A basic concept of the quality assurance on biotechnological products (biopharmaceuticals)

(バイオテクノロジー応用医薬品（バイオ医薬品）の品質確保の基本的考え方)

Introduction

This document provides general principle to ensure the quality of biotechnological products (hereinafter referred to as “biopharmaceuticals”) focusing on the elements peculiar to biopharmaceuticals on the basis of the recommendations in a series of so-called Q-quartet guidelines from ICH Q8 to Q11 and those in Q5A to Q5E and Q6B guidelines[1-6] on the quality of biopharmaceuticals. The general concepts for assurance of drug substances and drug products are described in the General Information, G10 “Basic Concepts for Quality Assurance of Drug Substances and Drug Products”.

The principles of this General Information apply to biopharmaceuticals: proteins and peptides, their derivatives, and products of which they are components. These proteins and polypeptides are produced from recombinant or non-recombinant cell-culture expression systems. The principles outlined in this document may also apply to other types of biotechnological/biological products.

In the case of biopharmaceuticals, an inherent degree of structural heterogeneity occurs in molecular structure due to the biosynthetic processes by living organisms to produce them. In addition to post-translational modification such as glycosylation, they may receive various modifications such as oxidation and deamidation during the production process and storage periods. Impurities that may remain in the drug substance of biopharmaceuticals include those with molecular diversity, such as proteins derived from cells used for production, and there is a risk of contamination such as viruses. Such quality profiles can vary due to various factors on the manufacturing process.

In order to ensure the quality of biopharmaceuticals, it is necessary to establish an appropriate quality control strategy in consideration of the above characteristics. “Basic Concepts for Quality Assurance of Drug Substances and Drug Products” described in General Information G10 is useful for this. First, the quality attributes are clarified by thorough characteristic analysis. Then, identify the critical quality attributes (CQAs) in consideration of the quality target product profile (QTPP), and construct a quality control strategy to keep the CQAs within the appropriate ranges, limits and distributions. If the manufacturing process is to be changed during the development period or the post-marketing period, conduct comparability exercise of before and after the change made in the manufacturing process, and check the validity of the change by verifying that the change will not have adverse impact on the quality, safety and efficacy of the drug product. New manufacturing and analytical technology of biopharmaceuticals are continuously being developed day by day, and desired to be utilized for continuous improvement of product quality throughout the product life cycle.

1. Quality evaluation and control of biopharmaceuticals

1.1. Quality evaluation

1.1.1. Characterization

Pharmaceutical characterization is an essential step in identifying CQAs and establishing quality control strategies. In the characterization of biopharmaceuticals, detailed analysis is performed as much as possible for the structure and physicochemical properties, biological activity, molecular variants of a desired product, process-related impurities, and so on. The desired product is the protein which has an expected structure and is expected from the DNA sequence, the protein which is expected from appropriate post-translational modification, and/or expected from the intended downstream processing/modification. Among the molecular variants of the desired product, those with properties comparable to the desired product with respect to biological activity, efficacy and safety can be classified as product-related substances. Otherwise, those without the same properties can be classified as product-related impurities. The active ingredient consists of the desired product and the product-related substances, and generally, the active ingredient of biopharmaceuticals has heterogeneity (Fig. 1).

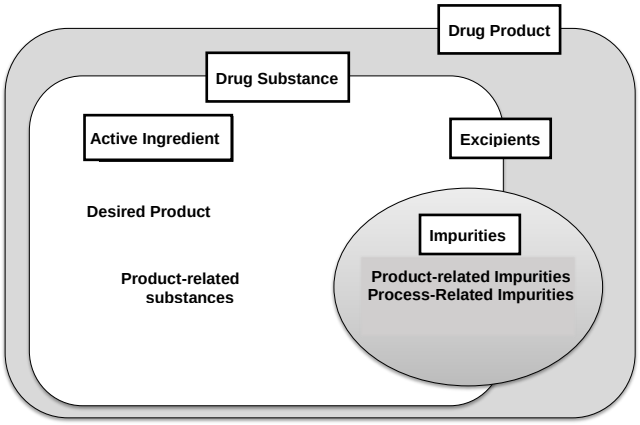

Fig. 1 Components of biopharmaceuticals.

*In biopharmaceuticals, the drug substance contains excipients such as buffer solution components for stabilization of the active ingredient.

a. Structure and Physicochemical Properties

Analyzes amino acid sequence and amino acid composition, terminal amino acid sequence, sulfhydryl group and disulfide bond, carbohydrate composition and structure, glycation, oxidation, deamidation, and so on. Oligosaccharides in glycoproteins are associated with stabilization of structure, biological activity, antigenicity and pharmacokinetics, and their profiles are susceptible to variations of manufacturing process. It is necessary to analyze in detail by monosaccharide analysis, oligosaccharide analysis/oligosaccharide profiling, glycopeptide analysis, glycoform analysis etc. Molecular variants such as oxidized and deamidated forms may be analyzed by peptide mapping. The charge profiles may be evaluated by ion exchange chromatography or isoelectric focusing. The molecular heterogeneity occurs not only during the culture process but also during subsequent manufacture and storage of the drug substance and drug product. Therefore, scientific understanding of the manufacturing process obtained by characterizing the degree of heterogeneity and profile, and evaluating the influence of the variations in process parameters on the degree of heterogeneity, will be effective to maintain the constancy of the manufacturing process. In addition, by setting specifications as needed, consistency between lots can be assured.

Physicochemical properties are analyzed in terms of molecular weight, molecular size, molar absorbance coefficient, etc. Information on the secondary structure and higher-order structure of the desired product can be obtained by spectroscopic methods such as circular dichroism, Fourier transform infrared absorption spectrum and NMR.

b. Biological activities

Biological activity is an indicator of the specific ability or capacity of a product to achieve a defined biological effect. It is difficult to determine higher-order structure by physicochemical analysis, because that an active ingredient of biopharmaceutical is a large molecule having complex structure, and is a mixture of various molecular species as mentioned above. Therefore, the confirmation that the biopharmaceutical has an expected structure is usually obtained by biological activity. Biological assays for measuring biological activity include biochemical assays (measurement of enzyme activity, measurement of binding activity, etc.), cell culture-based biological assays, animal-based biological assays, and so on. The tests are selected by considering characteristics of active ingredient, mechanism of action and its efficacy to the disease to be treated. For example, enzyme activity in the case of enzymes, cell proliferation activity in the case of growth factors, antigen binding activity, antigen neutralizing activity, antibody dependent cytotoxicity, and complement dependent cytotoxicity etc. in the case of antibodies, are evaluated.

In biological assays, the potency is expressed as a unit or relative activity (%) to the standard by comparing the response obtained from the sample to that from the standard. The potency is the quantitative measure of biological activity based on the attribute of the product which is linked to the relevant biological properties, and is expressed in "units". The biological activity used for potency measurement should, in principle, be the same as or similar to that expected in the clinical situation. The correlation between the expected clinical response and the activity in the biological assay should be established in pharmacodynamic or clinical studies.

c. Molecular Variants of desired product (product-related substances and product-related impurities)

In the characterization of drug substance and drug product, we analyze as much as possible such as the structure, biological activity and binding activity of the contained molecular variants. In products with large molecular masses and complex structures, it is often difficult to clearly separate the product-related substances and product-related impurities, and it is difficult to control the proportion of individual molecular variants in the drug substance and the drug product. In such cases, profiles (oligosaccharide profiles and charge profiles) obtained by appropriate analytical methods should be clarified. Typical molecular variants classified as product-related impurities are aggregates (multimers) and fragments. In addition, deamidated, isomerized, oxidized, mismatched S-S linked disulfide bond mismatched, glycated forms, etc. may be considered as impurities derived from the desired product. Aggregates and fragments are evaluated for their content by size exclusion chromatography, SDS polyacrylamide gel electrophoresis, SDS capillary gel electrophoresis, and so on.

d. Process-related impurities

The process-related impurities are classified into those derived from cell substrates (e.g., host cell proteins and host cell DNA), impurities derived from cell cultures (e.g., antibiotics and insulin), impurities derived from downstream processing such as extraction, separation, processing, purification and formulation steps (ligands solid support for chromatography such as protein A, enzymes, chemical modification reagents, solvents, etc.). Regarding process-related impurities, if it is possible to guarantee that impurities are constantly removed by the setting of in-process tests and/or control of process parameters, in some cases, it is not necessary to set specifications for drug substances and drugs product. Particular attention should be given to process-related impurities that exhibit pharmacological activity and may have immunogenicity.

1.1.2. Identification of CQA

For each quality attribute revealed by the pharmaceutical characterization, risk priority is estimated with respect to

the effect and uncertainty that their variation has on efficacy and safety, and then the CQAs to be controlled are identified. For example, in many cases, from the viewpoint of 1. biological activity or efficacy, 2. pharmacokinetics, 3. immunogenicity, 4. safety, if the product of the score of effect and the score of uncertainty for each attribute is above a certain value, that attribute is identified as CQA. Risk priority can also be estimated from the severity and probability of their impact on efficacy and safety.

1.2. Construction of quality control strategy

The quality control strategy defines a set of controls to bring the CQAs within appropriate limits, ranges and distributions. Quality control strategies include such as raw material control, manufacturing process control, specifications and stability testing. Acceptable ranges and target management criteria of CQAs are set based on characterization results, lot analysis results based on specifications, stability tests results, and clinical tests results, and so on. In stability testing, the analytical results of the quality attributes (i.e. the proportion of molecular variants such as fragments, oxidized forms and deamidated forms) and biological activity of the forced degradation samples in the stress stability testing and accelerated stability testing, are useful for setting the tolerance of each CQA and the control standard. In the course of developing the manufacturing process of biopharmaceuticals, identify the raw material characteristics and process parameters affecting the CQAs, and construct the control method of the manufacturing process so that the CQAs are within the target range. Based on these results, construct an appropriate control strategy consisting of raw material specifications, process parameter control, in-process testing, drug substance or drug product specifications.

1.2.1. Raw materials control

Raw materials for biopharmaceuticals include cell banks, media used in culture processes, media additives, resins used in purification processes, buffers, washing solutions, filters, etc. and they also include PEGylation reagents used in the modification process, additives used in the formulation process, and the like.

a. Evaluation and Control of Cell Bank (including evaluation of gene expression construct)

Cell substrates are generally controlled in a two-tiered cell bank, where Working Cell Banks are prepared from a Master Cell Bank, and their characteristics are clarified by conducting characterization and purity tests. Also, confirm that the cell substrate is appropriate for pharmaceutical production. In addition, the same evaluation is performed at the upper limit of in vitro cell age that can be used for production, and the stability of the cell substrate during the culture period is confirmed. In the purity test, it is evaluated that the cell bank is not contaminated with adventitious microbial contaminants (see 1.2.3 for virus). In the characterization, cell morphology, viable cell number, expression of target protein, etc. are evaluated. In the case of a cell line to which a gene expression construct is introduced, the gene expression construct should be evaluated for copy number and insertions or deletions, and cording sequence of desired protein, etc.

b. Control of other raw materials

Raw materials used in the manufacturing process are used after confirming that they fulfill the criteria for their intended use. When using raw materials derived from humans or animals, such as serum and enzymes, make sure that they meet the “Biological Raw Material Standards”.

1.2.2. Manufacturing process control

The manufacturing process of biopharmaceuticals consists mainly of drug substance process containing culture process and purification process, and formulation process. Because process parameters of culture process and purification process may affect the heterogeneity profile and impurity profile, etc., sufficient understanding of manufacturing process and the construction of appropriate control methods (setting and evaluation of process parameters, in-process test etc.) are essential for quality consistency. The constructed manufacturing process is qualified by process validation/ evaluation. Process validation is usually performed on a commercial-scale, however it can be performed on a small-scale model that has been qualified for investigating the ability to remove and inactivate virus and the number of reuses of purification columns and so on.

a. Process Parameter Control

The process parameters to be controlled and their control ranges in each manufacturing process are set based on the previous manufacturing results and univariate experiments, or on the relationships between the process parameters and the CQAs clarified by a systematic method. That is, when developing a manufacturing process, any method will clarify the degree of influence of each process parameter on each CQA, and the management range of each process parameter is set so that each CQA does not exceed the allowable range. As an example of process parameters to be controlled, in the culture process, temperature, medium additives concentration, dissolved oxygen concentration, dissolved carbon dioxide concentration, pH, stirring speed, culture time, etc., and in the purification process, column size, loading amount, buffer solution composition, flow velocity, etc. can be mentioned. The tolerance range of purification process parameters is set in consideration of the influence on the heterogeneity profile and the impurity removal efficiency. In addition, it is important to ensure that the characteristics of each process, such as cell density and

viability in the culture process and recovery rate in the purification process, fall within a certain range. Processes that have a particularly large impact on quality are regarded as critical processes. Major examples of critical processes include production culture processes, virus inactivation and removal processes, and affinity chromatography processes.

b. In Process Tests

In the quality control of biopharmaceuticals, in-process tests are considered possible or appropriate to control contamination with such as process-related impurities, viruses and adventitious infectious factors. Examples of in-process testing include adventitious virus test after production culture, filter integrity tests of virus removal filters and sterile filters, testing for process-related impurities such as host cell proteins and host cell DNA, bioburden test, and so on. In-process tests, as same as specifications, are also evaluated for validity by analytical method validation.

1.2.3. Evaluation and control of contaminant

Contaminants are substances that should not be present in manufacturing processes, such as adventitious chemicals, biochemical materials, or microorganisms. From the viewpoint of ensuring safety, contaminants should be strictly avoided, and after constructing an appropriate manufacturing process, and, as mentioned above, it should be appropriately controlled by raw material control, in-process testing or specifications.

Viruses may contaminate as an adventitious factor from production processes and may be present as an endogenous factor in cell substrates used. The following three major complementary approaches are taken as rational measures to prevent the virus contamination specific to products using biological origin and to ensure the safety. 1) Selecting and testing cell lines and other raw materials that contain media components to deny the presence of viruses that may be infectious or pathogenic to humans. 2) Assessing the capacity of production processes to clear infectious viruses. 3) Testing the product at appropriate steps of production for absence of contaminating infectious viruses. The details are described in General Information: “Basic Requirements for Viral Safety of Biotechnological/Biological Products listed in Japanese Pharmacopoeia”.

1.2.4. Specifications

a. Setting Basis of Specification

The items and test methods adopted for the specification differ depending on the quality control strategy established. It is necessary to clarify the setting basis of the tolerance limits/acceptance criteria. The tolerance limits/acceptance criteria are set on the basis of the data obtained from lots used in clinical trials, the data obtained from lots used to indicate the consistency of production, stability test data, and appropriate data in the product development stages. Additionally, the basis of the setting should be shown.

b. Description

It qualitatively defines the physical state (e.g., solid, liquid), color and transparency of a drug substance and drug product.

c. Identification test

Set up specific tests based on the structural features and specific properties of an active ingredient(s). In order to confirm the identity, not less than two types of tests (physical and chemical test, biological test, immunochemical test, etc.) are usually to be set up for a drug substance. For a drug product, one type of test may be sufficient, but some products may require more than one type of test.

d. Specific physical and/or chemical values

The quality attributes to be set as the specific physical and/or chemical values include oligosaccharide, charge, molecular mass/size, and so on. In the case where the product-related substances and the product-related impurities are difficult to separate and can not be set as the purity tests, the heterogeneity profiles are specified as the physical and/or chemical values. Typical examples include oligosaccharide profiles and charge profiles. In addition, set the specification for characteristics that are important in ensuring the quality of a drug substance and drug product. Examples of the items include pH and osmotic pressure, etc.

e. Purity test

Purity is usually assessed by a combination of analytical methods. In the selection and optimization of test methods for impurities, emphasis should be placed on separating or identifying the desired product and product-related substances from impurities (product-related impurities and process-related impurities).

f. Biological activities

Specifications for biopharmaceuticals should usually contain the tests for biological activity. Considering the action mechanism of the active ingredient, a suitable one of the methods used for the characterization is set as a biological activity test. The tolerance limit is expressed in units/mL when the potency in the solution is used as an index, and when using the potency per protein amount as an index, it is expressed in units/mg. The potency per amount of protein is called the specific activity. Besides these, the specific activity may be compared with a standard material, and this may be expressed as a percentage (%) to obtain a tolerance limit. In recent years, there has been an increasing number of cases where the ratio (%) of specific activity to a standard material is set as a tolerance limit without setting a unit.

g. Assay

The content of active ingredient contained in a drug substance and drug product is expressed as protein content (mass) or potency (unit). As it is an critical factor of product quality, measure it using an appropriate quantitative

method. For tests to determine the protein content, the methods described in the General Information “Total Protein Assay”, the method comparing peak areas with the standard material using HPLC, etc. are used. A biological activity test is used to determine the potency.

If physicochemical testing has provided sufficient physicochemical information on the product, including information on higher order structure, and proper correlation with biological activity has been well demonstrated, in addition, if the manufacturing experience is well established, biological activity tests for determining potency can be replaced by physicochemical testing. For insulins, etc., the content (unit) of the active ingredient in a sample is determined by comparing the peak area with the standard material indicated by the unit by the quantitative method using HPLC.

h. Tests for preparations

Conduct tests for preparations according to the dosage form. Since most biopharmaceuticals are injections, sterility test, bacterial endotoxin test, test for extractable volume of parenteral preparations, insoluble particulate matter test for injections and foreign insoluble matter test for injections, content uniformity test, etc. are conducted.

1.2.5. Stability testing

a. Conditions of stability testing

The shelf life of biopharmaceuticals is usually set based on the actual storage period of the product to be applied and on the results of long term testing at actual storage temperatures. The accelerated testing and the stress testing can provide supplementary information for setting the shelf life and useful information for elucidating the mechanism of quality change, as well as evaluating the validity of the analytical methods and the influence of storage conditions such as during transportation.

b. Attributes to be evaluated

During the storage of biopharmaceuticals, the bioactivity may decrease and the physicochemical properties may change, so it is necessary to comprehensively evaluate the quality characteristics by various analytical methods. In the stability testing, usually, adopting the appropriate attributes and tests used in characterization, evaluate the changes according to the characteristics of the product, such as biological activity, molecular heterogeneity, and product-related impurities.

2. Comparability of biopharmaceuticals subject to changes in their manufacturing process

When changing the manufacturing process of biopharmaceuticals, evaluation work on compatibility will be carried out in order to ensure the quality, efficacy and safety of the drug product produced by the changed manufacturing process. The demonstration of comparability does not necessarily mean that the quality attributes of the pre-change and post-change product are identical, but that they are highly similar and that the existing knowledge is sufficiently predictive to ensure that any differences in quality attributes have no adverse impact upon safety and efficacy of the drug product. However, where the relationship between specific quality attributes and safety or efficacy has not been established, it might be appropriate to include a combination of quality, nonclinical, and/or clinical studies in the comparability exercise.

2.1. Considerations for the comparability exercise

The extent to which the test to prove the compatibility before and after the change should be conducted is considered on the production step where the changes are introduced, on the potential impact of the changes on the quality characteristics, on the suitability of the analytical techniques used, and on the relationship between quality attributes and safety or efficacy based on overall nonclinical and clinical experience. The judgment of product comparability should be done by considering characterization data, stability data providing insight into potential product differences in the changes and the degradation of the protein, and data of lots used for demonstration of manufacturing consistency. The historical data that provide insight into changes of quality attributes with respect to safety and efficacy following manufacturing process change, and nonclinical or clinical characteristics of the drug product and its therapeutic indications should also be considered.

2.2. Quality considerations

By re-executing all or part of the characterization that has already been carried out (if it is a part, it is necessary to explain its appropriateness), compare the quality characteristics before and after the change directly, to obtain the data needed to determine the comparability. However, it is necessary to evaluate the meaning of the difference by additional characterization, such as when heterogeneity and/or impurity profile are found before and after the change. Even when evaluating the same quality attributes, it is necessary to apply multiple analysis methods and analysis methods having different measurement principles, and devise to be able to detect the change of the quality characteristics that may occur due to the change of manufacturing process. In addition, changes in the manufacturing process, even minor one, may affect the stability of the product, so when changing the manufacturing process that possibly affect the quality characteristics, also evaluate the influence on the stability of the product.

2.3. Manufacturing process considerations

Confirm that the process controls in the modified process provide at least similar or more effective control of the product quality, compared to those of the original process. A careful consideration of potential effects of the planned change on steps downstream and quality attributes related

to these steps is extremely important. The modified process steps should be re-evaluated and/or re-validated, as appropriate.

References

1) ICH: Guideline for Q5A (R1), Viral Safety Evaluation of Biotechnology Products Derived from Cell Lines of Human or Animal Origin.
2) ICH: Guideline for Q5B, Quality of Biotechnological Products: Analysis of the Expression Construct in Cells Used for Production of R-DNA Derived Protein Products.
3) ICH: Guideline for Q5C, Quality of Biotechnological Products: Stability Testing of Biotechnological/Biological Products.
4) ICH: Guideline for Q5D, Derivation and Characterization of Cell Substrates Used for Production of Biotechnological/Biological Products.
5) ICH: Guideline for Q5E, Comparability of Biotechnological/Biological Products Subject to Changes in Their Manufacturing Process.
6) ICH: Guideline for Q6B, Specifications: Test Procedures and Acceptance Criteria for Biotechnological/Biological Products.