BioPhorum

BEST PRACTICES GUIDE FOR EVALUATING LEACHABLES RISK FROM POLYMERIC SINGLE-USE SYSTEMS USED IN BIOPHARMACEUTICAL MANUFACTURING

CONNECT
COLLABORATE
ACCELERATE™

Contents

List of tables

Main authors

Amgen Inc.
Weibing Ding

AstraZeneca Biologics
Kuruppu Dharmasiri

Bayer
Alexandrina D. Rusu

Biogen
Shujian Yi

Bristol-Myers Squibb
Kathryn McGohan

Genentech Inc.
Diana Lok

Janssen
Aidan W. Sexton

Janssen R&D
Ping Wang

Lonza
Michael Jahn

Merck & Co. Inc., USA
Christopher Smalley

Pfizer
Dhaval Tapiawala

Pfizer
Gary L. Madsen

Regeneron Pharmaceuticals
Seamus O'Connor

Sanofi
Ken M. Wong

Shire
Laszlo Litauszki

BioPhorum
Gerry McAuley

Acknowledgements

We would like to thank the following people for their contribution to the development and review stages of this guide.

Sanofi Pasteur Ltd.
Anderson Wong

Eli Lilly
Deirdre Buckley
Fran Sexton

Amgen Inc.
Duncan Low

Genentech Inc | F. Hoffmann-La Roche Ltd.
Ekta Mahajan

Bristol-Myers Squibb
Holly Shackman

Merck
Irene Cecchini

Shantha (a Sanofi Company)
K.S. Jaganathan

Sanofi SCO
Luc Kupers

Bristol-Myers Squibb
Michael DiFiore

Shire
Naveen Pathak

Pfizer
Neal S. Adams
Neil Pothier

Lonza
Rob Grassi

GSK Vaccines
Silvio Tollis

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 110 manufacturers and suppliers deploying their top 5,000 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

Use of single-use systems (SUS) and SUS components in clinical and commercial biopharmaceutical manufacturing has increased rapidly in recent years. Polymeric SUS components offer significant advantages over conventional (i.e. reusable) components in terms of flexibility, speed and efficiency of operation. Extensive experience across the industry has demonstrated that SUS can be deployed safely in both clinical and commercial applications. However, there remain lingering concerns for implementing SUS components into a biopharmaceutical manufacturing process. One of these lingering concerns is the potential for compounds leaching from the polymeric component(s) and entering the process stream as a result, with potential negative impacts on product quality and/or process performance.

As such, there are regulatory guidelines and regulations for leachables from the US Food and Drug Administration (FDA), the European Medicines Agency (EMA), and the International Council on Harmonisation (ICH):

- 21 Code of Federal Regulations (CFR) 211.65(a) specifically states:

  "Equipment shall be constructed so that surfaces that contact components, in-process materials, or drug products shall not be reactive, additive, or absorptive so as to alter the safety, identity, strength, quality, or purity of the drug product beyond the official or other established requirements."

- Section 6.1.3 of the European Medicines Agency's 2016 *Guideline on process validation for the manufacture of biotechnology-derived active substances and data to be provided in the regulatory submission* states:

  "When single use equipment is used in evaluation studies, consideration should be given to leachables and extractables. Information should be provided on the nature and amount of potential leachables, and the removal of such impurities. Besides data, this normally includes a risk assessment."

- The ICH Q9 guideline on *Quality Risk Management* offers the following guidance:

  "It is important to understand that product quality should be maintained throughout the product lifecycle such that the attributes that are important to the quality of the drug (medicinal) product remain consistent with those used in the clinical studies." It should be noted that the recommendations in this paper are suggestions and organizations should devise strategies specific to their products in consultation with regulatory agencies based on process/product understanding, prior manufacturing experience, clinical safety and efficacy of the product and extent of unmet medical need.

Non-reactive and non-absorbing properties of materials are usually controlled by process design and by the selection of raw materials and manufacturing components.

While extractables studies and data are the responsibility of the SUS component manufacturers, it is the biopharmaceutical manufacturer's responsibility to qualify various materials used in the manufacture of a biological drug to ensure that the materials are appropriate and meet defined specifications for:

- toxicological risk: final drug product (FDP) safety
- process performance: impact on process performance, e.g. cell growth, etc.
- product quality: impact on FDP quality, e.g. stability, activity, etc.

Extractables are chemical entities that are extracted from a component of a process system into a solvent under controlled conditions that are usually more aggressive than normal operating conditions. These conditions can include increased time or temperature and generally involve the use of solvents that have properties (pH, polarity, ionic strength, concentration of dissolved components, etc.) selected to bracket the normal operating conditions. Although such aggressive conditions are not routinely encountered in biopharmaceutical manufacturing processes, extractables are nevertheless important because knowledge concerning extractables can help to identify the potential leachables that may enter a process stream.

Leachables, by contrast, are chemical entities that come from SUS components during normal use. Compounds observed in extractables studies do not necessarily become leachables under normal operating conditions. Leachables from SUS components can sometimes be found in the FDP, usually at trace levels relative to the drug substance (DS).

However, currently available regulations and guidelines do not provide details on how to design test plans, how to perform analyses or how to interpret extractables and leachables (E/L) profiles. Basic principles of risk management/assessment should be used to better identify, evaluate, communicate and mitigate the risks that extractables/leachables pose to product quality and patient safety.

The purpose of the BioPhorum Operations Group (BioPhorum) Leachables Best Practices Guide is to present a practical and adaptable approach for assessing leachables risks associated with SUS biopharmaceutical manufacturing processes. This guide will also aid in study design to assess polymeric SUS components, using appropriate analytical methodologies for detection of potentially leachable compounds. The methodology presented is intended to be robust and yet sufficiently flexible to be adapted to each company's needs.

Use of this guide will help to determine where there are risks that may require additional studies. This will lead to better understanding of patient risk, as a result of the use of polymeric SUS components in the drug manufacturing process under normal processing conditions. It is important to note that a risk-based approach does not necessarily require that leachables studies be conducted for all SUS.

Additionally, this guide presents parameters to consider when designing a study to ensure that maximum value is derived from the data generated. Using this guide, an end user can efficiently design studies that support a full range of manufacturing process conditions and that will provide a thorough understanding of leachables that may be present within products and in-process streams. This guide also presents analytical techniques and considerations to help end users effectively detect and identify leachable compounds occurring within products or in-process streams that have resulted from contact with SUS under conditions potentially experienced in actual manufacturing processes.

Taken together, these best practices will help to standardize approaches to identifying leachables risks, from a patient safety point of view, which warrant further study. Additionally, these practices will assist users in designing leachables studies efficiently, using appropriate analytical techniques to ensure that detection of any potential leachables can be optimized. Adoption of the best practices can also help manufacturers enhance the benefit of leachables studies performed, and ensure that product quality and patient safety are maintained following implementation of SUS in biopharmaceutical manufacturing processes.

Sections for risk assessment, study design and analytical methods are presented in the order in which they would typically be required during the assessment of a SUS for leachables. Risk assessment determines what data and testing are required. If a leachables study is determined to be necessary based on the risk assessment, the proposed study design outlines best practices for setting up and conducting the study. The outlined analytical techniques provide guidance for analyzing the resulting leachables study samples. Lastly, a brief list of some common concerns and considerations for each of these aspects is provided.

Appendix 1 contains a list of abbreviations and their explanations as used throughout this guide.

2.0

Risk assessment

Risk assessment uses a science-driven and risk-based approach to develop a comprehensive understanding of the risks associated with the use of polymeric manufacturing components utilizing process knowledge and prior experience of using SUS.

The principles of risk management are key to effective identification, evaluation and mitigation of extractables and leachables (E/L) risks impacting product quality and patient safety for polymeric SUS.

The scope of the risk assessment should be defined following the process in Appendix 2: An E/L risk assessment process flow diagram. The assessment should determine if the scope, i.e. the evaluation boundary of the risk assessment, is for the operation of one unit or for the overall process. A risk assessment should consider the cumulative use, i.e. all instances of use of a particular material. For example, if a bag or tubing is used in multiple different stages of a manufacturing process, then all such stages should be considered in determining the overall risk associated with the bag or tubing. Considerations for parameters to evaluate should include, but are not limited to, the total surface area of a component, the location of its use, process conditions, etc., as described later in this section. Once the assessment boundary has been determined, a comprehensive list of process/manufacturing components should be compiled from which to select the polymeric components that will be evaluated for leaching propensity.

The stage of drug development and the inherent material extractables risk level for each polymeric component should guide the extent of testing required for each material. It should also guide the level of detail necessary in the item's specification based on its intended use and available controls (i.e. purification steps) in the manufacturing process, e.g. polyethylene (PE) is generally a low-risk material of construction (MOC), while poly vinyl chloride (PVC) would generally be a high-risk MOC. Acceptance criteria for specified attributes on each material should be established. For example, for some materials all relevant attributes or acceptance criteria may not be known at the Phase 1 stage of development. At a minimum, the certificate of analysis (CoA) should be examined for each lot of material to ensure that it meets established acceptance criteria for specified attributes, e.g. United States Pharmacopeia (USP) Class VI compliance. However, as compared with Phase 1 and 2 where a CoA is recommended, a more detailed E/L assessment is required for the Phase 3 clinical and subsequent commercial phases of production.

Regardless of the drug development phase, the manufacturing of DS or FDP should involve collecting and evaluating the following minimum information on SUS polymeric components:

- MOC
- origin of material
- SUS storage and shipping conditions, and expiration dating (where relevant)
- sterilization requirements and methods used
- physical and chemical compatibility with manufacturing conditions
- USP Class VI compliance, USP <661.1>, CFR Part 21, Subparts 177, 178, 179 and 182; European Pharmacopoeia (EP) 3.1.1, EP 3.1.9, as applicable for tubing, such as ISO 10993
- extractables profile (vendor provided, historical database or a combination of both) (Ref. 3).

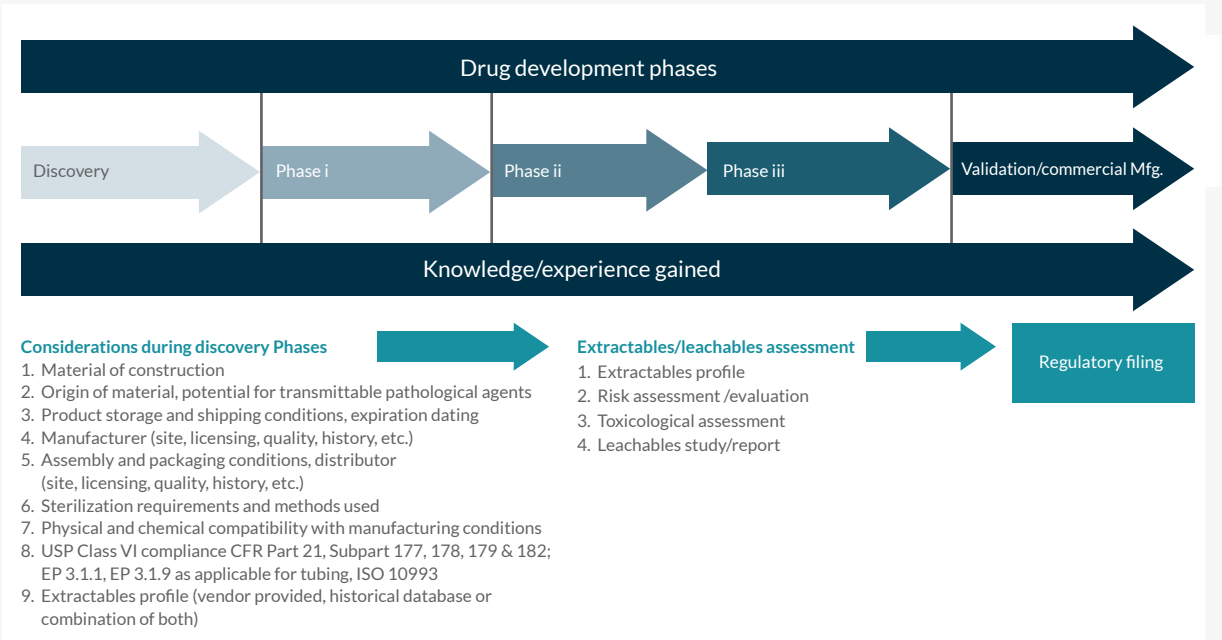
It is up to the end user, or individual company, to decide whether or not to perform an extractables risk assessment or a study for early phases (i.e. prior to Phase III). At a minimum, end users should consider these criteria for Phase I and Phase II (Figure 1). However, available extractables data from the vendor should be documented for early phase programs and this data should be collected as per the *BioPhorum Extractables* Protocol (Ref. 3). Once a risk assessment has been performed, the following aspects of the manufacturing operation should be considered when making a risk-based decision on whether to proceed with a leachables study:

- unit operations that impact on leachables levels (dilution, clearance)
- available knowledge from prior use of the components in similar processes within the organization that quantitatively demonstrated acceptable E/L risk
- evaluation of extractables data in higher-risk components, e.g. components used in post-clearance operations, such as final ultrafiltration/diafiltration (UF/DF), chromatographic purification, etc.

Two other considerations are worth mentioning for clarification:

- a leachables evaluation is usually not necessary for material manufacturing during early phases of clinical trial drug development
- any lot representative of the commercial process can be used for leachables studies, regardless of the development phase at which the lot was produced

Figure 1: Proposed approach for extractables and leachables at different phases of drug development



Once the risk assessment boundary has been defined (as per Appendix 2), based on the intended use of the polymeric parts, they can be divided into two general categories:

- components with process fluid contact
- components with no process fluid contact.

If the polymeric manufacturing components do not have any process fluid contact, no further action is needed for evaluating E/L. Such components do not represent a risk to patients or drug product (DP) quality and can be removed from the risk assessment.

2.1 Extractables and leachables propensity assessment

The scientific approach to extractables and leachables (E/L) risk assessment only determines the likelihood of a leachable migrating into the production stream of a pharmaceutical product and does not consider the toxicity of potential leachables. A toxicity assessment is performed separately and in addition to the leachables studies (as appropriate) that are required to mitigate risks associated with the use of SUS components.

The model proposed standardizes the risk assessment process. It employs a numerical scale for risk ratings at the individual consideration level and has been developed using the collective experience of a group of subject matter experts across the BioPhorum Extractables and Leachables workstream member companies. Risk comparability is achieved by assigning consistent definitions to the numbers. While the definitions and numerical scale can be adjusted for an individual organization's needs, the main considerations remain unchanged, resulting in a model that provides consistency, yet is adjustable to the overall risk level of the specific process. The model accepts the inherently subjective nature of any risk assessment and does not attempt to eliminate subjectivity completely. Rather, the model is a road map allowing biopharmaceutical developers/manufacturers to standardize their organization's approach, while still allowing individual end users to apply their perception of the risk to a product associated with a specific process. At the same time, the comprehensive approach of this model ensures that all the relevant scientific aspects are considered in each risk assessment.

The risk assessment model explicitly focuses on final drug product (FDP) safety with respect to patients and does not evaluate the potential impact of leachables on the manufacturing process itself. However, in addition to FDP safety considerations, leachables from SUS components may also represent a risk to the manufacturing process performance. For example, leachables from polymeric materials have been known to inhibit the growth of certain cell lines (Refs. 12 and 13). It is acknowledged that the risk to manufacturing process performance can be process-specific.

A process-specific risk assessment considers the manufacturing process progression as the raw materials enter DS or FDP, with in-process checks used to assure intermediate quality before the next processing step begins. It is presumed that any impurity entering the production stream can have negative impacts on product quality, and the severity of such a failure mode is assumed to be high. A process-specific assessment takes into account where along the production stream the component is used and assigns a risk rating accordingly. As the production process advances to the FDP state, the risk to the patient represented by any leachable increases.

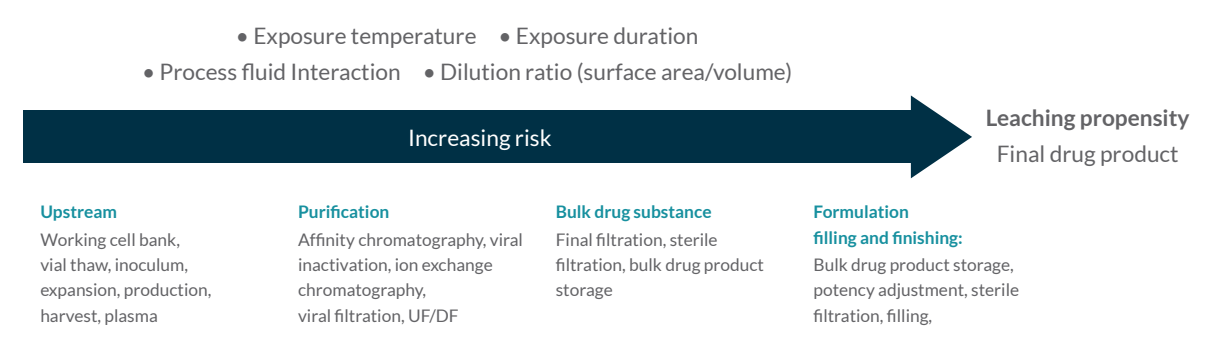
The risk assessment model considers the following process-specific criteria:

- all polymeric manufacturing components that contact the production stream (components that do not contact the production stream are excluded)
- proximity of the process step from raw materials to DS or DP.

2.1.1 Distance along production stream (DAS)

Polymeric components used in process steps closer to DS or DP will carry a higher risk rating than those used in upstream process steps. For example, a bag or filter used for final filtration of bulk drug substance (BDS) will have a much higher risk rating compared with components used in upstream process steps since there are no purification steps post-UF/DF.

Figure 2: Distance along production stream (DAS)



In addition, if sufficient institutional knowledge exists regarding the process, the risk assessment model may also consider the likelihood of removing a leachable during purification steps to bring its level to acceptable limits. A lower risk score would be associated with any purification step that could be justified as a leachables removal or dilution step, based on a scientific rationale/data, even with a high likelihood of leachables entering process streams from upstream polymeric components (Ref. 13). For example, the UF/DF step at the end of the purification process may be justified as a clearance step, then the polymeric components used in process steps upstream of the final UF/DF step may be given lower risk scores.

2.1.2 Processing conditions

The risk assessment model not only considers the composition of the polymeric components of SUS but also the processing conditions to which the component is exposed (see Table 1 and Appendix 2). These processing conditions include:

Exposure temperature

Higher temperatures may increase the possibility of leachables migrating into the process stream. Similarly, frozen liquids (i.e. below 0°C) have a lower propensity for leaching. However, one must not discount the impact of freeze-thaw cycles on the integrity of polymeric materials and attendant leachables risks. Separate consideration of conditions for liquids at <0˚C, 0–8˚C, >8–30˚C and >30˚C (typical temperature ranges in biologics/biopharmaceutical processes) is practical for use with aqueous systems because these conditions reflect common manufacturing practices.

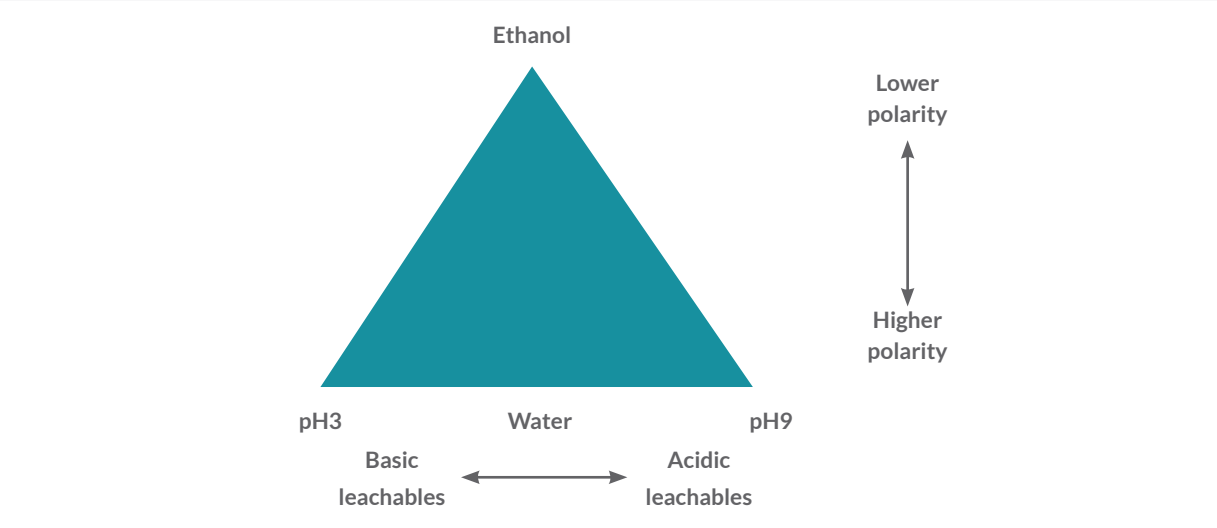
Exposure duration

Exposure duration also needs to be considered because the length of exposure may increase the propensity for leaching. Processing or exposure of transient durations of <60 minutes typically represent a low risk of leaching simply because there is limited time for migration of compounds from the SUS component to the process stream to occur. Applying a timescale in terms of minutes, hours, days or weeks provides a practical system for creating a risk profile associated with exposing a particular polymeric component to the process stream. Increased exposure duration customarily impacts semi- and non-volatile leachables migrating into the process stream. The concentration of volatile leachables may decrease in the process stream, depending on the specific processing conditions.

Process fluid interaction (PFI) or solvation power and solvent penetration

The solubility of chemicals originating from polymeric components in the process fluid is another important consideration for leachables risk assessment. Figure 3 illustrates the dependence of the solubility of leachables on the process fluid characteristics. Leachables that do not dissolve in the process fluid will partition, preferably to the manufacturing component and not to the process fluid. For organic chemicals, their degree of polarity plays a significant role in the partitioning phenomenon. Polar organics inherently tend to migrate more into polar aqueous fluids, while less-polar organics will dissolve to a greater extent in fluids that also have a lower degree of polar character. Fluid pH impacts the solubility of organic substances because pH can change the polarity of certain classes of organic entities. For example, carboxylic acids are more soluble in basic solutions, while organic amines will have increased solubility in acidic solutions. Surfactants (e.g. poloxamer, polysorbate, etc.) also strongly influence the propensity of polymers to leach.

Figure 3: Diagram of solubility dependence of leachables



A related consideration is the solvent's ability to penetrate the polymer. Increased penetration leads to enhanced migration. Increasing the solvation power of a process fluid for particular leachables and increasing the ability of the process fluid to penetrate the polymer both serve to increase the possibility of leachables entering the production stream. In addition, process stream liquids that are known to be good solvents for organic materials may be considered to represent higher risks of leaching. The same is true for process stream fluids that penetrate the polymeric portion of a single-use system. If applicable, vendor data can be used to help design mitigation strategies.

Dilution ratio or exposure surface area to process liquid volume ratio

The concentration of leachables in the process stream is dependent on both the amount of leachables that migrate and on the volume of liquid the polymeric component is exposed to. For simplicity, dilution ratio (DR) as used in this guide is the ratio of the exposure surface area (ESA) of the SUS component to the volume (V) of the process liquid. Consideration of the DR (exposure surface area to volume ratio) provides an appropriate representation of risk that combines the two dependencies and aids in normalizing the assessment of risk at various manufacturing scales. The basic concepts of DR are:

- higher surface areas provide more opportunity of leaching/Lower process volumes concentrate the leachables and represent a higher risk.
- higher extractables concentrations in the polymer provide higher propensities to leach.

Pre-treatment

Any pre-treatment of components, by steps such as autoclaving, gamma irradiation or water for injection (WFI) flushes, etc., may affect their leachables profile. Hence, the pre-treatment and its potential effect on SUS polymers need to be considered in any E/L risk assessment.

2.2 Model risk assessment process

The risks associated with a propensity for leaching or potential leachables extracted from polymeric components can be weighted in alignment with user's process understanding and experience. The BioPhorum model is presented here, which companies can adapt to their requirements (see Tables 1 and 2).

Table 1: Example leachables risk assessment model

Consideration	Ratings[a]		Weight[b]
Distance along the production stream (DAS)	1	Upstream: *e.g. working cell bank, vial thaw, inoculum, expansion, production, harvest, plasma and solution preparation*	0.40
	3	Purification: *e.g. filtration, chromatography, viral inactivation, viral filtration and UF/DF*	
	5	Bulk drug substance: *e.g. formulation, 0.22 µm filtration, BDS storage*	
	9	Final formulation, fill/finish: *e.g. bulk drug product storage, potency adjustment, sterile filtration and filling*	
Exposure temperature (ET)	1	<0°C	0.15
	3	0 to 8°C	
	5	>8°C to 30°C	
	9	>30°C	
Exposure duration (ED)	1	Transient (≤60 minutes)	0.15
	3	Short (≤24 hours)	
	5	Medium (≤7 days)	
	9	Long (>1 week)	
Process fluid interaction (PFI)	1	Limited penetration into polymeric component (i.e. water)	0.15
	3	Low solvation power or low penetration of polymeric component *e.g. neutral pH without organics, surfactants, etc.*	
	5	Medium solvation power or medium penetration of polymeric component *e.g. surfactant, low-concentration organics, high/low pH solutions without organics/detergents*	
	9	High solvation power or high penetration of polymeric component	
Dilution ratio (DR)	1	$<1 \times 10^{-03}$ m²/L	0.15
	3	1×10^{-02} to 1×10^{-03} m²/L	
	5	1×10^{-01} to 1×10^{-02} m²/L	
	9	$>1 \times 10^{-01}$ m²/L	

DAS = distance along production stream DR = dilution ratio ED = exposure duration ET = exposure temperature PFI = process fluid interaction.

[a]Parameter range definitions in this table represent examples only. Individual companies should develop their specific range definitions according to their internal policies/standard operating procedures.

[b]Weighting levels used in the table represent examples only. In this table, 0.40 is used for DAS rating, and 0.15 is used for all other considerations. Individual companies may use an equal weighting distribution or may assign weighting levels according to their internal policies

2.2.1 Disposable component qualification per risk categorization

The overall leachables risk rating (LRR) is calculated as shown in Table 2.

Table 2: Example leachables risk rating calculations

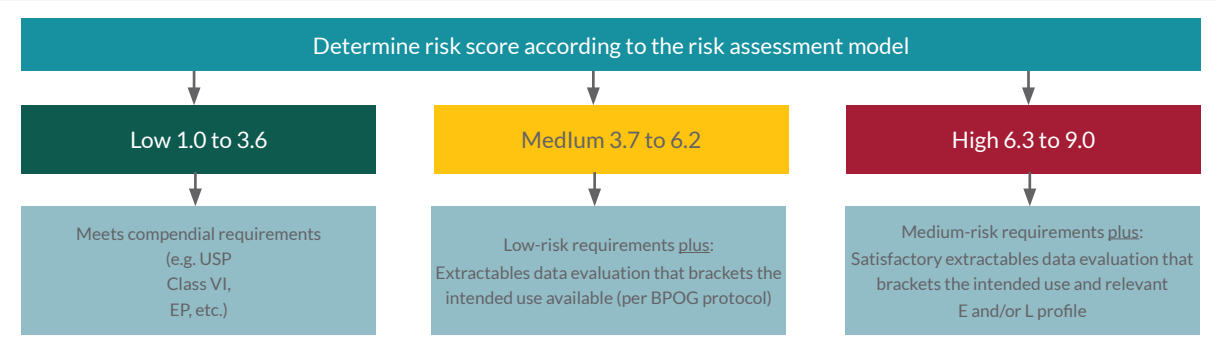
Leachables risk rating (LRR) calculation	Calculated by the following equation: LPR = DAS*Weight (0.4) + ET*weight (0.15) + ED*weight (0.15) + PFI*weight (0.15) + DR*weight (0.15) Possible range: 1.0 to 9.0
Leachables risk rating levels	6.3 to 9.0: High 3.7 to 6.2: Medium 1.0 to 3.6: Low

DAS = distance along the production stream; ET = exposure temperature; ED = exposure duration; PFI = process fluid interaction; DR = dilution ratio

Per the model, the overall leachables risk rating will be in the range of 1.0–9.0, allowing prioritization and translation into high, medium and low overall risk levels.

Once determined, the leachables risk level can be used to determine the testing requirements (i.e. a high risk level in one component in the process can prompt more in-depth testing, whereas another component with a lower risk may require a less comprehensive set of tests). Once the overall risk rating of the polymeric component of interest has been finalized and has been ranked as low, medium or high, the qualification requirements (such as those shown in Figure 4) should be established to fully qualify the disposable for its intended use.

Figure 4: Suggested requirements based on the risk categorization of polymeric components



For low-risk applications (LRR = 1.0–3.6), the minimum qualification requirements should be the meeting of relevant compendial test standards such as USP Class VI (also EP 3.1.9, if the component is silicone tubing).

For medium-risk applications (LRR = 3.7–6.2), supplier-provided extractables data (as per the BioPhorum Extractables Protocol) or in-house leachables simulation study data can be used to demonstrate risk control, where appropriate, for further evaluation in addition to the minimum qualification requirements.

For high-risk applications (LRR = 6.3–9.0), the minimum qualification requirements include those for medium-risk components as well as an additional, process-specific, leachables risk assessment. If the calculated per dose exposure level cannot be accepted from a safety perspective (based on a toxicological assessment of the extraction profile), a leachables study may be warranted to demonstrate risk control and/or establish the safety of the disposable component based on its leachables profile in the DP. This is only required in situations where extractables data from the supplier are either:

- not available
- do not correspond to current processing conditions in which the polymeric component in question is utilized.

If so, it will be necessary to generate extractables and/or leachables data prior to proceeding with the next step in the qualification process.

It is recommended that leachables data be generated utilizing worst-case conditions applicable to the intended process step – specifically with respect to the polymeric component process usage parameters such as exposure temperature (ET), exposure duration (ED), etc. Guidance for execution of this leachables testing step, including recommended process simulation conditions and analytical methodology, is indicated in Sections 2 and 3 below.

Once an appropriate extractables data set is available, a DP-specific safety assessment based on production batch size and dosing regimen should be conducted to evaluate the patient safety aspects of extracted compounds (Ref. 5). If certain extractables are above the safety concern threshold (SCT) after an initial worst-case assessment has been conducted, a leachables study may be necessary. Such a leachables study may include an examination of long-term leachables profile (e.g., product intermediate storage container) in order to fully qualify a high-risk component. Alternatively, another polymeric component with a different MOC may be considered for use.

The overall extractables profile of the SUS is used for a toxicological assessment of patient safety. If the calculated potential patient exposures of the potential leachables are lower than appropriate safety thresholds, e.g. threshold of toxicological concern (TTC), (based on ICH M7 guidelines, the standard reference at the time of preparing this guide), then the safety risk of the potential leachables from the material is considered to be acceptable with regard to patient safety, and no further leachables studies are required.

However, if the calculated potential patient exposures of the extractables are higher than the permissible daily exposure (PDE) for known compounds, or SCT for unidentified compounds, then the extractables should be positively identified (if not already achieved) and their toxicity profiles should be assessed. These high-risk components should be tested as per a leachables study plan, and a further safety risk assessment should be conducted based on the leachables profile.

If the toxicological risk assessment of extractables data determines that the maximum dosage of drug presents a safety risk, then a leachables study is necessary. The primary focus of the leachables testing will be to first determine the levels of these compounds in the process stream. The analytical methodology employed to detect and quantitate these compounds will be defined by the nature of the particular extractables compounds. Guidance for the design of such a leachables study can be found in Section 2.

Potential interactions between leachable compounds and the DS can be monitored through the stability monitoring program for that product.

The risk assessment model proposed (Tables 1 and 2) was applied to an example of a typical biologics manufacturing process as described in Appendix 3 (Ref. 6). The parameters used for the process and example assessment that are described in Appendix 4 were used to calculate the risk profile of different polymeric components used in the process. The calculated risk profile numbers identified "High", "Medium", and "Low" E/L risk classifications. For example, a sterilizing filter and a Bulk Drug Substance (BDS) storage bag were identified to have a high-risk classification for leaching, requiring an extractables profile to be obtained and evaluated further.

A toxicological evaluation of the extractables also needs to be completed for these two high-risk example components shown in Appendix 4. If the toxicological profile of the extractables represents an acceptable risk to patient safety then no further leachables studies are required and the extractables data can be used as part of the components' qualification package. However, if the toxicological profile represents a risk with regards to patient safety, then conducting a leachables study as described in Section 2 may be deemed necessary. A leachables safety risk evaluation based on the leachables profile would also need to be completed. From this latter study, if the toxicological profile of the leachables is considered to represent an acceptable risk to patient safety, then the leachables data should be used as part of the qualification package. However, if the toxicological profile of the leachables presents an unacceptable risk to patient safety, then an alternate polymeric component (i.e. material change) with a lower risk profile or a non-polymeric component must be considered. Alternatively, process conditions may be modified in order to lower the risk profile, if feasible.

3.0

Leachables study design

This section defines the best practices for developing a study design to perform leachables testing of SUS components. This testing process is typically conducted when components have been determined to carry a high risk and therefore require leachables testing, but the principles outlined can also be used to conduct a leachables study intended for other purposes. These practices are applicable to components that are in contact with the product, with in-process fluids or with both.

Leachables testing should be carried out using actual process fluids, DS or DP. However, there are situations where surrogate fluids may be used and/or specific analytical methods need to be developed to mitigate analytical interference from protein signals, e.g. in situations where there is a high concentration of protein in the in-process fluid.

Designing a robust leachables study is critical for deriving maximum value from the data generated by testing. The parameters utilized in a leachables study should support a full range of potential manufacturing processes and storage conditions to ensure that the resulting leachables profile is representative of the leachables profile that could exist within the in-process fluid after contact with the SUS. The key parameters to be considered in such a study are presented in Table 3.

Table 3: Key parameters for leachables testing of SUS components

Key parameter	Considerations
Negative control solution	Negative control solution to calculate background levels should be included for all tests using the same test setup minus the single-use component. Control solution should never be exposed to the test article. Control solution should be aliquoted from the test solution pool prior to contact with the test article and stored under the same conditions as the test solution for the duration of the study.
Sample handling	Where possible, the control material and (post-exposure) samples should be stored in clean, properly prepared containers. Both the control material and the post-exposure test samples should be stored in containers of the same materials of construction and under the same conditions, to ensure comparability of the test samples with the control. Materials may include: • stainless steel • glass – type 1 – low total organic carbon (TOC) • polytetrafluoroethylene (PTFE) Samples should be stored according to best practices for preferred time and temperature, based on the requirements for each individual analytical technique.
Test article	Test article should undergo the same sterilization/pretreatment as the components that will be used in the manufacturing process. When performing a leachables evaluation for a filter, flushing the filter per manufacturing procedure is appropriate. Special storage conditions in the manufacturing process (e.g. protected from light) should be replicated when possible. Report flushing, sterilization mode and actual condition/pretreatment in the study report, if applicable.

Table 3: Key parameters for leachables testing of SUS components (continued)

Key parameter	Considerations
Contact fluid (media, buffer, process intermediates, DS/ formulated bulk)	Use actual process fluids, including active pharmaceutical ingredients (APIs), when possible. Otherwise, select the surrogate fluids closest to the actual fluid matrix with justification. Keep in mind that additional process fluid will be needed for study controls and blanks. Consider whether there is any expectation that the process fluid might interfere with analytical techniques, e.g. protein peak overlay on a leachables peak. Consider using a worst-case or diluted matrix to mitigate the interference, or run a parallel test with a similar fluid that excludes the interfering species, e.g. buffer without API. If interference is expected, refer to the extractables data and analytical method best practices. Use sterile process fluids for the study when possible. If non-sterile solutions are used, take precautions to ensure that the test fluid is not compromised by microbial growth. Record and report test fluid ingredients and concentration, if applicable.
Sterilization/ pretreatment	Report flushing (filters), sterilization mode and actual condition/pretreatment in the study report, if applicable
Sample volume	The sample volume should be adequate to allow for the generation of a sufficient sample size to satisfy the requirements for all analytical testing. If exposure conditions are to be simulated, the component surface area used in the manufacturing process (or step) and the volume of process fluid that the component will be exposed to should be determined in advance to scale down appropriately. When multiple components of the same MOC are used in the process, use the component with the highest surface area to volume ratio to represent the worst case. Record and report the MOC, the component surface area and the solution volume utilized in the study report. Consider generating a retain sample to allow for repetition of analytical testing or for performance of additional analytical methods.
Incubation	The options for incubation include dynamic and static soaks. Dynamic soak is accomplished by agitating, e.g. on a rocker table, the filled and sealed test article for the duration of the study. Dynamic soaking is more useful for testing component/fluid combinations that are flow through, rather than components that make only static contact with the fluid. Static soak is accomplished by incubating a filled and sealed test article with a known volume of fluid for the duration of the study. Static incubation is useful for testing storage containers where there is no significant movement of the fluid within the component under in-process conditions. Incubation parameters should mimic actual in-process parameters. Record and report the incubation parameters along with a diagram or picture of the test set-up, if possible. Record the component dimensions, e.g. exposed surface area, and the volume of solution exposed. Record measurements (pH, weight, etc.) of the test sample and the process fluid before and after extraction to account for any evaporation that may occur during the test.
Contact duration	The exposure time should adequately cover the full range of potential contact time for in-process usage. An exposure time exceeding the actual storage condition by 10% is recommended as a worst-case measure. Record and report the exposure time. For long-term studies (≥6 months), consider testing at multiple time points over the course of the study. When multiple time points are used, a separate test article should be used for the tests at each independent time point to mitigate the risk of contamination during aliquoting for analysis. Additionally, it is best practice to have a separate control for each time point to account for any changes in the solution over time that are not related to the test article. Record and report the exposure time.
Temperature	Bracket the high end of the process temperature throughout the duration of the study. Record and report the study temperature.
Analytical methods	See Section 3 (Analytical method) for reference. Express analytical results in µg (leachable in solution)/mL. Results may be corrected for any significant evaporation.

3.1 Additional considerations in leachables study design

The parameters listed in Table 3 can be modified to meet the specific testing requirements of several component types.

For example, storage containers may be used for short-term storage of the media, solutions and/or buffers used in processing. They may also be used for the long-term storage of APIs. Based on the in-process usage of a particular container, a leachables study to investigate the container can be designed with the parameters described in Table 3. Short-term usage may not require multiple time points over the entire duration of the study, while long-term storage of APIs may require several time points to cover the full range of potential exposures.

Filters can be flushed prior to leachables testing as per manufacturing procedures. Filters can be filled, sealed and extracted for the relevant period of time with agitation. For a dynamic leachables study on a filter, recirculation can be performed through the filter.

The cumulative surface area of exposure of tubing can be significant. Therefore, when designing a leachables study, one must consider both the total exposed surface area of tubing and the volume of in-process fluid to appropriately scale down the length of tubing for a leachables study. If worst-case conditions are desired, a higher exposed surface area to volume ratio in-process should be used.

Also, for worst-case conditions, the exposure time for the tubing can be doubled to ensure that in-process conditions will be supported by the data generated.

Gaskets and connectors can be tested by means of full submersion in the test solution for the leachables study or the component can be exposed to the test solution as part of an assembly as it is used in-process. The exposed surface area to volume ratio should sufficiently mimic normal operating parameters.

Column tubes can be analyzed either as individual parts, or completely assembled as for use in pre-packed column assemblies. For studies of a tube only, a sample of the same MOC as the processing component can be used. For pre-packed columns, exposure of only the surfaces contacting the process fluids will best mimic in-process usage. Full submersion of a coupon of material would be considered the worst-case test because full submersion will test non-product contact surfaces as well as surfaces exposed during processing.

Filling manifolds can be studied as a whole component by filling, capping and storing the assembly for the appropriate length of time for the application in question. Alternatively, each component of the assembly can be studied individually by following the best practices for each specific component.

Appendices 5 and 6 provide a framework for reporting the experimental design details and for reporting the compounds detected in the leachables study, respectively.

4.0

Analytical methods

The goal of the analytical techniques discussed in this section is the detection and measurement of any compounds that have leached from SUS components that may end up in the process stream and/or final DS/FDP. These techniques may also be used to demonstrate whether leachable compounds are, or are not, present below a given identification concern threshold level. Leachable compounds detected may result from contact with SUS components used in the manufacturing process, intermediate storage containers or packaging/delivery system components. The analytes observed will most likely represent a subset of the list of extractables species that were previously determined through controlled extraction studies carried out on the process-contacting components. Additional compounds detected that were not identified as extractables may be the result of residual solvents or interactions of the DS/FDP complex matrix solution with the product contact material components.

4.1 Sample selection and handling

To derive a leachables profile for a process contact component, or to discover the source of a leachable compound detected in the fluid stream, analytical samples should be collected for testing from various locations in the manufacturing process. It is assumed that by this time the assessment team has already completed the processes outlined in Section 1 (risk assessment) and Section 2 (leachables study design), has decided (based on the results) that a leachables study is required and has designed an appropriate study. The sampling points and times should be outlined in a protocol that takes into account the representative in-use conditions described in Section 2. Samples should be collected and stored in inert containers such as glass (for organic species) or polytetrafluoroethylene (PTFE) (for inorganics) and tested within a reasonable amount of time. Samples that are difficult to manipulate for testing (e.g. due to inhomogeneity or adsorption issues) may be collected and stored in the same container that is used for the analysis. Additional (i.e. duplicate retain) samples should be collected and retained as a contingency for instrument failure or for future re-analysis. Appropriate storage of test samples should be considered when the testing will not be imminent.

4.2 Method overview

In terms of their ability to detect a broad range of
In terms of their ability to detect a broad range of compounds, the main analytical techniques applied to detect and measure leachables should be the same (or similar to) those used in extractables studies. It is assumed that, by the time such decisions are made, an acceptable extractables study has been conducted and therefore information on the potential leachables is available.

Target leachables methods may be used in conjunction with more comprehensive analytical techniques (e.g. headspace [HS]/gas chromatography [GC]/mass spectrometry [MS], GC/MS, liquid chromatography [LC]/MS or LC/UV, inductively coupled plasma mass spectrometry [ICP MS], inductively coupled plasma optical emission spectrometry [ICP OES] and inductively coupled plasma atomic absorption spectroscopy [ICP-AAS]) that are selected based on their appropriateness for the compounds and matrices in question. Appropriate analytical techniques and/or alternative specific detectors (such as GC/flame ionization detector (FID), GC/nitrogen-phosphorus detector(NPD), nuclear magnetic resonance (NMR), Fourier transform infrared spectroscopy (FTIR), and ion chromatography (IC) which may be used for the detection and quantification of specific compounds, should be selected from the options presented in Table 4. While the analytical techniques used for leachables analysis will target anticipated leachable compounds, these techniques should also focus on screening for unexpected leachable compounds as appropriate. Semi-quantitative data, including limit assays, may be reported in cases where quantitation is not possible (e.g. when exact standards may not be available for compound confirmation and quantitation).

Table 4: Overview of analytical techniques for leachables determination

Analyte class	Technique/method	Screening analysis	Targeted analysis	Identification	Quantification
Main techniques					
Organic volatiles	HS/GC/MS	X	X	X	X
	HS/GC/FID	X	X		X
Organic semi-volatiles	GC/MS	X	X	X	X
	GC/FID	X	X		X
Organic non-volatiles	LC/MS	X	X	X	X
	LC/PDA	X	X	X	X
Inorganic (trace elements and heavy metals)	ICP/MS	X	X	X	X
	ICP/OES	X	X	X	X
Other techniques					
Inorganic	AAS	X	X	X	X
Inorganic/organic	IC	X	X	X	X
Organic	GC/NPD	X	X	X	X
	NMR	X		X	
	FTIR	X		X	

AAS = atomic absorption spectroscopy FID = flame ionization detector FTIR = fourier transform infrared spectroscopy
GC = gas chromatography HS = head space IC = ion chromatography ICP = inductively coupled plasma LC = liquid chromatography
MS = mass spectrometry NMR = nuclear magnetic resonance NPD = nitrogen-phosphorus detector
OES = optical emission spectroscopy PDA = photo diode array

4.2.1 Gas chromatography methods

Analysis by gas chromatography with both headspace (for volatiles) and/or direct injection (for semi-volatiles) is recommended, where applicable. Appropriate sample preparation procedures (solid phase or liquid-liquid extraction, protein precipitation/separation, etc.) may need to be applied to separate the leachable compounds from the product matrix solution, then transfer the leachable compounds into a GC-compatible solvent, and finally concentrate the leachable compounds for analysis. Mass spectrometry (MS) is the preferred detection method for both identification and quantification. Alternative detectors for specific classes of compounds (e.g. NPD) or for quantification (e.g., FID) may be used in addition to MS detection if the SUS component materials and leachables require it.

4.2.2 Liquid chromatography methods

Analysis by liquid chromatography coupled with photodiode array (PDA) and mass spectrometric (MS) detection is recommended, where applicable. It is acknowledged that certain matrix solutions may present challenges (e.g. polysorbate-80 excipient). For this reason, appropriate sample preparation procedures need to be applied to:

1. minimize matrix interferences (e.g. by sample dilution)
2. separate the leachable compounds from the product matrix solution (e.g. solid phase or liquid-liquid extraction)
3. transfer leachable compounds into a solvent compatible with the analytical method, and finally to
4. concentrate the compounds for analysis.

Where appropriate, mass spectrometric analysis should be conducted in both positive and negative modes with electrospray ionization (ESI) as well as with atmospheric pressure chemical ionization (APCI). Running both positive and negative modes of ionization provides complementary data and allows detection of the maximum range of potential compounds leaching from the bulk component material as well as from additives and degradation products.

4.2.3 Elemental analysis methods

Trace elements and heavy metals are assessed by inductively coupled plasma mass spectrometry (ICP-MS) or by inductively coupled plasma optical emission spectrometry (ICP-OES). The samples should be analyzed intact unless dilution and acidification are needed to meet the required detection limits for all metals of interest. Appropriate sample pre-treatment (e.g. mineralization) should be applied to improve the detection of certain elements and to minimize matrix interferences. Full elemental screening analysis is part of an extractables study and is not generally performed as part of a leachables assessment unless their presence is suspected. Other detection methods such as atomic absorption spectroscopy (AAS) or atomic emission spectroscopy (AES) may be used for specific elements (e.g. Si) in cases where interferences cannot be avoided when using ICP-MS.

4.2.4 Additional methods

As in the case of extractables studies, additional analytical techniques should be used to supplement the data as needed and where applicable. Nevertheless, the conditions that were used in the extractables study should be similar to those in the analytical study at this stage, unless a change in conditions is justified by potential matrix interferences or by incompatibility of the final product with the analytical techniques. This approach provides better continuity between the data generated for extractables and the final leachables analysis, thus lowering the risk of an extractable (that in fact becomes a leachable) going undetected in the leachables analysis.

4.3 Method system suitability standard

Appropriate standards and reference materials (certified Reference Materials [CRM], if available) should be used in order to monitor the performance of the analysis, as well as to establish the level of the determined leachables species. If a specific compound reference material is not available, an appropriate known chemical species related to the component being examined or a chemically-similar compound may be used to produce a semi-quantitative value. Multiple reference materials may be used in the same analysis, to represent different classes of compounds.

4.4 Leachables identification and quantitation

The list of target compounds that are to be quantitatively/semi-quantitatively determined is established based on extractables study results, as well as from the leachables screening results. Use of the safety threshold concept is recommended for establishing the safety threshold for individual leachables, particularly for the identification and qualification of potentially mutagenic compounds. Results from extraction studies and leachables screenings can use a calculated analytical evaluation threshold (AET) that takes into account an appropriate safety threshold that is needed for a specific analyte, as well as the dose parameters for a given drug product (DP) and its route of administration. The threshold can be based on ICH M7 recommendations or product-specific safety thresholds (e.g. permitted daily exposure –(PDE)) that have been proposed for parenteral drugs (Ref. 5).

4.5 Validation/qualification

Quantitative methods for leachables should be validated or qualified, as appropriate, when monitoring a known leachable. Depending on the intended use, validation/qualification of quantitative methods may include the characteristics of accuracy, precision, specificity, robustness, detection limit, quantitation limit, linearity and range. Validation/qualification of limit test methods may include specificity and detection limit. Unexpected compounds observed above an evaluation threshold in the analysis may need to be validated/qualified into the same quantitative method for the remaining time points and target leachable compounds observed outside the range of the method may be appropriately diluted and retested.

4.6 Reporting of analytical data

Leachables data should be summarized into a report with representative chromatograms and raw data tables showing the results. Appendix 6 shows an example of a useful format for summarizing the leachables analytical data. This report should include:

1. amount and identity of known compounds
2. estimated amount and class of compound should be provided, for any unidentified compound
3. analytical method conditions for each technique
4. any additional discussion necessary to provide context to the results
5. analytical parameters and method performance criteria (i.e. sensitivity, accuracy and precision) for a variety of test methods (Appendix 7).

4.7 Leachables safety assessment reporting

The final leachables qualification report should convert the concentration results to µg/day for safety evaluation. The leachables safety assessment should only list compounds found above the AET/SCT and/or specific cutoff value of target leachables from the toxicological evaluation.

5.0

Key lessons and common pitfalls

This section presents guidance to the risk assessment team in the form of lists reflecting issues that commonly arise during the execution of the leachables assessment process for a SUS.

5.1 General lessons

- Educate end users, other pertinent personnel and suppliers on the differences between extractables and leachables (E/L).
- SUS component suppliers must provide the extractables data required.
- Drug manufacturers are responsible for deciding if leachables studies are required, as described above, based on a risk assessment.
- Leachables testing should be pursued only after exhausting the potential of available extractables data and patient safety data to predict leachables behavior and the risk classification indicates it is required.
- Do not assume the study protocol will be read and understood – question and check understanding with pertinent personnel before the work begins.
- Involve a toxicologist early in the leachables testing process once it is clear that testing will be required.
- Build a database from which information on other E/L work performed on different projects can be accessed.
- Understand where a component is used elsewhere in your company and use the knowledge gained from its use.
- Persist with reasonable requests for supplier information. Ensure suppliers understand the significance and importance of your requests for information. Generally, the more they understand the more open they will be.
- Do not be overly reliant on the semi-quantitative values for safety assessment.

5.2 Risk assessment

- Insist on components being tested in accordance with the complete BioPhorum Extractables Protocol – doing so provides a foundation of data with which to perform the risk assessment.
- Familiarize yourself with the suppliers' processes and procedures – if these are not sufficient to support a required leachables study, they must be changed. Alternatively, you should consider seeking a different supplier.
- Create a study design that is as simple as possible and one that is easy to follow consistently.
- Diligently identify in advance all SUS components contacting the process stream, regardless of apparent size/surface area and contact time.
- Do not rely blindly on vendor extractables data – instead, independently evaluate the fitness for purpose of such data.
- Do not set prescriptive actions when assigning risk levels – instead, first determine additional levels of review that may be needed before deciding on subsequent actions.
- Leverage existing knowledge when determining cut-off points for risk categories. Align with industry practices – do not make arbitrary choices. Make use of the BioPhorum Risk Assessment benchmarking data as your guide for each cut-off.
- Use a harmonized risk assessment process across the entire company.
- Provide a scientific rationale for not performing any potential test.
- Do not create rigid risk evaluation processes that drive study execution; maintain reasonable flexibility in your approach.
- Give scientific justification of materials used upstream of UF/DF (or other known clearance steps) to be classified as a lower risk.
- Perform quantitative assessments with consideration of (and normalization to) batch size and dosing level.
- Do not assume that a component with the same MOC from a different supplier will have the same extractables profile. The method of manufacture and quality of the base polymer resin materials varies from different sources used. This can impact the profile and therefore components from each supplier would need to undergo the same testing protocol. This is another reason for insisting that the BioPhorum Extractables Protocol be used.

5.3 Study design

- Align the leachables study with the BioPhorum Extractables Protocol as much as possible, using available extractables data to guide the sampling and analytical plan.
- Educate your toxicologist on the study purpose and methodology to ensure that it is fit for purpose.
- Decide if you are looking for specific leachables based on the extractables data and/or screening for any unexpected leachables that might be present.
- Define equipment and component requirements early and acquire these as soon as possible – failure to ensure their availability is a primary reason for study delays.
- Standardize sample containers used and the method for preparing these containers.
- Do not blindly adopt extreme worst-case conditions; maintain flexibility.
- Where possible, use inert tubing and pumps for recirculation set-up.
- Consider using actual process samples rather than material from scaled-down studies, when possible.
- Consider that you may need to accommodate a future technology transfer of the leachables study to full-scale commercial manufacturing; design a method that can be readily adapted to a larger scale.
- Include screening methods for leachables in lieu of a targeted leachables study.
- Communicate the study plan to all involved parties to get buy-in; maintain regular contact with team members to track actual progress.
- Remember to include personnel from operations and manufacturing support functions to ensure you understand all aspects of the process that the SUS component is used in.
- Review specific compounds of concern, i.e. impact to process, quality and/or safety prior to study initiation.
- Do not assume that a contract research organization (CRO) will measure all compounds that you considered 'standard' – explicitly state which compounds must be measured.
- Remember that your goal is the demonstration of toxicological safety; therefore, use of an alternative leachables mitigation strategy may be justifiable.

5.4 Analytical methods

- Know what you need from the data before approaching a CRO to perform leachables studies.
- Define the limits of detection (LOD) and limits of quantitation (LOQ) of your analytical methods. Persist in getting required data from vendors.
- Validate/qualify the analytical methods appropriately in consideration of the test objective.
- Use standard/control samples at the beginning and the end of each run.
- Use accurate mass detectors, even if only conducting a screening study.
- Use appropriate matrix controls to account for degradation of matrix components that could be mistaken for leachables.
- Non-specific analyses are typically not useful for leachables studies.
- Do not make assumptions without data; generate required data.
- Matrix interference from protein/excipients/process components can be significant; ensure that such effects on the methods planned are well understood before performing analysis.
- Determine analytical evaluation thresholds (AET) for your target compounds.
- Where possible, use an MS (or MSn) library to aid in the identification of compounds.

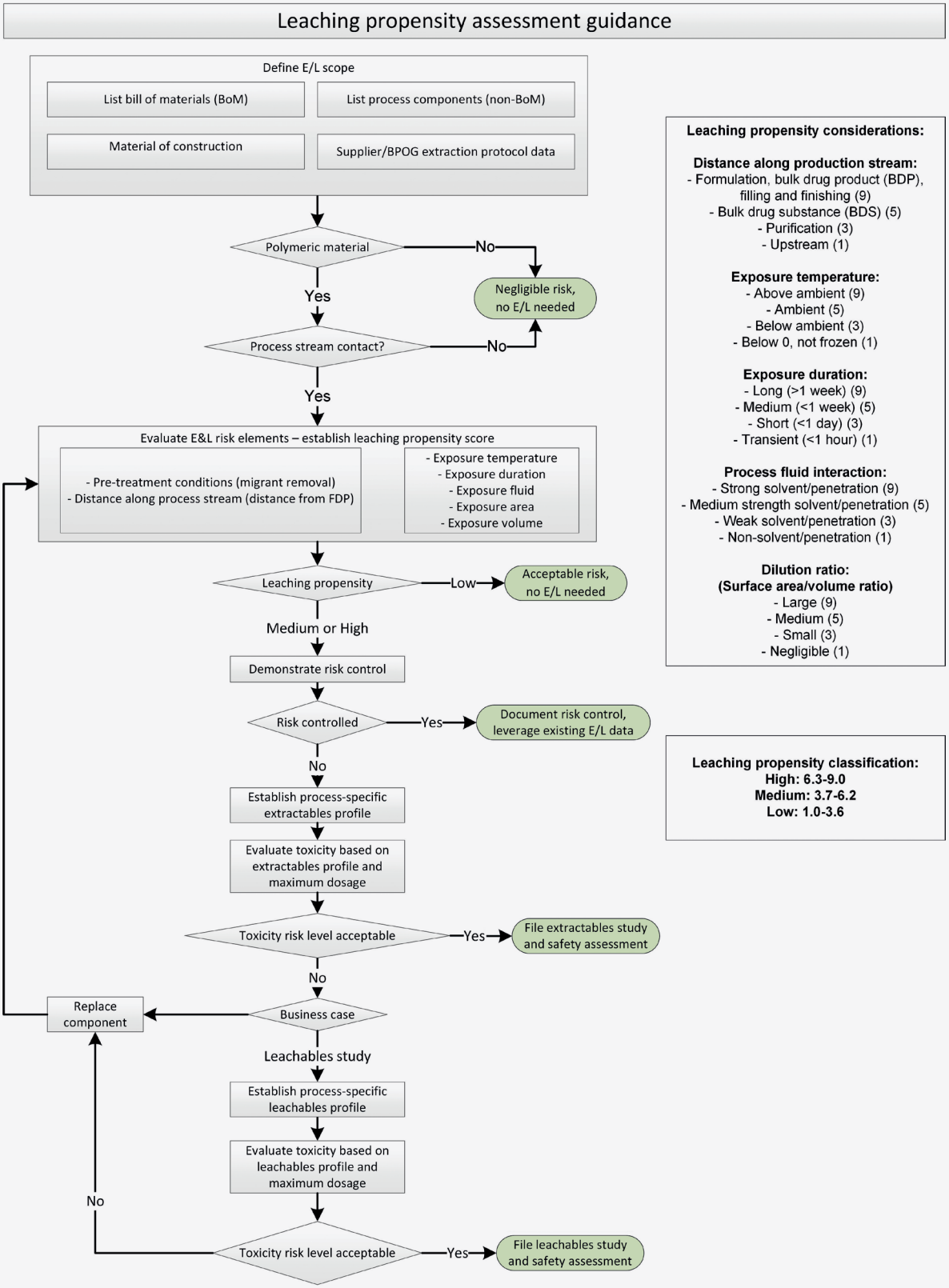
Appendix

Appendix 1: List of Abbreviations

Acronym	Definition
AAS	atomic absorption spectroscopy
ACN	acetonitrile
AES	atomic emission spectroscopy
AET	analytical evaluation thresholds
API	active pharmaceutical ingredient
APCI	atmospheric pressure chemical ionization
BDP	bulk drug product
BDS	bulk drug substance
BHT	butylated hydroxytoluene
BPA	bisphenol A
BPOG	BioPhorum Operations Group
CFR	Code of Federal Regulations
CoA	certificate of analysis
CRM	certified reference materials
CRO	contract research organization
DCM	dichloromethane
DAS	distance along the production stream
DF	diafiltration
DP	drug product
DR	dilution ratio
DS	drug substance
ED	exposure duration
EFA	effective filtration area
EMA	European Medicines Agency
E/L	extractables and leachables
EP	European Pharmacopoeia
ESA	exposure surface area
ESI	electrospray ionization
ET	exposure temperature
FDA	US Food and Drug Administration
FDP	final drug product
FID	flame ionization detector
FTIR	Fourier transform infrared spectroscopy
GC	gas chromatography
HPLC	high performance liquid chromatography
HS	headspace

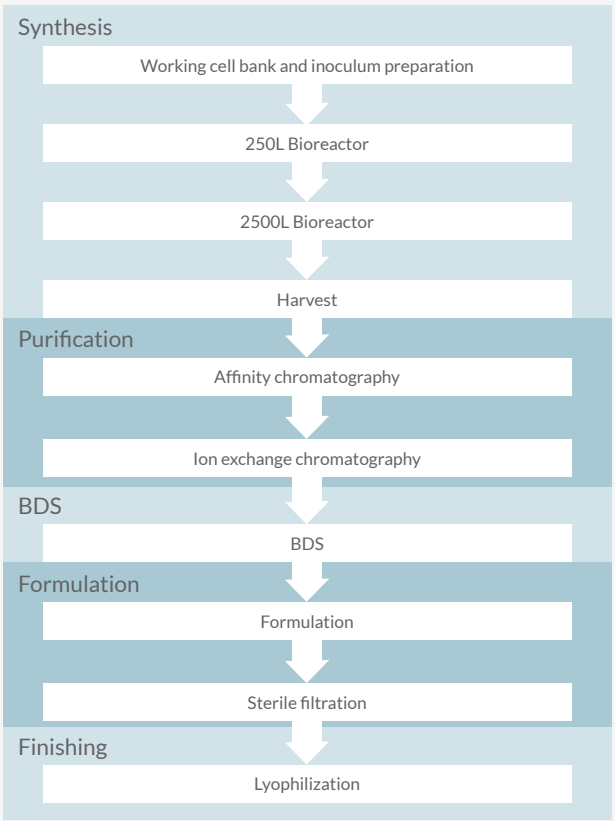
Acronym	Definition
IC	ion chromatography
ICH	International Council on Harmonisation
ICP-AAS	Inductively coupled plasma atomic absorption spectroscopy
ICP MS	Inductively coupled plasma mass spectrometry
ICP OES	Inductively coupled plasma optical emission spectrometry
ID	identification
LC	liquid chromatography
LOD	limit of detection
LOQ	limit of quantitation
LRR	leachables risk rating
MEK	methyl ethyl ketone
MeOH	methanol
MOC	material of construction
MS	mass spectrometry
NMR	nuclear magnetic resonance
NPD	nitrogen-phosphorus detector
OES	optical emission spectrometry
PDA	photodiode array
PDE	permissible daily exposure
PFI	process fluid interaction
PTFE	polytetrafluoroethylene
PVC	polyvinyl chloride
RSD	relative standard deviation
SCT	safety concern threshold
SOP	standard operating procedure
SUS	single-use systems
TFF	tangential flow filtration
TIC	total ion current
TOC	total organic carbon
TTC	threshold of toxicological concern
UF	ultrafiltration
UHPLC	ultra-high performance liquid chromatography
USP	United States Pharmacopeia
UV	ultraviolet
V	volume
WFI	water for injection

Appendix 2: An extractables and leachables (E/L) risk assessment process flow diagram



Appendix 3: Example list of operations used in leachables risk assessment for biological processes

For the risk assessment document, a real biologics process is used as an example to perform a risk assessment. The unit operations involved in the process are as follows:



Appendix 4: Example extractables/leachables propensity ratings for polymeric manufacturing components

Parameter and weight	Distance along process stream 0.40	Exposure temperature 0.15	Exposure duration 0.15	Solvation power and penetration 0.15	Dilution factor 0.15	Component E/L risk number overall	Risk classification
Component							
PCS tubing	3	5	3	3	3	3.3	Low
Ethylene glycol tubing	3	5	3	9	3	4.2	Medium
Connector	3	5	3	3	1	3.0	Low
10L carboy	1	5	5	3	1	2.5	Low
20L carboy	1	5	5	3	1	2.5	Low
LDPE bag	1	5	3	1	1	1.9	Low
Graduated cylinder	3	5	1	3	1	2.7	Low
PS bottle	1	5	9	3	1	3.1	Low
O-ring	1	5	3	3	1	2.2	Low
50L plastic bag, 3 ports	3	5	5	3	5	3.9	Medium
Acetic acid tubing	3	5	1	9	5	4.2	Medium
Filling tubing	9	5	3	3	5	6.0	Medium
Sterilizing-grade filter	9	5	5	3	9	6.9	High
In-process filter	3	5	5	3	9	4.5	Medium
BDS Storage bag	9	3	9	5	5	6.9	High

Leachables risk ratings: 6.3– 9.0: High; 3.7–6.2: Medium; 1.0–3.6: Low

Example definitions of extractables/leachables risk ratings

	Process stream rating	E&L risk rating - polymers			
Risk rating	**Distance along PS**	**Exposure temperature**	**Exposure time**	**Solvation power or Penetration**	**Dilution factor (Surface area/ process volume)**
1	**Synthesis:**	**Frozen**	**Transient**	**Non-solvent/no penetration**	**<1 x 10^{-3} m^2/L**
Example	Cell bank, vial thaw, inoculum, expansion, production, harvest, plasma thaw	<0°C	Minutes	Water for injection, inorganic buffer	Fittings, connectors, gaskets
3	**Purification:**	**Cold**	**Short**	**Low solvation power or penetration**	**1 x 10^{-3} to <1 x 10^{-2} m^2/L**
Example	Affinity chromatography, viral inactivation, ion exchange chromatography, viral filtration, UF/DF	0-8°C	Hours	20% protein solution, organic buffer	Short/high diameter tubing
5	**Bulk drug substance:**	**Ambient**	**Medium**	**Medium solvation power or penetration**	**1 x 10^{-2} to <1 x 10^{-1} m^2/L**
Example	BDS filtration, BDS Storage	8-30°C	Days	40% alcohol, 1% surfactant	Long, low diameter tubing
9	**Formulation, filling and finishing:**	**Above ambient**	**Long**	**High solvation power or penetration**	**>1 x 10^{-1}m2/L**
Example	Potency adjustment, sterile filtration, filling, lyophilization, FDP storage	>30°C	Weeks or longer	Isopropanol, ethanol	Filters, final container

Appendix 5: Example of study design parameters

Test article			
Number of test articles			
Materials of construction			
Part number			
Lot number(s)			
Expiration date			
Solution identity			
Pretreatment	Variable	Units	Value(s)
Gamma irradiation	Dose	kGy	
Autoclave	Time	Minutes	
	Temperature	°C	
	Number of cycles	#	
Pre-flush	Fluid identity	Name	
	Duration	Minutes	
	Temperature	°C	
	Volume	L	
Test conditions	Variable	Units	Value(s)
	Temperature	°C	
	Duration	Mins, hours, days	
	Solution contact surface area	cm^2	
	Solution volume	mL	
	Surface area to volume ratio	cm^2/mL	
	Solution/component weight (start)	g	
	Solution/component weight (end)	g	
	Solution pH (start)	pH	
	Solution pH (end)	pH	

Supporting information			
Bags	Film thickness	mm	
	Volume (capacity)	L	
Tubing	Wall thickness	mm	
	Internal diameter	mm	
	Length	mm	
Connectors	Internal diameter	mm	
Filters and TFF cassettes	EFA	m^2	
Filling needles	Internal diameter	mm	
Timing of irradiation	Time between manufacturing and gamma irradiation	Days (Lot 1) Days (Lot 2), if applicable	
	Time between gamma irradiation and incubation	Days (Lot 1) Days (Lot 2), if applicable	
Gamma irradiation	Typical dose range in manufacturing	kGy	

EFA = effective filtration area TFF = tangential flow filtration

Appendix 6: Example of leachables study results reporting format

ID of compound detected	Analytical method	LOQ	Process fluid name (e.g. buffer solution)		DS or DP solution name (e.g., buffer + API or DS; DP including excipients)	
			Control ($\mu g/cm^2$)	Day number	Test sample ($\mu g/cm^2$)	Day number
Compound 1						
Compound 2						
Compound 3						

API = active pharmaceutical ingredient; DS = drug substance; DP = drug product; LOQ = limit of quantitation.
Add as many rows as necessary.
Data could also be reported in mg/mL here.

Appendix 7: Recommended analytical techniques for Leachables identification and quantification

Outlined below are the example approaches for the major analytical techniques applied to the identification and quantification of leachables from SUS components. Parameters are shown as an example starting point and may be adjusted to fit specific product needs.

Appendix 7.1 LC-UV-MS: HPLC with UV photodiode array detection and mass spectrometry

Table A7-A: Example conditions and assay performance parameters for HPLC with UV PDA and mass detection

Standards	BPA and Irganox® 1010[a] (method sensitivity and range)
Limit of detection	BPA standard signal-to-noise ratio ≥3
Precision (UV)	SCT for each specific compound or 1µg/mL BPA, RSD ≤20% (n=6)
Spike recovery (UV)	80-120% or acceptable range for purpose
Column	C18
Mobile Phase A[b]	Acidified water or aqueous buffer
Mobile Phase B[b]	Acidified organic (ACN and/or MeOH) or as appropriate
PDA range	200-400nm or selected wavelengths
Mass spectrometric scan range	100-2000m/z or as appropriate for the instrument

ACN = acetonitrile; BPA = bisphenol A; HPLC = high-performance liquid chromatography; LC = liquid chromatography; MeOH = methanol; MS = mass spectrometry; PDA = photo diode array; SCT = safety concern threshold; RSD = relative standard deviation, UV = ultraviolet.

[a] Irganox is registered trademark of Ciba Specialty Chemical Corporation.

[b] Mobile phase additives may be selected to optimize both detector sensitivity as well as chromatographic performance based on the expected analytes.

Notes

- Other chromatographic instrumentation and conditions may be used to meet assay performance parameters, such as ultra-high performance liquid chromatography (UHPLC).
- Limit of quantitation (LOQ) in the matrix should be reported.
- Standards listed in the table are to demonstrate method sensitivity in the detectors and to demonstrate the chromatographic range for the observation of unexpected compounds; additional known extractables compounds should also 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 SCT or 1µg/mL for each specific compound or BPA in buffer or placebo.
- 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 ≥30% higher than in controls.
- Mass spectrometric detection may include +/- electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI), depending on the identified target compounds.
- 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 the area of lowest standard injection should be reported.
- Limit assays can also be set up for specific compounds, according to their accepted safety thresholds based on their toxicity and referring to the product posology.

Appendix 7.2 GC-MS: direct injection gas chromatography with mass spectrometry

Table A7-B: Example assay performance parameters for direct injection GC with mass detection

Standards	Eicosane and butylated hydroxytoluene (method sensitivity and range), or other suitable standard
Internal standard	phenanthrene-d_{10} or p-tertphenyl-d_{14} (or alternative appropriate standards to cover different analytes within the chromatographic run)
Limit of detection	30-600m/z or as appropriate
Scan range	BHT, standard signal-to-noise ratio ≥3
Precision (TIC)	SCT for each specific compound or 1µg/mL BHT, RSD ≤20% (n=6)
Spike recovery (TIC)	80-120% or acceptable range for purpose
Column	DB-624 (or equivalent)

BHT = butylated hydroxytoluene GC = gas chromatography MS = mass spectrometry RSD = relative standard deviation SCT = safety concern threshold TIC = total ion current.

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) in the matrix should be reported. Standards listed in the table are to demonstrate method sensitivity and chromatographic range; additional known extractables 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 SCT or 1µg/mL of a specific compound or butylated hydroxytoluene (BHT) in buffer or placebo.
- 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.
- Where quantitation is not possible, semi-quantitative values may be reported by reference to responses of suitable standards or referring to the response of the internal standard.
- For semi-quantitative analysis, results for peaks with a signal-to-noise ratio >10 or peaks above the area of lowest standard injection should be reported.
- Limit assays can also be set up for specific compounds, according to their accepted safety thresholds based on their toxicity and in reference to the product posology.

Liquid-liquid extraction procedure for direct injection

- Dichloromethane (DCM) is the preferred extraction solvent with the internal standard at 1µg/mL for screening or quantification of unknowns; a higher or lower level may be needed depending on established safety threshold levels for specific compounds.
- 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 1mL; repeat preparation if sample reaches significantly less than 1mL.
- Reconstitute concentrated extract for analysis with DCM to a known final volume.

Appendix 7.3 GC-MS: headspace sampling GC with mass spectrometry

Table A7-C: Example assay performance parameters for headspace sampling GC with mass detection

Standards	Toluene, MEK and octamethylcyclotetrasiloxane (method sensitivity and range), or other suitable standard
Internal standard	Toluene-d_8 or another appropriate standard
Limit of detection	MEK, standard signal-to-noise ratio ≥3
Scan range	30-400m/z or as appropriate
Precision (TIC)	SCT for each specific compound or 1ppm MEK, RSD ≤20% (n=6)
Spike recovery (TIC)	70-130%
Column	DB-624 (or equivalent)

GC = gas chromatography MS = mass spectrometry MEK = methyl ethyl ketone TIC = total ion current SCT = safety concern threshold
RSD = relative standard deviation

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) chromatogram to ensure no unexpected peaks are observed.
- Limit of quantitation (LOQ) should be reported.
- Standards listed in the table are to demonstrate method sensitivity and chromatographic range. Additional known extractables 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.
- Spiked concentration is safety concern threshold (SCT) or 1µg/mL of specific compounds and/or methyl ethyl ketone (MEK) in buffer or placebo.
- Control sample injections should be run to subtract matrix-associated peaks from consideration.
- eport sample compounds also observed in controls if they are ≥50% of the control amount.
- Where quantitation is not possible, semi-quantitative values may be reported by reference to responses of suitable standards referring to the response of the internal standard.
- For semi-quantitative analysis, results for peaks with a signal-to-noise ratio >10, or peaks above the area of lowest standard injection should be reported.
- Limit assays can also be set up for specific compounds, according to their accepted safety thresholds based on their toxicity and in reference to the product posology.

Appendix 7.4 Inductively coupled plasma with mass spectrometric detection (ICP-MS) or with optical emission spectrometric detection (ICP-OES)

The following points are valid for both quantitative and semi-quantitative analysis (i.e. total quantitative single point standard calibration).

- 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 the study material.
- Target level of limit of quantitation (LOQ) is 10ppb; LOQ may be lower or higher than 10ppb depending on the element being detected, the sample matrix and instrument parameters used; if LOQ >10ppb, justification should be provided.
- Report LOQ obtained for each element detected.
- Limit of detection (LOD) should be reported.
- Standard solutions containing detected elements should be used for recovery studies; recovery should be from 80–120%.
- Quantify detected elements based on calibration curves.
- For elements that have concentrations higher than the LOQ the final report should include "worst case" concentrations in µg/mL (if information on exact dosing is not available).
- For elements below LOQ, report LOQ and indicate lower than LOQ.
- Control sample injections should be run to subtract matrix-associated elements from consideration.

Appendix 7.5 Ion chromatography

Ion chromatography (or ion-exchange chromatography) is a process that allows the separation of ions and polar molecules based on their affinity to an ion exchanger and on their net surface charge. This technique is useful for analysis of inorganic ions that do not possess chromophores but are not amenable to analysis via ICP-MS or GC-MS techniques.
Ion chromatography can also be used to separate and detect different oxidation states of elemental impurities if necessary.

- Select anion exchange or cation exchange column based on expected polarity of molecules.
- Ionic strength and pH of the mobile phase is an important factor.
- Limit of quantitation (LOQ) should be reported.
- Instrument and analysis conditions should be optimized to achieve required sensitivity.
- Use known standards to demonstrate method sensitivity and chromatographic range; additional known extractables compounds may be prepared as standards to be injected for each unique material.
- An injection of standard should occur at least once for every 10 sample injections.
- Control sample injections should be run to subtract matrix-associated peaks from consideration.
- 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 the area of lowest standard injection should be reported.

References

1. European Medicines Agency, *Guideline on Plastic Immediate Packaging Materials*, 2005.

2. Chemistry Manufacturing and Control Documentations, Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER), *Guidance for Industry: Container Closure Systems for Packaging Human Drugs and Biologics*, 1999.

3. Ding W, Madsen G, Mahajan E, O'Connor S, Wong K, *Standardized Extractable Testing Protocol for Single-Use Systems in Biomanufacturing*, Pharmaceutical Engineering, 2014; 34(6), 1–11.

4. Ball D, Blanchard J, Jacopson-Kram D, et al., *Development of Safety Qualification Thresholds and their use in Orally Inhaled and Nasal Drug Product Evaluation*, Toxicol Sci. 2007; 97(2):226–36.

5. Broschard TH, et al., *Assessing Safety of Extractables from Materials and Leachables in Pharmaceuticals and Biologics – Current Challenges and Approaches*, Regulatory Toxicology and Pharmacology, Volume 81, November 2016, pages 201–211.

6. Ding W, *Determination of Extractables and Leachables from Single-Use Systems*, Chemie Ingenieur Technik, Wiley Publication, 2013, 85, No. I–2, pages 186–196.

7. ICH M7, *Assessment and Control of DNA Reactive (Mutagenic) Impurities in Pharmaceuticals to Limit Potential Carcinogenic Risk*, 2014.

8. ICH Q3D, *Guideline for Elemental Impurities*, 2014.

9 ICH Q9, *Quality Risk Management*, 2005.

10. European Medicines Agency, *Guideline on Process Validation for the Manufacture of Biotechnology-Derived Active Substances and Data to be Provided in the Regulatory Submission*, 2016.

11. Code of Federal Regulations, 21 CFR 211.65, 2016, Title 21 Volume 4.

12. Hammond M, et al., *Identification of a Leachable Compound Detrimental to Cell Growth in Single-Use Bioprocess Containers*, PDA J Pharm Sci and Tech, 67 (2013): 123–134.

13. Magarian N, et al., *Clearance of Extractables and Leachables from Single-Use Technologies via Ultrafiltration/Diafiltration Operations*, Biotechnol. Prog. (2016), 32:718–724

14. BPOG Extractables and Leachables Team, *Survey of Industry Leachables Best Practices Completed*, PDA Letter, March 2016, p 28–30, 34.

15. McGohan K, *Developing a Best Practice Guide for Leachables Risk Assessment*, Study Design, and Analytical Methods, BioPharma Asia, Vol 5 (4) July–August 2016, 6–11.