Sartoclean® CA

Extractables Guide

SARTORIUS

Table of Contents

Tables and Figures

Tables and Figures

1. Introduction

1.1 Background

Pharmaceutical and biopharmaceutical products are subject to precisely defined quality requirements.

The quality, efficacy, and safety of the final drug product can only be guaranteed if the entire production process is qualified and includes sufficient and reliable protection against contamination. The pharmaceutical and biopharmaceutical industry conducts comprehensive tests, both in the preliminary stage of process development and within the context of process monitoring and quality control to ensure the quality of its products.

Generally, all integrated parts of the production processes that are exposed to intermediates and drug product solutions are potential sources for impurities. Consequently, any single-use equipment or component with fluid contact, such as storage, mixing or bioreactor bags, tubing, connectors, valves, sensors, chromatography columns, filters, etc. should be checked for any potential compound – extractables – that can be released by the device. Current analytical methods are capable to detect such compounds at very low concentrations for subsequent evaluation.

Information about selected physicochemical characteristics and extractables profiles of a single-use device obtained from tests according to USP and EP monographs for Sterile Water for Injection (WFI) Results are typically summarized in the Validation Guides. This includes results of the amounts of the total organic carbon (TOC), the pH value, conductivity, and selected ionic species. An extractables study is required in addition to this information to allow a comprehensive evaluation of the single-use component.

1.2 Standardized Extractables Approach

Sartorius has developed a fully qualified extractables approach (SEA) for testing single-use devices used in the biopharmaceutical industry.[1] There is a significant overlap of the test practices between SEA and the upcoming USP <665>, allowing a straightforward combination of both approaches. In this context, the SEA approach was extended to fully adopt the requirements outlined in the upcoming USP <665>.[2] Specific elements of the SEA will remain part of the extractables protocol, such as extraction with pure water and pure ethanol. These solvents provide relevant baseline data for identification and scaling of extractables data and modelling of process equipment-related leachables (PERLs) as described below. Sartorius products will be tested using this combined approach and documentation will be updated accordingly. The respective documents can be used directly in submission documentation for example as a regulatory support file.

A component-based approach is applied, whenever practically applicable, to be able to perform scaling calculations to different sizes. In addition, the approach allows modeling of extractables data for complex assemblies. Several extraction solvents at different time points are tested to obtain the most complete extractables information which enables a full safety evaluation of the single-use device. The selected analytical methods used are in accordance with the USP <1663>. The solvent selection includes pure water and pure ethanol, 50 % ethanol, and | or high and low pH solutions. Certainly, the use of a pure organic solvent exaggerates common process conditions and, consequently, the number and quantity of extractables will be higher compared to aqueous extraction solutions. The main benefits of using pure ethanol are that it provides the best analytical conditions leading to the lowest level of non-identified or incorrectly identified compounds. Additionally, no sample preparation step before analysis is required which minimizes the potential loss of information and reduces the risks of missing a potential leachable.

The extraction and analytical conditions applied in this approach enable a full material characterization and safety evaluation of the single-use equipment tested.

[1] Pahl, I. et al. Development of a Standardized Extractables Approach for Single-Use Components - General Considerations and Practical Aspects. *Bioprocess Int.* 16, 2018

[2] Sartorius Statement on USP <665>: Extractables Strategy and the Upcoming USP 665_signed.pdf; June 2021

1.3 Extractables Guides

Sartorius provides documented extractables information for the majority of its single-use devices in Extractables Guides. These documents are controlled and quality approved. The data is regularly reviewed, and updates are detailed in the document version history section. The Extractables Guides should be used for the initial design qualification (DQ) and installation qualification (IQ) to assess material safety of the respective single-use equipment and for further process qualifications (PQ) including the design of a subsequent leachables study. Sartorius offers the opportunity to obtain a customized Extractables Safety Assessment Report as well a leachables study from its Confidence® Validation Services.

1.4 Update of Extractables Guides

The tremendous advances in analytical techniques over recent years coupled with today's more comprehensive understanding of extractables means it is necessary to review and update the Extractables Guides accordingly. In particular, today it is expected that extractables are measured – alongside previously used techniques – with high resolution mass spectrometry; and it is expected that suppliers provide databases for identification which enables the elucidation of a full extractables profile of a single-use component. These technical improvements allow a better understanding of the relationship between extractables profiles and the extraction conditions and test item. In this respect, Sartorius' approach can be regarded as a fully developed methodology which is used for generating Extractables Guides for new devices and to update also existing guides that have been available for many years.

It should be highlighted that the update or replacement of a legacy guide due to a change in extraction conditions or improvements in analytical methodology, does not influence the existing process qualification (DQ, IQ, PQ) of the single-use equipment for customers.

Further, an updated Extractables Guide is released when there are major changes in construction materials or their production parameters.

2. Objective and Methodology of the Tests

Single-use components or systems such as a filtration unit, a bag, or a complex assembly are constructed from various well-defined polymers. Each material has its own unique extraction profile and individual extractables can be assigned to the materials used. Such potential extractables are residual monomers, oligomers or degradation products of the polymer itself, stabilizers such as antioxidants, clarifying agents, or other processing aids. Different extraction solutions are applied to obtain the most comprehensive extraction of extractable compounds from different construction materials. The broad and complex spectrum of typical extractables represents an analytical challenge which is overcome by combining several orthogonal analytical tools. Analytical methodology is continuously optimized, and today, it even allows the detection of compounds which are only present at trace level concentrations.

To obtain conclusive data about the extractables from single-use equipment, studies should be based on worst-case conditions in terms of temperature, time, surface area to volume ratio (S/V), and extraction solutions. Potential pre-treatment methods such as gamma sterilization should be considered. Precisely what pre-treatment and extraction regime represent worst-case conditions in the pharmaceutical and biopharmaceutical industry remains a matter of general discussion and is dependent on the intended use. The following worst-case scenario is generally assumed: the respective single-use device is filled directly without flushing and all potential extractables are present in this volume. A high S/V such as 6:1 or 1:1 is used to obtain a relevant concentration of extractables in the extraction solvent. In case the S/V cannot be achieved because of the dimensions of the single-use component, the highest possible ratio is adjusted or test items such as dog bones identically manufactured, packed, and pretreated to the final product are used for the extraction study.

As mentioned, it is impossible to directly test all typical process solutions that a single-use product may encounter. Therefore, pure water, 50 % ethanol, and pure ethanol are chosen to create a database encompassing extractables that can be expected in an aqueous and organic extraction solution. Additionally, pH 3 solution and pH 10 solutions are used to mimic acidic and alkaline conditions, resp. An elevated temperature of 40 °C is selected because the extraction rate and final concentration of an extractables increases with temperature. Extraction times depend on the use of a single-use device, separated into two cases. Extraction at one or seven days is performed for devices typically used short-term where the extractables concentration is mainly controlled by diffusion. Single-use devices for long-term use are subjected to extraction conditions for 21 days and | or 70 days to ensure an exhaustive extraction with extractables concentration close to equilibrium.

Data from aqueous extraction solutions should be used to assess the probable leachables profile relevant for the majority of pharmaceutical and biopharmaceutical processes. Other solvents or extreme process parameters should be considered individually. For this purpose, a customer-specific process validation can be obtained via our Validation Services Confidence®.

A variety of different separation and detection techniques are used for comprehensive extractables analysis. Separation methods include reversed phase high performance liquid chromatography (HPLC) and | or ultra-high-performance liquid chromatography (UPLC), and gas chromatography (GC). The most versatile technique for the identification and quantification is mass spectrometry (MS) or high-resolution mass spectrometry (HRMS). An ultraviolet-visible (UV-Vis) detector is commonly used in liquid chromatography. Common techniques for the measurement of elements are inductively coupled plasma with optical emission spectrometry (ICP-OES) and | or mass spectrometry (ICP-MS). Within the scope of an extractables study, a combination of HPLC-UV and UHPLC-UV | HRMS, referred to as LC-MS in the following, together with GC MS is optimal to identify and semi-quantify or quantify individual organic substances. Additionally, short-chain carboxylic acids are measured by ion chromatography (IC) with a conductivity detector.

With the methods used, it is possible to determine volatile, semi-volatile, and non-volatile substances. For example, GC-MS perfectly combines the measurement of volatile compounds such as solvents using headspace (HS) sampling; and semi-volatile substances such as additives or polymer degradants using liquid injection. Further analytical work such as derivatization before GC-MS measurements can be performed to detect and quantify compounds which are difficult to analyze.

UV-detection is applicable for compounds possessing a chromophore such as aromatic compounds. At the same time, UV-inactive substances such as alkanes or alcohols are difficult to detect. Identification of a chromatographic peak is performed by comparison of the retention time of the peak with the retention time of an authentic reference standard.

LC-MS allows an effective and state-of-the-art analysis of diverse extractables. The effectiveness and analytical outcome of the LC-MS - especially for the suspect and non-target screening - is strongly related to the equipment, the experience of the user, and the manufactures software used for processing. In addition, it relies heavily on a comprehensive internal library. For routine extractables studies, two well-recognized ionization techniques have been established: Electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI). They enable the determination of the intact molecular ion together with its isotopic pattern and provide the possibility to calculate the molecule's formula. This can be helpful for determining and identifying unknowns. Quantities of extractables which are detected in the suspect and non-target screening are estimated using other analytical methods if justified. Using response factors only for the estimation of extractables quantities without justification is extremely difficult in LC-MS screening since these factors vary significantly. In this case, no quantitative estimation is performed.

ICP-MS and | or ICP-OES are used for the quantification of elements and are performed in accordance with guidelines ICH Q3D and USP <232>. Relevant elements beyond those mentioned in the guidelines are also determined.

Results of the analyses are controlled for plausibility using all available information. Sartorius continuously expands its material-specific internal library for this purpose which contains more than 700 identified individual compounds. Evidence of the identity and origin of extractables is derived from existing information about the raw materials used, the manufacturing process and the function of the identified chemical substances. The CAS number of the Extractables is provided as the unique identifier. The unique Sartorius ID (USID) is provided in case a CAS number was not assigned or is available in the chemical abstract service. Structural information can be provided on request.

Quantitative and semi-quantitative data of the extractables are provided wherever scientifically possible. The quantity per surface area or volume is provided within the Extractables Guide which can be used for scaling exercises and to estimate the concentration range of the extractable compound in a biopharmaceutical process solution.

3. Design of the Extractables Study

3.1 Product Information

Filter elements are manufactured in different formats such as filter cartridges and capsules (Midicaps®, Maxicaps®, and T-Style Maxicaps®) for use in different applications.

Extractables data for all different sizes of each format are required. A component-based approach was selected to give the highest flexibility with the most reasonable efficient analytical input. For this reason, an extraction is performed on one size of a single basic filter element with this being representative of the other sizes of the same type.

The different types of empty housings are also investigated separately and in addition to the basic filter elements. To calculate the expected extractables load of a capsule, the data from the representative basic filter element must be added to the data from the corresponding housing.

Additionally, to analyze the extractables profile of a filter, the different components must be tested after sterilization steps such as autoclaving or gamma irradiation. For an overview see Table 1.

Table 1: Overview of the Sartoclean® CA filter portfolio

				
	Basic Filter Element | Cartridges	Midicaps®	Maxicaps®	T-Style Maxicaps®
Autoclavable	×*	×	×	×

* Only cartridges are also suited for in-line steaming.

3.2 Component Approach

The quantity of extractable substances is proportional to the product contact area expressed by the effective filtration area (EFA) for a basic filter element or filter cartridge and the surface of the empty housing. This means that under non equilibrium conditions - one day extraction time - extractable results for one size of a filter element can be used to determine the amount of extractables from other sizes of the same type (same material) using the relationship of the surfaces. This is also valid for the membrane support material and the housing components. It is scientifically justified for Sartorius filter devices.[3]

In the context of a component approach, the basic filter elements and housings are investigated separately. Quantitative and semi-quantitative extractables values are available for all possible housing and basic filter element combinations. Based on the obtained extractables data and the surface relation, extractables quantities for all the different types and sizes can be calculated based on the samples tested.

The calculation of extractables for capsules must be performed by using the EFA and the housing surface in relation to the tested element. The required information of the different EFA and housing surfaces can be found in Table 3 to Table 5.

Terminology
In order to distinguish between the different components a clear terminology is required. The following terms are used in this document:

Basic filter element
A basic filter element consists of pleated membranes and fleeces, inner core, outer cage, and end caps. Basic filter elements are used for the manufacturing of the filter cartridges and capsules.

Filter cartridge
A filter cartridge is a basic filter element with adapters and a sealing ring (O-ring) to be used in stainless steel housings.

Adapters of the filter cartridges are not or only in negligible contact with the process fluid and are composed of the same material as the cartridge. Therefore, extractables data for the cartridge are regarded as equivalent to the corresponding basic filter element.

Housing
A housing is made of polypropylene and is used to manufacture a single-use capsule.

Capsule
A capsule is a basic filter element thermally welded into a housing.

[3] Pahl, I., et al. Using Extractables Data of Sterile Filter Components for Scaling Calculations. *PDA J. Pharm. Sci. Technol.* 73, 523–537, 2019

Table 2: Overview of the possible combinations for the different basic filter elements and housing types

Cartridges	Midicaps®	Maxicaps®	T-Style Maxicaps®
no housing			
Filter cartridge	**Basic filter element**	**Basic filter element**	**Basic filter element**
PP fleece	PP fleece	PP fleece	PP fleece
			
autoclavable | in-line steaming	autoclavable	autoclavable	autoclavable

Extractables data is obtained separately for the basic filter element and housing types.

Table 3: Effective filtration area (EFA)

Mini Cartridges and Cartridges	Size	EFA [cm²]	Employed in Housing Type
 	7	500	Midicaps®
	8	1,000	
	9	2,000	
	0	4,500	
	1	6,000	T-Style Maxicaps® | Maxicaps®
	2	12,000	
	3	18,000	

Table 4: Surface area of the Midicaps® housings

Midicaps® Housings	Size	Surface [cm²]
	7	190
	8	250
	9	350
	0	630

Table 5: Surface area of Maxicaps® housings*

T-Style Maxicaps® | Maxicaps®	Size	Surface [cm²]
	1	1,000
	2	1,700
	3	2,400

*Dimensions are identical for the Maxicaps® and T-Style Maxicaps® version.

3.3 Test Item Information

The components investigated together with the different sterilization methods applied are listed in Table 6. A size 9 basic filter element was extracted and investigated as representative size for the filter. For the Midicaps® housings the size 0 and for the Maxicaps® housing the size 1 (10") were chosen for the extractables study. Manufacturing, production steps, and packaging was identical to the final product. Extractables data for housings are available for pure ethanol and water extracts. The data packages for 50 % ethanol, pH3, and pH 10 are scheduled. The document will be updated after the set of data is generated and complete.

The extractable profile for the capsule can be calculated by combining the data from a basic filter element with the data from the appropriate housing. Possible combinations are given as examples in Table 7. Gaskets, if present, were removed before extraction. The surface area of the gasket in dynamic fluid contact is very low and negligible for an installed filter compared to the surface area of the whole device. In addition, such elastomeric materials are not in the scope for comprehensive Extractables testing of current standards such as USP <665> (Draft).

Table 6: Investigated components and pretreatment methods applied

#	Component Name	Size	Batch Number	Pretreatment
1	Sartoclean® CA Basic Filter Element	9	929009703	-*
2	Midicaps® Housing	0	601012015	-*
3	Maxicaps® Housing	1	160044883	-*
4	T-Style Maxicaps® Housing	1	160040383	-*

*Autoclaved during manufacturing at 121 °C and a minimum of 20 min.

Table 7: Examples of possible and allowed combinations of investigated components

Component	Type of Sartoclean® CA Cartridge or Capsule	Calculation Example
1+2	Sartoclean® CA Midicaps®	Size 9 shown in 3.4
1	Sartoclean® CA filter cartridge	Size 1 shown in 3.4
1+3	Sartoclean® CA Maxicaps®	Size 2 shown in 3.4
1+4	Sartoclean® CA T-Style Maxicaps®	Size 1 shown in 3.4

3.4 Example Calculations

The extraction took place under exaggerated conditions in terms of temperature, surface area to volume ratio (S/V), and an extraction time of 24 h. With this setup, the concentration of an extractables is controlled by its diffusion within the polymeric material. Therefore, it can be assumed that the quantity of an individual extractables correlates directly to the surface of the respective material.[4]

To calculate the concentration of an extractable which might be released into the process solution, the maximum quantity per contact surface area of this compound, the surface area of the single-use device which is in contact, and the volume of the process solution must be considered. The calculated results are rounded to two significant digits.

The data from the water extraction should be taken for estimating extractables for neutral aqueous process solutions such as buffers. High and low pH process solvents can be covered by pH 3 and pH 10 extractables data. For process solutions with a higher organic content, the data from the pure ethanol or 50 % ethanol extractions should be used for extractables evaluation.

Calculation example for a capsule: Size 9 Sartoclean® CA Midicaps®

To calculate the total amount of a selected extractable for a size 9 Midicaps®, the input of the Sartoclean® CA basic filter element and the corresponding Midicaps® housing have to be accounted for. An example calculation for one extractable (tris(2,4-di-*tert*-butylphenyl) phosphate, CAS 95906-11-9) is shown. The extractable data from the water extract is selected to mimic an aqueous process solution.

Input basic filter element

In the water extract of Sartoclean® CA basic filter no tris(2,4-di-*tert*-butylphenyl) phosphate was detected. Therefore, the total amount of this extractable per filter element is: 0 µg

Input housing

As shown in Table 32 tris(2,4-di-*tert*-butylphenyl) phosphate has a concentration of 0.24 µg/cm^2. A size 9 Midicaps® housing has a surface area of 350 cm^2. Therefore, the total amount of this extractable per housing is:

0.24 µg/cm^2 × 350 cm^2 = 84 µg

Total amount of tris(2,4-di-*tert*-butylphenyl) phosphate for a size 9 Sartoclean® CA Midicaps®

The total amount of tris(2,4-di-*tert*-butylphenyl) phosphate for a size 9 Midicaps® is the summarized value of the two components:

0 µg (basic filter element) + 84 µg (housing) = 84 µg

Example bulk concentration of Tris(2,4-di-*tert*-butylphenyl) phosphate for a size 9 Sartoclean® CA Midicaps®

In case of a sterile bulk filtration with an organic solvent with a total filtration volume of 50 L the following worst-case concentration of tris(2,4-di-*tert*-butylphenyl) phosphate in the bulk solution can be calculated to:

84 µg/50 L ~ 1.7 µg/L

[4] Pahl, I., et al. Using Extractables Data of Sterile Filter Components for Scaling Calculations. *PDA J. Pharm. Sci. Technol.* 73, 523–537, 2019

Calculation example for a filter cartridge: Size 1 Sartoclean® CA filter cartridge

To calculate the total amount of a selected extractable for a size 1 Sartoclean® CA filter cartridge, only the input of the basic filter element has to be taken into account (basic filter element and cartridge are equivalent, see section 3.2). An example calculation for one extractable (caprolactam, CAS 105-60-2) is shown. The extractable data from the basic extract is selected to mimic a high pH process solution.

Input basic filter element

As shown in Table 21 caprolactam has a concentration of 0.49 µg/cm2. A size 1 Sartoclean® CA filter element has an EFA of 6,000 cm^2. Therefore, the total amount of this extractable per basic filter element is:
0.49 µg/cm^2 × 6,000 cm^2 = 2,940 µg or ~2.9 mg

Total amount of caprolactam for a size 1 Sartoclean® CA filter cartridge

The total amount of caprolactam for a size 1 Sartoclean® CA filter cartridge is equal to the basic filter element = 2,940 µg or ~2.9 mg

Example bulk concentration of caprolactam for a size 1 Sartoclean® CA cartridge

In case of a sterile bulk filtration of a high pH aqueous solvent with a total filtration volume of 500 L the following worst-case concentration of caprolactam in the bulk solution can be calculated to:
2,940 µg/500 L ~ 5.9 µg/L

Calculation example for a capsule: size 2 Sartoclean® CA Maxicaps®

To calculate the total amount of a selected extractable for a size 2 Sartoclean® CA Maxicaps®, the input of the Sartoclean® CA basic filter element and the corresponding Maxicaps® housing have to be accounted for. An example calculation for one extractable (tris(2,4-di-*tert*-butylphenyl) phosphite, CAS 31570-04-4) is shown. The extractable data from the ethanol extract is selected to mimic an organic process solution.

Input basic filter element

As shown in Table 20 tris(2,4-di-*tert*-butylphenyl) phosphite has a concentration of 1.0 µg/cm^2. A size 2 Sartoclean® CA filter element has an EFA of 12,000 cm^2. Therefore, the total amount of this extractable per basic filter element is:
1.0 µg/cm2 × 12,000 cm^2 = 12,000 µg

Input housing

Tris(2,4-di-*tert*-butylphenyl) phosphite was detected in the ethanol extract of Maxicaps® housing with the concentration of 0.35 µg/mL as given in Table 42. A size 2 Maxicaps® housing has a surface area of 1,700 cm^2. Therefore, the total amount of this extractable per housing is:
0.35 µg/cm^2 × 1,700 cm^2 = 595 µg

Total amount of tris(2,4-di-*tert*-butylphenyl) phosphite for a size 2 Sartoclean® CA Maxicaps®

The total amount of tris(2,4-di-*tert*-butylphenyl) phosphite for a size 2 Maxicaps® is the summarized value of the two components:
12,000 µg (basic filter element) + 600 µg (housing) = 12,600 µg ~13 mg

Example batch concentration of tris(2,4-di-*tert*-butylphenyl) phosphite for size 2 Sartoclean® CA Maxicaps®

In case of a sterile bulk filtration with an organic solvent with a total filtration volume of 1,000 L the following worst-case concentration of tris(2,4-di-*tert*-butylphenyl) phosphite in the bulk solution can be calculated to:
13,000 µg/1,000 L = 13 µg/L

Calculation example for a capsule: Size 1 Sartoclean® CA T-Style Maxicaps®

To calculate the total amount of a selected extractable for a size 1 Sartoclean® CA T-Style Maxicaps®, the input of the Sartoclean® CA basic filter element and the corresponding T-Style Maxicaps® housing have to be accounted for. An example calculation for one extractable (stearyl alcohol, CAS 112-92-5) is shown. The extractable data from the ethanol extract is selected to mimic an organic process solution.

Input basic filter element

Stearyl alcohol was not detected in the ethanol extract of the Sartoclean® CA filter element. Therefore, the total amount of this extractable per basic filter element is: 0 µg

Input housing

As shown in Table 47 stearyl alcohol has a concentration of 0.73 µg/cm^2. A size 1 T-Style Maxicaps® housing has a surface area of 1,000 cm^2. The total amount of this extractable per housing is:
0.73 µg/cm^2 × 1,000 cm^2 = 730 µg

Total amount of stearyl alcohol for a size 1 Sartoclean® CA Maxicaps®

The total amount of stearyl alcohol for a size 1 T-Style Maxicaps® is the summarized value of the two components:
0 µg (Basic Filter Element) + 730 µg (Housing) = 730 µg

Example batch concentration of stearyl alcohol for size 1 Sartoclean® CA T-Style Maxicaps®

In case of a sterile bulk filtration with an organic solvent with a total filtration volume of 300 L the following worst-case concentration of stearyl alcohol in the bulk solution can be calculated to:
730 µg/300 L ~ 2.4 µg/L

3.5 Extraction Parameters and Equipment

Extraction is performed under defined conditions according to internal standard operation procedures from components "out-of-box", i.e. received as the final product in the final packaging without any additional rinsing. Water with the quality water for injection (WFI) and pure ethanol were used as extraction solvents for the housings. For the extractions of the basic filter elements pure ethanol, 50 % ethanol, water, and low and high pH solutions were used.

All extracted components usually had a storage time of less than six months. The extraction temperature was set to T = 40 ± 3 °C; extraction time was set to t = 24 h. Shaking at a minimum of 75 ± 5 rpm was applied to avoid concentration gradients in the extraction solution. To ensure that the solvent loss was less than 1 % the mass of the extraction unit (glass vessel or housing) was controlled before and after extraction.

For extraction of the basic filter elements, glass vessels were used which are designed to maintain an S/V ratio of 1:1 for a size 8 test item. The glass extraction vessels were placed in a temperature-controlled shaking water bath covered with a hood to ensure a constant extraction temperature in the glass vessels of T = 40 ± 3 °C. Blanks were prepared under the same conditions using the glass vessel and the extraction solution without the basic filter element. An illustration of the extraction set-up is shown in Figure 1.

For extraction of the housings, they were filled with the extraction solution until the S/V ratio of 1:1 was reached. Subsequently, the housings were sealed with polytetrafluoroethylene (PTFE) caps and placed into temperature controlled incubation shaker at T = 40 ± 3 °C. Blanks were prepared in a glass flask under identical conditions.

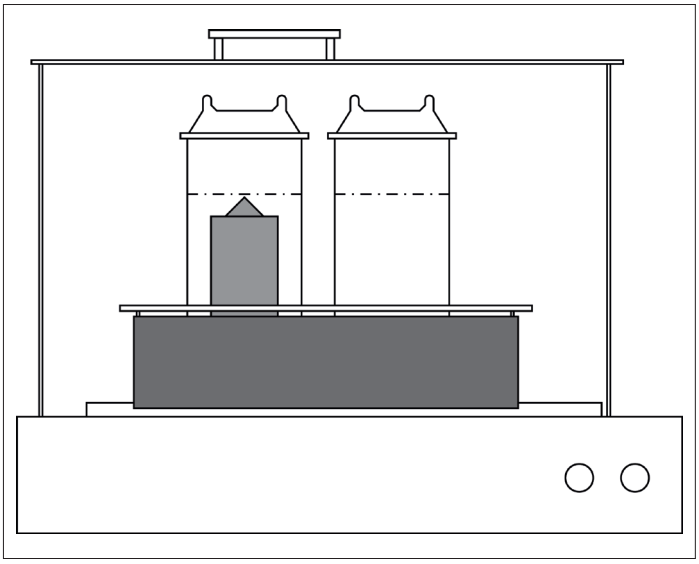

Figure 1: Extraction set-up for basic filter elements

3.6 Analytical Scheme and Processing Procedure

Extracts generated are qualitatively and quantitatively analyzed for extractables substances. The components are tested using the experimental set-up developed by Sartorius. Substances that can be expected as extractables are: Processing aids, polymer related compounds, or additives such as stabilizers. They can be released from the materials which are: the cellulose acetate (CA) membrane, the nonwoven polypropylene (PP), the polypropylene inner core and outer support cage for the cartridges or the polypropylene for the housings.

The extraction sample and corresponding sample blank are compared. Only peaks detected in the chromatograms of the sample extract and exceeding the blank value by 50 % are considered as relevant and are reported as extractables.

The mass spectra obtained after chromatographic separation by GC are evaluated by means of reference spectra of an internal spectrum library and the NIST Mass Spectral and Retention Index Library or an authentic reference standard.

If a substance is confirmed by HS GC-MS or GC-MS analysis, the authentic reference compound (if commercially available) is measured together with the internal standard and the response factor is determined. Subsequently, the concentration of this confirmed compound in a sample extract is calculated using the peak area ratios of the substance and the internal standard and corrected by the response factor (one-point type calibration).

The concentration of all other substances (without CAS number) is estimated from peak area ratios of standard substance and the peak in question (semi quantification). For this purpose, the following assumptions are made:

- The response factor of the compound in question and the internal standard in GC-MS are identical.
- The recovery rate of the compounds in the aqueous extract is 100 % after sample preparation.

Quantitative estimation by HPLC-UV is performed by comparing the measured peak with an authentic reference standard (internal standard mixture) and the calculated concentration is given in the corresponding HPLC-UV table. If a peak in question does not match to a peak of a known authentic reference standard (retention time does not fit) all available information about the test item materials are used to assign the peak to a potential chemical family and the concentration is estimated using the response from a reference compound.

For the LC-MS target analysis quantification is carried out by a calibration using authentic reference standards (for the list of targets see Table 15). For the suspect and non-target screening a visual comparison is performed of the base peak ion (BPI) chromatogram; in literature referred also as base peak chromatogram (BPC). Only the most dominant monoisotopic exact mass of the molecular ion adduct is reported. Identification is performed using an internal data base. Structural information is provided for compounds which are not identified in the suspect target screening if possible. A quantitative estimation is performed using information from other analytical methods if scientifically justified.

For ICP-MS the samples are acidified before measurement. An external calibration with different multi-element standard solutions is performed for quantification. Internal standards such as yttrium, rhodium, and lutetium are used for compensation of matrix effects.

The sensitivity of an analytical method depends strongly on the type of analyte, the sample matrix and the equipment itself. Therefore, reporting limits (RL) for analyte concentrations in the extracts are established to the lowest but reasonable level to allow a safe and reliable identification of the extractables and to enable comparability between laboratory results. The reporting limits are given in Table 9. They are transformed into the dimension of "µg/cm^2" by using the actual surface area to extraction volume ratio applied in the study.

Table 8: Analytical scheme for basic filter elements and housings

	GC-MS	HS GC-MS	HPLC-UV	LC-MS	ICP-MS
Water	×	×	×	×	×
50 % Ethanol*	×	-	×	×	-
Ethanol	×	-	×	×	-
pH 3*	×	×	×	×	×
pH 10*	×	×	×	×	×

*USP <665> testing is ongoing for housings.

Table 9: Reporting limits concentrations and quantities for the different analytical techniques

Analytical Technique	GC-MS	HS GC-MS	HPLC-UV	LC-MS	ICP-MS
Reporting Limit [µg/mL]	0.10	0.10	0.30	0.10	0.10
Reporting Limit [µg/cm²]	0.10	0.10	0.30	0.10	0.10

3.7 Sample Preparation

Ethanol extracts are used directly for each analysis without any dilution or concentration steps. Since ethanol is compatible with all analytical techniques no sample preparation or solvent change needs to be performed.

Aqueous extracts are used directly for HPLC-UV, LC MS, ICP-MS, and HS GC-MS. A liquid-liquid extraction (LLE) with dichloromethane prior to analysis is performed for the GC-MS analysis. The efficiency of the LLE is controlled by an internal extraction standard. The recoveries achieved after the sample preparation procedure are controlled by spiking an aqueous sample with common plastic additives. The recoveries in general are between 75 to 120 %.

3.8 Analytical Equipment

The following analytical equipment and parameters are used for analyses.

Table 10: GC-MS system and parameters

GC System	Clarus 600GC
MS System	Clarus 600T MS Turbo
Column	USP G27 column
Injector Temperature	250 °C
Column Temperature	35 to 300 °C
Carrier Gas (flow)	Helium (1 mL/min)
Injection Volume	1 µL (splitless)
Internal Standard	2-Fluorobiphenyl
Mass Range	35–700 m/z

Table 11: HS GC-MS system and parameters

GC System	Clarus 600GC
MS System	Clarus 600T MS Turbo
HS Sampler	Turbomatrix HS 40 Trap
Column	USP G27 column
Injector Temperature	250 °C
Column Temperature	35 to 300 °C
Carrier Gas	Helium (0.6 mL/min)
Injection Volume	Vial pressurize 3 min at 20 psi, decay time 1.5 min on carbon trap
Internal Standard	Toluene-d_8
Mass Range	30–300 m/z

Table 12: HPLC-UV system and parameters

System	Agilent 1200 infinity
Detector	VWD G 1314A, detection wavelength 220 nm
Column	USP L1 column
Mobile Phase	Gradient of acetonitrile and water
Injection volume	20 µL

Table 13: LC-MS system and parameters

LC System	Waters ACQUITY UPLC I-Class
MS System	Waters Xevo G2-XS Q-Tof (ESI mode)
Detector	PDA Detector, wavelength 220 nm
Column	USP L1 column
Mobile Phase	Gradient of acetonitrile and water with 10 mmol ammonium acetate
Injection Volume	1 µL
Mass Range	50–1,500 m/z

Table 14: ICP-MS element analysis

System	Agilent 7900
Plasma Gas	Argon
Internal Standard	Rhodium, Yttrium, Lutetium

The following elements have been analyzed according to the ICH Q3D "Guideline on Elemental Impurities" and the USP <232> "Elemental Impurities - Limits" extended by elements which might be relevant in biopharmaceutical manufacturing:

Ag, Al, As, Au, B, Ba, Bi, Ca, Cd, Co, Cr, Cu, Fe, Ge, Hg, Ir, K, Li, Mg, Mn, Mo, Na, Ni, Os, Pb, Pd, Pt, Rh, Ru, Sb, Se, Si, Sn, Sr, Ti, Tl, V, W, Zn, Zr

The compounds in Table 15 are routinely investigated in the LC MS target analysis and are quantified if present using a multi mix standard. They include relevant additives listed for example in current European Pharmacopoeia chapter 3.1.13 "Plastic Additives" and in United States Pharmacopeia <661.1> "Plastic Materials of Construction", degradants thereof, relevant REACH compounds, and additional commonly observed extractables. The list of targets can be extended and adjusted toward further, expected extractables.

Table 15: Compounds analyzed by LC-MS target analysis

Target Name	CAS Number
1,3,5-Trimethyl-2,4,6-tris(3,5-di-*tert*-butyl-4-hydroxybenzyl)benzene	1709-70-2
2-(*tert*-Butyl)-6-methyl-4-(3-((2,4,8,10-tetrakis(*tert*-butyl)dibenzo[d,f][1,3,2] dioxaphosphepin-6-yl)oxy) propyl)phenol	203255-81-6
2,4-Di-*tert*-butylphenol	96-76-4
2,6-Di-*tert*-butyl-4-methylphenol	128-37-0
2,6-Di-*tert*-butylphenol	128-39-2
3-(3,5-Di-*tert*-butyl-4-hydroxyphenyl) propionic acid	20170-32-5
3,3'-Bis(3,5-di-*tert*-butyl-4-hydroxyphenyl)-N,N'-hexamethylenedipropionamide	23128-74-7
3,9-Bis(octadecyloxy)-2,4,8,10-tetraoxa-3,9-diphosphaspiro[5.5]undecane	3806-34-6
Benzyl butyl phthalate	85-68-7
Bis(2,4-di-*tert*-butylphenyl)phosphate	69284-93-1
Bis(2-ethylhexyl) phthalate	117-81-7
Bis(2-methoxyethyl) phthalate	117-82-8
Bisphenol A	80-05-7
Caprolactam	105-60-2
Dibutyl phthalate	84-74-2
Dilauryl 3,3'-thiodipropionate	123-28-4
Diisobutyl phthalate	84-69-5
Distearyl 3,3'-thiodipropionate	693-36-7
Erucamide	112-84-5
Ethylene bis(stearamide)	110-30-5
Ethylene bis[3,3-bis(3-*tert*-butyl-4-hydroxyphenyl) butyrate]	32509-66-3
Octadecyl 3-(3,5-di-*tert*-butyl-4-hydroxyphenyl) propionate	2082-79-3
Octanoic acid	124-07-2
Oleamide	301-02-0
Palmitamide	629-54-9
Palmitic acid (C16:0)	57-10-3
Pentaerythritol tetrakis(3-(3,5-di-*tert*-butyl-4-hydroxyphenyl)propionate)	6683-19-8
p-Toluenesulfonamide	70-55-3
Stearamide	124-26-5
Stearic acid (C18:0)	57-11-4
Tris(2,4-di-*tert*-butylphenyl) phosphate	95906-11-9
Tris(2,4-di-*tert*-butylphenyl) phosphite	31570-04-4
Tris(3,5-di-*tert*-butyl-4-hydroxybenzyl) isocyanurate	27676-62-6

4. Sartoclean® CA Basic Filter Element

4.1 GC-MS Analysis of the Water, pH 3, pH 10, 50 % Ethanol, and Ethanol Extracts

The results of the GC-MS analyses of the different extracts are summarized in the following tables.

Table 16: GC-MS analysis of the water, pH 3, and pH 10 extracts of Sartoclean® CA basic filter element

RT [min]	Compound	CAS Number	Quantity/EFA [µg/cm²]		
			Water	pH 3	pH 10
11.27	Caprolactam	105-60-2	-	0.19	0.26

Table 17: GC-MS analysis of the 50 % ethanol and ethanol extracts of Sartoclean® CA basic filter element

RT [min]	Compound	CAS Number	Quantity/EFA [µg/cm²]	
			50 % Ethanol	Ethanol
10.55	Dodecane	112-40-3	-	0.11
11.09	Glycerol*	56-81-5	0.15	2.9
11.27	Caprolactam	105-60-2	0.24	0.59
11.53	Branched alkane	-	-	0.10
13.24	Tris(3,5-di-*tert*-butyl-4-hydroxybenzyl) isocyanurate	27676-62-6	-	0.28
13.60	Pentadecane	629-62-9	-	0.12
14.61	Diethyl terephthalate	636-09-9	-	0.92
15.23	Branched alkane	-	-	0.14
16.68	Branched alkane	-	-	0.14
17.98	Branched alkane	-	-	0.12

* Detected as tris TMS derivative (CAS 6787-10-6) after derivatization.

4.2 HS GC-MS Analysis of the Water, pH 3, and pH 10 Extracts

The results of the HS GC-MS analysis of the different extracts are summarized in the following tables.

Table 18: HS GC-MS analysis of the water, pH 3, and pH 10 extracts of Sartoclean® CA basic filter element

RT [min]	Compound	CAS Number	Quantity/EFA [µg/cm²]		
			Water	pH 3	pH 10
No peaks were detected at levels above the reporting limit.					

4.3 HPLC-UV Analysis of the Water, pH 3, pH 10, 50 % Ethanol and Ethanol Extracts

The results of the HS GC-MS analysis of the different extracts are summarized in the following tables.

Table 19: HPLC-UV of the water, pH 3 and pH 10 extracts of Sartoclean® CA basic filter element

RT [min]	Compound	CAS Number	Quantity/EFA [µg/cm²]		
			Water	pH 3	pH 10
No peaks were detected at levels above the reporting limit.					

Table 20: HPLC-UV analysis of the 50 % ethanol and ethanol extracts of Sartoclean® CA basic filter element

RT [min]	Compound	CAS Number	Quantity/EFA [µg/cm²]	
			50 % Ethanol	Ethanol
12.02	Ethylene glycol terephthalate (3:3)	16958-96-6	0.77*	-
17.88	Ethylene glycol terephthalate cyclic trimer	7441-32-9	-	5.1*
41.78	Tris(2,4-di-*tert*-butylphenyl) phosphate	95906-11-9	-	0.36
50.60	Tris(2,4-di-*tert*-butylphenyl) phosphite	31570-04-4	-	1.0*

*A reference of Tris(2,4-di-*tert*-butylphenyl) phosphate (CAS 95906-11-9) was used for the quantitative estimation (10 µg/mL equals 590 mAUs).

4.4 LC-MS Target Analysis of the Water, pH 3, pH 10, 50 % Ethanol, and Ethanol Extracts

The results of the LC-MS analyses of the different extracts are summarized in the following tables.

Table 21: LC-MS target analysis of the water, pH 3 and pH 10 extracts of Sartoclean® CA basic filter element

RT [min]	Compound	CAS Number	Quantity/EFA [µg/cm²]		
			Water	pH 3	pH 10
1.85	Caprolactam	105-60-2	0.41	0.41	0.49

Table 22: LC-MS target analysis of the 50 % ethanol and ethanol extracts of Sartoclean® CA basic filter element

RT [min]	Compound	CAS Number	Quantity/EFA [µg/cm²]	
			50 % Ethanol	Ethanol
1.85	Caprolactam	105-60-2	0.63	0.55
8.65	Palmitic acid (C16:0)	57-10-3	0.76	1.1
9.12	Stearic acid (C18:0)	57-11-4	0.27	0.68
9.61	Tris(3,5-di-*tert*-butyl-4-hydroxybenzyl) isocyanurate	27676-62-6	-	0.14
11.44	Tris(2,4-di-*tert*-butylphenyl) phosphate	95906-11-9	-	0.12
13.47	Tris(2,4-di-*tert*-butylphenyl) phosphite	31570-04-4	-	0.54

4.5 LC-MS Suspect and Non-Target Screening of the Water, pH 3, pH 10, 50 % Ethanol, and Ethanol Extracts

The results of the LC-MS suspect and non-target screening analyses of the different extracts are summarized in the following tables.

Dimethyl succinate-4-hydroxy-2,2,6,6-tetramethyl-1-piperidineethanol copolymer (65447-77-0) is the established USP plastic additive 11 and used as stabilizers in one of the construction materials. Degradants, such as low molecular weight oligomers, can be detected at trace level in LC-MS using ESI ionization because of excellent ionization efficiencies. They can be considered chemically as homologs of the plastic additive itself with a similar toxicological assessment.

Table 23: LC-MS suspect and non-target screening of the water, pH 3 and pH 10 extracts of Sartoclean® CA basic filter element

RT [min]	m/z ESI pos	m/z ESI neg	UV at 220 nm	Molecular Formula	Structural Suggestion	CAS Number	Quantity/EFA [µg/cm²]		
							Water	pH 3	pH 10
0.88	202.1802	-	no	$C_{11}H_{23}NO_2$	1-(2-Hydroxyethyl)-2,2,6,6-tetramethyl-4-piperidinol	52722-86-8	< 0.10	-	-

Table 24: LC-MS suspect and non-target screening of the 50 % ethanol and ethanol extracts of Sartoclean® CA basic filter element

RT [min]	m/z ESI pos	m/z ESI neg	UV at 220 nm	Molecular Formula	Structural Suggestion	CAS Number	Quantity/EFA [µg/cm²]	
							50 % Ethanol	Ethanol
0.88	202.1802	-	no	$C_{11}H_{23}NO_2$	1-(2-Hydroxyethyl)-2,2,6,6-tetramethyl-4-piperidinol	52722-86-8	< 0.10*	< 0.10*
3.09	316.2116	-	no	$C_{16}H_{29}NO_5$	Degradant of Dimethyl succinate-4-hydroxy-2,2,6,6-tetramethyl-1-piperidineethanol copolymer	USID-39	< 0.10*	< 0.10*
3.14	-	401.0878	yes	$C_{20}H_{18}O_9$	Ethylene glycol terephthalate (2:2)	23186-89-2	< 0.30**	< 0.30**
4.22	-	593.1297	yes	$C_{30}H_{26}O_{13}$	Ethylene glycol terephthalate (3:3)	16958-96-6	< 0.77**	-
4.89	279.0979	-	no	$C_{18}H_{15}OP$	Triphenylphosphine oxide	791-28-6	-	< 0.30**
5.06	219.1869	-	no	$C_{12}H_{27}OP$	Dimethyldecylphosphine oxide	2190-95-6	-	< 0.30**
6.72	594.1606	635.1406	yes	$C_{30}H_{24}O_{12}$	Ethylene glycol terephthalate cyclic trimer	7441-32-9	< 0.30**	5.1**
9.88	540.4257	-	no	$C_{31}H_{57}NO_6$	Degradant of Dimethyl succinate-4-hydroxy-2,2,6,6-tetramethyl-1-piperidineethanol copolymer	USID-199	< 0.10*	< 0.10*
10.58	440.4095	-	no	$C_{27}H_{53}NO_3$	2-(4-Hydroxy-2,2,6,6-tetramethylpiperidin-1-yl)ethyl palmitate (HTPEP)	USID-17	< 0.10*	< 0.10*
11.10	554.4416	-	no	$C_{32}H_{59}NO_6$	HTPEP methyl succinate	USID-203	-	< 0.10*
11.51	468.4408	-	no	$C_{29}H_{57}NO_3$	2-(4-Hydroxy-2,2,6,6-tetramethylpiperidin-1-yl)ethyl stearate (HTPES)	USID-18	< 0.10*	< 0.10*
12.79	422.3989	-	no	$C_{27}H_{51}NO_2$	2-(2,2,6,6-Tetramethyl-5,6-dihydropyridin-1(2H)-yl)ethyl palmitate	USID-201	-	< 0.10*
14.56	450.43018	-	no	$C_{29}H_{55}NO_2$	2-(2,2,6,6-Tetramethyl-5,6-dihydropyridin-1(2H)-yl)ethyl stearate	USID-202	-	< 0.10*

* Estimated from GC-MS analysis, ** estimated from HPLC-UV analysis.

4.6 Element Analysis of the the Water, pH 3 and pH 10 Extracts

The results of the element analysis of the different extracts are summarized in the following tables.

Table 25: ICP-MS analysis of the water, pH 3 and pH 10 extract extracts of the Sartoclean® CA basic filter element

Element	Symbol	CAS Number	Quantity/EFA [µg/cm²]		
			Water	pH 3	pH 10
Aluminum	Al	7429-90-5	-	-	0.26
Calcium	Ca	7440-70-2	0.20	0.13	0.10
Sodium	Na	7440-23-5	-	0.67	-

5. Midicaps® Housing

5.1 GC-MS Analysis of the Water and Ethanol Extracts

The results of the GC-MS analyses of the water and ethanol extracts are summarized in the following tables.

Table 26: GC-MS analysis of the water extract of Midicaps® housing

RT [min]	Compound	CAS Number	Quantity/Surface [µg/cm²]
No peaks were detected at levels above the reporting limit.			

Table 27: GC-MS analysis of the ethanol extract of Midicaps® housing

RT [min]	Compound	CAS Number	Quantity/Surface [µg/cm²]
No peaks were detected at levels above the reporting limit.			

5.2 HS GC-MS Analysis of the Water Extracts

The result of the HS GC-MS analysis of the water extract is summarized in the following tables.

Table 28: HS GC-MS analysis of the water extract of Midicaps® housing

RT [min]	Compound	CAS Number	Quantity/Surface [µg/cm²]
No peaks were detected at levels above the reporting limit.			

5.3 HPLC-UV Analysis of the Water and Ethanol Extracts

The results of the HPLC-UV analyses of the water and ethanol extracts are summarized in the following tables.

Table 29: HPLC-UV analysis of the water extract of Midicaps® housing

RT [min]	Compound	CAS Number	Quantity/Surface [µg/cm²]
No peaks were detected at levels above the reporting limit.			

Table 30: HPLC-UV analysis of the ethanol extract of Midicaps® housing

RT [min]	Compound	CAS Number	Quantity/Surface [µg/cm²]
No peaks were detected at levels above the reporting limit.			

5.4 LC-MS Target Analysis of the Water and Ethanol Extracts

The results of the LC-MS analyses of the water and ethanol extracts are summarized in the following tables.

Table 31: LC-MS target analysis of the water extract of Midicaps® housing

RT [min]	Compound	CAS Number	Quantity/Surface [µg/cm²]
No peaks were detected at levels above the reporting limit.			

Table 32: LC-MS target analysis of the ethanol extract of Midicaps® housing

RT [min]	Compound	CAS Number	Quantity/Surface [µg/cm²]
9.69	Erucamide	112-84-5	0.10
11.44	Tris(2,4-di-*tert*-butylphenyl) phosphate	95906-11-9	0.24

5.5 LC-MS Suspect and Non-Target Screening of the Water and Ethanol Extracts

The results of the LC-MS suspect and non-target screening analyses of the water and ethanol extracts are summarized in the following tables.

Table 33: LC-MS suspect and non-target screening of the water extract of Midicaps® housing

RT [min]	m/z ESI pos	m/z ESI neg	UV at 220 nm	Molecular Formula	Structural Suggestion	CAS Number	Quantity/Surface [µg/cm²]
No additional peaks were detected in the suspect and non-target screening.							

Table 34: LC-MS suspect and non-target screening of the ethanol extract of Midicaps® housing

RT [min]	m/z ESI pos	m/z ESI neg	UV at 220 nm	Molecular Formula	Structural Suggestion	CAS Number	Quantity/Surface [µg/cm²]
No additional peaks were detected in the suspect and non-target screening.							

5.6 Element Analysis of the Water Extracts

The result of the element analysis of the water extract is summarized in the following table.

Table 35: ICP-MS analysis of the water extract of the Midicaps® housing

Element	Symbol	CAS Number	Quantity/Surface [µg/cm²]
No elements were detected at levels above the reporting limit.			

6. Maxicaps® Housing

6.1 GC-MS Analysis of the Water and Ethanol Extracts

The results of the GC-MS analyses of the water and ethanol extracts are summarized in the following tables.

Table 36: GC-MS analysis of the water extract of Maxicaps® housing

RT [min]	Compound	CAS Number	Quantity/Surface [µg/cm²]
No peaks were detected at levels above the reporting limit.			

Table 37: GC-MS analysis of the ethanol extract of Maxicaps® housing

RT [min]	Compound	CAS Number	Quantity/Surface [µg/cm²]
10.80	Dodecane	112-40-3	0.11
35.05	Tris(2,4-di-*tert*-butylphenyl) phosphite	31570-04-4	0.13

6.2 HS GC-MS Analysis of the Water Extracts

The result of the HS GC-MS analysis of the water extract is summarized in the following table.

Table 38: HS GC-MS analysis of the water extract of Maxicaps® housing

RT [min]	Compound	CAS Number	Quantity/Surface [µg/cm²]
No peaks were detected at levels above the reporting limit.			

6.3 HPLC-UV Analysis of the Water and Ethanol Extracts

The results of the HPLC-UV analyses of the water and ethanol extracts are summarized in the following tables.

Table 39: HPLC-UV analysis of the water extract of Maxicaps® housing

RT [min]	Compound	CAS Number	Quantity/Surface [µg/cm²]
No peaks were detected at levels above the reporting limit.			

Table 40: HPLC-UV analysis of the ethanol extract of Maxicaps® housing

RT [min]	Compound	CAS Number	Quantity/Surface [µg/cm²]
No peaks were detected at levels above the reporting limit.			

6.4 LC-MS Target Analysis of the Water and Ethanol Extracts

The results of the LC-MS analyses of the water and ethanol extracts are summarized in the following tables.

Table 41: LC-MS target analysis of the water extract of Maxicaps® housing

RT [min]	Compound	CAS Number	Quantity/Surface [µg/cm²]
No peaks were detected at levels above the reporting limit.			

Table 42: LC-MS target analysis of the ethanol extract of Maxicaps® housing

RT [min]	Compound	CAS Number	Quantity/Surface [µg/cm²]
13.47	Tris(2,4-di-*tert*-butylphenyl) phosphite	31570-04-4	0.35

6.5 LC-MS Suspect and Non-Target Screening of the Water and Ethanol Extracts

The results of the LC-MS suspect and non-target screening analyses of the water and ethanol extracts are summarized in the following tables.

Table 43: LC-MS suspect and non-target screening of the water extract of Maxicaps® housing

RT [min]	m/z ESI pos	m/z ESI neg	UV at 220 nm	Molecular Formula	Structural Suggestion	CAS Number	Quantity/EFA [µg/cm²]
No additional peaks were detected in the suspect and non-target screening.							

Table 44: LC-MS suspect and non-target screening of the ethanol extract of Maxicaps® housing

RT [min]	m/z ESI pos	m/z ESI neg	UV at 220 nm	Molecular Formula	Structural Suggestion	CAS Number	Quantity/EFA [µg/cm²]
9.63	934.6403	915.5994	–	$C_{56}H_{84}O_{10}$	Pentaerythritol tris(3,5-di-*tert*-butyl-4-hydroxyhydrocinnamate	84633-54-5	< 0.30 *

* estimated from HPLC analysis

6.6 Element Analysis of the Water Extracts

The result of the element analysis of the water extract is summarized in the following tables.

Table 45: ICP-MS analysis of the water extract of the Maxicaps® housing

Element	Symbol	CAS Number	Quantity/Surface [µg/cm²]
No elements were detected at levels above the reporting limit.			

7. T-Style Maxicaps® Housing

7.1 GC-MS Analysis of the Water and Ethanol Extracts

The results of the GC-MS analyses of the water and ethanol extracts are summarized in the following tables.

Table 46: GC-MS analysis of the water extract of T-Style Maxicaps® housing

RT [min]	Compound	CAS Number	Quantity/Surface [µg/cm²]
No peaks were detected at levels above the reporting limit.			

Table 47: GC-MS analysis of the ethanol extract of T-Style Maxicaps® housing

RT [min]	Compound	CAS Number	Quantity/Surface [µg/cm²]
17.74	Stearyl alcohol	112-92-5	0.73
19.01	Stearyl acrylate	4813-57-4	2.1

7.2 HS GC-MS Analysis of the Water Extracts

The result of the HS GC-MS analysis of the water extract is summarized in the following table.

Table 48: HS GC-MS analysis of the water extract of T-Style Maxicaps® housing

RT [min]	Compound	CAS Number	Quantity/Surface [µg/cm²]
No peaks were detected at levels above the reporting limit.			

7.3 HPLC-UV Analysis of the Water and Ethanol Extracts

The results of the HPLC-UV analyses of the water and ethanol extracts are summarized in the following tables.

Table 49: HPLC-UV analysis of the water extract of T-Style Maxicaps® housing

RT [min]	Compound	CAS Number	Quantity/Surface [µg/cm²]
No peaks were detected at levels above the reporting limit.			

Table 50: HPLC-UV analysis of the ethanol extract of T-Style Maxicaps® housing

RT [min]	Compound	CAS Number	Quantity/Surface [µg/cm²]
No peaks were detected at levels above the reporting limit.			

7.4 LC-MS Target Analysis of the Water and Ethanol Extracts

The results of the LC-MS analyses of the water and ethanol extracts are summarized in the following tables.

Table 51: LC-MS target analysis of the water extract of T-Style Maxicaps® housing

RT [min]	Compound	CAS Number	Quantity/Surface [µg/cm²]
No peaks were detected at levels above the reporting limit.			

Table 52: LC-MS target analysis of the ethanol extract of T-Style Maxicaps® housing

RT [min]	Compound	CAS Number	Quantity/Surface [µg/cm²]
32.67	Distearyl 3,3'-thiodipropionate	693-36-7	0.68

7.5 LC-MS Suspect and Non-Target Screening of the Water and Ethanol Extracts

The results of the LC-MS suspect and non-target screening analyses of the water and ethanol extracts are summarized in the following tables.

Table 53: LC-MS suspect and non-target screening of the water extract of T-Style Maxicaps® housing

RT [min]	m/z ESI pos	m/z ESI neg	UV at 220 nm	Molecular Formula	Structural Suggestion	CAS Number	Quantity/Surface [µg/cm²]
No additional peaks were detected in the suspect and non-target screening.							

Table 54: LC-MS suspect and non-target screening of the ethanol extract of T-Style Maxicaps® housing

RT [min]	m/z ESI pos	m/z ESI neg	UV at 220 nm	Molecular Formula	Structural Suggestion	CAS Number	Quantity/Surface [µg/cm²]
20.67	699.5956	–	–	$C_{42}H_{82}O_5S$	Distearyl 3,3'-sulphinylbispropanoate	27141-32-8	< 0.10*

*estimated from GC-MS analysis

7.6 Element Analysis of the Water Extracts

The result of the element analysis of the water extract is summarized in the following table.

Table 55: ICP-MS analysis of the water extract of the T-Style Maxicaps® housing

Element	Symbol	CAS Number	Quantity/Surface [µg/cm²]
No elements were detected at levels above the reporting limit.			

8. Summary

Samples of pure ethanol, 50 % ethanol, water, pH 3 and pH 10 extracts were evaluated regarding extractables that might be associated with the use of Sartoclean® CA filter cartridges. Midicaps®, Maxicaps®, and T-Style Maxicaps® housings were extracted with pure ethanol and water. State of the art analytical techniques were used and included headspace GC-MS and GC-MS, LC-MS, HPLC-UV, and ICP-MS. The samples after the extraction were compared to the sample blank which had no contact with the components.

Extraction of the components was performed under exaggerated worst-case conditions with regard to temperature, time, and extraction solution. A significantly lower number and quantities of substances are likely to be released under pharmaceutical and biopharmaceutical process conditions. In addition, flushing prior to use can reduce the level of process equipment-related leachables (PERLs) significantly.

The extractables identified are summarized below. Always the highest quantity in µg/cm² is provided if the compound is found in multiple analytical techniques and extraction time points.

The harsh extraction conditions in combination with sophisticated analytical techniques and lowest possible reporting limits ensure to cover almost all compounds that are potentially released as PERLs or leachables. Depending on the risk classification of the single-use device in the process, it is recommended to perform simulation or leachables studies in addition to meet qualification requirements to fulfill regulatory expectations.

Toxicological information was taken from PubChem (https://pubchem.ncbi.nlm.nih.gov/) or from Registration Dossiers of European Chemicals Agency (https://echa.europa.eu/).

Cramer classes [Methods → Select a decision tree → Cramer rules] were determined using the Toxtree software "Estimation of Toxic Hazard - A Decision Tree Approach" version 3.1.0-1851-1525442531402 (www.ideaconsult.net).

Toxicological data for the extractable elements is taken from current ICH guideline Q3D (R1) on elemental impurities.

Additional information for the safety assessment of the single-use equipment such as compound-specific information on structural alerts, chemical-specific genotoxicity data, or the permitted daily exposure (PDE) can be purchased on request from Confidence® Validation Services.

Table 56: Overview of the compounds detected in the water extract

Compound	CAS Number	Quantity$_{max}$ [µg/cm²]	Component	Analytical Method	Toxicological Information	
					LD Value	Cramer Class
1-(2-Hydroxyethyl)-2,2,6,6-tetramethyl-4-piperidinol	52722-86-8	< 0.10	Basic filter element	LC-MS$_{screening}$	LD$_{50}$ (oral rat): > 2,000 mg/kg	III
Caprolactam	105-60-2	0.41	Basic filter element	LC-MS$_{target}$	LD$_{50}$ (oral rat): 2,210 mg/kg	III

Table 57: Overview of the elements detected in the water extract

Elements	CAS Number	Quantity$_{max}$ [µg/cm²]	Component	Toxicological Information	
				LD Value	Class acc. to ICH Q3D
Calcium	7440-70-2	0.20	Basic filter element	Not available	Other element

* Not classified due to low inherent toxicity, elements do not need to be included in risk assessments according to ICH Q3D (R1).

Table 58: Overview of the compounds detected in the pH 3 extract

Compound	CAS Number	Quantity$_{max}$ [µg/cm²]	Component	Analytical Method	Toxicological Information	
					LD Value	Cramer Class
Caprolactam	105-60-2	0.41	Basic filter element	LC-MS$_{target}$	LD$_{50}$ (oral rat): 2,210 mg/kg	III

Table 59: Overview of the elements detected in the pH 3 extract

Elements	CAS Number	Quantity$_{max}$ [µg/cm²]	Component	Toxicological Information	
				LD Value	Class acc. to ICH Q3D
Calcium	7440-70-2	0.13	Basic filter element	Not available	Other element*
Sodium	7440-23-5	0.67	Basic filter element	LD$_{50}$ (intraperitoneal mouse): 4,000 mg/kg	Other element*

* Not classified due to low inherent toxicity, elements do not need to be included in risk assessments according to ICH Q3D (R1).

Table 60: Overview of the compounds detected in the pH 10 extract

Compound	CAS Number	Quantity$_{max}$ [µg/cm²]	Component	Analytical Method	Toxicological Information	
					LD Value	Cramer Class
Caprolactam	105-60-2	0.49	Basic filter element	LC-MS$_{target}$	LD_{50} (oral rat): 2,210 mg/kg	III

Table 61: Overview of the elements detected in the pH 10 extract

Elements	CAS Number	Quantity$_{max}$ [µg/cm²]	Component	Toxicological Information	
				LD Value	Class acc. to ICH Q3D
Aluminum	7429-90-5	0.26	Basic filter element	LD_{50} (oral rat): > 10,000 mg/kg	Other element*
Calcium	7440-70-2	0.10	Basic filter element	Not available	Other element*

* Not classified due to low inherent toxicity, elements do not need to be included in risk assessments according to ICH Q3D (R1).

Table 62: Overview of the compounds detected in the 50 % ethanol extracts

Compound	CAS Number	Quantity$_{max}$ [µg/cm²]	Component	Analytical Method	Toxicological Information	
					LD Value	Cramer Class
1-(2-Hydroxyethyl)-2,2,6,6-tetramethyl-4-piperidinol	52722-86-8	< 0.10	Basic filter element	LC-MS$_{screening}$	LD_{50} (oral rat): > 2,000 mg/kg	III
2-(4-Hydroxy-2,2,6,6-tetramethylpiperidin-1-yl) ethyl palmitate (HTPEP)	USID-17	< 0.10	Basic filter element	LC-MS$_{screening}$	Not available	III
2-(4-Hydroxy-2,2,6,6-tetramethylpiperidin-1-yl) ethyl stearate (HTPES)	USID-18	< 0.10	Basic filter element	LC-MS$_{screening}$	Not available	III
Caprolactam	105-60-2	0.63	Basic filter element	LC-MS$_{target}$	LD_{50} (oral rat): 2,210 mg/kg	III
Degradant of Dimethyl succinate-4-hydroxy-2,2,6,6-tetramethyl-1-piperidineethanol copolymer	USID-39	< 0.10	Basic filter element	LC-MS$_{screening}$	Not available	III
Degradant of Dimethyl succinate-4-hydroxy-2,2,6,6-tetramethyl-1-piperidineethanol copolymer	USID-199	< 0.10	Basic filter element	LC-MS$_{screening}$	Not available	III
Ethylene glycol terephthalate (2:2)	23186-89-2	< 0.30	Basic filter element	LC-MS$_{screening}$	Not available	I
Ethylene glycol terephthalate (3:3)	16958-96-6	0.77	Basic filter element	HPLC-UV	Not available	I
Ethylene glycol terephthalate cyclic trimer	7441-32-9	< 0.30	Basic filter element	LC-MS$_{screening}$	Not available	III
Glycerol	56-81-5	0.15	Basic filter element	GC-MS	TD_{Lo} (oral human): 1,428 mg/kg	I
Palmitic acid (C16:0)	57-10-3	0.76	Basic filter element	LC-MS$_{target}$	LD_{50} (oral rat): > 10,000 mg/kg	I
Stearic acid (C18:0)	57-11-4	0.27	Basic filter element	LC-MS$_{target}$	LD_{50} (oral rat): 21,500 mg/kg	I

Table 63: Overview of the compounds detected in the ethanol extracts

Compound	CAS Number	Quantity$_{max}$ [µg/cm²]	Component	Analytical Method	Toxicological Information	
					LD Value	Cramer Class
1-(2-Hydroxyethyl)-2,2,6,6-tetramethyl-4-piperidinol	52722-86-8	< 0.10	Basic filter element	LC-MS$_{screening}$	LD_{50} (oral rat): > 2,000 mg/kg	III
2-(4-Hydroxy-2,2,6,6-tetramethylpiperidin-1-yl) ethyl palmitate (HTPEP)	USID-17	< 0.10	Basic filter element	LC-MS$_{screening}$	Not available	III
2-(4-Hydroxy-2,2,6,6-tetramethylpiperidin-1-yl) ethyl stearate (HTPES)	USID-18	< 0.10	Basic filter element	LC-MS$_{screening}$	Not available	III
Branched alkane	-	0.50	Basic filter element	GC-MS	Not available	Not applicable
Caprolactam	105-60-2	0.59	Basic filter element	GC-MS	LD_{50} (oral rat): 2,210 mg/kg	III
Degradant of Dimethyl succinate-4-hydroxy-2,2,6,6-tetramethyl-1-piperidineethanol copolymer	USID-39	< 0.10	Basic filter element	LC-MS$_{screening}$	Not available	III
Degradant of Dimethyl succinate-4-hydroxy-2,2,6,6-tetramethyl-1-piperidineethanol copolymer	USID-199	< 0.10	Basic filter element	LC-MS$_{screening}$	Not available	III
Diethyl terephthalate	636-09-9	0.92	Basic filter element	GC-MS	LD_{Lo} (intraperitoneal mouse): 1,111 mg/kg	I
Dimethyldecylphosphine oxide	2190-95-6	< 0.30	Basic filter element	LC-MS$_{screening}$	Not available	III
Distearyl 3,3'-sulphinylbispropanoate	27141-32-8	< 0.10	T-Style Maxicaps® housing	LC-MS$_{screening}$	Not available	III
Distearyl 3,3'-thiodipropionate	693-36-7	0.68	T-Style Maxicaps® housing	LC-MS$_{target}$	LD_{50} (oral rat): > 2,500 mg/kg	I
Dodecane	112-40-3	0.11	Basic filter element	GC-MS	LD_{50} (oral rat): > 5,000 mg/kg	I
		0.11	Maxicaps® housing			
Erucamide	112-84-5	0.10	Midicaps® housing	LC-MS$_{target}$	LD_{50} (oral rat): > 10,000 mg/kg	III
Ethylene glycol terephthalate (2:2)	23186-89-2	< 0.30	Basic filter element	LC-MS$_{screening}$	Not available	I
Ethylene glycol terephthalate cyclic trimer	7441-32-9	5.1	Basic filter element	HPLC-UV	Not available	III
Glycerol	56-81-5	2.9	Basic filter element	GC-MS	TD_{Lo} (oral human): 1,428 mg/kg	I
2-(2,2,6,6-Tetramethyl-5,6-dihydropyridin-1(2H)-yl)ethyl palmitate	USID-201	< 0.10	Basic filter element	LC-MS$_{screening}$	Not available	III
HTPEP methyl succinate	USID-203	< 0.10	Basic filter element	LC-MS$_{screening}$	Not available	III
2-(2,2,6,6-Tetramethyl-5,6-dihydropyridin-1(2H)-yl)ethyl stearate	USID-202	< 0.10	Basic filter element	LC-MS$_{screening}$	Not available	III
Palmitic acid (C16:0)	57-10-3	1.1	Basic filter element	LC-MS$_{target}$	LD_{50} (oral rat): > 10,000 mg/kg	I
Pentadecane	629-62-9	0.12	Basic filter element	GC-MS	LD_{50} (oral rat): > 5,000 mg/kg	I
Pentaerythritol tris(3,5-di-*tert*-butyl-4-hydroxyhydrocinnamate	84633-54-5	< 0.30	Maxicaps® housing	LC-MS$_{screening}$	Not available	II
Stearic acid (C18:0)	57-11-4	0.68	Basic filter element	LC-MS$_{target}$	LD_{50} (oral rat): 21,500 mg/kg	I

Compound	CAS Number	Quantity$_{max}$ [µg/cm²]	Component	Analytical Method	Toxicological Information	
					LD Value	Cramer Class
Stearyl acrylate	4813-57-4	2.1	T-Style Maxicaps® housing	GC-MS	LD_{50} (oral rat): > 2,000 mg/kg	I
Stearyl alcohol	112-92-5	0.73	T-Style Maxicaps® housing	GC-MS	LD_{50} (oral rat): > 5,000 mg/kg	I
Triphenylphosphine oxide	791-28-6	< 0.30	Basic filter element	LC-$MS_{screening}$	LD_{50} (oral mouse): 1,380 mg/kg	III
Tris(2,4-di-*tert*-butylphenyl) phosphate	95906-11-9	0.36	Basic filter element	HPLC-UV	Not available	III
		0.24	Midicaps® housing	LC-MS_{target}		
Tris(2,4-di-*tert*-butylphenyl) phosphite	31570-04-4	1.0	Basic filter element	HPLC-UV	LD_{50} (oral rat): 2,000 mg/kg	III
		0.35	Maxicaps® housing	LC-MS_{target}		
Tris(3,5-di-*tert*-butyl-4-hydroxybenzyl) isocyanurate	27676-62-6	0.28	Basic filter element	GC-MS	LD_{50} (oral rat): > 5,000 mg/kg	III

9. Document History

Version Number	Description of Change	Version Date
00	Initial release	Oct. 2021

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DIR No.: 2822632-000-00
Status 10 | 2021