



Validation guide summary

Accusil Platinum-cured silicone tubing



Contents

1 Introduction

2 Conditions of use

3 Chemical compatibility

4 Materials, manufacturing and regulatory compliance statements

- a. Materials of construction
- b. Manufacturing environment
- c. Country of origin
- d. Compliance declaration summary
- e. REACH legislation
- f. RoHS
- g. Storage conditions

5 Compendial and non compendial testing

- a. Summary table
- b. USP <87>
- c. USP <88>
- d. ISO 10993-4
- e. ISO 10993-5
- f. ISO 10993-6
- g. ISO 10993-10
- h. ISO 10993-11
- i. Kligman maximisation
- j. USP <85>
- k. USP<381>
- l. European Pharmacopeia 3.1.9
- m. USP <788>

6 Extractables

7 Conclusions

1. Introduction

Flexicon's Accusil™ platinum-cured silicone tubing is manufactured from a USP class VI raw material in an ISO 14644-1 Class 7 cleanroom operating within an ISO 9001 quality system.

Accusil is compliant with a list of compendial testing as summarised in Section 5A.

Accusil platinum-tubing has been exclusively designed for use in Flexicon peristaltic technology. When used together Accusil exhibits excellent dispensing accuracy of up to +/- 0.5%.

Accusil key features:

- **Laser Traceability: 100% traceability with laser etched lot number, product specification and use by date**
- **Cleanroom manufactured in an ISO 14644-1 Class 7 environment**
- **Double bagged for protection against contamination**
- **Fully post cured**
- **Suitable for gamma irradiation up to 50kGy**

Flexicon founded in 1986 in Denmark, is a company that specialises in the development and manufacture of aseptic filling systems for the biotechnology and pharmaceutical market with an emphasis on precision, efficiency and flexibility.

Watson-Marlow Fluid Technology Solutions (WMFTS) is the world leader in niche peristaltic and sinusoidal pumps and associated fluid path technologies. Founded on nearly 60 years of supplying engineering and process expertise and with over one million pumps installed worldwide, our pumps are tried, tested and proven to deliver. Flexicon has been a part of WMFTS since 2008.

Watson-Marlow 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.

Watson-Marlow Fluid Technology Solutions comprises ten established brands, each with their own area of expertise, but together offering our customers an unrivalled breadth of solutions for their filling and dispensing applications.

2. Conditions of use

Accusil tubing may be treated using either of the following sterilisation methods:

- **Gamma irradiated to 50kGy**
- **Autoclaved at 121C for up to 60 minutes**

2a Working temperature and pressure rating

The working temperature range of Flexicon Accusil™ silicone tubing is -20C to 80C (-4F to 176F).

3. Chemical compatibility

A general guide on chemical compatibility of Accusil tubing can be found on Watson-Marlow Fluid Technologies Solutions website wmfts.com/chemical

4. Materials, manufacturing and regulatory compliance statements

4a Materials of construction

Accusil is made of polydimethylsiloxane (PDMS)

4b Manufacturing environment

Accusil is manufactured according to the principles of GMP in an ISO 14644-1 class 7 cleanroom within a facility operating an ISO 9001 quality management system.

4c Country of origin

Accusil is manufactured at Bickland Water Rd, Falmouth, Cornwall, United Kingdom, TR11 4RU.

4d Compliance declaration summary

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

For full compliance statements please refer to the compliance summary sheet available from the website available on request.

Table 1: List of compliance statements for Accusil and substances **not** found in the processing of or raw materials for Accusil

Substances not present/Compliance statement	Raw material	Manufacturing process	Final product
Glycerin	-	-	-
Animal Derived Content	-	-	-
Melamine	-	-	-
Phthalates	-	-	-
Bisphenol A (BPA)	-	-	-
Latex	-	-	-
Heavy metals	-	-	-

'-' denotes not present or not added

4e REACH legislation

All raw materials, compounds used in the manufacturing process and the final Accusil product comply with the REACH regulation. None of the chemicals used in the manufacture of Accusil are on the candidate list of substances of 2008 or the list of Substance of Very High Concern (SVHC).

environment away from direct sunlight. The normal storage temperature is between -10C to 40C. Wherever possible, original packaging should be maintained. Stock should be rotated on a first in, first out (FIFO) basis. The performance of any tubing beyond its use by date, or which has not been stored according to the recommendations outlined above, cannot be assured.

4f RoHS

In compliance with the restriction of hazardous substances (RoHS) directives, no listed substances are used in the manufacture of Accusil.

4g Storage conditions

To maintain the performance of the tubing throughout its life, tubing should be stored in a cool, dry

5. Biocompatibility and physicochemical testing

5a Summary table

Table 2 contains a summary of all the compendial testing and ISO 10993 qualifications that Accusil has been evaluated for. Full test methods and results are available on request. All tests were carried out by third party contract labs.

Table 2: List of compendial and non compendial testing performed

Test reference	Test Description	Result
USP <87>	Biological reactivity test, <i>In Vitro</i>	PASS
USP <88>	Biological reactivity test, <i>In Vivo</i>	PASS
ISO 10993-4	Haemolysis Test—Autian method	PASS
ISO 10993-5	Biological evaluation of medical devices, tests for <i>In Vitro</i> 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
ISO 10993-10	Kligman maximisation—test for irritation and delayed type hypersensitivity	PASS
USP <85>	Limulus Amebocyte Lysate (LAL) bacterial endotoxin assay	REPORT
USP <381>	Physicochemical tests on elastomeric closure materials	PASS
E.P. 3.1.9	European Pharmacopeia 3.1.9 silicone elastomer for closures and tubing	PASS

Accusil Platinum-cured silicone tubing has passed a number of compendial and ISO testing, a summary of the results are disclosed within.

5b USP <87> Biological reactivity tests, *In Vitro*, post sterilisation samples

USP Biological reactivity tests, *In Vitro*, post sterilisation samples Samples of Accusil were gamma irradiated at 45–55kGy and tested in accordance with USP43, NF38, , Biological reactivity tests, *In Vitro*. The biological reactivity of a mammalian monolayer, L929 mouse fibroblast cell culture in response to Accusil was determined. Samples of Accusil tubing, positive control (rubber) and negative control articles were prepared at 37°C for 48 hours. Biological reactivity was rated on a scale ranging from Grade 0 (no reactivity) to Grade 4 (severe reactivity).

Results: No reactivity (grade 0) was exhibited by the cell cultures when exposed to Accusil. Therefore Accusil is not cytotoxic and passed the requirements of USP biological reactivity tests.

5c USP <88> Biological reactivity tests, *In Vivo*, post sterilisation samples

USP Class VI Plastics Test assesses the potential toxicity of a given test article by introducing a sample *In Vivo* systemically, intracutaneously and through implantation.

Samples of Accusil were tested in accordance with USP32, NF 27, <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 70C for 24 hours.

Results: Accusil extracts and implants showed no toxicity, therefore Accusil met the requirements of USP <88> Class VI biological reactivity tests.

5d ISO 10993-4 Haemolysis

The haemolysis test assesses the potential for indirect contact of a given sample with blood to cause the rupture of erythrocytes (red blood cells).

Samples of Accusil were tested in accordance with ISO 10993-4, 2002, Biological Evaluation of Medical Devices—Part 4: Selection of tests for interactions with blood.

Results: Accusil showed no signs of haemolytic activity. Therefore Accusil passed the requirements of ISO 10993-4.

5e ISO 10993-5 Biological evaluation of medical devices—tests for *In Vitro* cytotoxicity

ISO 10993-5 Biological evaluation of medical devices—tests for *In Vitro* cytotoxicity The biological reactivity of a cell culture, in response to extracts from Accusil was determined. The maintenance medium on the cell cultures was replaced by extracts of Accusil, or control article. The cell cultures were incubated for 48 hours at 37C ±1C. Biological reactivity was evaluated by a photo spectrometer at 450nm wavelength.

Results: Accusil showed no signs of cytotoxic activity. Therefore Accusil passed the requirements of ISO 10993-5.

5f 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.

Accusil was tested in accordance with ISO 10993-6. Strips of Accusil (1mm x 1mm x 10mm) and the standard negative control plastics were evaluated. The test sites were examined for inflammation, encapsulation, necrosis, haemorrhage and discolouration macroscopically.

Results: Accusil did not demonstrate any difference when compared to the control implant sites. Therefore Accusil passed the requirements for ISO 10993-6.

5g ISO 10993-10 Biological evaluation of medical devices, irritation

The intracutaneous test is designed to evaluate local responses to the extracts of Accusil following intracutaneous injection. Accusil was tested in accordance with the requirements of ISO 10993-10. Accusil tubing is extracted using 0.9% sodium chloride for injection and cottonseed oil at 70C for 24 hours.

Results: Accusil meet the requirements of ISO 10993-11 guidelines for the systemic injection test.

5h ISO 10993-11 Biological evaluation of medical devices, systemic toxicity

The systemic injection study is designed to screen Accusil tubing extracts for potential toxic effects as a result of a single dose systemic injection.

Accusil was tested in accordance with the requirements of ISO 10993-11. Accusil tubing is extracted using 0.9% sodium chloride for injection and cottonseed oil at 70C for 24 hours.

Results: Accusil meet the requirements of ISO10993-11 guidelines for the systemic injection test.

minutes. LAL-lysate was added to each sample well on an automated microtiter plate and then transferred to a plate reader where the absorbance of each well was read at 405 nm and 37 °C at set timepoints. A standard curve was generated using software based on reaction time versus endotoxin concentration which was then compared to the endotoxin concentration and reaction time of the sample extract.

Results: Accusil extracts had an EU/ml value of <0.011 EU/cm² which is comparable to the levels of endotoxin observed in water for injection.

5i ISO 10993-10 Kligman Maximisation Test

The purpose of this test is to detect the allergenic potential of a test article.

Accusil was tested in accordance with ISO 10993-10. Samples of Accusil were extracted in USP 0.9% Sodium chloride for injection and cottonseed oil at 70C for 24 hrs. The extracts were injected intradermally. After two weeks, an additional topical application was introduced to the site of intradermal injections.

Results: The skin sites that were exposed to the test articles and negative control showed no signs of erythema or edema. Therefore Accusil is deemed not to contain any allergic potential.

5k USP <381> Physicochemical tests on elastomeric closure material

Extracts of Accusil were prepared according to the requirements of USP 32, NF 27, Chapter 381 as directed under Physicochemical tests. The results of the tests are summarised in [Table 3](#) below.

Results: Based on the evaluation criteria mentioned below, Accusil tubing meets the requirements of the USP <381> section physicochemical tests.

5l European Pharmacopoeia 3.1.9

Extracts of Accusil were prepared in accordance with the requirements of European pharmacopoeia, 6.8, Chapter 3.1.9 Silicone elastomer for closures and tubing. The results of test are summarised in [Table 4](#) on the following page.

Results: Based on the results of the tests, Accusil tubing meets the requirements of EP 3.1.9 section Physicochemical tests.

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

USP Limulus amoebocyte lysate (LAL) bacterial endotoxin assay Endotoxins are lipopolysaccharide complexes in gram negative bacterial cell walls. The limulus amoebocyte lysate (LAL) Kinetic Chromogenic assay is used to determine the quantitative level of endotoxin present within our products. Accusil tubing was tested in accordance to the requirements of USP 85. Accusil (23.7 cm² surface area) was extracted in 50mL of LAL reagent water at room temperature for 60

Table 3: List of physicochemical tests that form USP <381>

Test	Test Result	Evaluation Criteria (Type one closures)	Result
Appearance of solution	0.121NTU	< 6NTU < less intense than matching fluid O	PASS
Acidity or alkalinity	0.05ml NaOH to produce blue colour	< 0.3ml NaOH to produce a blue colour	PASS
Heavy metals	Less intense colour than standard solution	Standard solution containing 2ppm Lead (Pb)	PASS
Reducing substances	<0.1 ml	< 3ml	PASS
Absorbance	0.05 Abs	< 0.2ppm	PASS
Extractable Zinc	0.003ppm	< 5ppm	PASS
Ammonium	Less intense colour than standard solution	Standard solution not more than 2ppm of Ammonium present	PASS
Volatile Sulfides	No black stain	No black stain	PASS

Table 4: List of physicochemical tests that form EP 3.1.9—Silicone elastomer for closures and tubing

Test	Test Results	Evaluation Criteria	Result
Appearance of solution	Clear, 0.68NTU	< 3NTU	PASS
Acidity	≤ 2.5ml 0.01M NaOH (blue colour)	2.5ml of 0.01M NaOH change to blue	PASS
Alkalinity	≤ 1.0ml 0.01M HCl (yellow changes to red)	1.0ml of 0.01M HCl change to yellow to red	PASS
Reducing substances	0.3ml	Diff. between sample and blank ≤ 1.0ml	PASS
Relative density	1.18	1.05 – 1.25	PASS
Volatile matter	0.20%	< 2% (Pt cured)	PASS
Mineral oils	No fluorescence	Less fluorescence than 1ppm standard	PASS
Substances soluble in hexanes	10.3mg	15mg (or 3%)	PASS
Phenylated compounds	Max absorbance < 0.01A.u.	Absorbance < 0.4A.u. (between 250 and 340nm)	PASS
Platinum	Less coloured than Pt reference (30ppm)	Less coloured than Pt reference (30ppm)	PASS

5m USP <788> Particulate testing/ microscopic particulate count analysis test

This test is used to determine a level of particulates measuring 10 micron (µm) or less and 25 micron (µm) that may be present in any given drug product. A length of Accusil tubing was filled with low particulate water and shaken 20 times. The extraction fluid was then recovered and the particles were measured using light obscuration.

Extracts from a Pumpsil tube contained 723 particles > 10µm and 11 particles > 25µm

to identify if there are any semi volatile compounds present in the extracts whilst volatile extractables can be detected using HS-GC/MS. Potential elemental impurities can be identified by ICP-MS.

The extracts were evaluated for the elemental impurities listed in the ICH Q3D and USP 232 guidelines.

Results: These studies have shown the extractables are indicative of the materials of construction. WMFTS can provide assistance in the evaluation of extractables data for risk assessment purposes.

6. Extractables

Sections of Accusil tubing were subjected to extraction in multiple solvents at controlled temperatures. The test material was extracted in a 6cm²:1mL surface area to volume ratio. The solvent extracts were then analysed using high pressure liquid chromatography-diode array detector-mass spectrometry (HPLC-DAD/MS), headspace gas chromatography mass spectrometry (HS-GC/MS) direct injection gas chromatography mass spectrometry (DI-GC/MS) and inductively coupled plasma-mass spectrometry (ICP/MS). HPLC-DAD/MS is used to detect the presence of non volatile and UV active extractables. DI-GC/MS can be used

7. Conclusions

Accusil has passed a number of pharmacopoeial and ISO testing, a summary of the results are disclosed within. For further information with full compliance statements and test reports, please fill in a request form available on the WMFTS website.

Compliance summary & full validation guide for Accusil are available on the wmfts.com website:

wmfts.com/accusil-validate

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