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▲⟨2800⟩ MULTI-INGREDIENT DIETARY SUPPLEMENT PRODUCTS—PRODUCT QUALITY TESTS

INTRODUCTION

Multi-ingredient dietary supplement products are products containing two or more dietary ingredients. They can be formulated as capsules, tablets, oral powders, oral liquids, or chewable gels (marketed as "gummies"). The definition and description of these dosage forms and brief information about their composition and manufacturing process can be found in *Pharmaceutical Dosage Forms* ⟨1151⟩.

This chapter focuses on the quality tests that are generally necessary to assess the quality of various dietary supplement multi-ingredient finished dosage forms for which individual monographs are not available. It provides a list of consolidated common product quality test requirements for multi-ingredient dietary supplements. Dietary supplement product quality tests can be divided into three categories: 1) universal tests that are applicable to all dietary supplement products, regardless of the dosage form type; 2) specific tests that should be considered for inclusion for specific types of finished products; and 3) product performance tests such as disintegration or dissolution to estimate the ability of the finished products to release dietary ingredients for potential absorption and ensure consistent product quality from batch to batch.

The quality tests listed in this chapter can be used by manufacturers to develop appropriate tests and specifications to assure the quality of multi-ingredient dietary supplement products. Available monographs for individual dietary ingredients and finished dietary supplement products can serve as a supportive starting point for considering quality tests for multi-ingredient dietary supplement products containing the same ingredients. Quality tests and acceptance criteria listed in individual monographs also can be helpful in establishing specifications for finished products containing different combinations of multiple ingredients.

This chapter is intended as an informational chapter, not as a requirement for compliance. It can be used for the dietary supplement finished dosage forms that contain a combination of botanical ingredients and ingredients other than vitamins and minerals. The chapter does not apply to finished dosage forms that contain probiotics or multi-vitamin or multi-mineral products, for which there are monographs.

DIETARY SUPPLEMENT PRODUCT QUALITY TESTS

Universal Tests

Universal tests should be applied to all dietary supplement dosage forms; they include *Identification*, *Strength*, and *Impurities* (organic, inorganic, and residual solvents). The tests for *Strength* should support the *Definition* (description) of the dosage form. A finished product *Definition* provides the official names of each dietary ingredient contained in the finished product and acceptance criteria for the strength. The acceptance criteria are typically specified as the percentage of the labeled amount of each dietary ingredient, which may be expressed in terms of active constituents, identified markers, salt, or free acid/free base. The *Definition* also may include statements about other added substances (e.g., preservatives).

IDENTIFICATION

Identification tests are provided as an aid to confirm that the finished product contains the labeled dietary substances. Identification tests must be specific to each dietary ingredient contained in the finished product and should be able to discriminate between closely related species and possible impurities or adulterants that are likely to be present. If a single test lacks specificity, two or more orthogonal tests should be used for identification. A specific identification test for polymorphic forms should be carried out, if applicable. Moreover, if the dietary substance is a salt, an appropriate identification test also should be included for the counterion. The following are suitable analytical identification methods.

Thin-layer chromatography (TLC) and high-performance thin-layer chromatography (HPTLC): These methods are frequently used in *USP* monographs for the identification of botanical articles by fingerprint (i.e., sequences of zones that have specific positions, colors, and intensity) comparison against that of a USP Reference Standard, monograph description, or a reference chromatogram. HPTLC in most cases can differentiate between closely related species as well as readily detect the presence of adulterants. Available TLC or HPTLC procedures and acceptance criteria should be verified for use in combinations of dietary ingredients. For some mixtures, new TLC or HPTLC procedures need to be developed and validated to establish new acceptance criteria for combined fingerprints. A general analytical procedure for the application of HPTLC to the identification of botanicals is described in *HPTLC for Articles of Botanical Origin* ⟨203⟩. More practical details can be found in *Identification of Articles of Botanical Origin by High-Performance Thin-Layer Chromatography Procedure* ⟨1064⟩.

High-performance liquid chromatography (HPLC), ultra-high performance liquid chromatography (UHPLC), and gas chromatography (GC): HPLC, UHPLC, and GC are generally used in *USP* monographs to separate and identify various ingredients by comparing the chromatographic retention time(s) of the main peak(s) of the test product with the retention time(s) of the peak(s) in the corresponding standard samples. In most cases, the preparations of standards and samples, as well as chromatographic conditions for identification tests are the same as those given in the sections of monographs for the quantitative determination of dietary ingredients or marker compounds. In some cases, the chromatographic retention time representing a particular ingredient may not be unique and therefore may not be sufficient to establish identity. This limitation can be eliminated, where applicable, by using two chromatographic procedures, where the separation is based on different principles, or by using spectral analysis of the peak [e.g., diode array detector (DAD), mass spectrometry (MS)], which would allow both chromatographic and spectral identification of an analyte.

Nuclear magnetic resonance (NMR): NMR spectroscopy is a technique of high specificity and may be used for structure identification. The basis for identification is provided by a comparison of the signals from the test sample with the expected signals from a qualified reference standard. It can be used in conjunction with the HPTLC procedure for more accurate identification testing. Detailed information on NMR instruments and NMR applications can be found in *Nuclear Magnetic Resonance Spectroscopy* ⟨761⟩ and *Applications of Nuclear Magnetic Resonance Spectroscopy* ⟨1761⟩.

DNA: DNA metabarcoding and species-specific polymerase chain reaction (PCR) can be an additional option to improve test specificity, even though these methods have many limitations when applied to highly processed botanical materials. DNA-based methods also have been shown to be efficient in distinguishing genuine plant materials from adulterants in complex botanical matrices. A shortcoming of DNA-based testing is that whereas it may confirm the correct species, it cannot confirm the correct plant part, and an orthogonal test may be needed. See *Identification of Articles of Botanical Origin* ⟨563⟩ for additional information.

STRENGTH

Strength is a test procedure that selectively and accurately quantifies the dietary ingredients or identified markers free from interference with other analytes, possible impurities, excipients, and potential degradation products (can be generated from stress tests using heat, light, acid and alkaline hydrolysis, oxidation). HPLC, UHPLC, and GC are the most widely used analytical techniques in *USP* monographs for the quantitative determination of dietary ingredients in finished products. The test procedures used for quantitation of dietary ingredients (or established markers) in multi-ingredient finished products should be appropriately verified (see *Verification of Compendial Procedures* ⟨1226⟩) or validated (see *Validation of Compendial Procedures* ⟨1225⟩). Specificity, linearity, precision, accuracy, and robustness are typical analytical characteristics used in method validation for dietary supplement products.

Multi-ingredient finished products are complex, and characteristics such as analyte solubility, excipient type, and micro-encapsulation of ingredients can complicate sample preparation and analysis. The extraction and separation of dietary ingredients in the matrices of dietary supplement finished products that contain significant quantities of protein, fats, or carbohydrates in addition to the mixture of dietary ingredients can be even more complicated. Therefore, sample preparation procedures must be carefully optimized for the recovery of dietary or marker compounds prior to method validation and use. In some cases, different sample preparations may need to be developed because of different solubility and pH stability of dietary ingredients in sample solutions.

IMPURITIES

Specifications for impurities are critical quality attributes of dietary ingredients and dietary supplements because they have the potential to affect the safety of dietary supplements.

Organic impurities: During product manufacture and over the shelf life of the product, degradation products can form. These degradation products can be a result of the degradation of the dietary ingredients or from interactions between the dietary ingredients and excipients, among other factors. The preparation processes of some ingredients—such as isolation, purification, fermentation, and synthesis—may also contribute to the formation of organic impurities. The analytical procedures and acceptance criteria should specifically limit impurities with potential toxicity.

Inorganic impurities: All dietary supplement finished dosage forms should meet the requirements of *Elemental Contaminants in Dietary Supplements* ⟨2232⟩. This chapter provides the analytical procedures and acceptance criteria for the four major elements of toxicological concern: arsenic, cadmium, lead, and mercury. Additionally, other elemental contaminants identified as potential reasonably anticipated contaminants (RACs) should be evaluated.

Residual solvents: Dietary supplement products should contain levels of residual solvents no higher than can be supported by safety data. See *Residual Solvents* ⟨467⟩.

Specific Tests

In addition to the *Universal Tests*, the following specific tests should be considered depending on the dietary supplement finished dosage form.

WEIGHT VARIATION

(TABLETS, CAPSULES, CHEWABLE GELS, ORAL POWDERS)

Weight variation tests provide limits for the permissible variations in the weights of individual tablets, capsules, or chewable gels and oral powders expressed in terms of the allowable deviation from the average weight of a sample. *Weight Variation* ⟨2091⟩ describes separate procedures and limits for hard shell capsules, soft shell capsules, uncoated tablets, coated tablets, and chewable gels that are intended for use as dietary supplements. Typical recommendations for oral powders can be found in the *USP* monograph *Vitamins with Minerals Oral Powder*.

DELIVERABLE VOLUME

(ORAL SOLUTIONS)

When the liquid dietary supplement formulation is packaged in a multiple-dose container, compliance with *Deliverable Volume* ⟨698⟩ is recommended.

ALCOHOL DETERMINATION

(ORAL SOLUTIONS)

If the liquid formulation contains a quantity of alcohol, *Alcohol Determination* ⟨611⟩ should be included. The limits may be an absolute concentration, in percentage, or relative to a labeled content.

WATER ACTIVITY

(CHEWABLE GELS)

Chewable gels are formulated with one or more gelling agents (e.g., gelatin, pectin, or starch), nutritive sweeteners (e.g., sucrose, fructose, or corn syrup), flavoring agents, non-nutritive sweeteners, colorants, and water. Determination of the optimum water activity specification is a critical quality attribute necessary to ensure that the product is shelf stable. Water activity can be used to predict and resolve problems with moisture migration, can identify whether microbial growth will be a concern (i.e., surface mold grown), and may be a predictor of shelf stability. It also can be used to select appropriate packaging. In general, the chemical stability of the chewable gel matrix can be compromised if the chewable gels are not properly prepared to the proper equilibrium; sometimes the disaccharides that are present can invert, causing the chewable gels to become soft and sticky. *Water Activity* ⟨922⟩ outlines the recommended methods to qualify, calibrate, and use water activity meters to accurately measure the water activity. The effects of water activity should be combined with pH to control microorganisms more effectively. Water activity and pH simultaneously can provide effective microbial control at levels that would typically be considered unsafe for either one alone.

pH

(CHEWABLE GELS, ORAL SOLUTIONS)

pH is an important factor that determines if a chewable gel product will support the growth of a pathogenic microorganism. See *USP* and *pH* ⟨791⟩ for pH measurement recommendations.

pH is also an important parameter for liquid (aqueous based) dietary supplement products because liquid formulations are sensitive to changes in pH due to exposure to atmospheric carbon dioxide. The pH of an oral liquid formulation can affect the taste and stability of the product.

WATER CONTENT

(ORAL POWDERS)

Special consideration should be given to oral powders for which water content has been shown to be a potential quality attribute. See *Water Determination* ⟨921⟩ for appropriate procedures. Water activity may also be a desired attribute for oral powders because the combination of water determination and water activity can sometimes be used to determine if a powder will be susceptible to case hardening.

MICROBIAL CONTENT

For dietary supplement products other than probiotics, the microbial load and presence of specific microorganisms in nonsterile preparations may affect the quality of the product and have the potential to adversely affect the health of the product users. The microbial specification needed for a particular product depends on the dosage form. *Microbial Enumeration Tests* ⟨2021⟩ provides tests for the estimation of the number of viable aerobic microorganisms present in nutritional supplements of all kinds, from raw materials to the finished forms. *Absence of Specified Microorganisms* ⟨2022⟩ describes the testing of nutritional and dietary articles for specified microorganisms whose absence is recommended by the guidance in *Microbiological Attributes of Nonsterile Nutritional and Dietary Supplements* ⟨2023⟩.

OTHER TESTS

Environmental contaminants: Tests for aflatoxins and tests for residual pesticides are described in *Articles of Botanical Origin* ⟨561⟩. It is manufacturers' responsibility to determine if these tests or tests for other contaminants such as those for other mycotoxins or pyrrolizidine alkaloids are necessary for the finished product. For dietary supplement dosage forms containing fish oil-based products, the test for *Limit of Dioxins, Furans, and Polychlorinated Biphenyls*, using the current revision of Methods No. 1613 and No. 1668 of the Environmental Protection Agency, should be included.

Acid Value, Anisidine Value, Peroxide Value: These tests in *Fats and Fixed Oils* ⟨401⟩ may be required for dietary supplement dosage forms containing oil-based products.

Adulterants: Intentional adulteration of dietary supplement products, especially botanicals—with less expensive ingredients, substituted species or markers, or pharmaceutical chemicals—is a significant concern. Sports performance enhancement, weight loss, and sexual enhancement are currently the major categories of adulterated products. Screening for the potential presence of pharmaceutical ingredients is essential for these types of products. See *Screening for Undeclared Drugs and Drug Analogues* ⟨2251⟩ for additional information.

Dietary Supplement Product Performance Tests

The absorption of dietary ingredients from a dietary supplement product after oral administration depends on the ability of the dosage forms to release the dietary ingredients. Disintegration and dissolution tests are quality control tools used to assess performance characteristics of dietary supplement finished dosage forms and ensure consistent product quality from batch to batch. These tests are very useful during dietary supplement product development for identifying critical manufacturing attributes such as the impact of ingredient properties and the impact of the manufacturing process on finished product performance. After the finished dosage form is released for marketing, disintegration and dissolution tests may be useful to detect problems that may arise because of use or misuse, or changes in coatings, lubricants, disintegrants, and other components. These performance tests are also useful to detect manufacturing process issues, such as over compression and over drying, that would affect the release characteristics of the final dosage forms. These tests are not intended to be used as a demonstration or as a surrogate for in vivo absorption, bioavailability, or effectiveness, unless an in vitro–in vivo correlation (IVIVC) has been established.

DISINTEGRATION

(TABLETS, HARD SHELL CAPSULES, DELAYED-RELEASE TABLETS, AND DELAYED-RELEASE SOFT SHELL CAPSULES)

Disintegration testing is used to determine whether tablet or capsule finished dosage forms disintegrate within the established time when placed in a liquid medium at the experimental conditions described in *Disintegration and Dissolution* ⟨2040⟩. Delayed-release tablets and delayed-release capsules are tablets or capsules that are formulated with acid-resistant or enteric coatings. For these products, when appropriate, a disintegration test with a stage using simulated gastric fluid followed by a stage using simulated intestinal fluid should be performed as directed in ⟨2040⟩. A rationale for using a disintegration test as a quality control test for performance characteristics of finished products should be justified.

The disintegration test is not applicable for chewable products or for those designed as extended-release dosage forms.

RUPTURE TEST

(SOFT SHELL CAPSULES)

For immediate release soft shell capsules, a rupture test is recommended as described in ⟨2040⟩. For soft gelatin capsules that do not conform to the rupture test acceptance criteria, papain or bromelain can be added to the *Medium*.

DISSOLUTION

(CAPSULES, TABLETS, CHEWABLE TABLETS, CHEWABLE GELS)

Dissolution tests are designed to assess in vitro release of dietary ingredients or identified markers from dosage forms. As a general rule, for products containing multiple ingredients, the ability of each ingredient to be released should be evaluated. If justified, the dissolution of one ingredient, usually the least soluble, can be evaluated. Chewable tablets should undergo dissolution testing as a product performance test because they might be swallowed without proper chewing. In general, the dissolution test conditions for chewable tablets should be the same as for the non-chewable tablets. See the FDA Guidance for Industry *Quality Attribute Considerations for Chewable Tablets* (*1*). The dissolution test is also recommended to evaluate the release of dietary ingredients from chewable gels, because in most cases the dietary ingredients added to the chewable gel matrix are encapsulated. In general, dissolution test conditions for tablets and capsules with similar ingredients should work for chewable gels.

The Dissolution Procedure: Development and Validation ⟨1092⟩, *Capsules—Dissolution Testing and Related Quality Attributes* ⟨1094⟩, *Oral Dosage Forms—Performance Tests* ⟨1711⟩, and ⟨2040⟩ provide approaches to the performance testing of oral dosage forms.

REFERENCES

1. US Food and Drug Administration. Guidance for Industry. Quality attribute considerations for chewable tablets. 2018. *www.fda.gov/files/drugs/published/Quality-Attribute-Considerations-for-Chewable-Tablets-Guidance-for-Industry.pdf*.

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