AOAC SMPR 2014.008

Standard Method Performance Requirements for Screening Method for Selected Adulterants in Dietary Ingredients and Supplements Containing Chondroitin Sulfate

Intended Use: Routine Surveillance of Dietary Ingredients and Products by a Trained Technician

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.]

2 Applicability

Screening method for selected adulterants (as identified in Annex I: Target Compounds) in dietary ingredients and supplements claiming to contain chondroitin sulfate.

3 Analytical Technique

Any analytical technique(s) that detects 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

Adulterant.—Any poisonous or deleterious substance which may render a product injurious to users under the conditions of use prescribed in the labeling thereof; or any valuable constituent that has been in whole or in part omitted or abstracted; or any substance that has been substituted wholly or in part; or a substance that has been added so as to increase the bulk or weight, or reduce the quality or strength, or make a product appear better or of greater value than it is. (United States *Code of Federal Regulations* Title 21 §402)

Chondroitin sulfate (CS).—CS salts consist 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)]}

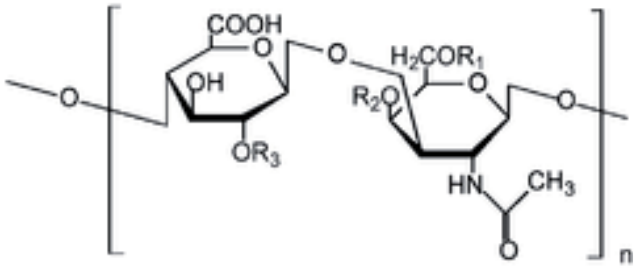

Figure 1. Chemical structure of the most abundant repetitive disaccharide unit in a chondroitin sulfate chain. Chondroitin-4-sulfate: R1 = H; R2 = SO3H; R3 = H. Chondroitin-6-sulfate: R1 = SO3H; R2, R3 = H. Chondroitin sulfate has also a linkage region to consisting of GlcAβ-1-3Galβ-1-3Galβ-1-4Xylβ-1-O-Ser, 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.

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.

Laboratory probability of detection (LPOD).—The POD value obtained from combining all valid collaborator data sets for a method for a given matrix at a given analyte level or concentration. [Appendix H: *Probability of Detection (POD) as a Statistical Model for the Validation of Qualitative Methods, Official Methods of Analysis of AOAC INTERNATIONAL* (2012) 19th Ed., AOAC INTERNATIONAL, Gaithersburg, MD, USA]

Probability of detection (POD).—(*Ibid*) The proportion of positive analytical outcomes for a qualitative method for a given matrix at a given analyte level or concentration.

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*

Table 1. Method performance requirements

Type of study	Parameter	Parameter requirements	Target test concn, % (w/w)	Minimum acceptable results
Single-laboratory validation	Matrix studies	Minimum of 33 replicates representing ideally all target compounds in Annex I and all matrix types listed in Annex II, spiked at or below the designated low-level target test concentration	≤5	90% POD[a] of the pooled data for all target compounds and matrixes
		High concentration. Minimum of five replicates per matrix type spiked at the designated high-level target test concentration.	ca 20	100% correct analyses are expected per matrix type[b]
		Zero concentration. Minimum of five replicates per matrix type that have tested negative with a second method and have not been spiked.	0	
Multilaboratory study	LPOD[c]	Use Appendix N: *ISPAM Guidelines for Validation of Qualitative Binary Chemistry Methods*	≤5	≥0.85
			ca 20	≥0.95
	$LPOD_{(0)}$[c]		0	≤0.05

[a] 95% confidence interval.

[b] 100% correct analyses are expected. Some aberrations may be acceptable if the aberrations are investigated, and acceptable explanations can be determined and communicated to method users.

[c] LPOD and $LPOD_{(0)}$ are not required for First Action *Official Methods of Analysis* approval.

8 Validation Guidance

Ideally all target compounds in Annex I and all matrixes in Annex II shall be evaluated.

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 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)

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.

9 Maximum Time-to-Result

No maximum time to result.

Approved by AOAC Stakeholder Panel on Dietary Supplements (SPDS). Final Version Date: September 5, 2014. Effective Date: October 16, 2014.

ANNEX I
Target Compounds

Carrageenan
Sodium hexametaphosphate
Sodium alginate
Propylene glycol alginate sulfate sodium
Over-sulfated polysaccharides

ANNEX II
Matrices

Tablets
Capsules
Softgels
Gelcaps
Gummies
Chewables
Liquids
Powders