LEPURE

Validation Guideline

LFRS Platinum Cured Silicone Tubing

Shanghai LePure Biotech Co., Ltd.

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1.Introduction

LFRS platinum cured silicone tubing (LFRS silicone tubing) is widely used in the pharmaceutical and biopharmaceutical fields - the transportation, sterilization, filling and packaging of ultrapure liquid, single-use system and other processes in drug production. LFRS silicone tubing products, qualification, manufacturing and release of which meet strict requirements and pass the quality control test, can provide safe and reliable single-use bioprocess products for end-users in the biopharmaceutical industry. This "Validation Guideline" provides technical parameters, regulatory compliance, and related physical and chemical tests of LFRS silicone tubing.

1.1 Quality assurance

The quality system conforms to ISO13485: 2016 standard and is applicable to all production facilities. The production head and the quality management head have relevant expertise and work experience, being capable of performing duties in production and quality management. The Company shall continuously conduct personnel training and qualification assessment for production, quality, management and technical personnel at all levels to ensure that employees have a clear understanding of required standards, and to measure the quality of employees through strict preliminary screening and continuous ability testing. The quality and safety of products are restricted and guaranteed by the supplier management policy, incoming material quality control, process monitoring and finished product inspection and release. At the same time, control management files and centralized document system shall be changed to ensure the consistency of products with processes to achieve continual improvement.

1.2 Material control

The quality of raw materials should be fully controlled to avoid the use of toxic or harmful additives, so as to control risks from the source. Raw materials and excipients for production should be controlled according to the risk level. All raw materials and excipients in contact with the liquid should be subject to the biocompatibility evaluation. Animal-derived materials should not be used, and all raw materials and excipients should be traceable.

1.3 Validation summary of LFRS silicone tubing

1.3.1 Physical Properties

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2. Physical Properties

2.1 Basic physical properties

Hardness test refers to the measurement of the hardness (Shore A) of materials. Tensile strength test refers to the measurement of the tension (MPa) required to stretch the material to its breaking point. Tensile elongation test refers to the measurement of the extensibility of materials-elastic coefficient or elastic modulus is used to measure the deformation tendency of materials subject to external forces.

The basic physical properties (hardness, tensile strength, tensile elongation) of LFRS silicone tubing are tested and shown in the following table:

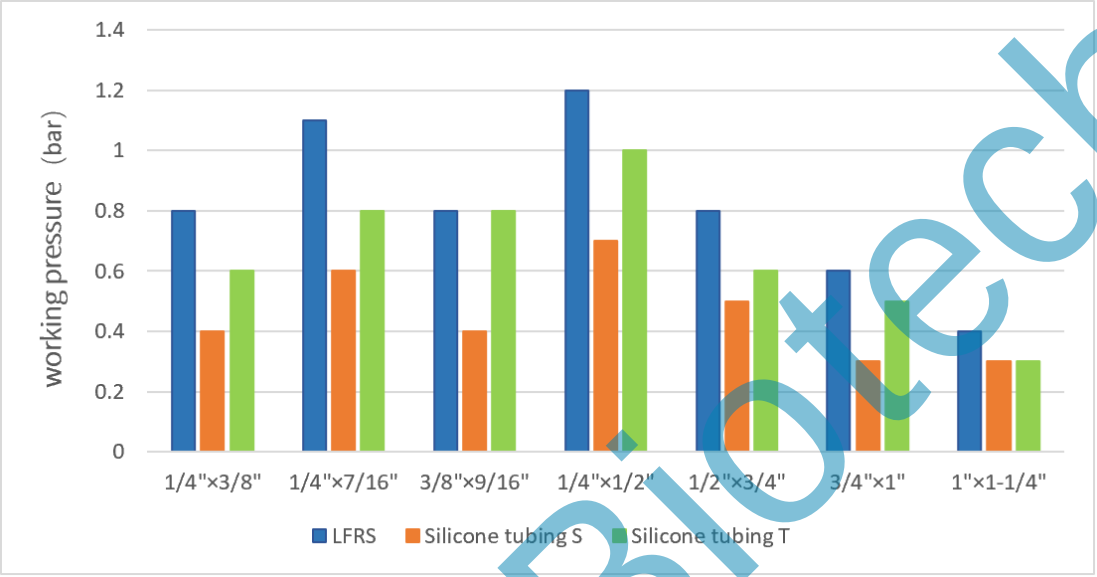
Table 1. Basic Physical Properties of LFRS Silicone Tubing

Test items	Test standards	Unit	Value
Hardness	ASTM D2240	Shore A	60±5
Tensile strength	ASTM D412	MPa	⩾6.0
Tensile elongation	ASTM D412	%	⩾300
Permanent set at break	ASTM D412	%	⩽10
Relative density	ASTM D792	g/cm^3	1.05~1.25
Tearing strength	ASTM D624	kN/m	⩾15

2.2 Study on withstand voltage performance

The purpose of this test is to verify the pressure resistance of the pipeline under various conditions, and to avoid pipe cracking and damage due to excessive pressure during actual operation. Evaluate the pressure tolerance of this series of products by testing some main pipelines and comparing with the data officially published by other commonly used silicone tubing.

Table 2 Comparison Table of Working Pressure of LFRS Silicone Tubing



3. Biocompatibility

Biocompatibility is the main factor affecting the biosafety of platinum cured silicone tubing. LFRS platinum cured silicone tubing was irradiated with 45-55 kGy gamma rays and tested according to USP and ISO 10993 related standards. The results of biocompatibility test are summarized in Table 3, and LFRS silicone tubing passes all tests.

Table 3. Biocompatibility Test Results of LFRS silicone tubing

Test standards	Test items	Test results
ISO 10993-4	Hemolysis test	Pass
ISO 10993-5	Cytotoxicity	Pass
ISO 10993-6	Implantation test	Pass
ISO 10993-10	Irritation and Sensitization tests	Pass
ISO 10993-11	Acute Systemic Toxicity test	Pass
USP<87>	Biological reactivity testing, in vitro	Pass
USP<88>	Biological reactivity testing, in vivo, class VI	Pass

3.1 Cytotoxicity test

According to the section of ISO 10993-5:2009 "Biological Evaluation of Medical Devices

- Part 5: Tests for In Vitro Cytotoxicity", the silicone tubing sample produced was tested to verify its biocompatibility.

The vigorous L-929 cells were cultured in the test sample extract for 48 h (37°C, 5% CO_2), and then observed in terms of the cell morphology and cell lysis to determine the potential cytotoxicity of the test sample. According to the test, the cytotoxic reaction grade was Grade 1 (the cytolysis proportion does not exceed 20%), indicating that the LFRS silicone tubing had no potential toxic effect on cells.

3.2 In vivo biological test

According to the section of ISO 10993-4:2009 "Biological Evaluation of Medical Devices - Part 4: Selection of Tests for Interactions with Blood" and ISO 10993-10:2010 "Biological Evaluation of Medical Devices - Part 10: Tests for Irritation and Skin Sensitization", the silicone tubing sample produced was tested to test its biocompatibility.

ISO 10993-4: In vitro tests were performed to evaluate whether the test sample has adverse effects on hemolysis performance. Prepare the sample by indirect contact method; add diluted blood in proportion and put the sample into the 37 °C water bath for an hour; take the sample from the water bath for centrifugation; absorb and dilute the supernatant before the measurement of absorbance value with a spectrophotometer (540 nm); and calculate the hemolysis rate and hemolysis index. Under this test condition, the hemolysis index of the test sample in indirect contact was less than 5%, and there was no hemolysis, indicating that the LFRS silicone tubing test sample had no effect on the hemolysis performance.

ISO 10993-10: The test sample was extracted with 0.9% sodium chloride injection (SC) and cottonseed oil (CSO), and the control solution without test sample was prepared by the same method. Inject test solution and control solution intracutaneously on both sides of the rabbit spine (for each test solution). Score the erythema and edema at the injection site at (24 ± 2) h, (48 ± 2) h and (72 ± 2) h after injection, and calculate the total average integration of intracutaneous reaction between the test sample and the corresponding control solvent to evaluate its biocompatibility. According to the test, both the final score of rabbit intracutaneous reaction in the SC and CSO test solutions of the silicone tubing were not greater than 1.0, which indicates that the extract of LFRS silicone tubing test sample did not cause intracutaneous irritation.

4. Particulate Matter

The purpose of insoluble particle test is to study the quantity of insoluble particles in LFRS silicone tubing and confirm whether it meets the "Enterprise Standard" of LePure Biotech.

The YBB 00272004-2015 "National Drug Packaging Material Standards" was adopted to

determine the insoluble particles in packaging materials. The sampling amount should not be less than 5 mL each time, and the data of first test among three times should be discarded. Test result: Particles ⩾ 5 μm: less than 50 pcs/mL, particles ⩾ 10 μm: less than 10 pcs/mL, and particles ⩾ 25 μm: less than 1 pcs/mL, indicating that the test result of insoluble particles in the LFRS silicone tubing conforms to the "Enterprise Standard" of LePure Biotech.

5. Endotoxin

The purpose of the test is to study the level of bacterial endotoxin in the LFRS silicone tubing (the endotoxin is an exogenous pyrogen, which can activate neutrophils to release an endogenous pyrogen and acts on the heat regulating center to cause a fever) and confirm whether it conforms to the 1143 test for bacterial endotoxin in the Chinese Pharmacopoeia (V4, 2020). The test sample was tested for bacterial endotoxin by gel-clot method, and all the test results of test solutions were negative. Under the test conditions, the endotoxin content of the test sample solution is lower than 0.25EU/mL, which meets the requirements of Chinese Pharmacopoeia (V4, 2020).

6. Chemical Compatibility

The chemical compatibility of this series of silicone tubing with 66 common reagents is listed in the following table.

Table 4. Chemical Compatibility with 66 Common Reagents

Chemical Material	Rating	Chemical Material	Rating
Acetaldehyde	Yes	Castor oil	Yes
Lactic acid, cold	Yes	Acetamide	Yes
Chlorine	No	Mercury	Yes
Acetic Acid	No	Chlorobenzene	No
Methyl alcohol	Yes	Acetic Anhydride	No
Chloroform	No	Natural gas	Yes
Acetone	Yes	Citric acid	Yes
Nitric acids	No	Acetylene	Yes
Cresols	No	Animal oil	Yes
Ammonia Gas	Yes	Cyclohexane	No
Lubricating oil	No	Ammonium hydroxide	No
Dextrose	Yes	Mineral oil	No

Amyl acetate	No	Ethers	No
Oxygen, cold	Yes	Amyl chloride	No
Ethyl acetate	No	Ozone	Yes
Aniline hydrochloride	No	Ethyl chloride	No
Paraffin	Yes	Phenylamine, cold	No
Ethylene glycol	Yes	Propane	No
Aromatic hydrocarbons	No	Ethylene	Yes
Rubber	Yes	Beer wort	Yes
Formaldehyde	Yes	Sea water	Yes
Beer	Yes	Fuel oils	No
Silicone oil	No	Benzene	No
Gasoline	No	Soap, mild	Yes
Blood	Yes	Glycerin	Yes
Sulphur dioxide	No	Boric acid	Yes
Glycols	Yes	Sulfuric acid	Yes
Butyl alcohol	N/A	Hydraulic oil	No
Urea	Yes	Cane sugar liquors	Yes
Hydrazine	No	Vegetable oils	Yes
Carbon dioxide	Yes	Hydrocyanic acid	Yes
Yeast	Yes	Carbon tetrachloride	No
Hydrofluoric acid	N/A	Potassium permanganate	Yes

7. Sterilization test

7.1 Gamma irradiation sterilization

Silicone tubing is a product that can provide high sterility guarantee. When the silicone tubing is provided in a sterile way, various expected microbial contamination should be minimized before sterilization. The LFRS silicone tubing is produced in an environment where microorganisms under control. The number of microorganisms carried before sterilization is controllable. The silicone tubing will become sterile products after the microorganisms are inactivated through the sterilization process.

Typical single-use system components were selected to test the microbial load and obtain the initial microbial load data. The relationship between the production process and

microbial load was analyzed to indicate that the higher the process complexity, the greater the quantity of microbial load. Single-use system products (including silicone tubing) were classified into a product family in the radiation process. The biological load was obtained through microbial test on the tubing. According to GB18280.2 and tables in GB18280.2-2015, it met the requirements. It was further validated by sterility test that LFRS silicone tubing was suitable for VDmax25 irradiation sterilization method, and the irradiation sterilization dose of the product was determined to be 25 - 40kGy.

7.2 Autoclave

The LFRS silicone tubing is tolerant to high pressure steam sterilization at 121°C for 30 minutes or 134°C for 10 minutes at most.

8. Production management

8.1 Batch management and traceability management

For the production of LFRS silicone tube, the operating procedures for dividing product batches have been established to ensure the homogeneity and consistency of the quality and characteristics of the same batch of products. The standard operating procedures for batch number and production date have been formulated to ensure that each batch of products has a unique batch number.

8.2 Packaging specification

The LFRS platinum cured silicone tubing with material tail number - F is standard 15m/roll, and the LFRS platinum cured silicone tubing with material tail number - H is standard 7.5m/roll, each roll of pipe is packed with double layer plastic bags, and the outer package is independent carton.

9. Statement

9.1 Shelf life statement

The LFRS silicone tubing should be protected from heavy pressure, scratches and collisions with sharp appliance, and should be stored in a cool, dry, well ventilated and clean environment without corrosive gas, with a shelf life of 5 years.

9.2 ADCF statement

The raw materials of LFRS silicone tubing are animal derived component free; During the production and processing, no animal derived components are added.

Shanghai LePure Biotech Co., Ltd.
Website: www.lepure-bio.com
Building 3, 410 Yunzhen Road, Songjiang, Shanghai, China 201600
E-mail: marketing @ lepure-bio.com