BioPhorum

BIOPHORUM BEST PRACTICES GUIDE FOR

EXTRACTABLES TESTING OF POLYMERIC SINGLE-USE COMPONENTS USED IN BIOPHARMACEUTICAL MANUFACTURING

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About BioPhorum

BioPhorum's mission is to create environments where the global biopharmaceutical industry can collaborate and accelerate its rate of progress, for the benefit of all.

Since its inception in 2004, BioPhorum has become the open and trusted environment where senior leaders of the biopharmaceutical industry come together to openly share and discuss the emerging trends and challenges facing their industry.

Growing from an end-user group in 2008, BioPhorum now comprises over 90 manufacturers and suppliers deploying their top 3,500 leaders and subject matter experts to work in seven focused Phorums, articulating the industry's technology roadmap, defining the supply partner practices of the future, and developing and adopting best practices in drug substance, fill finish, process development and manufacturing IT. In each of these Phorums, BioPhorum facilitators bring leaders together to create future visions, mobilize teams of experts on the opportunities, create partnerships that enable change and provide the quickest route to implementation, so that the industry shares, learns and builds the best solutions together.

1.0

Introduction

In 2014, BioPhorum (at that time BioPhorum Operations Group (BPOG)) published a standardized extractables protocol[1]. This protocol became widely referred to in the industry as the 'BPOG protocol'. The protocol became one key element of the BioPhorum disposables 5-year plan[2] seeking to accelerate the understanding and uptake of single-use systems (SUS). The vision was always to create a two-step process in which extractables testing was first standardized to allow generation of comparable data and second, that data was reviewed to understand what testing was necessary and sufficient. In 2019, five years after publication, three key pieces had aligned to allow this review to happen. First, a sufficient quantity of data had been generated to allow a review. Second, thinking around leachables risk had been explored and consolidated into a widely accepted best practice guide[3]. Third, the collaboration had evolved to include supply partners as an integral part of the BioPhorum team. Data and key insights into the practicalities of running the protocol were generously provided by multiple supply partners. A scientific review of the data was performed by a group of end-users and will be published separately. This work reports major changes to the 2014 protocol following that review and brings clarification in some areas. It represents the combined opinion of the biopharmaceutical manufacturers and supply chain. Most importantly, it provides significant assurance that the data generated by the revised protocol supports biomanufacturers in delivering safe medicines while eliminating testing that was not providing additional information. The net effect is to accelerate availability of extractables data, accelerate implementation of SUS in commercial production, and contain the costs of therapeutic manufacture.

General requirements for extractables and leachables (E&L) are already mandated by regulatory agencies[4, 5]. Biopharmaceutical companies must meet these requirements to demonstrate equipment suitability and compliance with good manufacturing practice (GMP) whether the equipment is of traditional design or is single use. As a biopharmaceutical company moves a new drug molecule candidate through the clinical development process, a position on the drug candidate and manufacturing process is developed and filed with regulatory agencies. This culminates in final process validation for commercial manufacturing and licensure. Regulatory guidance for process validation outlines three distinct stages: process design, process qualification, and process verification[6]. Equipment design data for bioprocessing components, whether of traditional or single-use design, is required at each stage. Extractables testing is a key element of SUS equipment design[7–9].

Reviewing data derived from extractables testing is the mechanism by which SUS suppliers ensure safety of the polymers and chemicals used in fabrication of their products. This data is also the best means for end-users to evaluate fitness of a given SUS component for use in their specific biomanufacturing processes. SUS extractables testing data and leachables evaluation are used by end-users to assess and control potential risks to patients that the use of SUS components in product manufacturing may pose[10, 11].

Extractables testing study data provided by SUS suppliers must be well documented, reproducible, and readily interpreted to enable biopharmaceutical companies to use a scientific and risk-based approach when determining the readiness of submissions to regulatory agencies. If inadequate extractables testing data is provided by an SUS supplier, the biomanufacturer may need to delay filing while conducting their own studies. This may result in the same components being tested multiple times or even being deselected, delaying the implementation of SUS in biomanufacturing.

1.1 Updates to BioPhorum extractables protocol

User experience prior to 2014 showed that most suppliers' extractables data packages were not adequate for component qualification and process evaluation. The extractables testing conducted was not consistent between suppliers and was not presented in a way that enabled users to interpret and compare test data from different SUS suppliers or qualify SUS equipment.

In response to this, a proposal outlining standardized methods for extractables testing of SUS components was published[1] by the BioPhorum extractables workstream based on the results of a survey of 17 companies across 26 sites.

After four years of using the standardized extractables protocol, a sufficient quantity of data had been generated to allow a review. The review performed by the BioPhorum extractables workstream was focused on assessing the extraction capability of the solvents and value of the recommended time points. The full review is published separately, but the outcome is incorporated in this document which replaces the former published extractables protocol. The main changes are:

- **Removal of 5M sodium chloride and 1% polysorbate 80 as extraction solvents** since these two solvents were shown to have low unique extraction capability.
- **Elimination of the time point zero interval** as it was shown that compounds observed at this time point were present at higher concentrations at later time points.
- **Elimination of elemental analysis of 50% ethanol extracts.**

These updated recommendations reflect the opinion of end-users at biopharmaceutical organizations that produce a diversity of biologic products in a variety of regulatory environments. The protocol gives guidance on the **suggested** methods for extractables studies, including sample preparation, extraction conditions, recording test article sampling conditions, and reporting data from analysis of extracts. **Flexibility is deliberately included.** Suppliers can alter many study parameters due to restrictions based on the use of SUS, physical form factor, chemical compatibilities, etc. **if valid justifications are provided.**

1.2 Application of extractables data

The extractables testing information package to be provided by a SUS supplier should not be passed directly to a regulatory agency, except where it is essential to include in the filing to justify leachable targets and test plans. Rather, the purpose of the information package is to allow the SUS end-user to rigorously estimate the types and amounts of leachables that could be generated by the SUS component during its intended use. This allows the assessment and control of risks to patient safety as well as demonstrating process compatibility[11–15].

Note: The final responsibility for confirming the safety and efficacy of a healthcare product remains with the biomanufacturer, who will take a scientific and risk-based approach to determining what additional studies should be conducted based on the application, point and phase of use. Consequently, the biomanufacturer remains responsible for evaluation of the extractables data with respect to the specific use of a component as well as in-process fluid contact and final container leachables testing[16–18].

The BioPhorum protocol clarifies what extractables testing component suppliers should perform to provide most value to their customers and facilitate this evaluation.

1.3 Scope

BioPhorum's standardized extractables testing protocol applies to, but is not limited to the following SUS components that come into contact with product or process fluids.

- Films used in bags for storage, mixing, or as bioreactors
- Tubing
- Tubing connectors and disconnectors
- Aseptic connectors and disconnectors
- Platinum-cured molded tube connectors
- Sterilizing-grade and process filters
- Tangential flow filtration cassettes
- Sensors
- Valves
- Elastomeric parts (e.g. gaskets, O-rings, diaphragms, and septa)
- Wetted polymeric surfaces of positive displacement and centrifugation pumps
- Chromatography column housings
- Impellers (e.g. in mixing bags, bioreactor bags)
- Filling needles

The standardized extractables testing protocol does not cover final container closure systems for drug products. Also, non-fluid contact SUS components, assorted polymeric auxiliary production aid items used extemporaneously for material dispensing or transfer of ingredients and multi-use polymeric components which are subjected to cleaning validation are not in scope. These include but are not limited to:

- Vent filters
- Filters using non-polymeric matrices/media (e.g. diatomaceous earth)
- Plugs and end caps
- Sample syringes
- Sampling accessories (e.g. syringes/needles)
- Pipette tips
- Vent valves
- Scoops
- Graduated cylinders
- Beakers
- Weighing dishes
- Chromatography resins
- Any non-fluid contact SUS component

For an assembly, the preferred approach is to provide extractables data for each component. The responsibility for combining and/or scaling the component extractables data to evaluate the extractables profile of the assembly remains with the end-user. A supplier of SUS assemblies is not required to generate extractables data for SUS components that are not manufactured by them if the assembly supplier provides end-users with adequate data from the actual manufacturer of the component. System integrators/ assemblers must however ensure that adequate extractables data is provided for each component that makes up an assembly. Therefore, they must either ensure that data provided for components used is sufficient or arrange for adequate data to be generated.

1.4 Component family and assembly family testing

It is not necessary to test each component if it belongs to a family of components (i.e. it is one of a number of components made in different sizes from the same materials using the same manufacturing process at the same manufacturer). An example is silicone tubing where the family may be platinum-cured silicone tubing of varying internal diameter or wall-thickness measurements. Platinum-cured silicone tubing manufactured by a different manufacturer is not considered to be part of the same family nor is peroxide-cured silicone tubing. The item that will give worst-case test results should be chosen to represent the product family.

A supplier should list all components that meet the family member criteria above as part of the component family. Component family information can be prepared and shared using the BioPhorum component family template **https://www.biophorum.com/bpog-extractables-component-family-template/**

Similarly, assembly families can be defined and documented using the BioPhorum assembly family template (https://www.biophorum.com/bpog-extractables-assembly-family-template/). An example of an assembly family is bags manufactured from the same film, ports, and tubing but in different bag sizes, with a different number of ports, or length of tubing. Extractables data should still be provided for each component in the assembly family to allow biomanufacturers to combine/scale data from components into different assemblies.

2.0

Extractables studies

Methods applied in SUS extractables studies are specific to each category of SUS components. One key aspect of extractables testing studies is ensuring that the SUS component is exposed to a volume of solvent sufficient to effectively model what occurs during the use of the component in biomanufacturing processes.

For most components, the ratio of a sample's surface area to the volume (cm^2/mL) of solvent to which it is exposed during testing should aim for 6:1 or higher[19].

One exception to this rule involves filters, for which the ratio of effective filtration area to solvent volume (cm^2/mL) should be minimally maintained at 1:1 or better. For any other SUS components for which the 6:1 (cm^2/mL) surface-area-to-volume ratio (SA/V) standard cannot be achieved, the component surface area exposed to a given solvent volume should be maximized, justified and documented. The justification of the final component surface-area-to-solvent-volume ratio used should be based on the component's intended use.

When performing extractables testing, the sample extraction setup listed in Table 1 for the various SUS component types should be used. Extraction solvents, exposure times, and exposure temperatures by SUS component type are listed in Table 2. The proposed study conditions along with the following instructions should be adhered to as closely as is practical.

- Negative controls to calculate background levels should be included for all tests, using the same test setup minus the test article. This is particularly relevant for test setups that require additional equipment to be used during extraction, e.g. extraction of tangential-flow filtration (TFF) cassettes. For negative control, polytetrafluoroethylene (PTFE) bottles are recommended for inorganic elemental analysis, while validated or qualified clean glass bottles, as well as PTFE bottles, are suitable for organic analysis.
- If an item is pre-treated before use in a process, the item should be pre-treated the same way, e.g. flushing and sterilization before start of extraction.
- When recirculation methods are used in extractables testing on filters, inert materials such as PTFE should be used where possible for the surfaces of pumps, tubing, and other components of the fluid supply system that contact recirculating fluids.
- During the extraction, part of the test solvent may evaporate. For this reason, the initial and final volume of the test solvent should be recorded. Solvent loss should be handled appropriately:

  ≤20% loss: correction can be performed but is not necessary

  >20–50% loss: corrections must be performed

  >50% loss: justification of the solvent loss and its handling must be provided
- If the SUS component is intended for use after gamma irradiation, then a gamma-irradiated test article should be used for the extraction study. The test article should be irradiated to attain a minimum dose within 10 kGy of the maximum-allowed dose (e.g. 45–55 kGy, if the maximum-allowed dose is 55 kGy). As many irradiation facilities have a standard dose range window of 15 kGy, it is allowed to exceed the required dose by five kGy (i.e. 45–60 kGy if the maximum-allowed dose is 55 kGy). Due to the degassing of volatile organic compounds from the gamma irradiated components, the time between gamma irradiation and the extraction test should be within a maximum of eight weeks to represent the typical worst-case scenario in which the equipment may be used for production.
- If the component is intended for use after autoclaving, then an autoclaved test article should be used for the extraction study. The test article should be autoclaved according to the component's product claim. The time between the autoclaving and the extraction test should be within 24 hours or as soon as practical. If the component can be either gamma irradiated or autoclaved, then separate studies for each condition should be performed.

2.1 Addressing variability

Demonstrating consistency of extracts from materials remains an important goal for biomanufacturers. It is recognized that variability may be introduced from multiple sources including:

- Raw material (resin) variability
- Variability due to manufacturing
- Variability during extraction
- Variability during analysis

The goal is to explore this variability to build the best picture possible of which compounds might realistically be extracted. The ideal study would therefore look at components manufactured from two resin lots with separate extractions. It is however recognized that due to resin lot sizes this may not be practical. Generation of extractables data should not be delayed unduly to achieve testing on multiple resin lots and consequently two areas of flexibility are offered. First, it is possible to use different components from the same family to achieve the testing of two resin lots. Second, in order of preference testing should be considered as follows:

- Two separate extractions from components in the same family manufactured from two different resin lots

Or, if this cannot be achieved:

- Two separate extractions from two components in the same family manufactured from the same resin lot in two different manufacturing events

Or, if this cannot be achieved:

- Two extractions from one lot of component

In all cases analytical methods should follow the standard procedures of your testing laboratory.

2.2 Extraction solvents, exposure times, and exposure temperatures

Testing SUS components with the solvents, exposure times, and exposure temperatures listed in Table 2 will provide extractables data applicable to most biomanufacturing processes.

These solvents, exposure times, and exposure temperatures represent reasonable worst-case conditions for most typical biomanufacturing applications.

The extraction model solvents included in the standardized protocol comprise a broad range of process fluids commonly used in bioprocesses: water for injection (WFI), 0.1M phosphoric acid (low pH), 0.5N NaOH (high pH) and 50% ethanol representing solvents with organic content, such as aliphatic alcohols, glycols, and surfactants.

The base and acid solvent recommendations are intended to bracket most pH ranges encountered in an end-user's processes. When the suggested testing solvent pH falls outside of the range of the single use component's recommended use, e.g. due to chemical compatibility issues, the polymer compatible pH range should be used for the testing and the justification should be stated in the Extractables Test Report (see section 2.4).

The 70-day data point specified for film, tubing, and ports is necessary to support the long-term storage of material in storage bags. Depending on factors used in the accelerated stability calculation, this can be up to three years shelf life at 0°C[20].

Table 1: Testing setup for various SUS components

Item	Considerations
Bag film, bottles, and carboys	• Film: weld into a bag of size sufficient to provide an adequate volume of extract for analysis but ≤ 5L if bag ports added for filling/emptying of the bag may be clamped off during incubation. • Remove excess air from the bag. • Record the volume of the bag/bottle/carboy. • Fill the bag/bottle/carboy with a volume of solvent sufficient to maintain ≥ 6:1 (cm^2/mL) SA/V ratio. • Filled bags should be laid flat and agitated at a minimum platform speed of 50 rpm • Record the solvent, actual SA/V ratio and concentration used, extraction time, and temperature (see Table 2). • Express analytical results in µg/cm^2 (inner surfa ce area of the bag/bottle/carboy).
Tubing	• Use a sufficient length of representative inner diameter (ID) tubing to provide an adequate volume of extract for analysis and preferably meet the 6:1 (cm^2/mL) SA/V ratio. • Record the total length, ID of tubing and area of fluid contacting surfaces. • Alternatively, small ID homogenous tubing can instead be submerged. • Place on an orbital shaker at a minimum of 50 rpm for the test time period. • Use pinch clamps (or equivalent) to close the ends of filled tubing. • Record the solvent, actual SA/V ratio and concentration used, extraction time, and temperature (see Table 2). • Express analytical results in µg/cm^2.
Bag ports	• Use a sufficient number of representative ID ports to provide an adequate volume of extract for analysis. • Record the surface area(s) of the bag port(s) used in the study and number of each size of port. • Submerge in a volume of solvent sufficient to maintain ≥ 6:1 (cm^2/mL) SA/V ratio. Alternatively, maximize the number of ports that can be submerged in a volume of solvent that provides an adequate volume of extract for analysis and note the resulting SA/V ratio. • Place on an orbital shaker at a minimum of 50 rpm for the test time period. • Record the solvent, actual SA/V ratio and concentration used, extraction time, and temperature (Table 2). • Express analytical results in µg/cm^2.
Molded stoppers	• Use a sufficient number of stoppers to provide an adequate volume of extract for analysis. • Submerge the stoppers in a volume of solvent sufficient to maintain ≥ 6:1 (cm^2/mL) SA/V ratio. Alternatively, maximize the number of stoppers that can be submerged in a volume of solvent that provides an adequate volume of extract for analysis and note the resulting SA/V ratio. • Place on an orbital shaker at a minimum of 50 rpm for the test time period. • Record the solvent, actual SA/V ratio and concentration used, extraction time, and temperature (see Table 2). • Express analytical results in µg/cm^2.
Impellers (e.g. in bioreactors, mixers)	• Use a sufficient size of coupon(s) to provide an adequate volume of extract for analysis. The coupon(s) need to be representative of the finished component, i.e. be manufactured under comparable conditions. • When multiple materials are included in the impeller, perform one extractables study per material or create a proportional mix of coupons of different materials. • Record the total surface area of the coupon(s). • Submerge the coupon(s) in a volume of solvent sufficient to maintain ≥ 6:1 (cm^2/mL) SA/V ratio. Alternatively, maximize the number of coupons that can be submerged in a volume of solvent that provides an adequate volume of extract for analysis and note the resulting SA/V ratio. • Place on an orbital shaker at a minimum of 50 rpm for the test time period. • Record the solvent, actual SA/V ratio and concentration used, extraction time, and temperature (see Table 2). • Express analytical results in µg/cm^2.
Tangential-flow filtration (TFF) cassettes	• Use cassettes with a nominal effective filtration area (EFA) ≥ 0.1 m^2. • Record nominal EFA of the cassette. • Expose fluid contact surfaces to a volume of solvent sufficient to maintain ≥ 1:1 (cm^2/mL) EFA-to-volume ratio under dynamic conditions. • Any required pre-flush, sanitization, or flush steps should be performed prior to extraction. • Record solvent, actual SA/V ratio and concentration used, extraction time, and temperature (see Table 2). • Express analytical results in µg/cm^2 of EFA.

Table 1: Testing setup for various SUS components (continued)

Item	Considerations
Tubing connectors and disconnectors, fittings, overmolded junctions	• Use a sufficient number of representative overmolded junctions, connectors, fittings or disconnectors to provide an adequate volume of extract for analysis. • Record the nominal length and ID/OD of each connector, fitting, overmolded junction or disconnector • Submerge in a volume of solvent sufficient to maintain ≥ 6:1 (cm^2/mL) SA/V ratio. Alternatively, maximize the number of connectors that can be submerged in a volume of solvent that provides an adequate volume of extract for analysis and note the resulting SA/V ratio. • Place on an orbital shaker at a minimum of 50 rpm for the test time period. • Record the solvent, actual SA/V ratio and concentration used, extraction time, and temperature (Table 2). • Express analytical results in µg/cm^2.
Aseptic connectors or disconnectors	• Use a sufficient number of representative ID, connectors or disconnectors from the same component family to provide an adequate volume of extract for analysis. Wider diameter connectors can be used to facilitate reaching an adequate volume of extract for analysis. • Record the nominal length and ID of each connector. • Fill the connectors or disconnectors with a volume of solvent sufficient to maintain ≥ 6:1 (cm^2/mL) SA/V ratio. Alternatively, completely fill the connectors and note the resulting SA/V ratio. • Use PTFE caps (or equivalent inert materials) to close ends of connectors or disconnectors. • Place on an orbital shaker at a minimum of 50 rpm for the test time period. • Record the solvent, actual SA/V ratio and concentration used, extraction time, and temperature (Table 2). • Express analytical results in µg/cm^2.
Sterilizing-grade and process filters	• Use filters with nominal effective filtration area (EFA) ≥ 0.1 m^2. • Record the nominal EFA of the filter. • Recirculate or fill with a volume of solvent sufficient to maintain ≥ 1:1 (cm^2/mL) EFA to volume ratio. • If the solvent is not recirculated through the filter, place the filter filled with test solvent on an orbital shaker at a minimum of 50 rpm for the test time period. Record the solvent , actual SA/V ratio and concentration used, extraction time, and temperature (see Table 2). • Express analytical results in µg/cm^2 of EFA.
Filling needles	• Use needles with the smallest ID available (or other representative ID). • Record the nominal ID/OD and total surface area of the needle. • Submerge the needles in a volume of solvent sufficient to maintain ≥ 6:1 (cm^2/mL) SA/V ratio or closest possible SA/V ratio. The SA/V ratio used needs to be justifiable based on the component's intended use. • Place on an orbital shaker at a minimum of 50 rpm for the test time period. • Record the solvent, actual SA/V ratio and concentration used, extraction time, and temperature (see Table 2). • Express analytical results in µg/cm^2.
Chromatography column housing	• Column testing to include only the housing of chromatography columns. • Use a sufficient size of coupon(s) to provide an adequate volume of extract for analysis. The coupon(s) need to be representative of the finished component, i.e. be manufactured under comparable conditions. • When multiple materials are included in the column, perform one extractables study per material or create a proportional mix of coupons of different materials. • Record the total surface area of the coupon(s). • Submerge the coupon(s) in a volume of solvent sufficient to maintain ≥ 6:1 (cm^2/mL) SA/V ratio. Alternatively, maximize the number of coupons that can be submerged in a volume of solvent that provides an adequate volume of extract for analysis and note the resulting SA/V ratio. • Place on an orbital shaker at a minimum of 50 rpm for the test time period. • Record the solvent, actual SA/V ratio and concentration used, extraction time, and temperature (see Table 2). • Express analytical results in µg/cm^2.
Small parts; (e.g. sensors, O-rings, gaskets, check valves, diaphragms, septa)	• Small parts include, but are not limited to, sensors, O-rings, gaskets, check valves, diaphragms and septa. • Use a sufficient number of components to provide an adequate volume of extract for analysis. • Record the total area of fluid contacting surfaces. • Immerse/fill the component(s) with a volume of solvent sufficient to maintain ≥ 6:1 (cm^2/mL) SA/V ratio or closest possible SA/V ratio. The SA/V ratio used needs to be justifiable based on the component's intended use. • Use PTFE caps (or equivalent inert material) to close the ends of tubes, sensors or valves. • Place on an orbital shaker at a minimum of 50 rpm for the test time period. • Record the solvent, actual SA/V ratio and concentration used, extraction time, and temperature (seeTable 2). • Express analytical results in µg/cm^2. If there are no other components in the family, the results can be expressed as µg/component (e.g. µg/sensor).

Note: Preferably accurate surface area information should be used for SA/V calculations. Where this is not possible a good estimate of surface area is acceptable.

Table2: Extraction solvents, exposure times, and exposure temperatures by SUS component type

Component type	Solvents				Time			
	50% ethanol	0.5N NaOH	0.1M phosphoric acid	WFI[a]	24 hours	7 days	21 days	70 days
					Temperature			
					40 °C			
Bag film, bottles, and carboys intended for long-term storage	X	X	X	X	X		X	X
Tubing intended for storage bags	X	X	X	X	X		X	X
Bag ports intended for storage bags	X	X	X	X	X		X	X
Molded stoppers	X	X	X	X	X		X	X
Bag film, bottles, and carboys	X	X	X	X	X		X	
Bag ports	X	X	X	X	X		X	
Impellers (e.g. in bioreactors, mixers)	X	X	X	X	X		X	
TFF cassettes intended for perfusion/continuous processing	X	X	X	X	X		X	
Tubing	X	X	X	X	X		X	
Tubing connectors and disconnectors, fittings, overmolded junctions	X	X	X	X	X		X	
TFF cassettes	X	X	X	X	X			
Aseptic connectors and disconnectors	X	X	X	X	X	X		
Sterilizing-grade filters/process filters	X	X	X	X	X	X		
Filling needles	X	X	X	X	X			
Chromatography column housing	X				X			
Small parts (e.g. sensors, O-rings, gaskets, check valves, diaphragms, septa)	X				X			

Abbreviations:
WFI = water for injection.
[a] Deionized water can be used for this purpose if WFI is not available.

2.3 Analytical methods

The goal of the analytical techniques used in extractables testing is to identify and quantitatively assess those compounds extracted from SUS components. The results can then be used for safety assessments[17, 18], aiding in the selection of the most appropriate components.

Extracts referenced in this section on analytical techniques are the solutions generated using solvents on SUS components during extractables testing studies.

The analytical techniques proposed are selected to detect a wide range of chemical compounds. An individual compound detected at a concentration of 0.1 µg/mL or greater should be reported and also **when possible** quantified and identified by using an authentic compound.

Quantitation can be performed by using an external authentic compound in a one-point calibration curve or a multi-point calibration curve. Alternatively, quantitation can be performed by adding an internal standard to the sample and using relative response factors (RRF) that are determined for each extractable compound in a separate experiment using an authentic compound. If an authentic compound is not available, a surrogate compound with a similar structure can be used. If the structure of the extractable is unknown, an assumption can be made that the response of the unknown extractable is identical to the response of the internal standard or a surrogate compound. Quantitation by authentic material, similar structure surrogate compound, or non-related standard should be noted. Compounds observed at a concentration below 0.1 µg/mL do not need to be reported.

Analysis by high-performance liquid chromatography (HPLC) or ultra-high-performance liquid chromatography (UHPLC) coupled with photodiode array (PDA) detection and mass spectrometry (MS) is required for all extractables testing. The dilution of extracts to mitigate matrix interference concentrations is acceptable.

Mass-spectrometric (MS) analysis should be conducted in both positive and negative mode with electrospray ionization (ESI) as well as atmospheric pressure chemical ionization (APCI) techniques. Using two ionization methods provides complementary data and allows detection of the maximum range of potential extractable compounds resulting not only from bulk component material, but also from additives and degradation products.

Gas chromatography (GC) with headspace inlets for volatiles and direct injection inlets for semi-volatiles is also required for all extractables testing. Mass-spectrometric detection should be performed in conjunction with either technique to permit compound identification via mass spectral libraries. Other detectors (e.g. flame ionization, nitrogen phosphorus, or nitrogen chemiluminescence) for specific classes of compounds may be used in addition to MS detection if required by the nature of the specific component materials and potential extractables involved.

The detected and identified compounds should be reported with a Chemical Abstracts Service (CAS) registry number and with International Union of Pure and Applied Chemistry (IUPAC) nomenclature or other relevant chemical name(s). Trade names can be added in brackets after the chemical name. Reporting of the chemical structure is optional. When identification is not possible then chemical class, empirical formula, molecular weight, or most abundant ions should be reported as applicable.

Inductively-coupled plasma mass spectrometry (ICP-MS) should also be performed to detect and quantify extractable elemental impurities. Optical emission spectroscopy (OES) is an alternate detection method that may be used provided specificity and required detection limits can be achieved. Extracts should preferably be analyzed intact. In cases where the extract matrix presents challenges, dilution of the extracts to mitigate matrix interference is acceptable. At a minimum, the amounts of all elements that are specified in USP <232>[21] or ICH Q3D[22] guidelines and are present in the extracts should be quantified and reported. Additionally it is also recommended to include iron, magnesium, and any other elements known to be used in the materials of construction. It is only required to report the results from the final extractables testing time point in WFI, acid, and base extraction solvents.

Additional analytical techniques, such as total organic carbon (TOC), pH and NVR can be used if deemed appropriate by the component manufacturer.

Outlined below are the ***recommended*** approaches for the four major analytical techniques applied to the identification and quantification of extractables from SUS components. Note: These are non-exhaustive proposals and not mandatory. Established methods and new analytical techniques can be used where appropriate.

2.3.1 Liquid chromatography mass spectrometry

Table 3: Assay performance parameters for HPLC with PDA and MS detection

Instrument settings	
Column	C18
Mobile Phase A	Acidified water
Mobile Phase B	Organic (e.g. Acetonitrile and/or acidified methanol)
PDA range	200–400 nm
MS scan range	100–2000 m/z
Method qualification	
Standards	Bisphenol A (BPA) [80-05-7] Pentaerythritol tetrakis(3,5-di-tert-butyl-4-hydroxyhydrocinnamate) (Irganox® 1010[a]) [6683-19-8] Other standard(s) suitable for MS detection can be added.
Precision/repeatability	1 mg/L (ppm) BPA in 50% ethanol. Pass criterion: relative standard deviation (RSD) ≤ 20% (n=6) (ultraviolet (UV)) Alternatively, other standard(s) suitable for MS detection can be used.
Accuracy/spike recovery	1 ppm BPA in 50% ethanol. Pass criterion: 80–120% (UV) Alternatively, other standard(s) suitable for MS detection can be used.
Limit of detection (LOD)	BPA in 50% ethanol, 0.5 N NaOH, 0.1 M H_3PO_4 , and WFI. Pass criterion: N/A. Record the LOD. Alternatively, other standard(s) suitable for MS detection can be used.
System suitability test	
Precision	1 ppm BPA in a suitable solvent. Pass criterion: RSD ≤ 20% (n=6) (UV) Alternatively, other standard(s) suitable for MS detection can be used.
Sensitivity	1 ppm BPA in suitable solvent. Pass criterion: signal-to-noise ratio S/N ≥ 3 (UV) Alternatively, other standard(s) suitable for MS detection can be used.
Retention time	1 ppm BPA, and Irganox® 1010 in a suitable solvent. Pass criterion: N/A Alternatively, other standard(s) suitable for MS detection can be used.

Abbreviations:
BPA = Bisphenol A, HPLC = high-performance liquid chromatography, LOD = limit of detection, MS = mass spectrometry, PDA = photo diode array, ppm = mg/L, RSD = relative standard deviation, S/N = signal-to-noise ratio, UV = ultraviolet [a]Irganox® is a registered trademark of BASF

Notes:

- Other chromatographic instrumentation and conditions may be used to meet assay performance parameters.
- Mass-spectrometric detection should be performed in both positive and negative ESI and APCI mode.
- The standards listed in Table 3 are intended to demonstrate method chromatographic range and sensitivity. Because BPA and Irganox® 1010 do not ionize well in all four MS detection modes, alternative standards can be added.
- The limit of detection (LOD) for the selected standard(s) should be reported in each of the extraction solvents.
- For sample bracketing, an injection of standard should occur at least once for every 10 sample injections, with a percent difference of ≤ 25%.
- Control sample injections should be run to subtract matrix-associated peaks from consideration.
- The levels of peaks from samples that are quantified/semi-quantified to ≥ 0.1 ppm and also observed at levels ≥ 3 times higher than in controls should be reported.

2.3.2 Gas chromatography mass spectrometry with direct injection

Table 4: Assay performance parameters for direct injection GC with MS detection

Instrument settings	
Column	DB-5MS (or equivalent)
MS scan range	30–800 m/z
Method qualification	
Standards	Butylated hydroxytoluene (BHT) [128-37-0] n-octane [111-65-9] and/or eicosane [112-95-8] Phenanthrene-d10 (D10) [1517-22-2] (internal standard)
Precision/repeatability	1 ppm BHT in 50% ethanol. Pass criterion: RSD ≤ 20% (n=6)
Accuracy/spike recovery	1 ppm BHT in 50% ethanol. Pass criterion: 80–120%
Limit of detection (LOD)	BHT in 50% ethanol, 0.5 N NaOH, 0.1 M H_3PO_4 , and WFI. Pass criterion: N/A. Record the LOD.
System suitability test	
Precision	1 ppm BHT in dichloromethane (DCM). Pass criterion: RSD ≤ 20% (n=6)
Sensitivity	1 ppm BHT in DCM. Pass criterion: S/N ≥ 3
Retention time	1 ppm BHT, D10, n-octane and/or eicosane in DCM Pass criterion: N/A

Abbreviations:
BHT = butylated hydroxytoluene, D10 = phenanthrene-d10, DCM = dichloromethane, GC = gas chromatography, LOD = limit of detection, MS = mass spectrometry, ppm = mg/L, RSD = relative standard deviation

Notes:

- Other chromatographic instrumentation and conditions may be used to meet assay performance parameters.
- The standards listed in Table 4 are intended to demonstrate the chromatographic range and method sensitivity. Additional standards may be added, as appropriate.
- The LOD for the selected standard(s) should be reported in each of the extraction solvents.
- For sample bracketing, an injection of standard should occur at least once for every 10 sample injections, with a percent difference of ≤ 25%.
- Control sample injections should be run to subtract matrix-associated peaks from consideration.
- The levels of peaks from samples that are quantified / semi-quantified to ≥ 0.1 ppm and also observed at levels ≥3 times higher than in controls should be reported.

Liquid-liquid extraction procedure for direct injection

- Use dichloromethane (DCM) as an extraction solvent and phenanthrene-d10 as an internal standard.
- Adjust the pH as needed.
- Extract the aqueous samples in 1:1 (v/v) ratio with DCM including internal standard; repeat the extraction three times on each aqueous sample aliquot.
- Combine DCM fractions and evaporate to an appropriate and known volume to concentrate sample and allow quantitation. Redo preparation if the sample is evaporated to dryness.

2.3.3 Gas chromatography mass spectrometry with headspace injection

Table 5: Assay performance parameters for headspace sampling GC with MS detection

Instrument settings	
Column	DB-624 (or equivalent)
MS scan range	30–400 m/z
Method qualification	
Standards	Methylethyl ketone (MEK) [78-93-3] Toluene [108-88-3] Octamethylcyclotetrasiloxane (D4) [556-67-2] Toluene-d8 [2037-26-5] (internal standard)
Precision/repeatability	1 ppm MEK in water Pass criterion: RSD ≤ 20% (n=6)
Accuracy/spike recovery	1 ppm MEK in water Pass criterion: 70–130%
Limit of detection (LOD)	MEK in 50% ethanol, 0.5 N NaOH, 0.1 M H_3PO_4, and WFI. Pass criterion: N/A. Note LOD.
System suitability test	
Precision	1 ppm MEK in water. Pass criterion: RSD ≤ 20% (n=6)
Sensitivity	1 ppm MEK in water. Pass criterion: S/N ≥ 3
Retention time	1 ppm MEK, Toluene, D4 in water Pass criterion: N/A

Abbreviations:
D4 = Octamethylcyclotetrasiloxane, GC = gas chromatography, MEK = methylethyl ketone, MS = mass spectrometry, ppm = mg/L, RSD = relative standard deviation

Notes:

- Other chromatographic instrumentation and conditions may be used to meet assay performance parameters.
- The LOD for the selected standard(s) should be reported in each of the extraction solvents.
- The standards listed in Table 5 are intended to demonstrate the chromatographic range and method sensitivity. Additional standards may be added, as appropriate.
- For sample bracketing, an injection of standard should occur at least once for every 10 sample injections, with a percent difference of ≤ 25%.
- Control sample injections should be run to subtract matrix-associated peaks from consideration.
- The levels of peaks from samples that are quantified/semi-quantified to ≥ 0.1 ppm and also observed at levels ≥ 3 times higher than in controls should be reported.

2.3.4 Detection of extracts by inductively-coupled plasma with mass spectrometric detection (ICP-MS)

As a minimum, the amounts of all metals appearing in extracts that are specified in USP <232>[21] or ICH Q3D[22] guidelines should be quantified and reported. It is also recommended to include iron, magnesium, and elements known to be used in the materials of construction.

- Instrument and analysis conditions should be optimized to achieve the required sensitivity.
- Control sample injections should be run to subtract matrix associated elements from consideration.
- Quantify the detected elements based on calibration curves.
- Standard solutions containing detected elements should be used for recovery study. The recovery should be from 80 to 120%.
- Report the LOD and limit of quantification (LOQ) obtained for each element included in the study.
- The reporting threshold for elements is targeted to be 20 µg/L (ppb). The LOD may be lower or higher than 20 µg/L, depending on the element being detected, the sample matrix, and instrument parameters used.
- For the elements that have concentrations higher than the LOQ, report both the concentrations and µg/cm^2.
- Any symbols to represent less than reporting or quantification limit must be accompanied by an appropriate footnote explaining the meaning of the symbol.

2.4 Reporting extractables testing data

Biomanufacturers and suppliers engaged in developing this work have agreed to the following standard for data reporting. Data reporting should consist of two documents – a written report with QA oversight and a standardized spreadsheet to facilitate use of the data by biomanufcturers. A template for the spreadsheet report, the BioPhorum extractables data summary (BEDS) template is available on the BioPhorum website along with further information about how to complete these documents **www.biophorum.com/bpog-extractables-test-report-template-jan-2019/**

This standardized extractables testing protocol provides suppliers with a set of procedures agreed to be representative of a comprehensive range of conditions by a broad group of biopharmaceutical companies. Using this protocol suppliers can prepare standardized extractables test reports for SUS components including, but not limited to, films, tubing, tubing connectors and disconnectors, aseptic connectors and disconnectors, sterilizing-grade and process filters, TFF cassettes, sensors, valves, chromatography columns, impellers, pump systems, and filling needles.

The extractables test report provides comprehensive information on the SUS component tested, including details of the testing setup, testing conditions and analytical methods applied, and identity and quantity of extracted compounds.

The extractables test report should include the following information for each extractables study.

1. **Title page**

   This should include:

   a. Report title, study identity, report date, report revision

   b. Name and location of the laboratory performing the testing and the name and location of the sponsor, if applicable

   c. Signatures

2. **Study summary**

   The summary for SUS components tested should consist of a:

   a. Short description of the background of the testing

   b. Short description of the testing and experimental setup

   c. Short summary of results

   d. Short conclusion

3. **Study design**

   The outline of the study design information must follow the BEDS template format. It includes information on:

   a. Test-article identity and traceability

   b. Pre-treatment(s) of the test article

   c. Extraction conditions, solvents and time points

   d. Analytical information

   e. Supporting information on the test item

4. **Summary tables**

   One summary table for organic compounds and one summary table for elements should be included in the report. The formats of the summary tables must follow the BEDS format. In addition to including the summary tables in the extractables test report, the summary tables need to be made available in Excel spreadsheet format.

   It is optional to report the structures of identified compounds.

5. **Results from analyses**

   Results from each individual analysis technique should be reported separately. It is highly recommended, but not mandatory, to follow the format provided in BEDS document.

6. **Analytical methods**

   Information on each individual analysis technique should be reported separately. It is highly recommended, but not mandatory, to follow the format provided in the BEDS document. Information on analytical methods should include:

   a. Method traceability

   b. Instrument settings

   c. Method qualification

   d. System suitability test

   e. Sample preparation

   f. Approaches for quantification and identification

7. **Deviations**

   Information on deviations from the study protocol or made during analysis and execution of the extractables study should be provided.

8. **Optional information**

   It is optional to include chromatograms or spectra in the report, but these should be available upon request. Chromatographic data should then be presented using the total ion current (TIC). Presentation of spectra is primarily of interest for unknown compounds.

The final reporting should be within the oversight of the company's quality management system and can be in one of the following formats (or a combination):

- Signed pdf print-out of the BEDS document and BEDS document provided as a spreadsheet file

  The generated pdf can be issued as final report. The title page can be modified to align with company branding and requirements for signatures, etc. It is also allowed to add additional pages to the report that are not part of the BEDS template.

- Signed pdf report and summary tables provided as BEDS document

  This reporting option can be used by suppliers/labs that prefer to report the information and data in text format. It is mandatory to use the format dictated by the BEDS template for the summary tables and the study design information. All other information can be entered in any format, as long as all information required by the BEDS template is provided in the report.

3.0

Conclusion

Working as a collaborative team has had huge benefits in the development of this standardized extractables testing protocol. This work represents the consensus position of world-leading experts from over 20 biomanufacturers and 13 SUS component or assembly manufacturers. Collaborating to develop this position has allowed a data-driven approach, putting patient safety as the foremost priority while balancing the potential impact of non-value-added testing to patient access. The protocol is practical, achievable and supported by key supply chain partners.

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Acronyms

Acronyms	Definition
BPA	Bisphenol A
BHT	Butylated hydroxytoluene
D10	Phenanthrene-d10
D4	Octamethylcyclotetrasiloxane
DCM	Dichloromethane
EFA	Effective filtration area
EMA	European Medicines Agency
GC	Gas chromatography
HPLC	High-performance liquid chromatography
ICH	International Council for Harmonisation
ID	Inner diameter
LOD	Limit of detection
LOQ	Limit of quantification
MEK	Methylethyl ketone
MS	Mass spectrometry
PDA	Photodiode array
PTFE	Polytetrafluoroethylene
RSD	Relative standard deviation
SA/V ratio	Surface-area-to-volume ratio
S/N	Signal-to-noise ratio
SUS	Single-use systems
USP	US Pharmacopeia
UV	Ultraviolet