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PHARMACEUTICAL INDUSTRY STANDARD

OF THE PEOPLE'S REPUBLIC OF CHINA

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YY/T 0962-2021

Replacing YY/T 0962-2014

Cross-Linked Sodium Hyaluronate Gel for Plastic Surgery

整形手术用交联透明质酸钠凝胶

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Cross-Linked Sodium Hyaluronate Gel for Plastic Surgery

1 Scope

This Standard specifies the requirements, inspection methods, packaging and information provided by the manufacturer of cross-linked sodium hyaluronate gel for plastic surgery (hereinafter referred to as cross-linked sodium hyaluronate gel).

This Standard applies to cross-linked sodium hyaluronate gel.

NOTE: Cross-linked sodium hyaluronate gel is suitable for filling of skin and subcutaneous tissue.

2 Normative References

The following documents are essential to the application of this document. For the dated documents, only the versions with the dates indicated are applicable to this document; for the undated documents, only the latest version (including all the amendments) is applicable to this document.

GB/T 16886.1 Biological Evaluation of Medical Devices - Part 1: Evaluation and Testing within a Risk Management Process

YY/T 1571 Tissue Engineering Medical Device Products - Sodium Hyaluronate

Pharmacopoeia of the People's Republic of China (IV Volumes) 2020 Edition

3 Terms and Definitions

For the purposes of this Document, the following terms and definitions apply.

3.1 Hyaluronic acid

A linear polysaccharide that is composed of disaccharide repeating structural units formed by connecting D-glucuronic acid and *N*-acetyl-D-glucosamine through *β*-(1-3) glycosidic bond. Each disaccharide unit is linked to another disaccharide unit by a *β*-(1-4) glycosidic bond. Hyaluronic acid generally exists in the form of sodium salt, namely sodium hyaluronate.

3.2 Cross-linking agent

Substance that is used for cross-linking of sodium hyaluronate.

3.3 Cross-linked sodium hyaluronate gel

The biological evaluation shall be carried out in accordance with the requirements of GB/T 16886.1.

5.19 Degradation properties

The degradation of cross-linked sodium hyaluronate refers to the degradation in vivo to the disappearance of the material under the local microscope by histological observation, excluding the further metabolic process of the material outside the implanted site. If the degradation time of the product is too long, other suitable methods can be used for degradation test.

6 Inspection Methods

6.1 Appearance

The cross-linked sodium hyaluronate gel is placed under the illumination of 1000lx~1500lx for random rotation observation, which shall comply with the provisions of 5.1.

6.2 Effective usage amount

Take out the cross-linked sodium hyaluronate gel in each single package as much as possible according to the normal use method; weigh it and then divide it by the density of the cross-linked sodium hyaluronate gel (ρ=1.01g/mL), which shall comply with the provisions of 5.2.

6.3 Particle size distribution

It shall be determined according to Determination Method of 0982 Particle Size and Particle Size Distribution - The Third Method (Light Scattering Method) – Wet Method in *Pharmacopoeia of the People's Republic of China (VI Volumes)* (2020 Edition), and shall comply with the provisions of 5.3.

6.4 Pushing force

Determined according to the method in Appendix A, it shall comply with the provisions of 5.4.

6.5 Infrared identification

Dry an appropriate amount of cross-linked sodium hyaluronate gel by freeze-drying method, ethanol precipitation and drying method or direct drying method (80°C and below); and then use potassium bromide to press into tablets; and then it shall be determined according to the 0402 Infrared Spectrophotometry in *Pharmacopoeia of the People's Republic of China (IV Volumes)* (2020 Edition), and shall comply with the provisions of 5.5.

6.6 Swelling degree

Determined according to the method in Appendix B, it shall comply with the provisions of 5.6.

6.7 Osmotic pressure

Direct sampling, it shall be determined according to the Determination Method of 0632 Molar Concentration of Osmotic Pressure in *Pharmacopoeia of the People's Republic of China (VI Volumes)* (2020 Edition), and shall comply with the provisions of 5.7.

6.8 pH value

The cross-linked sodium hyaluronate gel is diluted with purified water in an equal mass ratio; and it is determined according to the Determination Method of 0631 pH Value in *Pharmacopoeia of the People's Republic of China (VI Volumes)* (2020 Edition), and shall comply with the provisions of 5.8.

6.9 Sodium hyaluronate content

Determined according to the method in Appendix C, it shall comply with the provisions of 5.9.

6.10 Protein

Determined according to the method in Appendix D, it shall comply with the provisions of 5.10.

6.11 Total amount of heavy metals

It shall be determined according to the Second Method of 0821 Heavy Metal Inspection Method in *Pharmacopoeia of the People's Republic of China (VI Volumes)* (2020 Edition), and shall comply with the provisions of 5.11.

6.12 Residual amount of cross-linking agent

Determined according to the method in Appendix E, it shall comply with the provisions of 5.12.

If other cross-linking agents are used, limit requirements and inspection methods shall be provided.

The test methods for the residual amount of all cross-linking agents shall be able to detect the residual amount of cross-linking agent in the cross-linked sodium hyaluronate particles together.

6.13 Free sodium hyaluronate content

Determined according to the method in Appendix F, it shall comply with the provisions of 5.13.

6.14 Other additives

If other additives are added in the production process, their limit requirements and inspection methods shall be provided.

6.15 Sterile

It shall be inspected according to the 1101 Sterility Inspection Method in *Pharmacopoeia of*

Appendix A

(Normative)

Determination of Pushing Force

A.1 Principle

In this test, the injection core rod is pushed at a constant speed; and the injection needle is installed during the test to simulate the actual use situation. Push the core rod at a constant speed; the sample in the syringe is pushed out through the needle; and the push force curve is obtained. From the pushing force curve, the change of the pushing force during the extrusion process of the sample can be observed. If the pushing force is small, the sample is easily extruded; if the pushing force is large, the sample is difficult extruded. In addition, if the high and low drop of the pushing force is large, it shall indicate the sample has uneven dispersion or aggregation and concentration, which shall also affect the chirality during injection.

A.2 Instrument

Universal material testing machine.

A.3 Test method

Remove the outer packaging of the product; install the matching core rod and injection needle in the package; discharge a small amount of air at the front end of the syringe; and then install it on the testing equipment; and set the testing parameters of the testing equipment (universal material testing machine).

The test conditions are as follows:

a) test temperature: room temperature.

b) equilibration time at room temperature (for products stored in refrigerated conditions): take out the product and equilibrate at room temperature for 1h before testing.

c) test distance: set the test distance to full scale.

d) pushing speed: set the pushing speed to 30mm/min.

Test the pushing force according to the prescribed method; and record the maximum pushing force, the minimum pushing force and the average pushing force in the platform area of the pushing force curve.

Appendix C

(Normative)

Determination of Sodium Hyaluronate Content

C.1 Principle

After the hydrolysis of sodium hyaluronate, glucuronic acid reacts with carbazole reagent to produce reddish purple, the resulting color depth is proportional to the content of glucuronic acid.

C.2 Instrument

Electronic balance (with accuracy of 0.1mg), UV-Vis spectrophotometer, vortex mixer or equivalent equipment.

C.3 Preparation of solution

C.3.1 Carbazole ethanol solution with volume fraction of 0.125%

Weigh 0.125g of carbazole; add 100mL of absolute ethanol to dissolve. Transfer to a dark brown bottle and store in a dark place; it is valid for 15 days.

C.3.2 Glucuronic acid (GA) standard solution

Accurately weigh about 0.1g of glucuronic acid reference substance; put it in a 100mL volumetric flask; add water to dissolve and dilute to the mark; shake well; take as a stock solution; and store at 2 ℃ ~ 8 ℃. Before use, accurately take 5.0mL of the stock solution; put it in a 100mL volumetric flask; add water to make a solution containing 50μg per 1mL.

C.3.3 0.025mol/L sodium tetraborate sulfuric acid solution

Weigh 9.54 g of sodium tetraborate ($Na_2B_4O_7 \cdot 10H_2O$); add it to 1L of concentrated sulfuric acid; and cover it. Shake occasionally until the sodium tetraborate is completely dissolved. Stored at room temperature; it is valid for 12 months.

C.3.4 0.5mol/L sulfuric acid solution

Take 5 mL of 98% sulfuric acid; add it to a beaker containing 179 mL of water; and mix well.

C.3.5 1mol/L sodium hydroxide solution

Take 10 g of sodium hydroxide; add 250 mL of water; and stir to dissolve.

C.3.6 Test solution

Appendix E

(Normative)

Determination of Residual Amount of Crosslinking Agent 1,4-Butanediol Diglycidyl Ether (BDDE)

E.1 Enzyme-labelled method

E.1.1 Principle

This method is the one to detect the high sensitivity of the epoxy compounds. The epoxy compound reacts with nicotine and produces fluorescence under the action of excitation light of 370nm; and its fluorescence intensity is proportional to the amount of epoxy compound. The fluorescence intensity can be detected by fluorescence spectrophotometer at the emission wavelength of 430nm.

E.1.2 Instrument and reagent

Multifunctional microplate reader (or fluorescence spectrophotometer), electronic balance (with accuracy of 0.1mg), constant temperature water bath, and micro sample injector.

Hyaluronidase, BDDE, nicotinamide, acetophenone, potassium hydroxide, formic acid, ethanol.

NOTE: The above reagents are at least analytically pure.

E.1.3 Preparation of solution

E.1.3.1 2.0mg/mL BDDE standard stock solution

Accurately weigh 0.1g of BDDE into a 50mL volumetric flask; add purified water; mix well make constant volume; and set aside for later use.

E.1.3.2 125mmol/L nicotinamide solution

Accurately weigh 0.76g of nicotinamide into a 50mL volumetric flask; add purified water; make constant volume; mix well; and set aside for later use.

E.1.3.3 15% acetophenone solution

Pipette 1.5mL of acetophenone solution into a 10mL volumetric flask; add absolute ethanol to make the constant volume; and mix well for later use (due to the poor solubility of acetophenone in water, anhydrous ethanol is used here).

E.1.3.4 1mol/L potassium hydroxide solution

E.2.1 Principle

The separation principle of gas chromatography is to use the difference in the distribution of the components to be separated between the mobile phase and the stationary phase. When the two phases move relative to each other, the distribution of these components between the two phases is repeated. Even if the distribution coefficient of the components has only minor differences, and there can be significant differences as the mobile phase moves; and finally, these components are separated. Each component enters the detector successively; and the chromatographic signal is recorded by the data processing system.

E.2.2 Instrument and reagent

Gas chromatograph (FID detector), electronic balance (with accuracy of 0.1mg), 1,4-butanediol diglycidyl ether (BDDE), ethyl acetate (chromatographically pure), hyaluronidase (HAse).

E.2.3 Reference chromatographic conditions

Chromatographic column: DM-17 or DM-5 (30m×0.32mm×0.25μm) and other similar chromatographic columns.

Column temperature: the initial temperature is 200 °C; maintains for 5 min; and then increase to 280 °C at 20 °C/min; and maintain for 5 min.

Detector: FID.

Carrier gas: N_2.

Injection port temperature: 240°C.

Detector temperature: 280°C.

Carrier gas flowrate: 1.5mL/min.

Sample-injecting volume: 2μL.

Split ratio: 1:1.

E.2.4 Preparation of solution

E.2.4.1 Preparation of HAse solution

A fresh HAse solution is prepared by dissolving 25 mg of hyaluronidase in 10 mL of water.

E.2.4.2 Standard stock solution

Accurately weigh about 100mg of BDDE standard substance; put it in a 100mL volumetric flask; and dilute to the mark with water (stock solution A). Transfer 10mL of stock solution A to a 100mL volumetric flask; and dilute with water to the mark (stock solution B). Transfer

Appendix F

(Normative)

Determination of Free Sodium Hyaluronate Content

F.1 Principle

After the hydrolysis of sodium hyaluronate, glucuronic acid reacts with carbazole reagent to produce reddish purple; and the resulting color depth is proportional to the content of glucuronic acid.

F.2 Instrument

Electronic balance (with accuracy of 0.1mg), UV-Vis spectrophotometer, vortex mixer or equivalent equipment.

F.3 Preparation of solution

F.3.1 Carbazole ethanol solution with a volume fraction of 0.125%: Weigh 0.125 g of carbazole; add 100 mL of absolute ethanol to dissolve. Transfer to a dark brown bottle and store in a dark place; it is valid for 15 days.

F.3.2 Glucuronic acid (GA) standard solution: Precisely weigh about 0.1 g of glucuronic acid reference substance; put it in a 100 mL volumetric flask; add water to dissolve and dilute to the mark; shake well; take as a stock solution; store at 2 ℃ ~ 8 ℃. Before use, accurately pipette 5.0mL of the stock solution; put it in a 100mL volumetric flask; add water to make a solution containing 50μg per 1mL.

F.3.3 0.025mol/L sodium tetraborate sulfuric acid solution: Weigh 9.54 g of sodium tetraborate ($Na_2B_4O_7$•$10H_2O$); add it to 1L of concentrated sulfuric acid; and cover. Shake occasionally until the sodium tetraborate is completely dissolved. Store at room temperature; it is valid for 12 months.

NOTE: The reagents used in the test are analytically pure; and the sulfuric acid should be of guaranteed reagent.

F.4 Preparation of the test solution

Take an appropriate amount of cross-linked sodium hyaluronate gel and place it in an accurately pre-weighed volumetric flask, accurately weigh it (accurate to 0.1 mg); and record the gel mass as m_1; add an appropriate amount of purified water to dilute to the mark, and accurately weigh it (the mass of cross-linked sodium hyaluronate gel and water in the volumetric flask is recorded as m_2); so that the content of free sodium hyaluronate in the diluent is within the linear range of the standard curve; and fully shake and mix well. Then filter with slow filter paper; take 1mL of the filtrate and put it in a test tube as the test solution. Or centrifuge the diluent at 15000r/min

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