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Standardized Extractables Testing Protocol for Single-Use Systems in Biomanufacturing

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This article presents a concensus standardized extractables testing protocol for single-use systems in biomanufacturing.

The Need

General requirements for Extractables and Leachables (E&L) are already mandated by regulatory agencies.[1-2] Biopharmaceutical companies must meet these requirements in demonstrating equipment suitability and GMP compliance whether the equipment is of traditional design or single-use. However, because of the absence of specific regulatory requirements for extractables testing of Single-Use Systems (SUS) components, companies have needed to generate SUS extractables testing methods by extrapolating from their interpretation of regulatory requirements for existing container closure testing methods.

Extractables testing studies conducted by suppliers of SUS for biomanufacturing comprise filling or soaking SUS components in model solvents, and testing the resultant extracts for compounds that were released to the solvent by the treatment. Exposure times and temperature ranges are extended to exaggerate the chemical conditions of actual use. However, there are currently no industry standards for such studies, and while solvents used are often more aggressive than what is typical in biomanufacturing, the full range of conditions encountered by SUS components in actual use is not always represented. In addition, this lack of standardization in extractables testing creates difficulties for end-users in interpreting and comparing test data from different SUS suppliers.

Extractables testing study data provided by SUS suppliers must be well documented, reproducible, and readily interpretable in order for biopharmaceutical companies to use a scientific and risk-based approach in determining the readiness of various submissions to regulatory agencies. Current regulatory guidance[1-2] requires that biopharmaceutical manufacturers ensure the manufacturing systems do not adulterate the final drug product. The end users have used SUS extractables testing data and leachables evaluation to assess potential risks to patients of the use of these components in product manufacturing. If extractables testing data provided by an SUS supplier are not sufficient to perform adequate assessment of risks, it is the time-consuming process for the biopharmaceutical company to conduct their own studies to generate sufficient extractables testing data. This results in the same components being tested multiple times and delay in applications of SUS in biomanufacturing.

For a biopharmaceutical company to move a new drug molecule candidate through the clinical development process, the company first develops a position on the drug candidate that will be presented to regulatory agencies for concurrence. This position is applied to successive stages of the clinical development process, culminating 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.[3] 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. Reviewing data derived from extractables testing is the mechanism by which SUS suppliers ensure safety of the polymers 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 technology has numerous advantages for improving cycle time of biopharmaceutical products and in reducing overall manufacturing costs. Because it is in the interest of SUS suppliers, SUS end users, and patients in need of the biopharmaceutical products to accelerate the implementation of SUS components within the biopharmaceutical industry, a standardized extractables testing protocol with an agreed-upon set of testing methods to generate and analyze extracts is needed to establish common expectations among suppliers, end-users, and regulators on the type of extractables testing data to be generated.[4] The benefits of such standardization would include not only an enhanced ability of end-users to make informed choices when comparing SUS components from various suppliers, but also would assist SUS suppliers in more efficiently selecting materials in line with end-user needs and in controlling product variability.

BioPhorum Operations Group Extractables Work Group Proposal

The proposal outlining standardized methods for extractables testing of SUS components contained in this article was developed by the Extractables Work Group of the BioPhorum Operations Group (BPOG) and is based on results of a survey of 17 major BPOG member companies across 26 sites. As such, these recommendations reflect the broad SUS applications of end-users at biopharmaceutical organizations that produce a diversity of biologic products in a variety of regulatory environments. The protocol covers the methods used for extractables testing studies, including sample preparation, extraction conditions, recording test article sampling conditions, and reporting data from analysis of extracts.

Integration of these proposals by SUS suppliers into their existing product lifecycle management processes would be highly beneficial to suppliers to ensure that a comprehensive and consistent set of extractables testing data are readily available to biopharmaceutical end-users. A draft of the proposal was previously provided to 10 SUS equipment suppliers and 10 contract analytical testing laboratories for feedback on the methods proposed. Each responding organization was encouraged to provide both a written response as well as to participate in discussion forums with members of the BPOG Extractables Work Group.

Application of the Extractables Data

The extractables testing information package to be provided by an SUS supplier is not intended to be passed directly to a regulatory agency without a process- and product-specific evaluation. Rather, the purpose of the information package is to allow the SUS end-user to rigorously estimate the types and amounts of leachables that will be generated by the SUS component during its intended bioprocessing use in order to assess risks to patient safety and to demonstrate product compatibility, process performance, and fitness of the functional design for its intended purpose.[5-16] The use of standardized protocols also provides a baseline which can be used for comparative assessments of SUS from different suppliers as back-ups or alternate sources. Such an approach greatly facilitates the long-term success of SUS for biopharmaceutical manufacturing.

Note: the final responsibility for confirming the safety and efficacy of a healthcare product remains that of the end user, who should take a science and risk-based approach to determining what additional studies should be conducted based on the application, point and phase of use.

Scope

This BPOG's standardized extractables testing protocol applies, but is not limited, to the following SUS components that come into contact with product or process fluids. The standardized extractables testing protocol does not cover final container closure systems.

- Bags and films used for storage, mixing, or as bioreactors
- Tubing
- Tubing connectors and disconnectors
- Aseptic connectors and disconnectors
- Sterilizing-grade and process filters
- Tangential flow filtration cassettes
- Sensors
- Valves
- Elastomeric parts (gaskets, O-rings, diaphragms, and septum)
- Wetted polymeric surfaces of positive displacement pumps
- Chromatography columns
- Molded parts of mixers (e.g., impellers)
- Filling needles

A supplier of SUS assemblies is not required to generate extractables data for SUS components not manufactured by them as long as the assembly supplier provides end-users with data from the actual manufacturer of the component that complies with the standardized extractables testing protocol.

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 compo-

nent is exposed to a volume of solvent sufficient to effectively model what occurs during use of the component in actual biomanufacturing processes. For the majority of 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 be maintained at 6:1 or greater.[17] One important exception to this rule involves filters, for which the ratio of effective filtration area to solvent volume (cm^2/mL) should be 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, exposure of component surface area to solvent volume ratio should be maximized. In these exceptional cases, the final component surface area to solvent volume ratio arrived at should be justifiable based on the component's intended use.

When performing extractables testing, the sample extraction setups listed in Table A for the various SUS component types are used. Extraction solvents, exposure times, and exposure temperatures by SUS component type are listed in Table B. 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. For negative control, polytetrafluoroethylene (PTFE) bottles are recommended for inorganic elemental analysis, while validated or qualified clean glass bottles are suitable for organic analysis.
- If an item is pre-treated prior to actual use, the item should be pre-treated the same way before being used in extractables testing. For example, extractables testing results for a gamma-irradiated component cannot be used to represent the results of the same component after autoclaving.

Storage, Mixing, or Bioreactor Bags and Films
• Use a bag of size sufficient to provide an adequate volume of extract for analysis but ≤ 5L • Record the volume of the bag • Fill the bag with a volume of solvent sufficient to maintain 6:1 (cm^2/mL) surface area to volume (SA/V) ratio • Place on an orbital or rocker shaker at a minimum of 50 rpm[a] for the test time period • Record the solvent and concentration used, extraction time, and temperature (Table B) • Express analytical results in µg/cm^2 Note: Studies performed on 2D bags of same materials can represent other bag designs, e.g., 3D bags
Tubing
• Use a sufficient length of ½" ID (inner diameter) tubing to provide an adequate volume of extract for analysis • Record the total length and ID of tubing • Fill the tubing with a volume of solvent sufficient to maintain 6:1 (cm^2/mL) SA/V ratio • Use pinch clamps (or equivalent) to close the ends • Place on orbital shaker at a minimum of 50 rpm for the test time period • Record solvent and concentration used, extraction time, and temperature (Table B) • Express analytical results in µg/cm and µg/cm^2
Tubing Connectors or Disconnectors
• Use a sufficient number of ½" ID connectors or disconnectors to provide adequate volume of extract for analysis • Record length and ID of each connector • Submerge in a volume of solvent sufficient to maintain 6:1 (cm^2/mL) SA/V ratio • Place on orbital shaker at a minimum of 50 rpm for the test time period • Record the solvent and concentration used, extraction time, and temperature (Table B) • Express analytical results in µg/cm^2 and µg/unit
Aseptic Connectors or Disconnectors
• Use a sufficient number of ½" ID connectors or disconnectors to provide an adequate volume of extract for analysis • Multiple connectors can be used and extracts pooled for analysis • Record the 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 • 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 and concentration used, extraction time, and temperature (Table B) • Express analytical results in µg/cm^2 and µg/unit
Sterilizing-grade and Process Filters
• Use filters with Effective Filtration Area (EFA) ≥ 0.1 m^2 • Record the EFA of 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 and concentration used, extraction time, and temperature (Table B) • Express analytical results in µg/cm^2 of EFA and µg/unit
Tangential-flow Filtration Cassettes
• Use cassettes with an EFA ≥ 0.1 m^2 • Record EFA of cassette • Recirculate volume of solvent sufficient to maintain 1:1 (cm^2/mL) EFA to volume ratio • Any required preflush, sanitization, or flush steps should be performed prior to extraction • Record solvent and concentration used, extraction time, and temperature (Table B) • Express analytical results in µg/cm^2 of EFA and µg/unit
Sensors or Valves
• Use a sufficient number of ½" ID sensors or valves to provide an adequate volume of extract for analysis • Multiple sensors or valves can be used and extracts pooled for analytical purpose • Record the total surface area as the sum of tube and functional sensor surfaces for sensors; record the total surface area as the sum of valve diaphragm and tube surfaces for valves • Fill the sensor set or valve with a volume of solvent sufficient to maintain 6:1 (cm^2/mL) SA/V ratio or closest possible SA/V ratio • Use PTFE caps (or equivalent inert material) to close ends of tubes of sensor or valve • Place on an orbital shaker at a minimum of 50 rpm for the test time period • Record the solvent and concentration used, extraction time, and temperature (Table B) • Express analytical results in µg/sensor or µg/valve, and µg/cm^2
Chromatography Columns or Molded Parts of Mixers or Elastomeric Parts (gaskets, O-rings, diaphragms, and septum) or Wetted Polymeric Surfaces of Positive Displacement Pumps
• Use a sufficient size of coupon representing the finished column or molded parts of the mixer or elastomeric parts (gaskets, O-rings, diaphragms, and septum) to provide an adequate volume of extract for analysis • Record the total surface area of the coupon • Submerge the coupon in a volume of solvent sufficient to maintain 6:1 (cm^2/mL) SA/V ratio • Place on an orbital shaker at a minimum of 50 rpm for the test time period • Record the solvent and concentration used, extraction time, and temperature (Table B) • Express analytical results in µg/cm^2
Filling Needles
• Use needles with smallest ID available • Record the inner diameter and total surface area of the needle • Submerge needles in a volume of solvent sufficient to maintain 6:1 (cm^2/mL) SA/V ratio or closest possible SA/V ratio • Place on an orbital shaker at a minimum of 50 rpm for the test time period • Record the solvent and concentration used, extraction time, and temperature (Table B) • Express analytical results in µg/cm^2
Abbreviation: rpm = revolutions per minute or rocks per minute [a] 50 rpm at 20 mm radius

Table A. Testing setup for various SUS components.

- If the SUS component is intended for use after gamma irradiation, 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 to 55 kGy, if the maximum-allowed dose is 55 kGy). The irradiation facility (i.e,. irradiator design, equipment, and process) used should be validated according to ANSI/AAMI/ISO 11137-1:2006 and ANSI/AAMI/ISO 11137-3:2006. Due to the fact of degasing of volatile organic compounds from the gamma-irradiated components, the time between the gamma irradiation and the extraction test should be five weeks to represent the typical worst case production scenario.
- If the component is intended for use after autoclaving, an autoclaved test article should be used for extraction study. The test article should be autoclaved according to the component 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, separate studies for each condition should be performed.
- At least two samples of a component should be tested for extractables, each from different production lot.
- When recirculation methods are used in extractables testing on filters, inert materials such as PTFE should be used for 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 starting and end volume of the test solvent should be recorded. These values may be used in calculations for correction of analytical results, where appropriate.

Choice of Extraction Solvents, Exposure Times, and Exposure Temperatures

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

	Solvents						Time				
	50% Ethanol	1% PS-80	5M NaCl	0.5N NaOH	0.1 M Phosphoric acid	WFI[a]	Time 0 (≤ 30 min)	24 hours	7 days	21 days	70 days
							Temperature				
							Ambient (25°C)	40°C			
Storage, Mixing, and Bioreactor Bags	X	X	X	X	X	X	X	X		X	X[b]
Tubing	X	X	X	X	X	X	X	X		X	X[b,c]
Tubing Connectors and Disconnectors	X	X	X	X	X	X	X	X		X	
Aseptic Connectors and Disconnectors	X	X	X	X	X	X	X	X	X		
Sterilizing-grade Filters/Process Filters	X	X	X	X	X	X	X	X	X		
Tangential-flow Filtration Cassettes	X	X	X	X	X	X	X	X		X	
Sensors and Valves	X	X	X	X	X	X	X	X		X[d]	
Chromatography Columns; Elastomeric Parts (gaskets, O-rings, diaphragms, and septum); Wetted Polymeric Surfaces of Positive Displacement Pumps	X	X	X	X	X	X	X	X			
Molded Parts of Mixers	X	X	X	X	X	X	X	X		X	
Filling Needles	X	X	X	X	X	X	X	X			

Abbreviations: PS-80 = Polysorbate-80; WFI = water for injection; min = minute.
[a] Deionized water can be used for this purpose if WFI is not available.
[b] Duration, specified for testing storage bags and tubing, is necessary to support 3-year storage time at 0°C.
[c] Tubing is included because tubing sections are typically integrated with bags during storage.
[d] The 21-day time-point applies only to sensors used with bioreactors (e.g. for dissolved oxygen and pH).

Table B. Extraction solvents, exposure times, and exposure temperatures by SUS component type.

processes. Unique solvents and process conditions have been excluded. Solvents, exposure times, and exposure temperatures are recommended that represent reasonable worst-case conditions for most typical biomanufacturing applications. The initial time point at Time 0 (≤ 30 minutes) is based on well accepted analytical practices and sets a baseline for extraction kinetics evaluation. Studies have shown that the amount of some volatile extractable compounds decreased over time from Time 0 to 24 hours.[24] It is also critical for cell viability assessment as volatile leachable compounds from bags come in direct contact with process fluid immediately which can impact cell culture processes.[13] Protein stability can be impacted by change of pH or interaction of protein solutions with volatile and/or semivolatile leachable compounds on immediate contact. The 70-day data point specified for the storage bags and tubing is necessary to support long term storage of up to three years shelf life at 0°C storage condition. Tubing was included simply because tubing sections are typically integrated to the bag during storage. The combination of the temperature and time was established based on the ASTM F1980-07 Standard Guide for Accelerated Aging of Sterile Medical Devices.[18]

The common extraction model solvents included here comprise a broad range of buffer-based process fluids: Water For Injection (WFI), 0.1M phosphoric acid (low pH), and 0.5N NaOH (high pH). The choice of 50% ethanol was selected to represent organic solvents commonly used in bioprocesses such as aliphatic alcohols and glycols. Typical surfactant-containing aqueous solutions are represented by 1% Polysorbate-80. Polysorbate-80, even at very low concentration, facilitates the leaching of small-molecule, aromatic compounds.[25] The model solvent chosen to represent high salt concentrations in bioprocessing was 5M NaCl (high ionic strength). A review of available data packages and publications from SUS suppliers and end users indicated that certain chemicals observed in a NaCl extract were not detected in WFI extract.[6,19] In other cases, the same extractables were observed in both NaCl and WFI extracts, but at significantly higher concentrations in the former. The six solvents also effectively simulate protein solutions which typically involve high pH, low pH, salt, WFI, organic compound and surfactant.

The base and acid recommendations cover most pH ranges in user operational conditions. When the recommended pH range is outside of the single-use component's product claim due to chemical compatibility issue (e.g., polycarbonate-based aseptic connector is not compatible with 0.5N NaOH), the compatible pH range should be used for the testing and the justification should be stated in the Summary Extractables Statement.

Analytical Techniques

The goal of the analytical techniques used in extractables testing is to identify and quantitatively assess compounds resulting from the extraction of SUS components. The results can then be used for safety assessments.[20] In cases where quantitation is not possible, semi-quantitative values should be reported. Extracts referenced in this section on analytical techniques are the solutions generated by the use of solvents on SUS components during extractables testing studies.

The analytical techniques proposed in this article were selected to detect the widest possible range of chemical compounds. An individual compound detected at a concentration of 0.1 µg/mL or greater should be identified, confirmed and quantified by use of an authentic reference compound (e.g., extractables known to result from component raw materials). Compounds observed at a concentration below 0.1 µg/mL should be identified by mass spectral library match and confirmed with quantitation if an authentic reference compound is available. When an authentic reference compound is not available, a chemically similar compound may be used although this will result in semi-quantitative values in the results. (See Appendix: Recommended Analytical Techniques for Extractables Identification and Quantification).

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. It is acknowledged that certain extraction solvents may present challenges in detection (i.e., PS-80 extracts). Dilution of the extracts to acceptable matrix interference concentrations is acceptable in these cases (e.g., 0.1% PS-80).

Mass spectrometric analysis should be conducted in both positive and negative mode with Electrospray Ionization (ESI) as well as Atmospheric Pressure Chemical Ionization (APCI) techniques. Use of 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 from additives and degradation products as well.

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. Alternate detectors (e.g., nitrogen phosphorus, flame ionization, or nitrogen chemiluminescence) for specific classes of compounds may be used in addition to MS detection if required due to the nature of the specific component materials and potential extractables involved.

Inductively-Coupled Plasma Mass Spectrometry (ICP-MS) also should be performed to detect and quantify extract-

able metals. Optical Emission Spectroscopy (OES) as an alternate detection method may be used provided specificity and required detection limits can be achieved. Extracts should be analyzed intact unless dilution of the samples allows the required detection limits to be met for all metals of interest. In cases where the extract matrix would produce known interferences in detecting particular metals, a different isotope should be selected to minimize the interference. At a minimum, the amounts of all metals appearing in extracts that are specified in USP <232>,[21] EMEA,[22] and ICH guidelines[23] should be quantified and reported. While it is only required to record results from the final extractables testing time point, additional time points may be analyzed as necessary.

The detected and identified compounds should be named based on International Union of Pure and Applied Chemistry (IUPAC) nomenclature, and reported with Chemical Abstracts Service (CAS) registry number, empirical formulas, chemical structures, and molecular weights, when possible.

Additional analytical techniques should be used to supplement the required data, in particular, to determine the Total Organic Carbon (TOC) and pH of extracts when the test solvent does not interfere. Nonvolatile residue determination may be necessary in addition to the required analytical techniques when the test solvent is volatile. Resulting extractables testing data should be compiled into an extractables test report with representative chromatograms and raw data tables of the results. The extractables test report should include the amount and identity of known compounds and the estimated amount and class of compound for unknowns. The extractables test report also should include the analytical conditions for each technique as well as any additional discussion necessary to provide enough context such that

Test Article			
Number of Test Articles			
Part Number			
Lot Numbers			
Pretreatment[a]	**Variable(s)**	**Units**	**Value(s)**
Gamma irradiation	Dose	kGy	
Autoclave	Time, temperature, number of cycles	minutes, °C, #	
Pre-flush	Fluid identity, duration, temperature, volume	Name, minutes, °C, L	
Test Article Extraction Conditions	**Variable**	**Units**	**Value(s)**
	Temperature	°C	
	Duration	Minutes, hours, days	
	Solvent contact surface area	cm^2	
	Solvent volume	mL	
	Surface area to volume ratio	ratio	
Supporting Information			
Bags	Film thickness	mm	
	Volume (capacity)	L	
Tubing	Wall thickness	mm	
	Internal diameter	mm	
	Length	mm	
Tubing connectors and aseptic connectors	Internal diameter	mm	
	Length	mm	
Filters and TFF cassettes	EFA	m^2	
Filling needles	Internal diameter	mm	
2D and 3D bags	Time between film manufacturing and gamma irradiation	Days (Lot 1) Days (Lot 2) Days (Lot 3), if applicable	
All gamma-irradiated components	Time between gamma irradiation and extraction	days	
	Typical dose range during normal manufacturing	kGy	

Abbreviations: EFA = effective filtration area; TFF = tangential flow filtration.
[a] If none, indicate N/A (not applicable).

Table C. Information to be reported in summary extractables statement.

the results are readily interpretable by end-users. Specific analytical parameters and method sensitivity criteria are presented in the Appendix.

Extractables Test Report

This standardized extractables testing protocol provides suppliers with a set of procedures agreed upon as representative of a comprehensive range of conditions by a broad group of companies. Suppliers can then prepare standardized extractables test reports for SUS components, including, but not limited to, bags and films, tubing, tubing connectors and disconnectors, aseptic connectors and disconnectors, sterilizing-grade and process filters, tangential-flow filter cassettes, sensors, valves, chromatography columns, molded parts of mixers, and filling needles. The extractables test report provides comprehensive information on the SUS component tested, including materials of construction, 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. **Summary Extractables Statement**
   The summary extractables statement for SUS components tested should consist of:
   a. Summary of the materials of construction
   b. Testing setup
   c. Extraction conditions applied
   d. Analytical methods applied
   e. Identity and quantity of extracted compounds (analytical results)

2. **Details of pre-treatment methods**
   a. Gamma irradiation description includes the minimum and maximum dose allowed for the component during the manufacturing process and the actual dose used to gamma irradiate the component for testing.
   b. Autoclave description should include time (total and exposure) and temperature of each autoclave cycle. If multiple cycles are performed, the number of cycles also should be specified.
   c. Pre-flush description includes flush fluid used, temperature, time, and flush volume. Pre-flush typically applies to components such as tangential flow filtration cassettes.

3. **Time intervals between manufacture, irradiation, and testing for gamma-irradiated bag films**
   The time interval between when a bag film is manufactured and when gamma irradiation is applied should be recorded. The additives in polymers used to make bag films can oxidize over time, and the oxidized additives can generate different extractables compared to virgin additives. The time interval between gamma irradiation and extraction also should be reported.

4. **Thickness of the bag films and tubing**
   Multiple thicknesses of bag films are often available, e.g., 0.05 mm, 0.15 mm, and 0.5 mm. The thickness of the bag film or tubing should be reported.

5. **Composition of fluid-contacting surface materials**
   Materials comprising the surfaces that contact test solvents during testing, e.g., the inner surface of tubing or connectors, the interior of bioprocess bags as well materials of construction of other layers, or the fluid-contacting components of filters, should be specified.

6. **Traceability of components**
   The part numbers and lot numbers of test articles should be reported. These numbers should be traceable back to the lot numbers of resins used in manufacturing of the tested component.

For each extractables study, the following information in Table C is recorded and included in each summary extractables statement. Results of extract analysis (compound identities and amounts) are recorded separately.

Next Steps

The companies involved in the BPOG Extractables Work Group encourage the adoption by all SUS suppliers of the recommendations made in this article. Not only will adoption enable results from extractables testing on SUS components to be compared and used by SUS integrators and end-users, but also will simplify the approach of SUS suppliers to serve their markets. Such standardization will provide a set of common expectations for SUS component performance that SUS end-users, SUS suppliers, and regulators can reference as the current good extractables testing practice.

This standardized extractables testing protocol also will be made available to standard-setting organizations, such as ASTM and USP for consideration in developing a consensus standard. We expect that once a consensus standard has been agreed upon that a transition plan will be created with reasonable timeframes permitting suppliers to bridge any existing gaps between the new standard and their existing extractables testing and documentation procedures.

Appendix: Recommended Analytical Techniques for Extractables Identification and Quantification

Outlined below are the recommended approaches for the four major analytical techniques applied to the identification and quantification of extractables from SUS components.

1. Detection of Extracts by LC-UV-MS: HPLC with UV Photodiode Array Detection and Mass Spectrometry

Standards	Bisphenol A (BPA) and Irganox® 1010a (method sensitivity and range)
Limit of Detection	BPA, standard signal-to-noise ratio ≥ 3
Precision (UV)	1 ppm BPA, RSD ≤ 20% (n = 6)
Spike Recovery (UV)	80 - 120%
Column	C18
Mobile Phase A	Acidified water
Mobile Phase B	Organic (ACN and/or acidified MeOH)
PDA range	200 to 400 nm

Abbreviations: LC = liquid chromatography, MS = mass spectrometry, HPLC = high performance liquid chromatography, UV = ultraviolet, RSD = relative standard deviation, ACN = acetonitrile, MeOH = methanol
[a] Irganox is registered trademark of Ciba Specialty Chemical Corporation

Table D. Assay performance parameters for HPLC with UV photodiode array and mass detection.

Notes:

- Other chromatographic instrumentation, such as Ultra-High Performance Liquid Chromatography (UHPLC) and conditions may be used to meet assay performance parameters.
- Limit of Quantitation (LOQ) should be reported.
- Standards listed in the table are to demonstrate method sensitivity and chromatographic range. Additional known extractable compounds should be prepared as standards injected for each unique material.
- An injection of standard should occur at least once for every 10 sample injections.
- Spike is 1 ppm BPA in water and in 50% water/50% ethanol.
- Control sample injections should be run to subtract matrix-associated peaks from consideration.
- Report levels of peaks from samples that are also observed in controls ≥ 50% higher than in controls.
- Mass spectrometric detection is both +/- Electrospray Ionization (ESI) and Atmospheric Pressure Chemical Ionization (APCI).
- Mass spectrometric detection scan range is 100 to 2000 m/z.
- In cases where quantitation is not possible, semi-quantitative values may be reported by reference to responses of suitable standards.
- For semi-quantitative analysis, results for peaks with a signal-to-noise ratio > 10 or peaks above area of lowest standard injection should be reported.

2. Detection of Extracts by GC-MS: Direct Injection Gas Chromatography with Mass Spectrometry

Standards	n-Octane and butylated hydroxytoluene (method sensitivity and range)
Limit of Detection	BHT, standard signal-to-noise ratio ≥ 3
Precision (TIC)	1 ppm BHT, RSD ≤ 20% (n = 6);
Spike Recovery (TIC)	80 - 120%
Column	DB-5MS (or equivalent)

Abbreviations: GC = gas chromatography, TIC = total ion current, BHT = butylated hydroxytoluene, RSD = relative standard deviation

Table E. Assay performance parameters for direct injection GC with mass detection.

Notes:

- Other chromatographic instrumentation and conditions may be used to meet assay performance parameters.
- Chromatographic data should be presented using the Total Ion Current (TIC).
- Limit of Quantitation (LOQ) should be reported.
- Standards listed in the table are to demonstrate method sensitivity and chromatographic range. Additional known extractable compounds should be prepared as standards injected for each unique material.
- An injection of standard should occur at least once for every 10 sample injections.
- Spike is 1 ppm BHT in water and in 1% Polysorbate-80 extraction solvent.
- Control sample injections should be run to subtract matrix-associated peaks from consideration.
- Report levels of peaks from samples that are also observed in controls ≥ 50% higher than in controls.
- Mass spectrometric detection scan range is 30 to 600 m/z.
- In cases where quantitation is not possible, semi-quantitative values may be reported by reference to responses of suitable standards.
- For semi-quantitative analysis, results for peaks with a signal-to-noise ratio > 10 or peaks above area of lowest standard injection 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 pH as needed.
- Extract aqueous samples in 1:1 (v/v) ratio with DCM, including internal standard; repeat extraction three times

on each aqueous sample aliquot.
- Combine DCM fractions and evaporate to approximately 1 mL; repeat preparation if sample reaches significantly less than 1 mL.
- Reconstitute concentrated extract for analysis with DCM to final volume equal to original sample aliquot volume.

3. Detection of Extracts by GC-MS: Headspace Sampling GC with Mass Spectrometry

Standards	Methanol, MEK and octamethylcyclotetrasiloxane (method sensitivity and range)
Internal Standard	Toluene-d_8
Limit of Detection	MEK, standard signal-to-noise ratio ≥ 3
Precision (TIC)	1 ppm MEK, RSD ≤ 20% (n = 6)
Spike Recovery (TIC)	70 - 130%
Column	DB-624 (or equivalent)
Abbreviations: MEK = methylethyl ketone, RSD = relative standard deviation, TIC = total ion current	

Table F. Assay performance parameters for headspace sampling GC with mass detection.

Notes:
- Other chromatographic instrumentation and conditions may be used to meet assay performance parameters.
- Chromatographic data should be presented using the Total Ion Current (TIC).
- Limit of Quantitation (LOQ) should be reported.
- Standards listed in the table are to demonstrate method sensitivity and chromatographic range. Additional known extractable compounds should be prepared as standards injected for each unique material.
- An injection of standard should occur at least once for every 10 sample injections.
- Spike is 1 ppm MEK in water and in 1% Polysorbate-80 extraction solvent.
- Control sample injections should be run to subtract matrix-associated peaks from consideration.
- Report levels of peaks from samples that are also observed in controls ≥ 50% higher than in controls.
- Mass spectrometric detection scan range is 30 to 400 m/z.
- In cases where quantitation is not possible, semi-quantitative values may be reported by reference to responses of suitable standards.
- For semi-quantitative analysis, results for peaks with a signal-to-noise ratio > 10 or peaks above area of lowest standard injection should be reported.

4. Detection of Extracts by Inductively-Coupled Plasma with Mass Spectrometric Detection (ICP-MS)

- Instrument and analysis conditions should be optimized to achieve required sensitivity.
- Screen elements identified in ICH Q3D and USP <232>; where applicable, include silicon, tungsten and any additional elements known/suspected to be present in study material.
- The target level of Limit of Detection (LOD) is 20 ppb. The LOD may be lower or higher than 20 ppb depending on the element being detected, the sample matrix, and instrument parameters used. When the LOD is higher than 20 ppb, a justification should be provided.
- Report the LOD obtained for each element detected.
- Limit of Quantitation (LOQ) should be reported.
- Standard solutions containing detected elements should be used for recovery study; the recovery should be from 80 to 120%.
- Quantify the detected elements based on calibration curves.
- For the elements that have concentrations higher than DL, report the concentrations and µg/cm2.
- For the elements that are below DL, report the DL and indicate ND (not detected).
- Control sample injections should be run to subtract matrix associated elements from consideration.

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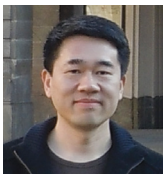

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