment or systems, as installed or modified, perform as intended throughout the anticipated operating ranges. *(2)*

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 API 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 affect quality and are therefore low risk. [Synonym: critical process parameter (CPP)] *(1)*

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.

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.

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.

Performance Parameter
An output variable or outcome that cannot be directly controlled but is an indicator that the process performed as expected. [Synonym: performance attribute]

Peak Asymmetry
A mathematical measure in a chromatogram of the HETP peak shape that is determined by measuring the front and back halves of a peak and is reflective of column efficiency. The ideal chromatogram contains a peak of perfect symmetry (see **Section 7.1** for further discussion).

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. *(2)*

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. *(2)*

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

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

Regeneration
Operation performed to remove residual proteins, impurities, or contaminants from the resin. [Synonym: strip]

Sanitization
A significant reduction in bioburden, achieved in chromatography by the use of bactericidal agents, such as sodium hydroxide (NaOH), hydrochloric acid (HCl), ethanol (EtOH), and isopropanol (IPA).

Scale-Down Model
A small-scale process step that has been demonstrated to be representative of a production-scale operation.

Set-Point
The value to which an operational parameter is set. The process should operate in a range around a given a set-point that is stated in the manufacturing procedures or batch records.

Slurry (noun)
A homogenous distribution of resin particles in a liquid.

Slurry (verb)
To mix resin particles in a liquid to achieve a homogenous liquid suspension.

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. *(2)*

Storage Solution
A solution typically selected to control bioburden during column storage.

Transition Analysis
Mathematical evaluation of the chromatogram tracing as the mobile phase changes from one solution to another. An alternative to HETP and peak asymmetry for evaluating column packing and performance.

Unit Operation
A discrete step or manipulation in a manufacturing process where process and operating parameters 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 pre-determined acceptance criteria. *(2)*

Wash
Step in a chromatography cycle designed to flush the column to remove substances acquired during a previous step (often used between the load and elution steps).

3.0 Chromatography Validation Prerequisites

The aim of process development is to establish the design space within which the process can operate in order to reproducibly manufacture a product that meets all critical quality attributes. Additionally, process development should establish a control strategy for maintaining the process within defined limits.

Process development activities should include identification and documentation of critical steps, operational parameters, and process controls. This could take the form of a process description identifying set-points, operating ranges, and proven acceptable ranges. Understanding how the operational parameters and ranges affect product quality and process performance will aid in managing risk, developing a process control strategy, and establishing acceptance criteria. ***(6,7)***

Knowledge of the biophysical characteristics of the protein of interest and the impurities associated with the feed material aids in the design of a protein purification process. Development studies are frequently performed using "platform" processes, as in the case with monoclonal antibodies (MAbs), or are developed heuristically based on experience. In some cases, experimental design studies (using limited design of experiments) are performed to define operating conditions.

In the latter part of the product development timeline, greater understanding is required to optimize the process. While "platform" processes are effective in quickly identifying process conditions that can deliver clinical-grade material, the operating ranges can be narrow and the process may lack robustness. In the later stages, more rigorous, product-specific studies are performed. These often include a variety of carefully designed studies to challenge parameters that could have significant impact on process performance.

High-throughput techniques and design of experiments are useful tools for exploring operational parameter ranges, their interactions, and their impact on product quality. ***(8,9,10)***

3.1 Process Risk Assessment

Risk assessments can be performed at different stages of the product lifecycle. A risk assessment may be used in early stages of process development and characterization to focus resources on the operating parameters or impurities most likely to present risk to product quality. In late stages of process development and characterization, risk assessments facilitate systematically

reviewing all operating parameters and ranges, assessing and identifying potential risks, as well as identifying any missing data needed to successfully transfer a process to production scale. Risks can be caused by using different controls, procedures, or instruments in production than those used in development. Risk assessment results may identify the need for additional characterization studies.

Once initial process development and characterization studies are complete and an understanding of process control for critical steps is established, an additional risk assessment may be used to identify and prioritize potential process risks and, if necessary, develop a strategy for risk mitigation. ***(11,12)*** Investigation of the impact of operating conditions beyond the normal operating range and identification of operating parameters' edge of failure are useful information when performing a risk assessment.