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**Determination Method for Chondroitin Sulfate and
Glucosamine Hydrochloride – Liquid Chromatography Method**

硫酸软骨素和盐酸氨基葡萄糖含量的测定 液相色谱法

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Determination Method for Chondroitin Sulfate and Glucosamine Hydrochloride – Liquid Chromatography Method

1 Scope

This Standard specifies the method for determining the content of chondroitin sulfate and glucosamine hydrochloride by liquid chromatography method.

This Standard applies to the determination of chondroitin sulfate and glucosamine hydrochloride extracted from cartilage tissues of cattle, pigs and sharks.

2 Normative References

The provisions in following documents become the provisions of this Standard through reference in this Standard. For dated references, the subsequent amendments (excluding corrigendum) or revisions do not apply to this Standard, however, parties who reach an agreement based on this Standard are encouraged to study if the latest versions of these documents are applicable. For undated references, the latest edition of the referenced document applies.

GB/T 6682 Water for analytical laboratory use - Specification and test methods (GB/T 6682-1992, neq ISO 3696:1987)

3 Principle

The chondroitin sulfate sample is uniformly dispersed with acetonitrile; dissolve in water; separate by liquid chromatography; measure at a wavelength of 192 nm; and quantify by the external standard method.

4 Reagents and Materials

4.1 Water: Grade-1 water specified in GB/T 6682.

4.2 Acetonitrile: Liquid chromatography grade.

4.3 Sodium pentane sulfonate: Purity $\geq 99.5\%$.

4.4 Standard product of chondroitin sulfate A: Purity is about 70%; and the rest is chondroitin sulfate C. See Appendix A for the structural formula.

4.5 Standard product of chondroitin sulfate B: Purity is about 90%; and the rest are chondroitin sulfate A and C. See Appendix A for the structural formula.

4.6 Standard product of chondroitin sulfate C: Purity is about 90%; and the rest is chondroitin sulfate A. See Appendix A for the structural formula.

4.7 Glucosamine hydrochloride: Purity $\geq$ 99.0%.

4.8 Standard solution: Weigh 25.0 mg of chondroitin sulfate standard A (or B, or C) which has been dried at 105°C for 4 h, and 25.0 mg of glucosamine hydrochloride in a beaker; add 5.0 mL of acetonitrile; shake to disperse evenly. Then add water; dissolve it with ultrasonic waves; transfer to a 50 mL volumetric flask; and dilute to the mark with water. The concentration of chondroitin sulfate is about 0.5 mg/mL; and the concentration of glucosamine hydrochloride is about 0.5 mg/mL.

4.9 Sodium pentane sulfonate solution: Weigh 0.87g of sodium pentane sulfonate solution and dissolve it in 500 mL of water. The concentration of this solution is 10 mmol/L.

5 Instruments and apparatuses

5.1 Ultrasonic generator.

5.2 Analytical balance: Sensitivity is 0.1mg.

5.3 High-performance liquid chromatography: Equipped with a diode array detector or an ultraviolet detector.

5.4 Filter membrane: polytetrafluoroethylene (PTFE) membrane, 0.2 μ m.

6 Test Procedures

6.1 Preparation of sample solution

When the sample is in a granular state, it needs to be ground into a powder in a mortar first.

The powdered samples are dried at 105°C for 4 h. Weigh 25.0 mg of sample into a 50 mL beaker; add 5.0 mL of acetonitrile; oscillate to disperse evenly. Then add water; dissolve it with ultrasonic waves; transfer to a 50 mL volumetric flask; dilute to the mark with water; and shake well. Before being measured by liquid chromatography, the sample solution is filtered with a 0.2 μ m filter membrane.

6.2 Chromatographic conditions

- a) Mobile phase: acetonitrile + sodium pentane sulfonate solution (10+90);
- b) Chromatographic column: C₁₈ (capped with polar small molecules); particle size 5μm; column length 250 mm; inner diameter 4.6 mm;

NOTE: NUCLEODUR C₁₈ Pyram column can be used, or other equivalent.

- c) Flow rate: 0.8 mL/min;
- d) Detection wavelength: 192 nm;
- e) Injection volume: 10μL.

6.3 Chromatographic determination

The standard solution and the sample solution are respectively injected into the liquid chromatograph for measurement; and record the chromatographic peak area. Chondroitin sulfate samples extracted from bovine cartilage tissue use standard product of chondroitin sulfate A; chondroitin sulfate samples extracted from porcine cartilage tissue use standard product of chondroitin sulfate B; and chondroitin sulfate samples extracted from shark cartilage tissue use standard product of chondroitin sulfate C. See Appendix B for typical chromatograms of standard solutions.

7 Calculation of Results

The content of the tested substance is calculated according to Formula (1):

$$W_i = \frac{A_i \times m_{is}}{A_{is} \times m} \times 100 \quad \dots\dots\dots (1)$$

Where:

W_i - the percentage of the tested substance in the sample, in %;

A_i - the chromatographic peak area of the tested substance in the sample solution;

m_{is} - the mass of the standard product of tested substance in the standard solution, in mg;

A_{is} - the chromatographic peak area of the standard product of tested substance in the standard solution;

m - the mass of the sample, in mg.

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