International Journal of Engineering & Scientific Research
Vol.12 Issue 08, Aug 2024
ISSN: 2347-6532 Impact Factor: 6.660
Journal Homepage: http://www.ijmra.us, Email: editorijmie@gmail.com
Double-Blind Peer Reviewed Refereed Open Access International Journal - Included in the International Serial Directories Indexed & Listed at: Ulrich's Periodicals Directory ©, U.S.A., Open J-Gage as well as in Cabell's Directories of Publishing Opportunities, U.S.A

Implementing a Lifecycle Approach for Biopharmaceutical Process Validation: A Risk-Based Strategy

Shikha Patel[*]
Krunal Soni[**]

Abstract

The lifecycle approach to process validation, endorsed by ICH, FDA, and EMA, has significantly enhanced validation programs. Beginning early in development and extending through product lifecycle, successful validation programs are built on solid corporate policies emphasizing quality management. Using a risk-based approach like Quality by Design (QbD), this paper reviews the application of quality target product profiles (QTPPs), critical quality attributes (CQAs), material attributes, and critical process parameters (CPPs) in ensuring product consistency. It focuses on validation strategies for biopharmaceutical processes, specifically therapeutic proteins and vaccines from recombinant cell culture systems. Excluding cell and gene therapies and synthetic peptides, it offers guidance on developing process validation master plans using a risk-based lifecycle approach, aiming to align with global regulatory expectations while supporting business practices.

Copyright © 2024 International Journals of Multidisciplinary Research Academy.*All rights reserved.*

Keywords:

Lifecycle Approach;
Quality by Design (QbD);
Critical Quality Attributes (CQAs);
Process Validation;
Biopharmaceutical Manufacturing.

Author correspondence:

Shikha Patel,
Sr. Manager, Quality Technical Operations
Editas Medicines, Cambridge, Massachusetts, USA
Email: shikha.patel@editasmed.com; shikhapatel87@gmail.com

1. Introduction

There have been major improvements in the design and execution of validation programs since the adoption of the life cycle concept by the International Council for Harmonization (ICH), U.S. Food and Drug Administration (FDA), and European Medicines Agency (EMA). The life cycle approach is thoroughly explained in the FDA's 2011 revision of the Guidance to Industry: Process Validation: General Principles and Practices and the European Commission's updated Annex 15: Qualification and Validation, Eudralex – Volume 4 *(1,2)*.

A successful validation program starts early in the process development life cycle and continues until the product's end of life. This program should be built on a solid corporate policy that outlines the organization's commitment to process validation principles. The policy must define the company's quality management philosophy and include components such as validation protocols and reports, requalification time frames, and the roles and responsibilities of key stakeholders*(7)*.

A risk-based approach, like Quality by Design (QbD), acknowledges the importance of process control strategies in maintaining product consistency and mitigating the risk of poor quality. This paper examines the use of quality target product profiles (QTPPs), critical quality attributes (CQAs), material attributes, and critical process parameters (CPPs). It also highlights management tools that can identify attributes related to the product and process and describes the connections between the product profile, quality attributes, and process parameters.

[*]Senior Manager, Quality Technical Operations, Editas Medicines, Cambridge, Masschussetts- USA.
[**]Senior Director, Quality, eGenesis, Cambridge, Masschussetts- USA.

1.1 Purpose and Scope

This article aims to help design and implement globally compliant validation programs to ensure that biotechnology-derived purified protein drugs are consistently produced and reliable. The approaches discussed are intended to add value, support good business practices, and meet current compliance and regulatory expectations.

This article focuses on validating biopharmaceutical processes used to manufacture therapeutic proteins, polypeptides, and vaccine drug substances. These substances are produced from recombinant cell culture expression systems and can be characterized using appropriate analytical procedures. The goal is to provide clear technical guidance for developing and designing a process validation master plan using a risk-based lifecycle approach and offer a comprehensive overview of strategies that may be used to validate a manufacturing process or unit operations. While the paper does not cover cell and gene therapy products, live-virus vaccines, biopharmaceuticals developed from oligonucleotides, or synthetic peptides, some concepts may still be applicable.

The strategies discussed primarily focus on the validation of non-sterile, low bioburden, well-characterized, protein-based drug substance processes. Specific aspects of process validation that are not unique to protein-based drug substances are not addressed in this paper.

2. Glossary of Terms

Action Limit
a limit that, when exceeded, indicates a process is outside of its normal operating range. A response to such an excursion should involve a documented investigation and corrective actions based on the results of that investigation.

Alert Level
An established level that, when exceeded giving an early warning of a potential drift from normal operating conditions; while not necessarily grounds for definitive corrective action, it typically requires follow up review.

Acceptance criteria
numerical limits, ranges, or other suitable measures for acceptance of the results of analytical procedures which the drug substance or drug product or materials at other stages of their manufacture should meet*(16)*. Exiting the acceptable range for a critical parameter during subsequent validation studies may result in questionable product quality that would require initiation of an investigation and possible batch rejection.

Continuous Monitoring
At leasttwo process unit operations conducted under pre-determined control conditions without process interruptions where real time process controls (PATs) maybe used to meet the process requirements.

Design of experiments (DOE)
A structured, organized method for determining the relationship between factors affecting a process and the output of that process*(8)*.

Design space
The multidimensional combination and interaction of input variables and process parameters that have been demonstrated to provide assurance of quality design space is proposed by the applicant and is subject to regulatory assessment and approval*(8)*.

Process Characterization Report
A report that includes results from a process characterization study with information on the performance of one or several unit operation(s).

Process Validation Master Plan
A document that defines the process validation scope and rationalizes for stage two and stage 3 and contains the list of process validation studies to be performed.

Process Performance Qualification Protocol
A written plan pre-approved by the quality unit that specifies critical steps, controls, and measurements. The process performance qualification protocol states how process performance qualification or other validation studies will be conducted, identifying sampling,assays, specific acceptance criteria, production equipment and operating ranges.

Process Performance Qualification Report
a report approved by the quality unit that summarizes specific tests performed, compare the test results with the protocol acceptance criteria, and addresses deviations encountered during this study.

Drug Product
a pharmaceutical product type that contains a drug substance, generally, in association with excipients*(16)*. The dosage form in the final immediate packaging intended for marketing*(17)*.

Installation Qualification (IQ)
documented verification that the equipment or systems, as installed or modified, comply with the approved design, the manufacturers recommendations, and/or user requirements*(17)*.

Operational Qualification (OQ)
documented verification that the equipment or systems, as installed or modified, perform as intended throughout the anticipated operating ranges*(17)*.

Critical Process Parameter (CPP)
an input process parameter that should be controlled within a meaningful operating range to ensure that drug substance critical 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.

Process Performance Qualification (PPQ)
the second stage of process qualification. It includes a combination of the actual facility, utilities, equipment, and trained personnel and the commercial manufacturing process, control procedures, and components to produce commercial batches. A successful PPQ will confirm the process design and demonstrate that the commercial manufacturing process performs as expected.

Process Validation
the documented evidence that the process, operated within established parameters, can perform effectively and reproducibility to produce an intermediate or drug substance meeting its predetermined specifications and quality attributes*(1,17)*.

Quality Target Product Profile (QTPP)
A prospective summary of the quality characteristics of a drug product that ideally will be achieved to ensure the desired quality, considering safety and efficacy of the drug product*(8)*.

Unit Operation (or Process Step)
a discrete step or manipulation in a manufacturing process where process and operating parameters are defined to achieve a specific process objective.

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*(17)*.

2.1 Abbreviations

CCS	Container Closure System
CMC	Chemistry, Manufacturing, And Controls
CPP	Critical Process Parameter
CQA	Critical Quality Attribute
DOE	Design Of Experiment
FMEA	Failure Mode Effects Analysis
IQ	Installation Qualification
OPV	Ongoing Process Verification
OQ	Operational Qualification
PAT	Process Analytical Technology
PPQ	Process Performance Qualification
TPP	Target Product Profile

3. Process Design

Before starting the Process Performance Qualification (PPQ) of a manufacturing process, several Good Manufacturing Practice (GMP) and process development tasks must be completed. This means that access must be validated, instruments calibrated, and support systems for production must be qualified with proper documentation in place. Additionally, the process itself should be fully developed, well-understood, and documented*(18,19)*.

At this point, the Quality Target Product Profile (QTPP) and Critical Quality Attributes (CQAs) are defined, and an initial risk assessment is conducted to categorize process parameters. This risk assessment helps in selecting parameters for process characterization studies. The written development and characterization report should include detailed study descriptions, justifications, data results, and recommendations. These reports need internal approval and must adhere to the company's standard operating procedures (SOPs). Although formal process validation is not typically required in Phase 1 of clinical development, exceptions exist for safety concerns like viral clearance validation. After Phase 1, and once the decision to continue product development is made, efforts to develop a commercial process must ensure compliance with all validation requirements. These documents are crucial for future process validation plans, protocols, and supporting pre-approval inspections (PIA).

3.1 Risk Assessments

Risk assessment is a crucial part of process validation and should be used from the beginning. The nature of the manufacturing process and unit operations influences the types and number of risk assessments and experimental studies conducted. Initially, a risk assessment is performed for each unit operation by examining its parameters and their potential impact on CQAs. The results guide further process development activities, prioritizing steps and parameters for optimization and characterization.

Before process validation, a quality risk assessment should examine:

- Impact on CQAs.
- Steps for viral clearance.
- Reduction or removal of bioburden and endotoxins.
- Product purity, integrity, stability, and homogeneity*(9,11,12)*.

3.2 Quality Target Product Profile and Critical Quality Attribute

The desired quality of the drug substance is determined based on its use in the drug product and its physical, chemical, biological, and microbial properties. The QTPP and potential CQAs of the drug product help identify the CQAs of the drug substance. After defining the QTPP, it's important to determine which attributes provide essential knowledge for process development and characterization, which then inform the control strategy. Consideration must also be given to impurities and excipients that may alter molecular properties. Drug substance CQAs typically include properties that affect identity, purity, biological activity, safety, and stability. Identifying CQAs should also consider contaminants as outlined in ICH guidelines*(15)*.

Quality attributes are ranked according to their severity on a critical continuum that reflects the complex structure-function relationships of biopharmaceutical products. Given the data required to define the QTPP and determine CQAs, these assessments should involve subject matter experts from outside the core CMC group. A thorough understanding of CQAs is essential as process development and characterization studies focus on this information.

3.3 Control Strategy

The main goal of process validation is to create a reliable manufacturing process and an effective control strategy that ensures the product remains of high quality. This section gives a straightforward summary of how to develop a control strategy for a biopharmaceutical product.

The information gathered about the product and process is used to build the control strategy, which is tested in later stages and then applied in the commercial process. A scientific and risk-based approach helps determine the best controls to ensure product quality based on the defined Critical Quality Attributes (CQAs). Risk assessments and process characterization studies often happen simultaneously and iteratively to aid in parameter classification and the development of the control strategy. The control elements can be used alone or in combination, depending on the identified risks:

- Controls for raw materials
- Procedural controls
- Controls for process parameters (such as set points, ranges, Critical Process Parameters (CPPs))
- In-process testing
- End-product testing
- Characterization testing
- Process monitoring
- Engineering controls (including facility and instrumentation)
- Stability testing

End-product testing is just one part of the control strategy, and it may not be necessary to test the final product for all CQAs. Understanding the process can justify using parametric controls, which can provide adequate control without the need for routine end-product testing.

3.4 Critical Process Parameters

Critical Process Parameters (CPPs) are variables within a process that, when altered, can impact a Critical Quality Attribute (CQA). Therefore, these parameters must be monitored and controlled to ensure the process consistently produces high-quality products. If data or analysis indicates that a process input parameter might affect CQAs, it is classified as a CPP. While immediate detection and redundant controls can minimize the chances of a parameter deviating from its normal range, the significance of its impact on the output determines its criticality. Regardless of the control level, parameters should be considered CPPs unless evidence shows they do not influence CQAs.

3.5 Process Characeterization

Process characterization comprises a set of documented laboratory studies in which parameters are purposely varied to determine the effect on product quality attributes and process performance. Parameter ranges that are explored during process characterization are generally

wider than those anticipated during routine manufacturing and may be chosen based on Process characterization involves a series of documented laboratory studies where parameters are intentionally varied to understand their effect on product quality and process performance. These studies typically explore broader parameter ranges than those used in regular manufacturing, based on equipment capabilities and past manufacturing data. Using Design of Experiments (DOE) methods helps in understanding interactions among multiple parameters, optimizing experiments, and applying statistical rigor to select parameter ranges. Characterizing the process includes identifying product quality attributes (CQAs) that affect safety, identity, strength, quality, and purity. Parameters influencing CQAs or operational reliability are examined during this phase.

Process characterization is a key part of quality risk management. The first step is identifying the risks associated with the parameters to be studied. Initially, high-risk parameters for each unit operation are confirmed. Then, detailed experiments are designed to gather additional data. The principles of process characterization can be applied to all stages of therapeutic product manufacturing, from cell banking to product packaging.

3.6 Criticality of Raw Materials

A material qualification program is essential for the raw materials used in initial manufacturing. At the minimum, vendors should be selected, and specifications established, especially for non-compendial grade materials. As the product progresses through clinical trials, a more comprehensive program should be defined, including qualifying new materials or alternate vendors, addressing material discrepancies, ensuring material stability, conducting vendor audits, and assessing supplier sustainability. A robust raw materials control program should be in place before proceeding with Process Performance Qualification (PPQ).

During early development stages, raw materials should be chosen through a defined process, considering various factors regarding their source and vendors:

- **Compendial Status:** Use compendial raw materials where available. For global distribution, consider multi-compendial materials. For materials that cannot be sourced as compendial grade, the manufacturer must define appropriate tests and specifications.
- **Vendor Selection:** Use raw materials from qualified vendors. Review the status of raw material vendors early to ensure they meet the company's quality system requirements. All vendors should be qualified by the time of PPQ.
- **Multiple Sources:** Identify multiple sources for raw materials to mitigate shortages and vendor issues. Including these sources in the validation process is wise for highly variable materials.
- **Source and Origin:** Prefer non-animal sourced materials when possible. Continuously review and document the source and origin for each raw material. Ensure compliance with procedures to prevent contamination with transmissible spongiform encephalopathy (TSE) agents. Cell culture source materials should also be tested for mycoplasma.
- **Testing of Critical Material Attributes:** Perform specific performance tests based on the potential variability of raw materials, as determined through risk assessment. For example, growth promotion tests for mammalian cell culture media can ensure required standards are met.

4. Process Qualifcation

This section provides an overview of the readiness assessment, process performance qualification (PPQ), and other validation studies. Process qualification is the stage where development and clinical batch manufacturing transition to regular commercial production. The goal is to prove that the process operates as intended and produces consistent commercial products. Figure 1 illustrates the general steps for preparing for process validation activities.

Before starting PPQ, a readiness assessment is necessary to ensure that all required information is available or will be completed on time. This assessment checks that the facility,

equipment, and trained staff are prepared to successfully conduct the qualification. It should include reviewing the process validation master plan, the quality system, and the ability to manage process variability through proper monitoring systems. Consistency checks during the readiness assessment help identify any potential issues that need to be addressed or accepted with a valid justification. This assessment determines if the plant is ready to proceed or if corrective actions are needed.

4.1 Equipment and Facilities

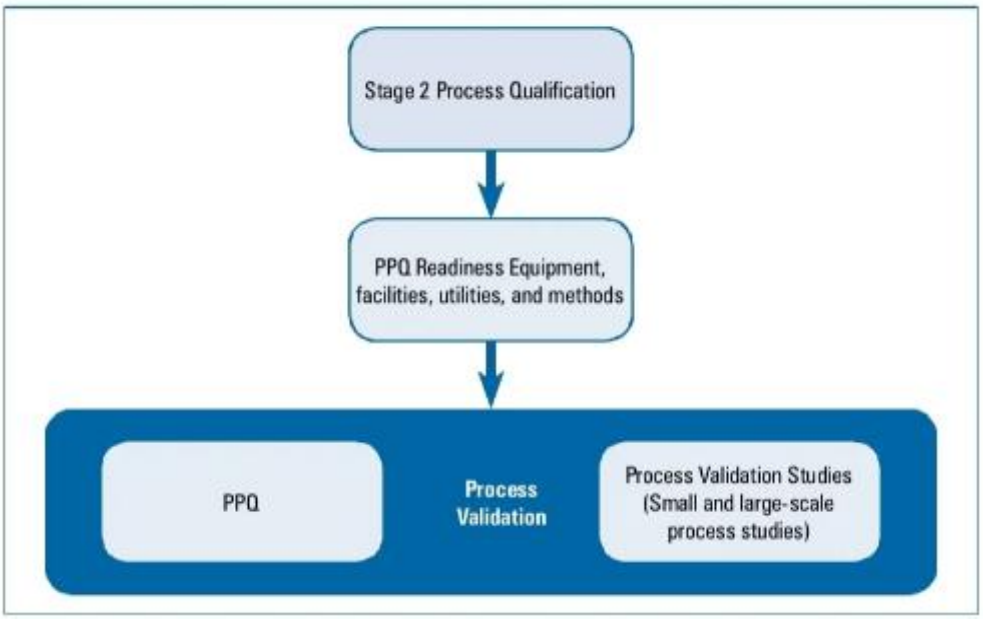
For GMP operations, equipment, utilities, and facilities should undergo qualification, which will be documented in the process validation master plan prior to PPQ batches and supporting validation studies (e.g. hold time, cleaning validation, etc.). The qualification bracket is a compilation of specifications, manufacturers documentation, and testing protocols performed on both the manufacturer and user sites qualification studies should always be designed and performed based on process parameters and intended use of equipment.

For GMP operations, equipment, utilities, and facilities must be qualified before PPQ batches and supporting validation studies (e.g., hold time, cleaning validation) can be conducted. This qualification is documented in the process validation master plan. The qualification process involves compiling specifications, manufacturer's documentation, and testing protocols performed both at the manufacturer's site and the user's site. These studies should be designed based on the process parameters and the intended use of the equipment.

The qualification life cycle is outlined in a comprehensive validation project master plan that must be completed before running and validating the process. A program for periodic qualification status assessment, preventive maintenance, instrument calibration, and change control should be established. The quality assurance unit must ensure that all participants in commissioning activities are fully trained. An in-house engineering department typically performs and documents all tests, then hands over the equipment package and documentation to the manufacturing and validation units.

Cleaning validation of product-contact equipment parts can be done alongside PPQ batches, with cleaning between batches, or separately with engineering batches using soiling techniques. To simplify manufacturing operations, improve contamination control, and reduce validation efforts, single-use systems can be a practical alternative to multiple-use equipment*(16,17)*.

Figure 1: Activities leading Upto Process Validation



4.2 Process Performance Qualification

This section covers the crucial aspects of process validation, detailing various approaches to Process Performance Qualification (PPQ) and other essential validation studies needed to complete a process validation package. The primary goal of PPQ is to demonstrate that the manufacturing process is consistent, clears contaminants, and meets predetermined acceptance criteria for Critical Process Parameters (CPPs), in-process controls, performance parameters, and Critical Quality Attributes (CQAs). Additional studies, such as the stability of in-process intermediates, solution stability studies, cell line characteristics, and shipping validation, are usually conducted separately from full-scale PPQ lots.

There are two main approaches to PPQ and process validation studies: prospective and concurrent. Prospective validation involves planning and completing the study before submitting an application for a new product or a product made under a revised manufacturing process. This approach is preferred in the biotechnology industry. Concurrent process validation, on the other hand, uses data collected during ongoing commercial manufacturing and requires regulatory approval. This method involves long-term monitoring and includes the same elements as prospective validation, but the replicate runs occur during commercial manufacturing, with results reviewed alongside product lot releases.

Grouping validation applies to equipment and processes that are physically and functionally similar. This method requires thorough documentation to ensure the equipment or processes are comparable. Grouping validation can be considered for equipment cleaning and operations(6,8).

4.3 Process Validation Studies

Process validation studies can involve a mix of full-scale and scale-down studies, depending on the appropriateness of the model and the study's nature. Full-scale studies are typically used to validate process consistency, performance, and concurrent lifetime studies of reusable membranes and column resins. Scale-down studies are performed when full-scale approaches are impractical. These studies help understand the stability of process intermediates without impacting full-scale operations. During these studies, it is crucial to control bioburden and endotoxin levels to ensure the results are not compromised by microbial activity. Full-scale studies are necessary to demonstrate microbial control.

The process validation program may include protocols on the following aspects:

- Cell bank and cell line stability
- Viral clearance (if applicable)
- Impurity clearance
- Process intermediate stability
- Process solution stability
- Drug substance fill, freeze, and storage
- Mixing studies (product and process)
- Chromatography resin and reusable filter membrane lifetime validation
- Leachable and extractable qualification
- Container closure integrity for drug substance.

4.4 Lifecycle Management

After a process is validated, the product lifecycle continues with routine monitoring and evaluation to ensure the process remains in control during commercial production. Continued process verification begins after successful validation with an approved process monitoring plan that includes the process inputs and outputs defining successful operation. Specific in-process controls are established to monitor the process.

Routine monitoring steps should be documented in the production records, and data trending should be done at regular intervals (e.g., quarterly for high production volume products or annually for low production volume products). Trending helps in the ongoing evaluation to ensure the process operates within its validated state. Knowledge gained from

continued process monitoring can improve overall process understanding, confirm or modify the control strategy, and provide early warnings when attributes start to drift. Statistical analysis and data trending should be used wherever possible to detect adverse process behavior.

When a process is transferred from the original site to another, technical adaptations and facility adjustments may be required. These adaptations must be fully evaluated using a risk-based approach similar to the one used for validation at the sending site. The process at the receiving site must achieve comparable outputs. Significant process modifications typically require separate regulatory filings.

If a product is manufactured at multiple sites, processes at all sites must remain harmonized. Any lifecycle activity or process improvement initiative should be evaluated for implementation across all manufacturing sites.

5. Conclusion

The adoption of the lifecycle approach to process validation by regulatory bodies such as ICH, FDA, and EMA has revolutionized the biopharmaceutical industry. By integrating this approach, organizations can ensure that their validation programs are not only compliant with regulatory expectations but also robust and adaptable throughout the product's lifecycle. The key to a successful validation program lies in its early inception during the process development phase and its continuity until the product's end of life. This requires a strong corporate policy that underscores the importance of quality management and clearly defines roles, responsibilities, and validation protocols.

Implementing a risk-based approach like Quality by Design (QbD) further strengthens the validation process. QbD emphasizes the necessity of understanding and controlling critical process parameters (CPPs) and critical quality attributes (CQAs) to ensure consistent product quality. The use of management tools to identify and connect these attributes with the product profile enhances the ability to maintain product consistency and mitigate risks. This paper has highlighted the significance of QTPPs, CQAs, and material attributes, and described how these elements are integral to developing an effective control strategy that assures product quality.

The focus on biopharmaceutical processes, particularly for therapeutic proteins and vaccines, illustrates the practical application of these concepts in real-world scenarios. The exclusion of cell and gene therapies, live-virus vaccines, and synthetic peptides underscores the specificity of the strategies discussed, though the fundamental principles of the lifecycle approach and risk management remain broadly applicable. By providing detailed guidance on developing process validation master plans, the paper aims to facilitate the design and implementation of globally compliant validation programs that not only meet regulatory standards but also support sound business practices.

Ultimately, the goal of process validation is to establish a reliable and repeatable manufacturing process that can consistently produce high-quality biopharmaceutical products. Through comprehensive risk assessments, thorough process characterization, and a robust control strategy, organizations can achieve this goal, ensuring that their products are safe, effective, and of the highest quality. The insights and strategies discussed in this article are invaluable in the development and validation of biopharmaceutical processes, providing a clear path towards achieving regulatory compliance and operational excellence.

References

[1] U.S. Food and Frug Administration. *Data Integrity and Compliance with cGMP: Questions and Answers, Guidance for Industry*; U.S. Department of Health and Human Services: Silver Spring, Md., 2018.

[2] International Conference for Harmonization. *Quality Guideline Q10: Pharmaceutical Quality System*; ICH: Geneva, 2008.

[3] U.S. Food and Drug Administration. *21CFR Part 211- Current Good Manufacturing Practice for Finished Pharmaceuticals, Subpart J- Records and Reports*; Government Publishing Office: Washington, D.C., 2005.

[4] U.S. Food and Drug Administration. Part 133- Drugs; Current Good Manufacturing Practice in Manufacture, Processing, Packing, or Holding. *Fed Regist* **1963**, 28 (120, Part II), 6385-87.

[5] U.S. Food and Drug Administration. Current Good Manufacturing Practice in Manufacture, Processing, Packing, or Holding. *Fed Regist* **1978,** 43 (44813, Book2), 45014-336.

[6] U.S. Food and Drug Administration. *FDA Compliance Program Guidance Manual*; 7346.832 (5/12/2010), Pre-approval Inspections. U.S. Department of Health and Human Services: Silver Spring, Md., 2010.

[7] International Conference for Harmonisation. *Quality Guidance Q12: Technical and Regulatroty Considerations for Pharmaceutical Product Lifecycle Management;* ICH: Geneva, 2017.

[8] European Commission. *Annex 11: Computerised Systems. EudraLex- Volume 4- Good Manufacturing Practice for Medical Products for Human and Veterinary Use*; European Commission: Brussels, 2011.

[9] Eurpoean Commisson. *Annex 15: Qualification and Validation; Eudralex- Volume 4- Good Manufacturing Practice for Medicinal Prducts for Human and Veterinary Use*. European Commissoin; Brussels, 2015.

[10] Shikha P, Krunal S (2024). International Journal of Management, IT & Engineeting. *Vol. 4, Issue 8: Data Integrity: Ensuring Quality and Safety Through Robust Controls and Risk Management*; IJMRA: Aug 2024; p 34.

[11] Parenteral Drug Association. *Technical Report 60: Process Validation: A Lifecycle Approach*; PDA: Bethesda, Md., 2013; p 102.

[12] Parenteral Drug Association. *Technical report 60-2: Process Validation: A Lifecycle Approach, Annex 1: Oral Sold Dosage/Semisolid Dosage Forms*; PDA: Bethesda, Md., 2017; p 40.

[13] Parenteral Drug Association. *Technical Report 60-3: Process Validation: A Lifecycle Approah, Annex 2: Biopharmaceutical Drug Substance Manufacturing*; PDA: Bethesda, Md., 2021; p22.

[14] Haider, S1. *Pharmaceutical Master Validation Plan: The Ultimate Guide to FDA, GMP, and GLP Compliance*. CRC Pess (St. Lucie Oress): Boca Raton, Fla., 2001; p 208.

[15] Bennan K, et al. *Evaluation of Extractables from Product-Contact Surfaces. BioPharm International 2002*, Dec 2002, 22-34.

[16] AM International. *ASTM E2500-13: Standard Guide for Specification, Design, and Verification of Pharmaceutical and Biopharmaceutical Manufacturing Systems and Equipment*; ASTM Intl: Wrst Conshohcken, Pa., 2013.

[17] U.S. Food and Drug Administration. *Guidance for Indusrty: PAT- A Framework for Innovative Pharmaceutical Development, Manufacturing, and Quality Assurance*; U.S. Department of Health and Human Services: Silver Spring, Md., 2003.

[18] World Health Organization. Annex 6: Good Manufacturing Practices for Sterile Pharmaceutical Products; WHO: Geneva, 2011.

[19] Brorson, K, et al. Identiifcation of Protein A Media Performance Attributes that can be Monitored as Surrogates for Retrovirus Clearance during Extended Resue. *J Chromatogr A* 2003, 989 (1), 155-63.

[20] U.S. Food and Drug Administration. *Guidance for Industry: Sterile Drug Products Produced by Aseptic Processing- Current Good Manufacturing Practice*, U.S. Department of Health and Human Services: Rockville, Md., 2004.

[21] Eurpoean Medicines Agency. *Guideline on Virus Safety Evaluation of Biotechnological Investifational Medicinal Prodcuts*; EMEA: Amsterdam, 2008.

[22] Webb, S D, et al. Freezing Biopharmaceuticals Using Commoon Techniques- and the Mgnitude of Bulk-Scale Freeze- Concenration. *BioPharm International* 2002, May 2002, 22-34.