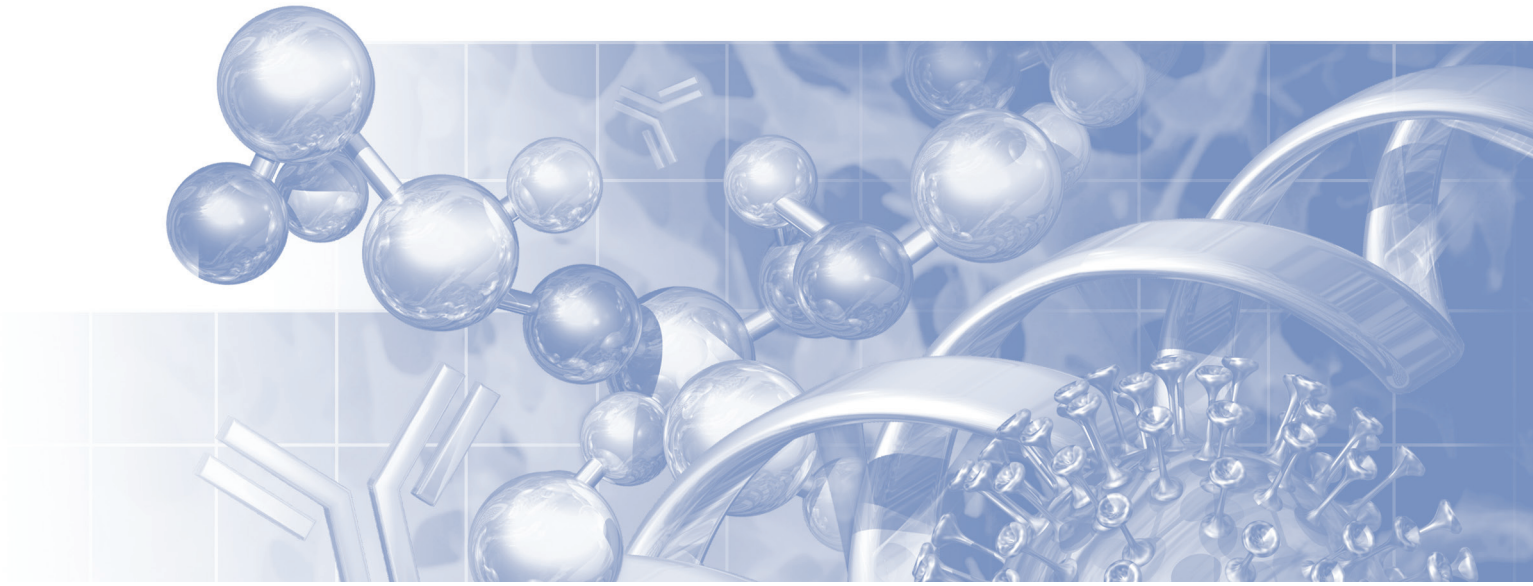
PALL Life Sciences

Validation Guide

USTR2366c(3)

Seitz® P-series Depth Filter Media



Filtration. Separation. Solution.SM

Contents

1. Overview

1.1 Introduction

Validation is defined as 'Establishing documented evidence which provides a high degree of assurance that a specific process will consistently produce a product meeting its predetermined specifications and quality attributes' (Guideline For Industry: Sterile Drug Products Produced by Aseptic Processing – Current Good Manufacturing Practice [US Department of Health and Human Services, Food and Drug Administration], September 2004).

This report contains validation data applicable to Seitz® P-series depth filter media, which are used in different formats and configurations such as:

- Flat sheets for use in filter presses
- Modules used in housings
- Filter capsules

This report contains a summary of tests carried out and results obtained by Pall. The data contained in this document are typical measured values, the correctness and reproducibility of which are controlled on a regular basis and confirmed by results of field applications.

This validation guide has been compiled for the users of Seitz P-series depth filter media as a basis and support for their own validation procedures.

The validation program included:

- Extractables tests:
- Conductivity and pH
- Total Organic Carbon (TOC)
- Extractable cations
- Total extractables
- Endotoxin levels
- ß-glucan levels
- Biological reactivity

1.2 Summary of Conclusions

1.2.1 Extractables Testing

Conductivity and pH value in Water For Injection (WFI)

After a WFI rinse volume of 50 L/m^2, the pH value of the depth filter effluent was between 5 and 7, meeting the requirements of the current USP.

The conductivity of the extract was < 20 µS/cm and can be reduced further with additional flushing. The conductivity of the EKXP grade media extract was

< 30 µS/cm and can be reduced further with additional flushing.

Total Organic Carbon (TOC) in WFI

Pall P-series depth filter media met the USP requirements of < 500 ppb (0.5 ppm) in WFI after a rinse volume of ≥ 100 L/m^2.

Extractable Cations (Al, Ca, Mg, Fe, Ni, Cu, Cr, As, Pb) in WFI and 40% Ethanol The typical values reached for extractable cations after a rinse volume of 50 L/m^2 are shown in Table 1.

Table 1
Extractable Cations in WFI and 40% Ethanol

	In WFI	In 40% Ethanol
Aluminum (Al)	< 0.05 ppm	< 0.02 ppm
Calcium (Ca)*	< 0.5 ppm	< 1 ppm
Magnesium (Mg)	< 0.1 ppm	< 0.2 ppm
Iron (Fe)	< 0.1 ppm	< 0.1 ppm
Nickel (Ni)	< 0.01 ppm	< 0.01 ppm
Copper (Cu)	< 0.1 ppm	< 0.1 ppm
Chromium (Cr)	< 0.01 ppm	< 0.01 ppm
Arsenic (As)	< 0.01 ppm	< 0.01 ppm
Lead (Pb)	< 0.01 ppm	< 0.01 ppm

** Ca value for EKX P in WFI is < 1 ppm and in 40% Ethanol < 2 ppm.*

Total Extractables 21 CFR 177.2260

The level of extractables of Seitz P-series depth filter media was significantly below the CFR limits and therefore met the requirements of 21 CFR 177.2260.

1.2.2 Endotoxin and ß-glucans Levels

Endotoxin
The endotoxin content of all sheets tested in human albumin without pre-rinsing was below the detection limit of 0.06 EU/mL.

After a rinse volume of 50 L/m^2 of WFI, the endotoxin content of the effluent of all tested sheets was below 0.02 EU/mL. Recovery rates indicate that there was no LAL-reactive material in the effluents.

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

1.2.3 Biological Reactivity Tests

All tested materials meet current USP requirements for:

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

Certificates and test reports are available upon request.

2. General Characteristics of Seitz P-series Depth Filter Media

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

EKX P EKS P EKM P

SUPRA EK 1 P KS 50 P

K 100 P

V 100 P

SUPRA 80 P

K 200 P

K 250 P

K 700 P

K 900 P

T 1500 P

All of these depth filter media grades are manufactured under special production conditions that assure a high level of purity. 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, Seitz P-series depth filter media can be distinguished by very low release of extractables and extremely low endotoxin levels. Furthermore, grades ranging from EKX P through SUPRA 80 P have been optimized for minimal ß-glucan content.

Seitz P-series depth filter media are therefore particularly suitable for critical applications in biotech and pharmaceutical production.

3. Typical Technical Data

Table 2
Test Parameters

Seitz P-series Depth Filter Media Grade	Permeability at 100 kPa ($L/m^2/min$)	Weight per Unit Area (g/m^2)	Thickness (mm)	Ash Content (%)	Adsorption of Azorubin Red (g/m^2)	Log Reduction Value (LRV)
EKX P	24	1550	3.6	62	2.5	9.0[1]
EKS P	29	1400	3.7	58	2.5	8.5[1]
EKM P	41	1400	3.7	51	2.5	8.0[1]
SUPRA EK 1 P	64	1300	3.6	47	10	7.5[2]
KS 50 P	93	1350	3.7	46	2.5	6.5[2]
K 100 P	149	1350	3.7	46	2.5	
V 100 P	149	1350	3.7	52	< 1	
SUPRA 80 P	159	1300	3.7	49	10	
K 200 P	217	1350	3.9	46	2.5	
K 250 P	535	1250	4.0	46	2.5	
K 700 P	935	1250	4.1	46	2.5	
K 900 P	1980	1200	4.1	46	2.5	
T 1500 P	7630	800	3.7	33		

[1] *Tested with Brevundimonas diminuta (ATCC 19146)*
[2] *Tested with Serratia marcescens (ATCC 14756)*

4. Extractables Tests

4.1 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 reduce extractable substances. The most common rinsing medium is WFI.

Many products filtered by Seitz P-series depth filter media contain alcohol in their formulation. Therefore, in addition to WFI, 40% ethanol was chosen as a second extraction medium.

The flux was adjusted to 500 L/m^2/h.

Samples for the determination of

- Conductivity
- pH
- TOC
- Cations (Al, Ca, Mg, Fe, Ni, Cu, Cr, As, Pb) were taken at rinse volumes of:
- 5 – 10 L/m^2
- After 50 L/m^2
- After 100 L/m^2

4.2 Conductivity and pH Value in WFI

4.2.1 Method

Conductivity and pH value were measured using a calibrated conductivity and pH meter.

4.2.2 Results

Table 3

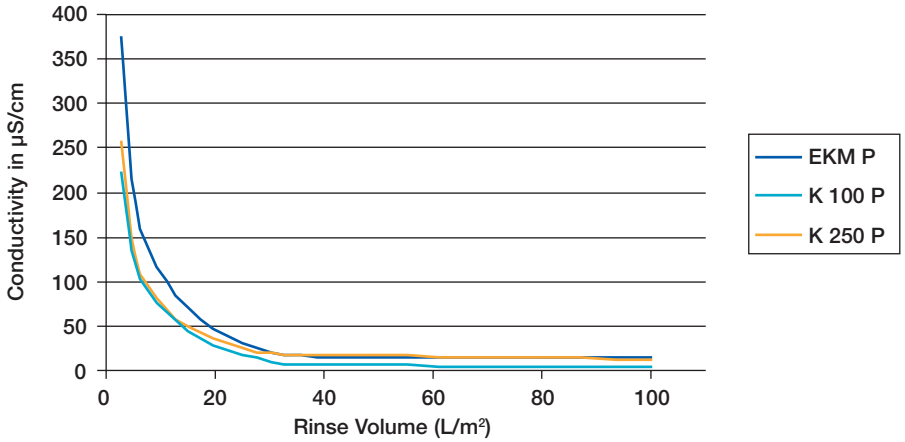
Conductivity Shift and pH Value (in WFI)

Seitz P-series Depth Filter Media Grade	Conductivity Shift in µS/cm Rinse Volume in L/m^2			pH Value Rinse Volume in L/m^2		
	5 – 10	50	100	5 – 10	50	100
EKX P	170	20	15	5.0	4.8	4.8
EKS P	133	6	3	6.0	6.0	6.0
EKM P	137	13	12	4.5	5.0	5.0
SUPRA EK 1	111	16	11	5.5	5.0	5.0
KS 50 P	82	4	2	6.0	6.0	6.5
K 100 P	88	4	2	5.0	5.5	5.0
SUPRA 80 P	110	10	7	5.5	5.5	5.5
V 100 P	12	1	1	6.0	6.0	6.0
K 200 P	113	7	4	5.0	5.0	5.0
K 250 P	92	15	9	4.5	5.0	5.0
K 700 P	87	13	10	4.5	5.0	5.0
K 900 P	62	10	9	4.5	5.0	5.0
T 1500 P	41	5	3	5.6	5.9	5.9

Control WFI sample: pH value 5.5

Figure 1

Effluent Conductivity in µS/cm versus Rinse Volume



4.2.3 Conclusion

After a WFI rinse volume of 50 L/m², the pH value of filter effluent was between 5 and 7, thus meeting the requirements of the current USP.

The conductivity of the extract was ≤ 20 µS/cm at this volume and can be reduced further with additional flushing.

4.3 Total Organic Carbon (TOC) in WFI

4.3.1 Method

TOC in WFI is the parameter used to characterize organic extractables from the filters. Measurement was performed using a TOC analyzer (Shimadzu Ltd., type 5000 A).

4.3.2 Results

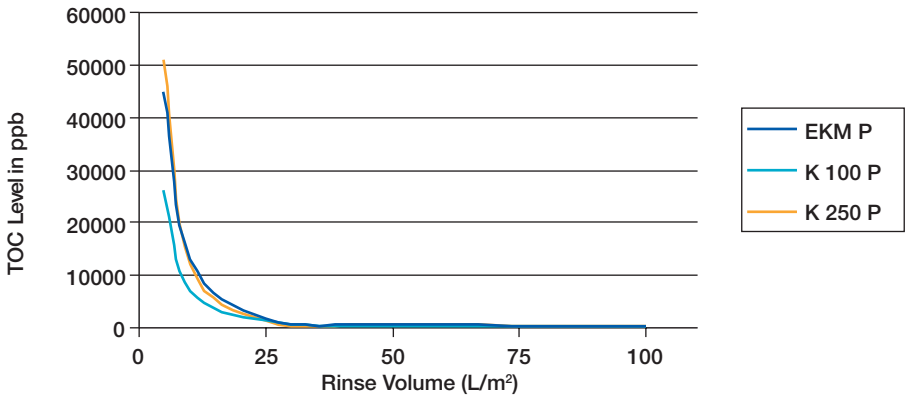
Table 4

TOC in WFI

Seitz P-series Depth Filter Media Grade	TOC level in ppm Rinse Volume in L/m²		
	5 – 10	50	100
EKX P	13	0.8	< 0.5
EKS P	7	0.6	< 0.5
EKMP	13	0.6	< 0.5
SUPRA EK 1	7	< 0.5	< 0.5
KS 50 P	5	0.6	< 0.5
K 100 P	7	< 0.5	< 0.5
SUPRA 80	4	< 0.5	< 0.5
V 100 P	2	< 0.5	< 0.5
K 200 P	15	0.6	< 0.5
K 250 P	12	0.6	< 0.5
K 700 P	11	0.5	< 0.5
K 900 P	8	0.5	< 0.5
T 1500 P	3	< 0.5	< 0.5

Figure 2

TOC Level in ppb after Rinsing with WFI



4.3.3 Conclusion

Seitz P series depth filter media meet the USP requirements of < 500 ppb (0.5 ppm) for TOC in WFI after a rinse volume of 100 L/m^2.

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

4.4.1 Method

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

4.4.2 Results

Table 5

Al (ppb) Level

Seitz P-series Depth Filter Media Grade	In WFI			In 40% Ethanol		
	Rinse Volume in L/m^2			Rinse Volume in L/m^2		
	5 – 10	50	100	5 – 10	50	100
EKX P	40	19	15	12	< 5	< 5
EKS P	16	< 5	< 5	< 5	< 5	< 5
EKM P	36	7	< 5	16	< 5	< 5
SUPRA EK 1	90	< 5	< 5	< 5	< 5	< 5
KS 50 P	< 5	< 5	< 5	< 5	< 5	< 5
K 100 P	14	< 5	< 5	8	< 5	< 5
SUPRA 80 P	< 5	< 5	< 5	< 5	< 5	< 5
V 100 P	37	13	8	19	< 5	< 5
K 200 P	38	7	< 5	18	< 5	< 5
K 250 P	66	17	14	31	< 5	< 5
K 700 P	58	10	10	31	5	< 5
K 900 P	69	9	< 5	45	5	< 5
T 1500 P	33	< 5	< 5	21	< 5	< 5

Figure 3

Al Level in ppb after Rinsing with WFI

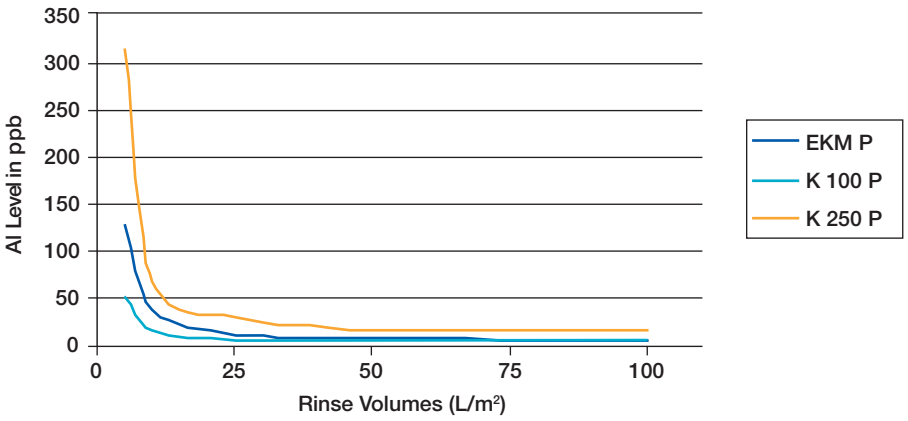


Figure 4

Al Level in ppb after Rinsing with EtOH

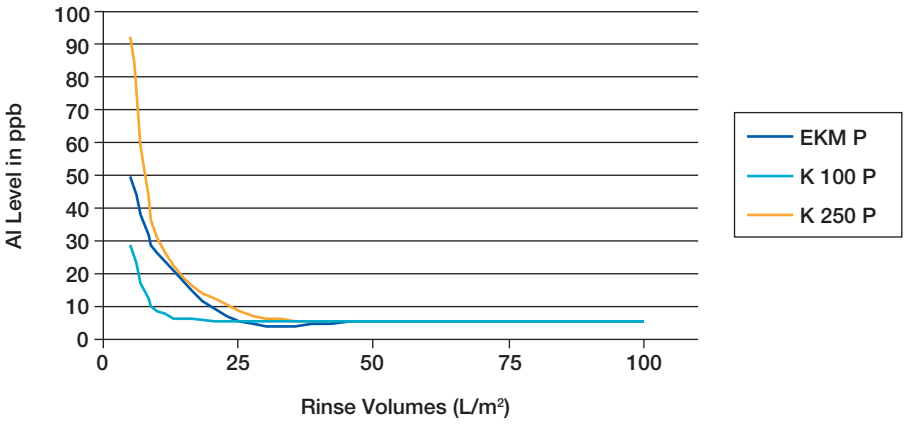


Table 6

Ca (ppm) Level

Seitz P-series Depth Filter Media Grade	In WFI			In 40% Ethanol		
	Rinse Volume in L/m²			Rinse Volume in L/m²		
	5 – 10	50	100	5 – 10	50	100
EKX P	18	0.53	0.14	7	0.71	0.41
EKS P	10	0.16	0.09	4	0.70	0.40
EKM P	10	0.20	0.08	4	0.80	0.50
SUPRA EK 1	7	0.15	0.05	4	0.70	0.30
KS 50 P	4	0.06	< 0.05	3	0.40	0.20
K 100 P	6	0.14	0.06	4	0.45	0.16
SUPRA 80 P	4	0.3	0.1	2	0.50	0.27
V 100 P	0.2	< 0.05	< 0.05	0.2	< 0.05	< 0.05
K 200 P	7	0.23	0.08	3	0.42	0.15
K 250 P	6	0.20	0.06	2	0.19	< 0.05
K 700 P	5	0.20	0.05	3	0.27	0.17
K 900 P	3	0.14	< 0.05	1	0.18	0.09
T 1500 P	2.1	0.08	< 0.05	0.9	0.10	< 0.05

Figure 5

Ca Level in ppm after Rinsing with WFI

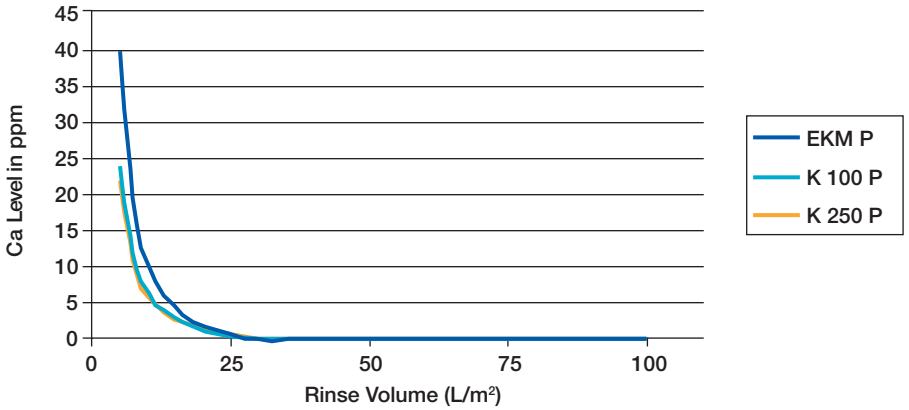


Figure 6

Ca Level in ppm after Rinsing with EtOH

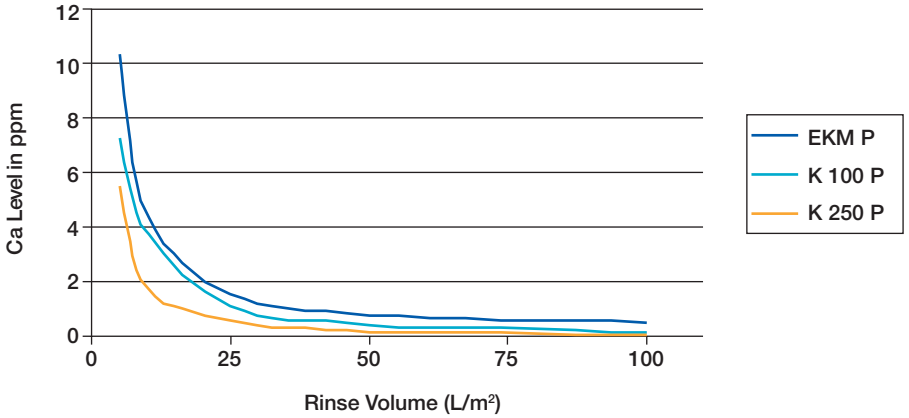


Table 7

Mg (ppm) Level

Seitz P-series Depth Filter Media Grade	In WFI			In 40% Ethanol		
	Rinse Volume in L/m²			Rinse Volume in L/m²		
	5 – 10	50	100	5 – 10	50	100
EKX P	2	0.05	< 0.05	2	< 0.05	< 0.05
EKS P	4	0.05	< 0.05	2	0.17	0.06
EKM P	2	< 0.05	< 0.05	2	0.07	< 0.05
SUPRA EK 1	2	< 0.05	< 0.05	2	0.08	< 0.05
KS 50 P	1	< 0.05	< 0.05	2	< 0.05	< 0.05
K 100 P	1	< 0.05	< 0.05	1	0.05	< 0.05
SUPRA 80 P	0.87	0.06	< 0.05	0.62	0.09	< 0.05
V 100 P	0.06	< 0.05	< 0.05	0.08	< 0.05	< 0.05
K 200 P	2	0.06	< 0.05	1	0.10	< 0.05
K 250 P	1	< 0.05	< 0.05	1	< 0.05	< 0.05
K 700 P	1	< 0.05	< 0.05	1	< 0.05	< 0.05
K 900 P	1	< 0.05	< 0.05	1	< 0.05	< 0.05
T 1500 P	0.48	< 0.05	< 0.05	0.34	< 0.05	< 0.05

Figure 7

Mg Level in ppm after Rinsing with WFI

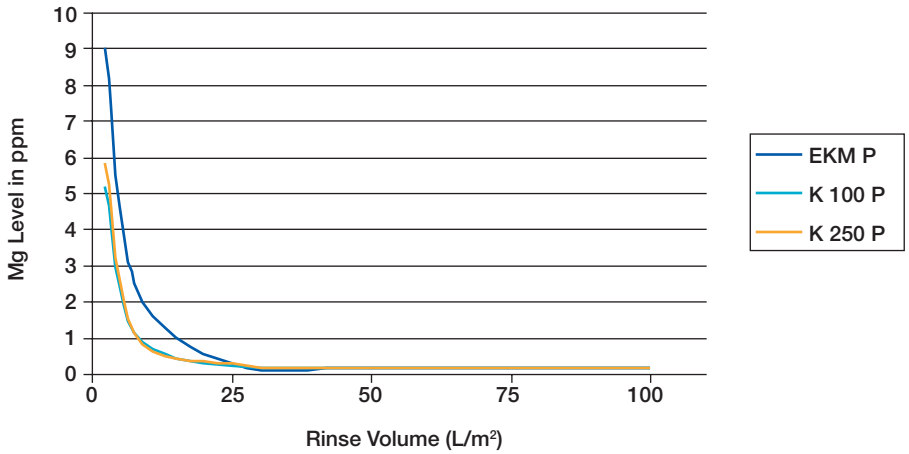


Figure 8

Mg Level in ppm after Rinsing with EtOH

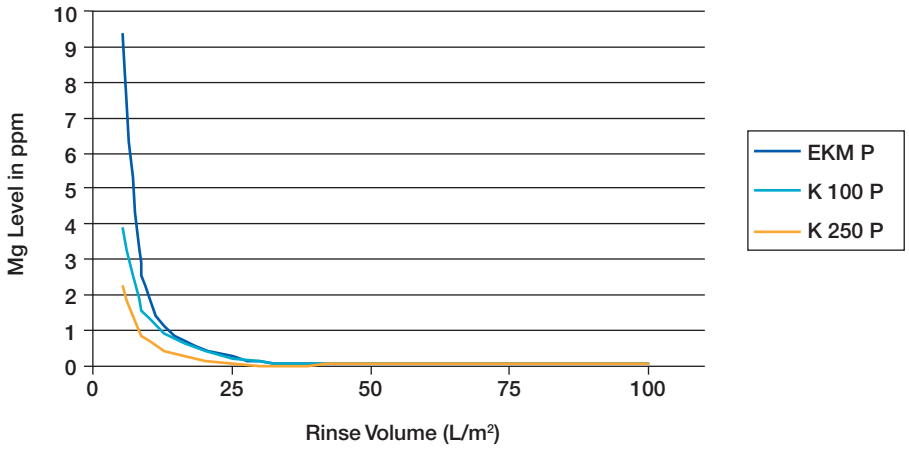


Table 8

Fe (ppm) Level

Seitz P-series Depth Filter Media Grade	In WFI			In 40% Ethanol		
	Rinse Volume in L/m²			Rinse Volume in L/m²		
	5 – 10	50	100	5 – 10	50	100
EKX P	0.05	< 0.05	< 0.05	0.05	< 0.05	< 0.05
EKS P	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
EKM P	0.1	< 0.05	< 0.05	0.06	0.06	0.06
SUPRA EK 1	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
KS 50 P	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
K 100 P	0.1	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
SUPRA 80 P	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
V 100 P	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
K 200 P	0.1	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
K 250 P	0.1	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
K 700 P	0.1	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
K 900 P	0.1	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
T 1500 P	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05

Table 9

Ni (ppb) Level

Seitz P-series Depth Filter Media Grade	In WFI			In 40% Ethanol		
	Rinse Volume in L/m²			Rinse Volume in L/m²		
	5 – 10	50	100	5 – 10	50	100
EKX P	< 5	< 5	< 5	< 5	< 5	< 5
EKS P	< 5	< 5	< 5	< 5	< 5	< 5
EKM P	< 5	< 5	< 5	< 5	< 5	< 5
SUPRA EK 1	17	< 5	< 5	< 5	< 5	< 5
KS 50 P	< 5	< 5	< 5	< 5	< 5	< 5
K 100 P	< 5	< 5	< 5	< 5	< 5	< 5
SUPRA 80 P	< 5	< 5	< 5	< 5	< 5	< 5
V 100 P	< 5	< 5	< 5	< 5	< 5	< 5
K 200 P	< 5	< 5	< 5	< 5	< 5	< 5
K 250 P	5	< 5	< 5	< 5	< 5	< 5
K 700 P	< 5	< 5	< 5	5	< 5	< 5
K 900 P	< 5	< 5	< 5	< 5	< 5	< 5
T 1500 P	< 5	< 5	< 5	< 5	< 5	< 5

Table 10

Cu (ppm) Level

Seitz P-series Depth Filter Media Grade	In WFI			In 40% Ethanol		
	Rinse Volume in L/m²			Rinse Volume in L/m²		
	5 – 10	50	100	5 – 10	50	100
EKX P	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
EKS P	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
EKM P	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
SUPRA EK 1	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
KS 50 P	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
K 100 P	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
SUPRA 80 P	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
V 100 P	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
K 200 P	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
K 250 P	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
K 700 P	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
K 900 P	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
T 1500 P	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05

Table 11

Cr (ppb) Level

Seitz P-series Depth Filter Media Grade	In WFI			In 40% Ethanol		
	Rinse Volume in L/m²			Rinse Volume in L/m²		
	5 – 10	50	100	5 – 10	50	100
EKX P	< 5	< 5	< 5	< 5	< 5	< 5
EKS P	< 5	< 5	< 5	< 5	< 5	< 5
EKM P	< 5	< 5	< 5	< 5	< 5	< 5
SUPRA EK 1	< 5	< 5	< 5	< 5	< 5	< 5
KS 50 P	39	< 5	< 5	< 5	< 5	< 5
K 100 P	< 5	< 5	< 5	< 5	< 5	< 5
SUPRA 80 P	< 5	< 5	< 5	< 5	< 5	< 5
V 100 P	< 5	< 5	< 5	< 5	< 5	< 5
K 200 P	< 5	< 5	< 5	< 5	< 5	< 5
K 250 P	< 5	< 5	< 5	< 5	< 5	< 5
K 700 P	< 5	< 5	< 5	< 5	< 5	< 5
K 900 P	< 5	< 5	< 5	< 5	< 5	< 5
T 1500 P	< 5	< 5	< 5	< 5	< 5	< 5

Table 12

As (ppb) Level

Seitz P-series Depth Filter Media Grade	In WFI			In 40% Ethanol		
	Rinse Volume in L/m²			Rinse Volume in L/m²		
	5 – 10	50	100	5 – 10	50	100
EKX P	< 5	< 5	< 5	< 5	< 5	< 5
EKS P	< 5	< 5	< 5	< 5	< 5	< 5
EKM P	< 5	< 5	< 5	< 5	< 5	< 5
SUPRA EK 1	< 5	< 5	< 5	< 5	< 5	< 5
KS 50 P	10	5	< 5	< 5	< 5	< 5
K 100 P	< 5	< 5	< 5	< 5	< 5	< 5
SUPRA 80 P	< 5	< 5	< 5	< 5	< 5	< 5
V 100 P	< 5	< 5	< 5	< 5	< 5	< 5
K 200 P	< 5	< 5	< 5	< 5	< 5	< 5
K 250 P	< 5	< 5	< 5	< 5	< 5	< 5
K 700 P	< 5	< 5	< 5	< 5	< 5	< 5
K 900 P	< 5	< 5	< 5	< 5	< 5	< 5
T 1500 P	< 5	< 5	< 5	< 5	< 5	< 5

Table 13

Pb (ppb) Level

Seitz P-series Depth Filter Media Grade	In WFI			In 40% Ethanol		
	Rinse Volume in L/m²			Rinse Volume in L/m²		
	5 – 10	50	100	5 – 10	50	100
EKX P	< 5	< 5	< 5	< 5	< 5	< 5
EKS P	< 5	< 5	< 5	< 5	< 5	< 5
EKM P	< 5	< 5	< 5	< 5	< 5	< 5
SUPRA EK 1	< 5	< 5	< 5	< 5	< 5	< 5
KS 50 P	< 5	< 5	< 5	< 5	< 5	< 5
K 100 P	< 5	< 5	< 5	< 5	< 5	< 5
SUPRA 80 P	< 5	< 5	< 5	< 5	< 5	< 5
V 100 P	< 5	< 5	< 5	< 5	< 5	< 5
K 200 P	< 5	< 5	< 5	< 5	< 5	< 5
K 250 P	< 5	< 5	< 5	< 5	< 5	< 5
K 700 P	< 5	< 5	< 5	< 5	< 5	< 5
K 900 P	< 5	< 5	< 5	< 5	< 5	< 5
T 1500 P	< 5	< 5	< 5	< 5	< 5	< 5

4.4.3 Conclusion

Typically, the following values for extractable cations were reached after a rinse volume of 50 L/m².

Table 14

Typical Extractable Cation Values Levels

	In WFI	In 40% Ethanol
Aluminum (Al)	< 0.05 ppm	< 0.02 ppm
Calcium (Ca)	< 0.5 ppm	< 1 ppm
Magnesium (Mg)	< 0.1 ppm	< 0.2 ppm
Iron (Fe)	< 0.1 ppm	< 0.1 ppm
Nickel (Ni)	< 0.01 ppm	< 0.01 ppm
Copper (Cu)	< 0.1 ppm	< 0.1 ppm
Chromium (Cr)	< 0.01 ppm	< 0.01 ppm
Arsenic (As)	< 0.01 ppm	< 0.01 ppm
Lead (Pb)	< 0.01 ppm	< 0.01 ppm

4.5 Total Extractables

4.5.1 Method

Under 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 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 in Table 15.

Table 15
Limitations for Different Extraction Media

Extraction Solvent	Extraction Conditions	CFR Limits
Deionized Water	100 °C	< 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 supporting data for the suitability of filters for pharmaceutical applications.

4.5.2 Results and Conclusion

The level of extractables of all Seitz P-series depth filters media was significantly below the CFR limits and therefore meets the requirements of 21 CFR 177.2260.

5. Endotoxin and ß-glucans

5.1 Endotoxin

The potential risk of a contamination by endotoxin in filter manufacturing is due mainly to water which has been exposed to and might contains gram negative bacteria. Therefore, the verification of low endotoxin levels of the filter extracts is an important issue during validation.

5.1.1 Method

Human Albumin – LAL Gel Clot Test

For the extraction and desorption of endotoxin from filters, human albumin could be demonstrated as being most effective. Thus, filtering a human albumin solution and demonstrating endotoxin levels are below detectable limit in the filtrate.

Filtration of a human albumin solution without pre-rinsing was performed and an endotoxin specific test was used to determine the level of endotoxin in EU/mL (endotoxin units/mL).

Water for Injection (WFI) – LAL Kinetic Chromogenic Test

A typical rinsing procedure of the filter after sterilization is performed with WFI. At a flux of 500 L/m^2/h, the recommended rinse volume is 50 L/m^2. After this rinse volume, a sample was taken of the WFI and tested in a chromogenic LAL test for endotoxin and LAL-reactive materials.

5.1.2 Results

Table 16

Endotoxin Level in Human Albumin without WFI Pre-rinse

Depth Filter Media Grade	Endotoxin Level in EU/mL
EKX P	< 0.06
EKS P	< 0.06
EKM P	< 0.06
SUPRA EK 1	< 0.06
KS 50 P	< 0.06
K 100 P	< 0.06
SUPRA 80 P	< 0.06
V 100 P	< 0.06
K 200 P	< 0.06
K 250 P	< 0.06
K 700 P	< 0.06
K 900 P	< 0.06
T 1500 P	< 0.06

Control sample human albumin: < 0.06 EU/mL.

Table 17

Endotoxin Level in WFI – Endotoxin Level after rinsing with WFI at 50 L/m²

Depth Filter Media Grade	Endotoxin Level in EU/mL
EKX P	< 0.02
EKS P	< 0.02
EKM P	< 0.02
SUPRA EK 1	< 0.02
KS 50 P	< 0.02
K 100 P	< 0.02
SUPRA 80 P	< 0.02
V 100 P	< 0.02
K 200 P	< 0.02
K 250 P	< 0.02
K 700 P	< 0.02
K 900 P	< 0.02
T 1500 P	< 0.02

Control sample WFI: < 0.02 EU/mL.

5.1.3 Conclusion

The endotoxin content of all tested sheets in human albumin without pre-rinsing was below the detection limit of of 0.06 EU/mL.

After a rinse volume of 50 L/m² of WFI, the endotoxin level of the effluent of all 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.

5.2 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 an 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.

5.2.1 Method

A typical rinsing procedure of the filter after sterilization is performed with WFI. At a flux of 500 L/m^2/h, the recommended rinse volume is 50 L/m^2. After this rinse volume, a sample was taken of the WFI and checked with a ß-glucan specific LAL test.

5.2.2 Results

Table 18

ß-glucans in pg/mL after rinsing with 50 L/m^2 of WFI

Depth Filter Media Grade	ß-glucans Level in pg/mL
EKX P	< 100 pg/mL
EKS P	< 100 pg/mL
EKM P	< 100 pg/mL
SUPRA EK 1 P	< 100 pg/mL
KS 50 P	< 100 pg/mL
K 100 P	< 100 pg/mL
SUPRA 80 P	< 100 pg/mL
V 100 P	< 100 pg/mL

5.2.3 Conclusion

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

6. Biological Reactivity Tests of Seitz P-series Depth Filter Media

6.1 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 USP Biological Reactivity Tests for Class VI (121 °C) plastics are 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.

- Acute systemic injection tests
- Intracutaneous tests
- Implantation tests

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

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

The extraction was performed at 121 °C 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.

6.2 Results

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

Table 19

Depth Filter Media Grades

EKX P	
EKS P	
EKM P	Representing also KS 50 P
SUPRA EK 1 P	
K 100 P	
SUPRA 80 P	
V 100 P	
K 250 P	Representing also K 200 P, K 700 P and K 900 P
T 1500 P	

All tes*ted mate*rials meet current USP requireme*nts for*:

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

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