Designation: E1298 – 06

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

This standard is issued under the fixed designation E1298; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

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.

[1] This guide is under the jurisdiction of ASTM Committee E55 on Manufacture of Pharmaceutical Products and is the direct responsibility of Subcommittee E55.04 on General Biopharmaceutical Standards.

Current edition approved Nov. 1, 2006. Published November 2006. Originally approved in 1998. last previous edition approved in 2000 as E1298 – 98 (2000). DOI: 10.1520/E1298-06.

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 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). The general considerations include the nature of the impurity, the amount of the impurity and the potential adverse effect of the impurity.

TABLE 1 Methods for Determination of Impurities and Contaminants

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

[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 *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.

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.

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