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Determination of Total Chondroitin Sulfate in Dietary Ingredients and Supplements

Stakeholder Panel on Dietary Supplements

Intended Use: Reference Method for Routine Analysis or Dispute Resolution

1 Purpose

AOAC *Standard Method Performance Requirements*SM (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 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*SM or AOAC *Official Methods of Analysis*SM, and can be used as acceptance criteria for verification at user laboratories. [Refer to Appendix F: *Guidelines for Standard Method Performance Requirements, Official Methods of Analysis of AOAC INTERNATIONAL* (2012) 19th Ed., AOAC INTERNATIONAL, Gaithersburg, MD, USA.]

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Developed by the Working Group for Chondroitin Sulfate and approved by the Stakeholder Panel on Dietary Supplements (SPDS).

2 Applicability

Quantitative determination of total chondroitin sulfate salts in dietary ingredients and dietary supplements.

3 Analytical Technique

Any analytical technique(s) that measures the analytes of interest and meets the following method performance requirements is/are acceptable. It is acceptable to have a different analytical method for each class of analytes.

4 Definitions

Chondroitin sulfate—Chondroitin sulfate salts consists mostly of the salts of the sulfate ester of *N*-acetylgalactosamine (2-acetamido-2-deoxy-d-galactopyranose usually abbreviated as GalNAc) and d-glucuronic acid copolymer. These hexoses are alternately linked –1,4 and –1,3 in the polymer. It is closely related to other glycosaminoglycans (GAGs), such as dermatan sulfate, hyaluronic acid, heparin, heparan sulfate, and keratan sulfate, which contain other hexosamine and/or glycuronic acid residues. Either of the residues can be sulfated at different positions. (*See* Figure 1.)

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 {United States 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.

Limit of quantitation (LOQ)—The minimum analyte concentration for which quantitative results may be obtained with 95% confidence.

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 the 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, and a protocol to demonstrate suitability.

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 of AOAC INTERNATIONAL* (2012) 19th Ed., AOAC INTERNATIONAL, Gaithersburg, MD, USA (http://www.eoma.aoac.org/app_f.pdf)

ISO Guide 34:2009 *General requirements for the competence of reference material producers*

8 Validation Guidance

All matrixes listed in Annex I must be evaluated for LOQ, repeatability, and recovery for First Action *Official Methods of Analysis* approval.

Candidate methods should be challenged with materials known to interfere with the assay.

Appendix D: *Guidelines for Collaborative Study Procedures to Validate Characteristics of a Method of Analysis, Official Methods of Analysis of AOAC INTERNATIONAL* (2012) 19th Ed., AOAC INTERNATIONAL, Gaithersburg, MD, USA (http://www.eoma.aoac.org/app_d.pdf)

Appendix K: *Guidelines for Dietary Supplements and Botanicals, Official Methods of Analysis of AOAC INTERNATIONAL* (2012) 19th Ed., AOAC INTERNATIONAL, Gaithersburg, MD, USA (http://www.eoma.aoac.org/app_k.pdf). Also at: *J. AOAC Int.* (2012) **95**, 268; DOI: 10.5740/jaoacint.11-447.

Appendix N: *ISPAM Guidelines for Validation of Qualitative Binary Chemistry Methods, Official Methods of Analysis of AOAC INTERNATIONAL* (2012) 19th Ed., AOAC INTERNATIONAL, Gaithersburg, MD, USA (http://www.eoma.aoac.org/app_n.pdf)

9 Maximum Time-to-Result

No maximum time to result.

ANNEX I

Matrices

Tablets

Capsules

Softgels

Gelcaps

Gummies

Chewables

Liquids

Powders

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Figure 1.

Chemical structure of one unit in a chondroitin sulfate chain. Chondroitin-4-sulfate: R1 = H; R2 = SO₃H; R3 = H. Chondroitin-6-sulfate: R1 = SO₃H; R2, R3 = H. Chondroitin-6-sulfate: R1 = SO₃H; R2, R3 = H. Chondroitin sulfate has also a linkage region to consisting of GlcA β -1-3Gal β -1-3Gal β -1-4Xyl β -1-O-Ser, and a capping trisulfated monosaccharide. Commercial chondroitin sulfate has a varying content of nonsulfated disaccharides and it may contain some degree of decarboxylation depending on the isolation and purification treatment. Sulfation position depends on the species from which it is derived, age of the animals and anatomic location of the cartilage.

Table 1

Method performance requirements

Type of study	Parameter	Minimum acceptable criteria	
Single-laboratory validation	Limit of quantitation	1% (w/w)	
	Analytical range	1–10% (w/w)	>10–100% (w/w)
	Repeatability (RSD _r)	3%	2%
	Recovery	92–105%	98–102%
Multi-laboratory validation	Reproducibility (RSD _R)	6%	4%