COMPLIANCE FOR BIOPHARMACEUTICAL LABORATORIES

Primer

The Measure of Confidence

Agilent Technologies

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Ludwig Huber

Agilent Technologies

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CONTENTS

PREFACE

Dr. Ludwig Huber

Chief advisor for global FDA and ISO 17025 compliance

This primer is intended to introduce biochemists and biologists working in biopharmaceutical development and manufacturing to FDA and equivalent international requirements. It is also useful for quality managers and staff and for everybody else involved in the registration process of biopharmaceuticals. The primer will also give strategies and specific recommendations for regulated laboratories on how to implement the requirements in the most cost-effective way.

In less than one day readers will learn about:

- FDA and equivalent international regulations related to biopharmaceutical development, clinical studies, and quality control in manufacturing
- Application and enforcement of regulations along the drug life cycle
- The registration process of biopharmaceutical drugs
- General quality assurance principles as they relate to laboratories
- Specific requirements for laboratories such as qualification of laboratory equipment, validation of analytical methods, testing and review, and approval of test results
- Setting specifications and acceptance criteria for biopharmaceutical drugs and drug substances
- Analytical methods and equipment typically used for testing of biopharmaceutical drugs and drug substances

The concepts and ideas expressed in this primer are my own and do not necessarily reflect official Agilent or Labcompliance policies.

Regulations and quality standards are quite dynamic. They are updated every couple of years. Guidelines for implementation, as developed by regulatory and industry task forces, are published more frequently. What is state-of-the-art today may not be appropriate tomorrow, especially in the rapidly changing area of biopharmaceutical development and analysis.

A timely update of all information is important and only possible using online information tools. To take this fact into account, I recommend a couple of websites with regular updates related to the topics of the primer:

http://www.fda.gov
Regulations and guidelines for the biopharmaceutical industry

http://www.ema.europa.eu
Website of the European Medicines Agency

http://www.ich.org
Website of the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH)

http://www.picscheme.org
Website of the Pharmaceutical Inspection Convention and Pharmaceutical Inspection Co-operation Scheme

http://www.usp.org
Website of the United States Pharmacopeia

http://www.who.org
Website of the World Health Organization

http://www.labcompliance.com
Website with tutorials, many references, and regular updates related to all quality and compliance issues in laboratories

Dr. Ludwig Huber

Chief advisor for global FDA and ISO 17025 compliance
Labcompliance
ludwig_huber@labcompliance.com

INTRODUCTION

Importance and Value of Regulations and Quality Assurance

While historically most drugs were based on chemical synthesis of small molecules, this has changed over the last two decades. An increasing number of pharmaceutical substances are directly extracted or derived from biological sources and/or produced using biotechnology processes.

Regulations and quality assurance principles play an important and increasing role in the pharmaceutical and biopharmaceutical industries. Companies with drugs in the development pipeline are advised to study, implement, and enforce regulations, otherwise they will not get approval to market the drug. Regulated work starts once a compound has been defined as a target to become a drug. Some companies may not have the financial resources to undergo all required steps of clinical studies and the marketing approval process to bring a drug to the market. Nevertheless, adhering to quality assurance principles is a significant benefit, because this increases the value of the company in a possible acquisition process and for getting studies subcontracted by other companies.

Regulations become global. With the high and ever-increasing costs – up to two billion dollars to develop and market a drug – companies must leverage the costs through selling the products to multiple countries. To market the drug, a company has to comply with the regulations of the target country. This is a challenge for the pharmaceutical industry and manufacturers of drugs, drug substances, and raw materials in countries without enforced regulations that conform to international standards. They may not have the knowledge, experience, and quality processes to comply with international regulations. However, more and more developing countries implement and enforce regulations that are based on the high standards of industrial countries.

Laboratories play a major role for both the development and manufacturing of drugs and drug substances, and they have to follow the same regulations as manufacturing facilities. The bottom line is that everybody working in biopharmaceutical laboratories should have a good understanding of regulations. This primer should help to achieve this goal.

Definitions

The terms ‘biologic product’, ‘products of pharmaceutical biotechnology’, and ‘biopharmaceuticals’ are sometimes used interchangeably and can mean different things to different people. However, a common understanding is of utmost importance to avoid endless discussions in meetings. Therefore, this section describes the most important definitions of the terms that are mentioned and used in this primer. The key outcome should be that all readers associate the terms used in the primer with the same meaning. For example, the primer will use the term ‘biopharmaceutical drug’ for all drugs that are based on large molecules.

Biologic product	Therapeutic product that is derived from biological processes
Biotechnology	Pharmaceutical product used for a therapeutic or medicinal product *in vivo* diagnostic purpose, which is produced in full or in part by biotechnological means.
Biopharmaceutical	Protein or nucleic acid–based pharmaceutical drugs are substances used for therapeutic or *in vivo* diagnostic purposes, which are produced by means other than extraction from natural (non-engineered) sources. In the context of this primer: all drugs that are based on large molecules.
Generic drugs	Copies of drugs that are based on expired patents
Biosimilar drugs (Europe) **Follow-on proteins (US)**	Copies of biopharmaceutical drugs that are based on expired patents
GxPs	Good Practices, where x can stand for: 'L' (Good Laboratory Practices), 'C' (Good Clinical Practices) and 'M' (Good Manufacturing Practices) This primer will make frequent use of GxPs.
cGMP	Good Manufacturing Practices (GMPs) in FDA terms are usually called cGMP, which stands for current Good Manufacturing Practices. 'Current' means that the industry should always implement the actual (current) FDA guidelines and other interpretations of the rules. In the context of this primer, GMP also includes cGMP.
FDA	FDA stands for Food and Drug Administration. Even though the US FDA is most well-known, there are other equivalent organizations in other countries. In the context of this primer, FDA means the US FDA.

Figure 1 Relevant definitions concerning biologics and compliance.

Resources

While the scope of this primer is to give an overview on compliance for biopharmaceutical laboratories, there are lots of resources available where readers can get more details. They come from regulatory agencies, joint industry/agency task forces, and from private authors. Regulatory and other official documents will be discussed in the next chapter.

There are many publications available from private authors that are published as traditional journal papers, online articles and traditional textbooks. For online articles, readers are referred to well-known internet search engines. This section gives an overview of textbooks published by private authors or organizations.

- Biopharmaceuticals, Biochemistry and Biotechnology[1].
  This book provides an overview of the science and applications of biopharmaceutical products. It has detailed information on regulatory requirements.
- The Pharmaceutical Regulatory Process[2].
  This book provides information on the pharmaceutical and biopharmaceutical development and marketing approval process.
- FDA Regulatory Affairs: A Guide for Prescription Drugs, Medical Devices and Biologics[3].
  This book provides details of the regulatory requirements and processes and has a chapter on biologics.
- Protein Pharmaceuticals[4].
  A section of this book discusses regulatory issues of parenteral protein formulations.
- Agilent Technologies has published primers on Analytical Instrument Qualification and System Validation[5], on Validation of Analytical Methods[6], and on Approaches for Quality by Design in Pharmaceutical Development[7]. Even though not developed specifically for biopharmaceutical laboratories, they are quite useful to get a good understanding about different compliance and quality assurance aspects in biopharmaceutical laboratories.

REGULATIONS AND GUIDELINES

1

The pharmaceutical industry is one of the most regulated industries. Drug development and manufacturing are controlled by government agencies through a set of laws, regulations, and guidance documents in all industrial countries and in an increasing number of developing countries. The most important underlying regulations are the so-called GxP regulations consisting of good laboratory practices (GLP), good clinical practices (GCP) and good manufacturing practices (GMP). In addition, there are special regulations for product labeling, the use of computers in a regulated environment, and for marketing authorization.

The main purpose of regulations is to ensure the quality, safety, and efficacy of drugs. For marketing authorization of new drugs, agencies evaluate study data and determine if the benefit of the drug is higher than the risk through insufficient drug safety. Regulations for the pharmaceutical industry in general follow modern quality system principles, with high focus on data accuracy, reliability, and integrity.

The philosophy of the regulatory process is very similar in most countries but implementation can be different. Laws are released by governments and regulatory agencies develop regulations with information on how to implement the laws. Regulatory agencies inspect related industrial establishments to check compliance of industry.

Having regulations in all industrial countries, and with an increasing tendency in developing countries, is a big challenge for the pharmaceutical industry. In the ideal world, regulations and the marketing authorization process should be the same. While this is not currently realistic and may not be in the near future, there are attempts to harmonize at least some aspects. Important organizations working toward global regulations and guidelines include the Pharmaceutical Inspection Convention Cooperation Scheme (PIC/S) and the International Conference for Harmonization (ICH). Pharmacopeias in the US, Europe, and Japan also have ongoing programs to harmonize test methodologies.

This chapter describes the roles of US FDA and European health agencies and lists the most important documents. It also describes the tasks and documents of other organizations and task forces with high impact on the pharmaceutical industry, such as PIC/S, ICH, and USP.

1.1 The United States

In the United States, pharmaceutical development and manufacturing are regulated by the Food and Drug Administration (FDA). The goal of FDA activities is to protect public health by assuring the safety, efficacy, and quality of human and veterinary drugs and biological products. Besides drugs, the FDA also controls food, tobacco, medical devices, and cosmetics. The FDA derives its statutory power from the Federal Food, Drug, and Cosmetic Act. The act has its origin in the Pure Food and Drugs Act from 1906, a law that prohibited interstate commerce of adulterated and misbranded food and drugs.

The first version of the FD&C was passed by Congress as early as 1938. This law, for the first time, required companies to prove the safety of new drugs before putting them on the market. It also added the regulation of cosmetics and therapeutic devices, and included general updates to improve consumer protection.

Amendments in 1962 required that all drugs be proven effective as well as safe and gave the FDA the authority to regulate prescription drug advertising. The Medical Device Amendment of 1976 gave the FDA authority to ensure the safety and effectiveness of medical devices, including diagnostic products.

Laws are quite general and usually don't state details of implementation and enforcement. For enforcement of laws, Federal Agencies such as the Food and Drug Administration (FDA) promulgate rules or regulations. These are published as the Code of Federal Regulation (CFR) in the Federal Register and inform the public and industry how the laws are implemented.

The most important FDA regulations for drug development and manufacturing are Good Laboratory Practices, Good Clinical Practice, and Good Manufacturing Practices (GxPs). Figure 2 shows these and other regulations for the pharmaceutical industry that are also important for the biopharmaceutical industry.

Part	Title	Applies To
11	Electronic Records & Signatures	All FDA Regulations
50	Protection of Human Subjects	GCP, clinical trials
56	Institutional Review Boards	GCP, clinical trials
58	Good Laboratory Practice	GLP studies, pre-clinical
210	Manufacturing/Distribution	cGMP, definitions
211	Finished Pharmaceuticals	cGMP, incl. quality control
312	Investigational New Drugs	GCP, clinical trials
314	New Drug Marketing Approval	Approval process
320	Bioavailability/Bioequivalency	Pre-clinical
511	New Animal Drugs for Investigational Use	Animal drugs
514	New Animal Drug Applications	Animal drugs
600	Biologic Products General	Biologic products
601	New Animal Drugs for Investigational Use	Biologic approvals
610	General Biological Products Standards	cGMP

Figure 2 Important FDA regulations for the (bio)pharmaceutical industry.

Manufacturing and marketing authorization of biologic products are published in Parts 600, 601, and 610. Investigational new biologics are governed by the same regulation as investigational new drugs.

Typically, regulations are not detailed enough for implementation and enforcement by the industry and FDA inspectors. Therefore, the FDA has developed inspection and industry guidances on many topics, which are available on the internet for the FDA staff and industry (www.fda.gov, search for FDA Guidance). They provide assistance to the regulated industry by clarifying requirements imposed by Congress or issued in regulations by the FDA, and by explaining how industry may comply with these statutory and regulatory requirements. They also provide specific review and enforcement approaches to ensure that FDA investigators implement the agency's mandate in an effective, fair, and consistent manner. While laws and regulations are mandatory for the industry, guidance documents are not. Industry can decide to use alternatives to comply with regulations.

Important FDA guidances related to biopharmaceutical laboratories are:

- Analytical Procedures and Methods Validation (draft)[8]
- Bioanalytical Method Validation[9]
- Microbiological Pharmaceutical Quality Control Labs[10]
- Biotechnology Inspection Guide[11]

Besides creating regulations, policies, and guidance documents, the FDA licenses and inspects manufacturing facilities, tests products, and evaluates claims and drug advertising.

The FDA consists of several centers that are responsible for different products such as food, drugs, cosmetics, and devices. The centers are headed by a commissioner who is appointed by the President. Biopharmaceutical drug evaluation and approvals are undertaken by the Center for Drug Evaluation and Research (CDER) and the Center for Biologics Evaluation and Research (CBER). CDER regulates the development and marketing process of mainly chemical-based drugs; CBER regulates biologics. For biopharmaceutical products, there is not a clear definition of what is regulated by CDER or CBER, and frequently decisions are made on a case-by-case basis. The regulatory process and requirements are similar for CDER and CBER, but the administrative details can be different. The main documents required for marketing approval are known as Investigational New Drug Application (IND), New Drug Application (NDA), and Biologic License Application (BLA).

1.2 Europe

Drugs in Europe are evaluated for marketing authorization through the European Medicines Agency (previously EMEA, now EMA). It is a decentralized agency of the European Union, with headquarters in London. Their main responsibility is the protection and promotion of public health. The scientific opinions of the EMA for medicines for human use are prepared by the Committee for Medicinal Products for Human Use (CHMP). As in the US, in Europe a drug must have a marketing authorization before it can be distributed. Marketing authorization can be applied for through a

1. central procedure,
2. mutually recognized procedure, or
3. national procedure.

The most common procedure is the centralized procedure. The national procedure is mainly used when marketing authorization is only applied for in a single country. When marketing has been approved in a single country, the applicant can nominate this country as a reference state, and using the mutual recognition procedure apply for approval in other countries. Unless there is a safety risk, states for which the mutual recognition is intended will accept the marketing authorization.

All marketing authorizations for biopharmaceuticals have to use the centralized procedure. For this procedure, product evaluation is made by the EMA. Within the EMA, the Committee for Medicinal Products for Human Use (CHMP) performs the actual assessment of the application and issues a scientific opinion. Based on this opinion, the EU decides if it will grant marketing authorization. GMP requirements for drugs, or so-called medicinal products in Europe, are laid down in the EU guide: The Rules Governing Medicinal Products in the European Union, Volume 4: Good Manufacturing Practices Medicinal Products for Human and Veterinary Use[12].

1.3 International Conference for Harmonization (ICH)

The ICH was initiated in 1990 to bring together the regulatory authorities of Europe, Japan, and the United States and experts from the pharmaceutical industries in the three regions to discuss scientific and technical aspects of product registration.

The ICH publishes guidelines that are either signed into law by member countries, e.g., in Europe, or recommended as guidelines by national authorities, e.g., by the US FDA.

The most important documents related to biopharmaceuticals are the ICH Q6B, Q5C, Q5D, Q5E, and S6 guidelines:

- ICH Q6B: Specifications: Test Procedures and Acceptance Criteria for Biotechnological/ Biological Products[13]
  The document provides guidance on justifying and setting specifications for proteins and polypeptides that are derived from recombinant or non-recombinant cell cultures. The scope is limited to well-characterized biotechnological products, although the concepts may be applicable to other biologicals.
- Q5C: Quality of Biotechnological Products: Stability Testing of Biotechnological/ Biological Products[14]
  This document augments other more generic ICH guidances on stability testing and deals with the particular aspects of stability test procedures needed to take account of the special characteristics of products in which the active components are typically proteins and/or polypeptides.
- Q5D: Quality of Biotechnological Products: Derivation and Characterisation of Cell Substrates Used for Production of Biotechnological/Biological Products[15]
  The objective of this guideline is to provide broad guidance on appropriate standards for the derivation of human and animal cell lines and microbial cells to be used to prepare biotechnological/biological products and for the preparation and characterization of cell banks to be used for production.
- Q5E: Comparability of Biotechnological/Biological Products Subject to Changes in their Manufacturing Process[16]
  The objective of this document is to provide principles for assessing the comparability of biotechnological/biological products before and after changes are made in the manufacturing process for the drug substance or drug product. The document does not prescribe any particular analytical, nonclinical, or clinical strategy. The main emphasis of the document is on quality aspects.
- S6: Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals[17]
  This guidance is intended primarily to recommend a basic framework for the preclinical safety evaluation of biotechnology-derived pharmaceuticals. It applies to products derived from characterized cells through the use of a variety of expression systems, including bacteria, yeast, insect, plant, and mammalian cells.
- S6 Addendum to Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals[18]
  The purpose of the addendum is to complement, provide clarification on, and update the following topics discussed in ICH S6: species selection, study design, immunogenicity, reproductive and developmental toxicity, and assessment of carcinogenic potential.
- Q2(R1): Validation of Analytical Procedures: Definitions and Methodology[19]
  This guidance is the international standard for setting parameters and procedures for the validation of analytical methods.

1.4 Pharmaceutical Inspection Convention and Pharmaceutical Inspection Co-operation Scheme (PIC/S)

PIC/S is one of the most important organizations in the area of global harmonization of GMP regulations and inspections. Its mission is "to lead the international development, implementation and maintenance of harmonized Good Manufacturing Practice (GMP) standards and quality systems of inspectorates in the field of medicinal products". This is to be achieved by developing and promoting harmonized GMP standards and guidance documents; training competent authorities, in particular inspectors; assessing (and reassessing) inspectorates; and facilitating the co-operation and networking for competent authorities and international organizations. As of January 2015 there are 45 participating authorities in PIC/S, including health agencies from all EU member countries, Australia, Singapore, Canada, and the US FDA. Several more organizations have applied for PIC/S membership. Most likely new member countries that don't have their own GMP regulations will comply with the PIC/S GMPs, which are very similar to the EU GMP Guide. For example, Switzerland, Singapore, and Australia have declared PIC/S GMP Guides as their national GMP regulation.

An important document related to biopharmaceutical drugs is the PIC/S Guide: Inspection of Biotechnology Manufacturers[20].

1.5 United States Pharmacopeia (USP)

USP develops methodology for specific applications and general chapters on different analytical aspects for FDA-regulated industry. According to section 501 of the Federal Food Drug and Cosmetic Act, USP methodologies constitute legal standards. For marketing authorization, manufacturers should meet USP standards for the drug substance, for excipients, and for the drug product, if available. USP has developed several general chapters related to quality control laboratories.

- Chapter <111> on "Design and Analysis of Biological Assays"[21]
  The aim of this chapter is to present a concise account of biometrical procedures for USP bioassays.
- Chapter <1058> on "Analytical Instrument Qualification"[22]
  This chapter provides a framework for the qualification of analytical instruments. It covers the complete process from writing specifications and installation to initial and ongoing testing and maintenance.
- Chapter <1226> on "Verification of Compendial Methods"[23]
  This chapter has been written for laboratories implementing compendial and standard methods. The recommendations are also useful for laboratories implementing validated methods from any other laboratory.
- Chapter <1224> on "Transfer of Analytical Procedures"[24]
  This chapter describes several approaches for qualified transfer of analytical procedures. The most common one is comparative testing.

The United States also develops and provides standards and certified reference material that can be used as quality control samples in routine analysis and for validating accuracy of analytical methods. Extensive reference material is available to characterize biopharmaceutical drugs.

2 REGISTRATION OF BIOPHARMACEUTICAL PRODUCTS

The drug discovery, development, and marketing authorization process is a long process that typically takes more than 10 years. The process can be divided into phases that are shown in Figure 3. It starts with basic research and discovery activities, the results of which are then used to define efficacy targets for the potential drug. The discovery phase often involves thousands or even tens of thousands of new chemical entities (NCEs) or new biological entities (NBEs) being screened for activity against a target disease. Successful NCEs/NBEs are then checked for their potential toxic effects, again in screening-type tests, reducing the number of potential drug substances taken forward to full development. Basic research and drug discovery activities are not regulated.

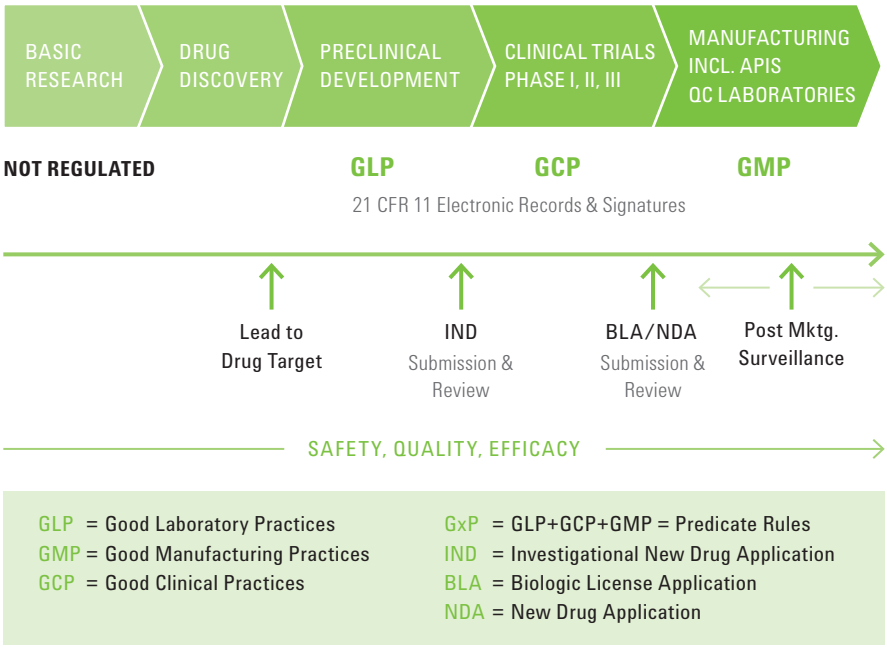

Figure 3 Pharmaceutical drug development and registration process.

Once a target compound has been identified to become a drug candidate, it goes through preclinical studies for initial safety tests. They are regulated through good laboratory practice regulations. Clinical trials are regulated through good clinical practice regulations and the manufacturing process through current good manufacturing practice regulations. Quality control laboratories are also regulated by GMP as well as the manufacturing process of drug substances (APIs). At the end of the preclinical studies, the regulated company submits an Investigational New Drug (IND) Application, and at the end of the clinical trials a New Drug Application (NDA) or New Biological License Application (NBA). The applications are reviewed by the FDA to decide if the drug can move to the next phase.

The BLA is not governed by the Food, Drug, and Cosmetic Act (FD&C Act), but by the Public Health Services Act (PHS Act). Requirements for investigational drugs and biologics are very similar and clinical trials are governed by the same FDA regulation 21 CFR 312.

Once the drug has been registered and is available on the market, health agencies regularly control compliance with GMP regulations through testing of products on the market and through inspections of manufacturing establishments. In case of non-compliance, agencies take enforcement actions. Examples are sending the company management a warning letter and shipment stop of products for companies in the US or an import alert for non-US based companies.

Throughout the development, the principles of the three pillars of GxPs are:

- Safety, to assure the maximum achievable protection against adverse events in relation to the benefit obtained by the drug.
- Quality, to assure high technical product excellence.
- Efficacy, to demonstrate the product effectiveness.

2.1 Preclinical Studies

Preclinical studies, sometimes also termed 'non-clinical' as they are not performed in humans, are regulated by GLP Principles. GLP study data are required for marketing authorization. The primary purpose of tests is to ensure minimum safety for human consumption during following clinical trials. In addition, preliminary efficacy information should be obtained. Typical tests are for toxicology, bioavailability, and pharmacology. In addition, preliminary information on stability should be obtained. First tests are done in test tubes (*in vitro*), followed by tests on animals (*in vivo*). The US regulation behind the GLPs is 21 CFR Part 58. International GLP regulations are based on GLP principles of the Organization for Economic Cooperation and Development (OECD)[25]. The regulation describes organizational aspects and conditions under which laboratory studies are planned, performed, monitored, recorded, and reported. The objective is to assure quality, traceability, integrity, and validity of test results.

GLPs govern laboratory facilities, animal housing facilities, personnel, and documentation. They require an increased formalization of the generation, management, and documentation of test data. For example, it should be possible to repeat an experiment based on documentation, even though the experiment was done five or more years ago.

2.2 Clinical Studies

Whereas the purpose of preclinical work is to develop adequate data to decide that the drug it is reasonably safe to proceed with human trials of the drug, clinical trials represent the ultimate premarket testing ground for new drugs. During these trials, an investigational compound is administered to humans and is evaluated for its safety and effectiveness in treating, preventing, or diagnosing a specific disease. The results of this testing will comprise the single most important factor in the approval or disapproval of a new drug.

Although the goal of clinical trials is to obtain safety and effectiveness data, the overriding consideration in these studies is the safety of those participating as subjects in the trials. CDER or CBER monitors the study design and conduct of clinical trials to ensure that people in the trials are not exposed to unnecessary risks. Clinical trials are done in three consecutive pre-approval phases, plus one optional post-approval phase.

Figure 4 shows the main objectives, types of subjects, number of subjects, and the expected outcome of the three phases. Within the given ranges, the information applies to drugs derived from small and large molecules.

Phase I clinical studies evaluate drug metabolism and structure-activity. It includes the initial introduction of an investigational new drug into humans. The studies are closely monitored and are usually conducted in healthy volunteer subjects but may also be conducted in patients. The studies are designed to determine the metabolic and pharmacologic actions of the drug in humans, the side effects associated with increasing doses, and, if possible, to gain early evidence on efficacy.

	Phase 1	Phase II	Phase III
Main Objective	Assess safety, explore efficacy, confirm safety in humans	Explore efficacy	Confirm safety and efficacy on wider range of population
Types of Subjects	Healthy males	Patients	Patients from multiple countries and multiple ages
Number of Subjects	10–100	50–500	2000 and more
Expected Outcome	Preliminary estimate of maximum dose	Determine dosage level for Phase III	Comprehensive understanding of maximum dose safety and efficacy

Figure 4 Clinical Phases I, II, and III.

During Phase I, sufficient information about the drug's pharmacokinetics and pharmacological effects should be obtained to permit the design of well-controlled, scientifically valid Phase II studies. The number of subjects typically is less than 100 and this phase can last up to one year.

Phase II includes the early controlled clinical studies conducted to obtain some preliminary data on the effectiveness of the drug for a particular indication or indications in patients with the disease or condition. This phase of testing also helps determine the common short-term side effects and risks associated with the drug. Phase II studies are typically well controlled, closely monitored, and conducted usually involving several hundred people. Phase II studies typically last up to two years. At the end of Phase II, the sponsor meets with the FDA to discuss data and plans for Phase III.

Phase III studies are performed after preliminary evidence suggesting effectiveness of the drug has been obtained in Phase II. They are intended to gather additional information about effectiveness and safety that is needed to evaluate the overall benefit–risk relationship of the drug. Phase III studies also provide an adequate basis for extrapolating the results to the general population. Studies usually include several hundred to several thousand people and last 3–5 years. Also, Phase III studies establish final formulation, marketing claims and product stability, packaging, and storage conditions.

In Phases II and III, CDER or CBER can impose a clinical hold if a study is unsafe or if the protocol is clearly deficient in design in meeting its stated objectives.

2.3
Marketing Authorization

Towards the end of clinical Phase III, companies initiate the marketing authorization process. This requires a thorough documentation of pre-clinical and clinical studies. Such documentation can easily be up to 100,000 or more pages when printed. Biologics are approved under the Public Health Service Act (PHS Act), not under the Food, Drug and Cosmetic Act (FD&C Act). FDA regulations to look at are CFR 314 for traditional drugs (NCEs) and 601 for biologic products (NBEs). The regulatory mechanism is designed to give the FDA sufficient information to make an evaluation of the new drug; the New Drug Application (NDA) for traditional drugs or the Biologic License Application (BLA) for biologics. Common Technical Documents guides from the ICH are recommended guidelines to study when submitting the documentation.

Steps for the marketing authorization process include:

- Applicant requests a pre-submission meeting with FDA to clarify any open questions.
- Applicant submits NDA (paper or electronic) to CDER or CBER, depending on whether authorization is for a traditional drug or biologic product.
- FDA conducts a preliminary technical screening mainly to check if the documents are complete. If 'complete' the FDA considers the application 'filed' and will begin the review process.
- A team of FDA technical specialists from different offices reviews the documents. Team members can be chemists, physicians, statisticians, or pharmacologists.
- If there are any easily correctable deficiencies, the FDA informs applicants about them.
- The applicant corrects the deficiencies and resubmits the NDA.
- FDA prepares preliminary review report. At this time FDA may also conduct GMP inspections.
- The final review is made under supervision of the appropriate Director, who may consult an advisory team.
- An action letter is issued within 180 days of the start of the review, unless an extension is agreed upon with the applicant.
- The approval is issued if submission is acceptable.

According to the Hatch–Waxman Act of 1984, traditional generic drugs don't have to go through the complete development process. The act authorized marketing upon approval of Abbreviated New Drug Applications (ANDAs). Submission documentation should include evidence that the active ingredient of the generic drug is the 'bioequivalent' of a drug previously approved by the FDA. The manufacturer does not have to submit preclinical and clinical safety and efficacy studies.

Current US law provides no generally abbreviated submission for bio-generic drugs or, as they are called by the FDA, follow-on biologics. Instead, manufacturers of biopharmaceuticals, depending on structural and functional analyses data, may have to perform full clinical trials and create a full BLA for these products. Between 2012 and 2014, the FDA has published several biosimilars draft guidances.[34] The situation is different in Europe, where abbreviated submissions are generally possible for biosimilar drugs, as they are called in Europe, but still some clinical studies should be conducted. In response to the need for guidance on what to do the European Medicines Agency has published recommendations for non-clinical and clinical studies, for example, for biological medicinal products containing monoclonal antibodies (mAb)[32].

2.4 Manufacturing

Manufacturing and associated quality control is governed by good manufacturing practice regulations. GMPs should ensure that APIs and drugs are manufactured according to quality and safety specifications. Related regulations in the US are CFR 210 with general requirements and 211 for finished drugs. GMPs for APIs typically follow ICH Q7.

GMPs cover all processes, such as:

- Manufacturing, packaging, and distribution
- Quality control laboratories
- Manufacturing of material for clinical trials
- Finished drugs and drug substances or APIs

GMP quality control testing includes raw material, intermediates and finished drugs, ongoing stability testing and ongoing dissolution testing. Detailed requirements for biopharmaceutical QC requirements are described in the next chapter.

2.5 Requirements for Electronic Records and Signatures

In 1997 the FDA released a specific regulation for electronic records and signatures: 21 CFR Part 11. The regulation applies whenever computers are used to generate, evaluate, archive, retrieve, and transmit data. The regulation should ensure that computerized records and signatures are as trustworthy as records and signatures on paper. The focus of the regulation and enforcement is on accuracy, authenticity, integrity, security, and availability of data. The regulation has detailed requirements to achieve these objectives. They include:

- Computer systems should be validated.
- Any changes to records should be recorded by the system independently from the operator.
- When copies are made, they should be accurate and complete, or preserve the content and meaning. This is especially important when electronic records are printed.
- Access to computer systems and data should be controlled by procedures and related software functionality.
- The computer should record the identification of the user.

REQUIREMENTS FOR BIOPHARMACEUTICAL LABORATORIES 3

Reliable and accurate data measured in biopharmaceutical laboratories are important to ensure that only safe and efficient drugs are authorized for marketing and released for product shipment. Therefore, biopharmaceutical development and QC laboratories have to follow GxP regulations to demonstrate quality of data. Requirements can be divided into two categories:

1. General quality system requirements.
   Apply to all regulated activities within a company, for example, control of documents, internal audits, and qualification of personnel. They are typically called management requirements.

2. Laboratory-specific requirements.
   Apply to specific situations in a laboratory, for example, validation of analytical methods, sampling, product testing, and review and approval of test reports.

This chapter describes the GxP requirements for biopharmaceutical laboratories. When reading through the chapter, scientists and professional analysts may consider many of the requirements to be common sense and that there should be no need for formal compliance. However, in a regulated world it is not enough to understand what should be done and it is even not enough to implement the requirements. Most important is to document what has been implemented. Inspectors simply consider everything not documented as not having been done.

While the listed requirements, in principle, apply to all phases of development and manufacturing, an incremental approach should be used for implementation along the phases from preclinical studies to finished drug QC laboratories. For example, in clinical Phase I it may be sufficient to create a document that describes why an analytical method is suitable for its intended use. On the other hand, in Phase III the statements must always be supported by experiments.

While QC laboratories controlling the quality of commercial drugs that are sampled from large repetitive batch production should comply with all GMP requirements listed in CFR Part 210 and 211 to the full extent, this is not always necessary for earlier phases. For example, the FDA has published a "Guidance for Industry: CGMP for Phase 1 Investigational Drugs"[30]. The guide also has a chapter on "Biological and Biotechnological Products". The approach described in the guidance reflects the fact that some manufacturing controls and the extent of quality controls needed to achieve appropriate product quality differ not only between investigational and commercial manufacture, but also among the various phases of clinical trials. For example, for Phase I, the FDA may not always expect a full implementation of analytical instrument qualification according to USP chapter 1058[22] with vendor assessment, but recommends focusing on calibration and system suitability testing. The guide also does not mention that formal failure investigations should be initiated in out-of-specification situations.

3.1 Compliance Overview

The overall impact of regulations on a biopharmaceutical laboratory can be best illustrated by looking at the whole sample/data workflow as shown in Figure 5. The upper part shows general quality assurance requirements that are applicable to regulated laboratories. The lower part of the figure shows a typical laboratory workflow of samples and test data, together with key requirements. The middle part shows requirements that are applicable to the entire sample or data workflow.

COMPLIANCE ACROSS THE LABORATORY

Non-conflicting organizational structure, document control, complaint handling, corrective & preventive actions, supplier & subcontractor management, internal audits, qualification of personnel

COMPLIANCE ACROSS ALL WORKFLOW STEPS

Validation of analytical methods & procedures	Equipment calibration testing & maintenance	Controlled environmental conditions

COMPLIANCE ACROSS THE SAMPLE & DATA WORKFLOW

Sampling	**Sample handling**	**Testing**	**Test reports**	**Record management**
Sampling plan & Sampling Documentation	Sample identification & protection of sample integrity	Monitoring quality of test results, handling OOS	Test Conditions, test results, review & approval	Ensure record integrity & security

Figure 5 Requirements for biopharmaceutical laboratories.

3.2 General Quality Assurance Requirements

Biopharmaceutical laboratories are expected to follow quality assurance practices that are commonly accepted in regulated industries. The objective of quality assurance has been defined in the EU Guide to GMP for Medicinal Products as "The sum... of the organized arrangements made with the object of ensuring that medicinal products are of the quality required for their intended use"[12].

Documentation

GxPs require that regulated documents be controlled from creation through disposal. The regulated industry uses different types of documentation, as illustrated in the documentation pyramid in Figure

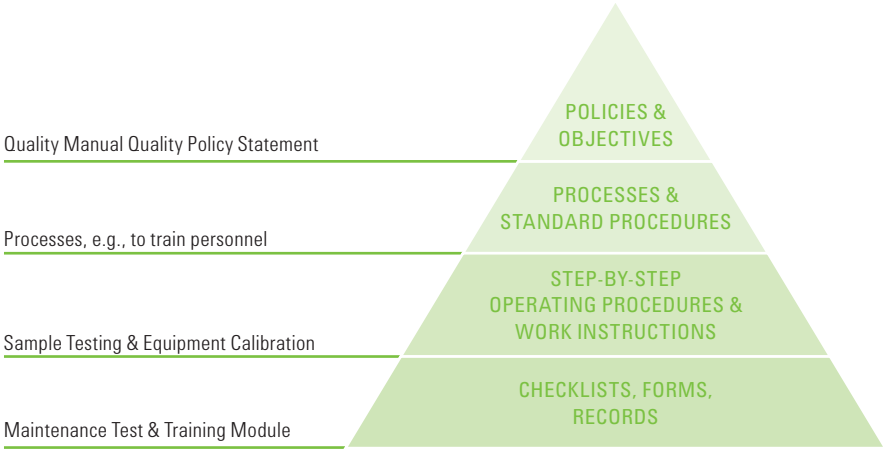

Figure 6 Documentation pyramid.

A policy documents the laboratory's intent to implement GxPs. The Quality Manual or Compliance Master Plan is the top tier of the document hierarchy. It describes the approaches to achieve quality data. It also includes policy statements describing the laboratory's intention to conform to GxP requirements. For example, a policy statement could be: *All personnel involved in regulated activities should be qualified for the assigned task, and the qualification should be documented.*

A generic procedure describes how various quality requirements can be achieved. For example, it describes how the requirement 'Personnel should be qualified for the assigned task' can be implemented. Standard operating procedures (SOPs) or Working Procedures are step-by-step instructions on how to exactly perform a specific task, such as calibrating a specific instrument.

Records are generated on a day-to-day basis. Examples include analytical results from product tests or calibration records of a balance. All documents should be properly controlled. For example, documents should be generated, reviewed, approved, and distributed following documented procedures and changes should be authorized and logged, and the updated document should get a new revision number or code.

Organization Structure and Responsibilities

GxPs require that organizational arrangements should be made so that departments with conflicting interests do not adversely influence quality and compliance of data. For example, finance and the QA department should operate independently from laboratory activities. Inspectors want to see organizational charts. Responsibilities of management and staff should be defined and annually reviewed. The Quality Assurance department should be responsible for setting up compliance systems. Implementation and maintenance of the system is the responsibility of each manager, supervisor and employee. Copies of job descriptions, job applications, resumes and annual reviews should be kept on file in the Human Resources and QA Department.

Qualification of Personnel

The single most important influential factor in acquiring accurate and reliable data is hiring, training and management of qualified people. Regardless of all the sophisticated equipment and validated methods in a laboratory, if people are not properly qualified and motivated to handle all laboratory activities, one will not obtain consistently reliable data. No/inadequate training of employees is one of the most frequently cited deviations in FDA inspectional observations, and warning letters and all GxP regulations require that all employees working in regulated environments should be qualified for their assigned task. Qualification can come through education, experience, or training. Important points include:

- For each employee there should be a job description with assigned tasks. Inspectors want to get documented evidence that the employee is qualified for the assigned task. This can be a biography with information on education and experience from previous jobs, or it can be training certificates.
- If there is not enough documented evidence that the person is qualified, the supervisor together with the employee develops a training plan to fill the gap.
- Once the training is completed, a certificate or other documentation should be issued to prove that the employee attended the training and that it was effective. In a laboratory, effectiveness of analysts can be demonstrated through running well-characterized samples with known amounts. The training was successful if the trainee gets the target results.
- Training should be delivered by qualified trainers. The qualification of the company providing the training and/or the trainer should be documented.
- Training should not only cover operational tasks but also GxP regulations.

- Training should be an ongoing effort.
- Training should be part of regular quality audits to verify that training programs and procedures for trainings are followed. This also includes verification that templates and checklists that are part of the procedure are used adequately.

Facilities and Environments

The laboratory should have a procedure to ensure that its facilities and environmental conditions do not adversely affect or invalidate sample handling, instrumentation, instrument calibration and qualification and product testing. The procedure should ensure that:

- Laboratories are equipped with climate and ventilation control and laboratory facilities meet the required environmental conditions, e.g., temperature and humidity, as specified by instrument manufacturers and as required for sample processing.
- Floors in the laboratories are constructed from a material that is resistant to most chemical spills and easily disinfected.
- Workbenches are constructed of material that is easily disinfected and impervious to most chemical spills.
- The laboratory is equipped with chemical hoods to capture hazardous materials used or produced in the analysis and to protect employees from hazardous concentrations of airborne toxic substances.
- An auxiliary power generating system is in place to provide emergency power for hazardous or sensitive operations.
- Laboratory storage areas provide proper storage of samples, standards, and reagents.
- Storage areas of sufficient size are present in the laboratory to ensure that glassware and portable instrumentation are properly stored.
- Separate areas are maintained for incompatible activities, and measures are taken to prevent cross-contamination.
- The laboratory areas are separated from other sections in the building such as Administration Services, Lunch Room, and Conference Rooms.
- Any additional laboratory conditions for specified analysis conditions are met, including hazardous biological and chemical material.
- Laboratories are cleaned and maintained according to a schedule.
- Supervisors implement environmental control programs in the laboratory.
- Supervisors recognize when environmental conditions are not met and adversely affect tests being performed. Analysis is not performed if monitoring reveals that required environmental conditions are not met.
- Laboratory employees plan and conduct laboratory operations in designated areas.
- Laboratory employees identify and suggest implementing any environmental controls needed to complete sampling and analysis, and ensure that these factors do not adversely affect the quality of test results.
- Laboratory employees properly handle and store hazardous waste as defined in the Hazardous Waste plan.

Internal Audits

Internal audits are a key element of any quality system. Their objective is to evaluate activities and existing documentation to check whether these meet predetermined internal and/or external standards and/or regulations or customer requirements. Besides checking compliance with internal and external standards, there is a second and even more important aspect of internal and external audits: they can be used to help improve processes and to establish a better system for the benefit of laboratory owners, employees and customers. If the procedure is done correctly, laboratory departments can learn extensively from auditors and inspectors because, as outsiders, they may contribute useful expertise and tips on how to improve certain quality and efficiency aspects.

Generally a laboratory is not audited for all items at once. Over a certain period of time, however, all items should have been checked in all laboratories. Therefore, audits should be conducted in accordance with a long-term plan. The objective is that all departments or laboratories are audited on all items over the planned period. Priorities of the audits can also be set based on current trends and regulatory focus.

There are two ways to achieve comprehensive coverage: the horizontal and the vertical approach. Using the horizontal approach, all departments are audited in detail for the same item at one particular time, e.g., for organization and analytical methods or equipment. In a subsequent audit, other items are checked. In a vertical audit, all or a selected number of different items are checked at one particular time. In practice not all laboratories are audited at the same time, but one after another according to an audit schedule.

3.3 Specific Quality Requirements Related to Product Testing

Sampling

GxPs require that sampling should be performed according to a sampling plan, and all sample details should be documented. Sampling of substances, materials, or products for subsequent testing should follow a well-documented procedure. Inspectors want to see a description of the sampling system, how sampling is performed, by whom and, if relevant, are SOPs available at the sampling location. Other requirements include:

- Sterile equipment and aseptic sampling techniques shall be used when necessary.
- The sample should be representative for each lot or batch.
- Sampling plans shall, whenever reasonable, be based on appropriate statistical methods.
- Cross-contamination from sample to sample should be avoided during sampling and sample transport and storage.
- Special care should be taken when sampling biopharmaceutical products so that the process stream is not contaminated through the sampling process.
- Samples should be uniquely identified and the sample integrity should be protected during transport and storage.
- Sampling data should be recorded, such as sampling procedure used, location, and the identification of the person who took the sample, equipment used for sampling, and environmental conditions, if relevant.
- A specific GMP requirement is to take enough reserve sample. The purpose is that these samples should be available in case the product testing and result are not in specification. Depending on the failure investigation result, the sample may have to be reanalyzed.

Handling of Test Items

Laboratories should ensure proper identification and protection of samples from the time the sample is taken until its disposal.

Receipt, protection, storage, processing, retention, and disposal should be described in a procedure. The procedure should include provisions for protection against deterioration, loss or damage during transportation, handling and storage. When samples require special environmental conditions for storage, environmental conditions should be controlled and monitored. A procedure should be developed for adequate sample handling. The procedure should ensure that:

- Each sample is uniquely identified through a sample number or code. The sample number is used to track the sample from the time the sample is collected until the analysis is completed. The sample number is also used to provide traceability between the sample and test results.
- When the sample arrives in the laboratory, it is physically inspected and abnormalities or any other special observations are recorded. The sample condition is compared with previously defined conditions and, in case of discrepancies, the laboratory consults the manager of the department where the sample came from. After consultation, it is decided whether to proceed with the test or take a new sample.
- Procedures for processing of samples are defined for each sample and followed.
- An appropriately identified reserve sample that is representative of each lot in each shipment is retained.
- The reserve sample consists of at least 1.5 times the quantity necessary for all tests to determine whether the product meets its established specifications.
- The reserve sample is stored in the same immediate container-closure system in which the drug product is marketed or in one that essentially has the same characteristics.

Testing

Each single test and series of tests should follow documented procedures. The procedure should ensure that:

- Only methods suitable for their intended use should be used for testing.
- Only qualified equipment is used for testing.
- Testing is performed by qualified people.
- The system is calibrated before the first sample run and within a series of runs sufficient calibrations are performed.
- Sufficient system suitability tests are conducted.
- Acceptance criteria are specified for each test.
- Test results are documented according to a protocol.
- Test procedures and parameters are documented with sufficient detail so that sample runs, including data evaluation, can be repeated based on this documentation.
- The quality of test results should be monitored.

Handling Out-of-Specification Test Results

The FDA requires that an investigation be conducted whenever an out-of-specification (OOS) result is observed. This includes laboratory testing during the manufacture of APIs, raw material and testing of finished products to the extent that GMP regulations apply. It also includes stability testing. OOS results include those that fall outside the specifications or acceptance criteria established in New Drug Applications, Biologic License Application, official compendia, or by the manufacturer. In case of an OOS situation, a failure investigation should be initiated. A laboratory should have an SOP to handle out-of-specification situations and failure investigations.

FDA and EMA also require conducting formal failure investigations for out-of-trend (OOT) situations. This is mainly to reduce or avoid out-of-specification (OOS) situations[26].

Data Validation and Reporting of Results

Tests results should be signed by the analyst and reviewed and approved by a second person. A reviewer can be the analyst's supervisor or a member of the QA staff. Review and reporting of test results should follow an SOP to ensure that:

- The analyst generating the analytical data has the primary responsibility for data correctness and completeness.
- The analyst is responsible for assembling the data package containing all relevant raw data needed for data interpretation and validation for each batch of sample processed. A data package typically includes: quantitative analysis reports, a list of instrument parameters and supporting graphics, for example, chromatograms and spectra.
- The package is reviewed and approved by a second person.

The information to be reviewed depends on the test. For chromatographic tests it should typically include:

- Conformance of test results to written specifications.
- Information on sampling and test method.
- Information on compound identity and strength.
- Completeness of the report header.
- The initials or signature of the person who performed the tests and the date(s) the tests were performed.
- Completeness of supporting material, e.g., chromatograms.
- Criteria for calibration and retention time windows.
- Proper documentation of manual integration results.
- Complete records of any modification of a method employed in testing.
- All calculations performed in connection with the test, including units of measure, conversion factors, and equivalency factors.
- Quality control or system suitability data.
- Electronic audit trail.
- Documentation of irregularities.

Reagents and Solutions

To ensure ongoing quality of reagents and solutions used for GxP studies, purchasing and testing should be handled by a quality assurance program. This should also include qualification of suppliers.

All reagents and solutions in the laboratory areas should be labeled to indicate identity, titer or concentration, storage requirements, and expiration date. Deteriorated or outdated reagents and solutions should not be used.

The expiration date depends on the nature of the chemical. Sodium chloride has practically no expiration date. In these cases it might be acceptable to indicate NONE or not applicable (N/A) on the label for expiration date. The laboratory must be prepared to justify this designation. Formal studies are not always required to justify assigned expiration dates. It is sufficient to assign expiration dates based on information from the supplier, literature references, and/or laboratory experience.

(Certified) Reference Material

Each laboratory should have a quality assurance program for reference material and standards, which should be part of the company or laboratory quality plan.

Steps in this program should include procedures for:

- The qualification of the supplier. Certification of ISO 9001 or an equivalent standard is strongly recommended; otherwise a direct audit is required.
- Frequency and types of checks of incoming material.
- Checks can include verification of identity and quantity.
- Registration of the material in a database.
- Handling and storing of the material.
- Preparation of internal reference material (IRM) and working standards from purchased material.
- Labeling, e.g., expiration date, storage conditions, toxicity.
- Regular checks of the material, e.g., for purity and amounts.
- Reference, primary, and working standards should be subjected to periodical intermediate checks according to a defined procedure.
- Actions to be taken in case the acceptance criteria are not met.
- Incoming tests when the reference material has been prepared and delivered from another laboratory in the same company (this also requires some checks).
- Disposal of used material.

For biopharmaceutical product testing, reference standards are often not commercially available. ICH Q6B recommends in this case an appropriately qualified in-house primary reference standard should be available, which should be prepared from lot(s) representative of production and clinical materials. An in-house working reference standard should be calibrated against the primary standard.

Retention and Retrieval of Records

GxPs have several paragraphs with details on how to store and retrieve records and data, for example, what should be archived and retention time.

The list of documents that should be archived includes everything from raw data to final results, but also protocols from meetings, if decisions related to the integrity of a study have been made.

GLPs require the assignment of a position for an archivist. This is either a part-time or full-time employee who is responsible for the archive. Some companies have a procedure that requires documents from an archive to only be checked out by the archivist or his designate. Whenever documents are taken out of the archive this should be documented, and the person who requests it should sign a statement that nothing has been changed, added, or deleted.

GLPs also specify for how long records and specimens should be retained. For example, in the US, material supporting FDA submissions should be retained until:

- two years after FDA approval, or
- five years after FDA submission.

However, this applies only for US FDA and retention times in other countries may be different. Two and five years may not look like a long time. However, two years after FDA approval and five years after an FDA submission can be a long time. Sometimes it may take ten or more years between the time GLP studies have been conducted and marketing is approved by the FDA. Required retention times for QC test records are shorter. According to most GMP regulations, required retention times are one year after the drug's expiration date. This typically means 6–7 years.

Equipment

GxPs require that analytical instruments used in generation, measurement, and evaluation of analytical data should be suitable for their intended use. This means instruments should be well designed, qualified, calibrated, or checked to ensure compliance with pre-determined specifications. Like with many other things, also in this area different organizations use different terms for the same task. Let's take the example of checking the precision of amounts against HPLC performance specifications. This is what usually is referred to by industry as equipment qualification. FDA terminology calls it equipment calibration, but inspectors ask for equipment qualification protocols. USP on the other hand calls it instrument qualification. This primer will use the USP terminology, which is widely used in the industry since the release of USP chapter <1058>.

The standard process for analytical instruments has been defined in the USP chapter "Analytical Instrument Qualification"[22]. It has not been created specifically for equipment used in biopharmaceutical laboratories, but the concept can be adapted accordingly. This section will give a brief overview on the USP process for qualification and maintenance. More detailed information is available in the primer from Agilent Technologies: Analytical Instrument Qualification and System Validation[5].

Analytical Instrument Qualification According to USP

Equipment qualification and validation of computerized systems cover the entire life of a product. It starts when somebody has a need for a specific product and ends when the equipment is retired. Because of the length of time and complexity, the process has been broken down into shorter phases, called lifecycle phases. Several lifecycle models have been described for qualification and validation. USP chose the 4Q model, which is widely used in pharmaceutical laboratories. The process is illustrated in Figure 7.

Figure 7 Analytical instrument qualification according to the 4Q model.

The entire qualification process is broken down into four qualification phases: design qualification (DQ), installation qualification (IQ), operational qualification (OQ), and performance qualification (PQ). The whole process for a specific project is outlined in the qualification plan and the results are summarized in a qualification report.

In the DQ phase the user writes requirement specifications for the equipment. This includes all functions the instrument should have and the performance specifications the equipment should meet as required for the intended application. Next the user compares his/her specifications with the vendor's specification sheet. As long as the vendor's specifications are equal to or better than what the user requires, the design is qualified for the intended use.

Also included in the DQ phase is a formal vendor assessment. This can be made based on experience with the vendor, through a mail audit or through a direct audit. The instrument is purchased and installed in the laboratory. During the IQ phase the shipment is compared with the purchase order for completeness and the vendor's installation instructions are executed. This may also include a check if the laboratory conforms to the vendor's environmental specifications, for example, humidity and room temperature. At the end of the installation process, the IQ protocol is completed recording the vendor, model, serial number, and other relevant information.

After the IQ phase has been completed, the instrument is tested against the functional and performance specifications as defined in the requirement specifications document. These OQ tests can be performed by a vendor representative or by the user. In any case, a user representative has to sign the OQ document. The instrument's OQ is repeated at regular intervals.

Requalification frequency depends on the instrument itself, the recommendations from equipment manufacturers, laboratory experience, and the extent of use. For instance, a pH meter should be calibrated before each use and the wavelength of an HPLC variable wavelength detector should be calibrated about every month or whenever the cell is removed and reinstalled. Typically, a complete chromatographic instrument OQ performance test should be done every 6 to12 months.

Also, in between the regular QQs the instrument performance should be checked on a day-by-day basis or whenever the instrument is used. This PQ test should be application specific and should focus on performance parameters that are most critical and are most likely to change over time. An example is the column performance in liquid chromatography and the UV detector baseline noise. Corresponding tests can be combined with system suitability tests (SST) that are required by USP to check the system performance for a specific application.

Validation of Software and Computer Systems

Software and computer systems used in GxP regulated environments should be validated. A similar approach is used as the one for equipment. The major differences are:

- More focus should be put on qualification of suppliers. Suppliers should provide documented evidence that the development followed a documented process and that the software has been validated as part of this process.
- Whereas for hardware equipment qualification all specifications are verified in the user's environment, this is not required for software. It is enough to verify a relatively small set of key software functions and to perform a complete system test.
- Many times users customize computer systems, for example, through report generators or when setting network configurations. Users should include these configurations in the requirement specifications document and should verify that the functions work properly.

More information on validation and examples of software and laboratory computer system validation is included in Reference 5.

Equipment Records and Other Documents

Written records should be maintained of all inspection, maintenance, testing, calibrating and/or qualification/validation operations. These records, containing the date of operation, should describe whether the maintenance operations followed written SOPs.

Written records should be kept of repairs performed on equipment as a result of failure and malfunction. Such records should document the nature of the defect, how and when the defect was discovered, and any remedial action taken in response to the defect. Remedial action should include a review of effects on data generated before the defect was discovered. All such records should be entered in an equipment logbook. The logbook should be maintained as long as the analytical data generated by the equipment.

(Preventive) Maintenance

Analytical instruments should be well maintained to ensure proper ongoing performance. Procedures should be in place for regular preventive maintenance of hardware to detect and fix problems before they can have a negative impact on analytical data. The procedure should describe:

- When maintenance should be done.
- How it should be done.
- What should be re-qualified after maintenance is done. For example, a PQ test should always be performed after instrument maintenance.
- How to document maintenance activities.

Planned maintenance activities should follow a documented instrument maintenance plan. Some vendors offer maintenance contracts with services for preventive maintenance at scheduled time intervals. A set of diagnostic procedures is performed and critical parts are replaced to ensure ongoing reliable system uptime. Unplanned activities that are necessary in addition to the planned activities should be formally requested by the user of the instrument or by the person who is responsible for the instrument. The reason for the requested maintenance should be entered as well as priority. All maintenance activities should be documented in the instrument's logbook.

Handling Defective and Non-Qualified Instruments

Defective and non-qualified instruments should be either removed from the laboratory area or clearly labeled as being defective or not qualified. Procedures should be available for most common problems, such as defective UV detector lamps. Procedures should also include information if and what type of requalification is required. Uncommon problems, for example, if an HPLC pump becomes defective without any obvious reason, should be handled through a special procedure that guides instrument users through the repair process and reinstallation. In this case, the impact of the failure on previously generated data should be evaluated.

3.4 Validation of Analytical Methods

GxPs require analytical methods to be validated to demonstrate suitability for their intended use. All methods used to check the quality, efficacy, and safety of drugs should be validated. Because of the importance of cleaning equipment in biopharmaceutical purification processes, the methods used to verify the effectiveness of cleaning should also be thoroughly validated. The ultimate objective of the method validation process is to provide evidence that the method does what it is intended to do, accurately, reliably, and reproducibly.

Regulatory agencies and other official organizations have developed several documents on analytical method validation. For example, the FDA has published a draft guidance on "Analytical Procedures and Methods Validation"[8] and a draft guidance on "Bioanalytical Method Validation"[9]. USP has a chapter "Verification of Compendial Methods". The reference document for validation of analytical methods is the ICH Q2(R1) guide "Validation of Analytical Procedures: Definitions and Methodology". All these official documents have been developed for validation of methods used for analysis of small molecules. The title of the FDA guidance on "Bioanalytical Methods Validation" may indicate that it applies to validation of biotechnology-derived products. However, the scope is the analysis of biological fluids, such as blood, and is recommended for use in preclinical and clinical studies. The concepts of the guidance cannot be transferred to biopharmaceutical quality control.

Currently it seems that there is no official guidance available for the validation of methods used for characterization of biopharmaceutical products and no such guidance is expected to come in the near future. It also is a fact that other than some relatively low molecular weight peptides, a full characterization of biopharmaceutical products is frequently impossible. Swartz and Krull[33] discussed this in detail. They also came to the conclusion that existing validation guidances have limitations in respect to the validation of methods used for biopharmaceutical drugs. For example, often reference standards are not available for accuracy determinations, which makes it difficult or impossible to quantitate impurities. And even with today's highly sophisticated instruments based on hyphenated techniques, it is not always possible to separate all proteins. The FDA seems to accept the limitations and does not expect the same clear validation results for complex protein biopharmaceuticals as for small molecule pharmaceuticals. The expectations are that unavoidable deficiencies of the ICH protocol requirements are documented and justified. Nevertheless, the industry is using the ICH Q2 guidance as reference to the fullest extent possible. This section will give a brief overview on method validation according to this guidance. More detailed information is available in the Agilent primer "Validation of Analytical Methods"[6]. For example, it includes definitions and detailed test parameters for all validation characteristics as required by ICH Q2.

Validation Parameters for Target Applications

The ICH Q2 describes validation parameters and gives recommendations on methods for validation. Validation parameters are listed in Figure 8. Robustness is not included in this list, but ICH expects tests to be done during method development. The FDA and other agencies expect that related robustness tests are included in the method validation package.

The concept of ICH is that it is not always necessary to validate all analytical parameters as listed in Figure 8. For example, if the method is to be used for qualitative trace level analysis, there is no need to test and validate the method's limit of quantitation, or the linearity, over the full dynamic range of the equipment. The extent of validation also depends on the lifecycle phase of the drug. While agencies expect full validation in clinical Phase III and for drug manufacturing control, most time-consuming tests, such as intermediate precision, reproducibility, and ruggedness are most likely not necessary in preclinical studies and for Phase I clinical studies. However, a statement is expected why the manufacturer believes that the method is suitable for its intended use.

According to ICH Q2, the selection of validation parameters and acceptance criteria should be based on regulatory requirements and should be justified and documented. ICH defines four different types of analytical procedures to be validated:

- Identification test
- Quantitation tests for impurities content
- Limit test for the control of impurities
- Quantitative tests of the active ingredient or other main components of the drug

Accuracy, any type of precision, and limits of detection and quantitation are not required if the analytical task is identification. For assays the major component or active ingredient to be measured is normally present at high concentrations; therefore, validation of limits of detection and quantitation is not necessary. For quantitative impurity tests all parameters should be validated apart from limit of detection. Limit tests only require validation of specificity and limit of detection.

Analytical Task	Identification	Impurity Testing Quantitative	Limit Tests	Assay
Accuracy	No	Yes	No	Yes
Precision				
Repeatability	No	Yes	No	Yes
Intermediate precision	No	Yes	No	Yes
Reproducibility	No	Yes	No	Yes
Specificity	Yes	No	Yes	Yes
Limit of detection	No	No	Yes	No
Limit of quantitation	No	Yes	No	No
Linearity	No	Yes	No	Yes
Range	No	Yes	No	Yes

Figure 8 ICH validation characteristics. (For a detailed description of terminology and methodology see Reference 19).

Strategies for Method Validation

The validity of a specific method should be demonstrated in laboratory experiments using samples or standards that are similar to unknown samples analyzed routinely. The preparation and execution should follow a validation protocol, preferably written in a step-by-step instruction format. Just like equipment qualification and computer system validation, method validation also is not a one-off event. It starts when somebody wants to implement a new method in a laboratory and ends when the method is no longer used. Because of the length of time and complexity, the process is broken down in phases. The process is illustrated in Figure 9.

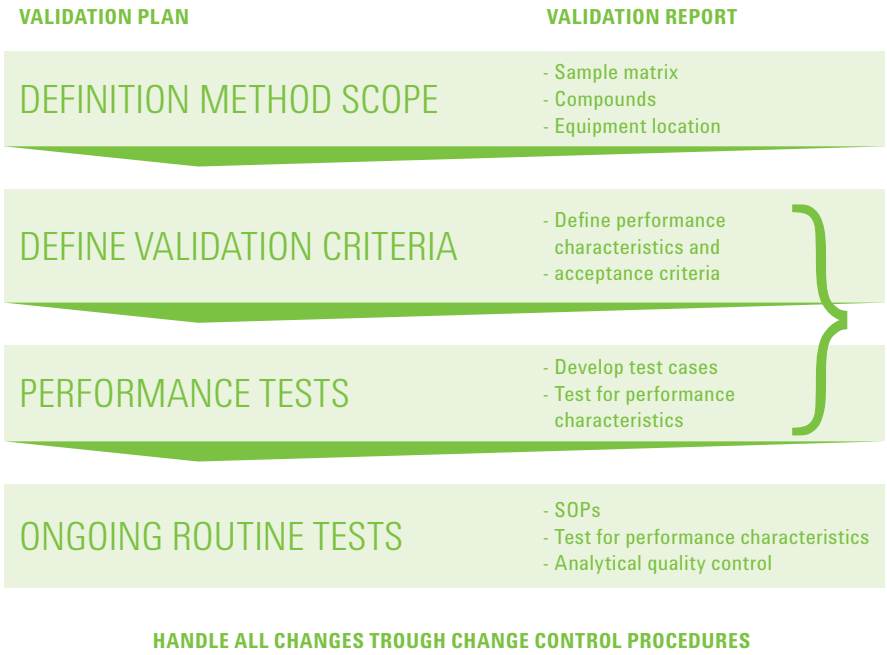

Figure 9 Method validation process.

First we develop a validation plan including owners, responsibilities, and deliverables. Next the scope of the method is defined. This includes the compounds with concentration range, the sample matrix, the specific equipment that should be used, and the location where the method should be used for sample analysis. Once we know what should be analyzed, performance characteristics, performance tests, and acceptance criteria are defined. Then test protocols are developed with all experimental details and tests executed according to the test protocols. Tests results are compared with acceptance criteria. As a last step, procedures are developed to use the method routinely and to verify ongoing system performance at the time of analysis. Tests may include system suitability testing and/or the analysis of quality control samples. All experimental conditions and validation results are documented in a validation report.

Verification of Compendial Methods

Laboratories working in regulated environments are recommended to use official methods as those developed and validated by recognized organizations such as the American Society for Testing and Materials (ASTM), or the USP.

For example, the US Food, Drug & Cosmetic Act requires FDA regulated industries to use compendial methods or demonstrate equivalency. These methods are validated so many analysts believe that the method can be used as it is without any further validation, verification or testing done in the laboratory. This is a wrong assumption. The FDA GMP regulation states in 21 CFR 211:194 a: "If the method employed is in the current revision of the United States Pharmacopoeia, or in other recognized standard references, or is detailed in an approved new drug application and the referenced method is not modified, a statement indicating the method and reference will suffice. The suitability of all testing methods used shall be verified under actual condition of use".

This makes it clear that official methods do not need to be validated as long as they are unchanged, but the laboratory should demonstrate that it is capable of successfully running the method. The question is how to do this: should some or all validation experiments be repeated, or are successful suitability tests or the analysis of quality control samples enough?

Help came from the USP through its chapter <1226>: Verification of Compendial Methods[23]. The given recommendations are not only useful to implement compendial methods but are useful for any method. The key recommendations are:

- Demonstrate the performance of the laboratory and system through system suitability tests.
- Assess the criticality and complexity of the method.
- Select the most critical performance characteristics of the method.
- Depending on the criticality and complexity of the method, repeat one to three of the most critical validation experiments.

Like the validation of methods developed in-house, the evaluation and verification of standard methods should also follow a documented process e.g., a validation plan or an SOP. Results should be documented in the validation protocol.

Transfer of Analytical Methods

When validated methods are transferred between laboratories, the receiving laboratory should demonstrate that it can successfully run the method. Typical instances of method transfers occur from the research and development (R&D) laboratory to quality control (QC), or from site A to site B, when a product line is moved, or from a sponsor company to a contract laboratory or from company X to company Y, when a product is purchased by another company. Until 2012, there was no official document available that could be used as a guidance on how the performance of the receiving laboratory could be demonstrated. This changed when USP released the general chapter <1224> "Transfer of Analytical Procedures"[24]. In 2014, a section on analytical method transfer was also added to chapter 6 of the EU GMP guide[26].

According to USP <1224>, successful method transfer can be demonstrated by several approaches. The most common one is comparative testing where a well-characterized sample is tested in the sending and in the receiving laboratory.

The key recommendations for implementation are:

- The transferring unit reviews the original validation package for compliance with ICH Q2 and performs missing validation experiments prior to the transfer process.
- The transferring laboratory defines one or more well-characterized samples and documents method parameters and acceptance criteria, for example, for accuracy of the method. The sample should cover the complete range as specified when the method was originally validated.
- Analysts from the transferring laboratory train analysts from the receiving unit.
- The samples are analyzed in the receiving laboratory and the results are compared with the acceptance criteria.
- The extent of testing and other transfer activities, and the implementation strategy should be based on risk assessment that considers the previous experience and knowledge of the receiving unit, the specifications of the product, and the complexity of the analytical procedure.
- Acceptance criteria for the transfer process should be based on studies executed during the original validation of the method.

Other approaches besides comparative testing include co-validation between sending and receiving laboratories, the complete or partial validation of the analytical procedures by the receiving unit according to ICH Q2, and the transfer waiver, which is a well-justified and documented omission of the transfer process.

New Developments

Since the release of ICH Q2 in the mid nineties, method validation processes have been well established without any significant change. This is changing now. Regulatory bodies and industry task forces proposed new concepts for method development and validation. This chapter will give an overview of two subjects: Quality by Design (QbD) and recommendations from new the FDA guidance and validation of analytical methods[8]. Both subjects are not regulations but show the trend in method development and validation, and may become part of formal guidance at any time.

Integrated Lifecycle and Quality by Design for Analytical Method Development and Validation

Quality by Design has long been used throughout the industry, e.g., the automotive industry. The concept has been adopted by the pharmaceutical industry since about 2002 when the US FDA promoted the concept as part of the Pharmaceutical cGMPs for the 21st Century program. The FDA has since initiated several follow-on initiatives:

- 2005: Implementation of a QbD pilot program to allow industry to submit information for a new drug application demonstrating use of QbD principles, product knowledge and process understanding.
- 2006: Merck & Co.'s Januvia became the first FDA-approved product based on QbD.
- 2007 and later: FDA presentations on QbD for analytical laboratories
- 2011: FDA and EMA announced a Collaborative QbD Application Review Pilot (extended in 2013)
- 2013 and beyond: FDA requires QbD elements in ANDAs and NDAs

The most important regulatory document related to QbD is ICH Q8: Pharmaceutical Development. The document defines QbD as "A systematic approach to development that begins with predefined objectives and emphasizes product and process understanding and process control, based on sound science and quality risk management".

This means in practice:

- The medicinal product is designed to meet patient needs and performance characteristics.
- The product is designed to consistently meet critical quality attributes.
- The impact of starting raw material and process parameters is understood.
- The process is evaluated and updated to allow for consistent quality over time.
- Critical sources of process are identified and controlled through appropriate control strategies.

Initially, the QbD focus of the FDA and the industry was on pharmaceutical development. However, a few companies reported on the application of QbD in analytical laboratories and FDA professionals presented on this topic at conferences. The highest application attention did get the integrated lifecycle approach for design, development, validation and routine use of analytical methods. With a slight modification, the ICH Q8 QbD definition can be easily adapted to analytical methods. In contrast to the ICH Q2, which is considered a one-time event and ensures the validated state right after initial validation and revalidation, QbD ensures long-term performance.

With the traditional ICH Q2-based validation process there is insufficient emphasis on robustness testing. As a result, there is also poor knowledge of critical parameters, which becomes especially obvious during method transfer. With QbD, methods are designed, developed and validated for highest reliability during routine use. This also means the method will work consistently within its design space, or within its target profile, no matter who the analyst is, which material is used (e.g., columns from different batches), and where the method is used. To maximize efficiency, QbD experiments only focus on critical operational parameters as defined by subject matter experts through risk assessment.

Key benefits of QbD for analytical methods include:

- Understanding, reducing and controlling sources of variability.
- Reducing analytical method-related out-of-specification and failure investigations.
- Lowering failure rates during method transfer.
- Facilitating continual method improvement.
- Eliminating regulatory re-approvals after changing method parameters within the predefined design space.

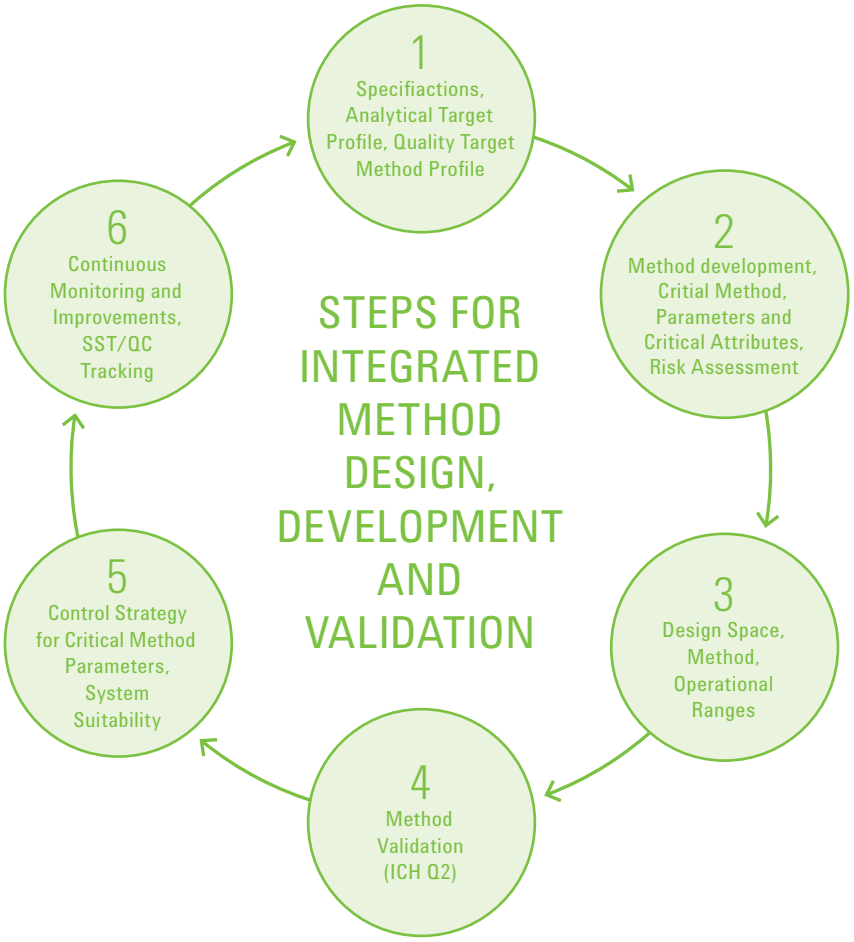

Figure 10 Steps for integrated method design, development, and validation.

Figure 10 illustrates the six-step QbD process as recommended for development and validation of analytical methods. Step one involves writing the design specifications known as the analytical target profile (ATP) and quality target method profile. These describe the performance criteria to which the method should conform. Examples of performance characteristics include the method's precision, accuracy, specificity, limit of quantitation, and linearity, Inputs typically come from the drug product development team, but may also come from quality control laboratories. Inputs for the method operational intent come from the end-user department, and include criteria such as ease-of-use, analysis cycle time, and acceptable solvents.

In step two, the method is designed and developed, critical method parameters and critical method attributes are identified, and a risk assessment is performed. Design elements include test conditions and material attributes such as sample matrix, sample stability, and sample solubility. The allowed environmental conditions such as humidity and temperature are also defined, as well as random effects such as different analysts, different instruments, and timing (e.g., day vs. night shift).

All design elements are sources of variability that can impact critical method attributes. The variables are documented in a fishbone-like diagram, and the impact of the variables on the critical method attributes is estimated through a risk assessment (Figure 11). The risk assessment is performed by a team of subject matter experts, e.g., in a brainstorming meeting. The team goes through the list of input parameters and identifies parameters that impact method performance. The outcome is a risk prioritization matrix that ranks the risk as high, medium or low.

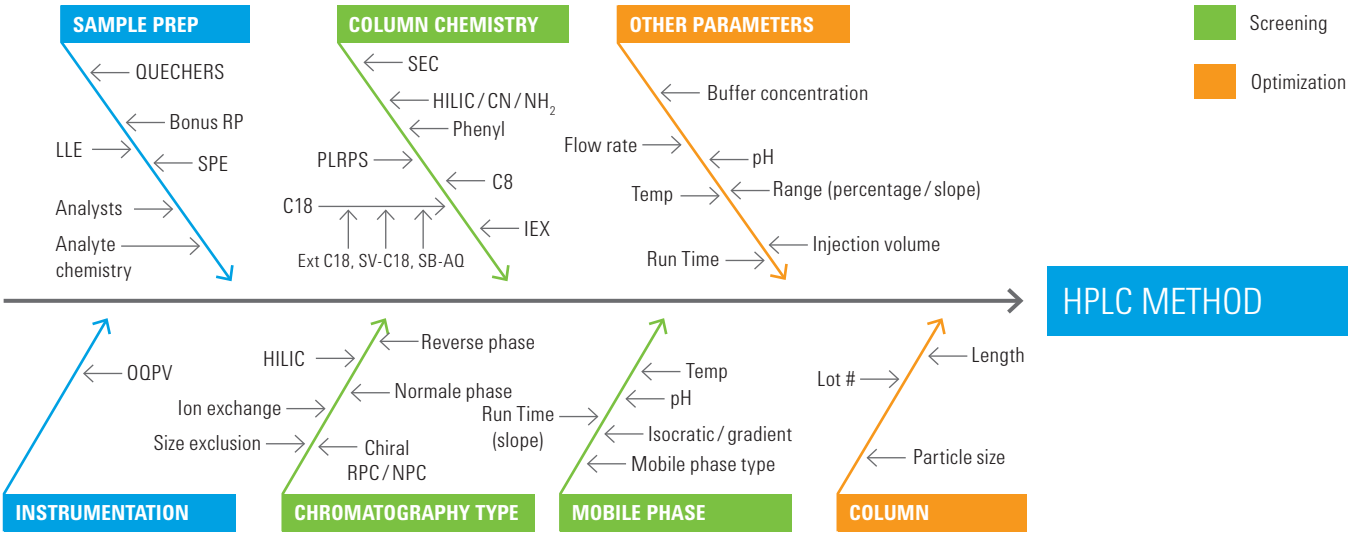

Figure 11 Example of a fishbone diagram showing input variables for the risk assessment.

Step three determines the design space and method operational ranges through Design of Experiment (DoE) studies. Critical method parameters and critical method attributes identified in step two as high risk are selected as variables for the DoE studies employing a multivariate experimental design approach. While the traditional approach alters two or more variables (e.g., % organic phase and column temperature in HPLC) individually, DoE studies change both variables at the same time to determine the impact on the critical method attributes.

Step four is the validation step, which verifies that the method conforms to the analytical target profile as defined in step one. The studies must follow validation requirements as defined in ICH Q2, if this is required or expected by regulations. A prerequisite for these studies is that equipment must be qualified, for example, according to USP chapter <1058>.

The purpose of step five is to control critical method attributes in order to assure that the method remains in a state of control. Examples for related activities are regular system suitability test runs with selected critical test parameters based on risk assessment and on the design space experiments. Running a certain percentage of the total runs as quality control samples is another alternative.

The last step is to continuously monitor the performance of the method during routine use. Method trend analysis on method performance should be performed at regular intervals to evaluate the need to optimize or change the analytical procedure. The appropriateness of the analytical methods should be periodically evaluated. Related information is obtained through actively collecting inputs from operators on the reliability and performance of the method, from customer complaints, from the outcome of regular method reviews and from tracking and trending results from quality control sample analyses and system suitability tests. Collected inputs should also include suggestions for improving the method.

Identified poor method performance, such as insufficient resolution between two peaks and insufficient method precision, should be addressed through appropriate follow-up. Related activities include identification of the root cause of poor performance, suitable corrective actions and preventive actions. If the method needs to be changed in order to deliver appropriate performance, and the required parameter to be changed is outside the method operational ranges, the process starts again at step 1 of Figure 10.

Recommendation from FDA New Draft Guidance on Method Validation

The guidance with the title "Analytical Procedures and Methods Validation for Drugs and Biologics[8]" was introduced as a draft in February of 2014. Once final, it will replace to current draft guidance from 2000 entitled "Analytical Procedures and Methods Validation". This sub-chapter provides an overview on the new guidance, with specific focus on what is new compared to the draft guidance from 2000.

The guidance complements the International Conference on Harmonisation (ICH) guidance Q2: Validation of Analytical Procedures: Text and Methodology (Q2(R1) for developing and validating analytical methods. It clearly states that ICH Q2 (R1) is considered the primary reference for recommendations and definitions on validation characteristics for analytical procedures. ICH validation characteristics are listed without any further information on test experiments and acceptance criteria.

The guidance is very much in line with modern approaches for method developments and validation; for example, it includes chapters on "Method Development" and "Lifecycle Management of Analytical Procedures." Both chapters together recommend the approach of Integrated Lifecycle Management for the design, development, validation and ongoing use of analytical methods. The Life Cycle Management chapter has subchapters on revalidation, on using alternative analytical procedures and on analytical method transfer. It is interesting that the guide only recommends revalidation after changes, e.g., after changing method parameters, equipment, and perhaps the manufacturing process, but it is silent about time-based revalidation, a practice frequently used by many companies. It is clear that time-based revalidation is replaced by the control and monitoring strategy that is part of Integrated Lifecycle Management.

The subchapter on method transfer refers to the USP chapter <1224> for additional guidance on this topic, but also has a recommendation that goes beyond this chapter. Related to comparative testing for stability indicating methods, it states "In cases where the transferred analytical procedure is also a stability indicating method, forced degradation samples or samples containing pertinent product-related impurities should be analyzed at both sites."

The guide does not specifically spell out the term "Quality by Design", but has several recommendations to implement items that are part of QbD, such as risk assessment, design of experiments, critical quality attributes, and multivariate experiments.

The method development section emphasizes robustness testing e.g.: "During early stages of method development, the robustness of methods should be evaluated" and "To fully understand the effect of changes in method parameters on an analytical procedure, you should adopt a systematic approach for method robustness study. You should begin with an initial risk assessment and follow with multivariate experiments."

The development section includes a statement that has triggered emotional discussion in method validation meetings. On the subject of submitting development data, it states "You should submit development data within the method validation section if they support the validation of the method". This practically means that analytical method development can fall in the category of a regulated activity, with all consequences, for example, the type and extent of documentation.

4 QUALITY CONTROL OF BIOPHARMACEUTICAL PRODUCTS

Like all other pharmaceutical products, also biopharmaceutical APIs and finished products undergo rigorous quality control in order to confirm conformance to pre-determined specifications. Products must be tested for identity, impurities, quantity, e.g., protein content, and stability to ensure safety and efficacy of the biopharmaceutical products. Thorough quality control is also required by all GMP regulations and is frequently subject to regulatory inspections.

Biopharmaceutical QC laboratories follow the same quality assurance principles as outlined in the previous chapter. For example, ICH Q6B requires analytical methods to be validated and either commercial or in-house standard reference material should be used for quality control. Products produced by biotechnology are regulated by the same GMPs as classical products. When necessary, special regulation and guidance documents have been developed to cover specifics of biopharmaceuticals, for example, FDA 21 CFR 610 and ICH Q6B.

The fundamental difference between QC of traditional pharmaceuticals and biopharmaceuticals lies in the methods that are used to determine the product characteristics: identity, potency, purity, and impurity profile. The reason is that the product characteristics are significantly different.

Because of higher complexity, the analysis of biotechnology-derived products involves more sophisticated analytical procedures. Molecules of proteins are an order of 2–3 times larger than molecules of traditional drugs. APIs are derived from living cells and typically include a complex pattern of product- and process-related impurities. In addition proteins undergo complex post-translational modifications, have a highly specific three- or sometimes four-dimensional structure, and have the potential for aggregation and/or adsorption.

In traditional pharmaceutical QC laboratories, relatively simple routine instruments such as HPLC with UV variable wavelength detection are used; in contrast, biopharmaceutical QC labs use sophisticated equipment only found in research and development of traditional drugs.

This chapter will give an overview of:

- ICH Q6B recommendations for product characterization.
- Analytical techniques used in biopharmaceutical QC laboratories.
- Specifications and acceptance criteria.

4.1 ICH Q6B Guidance – Overview

A comprehensive characterization profile is important for quality control of biopharmaceuticals. It allows setting specifications and acceptance criteria. ICH Q6B provides information for the development of such a profile.

It also has recommendations for setting and justification of specifications. In addition, it discusses considerations for analytical testing. The guide applies to proteins and peptides, their derivatives, and products of which they are components. The scope of the guidance is for proteins that are produced from recombinant cell culture expression systems, but principles outlined in the guidance may also apply to other product types, such as proteins and polypeptides isolated from tissues and body fluids.

The guide lists several options for testing, but does not give specific recommendations. The guidance recommends characterizing a product through physicochemical properties, biological activity, immunochemical activity, purity, and quantity.

Physicochemical Parameters

Physicochemical parameters include a determination of the primary structure, composition, and physicochemical properties. Information on the primary structure can be obtained from a combination of the results from peptide mapping, C- and N-terminal sequencing, and amino acid composition and sequence. Higher-order structures of the protein are also important, because they can significantly impact the activity of the drug. Data about these structures can be obtained by spectroscopic methods such as circular dichroism (CD) and NMR. Additional data on physicochemical characteristics should be collected, such as molecular weight or size, isoform pattern, extinction coefficient, and electrophoretic pattern. Data on identity, homogeneity, and purity can be collected, for example, by various liquid chromatographic methods, MS, or a combination of HPLC-MS.

Biological Activity

Determination of the biological activity is important because it determines the therapeutic effect of the drug. The biological activity data provides information on functionality and indirectly provides information on proper protein folding. Examples of procedures used to measure biological activities include animal-based biological assays, cell culture assays and biochemical assays. The results of biological assays should be expressed in units of activity calibrated against a commercially available or an in-house reference standard. In specific cases, the biological assay to measure the biological activity may be substituted by physicochemical tests. In this case results should be expressed in mass.

Immunochemical Properties

Immunological properties should be fully characterized when an antibody is the desired product. Binding assays of the antibody to purified antigens and defined regions of antigens should be performed to determine affinity, avidity, and immune reactivity. Immunochemical properties of a protein are used to establish its identity, homogeneity, or purity.

Quantity

The quantity of a protein in the product is typically determined by an ultraviolet spectrophotometry method. UV absorbance is best used with very pure proteins, since other proteins may interfere with the test results. The absorbance at the absorption maximum wavelength is determined, and the protein concentration is calculated using an empirical extinction coefficient.

Impurities

Impurities in protein-based drugs can be classified as either product- and process-related impurities. Process-related impurities stem from the cell, the cell culture, or the purification process. These impurities are the DNA and proteins of the host cell, the cell media components such as growth hormones, and substances that will be used in the downstream process or the purification process itself. Examples are enzymes, chemical and biochemical processing reagents, inorganic salts, ligands, and other leachables. Product-related impurities are modifications such as oxidation, isomerization, and post-translational modification such as deamidation, aggregation, and truncation.

4.2 Analytical Approaches

ICH Q6B lists possible analytical approaches for structural characterization, and for physicochemical properties. It also lists potential impurities, their sources and relevant analytical approaches. The guidance has a statement that the listed approaches are not recommendations and new analytical technology and approaches to existing technology are constantly being developed and should be applied when appropriate.

Since the release of the guidance back in 1999, analytical techniques have been substantially improved. For example, Krull and Rathore gave an update on Analytical Tools for Characterization of Biotechnology Products and Processes in 2010[30]. Most noticeable are the introduction of ultra high performance liquid chromatography (UHPLC) as a fast alternative to standard HPLC, introduction of sedimentation velocity analytical ultracentrifugation (SV-AUC) for quantifying low levels of aggregation in proteins as an alternative to size exclusion chromatography (SEC), and the routine use of hyphenated techniques such as UHPLC electrospray ionization (ESI)-MS-MS and size exclusion chromatography-multiangle laser light scattering (SEC-MALS).

Sophisticated software and databases are available to interpret and verify results, for example, the structure of carbohydrates can be determined by comparing actual NMR spectra with those available in a glycan database.[31]

Automated tools for sample preparation have led to an overall increase in sample throughput. Rathore[28] described several situations where he could improve the performance and speed of analyses with advanced technology. For example, through a combination of robotic liquid handling, automated sample preparation and rapid resolution HPLC (RR-HPLC), he was able to reduce the throughput for N-linked oligosaccharide mapping from one sample in five days to 30 samples in 24 hours.

Instrument suppliers introduced bio-inert systems to avoid adsorption of proteins and allow operation of the system over a wide pH range and with high salt tolerances. For example, Agilent Technologies has developed a bio-inert HPLC system with a stainless steel-free titanium-based pump, metal-free autosampler, metal-free detector flow cell, and metal-free capillaries and connections. Figure 12 shows a peptide map with a bio-inert HPLC system. Peptide mapping is a highly specific identity method that can be used to compare the protein structure of a specific lot of a product to a reference standard or to previous lots of the product to show conformance of lot-to-lot consistency.

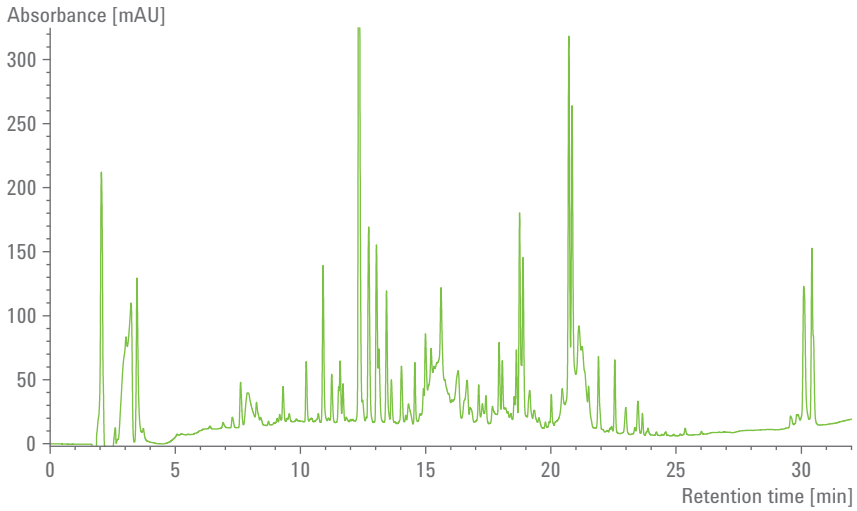

Figure 12 Separation of charge variants of monoclonal antibody using Agilent Bio Mab, 5 µm column, and Bio-inert LC System.

Methodology	Analytical Procedure
Amino acid sequence	Automated Edman chemistry/HPLC or MS/MS
Amino acid composition	Hydrolysis + reversed-phase HPLC post-column derivatization
N-terminal sequencing	Automated Edman chemistry and HPLC analysis
C-terminal sequencing	Combination of peptide mapping + ES-MS/MS, combined with enzymatic digestion and/or MW analysis
Peptide mapping	Fragmentation with selective enzymes or chemical degradation and analysis of the fragments with UHPLC, HPLC, or LC/MS
Disulfide bridges	Peptide mapping, digestion with a suitable enzyme and analysis with LC-ES/MS
Carbohydrate structure	Monosaccharide composition analysis using GC-MS, oligosaccharide pattern analysis using NMR through spectra comparison with known spectra

Figure 13 Methodology and analytical instrumentation for structural characterization and confirmation.

Despite advances in analytical instrumentation, it is still not possible to fully characterize all biotechnology-derived drugs. Therefore, the FDA and industry don't use the term "Fully Characterized Biological Drugs" but "Well Characterized Biological Drugs (WCBD)".

Figure 13 lists analytical approaches for structural characterizations. The list includes approaches from ICH Q6B and newer technologies. The best technical approaches will vary from product to product. Figure 14 lists analytical procedures for measuring physicochemical characteristics of drug substances and drug products.

Physicochemical Property	Analytical Procedure
Molecular weight or size	Size exclusion chromatography, SDS-PAGE, mass spectrometry, sedimentation velocity analytical ultracentrifugation
Isoform pattern	Isoelectric focusing, ion exchange chromatography, HPLC, SDS-PAGE
Extinction coefficient	UV/Vis spectrophotometry Maldi-TOF MS
Electrophoretic pattern (+ data on identity, capillary electrophoresis, homogeneity, purity)	Isoelectric focusing, SDS-PAGE, Western blot, capillary electrophoresis
Liquid chromatographic patterns (+ data on identity, ion exchange chromatography, homogeneity, purity)	Size exclusion chromatography, HPLC, ion exchange chromatography, affinity chromatography
Spectroscopic profiles	UV/Vis spectra Circular dichroism and NMR for higher-order structure

Figure 14 Analytical procedures to measure physicochemical characteristics.

4.3 Specifications for Routine Quality Control

Specifications, as defined in ICH Q6B, are the "list of tests, reference to analytical procedures, and appropriate acceptance criteria, which are numerical limits, ranges, or other criteria for the tests described". Setting, maintaining, and controlling specifications throughout the marketing of a product are requirements of all GMPs. Specifications should be available not only for drug substances and finished drugs but also for raw materials, excipients, in-process testing, and for stability testing. Drug product specifications are ultimately set to protect the patient from receiving a drug that is not fit for use. Specifications should be set by the drug manufacturer and should be justified.

ICH Q6B describes the approach for setting specifications and acceptance criteria but does not give exact numbers or even ranges for acceptance criteria. They are product specific and should be defined by manufacturers. The process to define acceptance criteria should follow documented procedures that preferably should include flowcharts with decision trees.

ICH Q6A has eight such decision trees for different scenarios[29]. Even though Q6A has not been specifically created for biopharmaceuticals, the concept can be transferred to biopharmaceuticals. An example is shown in Figure 15.

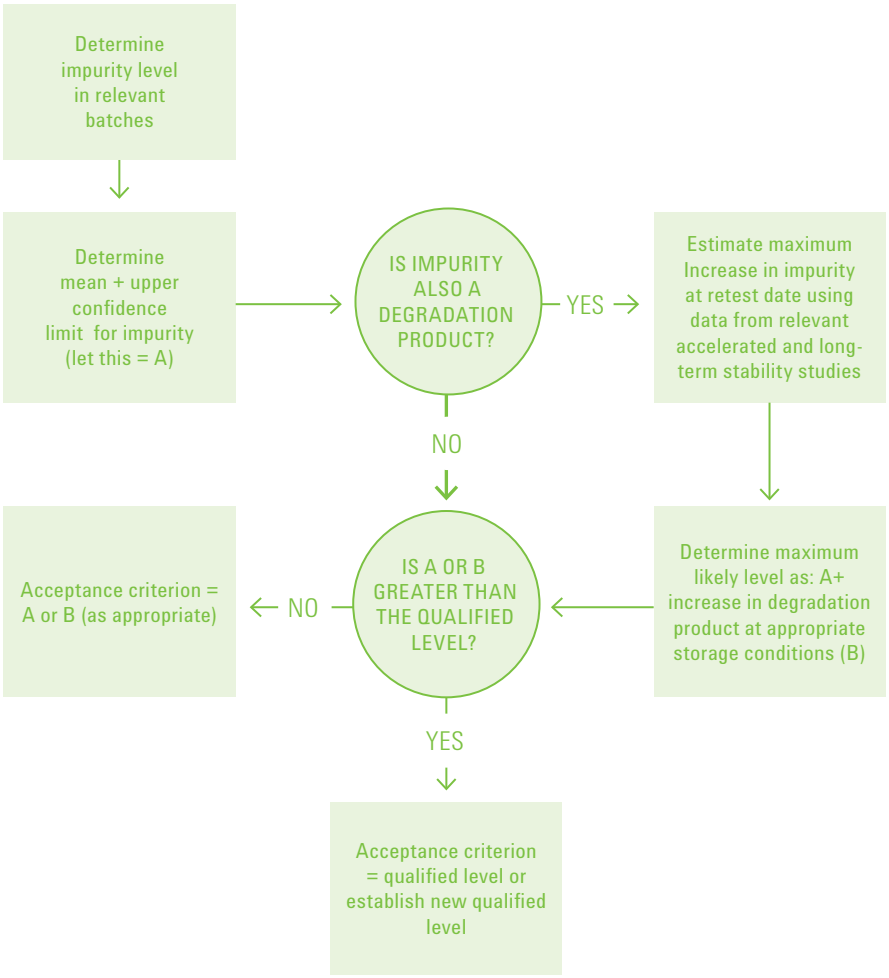

Figure 15 Case study for setting impurity specifications of drug products (taken from Reference 29).

Inputs for Specifications

Specifications and acceptance criteria should be linked to:

- Experience and safety data collected during preclinical and clinical studies. Without good data, especially on the impact of a drug's potency on the patient, safety specifications for potency are meaningless.
- Permitted daily exposure of the drug substance (usually expressed in mg/day). For example, impurity specifications should be set lower for drugs with high daily exposure permitted.
- Duration of the drug administration. For example, impurity specifications should be set lower for drugs that are administered permanently, e.g., to control blood pressure.
- Data obtained from manufactured lots used for preclinical and clinical studies.
- Data obtained from a sufficient number of lots manufactured across a representative range of process conditions used to demonstrate full-scale manufacturing consistency.
- Data collected during validation of the analytical procedure.

- Data collected during stability testing. Specifications used for product release may be different from shelf-life specifications. Release limits are internal or registered limits, which are based on stability studies and predict that the product will be fit for use during the shelf life. An expiry specification is the specification that is based on stability testing and predicts when a product is no longer fit for use. Stability testing should be performed according to ICH Q5C. Release specifications should be set so that the product is fit for use throughout its specified shelf life.
- Regulatory or compendial specifications, if available. These are specifications for which attributes and limits are well-defined.

Other recommendations are:

- Specifications should be set when a drug is filed and should be included in the NDA or BLA. At that time, limited data are available, especially on the constancy of the manufacturing process. Therefore, if more reliable data are available and the manufacturing constancy is improved, with no impact on a product quality such as safety and efficacy, the specifications should be updated.
- When limits on attributes are not completely available from clinical safety and efficacy data, adequate limits should be set that reflect process variation and batch-to-batch reproducibility.
- In addition to product specifications more narrow control and action limits should be set and monitored. Possible quality problems can be identified and corrected before an out-of-specification (OOS) situation occurs. This not only helps to reduce OOS situations and failure investigations, but also contributes to continuous process improvement.

Specifications for Drug Substances and Drug Products

Specifications should be set for drug substances (DS) and drug products (DP). Most specifications are similar but the focus for some specifications is different. For example, impurity testing of the DP refers to product and process impurities, whereas in testing the DS, the focus of impurity testing is on degradation products. The guide recommends checking DP and DS for appearance, identity, purity, impurities, potency, and quantity.

Appearance and General Tests

The physical state (e.g., solid, liquid), the color and other observations (e.g., the presence of visible particles) should be visually inspected and described for DS and DP.

Identity

This test should be highly specific. It should be based on unique aspects of the molecular structure. The test usually is qualitative.

Purity and Impurities

The main objective of DS is to separate the main compound(s) from product and process related impurities. The focus of DP is to separate the main compounds from degradation products that may be generated during storage and from excipients. The determination is usually based on a combination of methods.

Potency

A relevant, validated potency assay should be part of the specifications for DP. An alternative physicochemical and/or biological method may suffice for DS purity tests, if an appropriate potency assay is used for the DP. Similarly, an alternative physicochemical and/or biological method may suffice for the DP, if an appropriate potency assay is used for the DS. However, the rationale for such a choice should be provided.

Quantity

Quantity for DS and DP is usually based on mass content of the protein and determined through an appropriate assay.

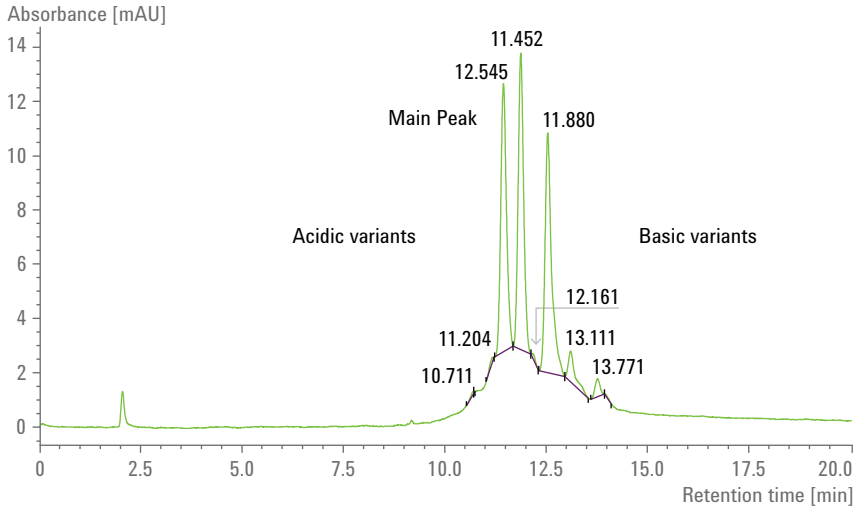

Figure 16 Separation of charge variants of monoclonal antibody using Agilent Bio Mab, 5 µm column, and Bio-inert LC System.

4.4 Methods to Confirm Specifications and Acceptance Criteria

Figure 16 is an example for the separation of charge variants of monoclonal antibody. Figure 17 shows examples of analytical procedures for a complete set of specifications for drug products. Routine evaluation of a drug's or drug substance's appearance, color, and visible and dissolved particles can be used to establish whether gross changes have occurred either during the purification process or during storage. The concentration of dissolved particles is determined through osmolality.

A variety of techniques are available for determination of identity and heterogeneity. The identity of proteins is typically established through a combination of several test methods. They include liquid chromatography patterns, charge pattern, molecular weight, and structure determinations. For example, molecular weights of both the intact molecule and any subcomponents can be determined by SDS-PAGE or SEC HPLC procedures. Comparing retention times, UV spectra, or MS spectra following an HPLC separation with those of reference standards is frequently used for identification of proteins. Glycosylation heterogeneity is determined through monosaccharide composition analysis with GC-MS or HPAEC-PAD and oligosaccharide pattern.

Some of the tests as described for identity determination are also useful for the determination of the purity profile and impurities. For example, product-related impurities such as degradants, truncated forms and isoforms are determined by HPLC, UHPLC, MS, LC/MS and with reducing and non-reducing SDS-PAGE. Dimers and higher aggregates are determined with size exclusion chromatography and analytical ultracentrifugation.

Downstream process-related impurities include residual solvents, enzymes, inorganic salts, and leachables such as heavy metals, plastics, resins, and others. GC headspace and ICP-MS/OES are listed as examples of residual solvent and trace metal analysis.

	Specification	Measurement
General Tests	pH	Calibrated pH meter
Identity and heterogeneity	Concentration of dissolved particles	Osmolality
	Charge pattern	Ion exchange chromatography (IEC)
	Molecular weight	
	Primary structure	
	Higher-order structure	NMR, circular dichroism
	Glycosylation heterogeneity	Monosaccharaide composition analysis with GC/MS or HPAEC-PAD and oligosaccharide pattern
	Amino-terminus of the protein for heterogeneity	N-terminal sequencing by automated Edman chemistry and HPLC analysis
	Identification of C-terminus and truncated version for heterogeneity	C-terminal sequencing through a combination of peptide mapping and ES-MS/MS
Purity and Impurities	Degradants, truncated forms, isoforms	SDS-PAGE (reducing and non-reducing) HPLC, UHPLC, LC/MS
	Deamidation products	Isoelectric focusing, IEC, peptide mapping
	Dimers and higher aggregates	SEC, analytical ultracentrifugation, SDS PAGE
	Post-translational modifications	Disulfide bridge analysis based on peptide mapping, digestion with a suitable enzyme and analysis with LC-ES/MS, C-terminal sequence analysis
	Host cell proteins	SDS-PAGE, immunoassays
	Related proteins	SDS-PAGE, immunoassays, HPLC, LC/MS
	Examples for process-related impurities	GC headspace and GC/MS headspace for residual solvents, ICP-MS and ICP-OES for trace metals
Potency	Adequate validated biological potency assay	Potency
Quantity	Protein content	UV scan

Figure 17 Examples of release tests for a biopharmaceutical drug.

Figure 18 shows an example chromatogram of monoclonal antibody monomer that is separated from its dimer by size exclusion chromatography.

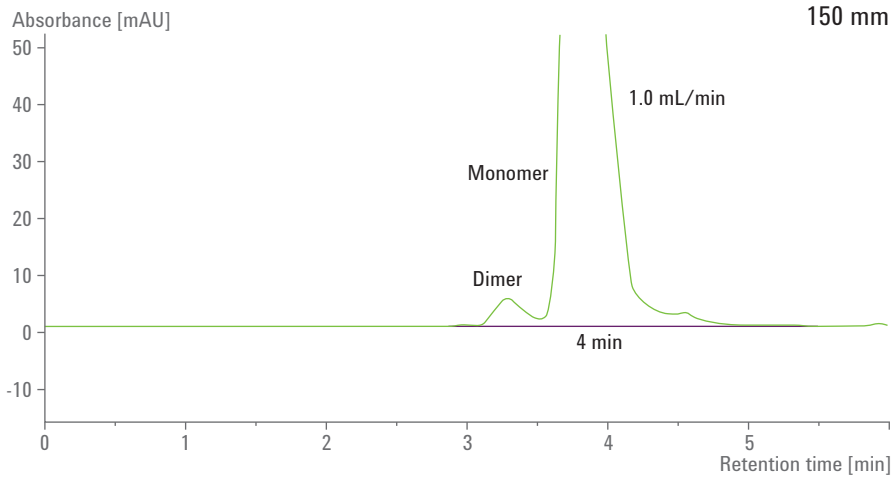

Figure 18 SEC profiles of monoclonal antibody with pH stress-induced aggregates (A) and overlay with intact IgG1 (B).

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GLOSSARY

ANDA	Abbreviated New Drug Application
API	Active Pharmaceutical Ingredients
ATP	Analytical Target Profile
BLA	Biologic License Application
CBER	Center for Biologics Evaluation and Research
CD	Circular Dichroism
CDER	Center for Drug Evaluation and Research
CFR	Code of Federal Regulations
CMA	Critical Method Attributes
CMC	Chemistry, Manufacturing, and Controls
CMP	Critical Method Parameters
DAD	Diode-Array Detector
DOE	Design of Experiments
DP	Drug Product
DS	Drug Substance
ELISA	Enzyme-linked Immunosorbent Assay
EMA	European Medicines Agency (previously EMEA)
EP	European Pharmacopeia
EU	European Union
FDA	Food and Drug Administration
GCP	Good Clinical Practice
GLP	Good Laboratory Practice
GMP	Good Manufacturing Practice
HHS	Department of Health and Human Services
HPAEC-PAD	High Performance Anion Exchange Chromatography with Pulsed Amperometric Detection
HPLC	High Performance Liquid Chromatography
ICH	International conference for Harmonization
IEF	Isoelectric Focusing
IND	Investigational New Drug (Application)
MALS	Multi-angle Light Scattering
NDA	New Drug Application
NBE	New Biological Entity
NCE	New Chemical Entity
PIC/S	The Pharmaceutical Inspection Convention and Pharmaceutical Inspection Cooperation Scheme
PTM	Post Translational Modification
QbD	Quality by Design
QA	Quality Assurance
QC	Quality Control
SDS-PAGE	Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis
SEC	Size Exclusion Chromatography
SEC-MALS	Size Exclusion Chromatography with Multi-Angle Laser Light Scattering
SOP	Standard Operating Procedure
USP	United States Pharmacopeia
WCBP	Well-characterized Biopharmaceutical Product

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