

Best Practices Guide

Enzyme Dietary Supplement Products

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1. INTRODUCTION AND SCOPE

Enzymes are proteins with highly specialized catalytic functions that are produced by all living organisms and are responsible for many essential biochemical reactions in microorganisms, plants, animals, and human beings. Enzymes are essential for all metabolic processes, but are not themselves alive. Although, like all other proteins, enzymes are composed of amino acids, they differ in function in that they facilitate biochemical reactions without undergoing change themselves. As highly efficient natural protein catalysts, enzymes help biochemical reactions take place quickly and efficiently. This catalytic capability is what makes enzymes unique. Enzymes not only work efficiently and rapidly, but they are also biodegradable.

Enzymes used in dietary supplements are usually referred to by their common names and may be of animal, plant, fungal, or bacterial origin. Most enzymes are very specific in their ability to catalyze only certain chemical reactions; this high degree of specificity and strong catalytic activity are the most important functional properties of enzymes. Commercial enzyme preparations usually contain several enzymes that have catalytic activities other than those for which they are standardized (often termed “side activities”).

Statement of Scope

This voluntary Best Practices Guide was prepared to help promote the safe production and use of enzyme-containing dietary supplements and to facilitate transparency and uniformity in the dietary supplement and enzyme industries. The Best Practices Guide was prepared with input from the dietary supplement and enzyme industries and takes into account the current U.S. laws and government regulatory requirements. It reflects the most up-to-date science and industry thinking with regard to the safe handling of enzyme-containing dietary supplements and will be updated as best practices evolve.

The Best Practices Guide is not intended to be a substitute for consultation with legal and regulatory counsel in all jurisdictions, including in the United States.

Examples of Types of Enzymes Commonly Used in Dietary Supplements

Examples of the most common types of enzymes used in dietary supplements follow. This list is not meant to be all-inclusive; other categories of enzymes may be safely used in dietary supplement products. It is the manufacturers’ responsibility to ensure that the enzymes used in their products are safe and in compliance with the applicable laws, regulations, and compendia.

1. *Proteases*: Enzymes that catalyze the hydrolysis of peptide bonds in proteins, yielding peptides and amino acids. Examples include Bromelain, Papain, Trypsin, Peptidase, Subtilisin, Serratiopeptidase, Nattokinase, and fungally derived proteases.
2. *Carbohydrases*: Enzymes that catalyze the hydrolysis of bonds in carbohydrates. Examples include Amylase, Glucoamylase, Cellulase, Invertase, Lactase, and alpha-Galactosidase.

3. *Lipases/esterases*: Enzymes that catalyze the hydrolysis of fats. Examples include pregastric esterase, pancreatic lipase, and fungally derived lipase.
4. *Other Enzymes*: Inclusive of nondigestive enzymes (e.g., catalase).

2. HANDLING, STABILITY, STORAGE, AND EXPIRATION DATING

Safe Handling Practices

Enzymes are biodegradable, water soluble, and generally nontoxic. However, health-related hazards such as skin irritation and respiratory sensitization may occur when working with enzymes if they are not handled correctly.

Skin irritation can occur from direct contact with proteolytic enzymes. Prolonged and direct exposure of skin or mucous membranes (particularly eyes and nose) to proteolytic enzymes may in some cases cause redness and itching of skin or mucous membranes. The irritation will cease shortly after the exposure to the proteolytic enzyme stops, and a full recovery is normally observed within a few days.

Respiratory sensitization, on the other hand, is a more serious health hazard, as it can develop into respiratory allergy. Naturally occurring substances such as pollen, house dust mites, animal hair, and fungi are known as common respiratory allergens and can cause allergies. They are all proteins, and they are all able to become airborne.

The symptoms associated with respiratory allergy are similar to those commonly related to hay fever and asthma:

- Persistent sneezing
- Blocked nose/sinus congestion
- Watery eyes/runny nose
- Breathing difficulties
- Coughing

Safe handling of enzyme preparations can be accomplished through proper work practices, engineering controls, and use of personal protective equipment. When working with these preparations, it is important to use work practices that minimize the generation of dust/aerosols or that result in inhalation or direct skin or eye contact.

Dust or aerosols are formed through high-energy operations such as weighing, mixing, grinding, washing with high pressure water or steam, and using compressed air for cleanup operations. Sweeping, blowing, splashing, steam cleaning, and high-pressure water flushing should be avoided. Mixing and grinding operations should be contained as much as possible, and the areas in which they take place should be provided with adequate local exhaust ventilation.

Direct skin contact may be avoided by wearing gloves appropriate for operations that have potential for enzymes to contact skin. It is advisable to wash enzyme-contaminated surfaces thoroughly with cold water before handling.

Firms should also become familiar with the Globally Harmonized System of Classification and Labeling of Chemicals (GHS). GHS information may be found at <http://www.osha.gov/dsg/hazcom/ghs.html>.

Use of Personal Protective Equipment

1. *Respiratory Protection:* Under most operating conditions involving enzymes, respiratory protection should be used in compliance with National Institute of Occupational Safety and Health (NIOSH) standards. When working with enzymes, there are some operations, such as spill cleanup, equipment cleaning, and equipment repairing, that may generate dust/aerosols that make respiratory protection necessary. Respiratory protection should also be used when indicated by your supervisor, safety professional, or medical personnel. The Occupational Safety and Health Administration (OSHA) respiratory protection standard (29 C.F.R. Part 1910 – see also <http://www.osha.gov/SLTC/respiratoryprotection/standards.html>) should be followed in the selection, training, and use of respirators. Use only NIOSH-approved respiratory protection.
2. *Protective Clothing and Gloves:* Protective clothing should be worn when there is a potential for skin or eye contact. This clothing may include gloves, aprons, safety glasses, and outer garments, such as coveralls or lab coats. Protective clothing is particularly important when working with proteolytic enzymes, which are known to cause skin irritation. Operations that require the use of protective clothing include spill cleanup, equipment maintenance, and equipment cleaning. Gloves should be worn when there is a potential for skin contact with any enzyme material. Absorbent liners or lined gloves are recommended to absorb perspiration. Protective clothing should be removed prior to leaving the work area and should not be worn into other areas of the facility (i.e., lunchroom, offices) or to the home. The OSHA personal protective equipment standard (1910.132-138) should be followed in selection, training and use of personal protective equipment. Consult the enzyme manufacturer and/or its SDS (MSDS) for additional information on the selection of personal protective equipment.

Maintenance and Spillage

All dietary supplement manufacturers must comply with the sanitation and maintenance requirements set forth in the U.S. Food and Drug Administration's (FDA) regulations for the current Good Manufacturing Practice in Manufacturing, Packaging, Labeling or Holding Operations for Dietary Supplements, 21 C.F.R. Part 111 (cGMPs). The recommendations that follow should be considered in light of the requirements in FDA's dietary supplement cGMPs when working with enzymes.

1. *Maintenance:* Whenever maintenance is to be performed on equipment that has been in contact with enzymes, the equipment should be cleaned before the work begins. Use wet washing (flooding, wiping) or a vacuum system equipped with a high-efficiency particulate air filter (HEPA) to clean equipment or spills. High-pressure cleaning (steam, air, or water) should

be avoided, since these operations are known to cause aerosol formation. Appropriate personal protective equipment (gloves, respirators, eye protection) should be used during maintenance operations.

2. *Spill Cleanup:* Spilled enzymes should be removed immediately by a central vacuum system, vacuums equipped with HEPA filters, mopping, low-pressure washing or as described on the manufacturer's SDS (MSDS). To minimize dust or aerosol formation during cleanup, do not sweep or use high water pressure, steam, or compressed air on spills. Use plenty of water in wet washing to flush all enzyme material away to minimize enzyme dust generation from dried material. Appropriate respiratory protection and protective clothing should be used during cleanup to avoid skin/eye contact and inhalation. Disposal of spilled material should be performed in compliance with federal, state and local regulations.

Personal Cleanliness

Personal cleanliness is essential to prevent irritation from proteolytic enzymes to skin and mucous membranes, and potential inhalation exposure from all enzyme types. The irritation response on skin is increased in the presence of moisture and when the natural oils of the skin are removed.

The following procedures are recommended to minimize potential irritation:

1. Hands should be washed with cool water and mild soap before leaving the work area and immediately after coming into contact with enzyme materials.
2. Change work clothes daily and whenever they are soiled with enzyme material. Do not wear work clothing, including shoes, home.
3. Avoid touching the face and eyes with enzyme-contaminated hands, gloves or clothing.
4. Wear absorbent liners or lined gloves to absorb perspiration, where appropriate.
5. After removing gloves, wash hands before touching anything.

Measuring Enzyme Levels in the Air

Air-monitoring techniques are available to measure the level of enzyme dust or mist in the air. Firms should identify whether air monitoring is needed based on the specific handling and operations they engage in that involve enzymes. The American Conference of Governmental Industrial Hygienists (ACGIH) has established a threshold limit value (TLV) for only one class of enzymes, subtilisins, of 60 ng/m³ as a ceiling limit. Both low-flow and high-flow air sampling methods are available for some enzymes. Contact the enzyme manufacturer for additional information regarding air monitoring techniques for a specific enzyme.

First-Aid Treatment

Firms should review and follow first-aid treatment recommendations contained in the SDS (MSDS) information for the enzymes they are using. The following first-aid steps are generally recommended for all enzymes:

1. *Skin Contact:* Most enzyme materials are water soluble; therefore, the exposed skin should first be thoroughly flushed with cool water and then washed with a mild soap and cool water. If clothes are contaminated, remove them, shower and change into clean clothes. Immerse the contaminated clothes in water and wash them thoroughly.
2. *Inhalation:* Remove the individual from exposure and monitor for irritation or allergic symptoms. Mild to severe symptoms may occur and may include any, or a combination of, the following: sneezing, nasal or sinus congestion, coughing, watery eyes, runny nose, tightness of the chest, hoarseness or shortness of breath, and/or asthma. If symptoms occur, consult a physician or, if severe symptoms are apparent, call 911. Symptoms may occur as late as two (2) or more hours after exposure, and anyone so exposed should be visually monitored over this time and not simply sent home.
3. *Eye Contact:* Rinse the eyes thoroughly with cool water for at least fifteen (15) minutes and then consult a physician.

Stability, Storage and Expiration Dating

The combination of moisture and heat can cause rapid deterioration of product integrity and enzyme activity levels. Although all enzymes lose potency over time at a rate dependent on storage conditions, enzyme products are sufficiently stable if kept cool and dry.

Each enzyme may have a unique stability profile under various conditions. Decisions regarding proper storage considerations should be based upon data and experience with each enzyme. Stability testing should be based on an appropriate and valid stability protocol suitable for enzyme proteins. Any stability analyses should be completed at the finished product stage, if possible (i.e., product is in the final primary package/enclosure system).

Accelerated stability testing is generally not recommended, though it may be appropriate under specific circumstances. In blends of multiple enzymes, it may be necessary to select appropriate enzyme “markers” of total enzyme activity due to the inherent limitations associated with assaying multi-enzyme blends, and a “worst-case” approach should be considered. Expiration dating, while not required under the FDA’s dietary supplement cGMPs, if included on the label should be supported by relevant data and experience with each enzyme. For most enzymes, appropriate storage conditions are <25°C and <40%RH. Stability programs should take into account the proteinaceous nature of enzymes and in particular the denaturation of enzymes at elevated temperatures.

Stability testing resources that firms may consider, include, but are not limited to:

- a) The International Conference on Harmonisation (ICH) – Quality guidance for stability testing. Note that the ICH guidance was developed for pharmaceutical products, and will require appropriate modification for dietary supplements that include enzyme ingredients.

The ICH Stability guidance documents may be downloaded at:

<http://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm065005.htm>

and/or

b) The NSF-DBA Stability Testing Guideline for Dietary Supplements, which may be found at:

http://www.nsf.org/newsroom_pdf/Stability_Testing_Dietary_Supplements.pdf.

3. IDENTITY SPECIFICATIONS AND TESTING

Enzyme-containing dietary supplements marketed in the United States must be produced in accordance with FDA's regulations for cGMPs (21 C.F.R. Part 111). 21 C.F.R. § 111.70(b) requires that "[F]or each component that you use in the manufacture of a dietary supplement, you must establish component specification as follows: (1) you must establish an identity specification; . . . (3) you must establish limits on those types of contamination that may adulterate or lead to adulteration of the finished batch of the dietary supplement to ensure the quality of the supplement."

Valid Scientific Method(s) to Identify a Component

Commercial enzyme preparations are extracts composed of a mixture of different proteins and are most often defined, identified, and labeled by their various enzymatic activities rather than by specific enzyme names. The total enzymatic activity does not necessarily come from one enzyme in the extract; sometimes several enzymes work in concert to achieve an overall activity and effect.

For example, the identity of a multi-enzyme preparation containing Papain and Bromelain, used as a component of a dietary supplement, may be specified as "protease" – the principal activity of the mixture – on the ingredient specification and the product label. Accordingly, the identification for such enzyme preparations can be verified by scientifically valid testing of enzymatic activity. If a dietary supplement manufacturer wishes to include the names of specific enzymes on the dietary supplement product label, the identity specification should be established for the labeled enzymes and "appropriate, scientifically valid methods" should be used to verify the identity specification of each labeled enzyme, which means that the method is "accurate, precise, and specific for its intended purpose."

Compendial methods of measuring and expressing enzyme activity are presented in the Food Chemicals Codex (FCC), United States Pharmacopoeia (USP), Federation Internationale Pharmaceutique (FIP), European Pharmacopoeia (Ph. Eur.) and Japan Pharmacopoeia (JP). Compendial methods should be adopted whenever possible.

For enzymatic activity not adequately covered by test methods in compendial sources, it is recommended that methods to measure activity have undergone scientifically sound development and validation procedures to ensure accuracy and reproducibility. A well-defined substrate with adequate lot-to-lot uniformity should be used. Validation of enzyme assays should document assay specificity, assay variability, assay linearity and assay sensitivity.

Where new internally developed or proprietary assay methods are used, it is recommended that enzyme dietary supplement manufacturers provide details of the method to their customers or a designated third party under the terms of an executed nondisclosure agreement. Whenever possible, such methods should utilize a commercially available analytical reference standard.

Enzyme Source Organisms

Source organisms used for enzyme production should comply with Joint FAO/WHO Expert Committee on Food Additives (JECFA) and Food Chemicals Codex standards. Enzymes used in dietary supplements may be derived from animal, plant, and microbial sources. Animal tissues used for the preparation of enzymes should comply with meat inspection requirements and be handled in accordance with good hygienic practices.

Plant materials and microorganisms used in the production of enzyme preparations should not leave any residues harmful to health in the processed and finished product under normal conditions of use.

Preparations derived from microbial sources should be obtained using a culture fermentation of a non-pathogenic and non-toxigenic strain and produced by controlled fermentation, thus preventing the introduction of microorganisms that could be the source of toxic materials and other undesirable substances.

Carriers, Diluents, and Processing Aids

The carriers, diluents, and processing aids used to produce enzyme preparations should be substances that are permitted for general use in foods.

Microbiological Testing

Enzyme preparations for use in dietary supplements should comply with the JECFA “General Specifications and Considerations for Enzyme Preparations Used in Food Processing,” which may be found at: http://www.fao.org/ag/agn/jecfa-additives/docs/enzymes_en.htm.

It is recommended that manufacturers of enzyme dietary supplements also reference approved/validated microbiological testing methods. Examples include, but are not limited to, Bacteriological Analytical Manual (BAM), USP/FCC and AOAC methods.

Preservatives

Any preservatives used in the final dietary supplement should be declared according to FDA labeling regulations in the United States and under applicable laws and regulations in other jurisdictions.

4. SAFETY AND TOXICITY TESTING

Enzyme preparations used in dietary supplements may contain other components including carriers, residual amounts of processing aids, and substances added as stabilizers, preservatives, or diluents. Production organisms should be non-pathogenic. Accepted microbiological techniques are used to exclude contaminating organisms and to avoid development of sub-strains from within the culture itself. *See above.*

Allergens

Enzymes are proteins and, as with any protein, may elicit an allergic reaction upon inhalation by sensitized individuals. While enzyme proteins are generally not considered food (ingestion) allergens, it is not appropriate to label enzyme-containing products as “allergen free” or “hypoallergenic.”

Diluents, carriers and other added agents may include Food Allergen Labeling and Consumer Protection Act of 2004 (FALCPA) listed allergens and should be labeled (i.e., enzymes standardized with lactose and SOD stabilized with wheat gliadin) as required by FALCPA.

Toxins and Toxicity Testing

Enzyme preparations for use in dietary supplements should comply with the JECFA “General Specifications and Considerations for Enzyme Preparations used in Food Processing,” which may be found at: http://www.fao.org/ag/agn/jecfa-additives/docs/enzymes_en.htm.

5. NDI Considerations

The Dietary Supplement Health and Education Act of 1994 (DSHEA) defines "new dietary ingredients" (NDI) as those dietary ingredients not marketed in the United States before October 15, 1994. Any enzyme 1) found to be a component of food that is not chemically altered; 2) grandfathered as a dietary supplement prior to October 15, 1994; 3) is Generally Recognized as Safe (GRAS) for its intended use; or 4) any enzyme, which is the subject of and meets the criteria of a food additive petition, may not require additional toxicological testing and may be exempt from the NDI process.

Enzymes found to fall outside these parameters may require registration and testing under the FDA’s guidance for NDIs. Manufacturers of enzyme dietary supplements should consult with their regulatory and legal counsel regarding NDI issues.

6. LABELING

Under 21 C.F.R. § 101.36(b)(3)(ii)(A), dietary ingredients without established daily requirements (RDI's and DRV's) must be labeled with appropriate metric units in the products nutrition labeling/supplement facts. Enzymes are proteins that function to catalyze specific chemical reactions. It is this catalytic activity rather than the presence of proteins for which enzymes are utilized as dietary supplements. Thus, providing a measure of potency (activity) in addition to mass (mg) is more informative for consumers. For compliant and meaningful labels, it is recommended that enzyme-containing dietary supplements be labeled with both metric measures and activity units either by individual enzyme or as proprietary blends of enzymes. Enzyme potency is determined through enzymatic activity assays.

Activity:

Enzyme activity is determined through enzymatic activity assays. Enzyme assays measure the ability of a given enzyme to catalyze a specific reaction under specific conditions including time, temperature, and pH. The assay units are typically expressed in unique acronyms or other nomenclature (HUT, PC, SU, ALU, etc.).

Enzyme-containing dietary supplements should be labeled with the appropriate enzyme assay units. Assay methods should be verified per cGMPs. Net weight of the supplement should be included along with the appropriate enzyme activity units in the supplement facts panel of the label per 21 C.F.R. § 101.36. Net quantity contents should be declared per 21 C.F.R. § 101.105.

Appropriate Assay(s) to Measure and Standardize Activity:

1. Published compendial methods, including (but not limited to) the FCC, USP and FIP, are the preferred methods to measure enzymatic activity for label declaration.
2. If no compendial method is available or applicable to the relevant enzyme activity, use of other methodologies published in peer-reviewed literature may also be used.
3. Nonpublished or proprietary methods may be necessary for some enzyme declarations. Companies promoting such methods should be able and willing to demonstrate that the methods are "appropriate, scientifically valid methods." *See also* Section 3, above.

Label Claims

It is the responsibility of the firm that is marketing the final enzyme-containing dietary supplement product under its brand (or private label) to ensure that specific enzymes listed on the label are present at the labeled activity.

Multiple Activities for a Single Enzyme

The best practice to prevent any consumer confusion is to list only one activity per enzyme on the final dietary supplement product label. If multiple activities for a single enzyme are included on the final dietary supplement product label, the label should clearly communicate to the consumer that the listed activities are from one enzyme only. The label should not represent (or imply) that there are additional ingredients that are not included in the finished dietary supplement product.

Total Activity for Enzyme Combinations

If the manufacturer of enzyme combination products wishes to label a “total activity” of the product the following should be taken into account. The measurable activity of individual enzymes varies strongly with the applied testing method. Compendial methods are dedicated to specific enzymes and use optimized conditions customized for the respective enzyme (including but not limited to substrate, temperature, pH and time). Therefore, the individual activity units of enzymes (e.g., USP units Trypsin and USP units Papain) cannot be simply added together to determine a total activity. Furthermore, in most cases a correlation factor (as for conversion of °C to °F) does not exist due to the differences in the dedicated methods for enzyme analytics. Consequently, for enzyme combination products a "total activity" cannot be calculated from the individual enzyme activities but only from a separate analysis of the final product. The manufacturer may select an appropriate method based upon the product characteristics.

Example: Total proteolytic activity: 900 USP units (trypsin)

7. CONTACT INFORMATION

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