WATSON MARLOW

Fluid Technology Group

bio PURE

Validation guide summary

Platinum-cured silicone transfer tubing

Contents

1. Introduction

Our factory in Seaford, Delaware USA produces a range of BioPure products including high purity silicone hose, gaskets and tubing for use in biopharmaceutical and pharmaceutical manufacturing. The facility contains 3,000 square feet of cleanroom manufacturing.

BioPure silicone transfer tubing is manufactured in ISO 14644-1 Class 7 cleanrooms according to GMP principles within a facility operating an ISO 9001 quality management system.

Silicone transfer tubing has a number of key features and benefits. These include:

- **USP Class VI, EP 3.1.9 compliance testing**
- **Animal Derived Component Free**
- **Autoclave and gamma stable**
- **Lot traceability via labeling and packaging**
- **Laser-marking available on request**

BioPure silicone transfer tubing is compliant with a range of compendial testing as detailed in Section 5A. Developed for the biopharmaceutical industry, silicone tubing is suitable for sterilisation by autoclave and gamma irradiation.

The Seaford factory was formed in 2003 and has been providing a range of single-use components to the biopharmaceutical industry for 18 years. It was acquired by Watson-Marlow Fluid Technology Group (WMFTG) in 2015.

WMFTG is a wholly owned subsidiary of Spirax-Sarco Engineering plc.

Spirax-Sarco's headquarters are in Cheltenham, England and is listed on the London Stock Exchange.

2. Conditions of use

BioPure silicone transfer tubing can be sterilised by autoclave (123°C, 30 minutes) and gamma irradiation up to 50 kGY

2a Working temperature and pressure rating

The working temperature range of BioPure silicone transfer tubing is –65°C to 254°C (–85°F to 490°F).

3 Chemical compatibility

Currently, there is no data available on the chemical compatibility of the finished BioPure silicone transfer tubing with regards to how it performs in the presence of different solvents. As such, it is recommended that the BioPure silicone transfer tubing is tested under the actual process conditions.

4 Materials, manufacturing and regulatory compliance statements

4a Compliance declaration summary

Table 1 details the different substances that are not present in the raw material, manufacturing process or final composition of BioPure silicone transfer tubing.

4b REACH legislation

All raw materials, compounds used in the manufacturing process and the final BioPure silicone transfer tubing complies with the REACH regulations.

None of the chemicals used in the manufacture of BioPure silicone transfer tubing are on the candidate list or the list of substances of very high concern (SVHC).

Table 1: List of compliance statements for BioPure silicone transfer tubing and substances not found in the processing of/ or raw materials for BioPure silicone transfer tubing

Named substance	Raw material	Manufacturing process	Final product
Animal Derived Content (ADC)	–	–	–
Phthalates	–	–	–
Bisphenol A (BPA)	–	–	–
Latex	–	–	–
Allergens per Annex II of Regulation (EU) No 1169/2011	–	–	–

'–' denotes not present or not added

4c RoHS

In compliance with the Restriction of Hazardous Substances (RoHS) directives, no listed substances are used in the manufacture of BioPure silicone transfer tubing.

4d Storage conditions

To maintain the performance of the components throughout their lifecycle, they should be stored in a cool, dry environment away from direct sunlight without exposure to chemicals and not subjected to stress. Normal warehouse conditions of 5C–40C (40F–86F) are acceptable. Wherever possible, original packaging should be maintained. Stock should be rotated on a first in, first out (FIFO) basis.

The performance of any component beyond its use by date, or which has not been stored according to the recommendations outlined above, cannot be assured.

5. Compendial and non compendial testing

5a Summary table

Table 2 (page 5) contains a summary of all the compendial testing and ISO qualifications that silicone transfer tubing has been evaluated for. Full test methods and results are available on request. All test work was performed on silicone transfer tubing samples and this was used to represent silicone transfer tubing product family as the components are manufactured using the same material at the same manufacturing site. The silicone transfer tubing was gamma irradiated from 45–55 kGy prior to testing.

5b USP <87> Biological Reactivity tests, *In Vitro*, Post Gamma Irradiation samples

USP 87 determines the biological reactivity of a cell culture in response to a given test article. Samples of silicone transfer tubing were gamma irradiated at 45–55 kGy and tested in accordance with USP 39, NF 34, <87>, Biological reactivity tests, In Vitro.

Extracts, positive control (rubber) and negative control articles were prepared at 37C (98.6F) for 48 hours. Biological reactivity was rated on a scale ranging from Grade 0 (no reactivity) to Grade 4 (severe reactivity).

Results: No reactivity was exhibited by the cell cultures when exposed to BioPure silicone transfer tubing. Therefore they passed the requirements of USP 87 biological reactivity tests and have no cytotoxic potential.

5c USP <88> Biological Reactivity tests, In Vivo, Post Gamma Irradiation samples

USP Class VI Plastics Test assesses the potential toxicity of a given test article systemically, intracutaneously and through implantation. Samples of silicone transfer tubing were gamma irradiated at 45–55 kGy and tested in accordance with USP 39, NF 34 <88>, Biological reactivity tests, In Vivo. This included the immersion of the test articles in the following solutions: USP 0.9% sodium chloride, cottonseed oil, 1 in 20 ethanol in NaCl and polyethylene glycol 400 at 121C (250F) for 1 hour.

Results: BioPure silicone transfer tubing extracts and implants showed no toxicity. Therefore, they passed the requirements of USP <88> biological reactivity tests.

5d ISO 10993–5 Biological evaluation of medical devices – part 5: tests for In Vitro cytotoxicity

The biological reactivity of a cell culture, in response to extracts from silicone transfer tubing was determined. The maintenance medium on the cell cultures was replaced by extracts of silicone transfer tubing or control article.

The cell cultures were incubated for 48 hours at 37±1C (98.6F±33.8F). Biological reactivity was evaluated by a photo spectrometer at 450 nm wavelength.

Results: BioPure silicone transfer tubing showed no signs of cytotoxic potential. Therefore they passed the requirements of ISO 10993–5.

Table 2: List of compendial and non compendial testing performed

Test reference	Test description	Result
USP <87>	Biological reactivity test, *In Vitro*	PASS
USP <88>	Biological reactivity test, *In Vivo*	PASS
ISO 10993–5	Biological evaluation of medical devices, tests for *In Vitro* cytotoxicity	PASS
ISO 10993–6	Biological evaluation of medical devices, implantation	PASS
ISO 10993–10	Biological evaluation of medical devices, irritation	PASS
ISO 10093–11	Biological evaluation of medical devices, systemic toxicity	PASS
E.P. 3.1.9	European Pharmacopeia 3.1.9 silicone elastomer for closures and tubing	PASS
USP <381>	Elastomeric closures for injections - physiochemical tests	PASS
USP <85>	Limulus amebocyte lysate (LAL) bacterial endotoxin assay	REPORT
ISO 10993-11	Systemic toxicity, pyrogen test	PASS

BioPure silicone transfer tubing has passed a number of compendial and ISO testing, a summary of the results are disclosed within.

5e ISO 10993–6 Biological evaluation of medical devices, implantation

The purpose of this test is to evaluate the solid material in direct contact with living tissue.

Silicone transfer tubing and the negative control plastics were tested. The test sites were examined for encapsulation, necrosis, haemorrhage and discolouration macroscopically.

Results: BioPure silicone transfer tubing did not demonstrate any remarkable difference as compared to the control implant sites when in contact with tissue for two weeks.

Table 3: List of physiochemical tests that form EP 3.1.9 – Silicone elastomer for closures and tubing

Test	Test Results	Evaluation Criteria	Result
Appearance of solution	Clear as water 0.3 NTU	Clear as water < 3 NTU	PASS
Acidity or alkalinity	0.0 mL of 0.01 M NaOH (blue colour) 0.0 mL of 0.01 M HCl (yellow changes to orange)	≤ 2.5 mL of 0.01 M NaOH change to blue ≤.0 mL of 0.01 M HCl change to yellow to orange	PASS
Relative density	1.16 g/mL	Between 1.05 – 1.25 g/mL	PASS
Reducing substances	0.5 mL	Diff. between sample and blank ≤ 1.0 mL	PASS
Substances soluble in hexanes	12.4 mg	≤ 15 mg (or 3%)	PASS
Volatile matter	0.17 %	< 2 % (Pt cured)	PASS
Mineral Oils	Less fluorescence than 1 ppm standard	Less fluorescence than 1 ppm standard	PASS
Phenylated compounds	< 0.4 AU from 250 to 340 nm	Absorbance < 0.4 A.u. (between 250 and 340 nm)	PASS
Platinum	0.0 ppm	Less coloured than Pt reference (30 ppm)	PASS

5f 10993–10 Biological evaluation of medical devices, irritation

The intracutaneous test is designed to evaluate local responses to the extracts of silicone transfer tubing following intracutaneous injection. Silicone transfer tubing is extracted using 0.9% sodium chloride for injection, cottonseed oil, 1 in 20 ethanol in NaCl or polyethylene glycol 400 at 121C for 1 hour.

Results: BioPure silicone transfer tubing meet the requirements of ISO10993–10 guidelines for the intracutaneous injection test.

5g ISO 10993–11 Biological evaluation of medical devices, systemic toxicity

The purpose of the systemic injection study is designed to screen test articles (silicone transfer tubing) extracts for potential toxic effects as a result of a single dose systemic injection. Silicone transfer tubing is extracted using 0.9% sodium chloride for injection, cottonseed oil, 1 in 20 ethanol in NaCl or polyethylene glycol 400 at 121C for 1 hour.

Results: BioPure silicone transfer tubing meet the requirements of ISO 10993–11 guidelines for the systemic injection test toxicity.

5h European Pharmacopeia 3.1.9

Extracts of silicone transfer tubing were prepared in accordance with the requirements of European pharmacopoeia, 2009, Chapter 3.1.9 Silicone elastomer for closures and tubing. The results of test are summarised in *Table 3*.

Results: Based on the results of the tests, BioPure silicone transfer tubing meet the requirements of EP 3.1.9 section Physiochemical tests.

5i USP <381> Elastomeric closures for injections - physiochemical tests

Extracts of BioPure silicone transfer tubing were prepared according to the requirements of USP 32, NF 27, Chapter 381 as directed under physiochemical tests. The results of the tests are summarised in the *Table 4*.

Results: Based on the evaluation criteria mentioned below, BioPure silicone transfer tubing meets the requirements of the USP <381> section physiochemical tests.

Table 4:

Test	Test Results	Evaluation Criteria	Result
Appearance of Solution Turbidity/Opalescence: Visual Comparison	Solution S is not more opalescent than Reference Suspension B and the Reference Suspension C	For Type I Closures: The Sample Solution is not more opalescent that Reference Suspension B	PASS
		For Type II Closures: The Sample Solution is not more opalescent than Reference Suspension C	PASS
Appearance of Solution Turbidity/Opalescence: Instrumental Comparison	The turbidity of Solution S is 0.291 NTU	For Type I Closures: The turbidity of the Sample Solution (corrected for the blank solution) is not more than (NMT) that for Reference Suspension B (6 NTU)	PASS
		For Type II Closures: The turbidity of the Sample Solution (corrected for the blank solution) is not more than (NMT) that for Reference Suspension C (18 NTU)	PASS
Appearance of Solution Color	Less intense than the color standard	The Sample Solution is not more intensely colored than the color standard	PASS
Acidity or Alkalinity	0.0 mL of 0.01 N Sodium Hydroxide	Not more than 0.3 mL 0.01 N sodium hydroxide produces a blue color, or Not more than 0.8 mL 0.01 N hydrochloric acid produces a yellow color, or No titration required	PASS
Absorbance	0.03 AU Between 220 nm and 360 nm	Not more than 0.2 AU between 220 nm and 360 nm for Type I closures	PASS
		Not more than 4.0 AU between 220 nm and 360 nm for Type II closures	PASS
Reducing Substances	Difference in Titration Volume= 0.1 mL	The difference between the titration volumes is not greater than 3.0 mL for Type I closures	PASS
		The difference between the titration volumes is not greater than 7.0 mL for Type II closures	PASS
Ammonium	The yellow color of the Test Solution is no darker than the Ammonium Standard Solution;< 2.0 ppm of NH_4	Any yellow color in the Test Solution is no darker than the Ammonium Standard Solution corresponding to not more than 2.0 ppm of NH_4 in Solution S	PASS

5j USP <85> Limulus amebocyte lysate (LAL) bacterial endotoxin assay

Endotoxins are lipopolysaccharide complexes in gram negative bacterial cell walls. The limulus ameobocyte lysate (LAL) gel clot test is used to detect and quantify endotoxin levels in test samples.

BioPure silicone transfer tubing was tested in accordance of USP 85. BioPure silicone transfer tubing (1 meter of tubing) was filled with 70.0mL of SWFI heated to 37 ± 1ºC.The SWFI remained in contact with the fluid pathway at room temperature for 60 ± 2 minutes.

A product sample was prepared from the BioPure silicone transfer tubing extract and the endotoxin standard. LAL was added to the samples, which were incubated at 37 ± 1ºC for 60 ± 2 minutes.

Results: BioPure silicone transfer tubing extracts had an EU/mL value of <0.06.

5k ISO 10993-11 systemic toxicity, pyrogen test

The purpose of the test was to detect the risk to a patient of a febrile reaction as a result of the administration of the test article extract. Samples of BioPure silicone transfer tubing were extracted using USP 0.9% Sodium Chloride for Injection (NaCl) at 70 ± 2ºC for 24 ± 2 hours.

Results: BioPure silicone transfer tubing meet the requirements of ISO 10993-11 guidelines for the systemic injection test.

6. Total Organic Carbon (TOC)

TOC is an analytical technique used to measure the level of organic molecules or contaminants present or extracted into purified water. For this TOC test the extraction procedure performed on a BioPure platinum cured silicone tubing sample is in accordance with USP <643>.

7. Extractables testing

Sections of BioPure silicone transfer tubing were subjected to extraction in multiple solvents at controlled temperatures. Extractions were performed using four (4) solvents: (1) purified water; (2) 50% (v/v) ethyl alcohol in water (50% EtOH); (3) 0.5 N Sodium Hydroxide (NaOH); and (4) 0.1 M Phosphoric acid (H3PO4). Extractions were performed under aggressive conditions of 40 ± 2 ° C for 24± 2 hours and 21 ± 1 days. The resulting extracts were analyzed by a variety of analytical techniques, to identify and quantify materials that may have migrated from the product contact material into the solution of interest. Inductively Coupled Plasma/Mass Spectrometry (ICP/MS) was used to analyze the extract samples for metals. Gas Chromatography/Mass Spectrometry (GC/MS) was used to analyze the extract samples for Volatile Organic Compounds (VOC) and Semi-Volatile Organic Compounds (SVOC). Liquid Chromatography/Mass Spectrometry (LC/MS) was used to analyze the extract samples for target Non-Volatile Organic Compounds (NVOC) and organic acids. An LC chromatogram utilizing a UV detector was obtained to determine the presence of peaks corresponding to non-target NVOC.

Results: These studies have shown that there are low levels of extractables from the tubing extracts of purified water, 50% (v/v) ethyl alcohol in water (50% EtOH), 0.5 N Sodium Hydroxide (NaOH), and 0.1 M Phosphoric acid (H3PO4). WMFTG can provide further information and assistance in the evaluation of extractables data for risk assessment purposes.

8. Conclusions

BioPure silicone transfer tubing have been shown to pass a number of compendial and ISO testing summarised in this guide. For further information with full compliance statements and compendial test reports, please contact your WMFTG representative.

The compliance summary and the full validation guide for BioPure silicone transfer tubing are available by filling in a request form on the wmftg.com website:

watson-marlow.com/biopure-validation/

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