Opta® SFT

Extractables Guide

SARTORIUS

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1. Introduction

1.1 Background

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

The quality and efficacy 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 such as storage, mixing or bioreactor bags, tubing, connectors, valves and sensors, chromatography columns, filters, etc. which are in contact with process fluids should be checked for any potential compound – extractables – that can be released by the device into the process stream and the final drug product. Current analytical methods are a means to detect such process-related impurities 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) are typically summarized in its Validation Guide. This includes results of the amounts of non-volatile residues (NVR) and total organic carbon (TOC) together with the pH value, conductivity, and selected ionic species. An extractables study is required in addition to this information for a comprehensive evaluation of the device.

1.2 Standardized Extractables Approach

Sartorius has developed a fully qualified extractables approach for testing single-use devices used in the biopharmaceutical industry.[1] The data obtained by this approach can be used directly in submission documentation for example as a regulatory support file.

A component-based approach whenever possible is applied 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 recommendations of the authorities provided for example in USP <1663>. The solvents selected include pure water and ethanol and | or high and low pH solvents.[2] 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 being 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] Dorey, S. et al. Theoretical and Practical Considerations When Selecting Solvents for Use in Extractables Studies of Polymeric Contact Materials in Single-Use Systems Applied in the Production of Biopharmaceuticals. *Ind. Eng. Chem. Res.* 57, 7077-7089, 2018

1.3 Extractables Guides

Sartorius provides documented extractables information for the majority of its single-use devices in its 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 also to update 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 an Opta® SFT Sterile Connector, 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.

In order 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 (SA/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. Opta® SFT are almost exclusively sterilized by gamma-irradiation which is considered as the worst-case sterilization method compared to autoclaving. 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 SA/V such as 6:1 or 1:1 is used to obtain a relevant concentration of extractables in the extraction solvent. 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 and pure ethanol are chosen to create a database encompassing extractables that can be expected in an aqueous and organic extraction solution. Additionally, 1 M NaOH and 1 M HCl solutions – if applied – are used to mimic strong alkaline and acid conditions. 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 to 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 from our Confidence® Validation Services.

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-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 in order 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 500 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

Opta® SFT Sterile Connector are manufactured in different sizes for use in different applications. For an overview see Table 1.

Extractables data for all different sizes are required. Sartorius' extractables approach gives the highest flexibility with the most reasonable efficient analytical input. For this reason, an extraction is performed on a defined dog bone maintaining the SA/V ratio of 6:1. Seals are not considered since of limited fluid contact. The thermoplastic seal elastomers are of medical grade and fulfill the highest classification of pharmaceutical polymers USP class VI and|or comply with FDA 21 CFR 177.2600. In addition, the elastomer material used are not in the scope for Extractables testing of current standards such as USP <665> (Draft).

Extractables data can be used to asses all different sizes of available Opta® SFT products. Opta® SFT sterile connectors are usually sterilized by gamma irradiation. This sterilization method is accepted as worst-case in comparison with autoclaving. Therefore, extractables data is generated from test samples gamma irradiated at the maximum dose. Data is also valid for autoclaved Opta® SFT sterile connectors.

Table 1: Dimensions and inner surface area for available Opta® SFT connectors

Fully Assembled	Length [cm]	Inner Surface Area [cm^2]
Small Bodies Opta® SFT-I		
½" HB	18.27	68
⅜" HB	16.70	45
¼" HB	15.58	27
Small Bodies Opta® SFT-D		
½" HB	17.17	53
⅜" HB	17.1	41
¼" HB	17.17	26
Large Body Opta® SFT-I		
¾" HB	20.55	106

Table 2: Product ordering information

Product Code	Description	Pack Size
Opta® SFT-I Small body for assembly with silicone tubing		
640MS014M---D	OPTA® SFT-I Sterile Connector, ¼" Hose Barb, Male Small Connector Body.	10
640FS014M---D	OPTA® SFT-I Sterile Connector, ¼" Hose Barb, Female Small Connector Body.	10
640MS038M---D	OPTA® SFT-I Sterile Connector, ⅜" Hose Barb, Male Small Connector Body.	10
640FS038M---D	OPTA® SFT-I Sterile Connector, ⅜" Hose Barb, Female Small Connector Body.	10
640MS012M---D	OPTA® SFT-I Sterile Connector, ½" Hose Barb, Male Small Connector Body.	10
640FS012M---D	OPTA® SFT-I Sterile Connector, ½" Hose Barb, Female Small Connector Body.	10
Opta® SFT-I Large body for assembly with silicone tubing		
640ML034M---D	OPTA® SFT-I Sterile Connector, ¾" Hose Barb, Male Large Connector Body.	10
640FL034M---D	OPTA® SFT-I Sterile Connector, ¾" Hose Barb, Female Large Connector Body.	10
Opta® SFT-D Small body for assembly with TPE tubing		
641MS014M---D	OPTA® SFT-D Sterile Connector, ¼" Hose Barb, Male Small Connector Body.	10
641FS014M---D	OPTA® SFT-D Sterile Connector, ¼" Hose Barb, Female Small Connector Body.	10
641MS038M---D	OPTA® SFT-D Sterile Connector, ⅜" Hose Barb, Male Small Connector Body.	10
641FS038M---D	OPTA® SFT-D Sterile Connector, ⅜" Hose Barb, Female Small Connector Body.	10
641MS012M---D	OPTA® SFT-D Sterile Connector, ½" Hose Barb, Male Small Connector Body.	10
641FS012M---D	OPTA® SFT-D Sterile Connector, ½" Hose Barb, Female Small Connector Body	10

3.2 Scaling Approach

The quantity of extractable substances is proportional to the product contact area expressed by the inner contact area for Opta® SFT. This means that under non equilibrium conditions – one day extraction time – extractable results for one size of an Opta® SFT device 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 scientifically justified for Sartorius filter devices[3] and is applicable to Opta® SFT.

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 by using the inner surface in relation to the tested element. The required information about the inner surface is provided in Table 1.

3.3 Test Item Information

It was required to prepare dogbones to match the aimed surface area to volume ratio of 6:1 cm^2/mL. The dog bones are manufactured from the identical raw material, polycarbonate resin, and using identical manufacturing parameters as for the Opta® SFT connectors. No additional additives such as mold releasing agents are used. The dogbones are representative for the Opta® SFT connectors. In Table 3 the materials information is listed.

Table 3: Investigated Opta® SFT material and pretreatment methods applied

Component Name	Surface Area [cm^2]	Batch Number	Pretreatment
Opta® SFT	55	89853	Gamma sterilized at 51 kGy

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

3.4 Example Calculations

The extraction took place under exaggerated conditions in terms of temperature, surface area to volume ratio (SA/V), and an extraction time of seven days. 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 have to be considered. The calculated results are rounded and presented with two significant digits.

The data from water extraction should be taken for estimating extractables for aqueous process solutions such as buffers or high and low pH solvents. For process solutions with a higher organic content, the data from the ethanol extraction should be used for extractables evaluation.

Calculation example for a Small Body Opta® SFT-I ½" HB – organic solvent
An example calculation is shown for the extractables compound Stearic acid (CAS 57-11-4).

The input of the tested Opta® SFT material is considered to calculate the total amount of this compounds for the Small Body Opta® SFT-I ½" HB. The extractable data of the ethanol extraction is selected to mimic a process medium with a high content of an organic solvent.

Input test item
The highest quantity per surface area of the extractables compound is 0.043 µg/cm^2 measured by LC-MS, see Table 19. A Small Body Opta® SFT-I ½" HB has an inner contact surface area of 68 cm^2. Therefore, the total amount of this extractable per basic filter element is:

0.043 µg/cm^2 × 68 cm^2 ~ 2.9 µg

Example bulk concentration
In case of a transfer of a bulk volume of 10 L the following worst case concentration of Stearic acid in the organic bulk solution can be calculated to:

2.9 µg/10 L = 0.29 µg/L

Calculation example for a Large Body Opta® SFT-I ¾" HB – aqueous solvent
An example calculation is shown for the extractables compound Formic acid (CAS 64-18-6).

The input of the tested Opta® SFT material is considered to calculate the total amount of this compounds for the Large Body Opta® SFT-I ¾" HB. The extractable data of the water extraction is selected to mimic an aqueous process solution.

Input test item
The highest quantity per surface area of the extractables compound is 0.12 µg/cm^2 measured by LC-MS, see Table 24. A Large Body Opta® SFT-I ¾" HB has an inner contact surface area of 106 cm^2. Therefore, the total amount of this extractable per basic filter element is:

0.12 µg/cm^2 × 106 cm^2 ~ 13 µg

Example bulk concentration
In case of a transfer of a bulk volume of 500 L the following worst case concentration of Formic acid in the aqueous bulk solution can be calculated to:

13 µg/500 L = 0.026 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

3.5 Extraction Parameters and Equipment

Extraction is performed under defined conditions according to internal standard operation procedures. Water with the quality water for injection (WFI) and pure ethanol were used as extraction solvents.

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 seven days. Shaking at a minimum of 75 ± 5 rpm was applied to avoid concentration gradients in the extraction medium. 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 dog bones, glass vessels were used which are designed to maintain an SA/V ratio of 6:1. The glass extraction vessel 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 medium without the test specimen.

3.6 Analytical Scheme and Processing Procedure

Extracts generated are qualitatively and quantitatively analyzed for extractables substances. The Opta® SFT polycarbonate material is tested using the experimental set-up developed by Sartorius. Substances that can be expected from polycarbonate as extractables are: Processing aids, polymer related compounds, or additives such as stabilizers.

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 12. 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 5. 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 4: Analytical scheme

	GC-MS		HS GC-MS		HPLC-UV		LC-MS		ICP-MS		IC	
	ethanol	water	ethanol	water	ethanol	water	ethanol	water	ethanol	water	ethanol	water
Test item	×	×	-	×	×	×	×	×	×	×	×	×

Table 5: Reporting limits for the different analytical techniques

Analytical Technique	GC-MS	HS GC-MS	HPLC-UV	LC-MS	ICP-MS	IC
Reporting limit [µg/mL]	0.1	0.1	0.3	0.1	0.1	0.1
Reporting limit [µg/cm²]	0.02	0.02	0.05	0.02	0.02	0.02

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, IC 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 the analyses of the water and ethanol extracts.

Table 6: 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 7: 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 8: 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 9: 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 10: 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 additional 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.

Table 11: IC system and parameters

System	ICS-5000 Thermo Scientific	
Column	AS15 and AS 19 IC Dionex	
Targets	**Target name**	**CAS number**
	Formic acid	64-18-6
	Acetic acid	64-19-7
	Propanoic acid	79-09-4
	Butanoic acid	107-92-6
	Pentanoic acid	109-52-4
	Hexanoic acid	142-62-1
	Isobutyric acid	79-31-2
	Isovaleric acid	503-74-2
	Lactic acid	50-21-5
	Maleic acid	110-16-7
	Acrylic acid	79-10-7

The compounds in Table 12 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 12: 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] fvedioxaphosphepin-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
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. Results Opta® SFT

4.1 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 13: Results GC-MS analysis of the water extract of Opta® SFT

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

Table 14: Results GC-MS analysis of the ethanol extract of Opta® SFT

RT [min]	Compound	CAS Number	Quantity/EFA [µg/cm²]
7.79	Cyclohexanone	108-94-1	0.032
8.26	Phenol	108-95-2	0.053
9.69	Nonanal	124-19-6	0.027

4.2 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 15: HS GC-MS analysis of the water extract of Opta® SFT

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

4.3 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 16: HPLC-UV analysis of the water extract of Opta® SFT

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

Table 17: HPLC-UV analysis of the ethanol extract of Opta® SFT

RT [min]	Compound	CAS Number	Quantity/EFA [µg/cm²]
8.63	Chlorobenzene*	108-90-7	0.40

* solvent related to manufacturing process of polycarbonate, class 2 solvent according to *ICH guideline Q3C (R6) on impurities: guideline for residual solvents* with PDE of 3.6 mg/day

4.4 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 18: LC-MS target analysis of the water extract of Opta® SFT

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

Table 19: LC-MS target analysis of the ethanol extract of Opta® SFT

RT [min]	Compound	CAS Number	Quantity/EFA [µg/cm²]
7.22	2,4-Di-*tert*-butylphenol	96-76-4	0.022
9.10	Stearic acid	57-11-4	0.043
9.69	Erucamide	112-84-5	0.025

4.5 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 20: LC-MS suspect and non-target screening of the water extract of Opta® SFT

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 21: LC-MS suspect and non-target screening of the ethanol extract of Opta® SFT

RT [min]	m/z ESI pos	m/z ESI neg	UV at 220 nm	Molecular Formula	Structural Suggestion	CAS Number	Quantity/EFA [µg/cm²]
7.91	486.1912	-	-	$C_{29}H_{24}O_6$	2,2-Bis(4-hydroxyphenyl)propane carbonate (1:2) – diphenyl	20325-64-8	< 0.050*
8.62	-	255.2329	-	$C_{16}H_{32}O_2$	Palmitic acid	57-10-3	0.027
8.81	740.2853	-	-	$C_{45}H_{38}O_9$	2,2-Bis(4-hydroxyphenyl)propane carbonate (2:3) – diphenyl [5]	USID-146	< 0.050*
9.28	994.3813	-	-	$C_{61}H_{52}O_{12}$	2,2-Bis(4-hydroxyphenyl)propane carbonate (3:4) – diphenyl [5]	USID-147	< 0.050*

*estimated from HPLC-UV analyses

[5] See publication Bignardi, C. et al. Targeted and untargeted data-dependent experiments for characterization of polycarbonate food-contact plastics by ultra-high performance chromatography coupled to quadrupole orbitrap tandem mass spectrometry. *J. Chromatogr.* A, 1372, 133–144, 2014

4.6 Element Analysis of the Water and Ethanol Extracts

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

Table 22: ICP-MS analysis of the water extract of Opta® SFT

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

Table 23: Results ICP-MS analysis of the ethanol extract of the Opta® SFT

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

4.7 Ion Chromatography of the Water and Ethanol Extracts

The results of the ion chromatography of the various extracts at the associated time points are summarized in the following tables.

Table 24: Results IC analyses of the water extract of the Opta® SFT

Carboxylic Acid	CAS Number	Quantity/Surface [µg/cm²]
Formic acid	64-18-6	0.12
Acetic acid	64-19-7	0.070

Table 25: Results IC analyses of the ethanol extract of the Opta® SFT

Carboxylic Acid	CAS Number	Quantity/Surface [µg/cm²]
Acetic acid	64-19-7	0.17

5. Summary

Samples of water and ethanol extracts were evaluated regarding extractables that might be associated with the use of Opta® SFT. State of the art analytical techniques were used and included headspace GC-MS and GC-MS, LC-MS, HPLC-UV, and ICP-MS. The water and ethanol 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 medium. Significantly fewer types 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 toxicological 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 26: Overview of the compounds detected in the water extracts

Compound	CAS Number	Quantity$_{max}$ [µg/cm²]	Analytical Method	Toxicological Information	
				LD Value	Cramer Class
Acetic acid	64-19-7	0.070	IC	LD_{50} (oral rat): 3,310 mg/kg	I
Formic acid	64-18-6	0.12	IC	LD_{50} (oral rat): 1,100 mg/kg	I

Table 27: Overview of the elements detected in the water extracts

Element	CAS Number	Quantity$_{max}$ [µg/cm²]	Toxicological Information	
			LD Value	Class acc. to ICH Q3D
No elements were detected at levels above the reporting limit.				

Table 28: Overview of the compounds detected in the ethanol extracts

Compound	CAS Number	Quantity$_{max}$ [µg/cm²]	Analytical Method	Toxicological Information	
				LD Value	Cramer Class
2,4-Di-*tert*-butylphenol	96-76-4	0.022	LC-MS$_{target}$	LD_{50} (intraperitoneal mouse): 25 mg/kg	I
Acetic acid	64-19-7	0.17	IC	LD_{50} (oral rat): 3,310 mg/kg	I
2,2-Bis(4-hydroxyphenyl)propane carbonate (1:2) – diphenyl	20325-64-8	< 0.050	LC-MS$_{screening}$	not available	III
2,2-Bis(4-hydroxyphenyl)propane carbonate (2:3) – diphenyl	USID-146	< 0.050	LC-MS$_{screening}$	not available	III
2,2-Bis(4-hydroxyphenyl)propane carbonate (3:4) – diphenyl	USID-147	< 0.050	LC-MS$_{screening}$	not available	III
Chlorobenzene	108-90-7	0.40	HPLC-UV	LD_{50} (oral rat): 1,110 mg/kg	III
Cyclohexanone	108-94-1	0.032	GC-MS	LD_{50} (inhalation rat): > 6.2 mg/L air (4 h)	II
Erucamide	112-84-5	0.025	LC-MS$_{target}$	LD_{50} (oral rat): > 10,000 mg/kg	III
Nonanal	124-19-6	0.027	GC-MS	LD_{50} (oral rat): 5,000 mg/kg	I
Palmitic acid (16:0)	57-10-3	0.027	LC-MS$_{screening}$	LD_{50} (oral rat): > 10,000 mg/kg	I
Phenol	108-95-2	0.053	GC-MS	LD_{50} (oral rat): 317 mg/kg	I
Stearic acid	57-11-4	0.043	LC-MS$_{target}$	LD_{50} (oral rat): 21,500 mg/kg	I

Table 29: Overview of the elements detected in the ethanol extracts

Elements	CAS Number	Quantity$_{max}$ [µg/cm²]	Analytical Method	Toxicological Information	
				LD Value	Class acc. to ICH Q3D
No elements were detected at levels above the reporting limit.					

6. Document History

Version Number	Description of Change	Version Date
00	Initial Release Extractables data according to the Sartorius Extractables Approach published in 2018. The release of the new extractables guide does not reflect a change in material or manufacturing processes. Previous Extractables Guide Valid_Extractables_Opta_SFT_SLO5702-e.	Feb. 2021

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DIR No.: 2692756-000-00
Status 03|2021