Life Sciences

Validation Guide

USTR 2404

Pall® SUPRAdisc HP Depth Filter Media

Filtration. Separation. Solution.SM

1. Introduction

Pall SUPRAdisc HP modules consist of two, graded depth filter media combined in one unit. This technology enhances process clarification steps such as whole cell and cell lysate separations and other fermentation applications. Additionally, other difficult to filter process fluids such as supernatants and complex nutrient media can benefit from Pall's **SUPRAdisc** HP technology.

The purpose of this report is to summarize the tests that were performed to qualify and to quantify the extractables of these media under certain conditions.

The data contained in this document represent typical values, the accuracy and reproducibility of which are controlled on a regular basis and confirmed by results of field applications.

This test report has been compiled for the users of **Pall SUPRAdisc** HP depth filters as a basis and support for their own validation procedures.

The test program included:

- Extractables testing
  - Conductivity
  - pH
  - TOC (Total Organic Carbon)
  - Extractable Cations
  - Total Extractables according to 21 CFR 177.2260
- Endotoxins
- ß-glucans
- Biological reactivity tests

1.1 Summary of Conclusions

1.1.2 Extractables

Conductivity and pH Value in Water For Injection (WFI)

After a rinsing volume of 100 L/m^2 the pH value of WFI was between 5 and 7, meeting the requirements of the current USP.

The conductivity of the effluent was < 20 µS/cm at this volume and can be lowered further by additional flushing.

TOC (Total Organic Carbon) in WFI

Pall SUPRAdisc HP depth filter media meet the USP requirements of ≤ 500 ppb (0.5 ppm) for TOC in WFI after a rinsing volume of ≥ 100 L/ m^2.

Extractable Cations (Al, Ca, Mg, Fe, Ni, Cu, Cr, As, Pb) in WFI and 40% Ethanol

Typically the following values for extractable cations were reached after a rinsing volume of 100 L/m².

	in WFI	in 40% Ethanol
Al	< 0.05 ppm	< 0.02 ppm
Ca	< 0.5 ppm	< 1 ppm
Mg	< 0.1 ppm	< 0.2 ppm
Fe	< 0.1 ppm	< 0.2 ppm
Ni	< 0.01 ppm	< 0.01ppm
Cu	< 0.1 ppm	< 0.1 ppm
Cr	< 0.01 ppm	< 0.01 ppm
As	< 0.01 ppm	< 0.01 ppm
Pb	< 0.01 ppm	< 0.01 ppm

Total Extractables according to 21 CFR 177.2260

The total extractables of all **Pall SUPRAdisc** HP depth filter media were significantly below the CFR limits and therefore met the requirements of 21 CFR 177.2260.

A detailed report is available by contacting Pall Corporation.

1.1.3 Endotoxins and ß-Glucans

Endotoxins

The endotoxin content of all tested **Pall SUPRAdisc** HP depth filter media in human albumin without pre-rinsing was below the detection limit of 0.06 EU/mL.

After a rinsing volume of 100 L/m² of WFI, the endotoxin content of the effluent of all tested **Pall SUPRAdisc** HP depth filter media was below 0.02 EU/mL. Recovery rates indicated that there was no LAL-reactive material in the effluents.

After the recommended rinsing procedure with WFI, ß-glucan levels of the effluents of all tested **Pall SUPRAdisc** HP depth filter media were below 100 pg/mL.

1.1.4 Biological Reactivity Tests

As referenced in Pall publication USTR 2366, all tested materials which are the components of **SUPRAdisc** HP media, met current USP requirements for:

- Cytotoxicity *(in vitro)*
- Class VI (121 °C) Plastics *(in vivo)*
- Hemolysis *(in vitro)*

Copies of certificates and test reports are available upon request.

2. General Characteristics of Pall SUPRAdisc HP Depth Filter Media

This test report describes the following depth filter media grades, listed with increasing permeability:

- PDD1
- PDE2
- PDH4
- PDK5

All of these depth filter media combinations are manufactured under special production conditions that guarantee the highest purity possible. These conditions include such things as:

1. Specific cleaning and disinfection of the manufacturing line
2. The use of RO-water for final rinsing.

Through the use of special production methods, **Pall SUPRAdisc** HP depth filter media can be distinguished by very low release of extractables and extremely low endotoxin levels. Furthermore, the grades PDD1 and PDE2 have been optimized for minimal ß-glucan content.

Pall SUPRAdisc HP depth filter media are therefore particularly suitable for the critical applications in biotechnology and pharmaceutical production.

3. Extractables

3.1 Method

In the filtration of biotech and pharmaceutical products, it is essential that product composition is not changed by filtration. An appropriate rinsing procedure after sterilization was therefore used to remove any extractable substances. The most common rinsing medium is Water for Injection (WFI).

Many products filtered by **Pall SUPRAdisc** HP depth filter media contain alcohol in their formulation. Therefore, in addition to WFI, 40 % ethanol was chosen as a second extraction medium.

For the extraction curves discussed in the following chapter, a filter with an effective filter area of 670 cm^2 (0.72 ft^2) was used. The flow rate was adjusted to 500 $L/m^2/h$.

Samples for the determination of

- Conductivity
- pH
- TOC (Total Organic Carbon)
- Cations (Al, Ca, Mg, Fe, Ni, Cu, Cr, As, Pb)

were taken at a rinsing volume of:

- 5 – 10 L/m^2
- After 50 L/m^2.
- After 100 L/m^2.

3.2 Conductivity and pH Values

3.2.1 Method

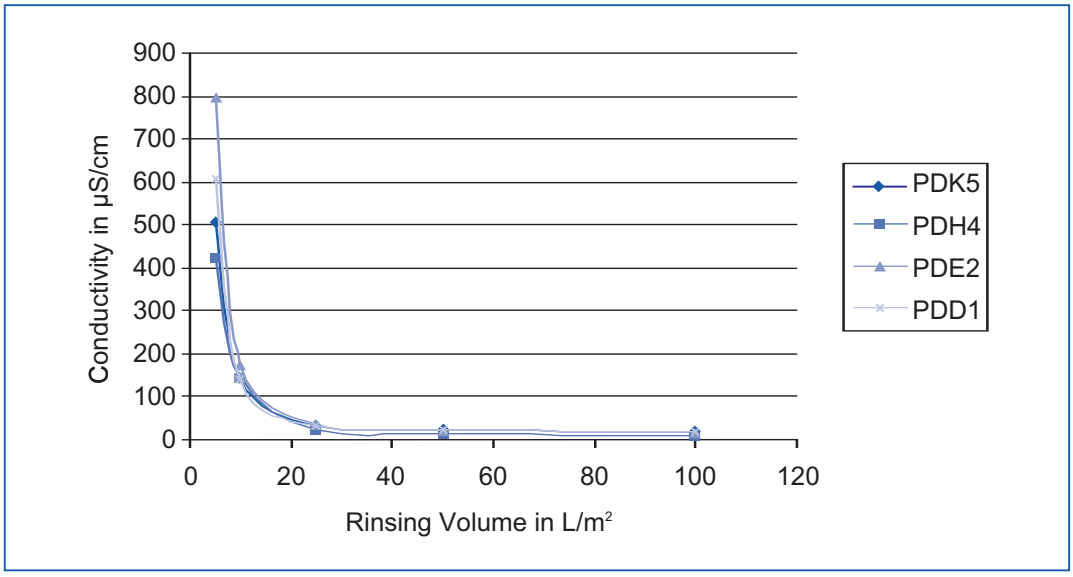
Conductivity and pH-values were measured by calibrated conductivity and pH-meter.

3.2.2 Results

Table 1: Conductivity and pH-values

Type	Conductivity in µS/cm			pH-Value		
	Rinsing volume L/m²			Rinsing volume L/m²		
	5-10	50	100	5-10	50	100
PDK5	144	21	17	5.5	6.0	6.0
PDH4	143	13	9	4.5	4.5	5.0
PDE2	177	25	18	5.0	5.0	5.0
PDD1	140	23	16	5.0	5.0	5.5

Figure 1 Conductivity in µS/cm at different Rinsing Volumes



3.2.3 Conclusion

After a rinsing volume of 100 L/m² the pH value of WFI was between 5 and 7, meeting the requirements of the current USP.

The conductivity of the extract was < 20 µS/cm at this volume and can be lowered further by additional flushing.

3.3 TOC (Total Organic Carbon) in WFI

3.3.1 Method

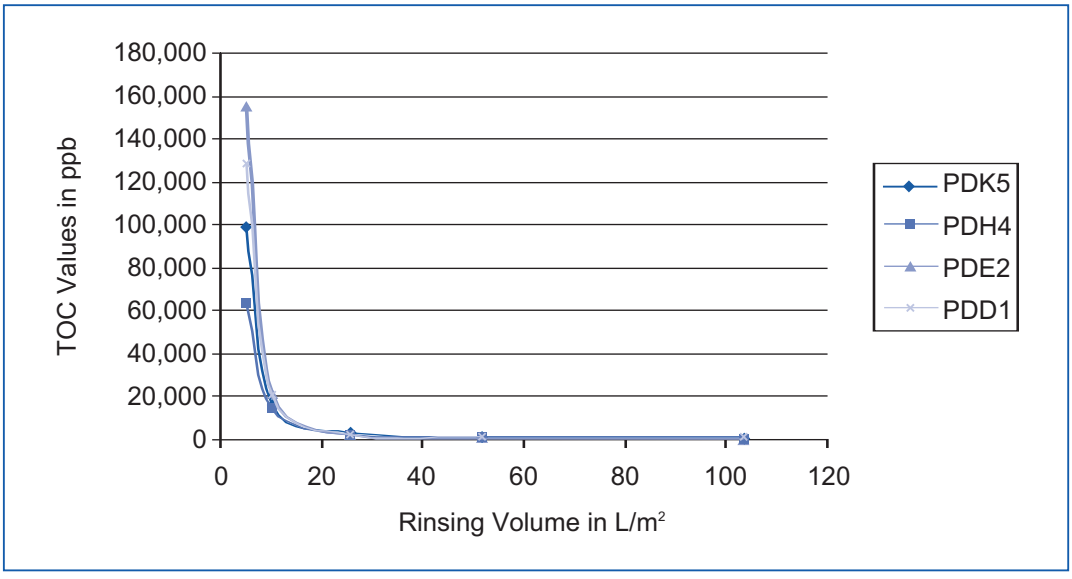
TOC (Total Organic Carbon) in WFI is the parameter characterizing organic extractables from the filters.

Measurements were performed using a TOC analyzer (Shimadzu Ltd., type 5000 A).

Table 2: TOC in WFI

Type	TOC ppm		
	Rinsing volume L/m²		
	5-10	50	100
PDK5	16	0.8	< 0.5
PDH4	14	0.9	< 0.5
PDE2	22	0.7	< 0.5
PDD1	21	0.8	0.5

Figure 2 TOC Values in ppb after Rinsing with WFI



3.3.2 Conclusion

Pall SUPRAdisc HP depth Filter media meet the USP requirements of ≤ 500 ppb (0.5 ppm) for TOC in WFI after a rinsing volume of 100 L/m².

3.4 Extractable Cations (Al, Ca, Mg, Fe, Ni, Cu, Cr, As, Pb) in WFI and 40 % Ethanol

3.4.1 Method

Cations (Al, Ca, Mg, Fe, Ni, Cu, Cr, As, Pb) extracted in ethanol and WFI were determined by AAS (atomic adsorption spectroscopy) by flame or graphite tube technique.

3.4.2 Results

Table 3: Al (ppb)

Type	in WFI			in Ethanol		
	Rinsing volume L/m²			Rinsing volume L/m²		
	5-10	50	100	5-10	50	100
PDK5	118	34	16	28	8	6
PDH4	118	18	13	29	8	<5
PDE2	155	38	30	25	<5	<5
PDD1	106	34	23	20	10	5

Figure 3 Al-Values in ppb after Rinsing with WFI

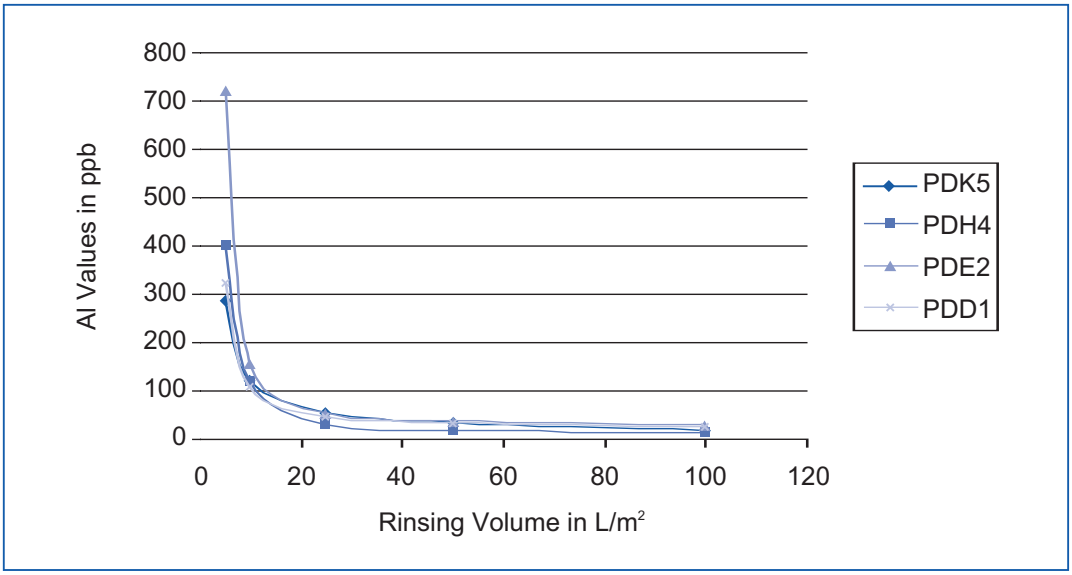


Figure 4 Al-Values in ppb after Rinsing with EtOH

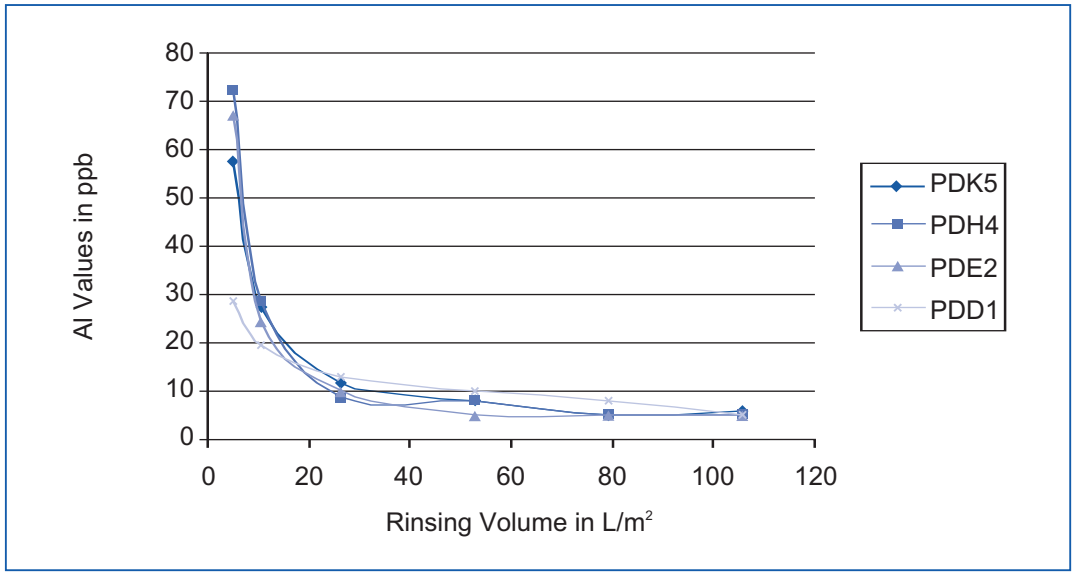


Table 4: Ca (ppm)

Type	in WFI			in Ethanol		
	Rinsing volume L/m²			Rinsing volume L/m²		
	5-10	50	100	5-10	50	100
PDK5	9	0.50	0.21	8	0.76	0.26
PDH4	10	0.28	0.16	6	0.60	0.24
PDE2	14	0.53	0.24	2	0.82	0.40
PDD1	10	0.60	0.24	4	2	0.80

Figure 5 Ca-Values in ppm after Rinsing with WFI

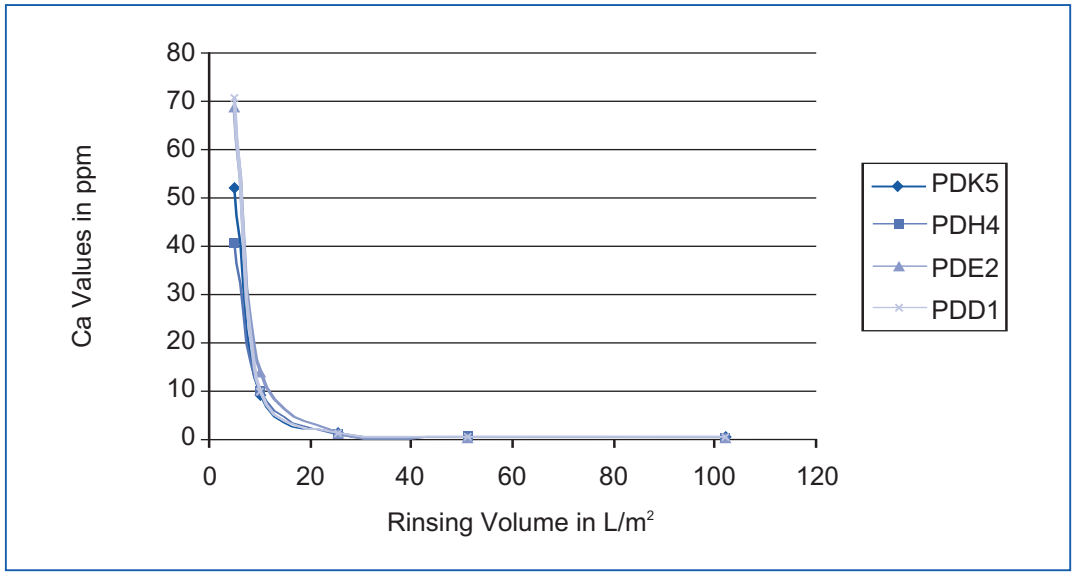


Figure 6 Ca-Values in ppm after Rinsing with EtOH

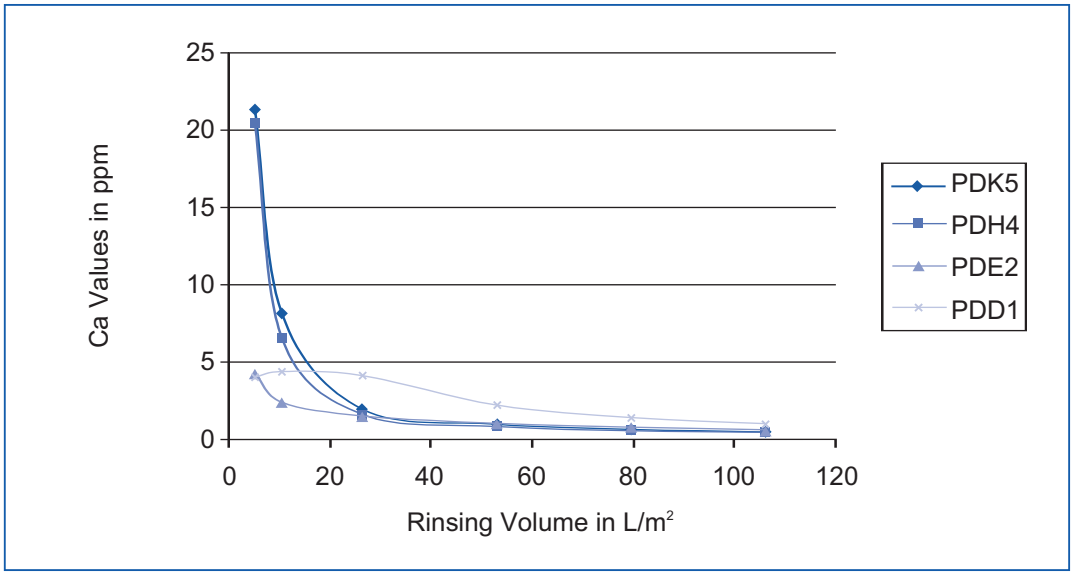


Table 5: Mg (ppm)

Type	in WFI			in Ethanol		
	Rinsing volume L/m²			Rinsing volume L/m²		
	5-10	50	100	5-10	50	100
PDK5	2	0.06	< 0.05	2	< 0.05	< 0.05
PDH4	2	0.06	< 0.05	2	0.09	0.08
PDE2	3	< 0.05	< 0.05	3	0.15	0.07
PDD1	2	< 0.05	< 0.05	3	0.14	0.06

Figure 7 Mg-Values in ppm after Rinsing with WFI

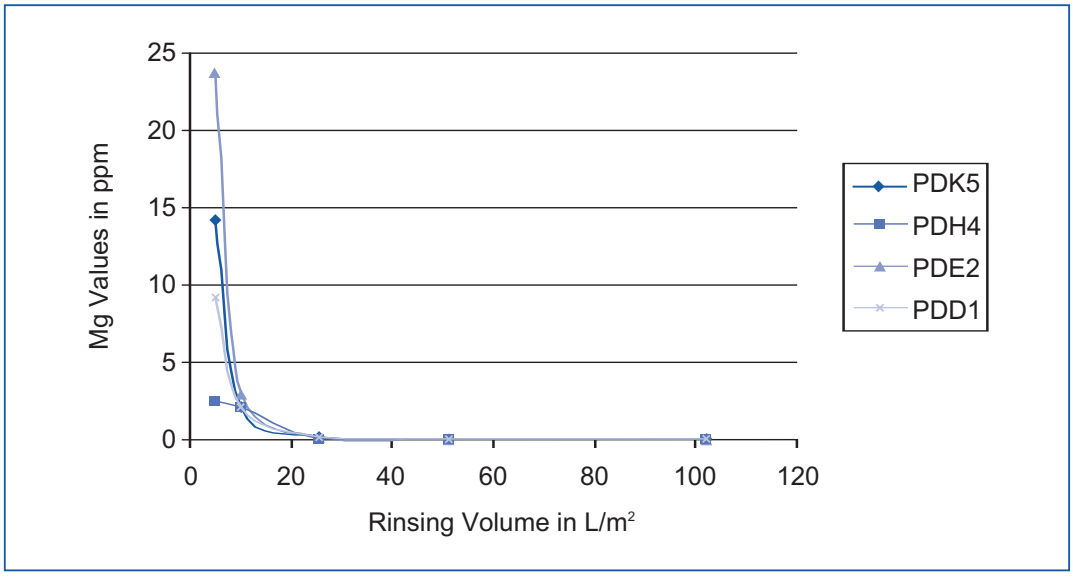


Figure 8 Mg-Values in ppm after Rinsing with EtOH

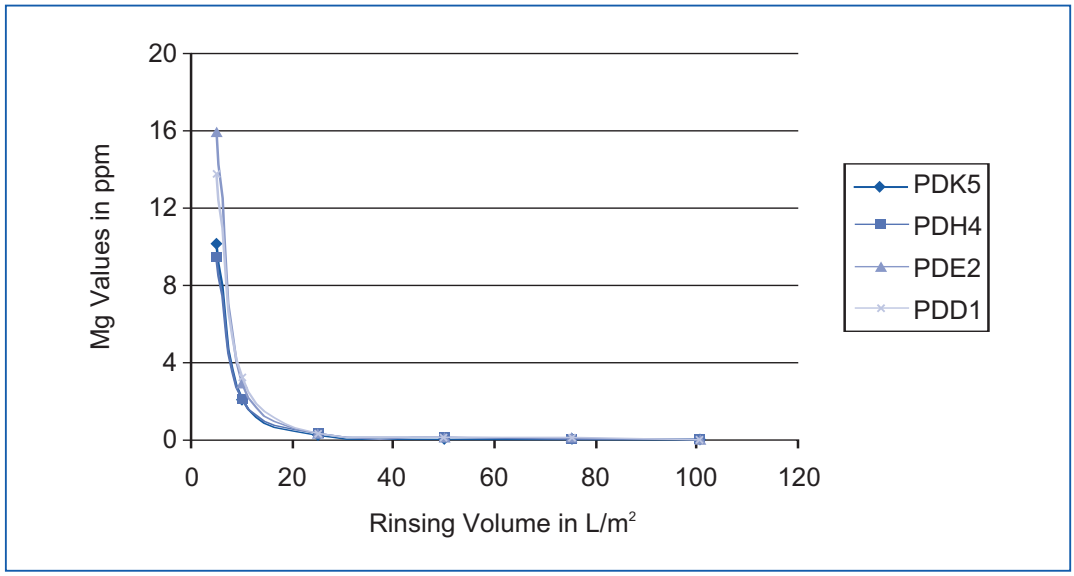


Table 6: Fe (ppm)

Type	in WFI			in Ethanol		
	Rinsing volume L/m²			Rinsing volume L/m²		
	5-10	50	100	5-10	50	100
PDK5	0.14	< 0.05	< 0.05	0.21	0.16	0.12
PDH4	< 0.05	< 0.05	< 0.05	0.17	0.15	0.09
PDE2	0.11	< 0.05	< 0.05	0.14	0.06	< 0.05
PDD1	0.10	< 0.05	< 0.05	0.13	0.07	0.07

Table 7: Ni (ppb)

Type	in WFI			in Ethanol		
	Rinsing volume L/m²			Rinsing volume L/m²		
	5-10	50	100	5-10	50	100
PDK5	< 5	< 5	< 5	< 5	< 5	< 5
PDH4	< 5	< 5	< 5	< 5	< 5	< 5
PDE2	< 5	< 5	< 5	< 5	< 5	< 5
PDD1	5	< 5	< 5	< 5	< 5	< 5

Table 8: Cu (ppm)

Type	in WFI			in Ethanol		
	Rinsing volume L/m²			Rinsing volume L/m²		
	5-10	50	100	5-10	50	100
PDK5	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
PDH4	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
PDE2	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
PDD1	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05

Table 9: Cr (ppb)

Type	in WFI			in Ethanol		
	Rinsing volume L/m²			Rinsing volume L/m²		
	5-10	50	100	5-10	50	100
PDK5	< 5	< 5	< 5	< 5	< 5	< 5
PDH4	< 5	< 5	< 5	< 5	< 5	< 5
PDE2	< 5	< 5	< 5	< 5	< 5	< 5
PDD1	< 5	< 5	< 5	< 5	< 5	< 5

Table 10: Pb (ppb)

Type	in WFI			in Ethanol		
	Rinsing volume L/m²			Rinsing volume L/m²		
	5-10	50	100	5-10	50	100
PDK5	< 5	< 5	< 5	< 5	< 5	< 5
PDH4	< 5	< 5	< 5	< 5	< 5	< 5
PDE2	< 5	< 5	< 5	< 5	< 5	< 5
PDD1	< 5	< 5	< 5	< 5	< 5	< 5

Table 11: As (ppb)

Type	in WFI			in Ethanol		
	Rinsing volume L/m²			Rinsing volume L/m²		
	5-10	50	100	5-10	50	100
PDK5	< 5	< 5	< 5	< 5	< 5	< 5
PDH4	< 5	< 5	< 5	< 5	< 5	< 5
PDE2	< 5	< 5	< 5	< 5	< 5	< 5
PDD1	< 5	< 5	< 5	< 5	< 5	< 5

3.4.3 Conclusion

Typically the following values for extractable cations were reached after a rinsing volume of 100 L/m².

	in WFI	in 40% Ethanol
Al	< 0.05 ppm	< 0.02 ppm
Ca	< 0.5 ppm	< 1 ppm
Mg	< 0.1 ppm	< 0.2 ppm
Fe	< 0.1 ppm	< 0.2 ppm
Ni	< 0.01 ppm	< 0.01ppm
Cu	< 0.1 ppm	< 0.1 ppm
Cr	< 0.01 ppm	< 0.01 ppm
As	< 0.01 ppm	< 0.01 ppm
Pb	< 0.01 ppm	< 0.01 ppm

3.5 Total Extractables according to 21 CFR 177.2260

3.5.1 Method

Title 21 "Food and Drugs"(2) chapter 177 the Code of Federal Regulations of the FDA (Food and Drug Administration) is concerned with indirect food additives: polymers.

Subpart C: Substances for use only as components of articles intended for repeated use.

The regulatory document 177.2260 refers to resin-bonded filters and states the limits for extractables in different extraction media and under different extraction conditions. The following limitations are stated here.

Extraction Solvent	Extraction Conditions	CFR Limits
deionized water	100 °C (212 °F)	≤ 4 % by weight of the filter
50 % ethanol	room temperature	≤ 4 % by weight of the filter
5 % acetic acid	room temperature	≤ 4 % by weight of the filter
n-hexane	reflux	≤ 4 % by weight of the filter

Although these regulations are not primarily intended for pharmaceutical products, they provide additional supportive data for the suitability of filters for pharmaceutical applications.

3.5.2 Results and Conclusion

The total extractables of all **Pall SUPRAdisc** HP depth filter media was significantly below the CFR limits and therefore meet the requirements of 21 CFR 177.2260.

A detailed report and further validation information are available by contacting Pall Corporation.

4. Endotoxins and ß- Glucans

4.1 Endotoxins

The potential risk of contamination by endotoxins in filter manufacturing is caused mainly by water. Therefore the verification of low endotoxin levels of the filter extracts is an important issue during validation.

4.1.1 Methods

4.1.1.1 Water for Injection, Kinetic Chromogenic LAL Test

A typical rinsing procedure of the filter after sterilization was performed with Water for Injection. The flow rate was 500 L/m²/h, the rinsing volume was 100 L/m² filter area. After this rinsing volume, a sample is taken and tested in a kinetic chromogenic LAL test for endotoxins and LAL-reactive material filtrate.

4.1.1.2 Human Albumin, LAL Gel Clot Test

Double layers of each media type were rinsed with a 5 % human albumin in physiological saline. The filtrate is checked for endotoxins content in the LAL gel clot test.

4.1.2 Results

4.1.2.1 In Water for Injection

All versions of **Pall SUPRAdisc** HP depth filter media are optimized regarding endotoxins, PDD1 was taken as representative example.

Pall SUPRAdisc HP Depth Filter Media Grade	Endotoxin Content in EU/mL
PDD1	< 0.02

After a rinsing volume of 100 L/m² of WFI, the endotoxin content of the effluent of the tested sheets was below 0.02 EU/mL in the LAL chromogenic test. Recovery rates indicate that there was no LAL-reactive material in the effluents.

4.1.2.2 In Human Albumin

For all media types contained in **Pall SUPRAdisc** HP depth filters the endotoxins content of the filtrate is < 0.06 EU/mL corresponding to a content < 0.4 EU/g filter mass.

4.2 ß-Glucans

ß-glucans occur in nature in molds, bacteria, algae, yeast and wood cellulose. In the production process of filter sheets, cellulose as a main raw material can contain ß-glucans (1.3-ß-D-glucan) as impurity. ß-glucans act as immunostimulatory agents and also give positive reactions in LAL tests but they show no fever reaction, unlike endotoxins. Cellulosic filters are a potential source for ß-glucans and thus need to be characterized for this type of extractable substances. Over the years, sensitive and specific detection methods mainly based on the LAL-test have been developed and qualified.

4.2.1 Method

A typical rinsing procedure of the filter after sterilization was performed with water for injection. At a flow rate of 500 L/m²/h, the recommended rinsing volume is 100 L/m² filter area. After this rinsing volume, a sample was taken of the WFI and checked in a ß-glucan specific LAL test.

4.2.2 Results

The versions PDE2 and PDD1 are optimized regarding ß-glucans. PDD1 was taken as representative example, the ß-glucan content was < 100 pg/mL.

Pall SUPRAdisc HP Depth Filter Media Grade	ß-Glucan content in pg/mL
PDD1	< 100 pg/mL

4.2.3 Conclusion

After the recommended rinsing procedure, ß-glucan levels of the effluents of the tested filter sheets were below 100 pg/mL.

5. Biological Reactivity Tests of Pall SUPRAdisc HP Depth Filter Media

5.1 Method

According to USP, the biocompatibility of a material can be checked in biological reactivity tests either *in vitro* or *in vivo.*

The cytotoxicity test is an in vitro reactivity test and designed to determine the biological reactivity of mammalian cell cultures following contact with specific extracts prepared from the materials under test.

The test for Class VI-121 °C plastics is a combination of *in vivo* tests that are designed to determine the biological response of animals to specific extracts prepared from the material under test. The USP defines six plastic classes based on the response to these tests for which extracts, materials and routes of administration are specified. In testing of filter sheets, the following tests were performed

- An acute systemic injection test
- An intracutaneous test
- An implantation test.

As extraction media for systemic and intracutaneous injection, the following media were used:

- Saline
- Saline in alcohol
- Polyethylene glycol 400
- Sesame oil.

The extraction is performed at 121 °C (250 °F) for 1 hour.

The hemolysis test is an *in vitro* test where the extracts of a test material are evaluated to determine whether the presence of any leachable chemical would cause *in vitro* red blood cell hemolysis.

5.2 Results

All **Pall SUPRAdisc** HP depth filters are a defined combination of specific **Pall** P-grade depth filter sheets. Detailed information about these P-grade sheets are available in Pall publication 'USTR 2366 Pall-P-grade Depth Filter Media Validation Guide'.

Extract of the results of the biological reactivity tests:

The following grades of P-series depth filter media were chosen as representatives because of their composition.

- EKS P
- EK M P representing also KS 50 P
- SUPRA EK 1P
- K 100 P
- SUPRA 80 P
- K 250 P representing also K 200P, K 700 P and K 900 P

All tested materials which are the components of Pall **SUPRAdisc** HP media meet current USP requirements for:

- Cytotoxicity *(in vitro)*
- Class VI (121 °C) Plastics *(in vivo)*
- Hemolysis *(in vitro)*

Copies of certificates and test reports are available upon request.

Notes

Notes

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