NSF International Standard for Dietary Supplements —
Dietary supplements

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6 Test methods used by testing laboratories for identification and quantification of ingredients – raw materials and finished products

6.1 Identification test methods

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6.2 Quantification test methods

6.2.1 Botanicals

If declared on the label, the identity of marker constituents shall be evaluated in accordance with the methods in table 4. If no method exists or if improved technology allows for a more accurate and precise method to be developed, one may be developed. The use of any new method shall require that a validation be performed, following the principles of the AOAC Single Lab Validation Guideline as a minimum, which includes an evaluation of specificity, linearity, reproducibility, accuracy, spike recovery, and method detection limit (if applicable). More rigorous validation could follow according to the guidelines of ICH, FDA, GLP, CEN, and/or AOAC, as appropriate.

6.2.2 Vitamins

The quantity of vitamins shall be evaluated in accordance with the methods listed in the USP-NF. If no method exists or if improved technology allows for a more accurate and precise method to be developed, one may be developed. The use of any new method shall require that a validation be performed, following the principles of the AOAC Single Lab Validation Guideline as a minimum, which includes an evaluation of specificity, linearity, reproducibility, accuracy, spike recovery, and method detection limit (if applicable). More rigorous validation could follow according to the guidelines of ICH, FDA, GLP, CEN, and/or AOAC, as appropriate.

6.2.3 Minerals

The quantity of minerals shall be evaluated in accordance with the methods listed in the USP-NF. If no method exists or if improved technology allows for a more accurate and precise method to be developed, one may be developed. The use of any new method shall require that a validation be performed, following the principles of the AOAC Single Lab Validation Guideline as a minimum, which includes an evaluation of specificity, linearity, reproducibility, accuracy, spike recovery, and method detection limit (if applicable). More rigorous validation could follow according to the guidelines of ICH, FDA, GLP, CEN, and/or AOAC, as appropriate.

6.2.4 Other dietary supplement ingredients

An effort shall be made to seek out the most appropriate method to confirm claims for the product under evaluation. The source of these methods may include AOAC International, USP-NF, AHP, European, German, Japanese monographs, INA, etc. The use of any new method shall require that a validation be performed, following the principles of the AOAC Single Lab Validation Guideline as a minimum, which includes an evaluation of specificity, linearity, reproducibility, accuracy, spike recovery, and method detection limit (if applicable). More rigorous validation could follow according to the guidelines of ICH, FDA, GLP, CEN, and/or AOAC, as appropriate.

6.2.5 Quality assurance for quantitative test methods

Many of the quantitative test methods for dietary supplement samples are performed utilizing chromatographic procedures. The typical quality assurance criteria that are applied are described in the following sections, however, some methods may have unique criteria which would be defined within the laboratory standard operating procedures or other reference method. For example, non-chromatographic test methods (such as titration and potentiometric techniques, uv-visible and gravimetric procedures, micro-assays, etc.) would employ quality assurance steps as applicable to the situation.

6.2.5.1 Calibration

Quantification test methods shall be performed using certified reference standards as calibration standards. The standards are typically purchased as single chemicals with greater than 95% purity. If a high-purity standard is not available, a lower-purity material shall be used if there is a means by which the actual purity can be measured (e. g., uv absorbance).

6.2.5.1.1 Multi-level calibration curves

Multi-level calibration curves shall be prepared with a minimum of three concentration levels such that any sample preparations under evaluation would be bracketed by a calibration standard. Curves shall give a correlation ~~coefficient~~ coefficient (r) of 0.995 or higher.

6.2.5.1.2 Single-level calibrations ~~curves~~

If a single level calibration is employed, the standard shall be run in triplicate and the relative standard deviation between these runs shall not exceed 2%. The detector response of the prepared sample shall be within 90% -%110 of that of the standard.

6.2.5.1.3 Blanks

A method/reagent blank shall be included in each analytical run. The blank response for the analyte of interest shall not be greater than one half the response of the lowest calibration standard for multi-level calibration curves. For single-level calibrations, the blank response for the analyte of interest shall not exceed 5% of the sample response.

6.2.5.1.4 Reproducibility/accuracy

All unfamiliar matrices shall be prepared in triplicate.

Whenever possible, two additional preparations shall be spiked with the reference standard(s) to assess recovery/accuracy. The recovery in the range of 70-130% of the theoretical spike value is considered acceptable.

The reproducibility between the two spiked samples as measured by ~~percent~~ relative percent difference (RPD) shall be no greater than 20%. The reproducibility of the method is also evaluated by the percent relative standard deviation (%RSD) of the triplicate sample preparations, which should not exceed 25%,

NOTE – When spiking with the reference standard is price prohibitive, a control sample with a known result shall be tested as part of the analysis run; this shall include a certified reference material or a sample that has been analyzed in the past.

6.2.5.1.5 Continuing Calibration Verification (CCV)

In order to assess instrument stability, a Continuing Calibration Verification (CCV) or bracketing standard~~s~~ shall be run after every 10 sample preparations and/or at the end of the run. The recovery for the CCV shall be between ~~within the uncertainty of the method for the data to be acceptable~~80-120% of the theoretical standard value. CCV standards, which are run to confirm an existing calibration, must show recovery of 90-110%. If the result falls outside this range, a new calibration shall be run.

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