

Validation Guide

Pharbeta[®] PES-A Filter Cartridges

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Part I.Introduction

This report contains validation data applicable to Darlly Pharbata®PES-A (0.22μm) PES filter cartridges. The product are used for reducing bio-burden and turbidity in a widely range of filtration processes. It provide an excellent service time and high initial flow rate in liquid filtration of pharmaceutica.

Validation data contains: Bacterical Challenge、Extractables、Biological Safety Tests..ect. All of the Validation tests abide by ISO 9001 quality system standards.

This report is designed to assist the filter user in meeting the validation requirements of regulatory authorities within the pharmaceutical industry.



Part II. Studies on Removal Efficiency

Microbial Validation using *Brevundimonas diminuta* (ATCC 19146) Liquid Challenge Tests.

1. Introduction

The FDA guidelines on Sterile Products Produced by Aseptic Processing (1987) state, “A sterilizing filter is one which, when challenged with the micro-organism *Pseudomonas diminuta* (P. diminuta), at a minimum concentration of 10^7 organisms per cm^2 of filter surface, will produce a sterile effluent”.

In order to meet the requirements of this guideline, liquid challenge tests using *Brevundimonas diminuta* (ATCC 19146) were performed with Pharbeta® PES-A (0.22 μm) PES filter cartridges using a minimum of 1×10^7 colony forming units (CFU)/ cm^2 of effective filtration area.

The correlation between microbial retention and a non-destructive integrity test is also an important aspect of the validation of sterilizing grade filters. The FDA guideline further states, “After a filtration process is properly validated for a given product, process and filter, it is important to assure that identical filter replacements (membrane or cartridge) used in production runs will perform in the same manner. One way of achieving this is to correlate filter performance data with filter integrity testing data”. The integrity tests used during this validation study were the Forward Flow and Bubble Point tests.

The Forward Flow Integrity Test

In the Forward Flow test, a filter is wetted with a suitable test liquid and a pre-determined gas pressure is applied to the upstream side of the filter assembly. After a stabilization period, the gas flow through the wetted membrane can be measured manually on the downstream side or on the upstream side, using sensitive flow measurement equipment such as the integrity test devices.

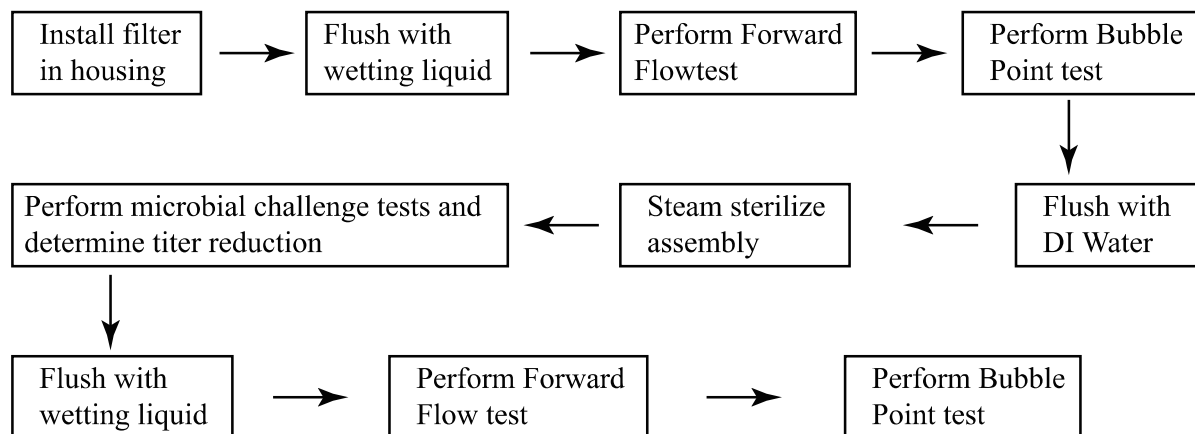
The Bubble Point Integrity Test

In the Bubble Point test, a filter is wetted with a suitable test liquid. After a stabilization period, increasing the gas pressure to the upstream side of the filter assembly, using sensitive flow measurement equipment such as the integrity test devices to test the change point of the gas flow rate.

The aims of this series of tests were to:

- ★ Determine the microbial removal efficiency of Pharbeta® PES-A (0.22 μm) PES filter cartridges in liquid challenge tests using *Brevundimonas diminuta* (ATCC 19146)
- ★ Correlate non-destructive Forward Flow and Bubble Point integrity tests with destructive challenge tests
- ★ Determine integrity test parameters

2. Summary of Methods



Pharbeta®PES-A (0.22µm) filter Cartridges (different S/N) with a range of Forward Flow and Bubble Point values were selected from manufacturing lots and subjected to microbial challenge tests using anaqueous suspension of *Brevundimonas diminuta* (ATCC 19146).

The filter sample was installed in a housing and tested for integrity by the Bubble Point and Forward Flow method, prior to being autoclaved at 121°C (250°F) for 30 minutes. The filter assembly was then aseptically connected to a pre-sterilized challenge apparatus.

An aqueous suspension of *B. diminuta* was passed through the filter to achieve a challenge level of $>1 \times 10^7$ CFU colony forming units (CFU) per cm² of effective filtration area. A total challenge per filter (10inch, 0.65m²) of $>1 \times 10^{11}$ CFU was achieved in all tests. On completion of the challenge, a second Bubble Point and Forward Flow test was performed.

During the challenge test, the entire filter effluent was passed through a 0.22 µm-rated analysis disc on the downstream side of the test filter assembly. The filter disc was incubated on TSA and, following incubation, the disc was examined to determine if bacteria had passed through the test filter during the challenge. The titer reduction (TR) for each filter was determined as follows:

$$TR = \frac{\text{Total number of organisms influent to the filter}}{\text{Number of colonies recorded on the downstream analysis disc}}$$

When no colonies were detected downstream, the titer reduction was expressed as:

$>$ Total number of organisms influent to the filter (e.g. $>1 \times 10^{11}$)

Please contact Darby if a more detailed description of the test methods is required.

3.Test Results

Forward Flow Correlation

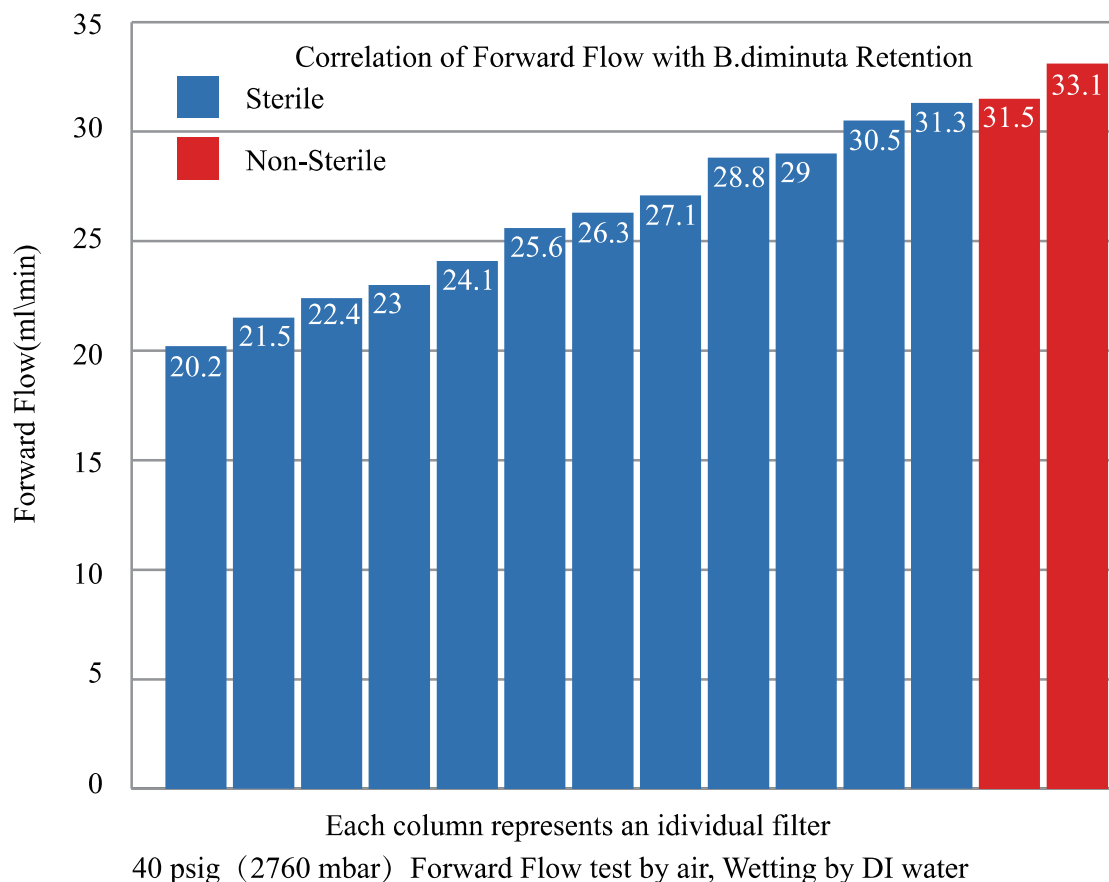
The Forward Flow and B. diminuta retention results are shown in Table (1),and presented graphically in Figure (1).

It was found that all of the 12 filterswith Forward Flow values ≤ 31.3 ml/min gave sterile effluent when challenged with $>1 \times 10^{11}$ CFU of B. diminuta per filter. Two filters with Forward Flow values 31.5ml/min, 33.1ml/min gave titer reductions between 5.9×10^{10} and 1.3×10^{10} .

Table (1) Correlation of Forward Flow with B. diminuta Retention for Pharbata®PES-A (0.22 μ m)Filters

Filter Serial Number	Forward Flow (ml/min)	Sterile Effluent	Titer Reduction
170105112	20.2	Yes	$> 1 \times 10^{11}$
170105199	21.5	Yes	$> 1 \times 10^{11}$
170104100	22.4	Yes	$> 1 \times 10^{11}$
170105322	23.0	Yes	$> 1 \times 10^{11}$
170104450	24.1	Yes	$> 1 \times 10^{11}$
170104371	25.6	Yes	$> 1 \times 10^{11}$
170104483	26.3	Yes	$> 1 \times 10^{11}$
170105391	27.1	Yes	$> 1 \times 10^{11}$
170104176	28.8	Yes	$> 1 \times 10^{11}$
170105286	29.0	Yes	$> 1 \times 10^{11}$
170105224	30.5	Yes	$> 1 \times 10^{11}$
170104157	31.3	Yes	$> 1 \times 10^{11}$
170104252	31.5	No	5.9×10^{10}
170105248	33.1	No	1.3×10^{10}

Figure (1) Correlation of Forward Flow with B.diminuta Retention for Pharbета®PES-A (0.22μm) Filters



Bubble Point Correlation

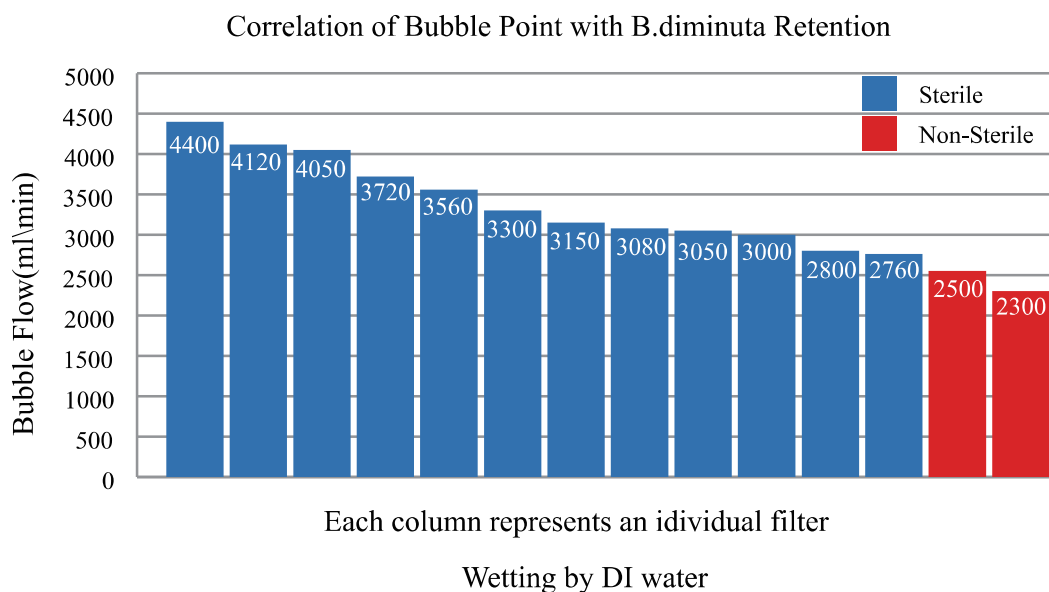
The Bubble Point and B. diminuta retention results are shown in Table (2), and presented graphically in Figure (2). It was found that all of the 12 filters with Bubble Point values ≥ 2760 mbar gave sterile effluent when challenged with $>1 \times 10^{11}$ CFU of B. diminuta per filter. Two filters with Bubble Point values 2550 mbar and 2300 mbar gave titer reductions between 5.6×10^{10} and 3.5×10^9 .

Table (2) Correlation of Bubble Point with B.diminuta Retention for Pharbета®PES-A (0.22μm) Filters

Filter Serial Number	Bubble Point (mbar)	Sterile Effluent	Titer Reduction
170307420	4400	Yes	$> 1 \times 10^{11}$
170306184	4120	Yes	$> 1 \times 10^{11}$
170303925	4050	Yes	$> 1 \times 10^{11}$

170308247	3720	Yes	$> 1 \times 10^{11}$
170302046	3560	Yes	$> 1 \times 10^{11}$
170308100	3300	Yes	$> 1 \times 10^{11}$
170301619	3150	Yes	$> 1 \times 10^{11}$
170303592	3080	Yes	$> 1 \times 10^{11}$
170302579	3050	Yes	$> 1 \times 10^{11}$
170305909	3000	Yes	$> 1 \times 10^{11}$
170305974	2800	Yes	$> 1 \times 10^{11}$
170305200	2760	Yes	$> 1 \times 10^{11}$
170304557	2550	No	5.6×10^{10}
170304766	2300	No	3.5×10^{10}

Figure (2) Correlation of Bubble Point with B.diminuta Retention for Pharbata®PES-A (0.22µm)Filters



4.Conclusions

Based on the results of the validation study, both the Forward Flow and Bubble Point test methods were demonstrated to be suitable non-destructive integrity tests for Pharbета®PES-A (0.22µm) filter cartridges. Integrity test parameters for Pharbета®PES-A (0.22µm) filters were set as follows:

Forward Flow Integrity Test Parameters for Pharbета®PES-A (0.22µm) PES Cartridge Filters (10inch, 0.65m²)

Wetting liquid	DI Water
Test gas	Air
Test pressure	2760mbar(40 psi)
Temperature	20°C±2°C
Maximum allowable Forward Flow limit	25mL/min

Bubble Point Integrity Test Parameters for Pharbета®PES-A (0.22µm) PES Cartridge Filters (10inch, 0.65m²)

Wetting liquid	DI Water
Test gas	Air
Temperature	20°C±2°C
Minimum allowable Bubble Point limit	3320 mbar(40 psi)

These Forward Flow and Bubble Point integrity test parameters:

- ★ Incorporate a safety margin.
- ★ Provide a high level of assurance of retention of *Brevundimonas diminuta*(ATCC 19146) when challenged with $> 1 \times 10^7$ CFU/cm² of effective filtration area.
- ★ Confirm that Pharbета®PES-A (0.22µm) filters satisfy the requirements of sterilizing grade filters as described in the FDA guidelines for aseptic processing (1987).

Please note: Integrity test values are continually reviewed and monitored during routine production tests. Test values are issued and controlled by Darlly.

Please contact Darlly for further details.

Part III. Validation of Physical Characteristics

1. Resistance to Steam Sterilization

1.1 Introduction

The aim of these tests was to determine the effects of steam in place sterilization on the integrity of Pharbata®PES-A (0.22µm) filtercartridges.

1.2 Summary of Methods

During the tests, typical productionfilters (10inch, 0.65m²) installed in a stainless steel housing were steamed in place using saturated condensate-free steam.

In each series of tests the following was performed:

- ★ Steam pressure and flow were held constant during the sterilization period.
- ★ Test filter cartridges were Forward Flow integrity tested at appropriate intervals.
- ★ After each steam in place cycle the filters were cooled by passing dry compressed air through the test filter.

1.3 TestResults

During this study, filters were steamed using high initial differential pressures (1000 mbar (14.5 psig)) at 121°C (249.8°F). The tests were performed in 30 minutes cycles in the forward (out to in) direction. These tests were performed in order to simulate steam conditions where transient high differential pressures (> 300 mbar (4.3 psig)) may occur during the steam sterilization cycle. At appropriate intervals, the filters were Forward Flow integrity tested and the results are shown in Table (3).

All filters passed the Forward Flow integrity test (2760mbar, DI water) after exposure to 30 minutes steam cycles 30 times.

Table (3) Effect of High initial Differential Pressures on Forward Flow Values

Filter Serial Number	Forward Flow(ml/min) after the following Number of 30 minutes Steam Cycles							
	0	1	2	3	5	7	9	10
170211051	20.1	21.1	20.7	19.9	18.0	19.5	19.9	22.2
170210102	19.8	22.9	16.2	19.8	21.2	22.4	24.3	24.7
170210099	17.8	24.4	19.5	18.3	17.9	21.9	22.1	20.4
170210124	22.3	19.5	18.5	19.8	21.0	20.1	21.1	22.5

Wetting by DI water before steam sterilizing, Limit value: 25mL/min

1.4 Conclusions

The data presented in this section support the following conclusions:

Pharbeta®PES-A (0.22µm) PES filtercartridges are robust and capable of withstanding multiple steam sterilization cycles, where the differential pressure may exceed 300 mbar (4.3 psig) in the forward direction, as demonstrated by exposing filters to high transient differential pressures of up to 1000 mbar (14.5 psig) during steaming at 121°C (249.8°F).

2. Water Flow/Differential Pressure Measurements

2.1 Introduction

The aim of these tests was to determine the water flow rates at set differential pressures across Pharbeta®PES-A (0.22µm) filter cartridges.

2.2 Summary of Methods

The tests were performed on standard production filters (10inch, 0.65m²). Test filters were installed in a stainless steel housing and flushed with DI water to pre-wet the filter membranes. Pre-filtered deionized water was then pumped through the assembly in the normal flow(out to in) direction. Pressure transducers on the upstream and downstream side of the test filter housing were monitored to calculate the differential pressure at different water flow rates.

Further measurements were taken with the housing only (no filter installed). The housing-only results were subtracted from the filter assembly results in order to provide flow/pressure characteristics for the filter only. All data were corrected to a standard temperature of 25°C (77°F).

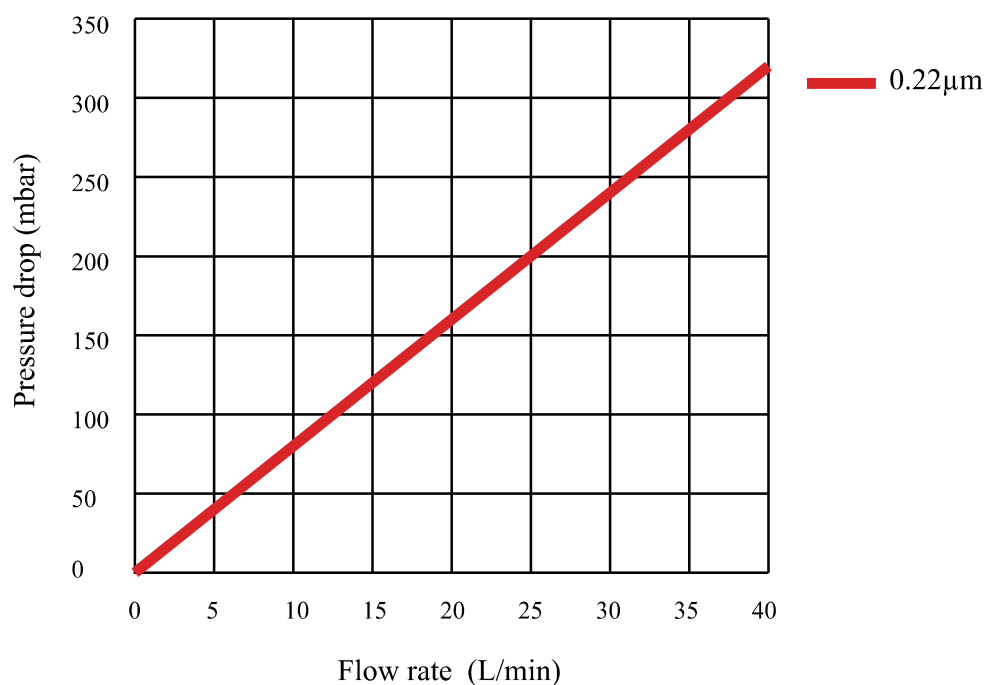
Please contact Darlly if further details about the test methods are required.

2.3 Test Results

The water flow measurements through typical filters(10inch, 0.65m²) at differential pressure are shown in figure (3), The water flow rates at 300 mbar (4.3psig) differential pressure for Pharbeta®PES-A (0.22µm) filter cartridges were found to range between 35 ~ 40L/min, These data can be used to form the basis of sizing filter systems using Pharbeta®PES-A (0.22µm)PES filter cartridges.

Note: The differential pressures quoted are for liquids with a viscosity of 1cP. Differential pressures for liquids at other viscosities can be estimated by multiplying the differential pressure by the viscosity in cP. To obtain the total pressure drop of a complete filter assembly the housing pressure drop must also be added. Please contact Darlly for further details

Figure (3) FlowRate/Pressure Drop



3. Resistance to Differential Pressure

3.1 Introduction

The aim of these tests was to determine the effects of Differential Pressure on the integrity of Pharbata®PES-A (0.22μm) filter cartridges.

3.2 Summary of Methods

The tests were performed on standard production filters (10inch, 0.65m²). Test filters were installed in a stainless steel housing and flushed with DI water at differential temperature/Pressure for 2 hours, Test the integrity of the filters.

3.3 Test Results

The Results of integrity (Forward Flow, 2760mbar, wetting by DI water) at differential Temperature/Pressure are shown in Table (4) .

Table (4) Pressure/Temperature

Temp FF (ml/min) ΔP (psig)	0 mbar	1000 mbar	2000 mbar	3000 mbar	4000 mbar	5000 mbar
Forward20°C	18.3	19.4	20.1	20.6	21.4	21.0
	17.9	18.5	19.9	20.2	22.1	23.4
Forward80°C	23.7	24.5	24.9	N/A	N/A	N/A
	24.1	24.9	24.8	N/A	N/A	N/A

Safety Limit value: 25ml/min

The data presented in this section support the following conclusions:

Maximum Forward ΔP at 20 °C $\leq 5000\text{mbar}$ (72.5 psig)

Maximum Forward ΔP at 80 °C $\leq 2000\text{mbar}$ (29 psig)

Please contact Darllly for further details.

Part IV. Extractables Testing

1.Introduction

The aim of this series of tests was to quantify the material, which can be extracted from Pharbata®PES-A (0.22μm) filter cartridges using Ultrapure water and ethanol.

2.Summary of Methods

Preparation of Filter Samples

Extractables tests were performed on typical production filter cartridges (10inch, 0.65m²), which had been autoclaved in order to maximize the quantity of any extractable material present. The filters were wrapped in aluminium foil and autoclaved for half hour at 121°C (250°F), using a slow exhaust cycle. Visible droplets of water remaining on the filter elements were allowed to evaporate at room temperature before the extraction was performed.

Extraction Procedure

Dynamic extraction tests were performed. The test filters were immersed in 1800 ml of extraction fluid in a clean measuring cylinder, Use Ultrapure water flush the filters for 8 hours.

Analysis of Material Extracted

After the extraction, 1500mL of the extraction liquid was evaporated to dryness and the non-volatile extractables were determined gravimetrically.

The material extracted by ethanol were analyzed by Fourier Transform Infra Red spectroscopy.

Please contact Darilly if further details of the test methods are required.

3.Test Results

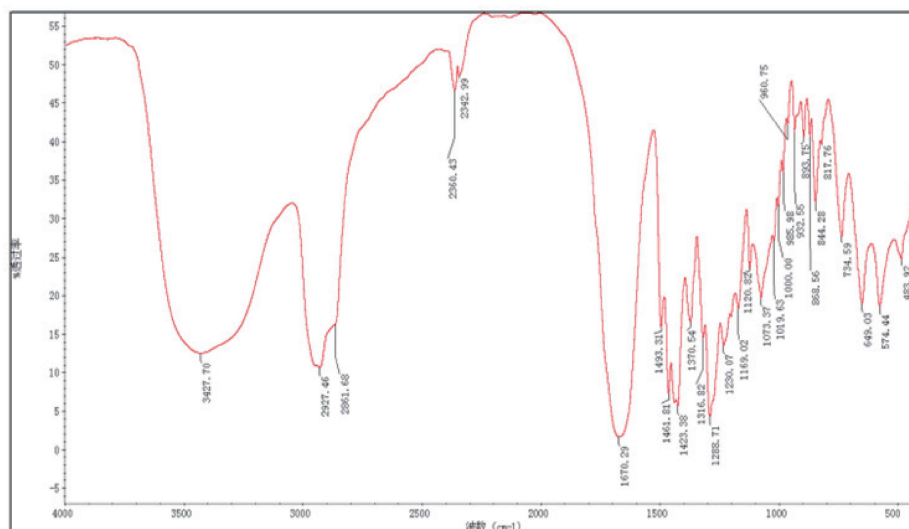
Table (5) shows the levels of extractables obtained using typical production (10inch, 0.65m2)Pharbeta®PES-A (0.22μm) filter cartridges.

Infra red spectra of ethanol extracts from Pharbeta®PES-A (0.22μm) filter cartridges (see Figure (4)) showed close similarities with those of polypropylene.

Table (5) Non-volatile Extractables using Typical (10") Pharbeta®PES-A (0.22μm) PESfilter cartridges

Extraction Fluid	Filter Serial Number	Residue
Ultrapure Water	170509294	13.4mg
	170509233	11.9mg
	170509248	12.9mg
Ethanol	170601254	34.5mg
	170601208	38.1mg
	170601285	36.7mg

Figure (4) Infra Red Spectrum of Ethanol Extracts from Pharbeta®PES-A (0.22μm)Filter cartridges



4. Conclusions

The levels of extractables determined for Pharbета®PES-A (0.22µm) filter cartridges were dependent on the solvent used. For most solvents tested, the gravimetric extractables were found to be extremely low. The results reported are typical for production elements.

Actual service will impose different conditions, such as different exposure times, temperature, liquid purity etc. Evaluation under process conditions is therefore also recommended.

Part V. Biological Safety Testing

1. Introduction

The purpose of these tests was to evaluate the biological suitability of the materials of construction of the Pharbета®PES-A (0.22µm) filter cartridges. The materials of construction of Pharbета®PES-A (0.22µm) PES filter cartridges are as follows:

Filter membrane medium:	PES membrane
Support/Drainage layers:	Polypropylene
Endcap/Adapter:	Polypropylene
Core/Cage:	Polypropylene/Polysulfone

2. Summary of Methods

The tests were performed in accordance with the Biological Reactivity Tests in vivo for Class VI Plastics (121°C) as described in the current United States Pharmacopeia. The tests were conducted EPIN L laboratories.

The testing procedures described in the USP include:

- ★ Injection of extracts of plastic materials
- ★ Implantation of the solid material into animal tissue

The four extracting media listed in the USP simulate parenteral solutions and body fluids. These include:

- ★ Sodium Chloride Injection
- ★ 1 in 20 Solution of Alcohol in Sodium Chloride Injection
- ★ Polyethylene Glycol 400
- ★ Vegetable Oil (sesame or cottonseed oil)

The USP states that extracts may be prepared at one of three standard conditions: 50°C (122°F) for 72 hours, 70°C (158°F) for 24 hours, or 121°C (250°F) for 1 hour. The most stringent condition not resulting in physical changes in the plastic is recommended, therefore the filters were extracted at 121°C (250°F).

Acute Systemic Injection Tests

An Acute Systemic Injection Test was performed to evaluate the potential of a single injection of an extract to produce systemic toxicity. Sodium Chloride Injection and 1 in 20 Solution of Alcohol in Sodium Chloride Injection were injected intravenously. Vegetable oil extract and Poly-ethylene Glycol 400 extract were injected intraperitoneally.

Intracutaneous Tests

An Intracutaneous Test was performed to evaluate the potential of a single injection of an extract to produce tissue irritation. All four of the extracts listed above were used for these tests.

Implantation Tests

Implantation tests were also performed, in order to subject the materials of construction to the most stringent conditions included in the USP. Each of the components of the Pharbeta®PES-A (0.22µm) filter cartridges was implanted separately.

3. Conclusions

Pharbeta®PES-A (0.22µm) filter cartridges meet the requirements of the USP for Class VI (121°C) Plastics. Figure (5) show a copy of the test certificate. Please contact Darly if copies of the test reports are required.

Figure (5) USP VI (121°C) Biological Safety Testing Report



Epin Suzhou Ltd.

TEST RESULT CERTIFICATION

Sponsor:	Hangzhou Darlly Filtration Equipment Co., Ltd.	Protocol effective date:	2018-03-19
		Technical completion date:	2018-04-13
		Report signature date:	2018-04-27
Address:	Xindeng Industrial Park, Fuyang, Zhejiang Province, 311404, China	Study number:	MED201801099-11-EN
			MED201801099-17-EN
			MED201801099-27-EN

Test Article:	PES Filter Cartridge	Ratio:	0.2g:1mL
Lot#:	P8B01001	Vehicles:	0.9% sodium chloride for injection (SC)
Model/Size:	Not supplied by sponsor		Corn oil (CO)
Physical State:	Solid		1:20 Alcohol in SC (AL)
Color:	White		Polyethylene glycol 400 (PEG-400)
Study:	Biological Test for Plastics, Class VI	Temp/Time:	121°C for 1h

1. REFERENCE:

USP 40, NF35, 2017, <88> BIOLOGICAL REACTIVITY TESTS, IN VIVO

2. GENERAL PROCEDURE:

The test article extracts and corresponding blank were injected systemically and intracutaneously in mice and rabbits, respectively. The injections were in the amounts and routes set forth by USP 40; including the further dilution of the extracts prepared with PEG-400. The animals were observed for signs of toxicity and skin reactivity for up to 72 hours post treatment. In addition, the test article components were implanted into the paravertebral musculature of rabbits for 2 weeks and observed for signs of hemorrhage, inflammation, necrosis, discoloration, and encapsulation, or infection.

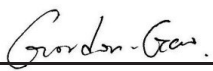
3. RESULTS:

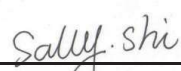
None of the mice injected with the test article extracts exhibited any signs of toxicity in the Systemic Injection Test. In addition, none of the rabbits injected intracutaneously with the test article extracts exhibited any signs of erythema, edema or clinical toxicity. In both the Systemic and intracutaneous Tests the controls were normal through 72 hours. No macroscopic reaction of the test article, implanted in the rabbit muscle for 2 weeks, was observed

4. CONCLUSION:

The test article meets the requirements of USP 40, NF 35, 2017, for the Biological Test for Plastics, Class VI-121°C

5. AUTHORIZED PERSONNEL:


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Study Director


Sally Shi
Quality Assurance



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