

BIOLOGICS AND BIOTECHNOLOGY DRUG SUBSTANCES OR PRODUCTS

INTRODUCTION

This chapter of the guideline provides guidance to Sponsors of Requests for Revision for new monographs for biologics and biotechnology drug substances and products. A Request for Revision to create a new USP monograph for these product types should include name, description, definition, other requirements (packaging and storage, labeling, USP Reference Standards), and the substance and product specifications. Both specifications should include universal tests and may include specific tests as well. In some instances, one or more of the universal tests may not be applicable to a drug substance or product. For example, the monograph for pancreatin does not have tests for impurity and quantitation. In such cases, appropriate justification should be provided for not including a universal test(s). Specific tests should be included when they have an impact on the quality of the drug substance for release and/or compendial testing. Taken as a whole, a specification should be stability-indicating, using either one or more procedures for quantitation that are stability-indicating, or a procedure for quantitation that is not stability-indicating with an accompanying stability-indicating procedures for impurity testing.

Although the number of manufacturers of any biologics and biotechnology drug product tends to be limited, these products usually are marketed globally. It is advantageous to have common international standards for each product. In order to help accomplish this, U.S. manufacturers are encouraged to develop USP monographs for their biologics and biotechnology products as a means of developing a uniform global standard for each product and, in turn, have a leadership role in the process. The European Pharmacopoeia recently has published several monographs of biologics and biotechnology drug substances and products. In many cases, the specifications, tests, procedures, and acceptance criteria do not conform to those contained in the biological license approved by the FDA. Ostensibly, the USP is a national pharmacopeia; however, its standards are recognized in numerous countries. Monographs in USP may help U.S. manufacturers minimize regulatory impact on global distribution of their products. An article recognized in the United States Pharmacopeia can be described as “USP” on its label.

SCOPE

This chapter covers proteins, peptides, carbohydrates, lipids, and their mixtures, but does not include vaccines, cells, blood and cellular blood components, or gene therapy, cell therapy, and tissue engineering ingredients and products. A compendial monograph is the minimum requirement to establish the identity of an active ingredient or product, but is not necessarily sufficient for full characterization.

NEW MONOGRAPH FOR A BIOLOGICS AND BIOTECHNOLOGY DRUG SUBSTANCE

Name The name in a Request for Revision is designated using the United States Adopted Name (USAN) as outlined under the General Chapter Nomenclature <1121>.

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When a USAN name is unavailable, the Sponsor is expected to petition the USAN Council in a timely manner. Where the USAN name is in dispute, all names under consideration should be included in the Request for Revision. At times, a nonproprietary proper name (often referred to as a generic name) can be selected by an applicant working with FDA. If a substance is available from alternative sources and/or is manufactured by alternative technologies, the source or technology of manufacture should be included, e.g., Antihemophilic Factor Plasma Derived and Antihemophilic Factor Recombinant.

Description Requests for Revision for biological/biotechnology-derived drug substances should include the structure, molecular weight, the Chemical Abstracts Service (CAS) registry number (American Chemical Society), and alternate names, if known.

Structure Requests for Revision for ingredients that are not mixtures should include a structure. For mixtures, include the structure of each ingredient, if known. For proteins and peptides, the structure should include an amino acid sequence, starting with N-terminal and ending in C-terminal, with disulfide bonds indicated by lines, and sites for glycosylation or other post-translational modifications identified. For oligosaccharides, the structure should include the monosaccharide sequence, starting with the nonreducing terminus (or termini), and the type of the glycosidic linkages identified (such as, 1,1,2). For polydispersed substances, such as heparin and dextran, the Request for Revision should include the structure of the repeating monomer unit, with the linkages fully described. For lipids, the structures should consist of the complete chemical structure of the glyceride or phosphoglyceride moiety and the fatty acid moiety, together with the linkages indicated. The structure of lipooligosaccharides or lipopolysaccharides should indicate the structures of both lipid and oligosaccharide or polysaccharide moieties, with the linkages indicated.

Molecular Weight Requests for Revision for substances that are not mixtures or polydispersed should include molecular weight. Where possible, the molecular weight should be based upon the sequence. For polydispersed substances, indicate the acceptable range of the average molecular weight.

CAS Number If it is available, the Request for Revision should include the CAS registry number. Where more than one CAS number has been used to describe the molecule, the Request for Revision should include them all.

Physical Form The Request for Revision should include the product's physical form, e.g., crystalline, lyophilized powder, solution, suspension, or emulsion.

DEFINITION

The Definition section of a monograph includes functional properties, chemical composition, and, when needed, performance characteristics for the biological/biotechnological substance. If the substance is manufactured by recombinant

technology, the Request for Revision should include the name and origin of the cell line. If the substance is obtained via bacterial fermentation, the Request for Revision should include the bacterial name and strain, as well as any genetic modification. Where a manufacturer relies upon techniques that might require disclosure of proprietary information to assess these performance characteristics, the technique (e.g., ELISA) and its acceptance criteria, but not the detailed procedure, should be provided in the Definition.

The Request for Revision should include a brief description of the production method, e.g., synthetic procedures, recombinant processes, extractions from animal or human materials, etc. For substances that consist of more than one component, a minimum potency or range must be included in the definition for each component. Ratios between components should be indicated for mixtures.

The Request for Revision should provide acceptance criteria for the Potency test. The potency of the substance may be expressed in USP Units per mg, especially for natural-sourced substances and mixtures. Because USP Units are based on USP Reference Standards and specified assays, they generally are not equivalent to International Units (IU) for a particular substance, and no official conversion parameter can be indicated to convert a USP Unit into an IU. However, if the standard and the assay procedure are the same, the Request for Revision should indicate that the USP Unit and the IU are equivalent.

For biotechnology-derived substances, the Definition should include a statement on limits for host cell DNA and host cell protein impurities. These limits are process-specific, and quantitative procedures are not included in monographs. Therefore, the determination of these impurities is performed by privately validated procedures and approved as part of the regulatory process.

OTHER REQUIREMENTS

Packaging and Storage Packaging and storage statements in the Request for Revision should conform to general statements in the *General Notices and Requirements* section of *USP-NF*. The Request for Revision should include specific packaging and storage statements. It also should include the type of closure and container used—glass or plastic—for substance storage. If either glass or plastic can be used, no specific statement about container type is needed. Storage conditions, including refrigerator or freezer storage temperatures with ranges, or protection from light, if warranted, should be indicated.

Labeling As defined in *General Notices and Requirements*, labeling includes both labels and labeling. The Request for Revision should provide the text that must be included both on label and in labeling. For a substance that can be produced via a number of methods, the production method should be indicated in the labeling. If the substance can be obtained from a variety of animals and/or cell lines, the Request for Revision should indicate the source(s). Similarly, if the material can be obtained from

different tissues, the Request for Revision should indicate the substance's source tissue, particularly if the same substance from different tissues has different potencies.

Reference Standards All official Reference Standards needed in order to conduct the substance's monograph tests should be listed. For example, if the proposed monograph includes a bacterial endotoxins test, the Request for Revision should cite General Chapter *Bacterial Endotoxins Test* <85> and include the *USP Endotoxin RS* (see *General Notices and Requirements* and General Chapter *USP Reference Standards* <11>). A current list of available official USP Reference Standards is provided in *PF* and in USP catalogues. Sponsors should indicate special handling conditions, if any, for a Reference Standard used in a monograph test. The Request for Revision should indicate when a reference standard other than those available from USP is used for a particular monograph test, e.g., WHO, etc.

SPECIFICATION

While USP intends to develop monographs that are as comprehensive as possible, it recognizes that some proprietary information may be needed in a Request for Revision. When proprietary information is provided and so identified, USP treats it confidentially (see *USP Document Disclosure Policy* in the current *USP-NF*). Nonetheless, specification procedures should be detailed sufficiently to allow a competent analyst, with the appropriate equipment and reagents, to conduct the analysis. Where this is not the case, the general technique and acceptance criteria should be provided in the Definition (see above).

Identification of a Biologics and Biotechnology Substance

The Identification test is one of the most important, variable, and flexible tests in *USP-NF* monographs. Validation of procedures used in the Identification test should conform to General Chapter *Validation of Compendial Methods* <1225>). The Request for Revision should include one or more procedures that are highly specific based on unique aspects of the substance's molecular structure and/or other specific properties. Several procedures, including physicochemical, biological and/or immunological, or cell-based procedures may be necessary for the Identification test. Typical procedures may include chromatography (GC, HPLC), electrophoresis [Polyacrylamide Gel Electrophoresis (PAGE)], Isoelectric Focusing (IEF), Capillary Electrophoresis (CE), other), immunological methods [immunodiffusion, immunoelectrophoresis, dot-blot, Western blot, Enzyme-Linked Immunosorbent Assay (ELISA), Radioimmunoassay (RIA)], amino acid analysis, N-terminal or C-terminal sequencing, peptide mapping, suitable fingerprinting analysis, enzyme activity, bioidentity test, and/or cell-based procedures. The Identification test does not include procedures to determine purity, which are found in the Impurity test.

Chromatography Liquid chromatography, particularly HPLC, commonly is used as an Identification test for ingredients. HPLC is used for a wide range of procedures, including peptide mapping, amino acid analysis, fingerprinting, and comparing the ingredient's

chromatograms to those of the corresponding Reference Standard. Gas chromatography (GC) is used more commonly for lipids or lipid-like molecules. Both HPLC and GC are separation techniques that permit selective elution of the molecule of interest (see General Chapter *Chromatography* <621>).

Electrophoresis Electrophoretic procedures include PAGE, under both non-denaturing (native) or denaturing [in the presence of sodium dodecyl sulfate (SDS) or urea] conditions, isoelectric focusing (IEF/using either a slab-gel or a gel-filled capillary), and capillary electrophoresis. Like chromatography, electrophoresis also involves selective separation of molecules. Electrophoretic procedures are used in the Identification test by comparing the electrophoregrams of the substance to those of the corresponding Reference Standard. Requests for Revision using native PAGE in the Identification test should refer to General Chapter *Electrophoresis* <726>. Requests for Revision relying on SDS-PAGE should refer to General Chapters *Biotechnology-Derived Articles* <1045>, and *Biotechnology Derived Articles–Tests* <1047>. Polyacrylamide gel electrophoresis in the presence of urea (Urea-PAGE) and SDS-PAGE commonly are used for separating small peptides, including products of protease digestion of a protein (see Peptide Mapping and Fingerprinting). Requests for Revision relying on IEF should refer to General Chapter *Biotechnology Derived Articles–Tests* <1047>. IEF separates proteins and peptides according to their isoelectric points, which depend upon their amino acid sequences and three-dimensional structures. IEF is particularly useful in separating protein isoforms, glycoforms, or other molecular variants arising through post-translational modification. IEF can be performed in a polyacrylamide slab gel (PAGE-IEF or conventional IEF) or in a gel-filled capillary (capillary IEF, CIEF). Requests for Revision relying upon capillary electrophoresis should refer to General Chapter *Biotechnology Derived Articles–Tests* <1047>. Capillary electrophoresis uses one or more capillaries as migration channels for electrophoresis and increasingly has become the procedure of choice when an electrophoretic separation method is needed. This is because CE is easier to perform, requires less time, and allows better precision and robustness than PAGE. The previously described PAGE variations also can be done by CE (that is, native CE, SDS-CE, and CIEF).

Amino Acid Analysis This procedure determines amino acid composition, or protein or peptide content. Because each protein or peptide has a unique amino acid sequence and, therefore, amino acid composition, this method can be used as an Identification test (see General Chapter *Biotechnology Derived Articles–Tests* <1047>).

Fingerprint A biologics and biotechnology substance Identification test may include a fingerprint procedure. This procedure type is comprised of a number of different approaches, depending upon the product type. These include a protein peptide map (see below), an oligosaccharide or polysaccharide monosaccharide composition profile for an oligosaccharide or a polysaccharide (typically by HPLC or CE), or a lipid carboxylic acid profile, using HPLC or GC.

Peptide Mapping The peptide mapping procedure probably is the most widely used Identification test for proteins and peptides (see General Chapter *Biotechnology Derived*

Articles–Tests <1047>). It is a powerful procedure capable of identifying a single amino acid change. When included in the Identification test, it requires comparison to the appropriate USP or other reference standard peptide map. A peptide map confirms the protein's primary structure and thus demonstrates process consistency and genetic stability.

N- and C-Terminal Sequencing Requests for Revision for a substance may rely upon either N- or C-terminal sequencing procedures in the Identification test (see General Chapter *Biotechnology Derived Articles <1045>*). N-terminal sequencing is easier to perform and can be done in an automated format. C-terminal sequencing is more difficult and can produce erratic results. The Request for Revision should indicate which type of sequencing is utilized.

Immunochemical Procedures These procedures are based upon specific binding between an antibody (mono- or polyclonal) and antigenic epitope(s) of a protein or carbohydrate biologics and biotechnology substance (antigen). The specificity and high affinity of interaction permit such methods to be used in the Identification test. Several alternative approaches are possible, e.g., immunodiffusion, immunoelectrophoresis, dot-blot, Western blot, ELISA, RIA. The procedure selected for a monograph depends upon a variety of factors, including the antibody's specificity, binding affinity, nature, and sample availability. ELISA and RIA more frequently are used for quantitative determination of antigen content but may be used in the Identification test. ELISA and RIA techniques are discussed in General Chapter *Biotechnology-Derived Articles <1045>*. For many substances, the antibody/antiserum used in an immunochemical procedure is raised and qualified in-house and is not available commercially. If an antibody/antiserum used in the Identification test is not commercially available, the Request for Revision should provide a detailed protocol for raising the antibody/antiserum.

Enzyme Activity Because enzymes have specific activity toward a well-defined substrate and the reaction can be inhibited by a specific factor, an enzyme activity procedure may be useful in the Identification test. These reactions may be sensitive to an enzyme's structural alteration to the extent of distinguishing a change in one amino acid residue at the active site. An enzyme activity procedure may be used for an enzyme, a substrate, or a specific inhibitor.

Bioidentity If a suitable physicochemical or immunochemical procedure is not available or deemed unsuitable, the Sponsor may include a bioidentity procedure as the Identification test. The bioidentity procedure, usually a biological activity procedure, is designed to ensure that the biologics and biotechnology substance has a biological activity of a given magnitude. This procedure can be performed in vivo, using an animal model, or in vitro, using either defined (non-bioengineered) or bioengineered cell lines.

Other Procedures The Request for Revision may include other procedures, e.g., colorimetry, spectrophotometry, Nuclear Magnetic Resonance (NMR) Spectroscopy,

Mass Spectrometry (MS), for the Identification test, with appropriate rationalization and validation data.

Identification of Counter-ions In a few instances, identification of a counter-ion may be necessary as part of an Identification test, e.g., Heparin Calcium (see General Chapter *Identification Tests—General* <191>). For procedures not included in General Chapter <191>, the Request for Revision should include a complete description of reactions and acceptance criteria. The Request for Revision also should include information on reagent purity, solution concentrations, and the procedure's relative sensitivity and specificity. Validation data should show procedure acceptability for the drug substance (see General Chapter *Validation of Compendial Methods* <1225>).

Purity The determination of absolute or relative purity of a biologics and biotechnology drug substance presents considerable analytical challenges, and the results generally are method dependent. The analytical procedure of choice should allow complete separation of the desired product and product-related substances from impurities (see below). Normally, it is very difficult to confirm complete separation by one procedure. Thus, a biologics and biotechnology drug substance's purity generally is assessed by a combination of analytical procedures.

Due to the unique biosynthetic production processes and molecular characteristics of biologics and biotechnology drug substances, they can include several molecular entities or variants. When these molecular entities are derived from anticipated post-translational modification, they are part of the desired product. When variants of the desired product are formed during the manufacturing process and/or storage and have properties comparable to the desired product with respect to activity, safety, and efficacy, they are considered product-related substances, not impurities. Where possible, a Request for Revision Sponsor should select an appropriate set of procedures—not just one—that justifies their use for determining the purity of a biologics and biotechnology drug substance. In addition, individual or collective acceptance criteria should be set for product-related substances, as appropriate.

Impurities The Request for Revision for a new biologics and biotechnology drug substance monograph should include a procedure and limits for any impurities that affect or have the potential to affect the drug substance's safety or efficacy, and may require identification of a counter-ion as part of an Identification test. USP will follow nomenclature and approaches for impurities shown in Table 1. The Request for Revision will include only procedures that control actual but not theoretical impurities. When different source and manufacturing methods result in different impurity profiles, different sets of procedures may be needed. The acceptance criteria for impurities included in a Request for Revision should be based upon data obtained from lots used in preclinical and clinical studies and manufacturing consistency lots.

To identify and quantify impurities, an external rather than an internal standard is preferred because internal standards may obscure other impurities. When possible, official USP impurity Reference Standards are the best option when one is quantifying

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identified impurities. If such standards are unavailable, the Request for Revision may include relative response factors and relative response times for a utilized chromatographic or electrophoretic method.

A Request for Revision should include a list of specified impurities by name and/or type, sub-classification (process- or product-related), relative retention time, relative response factor, acceptance criteria, approximate Limit of Quantification, Limit of Detection, as applicable, and toxicity, if known. The Request for Revision also should include system suitability criteria sufficient to ensure that the system (equipment and reagents) is capable of performing the procedure, and results from typical batches (usually, three batches are sufficient).

Acceptance criteria should comply with the ICH Q6B Guidance (Specifications: Test Procedures and Acceptance Criteria for Biotechnological/Biological Products) and should be provided for each specified impurity, any unspecified impurity (as appropriate), and total impurities as a percent of the total substance.

Table 1. Impurity Tests

Impurity Type	Impurity Sub-Classifications	Traditional USP Test(s)	New USP Tests
Process-related Impurities	Host cell/source-derived: proteins and nucleic acids (DNA & RNA)	None	None: The tests and reagents are typically proprietary; limits included in the Definition
	Cell culture-related: inducers, antibiotics, media components	None	None: The tests and reagents are typically proprietary; limits included in the Definition
	Downstream-derived: Impurities, including ligands, monoclonal antibodies, resins, residual solvents/moisture	Particulate matter (for injections), Residual moisture, Loss on drying	Specified impurities
Product-related Impurities	Degradation products, truncated forms, aggregates	Chromatographic Purity, Related substances, Residual protein	Specified and Unspecified impurities
	Molecular variants (post-translational modification, mismatched disulfide bonds)		

Process-related Impurities A biologics and biotechnology drug substance may contain impurities derived from the manufacturing process. Such impurities may be classified into three major categories: host cell/source-derived, cell culture-derived, and downstream-derived (see Table 1). These materials are expected to be removed by the downstream process. However, traces almost invariably are present in drug substances.

Host cell/source-derived impurities include proteins, nucleic acids, and carbohydrates derived from host cell, source tissues, or body fluids. The nucleic acid includes host cell genomic, inserted vector, total DNA, and total RNA. For host cell proteins, a sensitive assay, e.g., immunoassay, capable of detecting a wide range of protein impurities generally is used. Cell culture-derived impurities also include fermentation-derived impurities, such as inducers, antibiotics, sera, and other media components.

Downstream-derived impurities include enzymes, chemical or biochemical processing reagents (e.g., cyanogen bromide, guanidine, and oxidizing and reducing agents), inorganic salts (e.g., heavy metals, arsenic, and nonmetallic ions), solvents, carriers, ligands (monoclonal antibodies), and leachables.

Heavy Metals and Other Residual Metals A test for heavy metals may be included in the Request for Revision if they affect or have the potential to affect the safety or efficacy of the drug substance. The Heavy Metals test is described in the General Chapter *Heavy Metals* <231>. The Request for Revision should include validation data to ensure that the proposed methods have the requisite selectivity and quantification limit to support proposed acceptance criteria. Validation for heavy metals should consist of a Limit of Detection and Specificity. If a method other than those described in General Chapter <231> is used, the Request for Revision should include sample preparation, experimental procedure, acceptance criteria, and validation consistent with General Chapter *Validation of Compendial Methods* <1225>.

Procedures for other residual metals are described in the following General Chapters: *Aluminum* <206>, *Iron* <241>, *Lead* <251>, *Mercury* <261>, *Selenium* <291>, and *Zinc Determination* <591>. The Request for Revision should include validation data to ensure that the proposed methods have the requisite selectivity and quantification limit to support the proposed acceptance criteria. Other methods such as atomic absorption or inductively coupled plasma may be included, but a more complete rationale for the procedure and full validation as specified in General Chapter *Validation of Compendial Methods* <1225> should be provided.

Residual Moisture or Loss on Drying The Request for Revision for solid (dried by lyophilization or other procedure) biologics and biotechnology drug substances should include a procedure for residual moisture, where relevant. Three procedures are described in the General Chapter *Water Determination* <921>. The actual procedure of choice depends upon the nature of the substances. If none of the procedures described in General Chapter <921> is suitable, an alternative procedure can be included in the Request for Revision. In such cases, the reason for choosing an alternative procedure should be provided. For example, gas chromatography also has been used for the determination of water for many biologics and biotechnology. Whatever procedure is chosen, a validation package consistent with the requirements of General Chapter *Validation of Compendial Methods* <1225> should be provided with the Request for Revision.

When alcohol or other organic solvents are used in the purification of a biological/biotechnological substance, the loss on drying is not always due entirely to water. Under such circumstances, a Request for Revision may instead contain a *Loss on Drying* procedure, as described in General Chapter *Loss on Drying* <731>.

Product-Related Impurities Product-related impurities are molecular variants of the desired substance that co-purify with it. These include: a) intermediates, b) truncated forms produced during manufacturing or storage brought about by proteolysis, oxidation, pH, light (including degradants), c) modified forms, including isoforms, glycoforms, other molecular variants produced by post-translational modifications, formation of mismatched disulfide linkages, oxidation products, and d) aggregates. Typical procedures for the detection and quantitation of intermediates, truncated and

modified forms include HPLC, SDS-PAGE, peptide mapping, CE, and mass spectrometry. Typical procedures for detection and quantitation of aggregates include size exclusion chromatography, often with on-line light scattering, PAGE under native conditions, or CE. The Request for Revision should contain procedures and limits for these impurities, as well as for process-related impurities, particularly if they affect or could affect the product's safety and efficacy. When an impurity is known, it should appear as a requirement in the monograph as a specified impurity with its own test, separate from unspecified impurities. Acceptance criteria for both specified and unspecified impurities should be provided as appropriate. For example, the monographs for Insulin and Insulin Human include procedures and acceptance criteria for limit of high molecular weight proteins and related compounds.

Quantitation The Request for Revision should include a suitable procedure for quantitation of a biologics and biotechnology drug substance. A Quantitation test procedure determines the content (mass) of a drug substance and can be performed by a suitable physicochemical procedure, e.g., colorimetry, spectrophotometry, HPLC, or electrophoresis.

Wherever possible, the Quantitation test should be stability indicating. Generally, separation-based procedures such as HPLC, CE, PAGE, and SDS-PAGE can be stability indicating. In contrast, colorimetric and spectrophotometric procedures are not usually stability indicating. When the proposed Quantitation test procedure is not stability indicating, a separate stability-indicating procedure should be provided to monitor product-related impurities (see Impurity test).

The acceptance criteria for the Quantitation test should be related directly to the precision or relative standard deviation (RSD) of the analytical procedure. Validation of procedures used in the Quantitation Test should be based upon recommendations in General Chapter *Validation of Compendial Methods* <1225>. Data from representative analyses should be included for at least three batches of the drug substance.

Chromatography Both GC and LC procedures may be used for the Quantitation test, depending upon the nature of the molecule. To ensure a smooth transfer of procedures to a compendial standard, the Request for Revision should include several important pieces of additional information beyond those noted generally for the Quantitation test. These include the brand and size of the analytical column, alternative columns, mobile phase and column temperature control, and solution stability. A critical piece of information is the system suitability parameters. They usually are obtained through carefully completed validation studies and should be defined clearly in a Request for Revision.

Electrophoresis Normally, CE-based procedures have better precision, accuracy, robustness, and ruggedness than native or SDS (sodium dodecyl sulfate)-PAGE, and in many instances constitute the procedure of choice when an electrophoretic procedure is necessary. Like chromatographic procedures, a proposed CE-based method should include information about the brand, internal diameter, and material of the capillary,

nature and chemical type (structure, if possible) of the coating, voltage, and temperature control.

Total Protein Content The Request for Revision for a protein drug substance should contain a Total Protein Content test procedure as part of the Quantitation test. This procedure is necessary because the potency or the biological activity is expressed in specific activity units (per mass unit, such as milligram or microgram). This procedure should not be confused with the procedure for residual or host-cell protein content, which is a procedure used in the Impurity test for residual proteins. Seven procedures for the Total Protein Content test are described in the General Chapter *Biotechnology-Derived Products—Tests* <1047>. The choice of procedure depends upon the nature of the biologics and biotechnology substance, the availability of instrumentation, and other factors. Validation of the procedure should be based upon recommendations in General Chapter *Validation of Compendial Methods* <1225>. Data from representative analyses should be included for at least three batches of the drug substance.

Potency Test The Request for Revision should include a suitable validated procedure for the determination of potency of a biologics and biotechnology drug substance. Potency of a biologics and biotechnology drug substance provides an assessment of its biological property(ies) that describes the drug substance's specific ability or capacity to achieve a defined biological effect. Examples of the procedures that can be used to measure potency include: 1) animal-based biological procedures, which measure an organism's biological response to the drug substance; 2) cell culture-based biological procedures, which measure biochemical and physiological response at the cellular level; 3) biochemical procedures, which measure biological activities or biological responses induced by immunological interactions; and 4) ligand or receptor binding assays, which are based upon in vivo attribute(s) of the drug substance.

Potency, expressed in units, is the quantitative measure of biological activity based upon the drug substance's attribute(s) and is linked to the relevant biological properties, whereas quantity, expressed in mass, is a physicochemical measure of the content. The Potency test results should be expressed in units of activity calibrated against the USP Reference Standard for the drug substance, where available, or against a suitable reference standard, including characterized and qualified in-house reference standards.

Often, for complex molecules, the physicochemical information may be extensive but unable to confirm the higher-order structure. The Potency test, with appropriately established acceptance criteria, assesses higher-order structure of a biologics and biotechnology drug substance. Suitably chosen, a Potency test procedure can be stability-indicating. Activity-based procedures, such as enzyme assay, ligand binding, and cell culture-based procedures can be stability-indicating. ELISA procedures may or may not be stability indicating, depending upon whether the modification affects the three-dimensional structure of the antigenic epitope(s). Combining an applicable Potency test procedure with a physicochemical or biological assay for the determination of content permits measurement of specific activity.

ADDITIONAL TESTS

Microbial Limit Test The Sponsor should consult Decision Trees 6 and 8 of the ICH Q6A Guidance to determine whether a Microbial Limit test is required in a Request for Revision. Microbial limits consist of a *Total aerobic microbial count* and *Total combined yeast and mold count* procedures. When appropriate, absence of specific objectionable microorganisms should be included in the Request for Revision. Acceptance criteria should be established according to recommendations in General Chapter *Microbiological Attributes of Nonsterile Pharmaceutical Products* <1111>.

If the finished product made with a biologics and biotechnology drug substance is nonsterile, control of bioburden is critical to allow conformance to microbial limits for the product as required by the ICH Q6A Guidance and the General Chapter *Microbiological Attributes of Nonsterile Pharmaceutical Products* <1111>. Thus, the Microbial Limit test should be included for biologics and biotechnology drug substances used to make nonsterile products. If the test is not included in the Request for Revision, its omission should be justified.

Bacterial Endotoxins Test When the biologics and biotechnology drug substance is to be used in a sterile injection, a Bacterial Endotoxins test should be included in the Request for Revision (see General Chapter *Bacterial Endotoxins Test* <85>). The bacterial endotoxins limit must be expressed in USP Endotoxin Units. No bacterial endotoxins requirement is needed if the biologics and biotechnology substance is used for finished products that are nonsterile.

Sterility Test The Sterility test is required for a biologics and biotechnology drug substance when it is stored for an extended period of time before formulation into a product. Under such circumstances, the Sponsor must include the Sterility test in the Request for Revision. Procedures and other information for the test are described in General Chapter *Sterility Tests* <71>. The Request for Revision should indicate whether a membrane filtration (method of choice) or direct inoculation procedure is employed. The direct inoculation method is used if the membrane filtration procedure is not applicable.

Safety Test *USP-NF* monographs generally do not deal with safety. Thus, a Request for Revision should not, in most circumstances, include procedures for a Safety test. However, for certain animal- or human-derived biologics and biotechnology drug substances, a general Safety test is required by 21 *CFR* 610.11 as a check for overall final product safety. The Request for Revision for such articles should include a procedure(s) described in *Safety Tests—Biologicals* section in General Chapter *Biological Reactivity Tests, In Vivo* <88>. However, the Sponsor also should consult 21 *CFR* 601.2(c)(1) for specific exclusion of Safety test requirements. If the exclusion applies sponsors do not need to include a Safety test in the Request for Revision.

Additional Tests A Request for Revision may contain other tests, if required to address the quality, potency, and purity of a biologics and biotechnology drug substance.

NEW MONOGRAPH FOR A DRUG PRODUCT

Description A qualitative description of the dosage form should be provided (e.g., color, appearance, clear liquid, suspension) in the beginning of a monograph. The Description should include the Official Name, Labeling, and Packaging and Storage statements, as discussed under the Drug Substance section of this guideline. The Request for Revision should also contain additional information about the formulation, dosage, and approved usage. This information is usually available from the package insert and will assist USP staff and Expert Committee members in evaluating the Request for Revision.

Official Title The Official Title of a drug product is generally a combination of the active ingredient or moiety, a description of the dosage form type, and the mode of administration. Because dosage forms may differ in formulation, release characteristics, and dose, depending upon route of administration and other factors, USP assigns them different official names and thus different monographs.

Labeling Monograph labeling statements (see General Notices) that are intended to affect the packaging usually are added only when there is a substantial risk to the public health. These statements indicate a requirement for specific packaging elements or cautionary statements (such as *Dilute before use*). The labeling section of a package insert may also contain required labeling to indicate which compendial tests and/or procedures in the drug product monograph are applicable.

DEFINITION

A Request for Revision for biologics and biotechnology drug product should indicate the type of dosage form, e.g., injection, tablet, and describe the dosage form in detail, including the active ingredient(s), excipient(s), and antimicrobial preservative(s), with concentration(s). For a liquid formulation, the medium in which the ingredients are dissolved should be described, including buffer composition and pH, if applicable. Sterility information should be included, where applicable. If there is no USP monograph for the corresponding drug substance, the Request for Revision also should include information about: ingredient(s) origin, such as human, animal, yeast, bacterial (name and strain); or, where applicable, the expression system (host cell name and strain); and a brief outline of the manufacturing method, e.g., fermentation, synthesis, recombinant technology, or purification from animal or human-derived tissues. In general, the information should not be less than that provided in the label and product literature.

The definition also should include the biological potency or content of the active ingredient(s) expressed as a range around the label claim, e.g., 80.0 percent to 125.0 percent. For products composed of more than one active ingredient, the potency or

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content of each ingredient should be indicated as above. In some cases, this also may be expressed as “not less than” a certain value, if appropriate. The potency of an active ingredient may be expressed in USP Units per mass unit (e.g., milligram or microgram). Because USP Units are based upon a USP Reference Standard and a specified assay, a USP Unit is not equivalent to an International Unit (IU) for an ingredient, and no official conversion parameter can be indicated to convert a USP Unit into an IU. However, if the standard and the assay procedure are the same, the Request for Revision should indicate that the USP Unit and IU are equivalent.

OTHER REQUIREMENTS

Packaging and Storage Packaging and storage statements in a Request for Revision should conform to the requirements described above for the drug substance.

Labeling The Request for Revision should include approved labeling and provide information requested for the biologics and biotechnology substance.

USP Reference Standards A Request for Revision should list the USP Reference Standards that will be used in different procedures in the monograph, as described in the substance section of this guideline.

SPECIFICATION

A Request for Revision for a biologics and biotechnology drug product should contain much of the same material submitted for the drug substance monograph. It is possible to have several drug product monographs for different dosage forms that correspond to one drug substance. When a Request for Revision for a drug product monograph is submitted for which the corresponding drug substance monograph is already in *USP*, the relationship between the specification of the substance and proposed product monographs should be considered. A *USP* drug product specification will include universal tests and specific tests, as needed. The type of dosage form determines which additional specific tests to include (Table 2).

Table 2. Dosage Form Specific Tests

Dosage Form	Expected Specific Tests	Other Specific Tests
Injection, Injectable Suspension, and For Injection	pH Sterility Bacterial endotoxins/Pyrogen Particulate matter Fill volume	Antimicrobial preservative content (multi-dose) Osmolality Particle size distribution
Oral Solids	Dissolution Uniformity of dosage units	Disintegration Friability Moisture content Microbial limits Hardness

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Dosage Form	Expected Specific Tests	Other Specific Tests
Oral Liquids	pH Antimicrobial preservative content	Microbial limits
Inhalation/Nasal spray	pH Antimicrobial preservative content	Solubility Microbial limits

Identification Test The Request for Revision should include one or more procedures for the Identification test that are sufficiently specific to allow unequivocal identification of the active ingredients in the product(s). In general, the procedures used for the Identification test of the biological substance also can be used for the biological product. Several procedures, including physicochemical, biological, and/or immunochemical, may be necessary for the Identification test. Typical procedures may include chromatography (GC, HPLC), electrophoresis (PAGE, IEF, CE, other), immunochemical methods (immunodiffusion, immunoelectrophoresis, dot-blot, Western blot, ELISA, RIA), amino acid analysis, N- or C-terminal sequencing, peptide mapping, suitable fingerprinting analysis, enzyme activity, and bioidentity test, provided that excipients in the dosage form are non-interfering. When an excipient interferes with an Identification test procedure, additional or alternative tests may be required. For example, it may not be possible to perform a “fingerprint” test (peptide mapping) for a therapeutic protein if human albumin is used as an excipient. In such cases, another procedure for the Identification test should be included in the Request for Revision. Where required, the Identification test can be a combination of two or more procedures mentioned above, e.g., HPLC-MS. Validation of an Identification test procedure is described in the General Chapter *Validation of Compendial Methods* <1225>.

Impurities In general, the Impurity test of a biologics and biotechnology drug product monograph is intended to limit only specified impurities that may increase during shelf-life (e.g., aggregates, degradants). These impurities are identified through suitable stability studies. For new drug product monographs, USP will follow the nomenclature and approaches shown in Table 1. Where different dosage forms yield different impurity profiles, appropriate Impurity test procedures for each dosage form should be included in the Request for Revision. In this case, the Request for Revision should indicate the procedure to be provided in product labeling. However, if there is no *USP* monograph for the corresponding substance, the Request for Revision for the product should contain the procedures for Product- and Process-Related Impurities, as described in the drug substance section above. Validation of the Impurity test procedures should be based upon recommendations in General Chapter *Validation of Compendial Methods* <1225>. Data from representative analyses should be included for at least three typical production lots. In addition, the Request for Revision should include data from accelerated stability studies to identify potential degradants.

Quantitation Test The Request for Revision should include suitable procedures for the quantitation of the active ingredient(s) and relevant excipients. Excipients are relevant if they have a physiological, biochemical, biological, clinical, stability-attributing, or other known role in the formulation. When possible, the Quantitation test procedures should be stability indicating. Additional information about the Quantitation test is provided in the drug substance section of this guideline.

The procedure for the Quantitation test evaluates the average drug product, with individual units combined following an approved sampling procedure, and a portion tested. The calculation then allows the evaluation of the acceptance criteria as required by the Definition. The acceptance criteria should take into account such variables as manufacturing variability, propagation of experimental errors for each ingredient to the drug product, experimental error of the assay procedure, and sampling errors. The large number of variables may lead to wider acceptance criteria for drug product monographs. Validation of the procedures should be based upon recommendations in General Chapter *Validation of Compendial Methods <1225>*. Data from representative analyses should be included for at least three typical production lots.

Total Protein Content The Request for Revision for a protein drug product should contain a procedure to allow determination of total protein content as part of the Quantitation test. A procedure(s) for the Total Protein Content is necessary because the potency or biological activity is expressed in specific activity units per mass unit, e.g., milligram or microgram. Seven procedures to allow determination of Total Protein Content are described in detail in the General Chapter *Biotechnology-Derived Products—Tests <1047>*. The choice of a procedure depends upon the nature of the biologics and biotechnology product, the availability of instrumentation, and other factors. Validation of the procedures should be based upon recommendations in General Chapter *Validation of Compendial Methods <1225>*. Data from representative analyses should be included for at least three typical production lots.

Potency Test The Request for Revision should include a suitable validated procedure for quantitative determination of active ingredient's biological potency of the active ingredient in a biologics and biotechnology Drug Product. If there is more than one active ingredient in a product, the Request for Revision should include procedures for each. The potency provides an assessment of the active ingredient biological property(ies) in a product, which describes its specific ability or capacity to achieve a defined biological effect. If the active ingredient(s) has an enzymatic, immunologic, or specific ligand-binding activity that is related to its biological function, a corresponding enzymatic, immunologic, or specific ligand-binding procedure may be included in the Request for Revision as a surrogate to a functional test. The procedures that can be used to measure potency are described above for drug substances.

The results of the potency test should be expressed in units of activity calibrated against the USP Reference Standard for the corresponding drug substance, where available, or against a suitable reference standard, including characterized and qualified in-house reference standards. Often, for complex molecules, the information obtained by the

physicochemical measurements may be extensive, but unable to confirm the higher order structure. The potency test, with appropriately established acceptance criteria, can confirm consistent higher order structure and provide useful structural and stability information for a biologics and biotechnology drug product. Furthermore, appropriately chosen, a potency assay can be stability indicating. Compared with the potency of the corresponding drug substance, the potency of a product's active ingredient often can provide useful information regarding the excipients' effect(s) on the active ingredients.

In many cases, a ratio of the values obtained from a functional procedure to those from a physicochemical procedure, i.e., activity to mass ratio, provides appropriate potency information. Based upon the results of the physicochemical procedures for determining content and potency measurement, the specific activity or potency per unit mass can be calculated. Where this is not possible, the potency can be expressed per unit dose of the product.

OTHER SPECIFIC TESTS

A Request for Revision should list specific tests, when needed. The following specific tests usually are required, depending upon the dosage form (see Table 2).

Uniformity of Dosage Units The Uniformity of Dosage Units test is intended primarily for solid oral dosage forms. It is performed by evaluating at least 10 units, using either the weight variation procedure or the content uniformity procedure. The requirements and criteria to determine which procedure to use are included in General Chapter *Uniformity of Dosage Units* <905>. Where the weight variation procedure is indicated, the Request for Revision should include typical data for several batches. Where the content uniformity procedure is indicated, and the procedure used in the Quantitation test is employed to measure content, the Request for Revision should state that acceptance criteria “meet the requirements.” When use of the Quantitation test procedure is proposed, the validation data should support this additional use. When a procedure other than that of the Quantitation test is proposed, validation criteria required for a quantitative assay should be evaluated and included in the Request for Revision, with particular emphasis on precision.

pH The pH test is used primarily for injections and other liquid formulations. A pH test also can be stability indicating. A major component of the procedure employed in the pH test is sample preparation. The Request for Revision, therefore, should include information about the final sample concentration, temperature, proposed acceptance criteria, and data for three typical production lots (see General Chapter *pH* <791> for details).

Antimicrobial Preservative For drug products containing a preservative, the Request for Revision should include a procedure to measure content, as described in General Chapter *Antimicrobial Preservative Content Test* <341>. For preservatives and/or procedures not included in General Chapter *Antimicrobial Preservative Content Test* <341>, the Request for Revision should include a detailed description of the procedure,

including analytical method validation as required by General Chapter *Validation of Compendial Methods* <1225>, acceptance criteria, and data from at least three typical production lots. In addition, the Request for Revision should include data to support antimicrobial preservative effectiveness, as described in General Chapter *Antimicrobial Effectiveness Testing* <51>. The acceptance criterion is based upon the minimal amount that has been shown effective.

Sterility The Sterility test is applicable only to products labeled sterile. Procedures and other information for the test are described in General Chapter *Sterility Tests* <71>. The Request for Revision should indicate whether a membrane filtration (method of choice) or direct inoculation procedure is employed. The direct inoculation method is used if the membrane filtration procedure is not applicable.

Bacterial Endotoxins/Pyrogen For injections, the Request for Revision should include a Bacterial Endotoxins test procedure. The Request for Revision should include validation data that assess applicability of the procedure selected for the proposed drug product (see General Chapter *Bacterial Endotoxins Test* <85> for validation of the Bacterial Endotoxins test procedure). The endotoxins limit must be expressed in USP Endotoxin Unit, should be calculated based upon the maximum dose of the injection to be given to a patient within a one-hour period, and is expressed on a patient's per kilo basis. If a Bacterial Endotoxins test procedure cannot be validated for a biologics and biotechnology product, the Request for Revision should include a Pyrogen test (see General Chapter *Pyrogen Test* <151> for details).

Particulate Matter The Particulate Matter test is used for injections, injectable suspensions, or products for injection, which must meet acceptance criteria provided in General Chapter *Particulate Matter in Injections* <788>.

Residual Moisture A Request for Revision for tablets and capsules should include procedures to test for residual moisture determination, as required by the General Chapters *Containers* <661> and *Containers, Permeation* <671>. Three methods are indicated in General Chapter *Water Determination* <921>. The method of choice depends upon the product's nature. If none of the methods described in General Chapter *Water Determination* <921> is suitable for a biologics and biotechnology product, an alternative method can be included in the Request for Revision. In such cases, a rationale for the alternative method should be provided in the Request for Revision. For example, gas chromatographic methods also have been used for determining water for many biologics and biotechnology products.

Loss on Drying Where Loss on Drying is not due entirely to water, the Request for Revision instead may contain a Loss on Drying test (see General Chapter *Loss on Drying* <731>). Whatever procedure is chosen, a validation package consistent with the requirements of General Chapter *Validation of Compendial Methods* <1225> should be included in the Request for Revision.

Performance Tests These tests measure the amount of active ingredient released, or the degree of disintegration, at a specified time or times for solid oral dosage forms. For these dosage forms, dissolution or disintegration procedures are used, as described in General Chapters *Disintegration* <701>, *Dissolution* <711>, and *Drug Release* <724>. Information about media preparation is provided in the Reagents section of *USP-NF*. Conformance to a USP Performance test is intended to reflect expected performance over the product's shelf life.

Dissolution For specific information not covered in General Chapter *Dissolution* <711>, the Request for Revision should include: a detailed sampling procedure; dissolution conditions (medium, volume, apparatus, and temperature); sample analysis procedure (LC, UV-Vis); analytical method validation, as required by General Chapter *Validation of Compendial Methods* <1225>; acceptance criteria; and dissolution profiles of at least three production lots. In general, the Dissolution test should use conditions that differentiate between acceptable and unacceptable formulations, and the profiles should include several data points. Typically, the least vigorous conditions are those that provide the greatest differentiation, and are, therefore, preferred.

Disintegration A Disintegration test procedure usually is not acceptable as a means of satisfying the Performance test for a biologics and biotechnology drug product.

Drug Release This performance test parallels the Dissolution test, but is intended for modified-release drug products. For information not covered in General Chapter *Drug Release* <724>, Requests for Revision should describe this procedure, including multiple sampling times and multiple acceptance values. Multiple dissolution conditions may be required to represent varying gastrointestinal conditions. Sampling times and acceptance criteria may vary depending upon the procedure and the product. Typically, sampling times for the drug release procedure will include an early time to demonstrate absence of dose dumping, a time that covers the total dosing interval, and at least one time between the two extremes. The Request for Revision should include a detailed sampling plan, proposed acceptance criteria, and dissolution profiles over at least three production lots.

ADDITIONAL TESTS

The Request for Revision may include additional tests, as appropriate. The rationale for inclusion of tests should be provided, with full validation data, in conformance with the General Chapter *Validation of Compendial Methods* <1225>.

REFERENCE STANDARD MATERIAL

Most USP tests require comparison to one or more official USP Reference Standards (RS). USP monographs and General Chapters, therefore, include not only the test procedures, but also refer to needed RS for these procedures. Further information is provided in General Chapter *Reference Standards* <11>. A Request for Revision should define the need for RS material and should be accompanied by a sufficient quantity of

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candidate material, together with characterization data, stability data, storage conditions, and other relevant data. Sponsors can determine the amount of material and timing of material receipt, working with appropriate USP staff. USP will evaluate the Request for Revision to determine if more or fewer RS are needed. Based upon this review, USP subsequently tests collaboratively, labels, and packages candidate material(s). Test results are reviewed by the RS Committee of the Council of Experts. If approved, the candidate material will become official USP RS.

REAGENTS

USP monographs occasionally may refer to commercially available unofficial reference standards, in which case they are listed in the *USP–NF* Reagents section. This section describes the grade and purity of commercial material necessary to complete the procedure referencing the reagent. The addition of or revision to a reagent in the *USP–NF* reagent section generally is completed by USP staff, without collaborative testing or evaluation by the USP Reference Standard Committee. When a specific grade of material is required and is commercially available, the Request for Revision should include company, catalog number, CAS number, and reagent description. USP staff will work with the reagent vendor to create an appropriate description and any needed additional testing. Changes to reagents should include the same elements as a monograph revision, but the validation data only need show that the change is necessary and demonstrate equivalency.