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3 **Quantitation of Collagen**

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5 **Intended Use:** Reference method for cGMP compliance.

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7 **1. Purpose:** AOAC SMPRs describe the minimum recommended performance characteristics to be  
8 used during the evaluation of a method. The evaluation may be an on-site verification, a single-  
9 laboratory validation, or a multi-site collaborative study. SMPRs are written and adopted by AOAC  
10 Stakeholder Panels composed of representatives from the industry, regulatory organizations,  
11 contract laboratories, test kit manufacturers, and academic institutions. AOAC SMPRs are used by  
12 AOAC Expert Review Panels in their evaluation of validation study data for method being considered  
13 for *Performance Tested Methods* or *AOAC Official Methods of Analysis*, and can be used as  
14 acceptance criteria for verification at user laboratories.

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16 **2. Applicability:**

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

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21 **3. Analytical Technique:**

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

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25 **4. Definitions:**

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27 **Collagen**

28 A triple helix protein that generally consists of two identical chains ( $\alpha 1$ ) and an additional chain that  
29 differs slightly in its chemical composition ( $\alpha 2$ ). The amino acid composition of collagen is notable  
30 for its particularly high hydroxyproline content. The three most common types of collagen are: type  
31 I, found in skin, tendon, vascular ligature, organs, bone (main component of the organic part of  
32 bone); type II, found in cartilage (main collagenous component of cartilage); and type III, found in  
33 reticular fibers.

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35 Structures:

36 [http://www.sigmaaldrich.com/life-science/metabolomics/enzyme-explorer/learning-](http://www.sigmaaldrich.com/life-science/metabolomics/enzyme-explorer/learning-center/structural-proteins/collagen.html)  
37 [center/structural-proteins/collagen.html](http://www.sigmaaldrich.com/life-science/metabolomics/enzyme-explorer/learning-center/structural-proteins/collagen.html)

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39 **Dietary Ingredients**

40 A vitamin; a mineral; an herb or other botanical; an amino acid; a dietary substance for use by man  
41 to supplement the diet by increasing total dietary intake; or a concentrate, metabolite, constituent,  
42 extract, or combination of any of the above dietary ingredients.<sup>1</sup>

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44 **Dietary supplements**

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<sup>1</sup> Federal Food Drug and Cosmetic Act §201(ff) [U.S.C. 321 (ff)]

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

#### 48 49 **Hydrolyzed Collagen**

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

#### 53 54 **Limit of Quantitation (LOQ)**

55 The minimum concentration or mass of analyte in a given matrix that can be reported as a  
56 quantitative result.

#### 57 58 **Quantitative method**

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

#### 62 63 **Repeatability**

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

#### 67 68 **Reproducibility**

69 The standard deviation or relative standard deviation calculated from among-laboratory data.  
70 Expressed as the reproducibility standard deviation ( $SD_R$ ); or % reproducibility relative standard  
71 deviation (%  $RSD_R$ ).

#### 72 73 **Recovery**

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

### 76 77 **5. Method Performance Requirements:**

78 See table 1.

### 79 80 **6. System suitability tests and/or analytical quality control:**

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

### 83 84 **7. Reference Material(s):**

85 Refer to Annex F: *Development and Use of In-House Reference Materials* in Appendix F: Guidelines  
86 for Standard Method Performance Requirements, 19<sup>th</sup> Edition of the AOAC INTERNATIONAL Official  
87 Methods of Analysis (2012). Available at: [http://www.eoma.aoac.org/app\\_f.pdf](http://www.eoma.aoac.org/app_f.pdf)

88  
89 *Identify suitable materials for method validation*

### 90 91 **8. Validation Guidance:**

92 Requirement for consideration as an AOAC *Official Methods of Analysis*:

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94 Data demonstrating that a candidate method is able to: Separate a combination of native collagen type I, II  
95 and III and/or hydrolyzed collagen type I, II and III. Quantify each individual collagen type both native and  
96 hydrolyzed.

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100 [Appendix D](http://www.eoma.aoac.org/app_d.pdf): Guidelines for Collaborative Study Procedures To Validate Characteristics of a Method  
101 of Analysis; 19<sup>th</sup> Edition of the AOAC INTERNATIONAL Official Methods of Analysis (2012). Available  
102 at: [http://www.eoma.aoac.org/app\\_d.pdf](http://www.eoma.aoac.org/app_d.pdf)

103  
104 [Appendix F](http://www.eoma.aoac.org/app_f.pdf): Guidelines for Standard Method Performance Requirements; 19<sup>th</sup> Edition of the AOAC  
105 INTERNATIONAL Official Methods of Analysis (2012). Available at:  
106 [http://www.eoma.aoac.org/app\\_f.pdf](http://www.eoma.aoac.org/app_f.pdf)

107  
108 [Appendix K](http://www.eoma.aoac.org/app_k.pdf): Guidelines for Dietary Supplements and Botanicals; 19<sup>th</sup> Edition of the AOAC  
109 INTERNATIONAL Official Methods of Analysis (2012). Available on line at:  
110 [http://www.eoma.aoac.org/app\\_k.pdf](http://www.eoma.aoac.org/app_k.pdf)

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112 **9. Maximum Time-To-Result:** None

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**Table 1: Method performance requirements**

Parameter	Criteria
Analytical Range (%)	1 – 100
LOQ (%)	0.5
Recovery (%)	90-110
% RSD <sub>r</sub>	≤ 5
% RSD <sub>R</sub>	≤ 10

**Table 2: Matrices**

tablets  
capsules  
softgels  
powders  
liquids  
chewables