Method Name: Determination of Total Chondroitin Sulfate

Purpose: AOAC SMPR’s 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.

Approved by: Stakeholder Panel on Dietary Supplements (SPDS)

Final version date:

Effective date:

Intended Use: Reference method for dispute resolution.

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

2. 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.

3. Definitions:

Chondroitin Sulfate

Chondroitin Sulfate Sodium consists mostly of the sodium salt of the sulfate ester of N-acetylglactosamine (2-acetamido-2-deoxy-β-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 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.
Chondroitin sulfate has also a linkage region to consisting of GlcAβ-1-3Galβ-1-4Galβ-1-4Xylβ-1-O-Ser, and a capping trisulfated monosaccharide. Commercial chondroitin sulfate has a varying content of non-sulfated 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 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. ¹

**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 relative 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.

¹ *New Dietary Ingredients in Dietary Supplements - Background for Industry*: FDA.
4. **Method Performance Requirements:***

<table>
<thead>
<tr>
<th>Validation Type</th>
<th>Limit of Quantitation (LOQ)</th>
<th>1% (w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Analytical Range</strong></td>
<td>1-10% (w/w)</td>
<td>&gt;10-100% (w/w)</td>
</tr>
<tr>
<td><strong>Repeatability (RSD_r)</strong></td>
<td>≤ 3%</td>
<td>≤2%</td>
</tr>
<tr>
<td><strong>Recovery</strong></td>
<td>92-105%</td>
<td>98-102%</td>
</tr>
</tbody>
</table>

| Multi-laboratory study   | Reproducibility (RSD_R)     | ≤ 6%     | ≤4%      |

5. **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.

6. **Reference Material(s)**


7. **Validation Guidance:**

All matrices listed in Annex II 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 N:** ISPAM Guidelines for Validation of Qualitative Binary Chemistry Methods of the 19th edition of the AOAC INTERNATIONAL Official Methods of Analysis. Available at http://www.eoma.aoac.org/app_n.pdf

8. **Maximum Time-To-Result:** No maximum time to result.
| Annex I: Matrices |
|---|---|
| Tablets |
| Capsules |
| Softgels |
| Gelcaps |
| Liquids |
| Powders |
| Extracts |