AOAC SMPR 2016.005

Standard Method Performance Requirements (SMPRs®) for Quantitation of Collagen

Intended Use: Reference method for cGMP compliance

1 Purpose

AOAC SMPRs describe the minimum recommended performance characteristics to be used during the evaluation of a method. The evaluation may be an on-site verification, a single-laboratory validation, or a multi-site collaborative study. SMPRs are written and adopted by AOAC stakeholder panels composed of representatives from the industry, regulatory organizations, contract laboratories, test kit manufacturers, and academic institutions. AOAC SMPRs are used by AOAC expert review panels in their evaluation of validation study data for method being considered for *Performance Tested Methods*℠ or AOAC *Official Methods of Analysis*℠, and can be used as acceptance criteria for verification at user laboratories.

2 Applicability

The method will be able to identify and quantify individual native (undenatured) and hydrolyzed collagen type I, II, and III if one or multiple types are present in dietary ingredients and dietary supplement finished products.

3 Analytical Technique

Any analytical technique(s) that measures the analytes of interest and meets the following method performance requirements is/are acceptable.

4 Definitions

Collagen.—A triple helix protein that generally consists of two identical chains (α1) and an additional chain that differs slightly in its chemical composition (α2). The amino acid composition of collagen is notable for its particularly high hydroxyproline content. The three most common types of collagen are: type I, found in skin, tendon, vascular ligature, organs, bone (main component of the organic part of bone); type II, found in cartilage (main collagenous component of cartilage); and type III, found in reticular fibers. *Structures*.—http://www.sigmaaldrich.com/life-science/metabolomics/enzyme-explorer/learning-center/structural-proteins/collagen.html.

Dietary ingredients.—A vitamin; a mineral; an herb or other botanical; an amino acid; a dietary substance for use by man to supplement the diet by increasing total dietary intake; or a concentrate, metabolite, constituent, extract, or combination of any of the above dietary ingredients {Federal Food Drug and Cosmetic Act §201(ff) [U.S.C. 321 (ff)]}.

Dietary supplements.—A product intended for ingestion that contains a "dietary ingredient" intended to add further nutritional value to (supplement) the diet. Dietary supplements may be found in many forms such as tablets, capsules, softgels, gelcaps, liquids, or powders.

Hydrolyzed collagen.—Peptides and polypeptides rich in hydroxyproline, produced by breaking down the molecular bonds of native collagen strands using one or more combinations of physical, chemical, or biological methods.

Table 1. Method performance requirements

Parameter	Criteria
Analytical range, %	1–100
LOQ, %	0.5
Recovery, %	90–110
RSD_r, %	≤5
RSD_R, %	≤10

Limit of quantitation (LOQ).—The minimum concentration or mass of analyte in a given matrix that can be reported as a quantitative result.

Quantitative method.—Method of analysis whose response is the amount of the analyte measured either directly (enumeration in a mass or a volume), or indirectly (color, absorbance, impedance, etc.) in a certain amount of sample.

Repeatability.—Variation arising when all efforts are made to keep conditions constant by using the same instrument and operator and repeating during a short time period. Expressed as the repeatability standard deviation (SD_r); or % repeatability relative standard deviation (%RSD_r).

Reproducibility.—The standard deviation or relative standard deviation calculated from among-laboratory data. Expressed as the reproducibility standard deviation (SD_R); or % reproducibility relative standard deviation (%RSD_R).

Recovery.—The fraction or percentage of spiked analyte that is recovered when the test sample is analyzed using the entire method.

5 Method Performance Requirements

See Table 1.

6 System Suitability Tests and/or Analytical Quality Control

Suitable methods will include blank check samples, and check standards at the lowest point and midrange point of the analytical range.

7 Reference Material(s)

Refer to Annex F: *Development and Use of In-House Reference Materials* in Appendix F: *Guidelines for Standard Method Performance Requirements*, *Official Methods of Analysis* (2016) 20th Ed., AOAC INTERNATIONAL, Rockville, MD, USA (http://www.eoma.aoac.org/app_f.pdf)

Identify suitable materials for method validation.

8 Validation Guidance

Requirement for consideration as an AOAC *Official Method of Analysis*:

Data demonstrating that a candidate method is able to: Separate a combination of native collagen type I, II, and III and/or hydrolyzed collagen type I, II, and III in the matrices listed in Table 2. Quantify each individual collagen type both native and hydrolyzed.

Appendix D: *Guidelines for Collaborative Study Procedures to Validate Characteristics of a Method of Analysis, Official Methods of Analysis* (2016) 20th Ed., AOAC INTERNATIONAL, Rockville, MD, USA (http://www.eoma.aoac.org/app_d.pdf)

Appendix F: *Guidelines for Standard Method Performance Requirements, Official Methods of Analysis* (2016) 20th Ed., AOAC INTERNATIONAL, Rockville, MD, USA (http://www.eoma.aoac.org/app_f.pdf)

Table 2. Matrixes

Tablets
Capsules
Softgels
Powders
Liquids
Chewables

Appendix K: *Guidelines for Dietary Supplements and Botanicals*, *Official Methods of Analysis* (2016) 20th Ed., AOAC INTERNATIONAL, Rockville, MD, USA (http://www.eoma.aoac.org/app_k.pdf)

9 Maximum Time-to-Result

None

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