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Replace QB/T4576-2013

Sodium Hyaluronate
透明质酸钠

(English Translation)

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Foreword

SAC/TC64/SC5 is in charge of this English translation. In case of any doubt about the contents of English translation, the Chinese original shall be considered authoritative.

This document is drafted in accordance with the provisions of GB/T 1.1-2020 Standardization Guidelines Part 1: Rules for the Structure and Drafting of Standardization Documents.

This Standard replaces QB/T4576-2013 Sodium Hyaluronate.

Compared with QB/T4576-2013, the main technical changes in this document, except for editorial modifications, are as follows:

——changed "relative molecular weight" and "structural formula" (see 4.2, 4.3, 2013 version 4.2, 4.3);

——Deleted the product categories (see 5 in the 2013 edition);

——added "identification" and test method (see 5.1, 6.2);

——deleted the content of "net content" (see the 2013 edition of the 5.1);

——deleted the "transmission" and "the physical and chemical requirements" indicators (see 2013 edition of the 6.2);

——added the physical and chemical indicators of product classification (see 5.3);

——changed the "lead" and "arsenic" index requirements, increase the total number of colonies, indicators and requirements of coliform bacteria, delete the "salmonella" indicators requirements (see 5.4, version 6.3, 2013);

——added the measuring method for the determination of Sodium Hyaluronate (see appendix B).

Sodium Hyaluronate

1. Scope

This document specifies the identification, sensory, physical and chemical, safety and other requirements of sodium hyaluronate, describes the corresponding test methods, specifies the inspection rules and marking, packaging, transportation, storage content, and gives the molecular formula, structural formula and relative molecular mass information.
This document applies to the production, testing and marketing of sodium hyaluronate produced by fermentation of Streptococcus equi epizoosis subspecies with glucose, yeast powder and peptone as raw materials.

2. Standardization of reference document

The contents of the following documents constitute essential provisions of the text through normative references in the text. Where a reference file with a date is noted, only the version corresponding to the date applies to this file; For undated references, the latest version is (including all amendment orders) applied to this document.

GB/T191 Package storage and transportation graphic symbol
GB/T 601 Chemical reagent preparation of standard titration solution
GB/T 602 Chemical reagents preparation of standard solutions for the determination of impurities in chemical reagents
GB/T 603 Chemical reagent preparation of preparations and products used in test methods
GB 4789.2 National standard for food safety Microbiology test of food——determination of the number of colonies
GB 4789.3-2016 National Standard for Food safety Food microbiology inspection coliform count
GB 4789.15 National Standard for food safety Food microbiology test mould and yeast count
GB 5009.3-2016 National Standard for Food safety -- Determination of moisture in food
GB 5009.4-2016 National Standard for Food safety -- determination of ash content in food
GB 5009.11 National standard for Food safety -- Determination of total arsenic and inorganic arsenic in food
GB 5009.12 National standard for Food safety -- Determination of lead in food f
GB/T 6682-2008 Analysis of laboratory water specifications and test methods
GB 7718 National standard for Food safety -- General Rules for labelling pre-packaged food

3. Terms and definitions

The following terms and definitions apply to this document.

3.1 Sodium hyaluronate

A disaccharide structural unit formed by β-D-N-acetylglucosamine and β-D-glucuronic acid linked by β-1, 3-glycosidic bonds, and then linked by β-1, 4-glycosidic bonds, a sodium salt of a chain polymer acid mucopolysaccharide hyaluronic acid.

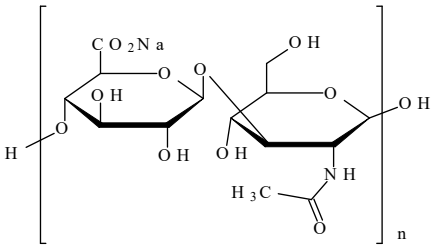
Note: Commercial sodium hyaluronate is also known as "hyaluronic acid", "hyaluronic acid", etc.

4. Molecular formula, relative molecular weight and structural formula

4.1 Molecular formula: $(C_{14}H_2ONNaO_{11})_n$

4.2 Relative molecular weight: 80 200-4 010 000(International Table of Relative Atomic Mass 2021)

4.3 Structural formula



5. Requirement

5.1 Identify

5.1.1 Infrared Spectrum

It accords with the infrared absorption spectrum characteristics of sodium hyaluronate. The infrared absorption spectra were consistent with the standard infrared absorption spectra of sodium hyaluronate (FIG. 1).

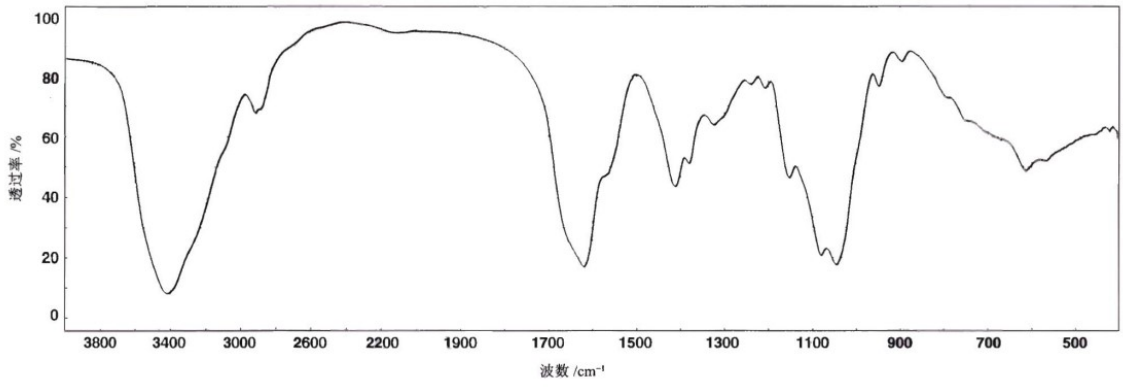


Figure 1 Standard infrared absorption spectrum of sodium hyaluronate

5.1.2 Sodium identification

In line with the characteristics of sodium salt flame reaction, that is, the flame is bright yellow.

5.2 Sensory requirements

The product is white or white-like powder or particle, no normal vision visible impurities.

5.3 Physicochemical indexes

Shall comply with the requirements of Table 1.

Table 1 Physical and chemical indexes

Item		index		
		premium grade	Firsts grade	qualified product
Sodium hyaluronate(on dry basis), g/100g	≥	95	90	87
moisture, g/100g	≤	10.0		
ash, g/100g	≤	13.0		
pH (0.1% aqueous)		6.0~8.0		

5.4 Safety index

Shall comply with the provisions of Table 2.

Table 2 Safety indicators

Item		Index
Lead (Pb) / (mg/kg)	≤	0.5
arsenic (As) / (mg/kg)	≤	0.3
Molds and yeasts/ (CFU/g)	≤	100
aerobic bacterial count/ (CFU/g)	≤	1000
coliform/ (MPN/g)	≤	3.0

6. 6 Experimental method

6.1 General requirements

Reagents and water used in this document, where no other requirements are indicated, shall all refer to pure reagents for analysis and tertiary water as specified in GB/T6682-2008. The standard titration solution, the standard solution, preparation and product for the determination of impurities used in the test are prepared per the provisions of GB/T 601, GB/T 602 and GB/T 603-2023, if no other requirements are indicated. The solution used in the test refers to an aqueous solution when the type of solvent is not indicated.

6.2 Identify

6.2.1 Infrared Absorption Spectrum

Potassium bromide tablet pressing method was adopted, the samples were dried in 105℃ oven for 2 h, and the transparent tablets were formed by pressing device under atmospheric pressure or vacuum conditions. The scanning range was 4000cm-1~400cm-1 by infrared spectrometer. The infrared absorption spectra of samples were compared with standard infrared absorption spectra.
Note: Base on equipment conditions, direct injection can be used.

6.2.2 Sodium

The platinum wire was moistened with hydrochloric acid and burned on the flame until colorless. Then the platinum wire was moistened with hydrochloric acid and dipped in a little sodium hyaluronate sample and burned on the flame to observe the color of the flame.

6.3 Sensory

Take appropriate samples and observe the color and shape of the samples with naked eyes under natural light.

6.4 Sodium hyaluronate content (on dry basis)

The determination shall be carried out in accordance with Appendix A or Appendix B, of which Appendix A is the arbitration method.

6.5 Moisture

The measurement shall be carried out according to the steps of the first method "direct drying method" stipulated in GB 5009.3-2016, where the drying time is 4h.

6.6 Ash Content

Determination was made according to the first method "total ash" specified in GB 5009.4-2016.

6.7 pH

6.7.1 Reagent and solution

6.7.1.1 CO_2 free distilled water: prepared in accordance with5.1.1.7 in GB/T 603-2023.

6.7.2 Instruments and equipment

pH meter: The accuracy was 0.01.
Mixer.
Constant temperature water bath pot.

6.7.3 Determination procedure

Weigh 0.1g sodium hyaluronate sample (accurate to 0.01g) and add 100 mL of carbon-dioxide-free distilled water, and dissolve it by magnetic stirring or heating

in a 40 ℃ water bath. The pH of the solution was measured with a pH meter. The result retainsone decimal place.

6.8 Lead(Pb)

Shall be determined according to the method specified in GB 5009.12.

6.9 Arseni(As)

Shall be determined according to the method specified in GB 5009.11.

6.10 Molds and yeasts

Determination shall be made according to the method specified in GB 4789.15. If the sample is insoluble, an appropriate amount of sterile hyaluronidase can be added to help the sample dissolve.

6.11 Aerobic bacterial count

Shall be determined according to the method specified in GB 4789.2.If the sample is insoluble, an appropriate amount of sterile hyaluronidase can be added to help the sample dissolve.

6.12 Coliforms

According to the MPN method specified in GB 4789.3-2016.If the sample is insoluble, an appropriate amount of sterile hyaluronidase can be added to help the sample dissolve.

7. Inspection rules

7.1 General Requirement

The products shall be inspected in accordance with the provisions of this document, and the qualified products shall be released only with the quality certificate issued by the quality testing department of the manufacturer.

7.2 Batch

With the same feeding production, the same specification, and the same variety of uniform quality products for a batch.

7.3 Sampling and retention of samples

7.3.1 Sampling

When drawing samples from a whole lot, a number of packing units should be drawn from the whole lot, followed by a uniform sample drawn from the extracted packing units. Sampling quantity is performed according to Table 3.

Table 3 Product sampling table

Lot range (minimum outer packing unit)	Number of samples drawn (minimum outer packing	Number of packaging per sample[a] (bottle, bag)

	unit)	
＜100	2	1
100~500	4	1
＞500	6	1
[a]The number of unit packages refers to small units in large packages		

7.3.2 Sampling

For sampling, insert a clean, dry sampling tool two-thirds of the way into the package. Sample 100 g in each bag. Quickly mix the sample and divide it by four parts. Then divide it into two clean and dry sampling bags and seal them. Label one copy for inspection and one copy sealed for future reference.

7.4 Ex-factory inspection items

Ex-factory inspection items are sensory, sodium hyaluronate content (based on dry basis), pH, moisture, total number of colonies, mold and yeast and coliform.

7.5 EC Type-examination certificate

The type test items are all items specified in the requirements of this document. In general, type test is carried out once every 6 months. Type test should also be carried out under any of the following circumstances:

a) When there is a great change in raw and auxiliary materials;
b) When changing key processes or equipment;
c) When the production of a new trial-made product or a normally produced product is resumed after three months of suspension;
d) When there is a big difference between the results of the outgoing inspection and the last type inspection result;
e) When it is necessary for the State supervisory and administrative authority to conduct random inspection according to relevant regulations.

7.6 Criterion rule

Inspection result If any product is found not in conformity with the requirements of this document, samples should be taken from the package of twice the amount for reinspection. The reinspection result shall prevail. If there is still one non-conforming item, the batch of products will be judged as non-conforming. If two or more items of products are found to be nonconforming to the requirements of this document, the batch of products shall be judged as nonconforming products.

8. 8 Marking, Packaging, Transportation and Storage

8.1 Packaging and marking

The outer packing mark shall comply with the provisions of GB/T 191. Pre-packaged product labels shall comply with GB 7718. Marks with special requirements shall be marked according to the requirements of the demander.

8.2 Transportation

The means of transport shall be clean; It is prohibited to mix with poisonous, harmful, corrosive and smelly articles, and should avoid moisture, pressure and exposure to the sun; Loading and unloading should be handled with care.

8.3 Storage

Products should be stored in dry, ventilated room temperature warehouse, strictly prohibited with toxic and polluted articles or other sundries.

Appendix A

(annex normative)

Determination of Sodium hyaluronate-Spectrophotometer method

A.1 Principle

Hyaluronic acid contains the same molar ratio of N-acetylglucosamine and glucuronic acid. The glucuronic acid can be separated by acid hydrolysis of sodium hyaluronate with sulfuric acid using borax as catalyst. Glucuronic acid reacts with carbazole to form an organic complex that shows a characteristic purple color and its absorbance is proportional to the concentration of glucuronic acid. The content of sodium hyaluronate can be determined by the content of glucuronic acid.

A.2 Reagent and solution

A.2.1 Standard: D-glucuronic acid, CAS 6556-12-3, purity≥98%.
A.2.2 Concentrated sulfuric acid: GR.
A.2.3 Carbazole.
A.2.4 Borax.
A.2.5 Anhydrous ethanol.
A.2.6 Carbazole ethanol solution (1.25 g/L) : Weigh 0.125 g carbazole (accurate to 0.001 g) and dissolve in 100 mL anhydrous ethanol in a brown bottle. 4℃~8℃ explosion-proof refrigerator storage, valid for 2 months; Or store at room temperature away from light, valid for 15 days.
A.2.7 D-glucuronic acid standard solution (0.2 mg/mL) : Accurately weigh 20 mg D-glucuronic acid standard product (which needs to be converted to 0.0001g according to the actual purity), put it in a 100 mL volumetric bottle, dissolve it with water, fix the volume to the scale, shake well and set aside.
A. 2.8 Borax-sulfuric acid solution (0.025 mol/L) : weigh borax 4.77 g (0.001 g) dissolved in 500 mL of sulfuric acid, save in fine glass bottle mouth sealed for later use.

A. 3 Instruments and equipment

A.3.1 Spiral Mixer.
A. 3.2 Spectrophotometer, can be determined under the 530 nm wavelength absorbance.

A. 4 Preparation of standard curve

Respectively measuring glucuronic acid standard solution (n.) 2.7 0.5 mL, 1.0 mL and 1.5 mL, 2.0 mL and 2.5 mL, put in 10 mL volumetric flask, add water dilute to scale, Standard solutions with concentrations of 10 μg/mL, 20 μg/mL, 30 μg/mL, 40 μg/mL and 50 μg/mL were obtained. Take 6 test tubes with plug scale and add 1.0 mL of standard solution of different concentration respectively, then add 5.0 mL of borax sulfide solution respectively, and put them in ice bath to cool for 15min. Shake gently and mix thoroughly with a vortex mixer. Heat the tube in boiling water for 10 minutes, then cool it to room temperature in an ice bath or running water. Add 0.2 mL carbazole solution (A.2.6) into the cooled test tube, mix well, heat in

the boiling water bath for 15 min, and then cool to room temperature. The absorption value was measured at 530 nm with a 10 mm cupola, and the standard curve was drawn with the absorption value as the ordinate and the concentration as the horizontal coordinate.

A. 5 Procedure

A.5.1 According to samples from sodium hyaluronate is about 0.1 g (m, accurate to 0.0001 g) to plug in the conical flask, add water to 100 g (w1, accurate to 0.01 g), magnetic stir until fully dissolved. Then weigh the above solution about 4.0g (w2, accurate to 0.01g) into a 50 mL (V) volumetric bottle, dilute it with water to the scale, and shake well.

A. 5.2 Learn l mL sample solution, add 5 mL borax sulfur acid, buy cooled in ice bath for 15 min. Shake gently and mix thoroughly with a vortex mixer. Heat the tube in boiling water for 10 minutes, then cool it to room temperature in an ice bath or running water. Add 0.2mL of carbazole test solution, mix well, heat in boiling water bath for 15 min, then cool to room temperature. The absorbance was measured at 530nm by spectrophotometer with l cm cupola.

A. 6 Calculation

Sodium hyaluronate content (on a dry basis) is calculated according to equation (1).

$$X_2 = \frac{c \times w_1 \times 10^{-6} \times V \times 100 \times 401.3}{w_2 \times m \times (1 - w_3/100) \times 194.1} \quad \text{.....(1)}$$

Where:

X_2——Sodium Hyaluronate content in the sample (dry basis), in the unit of grams per hundred grams (g/100 g);

c——ccording to the sample absorbance, find out the corresponding glucuronic acid concentration from the standard curve, in the unit of microgram per milliliter (μg/mL);

w_1——the quality of the sample solution, in the unit of gram (g);

10^{-6}——micrograms and gram conversion coefficient;

v——the capacity of the sample volume, in the unit of mL (mL);

100——Gram and 100 gram conversion factors;

401.3——The molecular weight of sodium hyaluronate;

w_2——solution weight, in the unit of gram (g);

m——the sample quality, in the unit of gram (g);

w_3——sample moisture, in the unit of grams per hundred grams (g/100g);

194.1——The molecular weight of D-glucuronic acid.

Results retained to 1 decimal places.

7 Precision

Under the condition of the repeatability of the absolute difference between the two independent determination results should not exceed 2% of the arithmetic mean

Appendix B

(annex normative)

Determination of Sodium hyaluronate-HPLC method

B.1 Principle

Hyaluronidase can act on the β-1,4-glucoside bond of sodium hyaluronate to hydrolyze sodium hyaluronate to produce n-acetylglucosamino-glucuronic disaccharide. The content of N-acetylglucosamino-glucuronic disaccharide was determined by high performance liquid chromatography. The standard curve was drawn with the concentration of sodium hyaluronate as the abscissa and the peak area of N-acetylglucosamino-glucuronic acid produced by sodium hyaluronate hydrolysis as the ordinate. The content of sodium hyaluronate in the sample can be calculated by the peak area of N-acetylglucosamino-glucuronic disaccharide produced by hydrolysis.

B. 2 Reagents and materials

B. 2.1 Sodium Hyaluronate reference material (CAS: 9067-32-7, Purity of 99% or higher)

B. 2.2 Hyaluronidase (not less than 4000 IU/mL)

B. 2.3 Sodium phosphate dibasic dihydrate $NaH_2PO4•2H_2O$

B. 2.4 Sodium phosphate dibasic dodecahydrate $Na_2HPO_4•12H_2O$

B. 2.5 Phosphate H_3PO_4

B. 2.6 Phosphate buffer solution (0.2 mol/L, pH6.0) : Weigh 27.4 g of sodium dihydrogen phosphate dihydrate (B.2.3) and 8.8 g of disodium phosphate dihydrate (B.2.4) in 1000 mL beek, dissolve in water, transfer to 1000 mL volumetric bottle, adjust pH 6.0 with 1mol/L of phosphoric acid solution or 1mol/L of sodium hydroxide solution, add water for constant volume, and shake well.

B. 2.7 Sodium Hyaluronate reference substance solution (1.0 mg/mL) : according to take 50 mg Sodium Hyaluronate reference substance (should be according to the actual conversion purity, accurate to 0.1 mg) in the volumetric flask, add 40 mL dissolved phosphate buffer solution, ultrasonic and dissolves adequately, phosphate buffer solution constant volume and shake well.

B. 2.8 Hyaluronidase solution (1000 IU/mL) : move adequate hyaluronidase (b.2.2) in 10 mL volumetric flask and dissolved phosphate buffer and constant volume. Use as you go.

B. 2.9 Mobile phase (1%) phosphoric acid solution: move in 10.0 mL phosphate (b. 2.5) in 800 mL water, blending after transfer into 1000 mL volumetric flask, the capacity and the water blending.

B. 3 Instruments and equipment

B.3.1 High Performance Liquid Chromatography with UV detector.

B.3.2 Column: sulfonated-diphenyl ethylene divinyl benzene copolymer strong cation exchange chromatography column (300 mm x 8 mm), or other equivalent chromatographic column.

B.3.3 chromatographic conditions: Flow 0.6 mL/min; injection Vol:20 μL; Column temperature: 40 ℃; Wavelength: 232 nm.

B. 4 Procedure

B.4.1 Preparation of standard curve

Move Sodium Hyaluronate reference substance solution respectively 0.05 mL and 0.1 mL, 0.2 mL and 0.5 mL and 1.0 mL and 2.0 mL in 15 mL plug calibration tube and phosphate buffer solution (b.) 2.6 to 9 mL, Then 1mL hyaluronidase (B.2.8) was added, mixed, and enzymolized in water bath at 37℃~42 ℃ for 1 h. The reaction was terminated by boiling water bath for 2 min. After cooling to room temperature, they were filtered with a 0.22μm filter membrane and analyzed according to the chromatographic conditions described in B.3.3 to record the peak area. The concentration of control solution was 0.005 mg/mL, 0.010 mg/mL, 0.020 mg/mL, 0.050 mg/mL, 0.100 mg/mL and 0.200 mg/mL, respectively. The standard curve was drawn with the concentration of sodium hyaluronate working solution as the abscissa and the peak area as the ordinate.

B.4.2 Sample preparation

According to samples from 0.1 g (0.001 g) into 100 mL volumetric flask, add 80 mL after phosphate buffer solution, 42 ℃ water bath ultrasonic to fully dissolving phosphate buffer solution constant volume.

B.4.3 Enzymatic Hydrolysis

Take 0.5 4.3 mL b. 4.2 after processing of the sample solution to 15 mL plug calibration test tubes, add 8.5 mL buffer solution. Then add 1.0 mL hyaluronidase solution, and mix well, enzymatic hydrolysis in 37℃~42 ℃ water bath for 1 h. Then the reaction was terminated by boiling water bath for 2 min. Cool to room temperature.

B.4.4 Determination

Move adequate b. 4.3 after enzymolysis liquid by 0.22 mu m filter membrane filtration. The filtrate was analyzed according to th. e chromatographic conditions in B.3.3 and the peak area was recorded.

B.5 Calculation

Sodium hyaluronate content (on a dry basis) is calculated according to equation (2) :

$$X = \frac{c \times V \times f \times 100}{m \times (1 - w_3/100) \times 1000} \quad \text{.....(2)}$$

Where:

X——Sodium hyaluronate content (measured on a dry basis), in the unit of grams per 100 grams (g/100g);

c——according to peak area of the sample solution by standard curve to calculate samples of sodium hyaluronate in the solution, in the unit of mg per milliliter (mg/mL);

V——the volume of the sample after dissolving, in the unit of mL;

f——sample solution diluted multiples;
100——g and hectogram conversion factor;
m——the sample that the quantity, in the unit of gram (g);
w_3——sample moisture, in the unit of grams per hundred grams (g/100 g);
1000——mg and scaling factor.
The calculation results with repeatability obtained under the condition of two independent determination results of arithmetic mean, said keep a decimal places.

B.6 Precision

Under the condition of the repeatability of the absolute difference between the two independent determination results do not exceed 2% of the arithmetic mean.

Appendix C

(informative)

Reference Chromatography

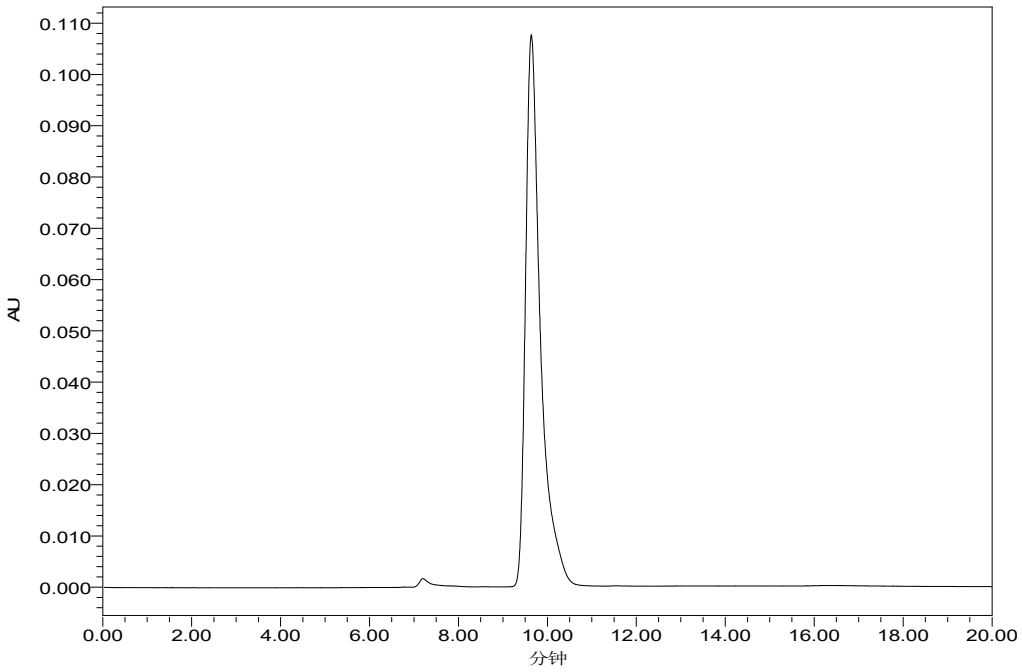


Figure C.1 Chromatogram of sodium hyaluronate standard solution

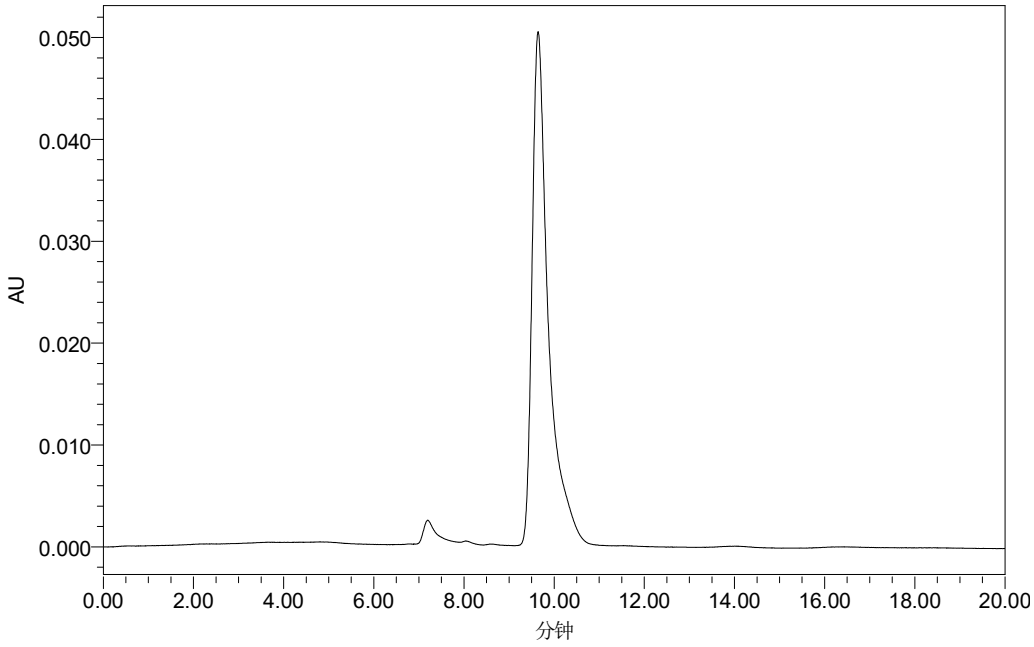


Figure C.2 Chromatogram of sodium hyaluronate sample solution