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Standard Guide for Determination of Purity, Impurities, and Contaminants in Biological Drug Products[1]

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INTRODUCTION

The purity of biological drug products historically has been significantly lower than that of other pharmaceutical drug products. This is a consequence of the structural complexity of biological drug products as well as the fact that, until recently, these products were obtained only with great difficulty and at high cost from natural sources such as human or animal serum or tissue. Although many of these products were of low purity, long-term use in humans proved their safety and efficacy. The development of recombinant DNA (rDNA) technology and the parallel development of sophisticated preparatory, analytical, and immunological methods, have resulted in the ability to produce high purity biological drug products. It should be recognized that the standards for purity of rDNA-derived drugs are comparable to those established for United States Pharmacopeia (USP)-quality drug substances. For example, the purity of an rDNA-derived drug substance may exceed 97 % and impurities, (see Section 4) such as host cell proteins are separately quantitated in the parts per million range (via immunoassay).

1. Scope

1.1 This guide covers the concepts of purity, impurity, and contamination in biological drug products.

1.2 This guide suggests methods for determination of impurities and contaminants in such products.

1.3 This guide is arranged as follows:

1.4 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.*

2. Terminology

2.1 *Definitions:*

2.1.1 *contaminants*—all adventitious substances or microorganisms present in raw materials, bulk drugs, or final products.

2.1.2 *deleterious impurities*—impurities that might be a health or safety concern, particularly with respect to toxicity, carcinogenicity, or immunogenicity. Deleterious impurities must be controlled and their levels determined using suitable analytical methods.

2.1.3 *impurities, of a biological drug product*—all process-related (nonadventitious) substances present in the raw materials, bulk drug, or final drug product that are not considered to be the active material, additives, or excipients.

2.1.4 *innocuous impurities*—impurities that are not a health or safety concern in the product. The route of administration of the drug may be a significant criterion in the determination of whether an impurity is innocuous.

2.1.5 *purity, of a biological drug product*—the measure of the biologically active drug in relation to the total substances (not including additives) present in the drug product, usually expressed on a percentage basis.

3. Significance and Use

3.1 This guide suggests analytical methods generally applied within the pharmaceutical industry to identify and quantitate the level of impurities and contaminants present in the

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preparation of a biological drug product. These methods are not intended to be all-inclusive. The methods used by an individual manufacturer must be specific to the product and process of production.

4. Purity

4.1 *General Considerations*—Numerous considerations are involved in determining purity acceptability criteria, and these criteria are ultimately determined on a case-by-case basis. In each individual case, the risk-to-benefit ratio must be evaluated in determining an acceptable level of purity and the acceptable types of impurities of any new drug product. For example, the same purity requirements are not necessarily applied to a vaccine, which may be administered in one or two injections, as to a chronic care product, which may be administered several times per week for many years. It is not difficult with currently available technology to achieve a purity level of 97 % (w/w). At this level of purity, drug doses of 0.01, 0.1, and 1 mg/kg in a 70-kg patient correspond to impurities of 21 µg, 210 µg, and 2.1 mg/dose, respectively. At a purity of 99.99 %, these figures decrease to 0.07 µg, 0.7 µg, and 7 µg, respectively. Even these latter levels of impurities may be significant if they are repeatedly administered for prolonged periods. Although regulatory guidance on acceptable purity levels has been difficult to obtain, the lack of a database for rDNA-derived products has given rise to a generally more conservative approach than that which has been used previously for human biologics or blood-derived products. Also, the purity of a biological drug substance cannot be defined without specifying the assay method used. Various levels of purity may be obtained by a variety of analytical methods. It is, therefore, extremely important to use analytically valid methods for the estimation of purity.

4.2 *Estimation of Purity*—Purity may be estimated by two basic methods: weight percentage correlations or relative response measurements. A recognized reference standard is required for quantitation when using weight percentage methods while data may be obtained independently of a reference standard when using relative response measurements. However, the accuracy and precision of the latter type of measurement is usually less than that obtained using the reference standard technique. Relative response measurements are often employed for the measurement of unknown impurities for which no reference standard is available (see Table 1).

5. Impurities

5.1 *General Considerations*—Impurities may include substances derived from the active drug substance as well as nondrug-related components such as host cell proteins or nucleic acids. Impurities may include those proteins that have undergone a chemical or physical change at one or more sites in the molecule. The determination of whether such changes in a molecule result in impurities must be made based on data that demonstrate whether or not these molecules have the same, similar, or different properties compared to the intact drug molecule (see Table 1).

5.2 *Major and Minor Impurities*:

5.2.1 Some distinctions have been made between major and minor impurities.[2] Although these distinctions were generated to address more conventional drugs, they are likely to form the basis for consideration of biological drugs, whose numbers will increase as rDNA products begin to reach the marketplace in larger numbers.

TABLE 1 Methods for Determination of Impurities and Contaminants

Common Impurities or Contaminants	Detection Method
Endotoxin	LAL,[A] rabbit pyrogen
Host Cell Proteins	SDS-PAGE,[B] immunoassays
Other Protein Impurities	SDS-PAGE, HPLC,[C] immunoassays
DNA	DNA hybridization, UV spectrophotometry
Mutants	HPLC-tryptic mapping
Formyl methionine	HPLC-tryptic mapping
Oxidized methionines	Amino acid analysis, HPLC-tryptic mapping, Edman degradation analysis
Proteolytic clips	IEF,[D] SDS-PAGE (reduced), HPLC
Deamidation	IEF (standard comparison), HPLC
Microbial (bacteria, yeast, and fungi)	Microbiological Testing (sterility, bioburden)
Mycoplasmas	Modified 21CFR Method,[E] DNAF[F]
Viruses (endogenous and adventitious)	CPE,[G] Had,[H] electron microscopy, reverse transcriptase activity

[A] Limulus amebocyte lysate.
[B] Sodium dodecyl sulfate-polyacrylamide gel electrophoresis.
[C] High performance liquid chromatography.
[D] Isoelectric focusing.
[E] Draft guidelines.
[F] DNA binding fluorochrome.
[G] Cytopathic effect.
[H] Hemadsorption.

5.2.1.1 A major or significant impurity is one present at 0.5 to 1 % or greater. Levels of such an impurity should be kept as low as technically possible. The manufacturer may consider obtaining toxicologic, pharmacologic, and immunologic data on major impurities.

5.2.1.2 A minor impurity is one present at less than 0.5 %.[2] Recommended actions for impurities below this level are mainly concerned with identification. Identification (that is, molecular weight determinations) of minor impurities may be accomplished by utilizing some of the assays outlined in this guide or additional assays if necessary.

5.3 *Nature of Impurities and the Consequences of Their Presence*:

5.3.1 Because the products from rDNA are derived from living organisms, any host cell or host cell component is an impurity. In addition, other substances present during the growth of the organisms and purification of the product are impurities. Such substances include antibiotics, media, reagents, and column materials.

5.3.2 Impurities that may be associated with fermentation processes include proteins, nucleic acids, carbohydrates, lipids, and endogenous viruses. It is possible that these impurities may remain associated with the product and the characterization of the product should address this possibility.

5.3.3 Protein impurities are the most common impurities.

[2] Wolters, R. J., "Bulk Drug Substances and Purity: A Regulatory Viewpoint," *Pharmaceutical Technology,* October 1984, pp. 35–38.

These may arise from fermentation, media, or the host organism. The acceptable level of these impurities must be established based on toxicological and immunological studies.

5.3.4 Damaged, mutated, or modified product (for example, disulfide isomers) can result from either fermentation or handling during recovery. Because such substances may have altered pharmacokinetic properties, biological potency, or enhanced immunogenicity (potentially leading to antibodies against the product itself), these impurities could be of major concern. Characterization and quantitation of these impurities should address their pharmacology.

5.3.5 Concern about nucleic acid impurities arises from the possibility of cellular transformation events in a recipient. The use of eukaryotic cells as host cells for pharmaceutical production (whether using rDNA methods or not), has recently been examined in great detail and, by consensus, is acceptable if adequate testing is performed.[3] Part of this testing involves process and product characterization in removing host cell and vector DNA. In general, a process validation study that demonstrates DNA removal and provides final product analysis estimates of less than 10 pg/dose of host/vector DNA is both acceptable and achievable. Less or more stringent requirements may be needed in special cases.

5.3.6 Concerns related to viruses as impurities refer entirely to endogenous viruses that might be present in the host cell. Adequate characterization of the master cell bank and monitoring of the harvest supernatants together with well-designed and validated purification processes can identify and remove viral impurities. Incorporation of deactivation steps in the process is also desirable in order to achieve adequate safety margins even if such steps do not improve the product yield or purity.

6. Contaminants

6.1 *General Considerations*—Contaminants may be divided into biological adventitious agents and chemical adventitious agents.

6.1.1 Biological adventitious agents include bacteria, fungi, mycoplasmas, and viruses.

6.1.2 Chemical adventitious agents include allergens and chemical species accidentally introduced into the material (for example, petroleum oil, heavy metals, residual solvents, column materials, cleaning reagents, and other products manufactured in the same facility).

6.2 *Effects of Contaminants*:

6.2.1 Contaminants may present a known health or safety risk. It is important to recognize that good manufacturing practices, (GMP) exist in the pharmaceutical industry to prevent or eliminate contaminants.

6.2.2 Contaminants may:

6.2.2.1 compromise host cell function prior to expression of the product,

6.2.2.2 cause degradation of the product after expression,

6.2.2.3 raise safety or efficacy issues if present in the final product, and

6.2.2.4 exist as a source of extraneous material, either innocuous or deleterious.

7. Methods for Determining Impurities and Contaminants

7.1 The methods outlined in Table 1 are some of the methods that may be used to determine and quantify the level of impurities and contaminants present in a biological bulk drug substance or final product, or both.

7.2 Some methods are rather general in applicability, for example, limulus amebocyte lysate, (LAL) rabbit pyrogen, sterility testing, and electron microscopy. Many of these methods are general procedures used in many laboratories but they must be tailored to the specific product and process being evaluated. For example, the applicability of various high performance liquid chromatography, (HPLC) techniques may vary because of the size and hydrophobicity of the molecule being analyzed. It is also possible for manufacturers of similar or identical biological drug products to use entirely different manufacturing techniques and processes. These differences will probably necessitate the use of different assays to determine the level or presence of impurities and contaminants.

[3] Quinnan, G. V., Jr., "Protein Contaminants in Biologic Products Derived from Cell Substances, in Abnormal Cells, New Products and Risk," *Proceedings*, National Institutes of Health, Bethesda, MD, 1985, p. 41.