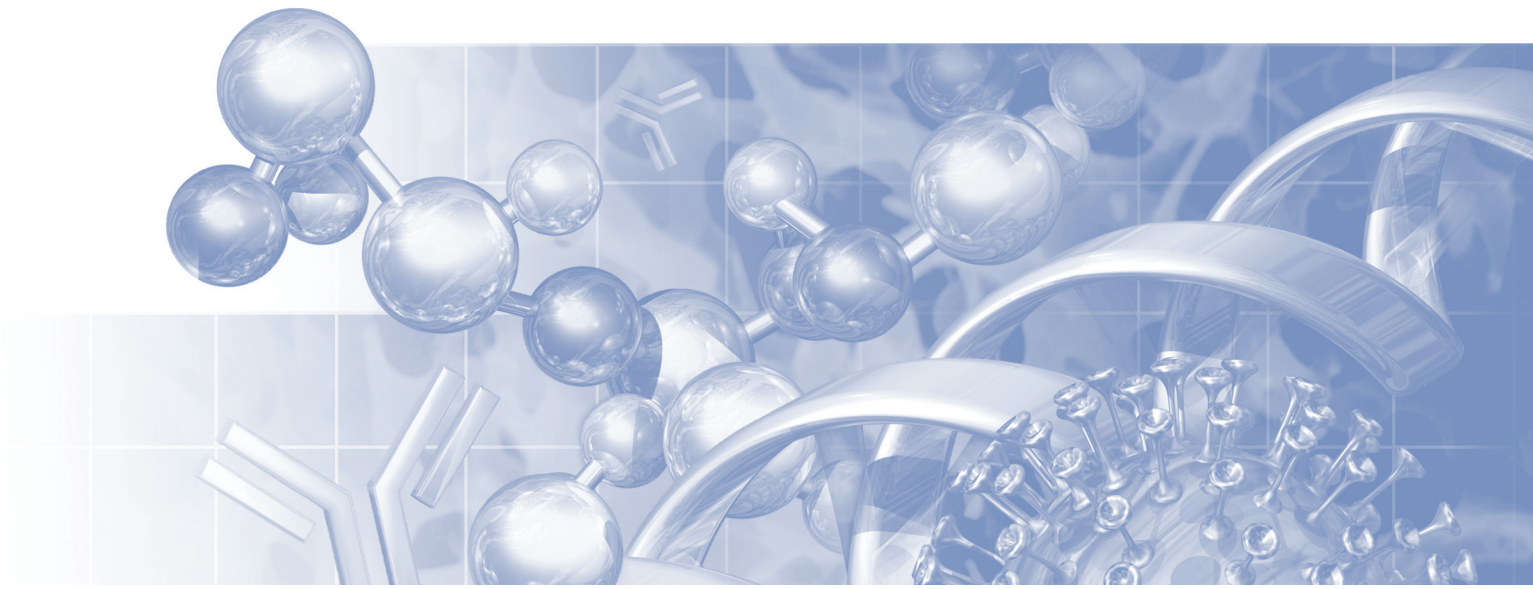
PALL Life Sciences

Technical Report

USTR 2859

Extractables and Leachables from Single-use Systems



*Filtration. Separation. Solution.*SM

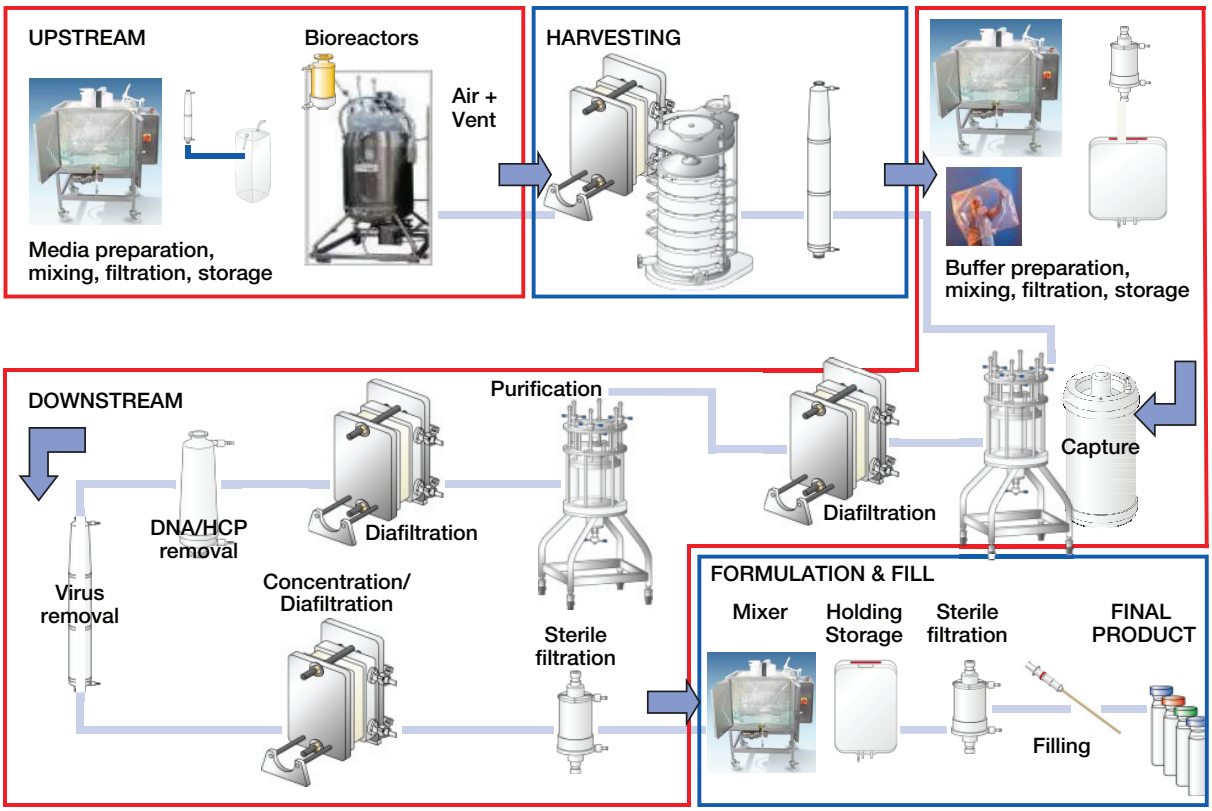
Contents

1. Introduction

Recent developments in bioprocessing equipment have expanded considerably the range and scale of unit operations incorporating single-use technology, offering pharmaceutical manufacturers a practical choice of cleanable or single-use systems from upstream and downstream processing, through formulation and filling. Some examples of this wide range of applications are shown in Figure 1, starting with upstream media preparation and bioreactors followed by harvesting and fluid clarification with disposable depth filters. The subsequent purification steps include buffer preparation, membrane chromatography and single-use ultrafiltration and diafiltration. Virus filtration and removal of DNA and HCP are also essential operations in many bioprocesses followed by final stages of concentration, diafiltration, sterile filtration and single-use formulation and filling.

Figure 1

Single-use Bioprocess Applications



For these and many other processes, Pall Life Sciences is a major supplier of components and systems including:

- Allegro™ 2D and 3D biocontainers
- Allegro mixers
- Mustang® membrane chromatography
- Kleenpak™ sterile connectors and disconnectors
- Allegro single-use needles
- Kleenpak, Novasip™ and Kleenpak Nova pleated capsule filters
- Stax™ depth filter capsules and systems
- Allegro fully-assembled single-use systems for fluid transfer, direct flow filtration, tangential flow filtration, mixing
- Allegro fully assembled formulation systems and filling lines

In this technical report, we describe:

1. Why extractables and leachables data are important and the regulatory concerns.
2. How Pall's Scientific and Laboratory Services has successfully applied a risk-based approach to characterization of extractables and leachables for end users to incorporate in their regulatory submissions.
3. How end users can obtain relevant and useful data acceptable to regulatory agencies based on:
   - Generic extractables data
   - User-specific extractables and leachables studies for process validation
   - Interpretation of generic and user-specific data.
4. How to minimize project time and costs for extractables and leachables studies.
5. Why and how Pall, as a service provider, can assist end users via its Validation, Engineering and Technical Support Services and in-house capabilities.

2. Users' Perspective

Despite the many advantages of single-use systems for safety, flexibility, reduced capital and operating costs and many others, there are also a number of reasons cited by the industry that restrict the incorporation of single-use technologies. In a recent survey of biopharmaceutical manufacturing conducted by Bioplan Associates [1], the most common factor cited to restrict the use of disposables was concerns over leachables and extractables. 65% of respondents agreed or strongly agreed that leachables and extractables were a concern.

3. Regulators' Perspective

Regulatory authorities around the world have also expressed their concerns on this topic. For example, in a presentation by Rabia Ballica of the FDA [2], it was stated that there were potential challenges from a product perspective because "extractables and leachables from product-disposables contact surfaces may impact on product quality and efficacy". Similarly, Destry Sillivan of the FDA CBER [3] stated in a CBER/DMPQ Communication to Regulated Industry: "…with respect to extractables and leachables data… it is ultimately your responsibility to assess this data and its applicability to your products and process. CBER recommends a risk-based approach be taken in evaluating extractables and leachables, where you take multiple aspects into account – for example, indication, safety issues, product characteristics, dosage, formulation, stability profile, etc."

So why is there concern over extractables and leachables? In fact, the primary concern is that leachables are chemicals that can migrate into the drug product from process equipment and could potentially affect:

- Safety – by increasing the toxicity of the drug
- Quality – by raising impurity levels
- Potency – by inactivating drug components (e.g. by oxidation or precipitation).

In biologicals, there is the concern that drug components such as proteins could mask some leachables and interfere with their analysis. For example, the non-volatile components in the drug components could interfere with quantitative non-volatile residue measurement and proteins could elute at the same time as leachables during the liquid chromatography analysis. This possibility leads us to look at extractables using model solvents that will have minimum interference with the analytical methods and under extraction conditions that are more aggressive to identify fully the chemicals that are extracted from process equipment. In this way, we can investigate migrant compounds that could potentially become leachables in the drug product.

4. Determination of Extractables and Leachables from Single-use Systems

Before embarking on a program for measurement of extractables and leachables from single-use systems, it is important to be aware of the wealth of information available on regulations and guidance. Because leachables can cause safety, quality and efficacy issues for final drug products, the FDA, EMA and ICH have clear regulations and guidelines for them, as shown in Table 1.

Table 1

Regulations and Guidance Applicable to Extractables and Leachables

International Standards
• ICH Q1A: Stability testing of new Drug Products and Substances: Container closure system
• ICH Q3A, Q3B: Impurities in New Drug Substances and Products: Threshold (%) depending of the max daily dose
• ICH Q3C = E.P.5.4 = USP<467> (draft): Guidelines for residual solvents
• ICH Q7A: Good Manufacturing Practice Guidance for Active Pharmaceutical Ingredients
• ISO 10993 part 13: Identification and quantification of degradation products from polymeric medical devices
• ISO 15747 (2003): Plastics containers for intravenous injection

USA and North America	Europe
• 21 CFR Part 211.65: Equipment construction	• EU GMP, Medicinal products for human and veterinary use, European Commission, Volume 4, chapter 3, paragraph 3.39
• 21 CFR Part 211.94: Drug Containers	• EMEA/205/04 Guideline on Plastic Immediate Packaging Materials
• Canadian Food and Drug Regulations, GMP, *Part C, Div. 2, Section C.02.005:* Equipment	
• FDA CDER/CBER Guidance for Industry: Container Closure Systems for Packaging Human Drugs and Biologics	

Other References
• ASTM E2097-00 (2006): Standard guide for determining the impact of extractables from non-metallic materials on the safety of Biotechnology Products
• BPSA Guides to Extractables from Single-use components and systems (2008, 2010)
• PDA Technical Report on Single-use Manufacturing (in development)
• PQRI publication: Safety thresholds and best practices for extractables and leachables in Orally Inhaled and Nasal Drug Products (2006); In Parenterals and Ophthalmic Drug Products (in development)

The regulations and guidelines do not, however, tell us how to design the test, how to perform analyses and how to interpret the data. That is where the industry groups come into play. The BioProcess Systems Alliance (BPSA) has published detailed recommendations on how to perform extractables studies on single-use systems. The Parenteral Drug Association (PDA) is also working on a technical report on single-use systems that will provide general recommendations for determination and application of extractables and leachables data. The Product Quality Research Institute (PQRI) has published recommendations for safety concern threshold of container/closure leachables in inhalation and nasal drug products and they are currently working on similar recommendations for parenteral and ophthalmic drug products.

This type of documentation provides a firm basis on which to plan a process-specific evaluation but before doing so, we need to be clear on the definitions of extractables and leachables to ensure that meaningful data are obtained and interpreted correctly. Many different versions have been published but the BPSA definitions are focussed specifically towards process equipment.

Extractables

The BPSA defines extractables as “Chemical compounds that migrate from any product-contact material, including elastomeric, plastic, glass, stainless steel or coating components when exposed to an appropriate solvent under exaggerated conditions of time and temperature”. Extractables can show the potential for a material to release leachables into a drug product or process fluid [4].

Leachables

The BPSA defines leachables as “Chemical compounds, typically a subset of extractables, that migrate into the drug formulation from any product-contact material, including elastomeric, plastic, glass, stainless steel or coating components as a result of direct contact with the drug formulation under normal process conditions or accelerated storage conditions and are found in the final drug product”[4].

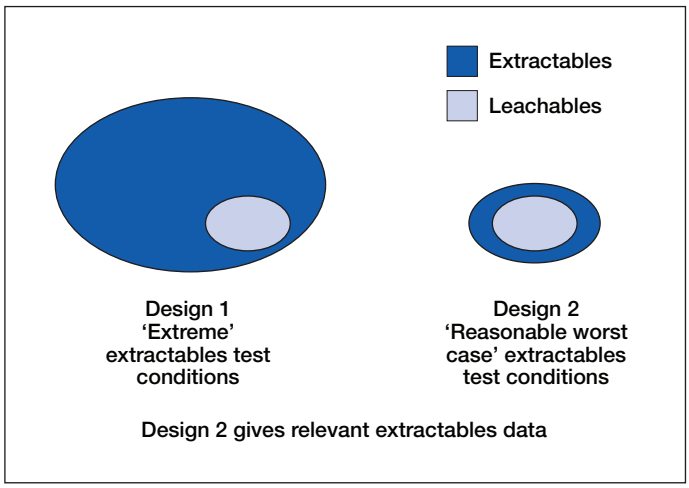
There are two key aspects to consider:

1. Extractables tests are performed using a model solvent whereas leachables studies use the actual drug product or process fluid.
2. Extractables are obtained under exaggerated or aggressive conditions but leachables tests use normal process conditions.

We then need to understand the relationship between extractables and leachables. Typically, leachables are shown as a sub set of extractables as explained by the diagram in Figure 2. In some cases, due to the interaction of process fluid or drug product with process equipment, some leachable compounds are not part of the extractables.

Figure 2

Typical Relationship between Extractables and Leachables



If extreme extractable conditions are used, such as with very aggressive solvents and very high temperatures, a large quantity of extractables will be obtained as shown in Design 1. The offset positioning of the extractables also indicates that many of the extractable compounds are not related to the leachables. Full chemical and physical analysis of these unrelated extractables takes time and may not be necessary for a specific process.

In Design 2, reasonable worst case test conditions have been used and the extractables results are more relevant to the process. So our primary objective is to obtain relevant extractables data that can then be used to represent the leachables, if a scientific justification can be made.

How can we obtain the relevant extractables data? The best place to start is the supplier of the process equipment. The BPSA has advocated that suppliers perform extensive studies on their disposable products and make the data available to users. If the data is not available or suitable for your process, then a process- and product-specific study needs to be performed. If the extractables study is performed properly, a leachables test may not be necessary if supported by a sound scientific justification. In some cases, if there is a possibility that some leachable compounds may not be part of extractables, the actual leachables study should be performed.

5. Pall's Approach to Generating Extractables Data for Single-use Systems

As a major supplier of filters and single-use systems, Pall recognized the importance of extractables from disposables many years ago and embarked on a systematic study program. Based on Pall's extensive experience, a practical and science-based approach for single-use systems was developed.

This study was divided into two parts.

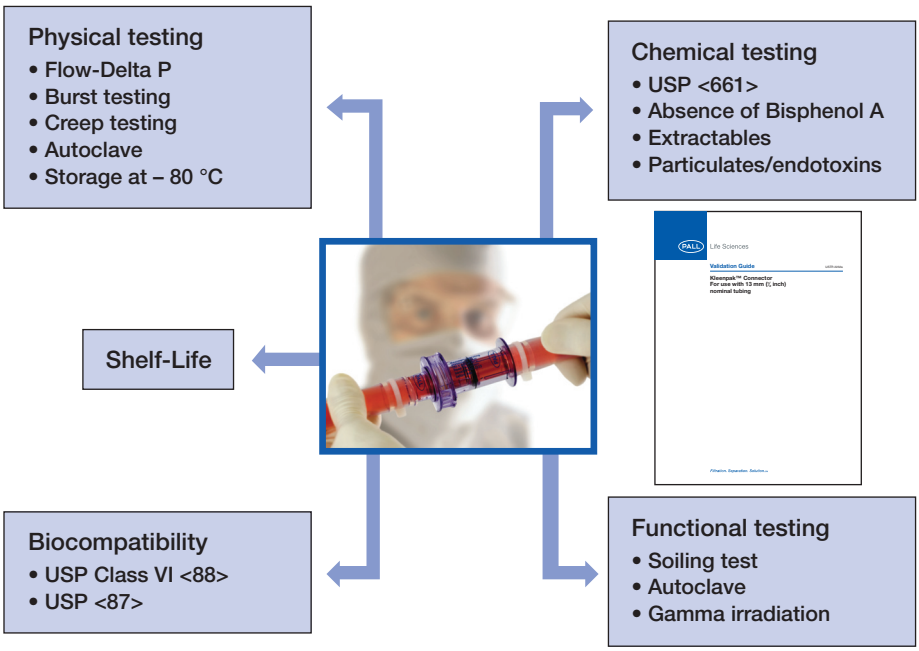
5.1. Individual Components

As part of Pall's validation approach, we generate extractables data for all our existing components as well as for the newly developed ones. As a system integrator, we also generate the same data for our standard components (such as tubing and connectors) supplied by other suppliers. We studied individual components separately using qualified or validated analytical methods to identify and quantify extractables under reasonable worst-case conditions. The data was then used to build a library of extractable compounds. The first set of components tested included Kleenpak sterile connector, Allegro biocontainers, Kleenpak capsule filters and thermoplastic tubing.

An extractables study on a single-use component is part of a more extensive qualification and validation program for that component. For example, the validation strategy for sterile connectors, presented in Figure 3, shows the extractables testing as an essential part of the Chemicals Testing program, which together with Physical and Functional Testing, Biocompatibility and Shelf Life studies form the basis of the Validation Guide. This guide, which is available to users, can be an important part of the process validation documentation for submission to regulatory agencies.

Figure 3

Validation Strategy for Pall Kleenpak Sterile Connectors



5.2. Multi-component Systems

Having completed extractables studies on the individual single-use components, we then designed model multi-component systems for further evaluation. One example, shown in Figures 4a and 4b, contained four types of single-use components – Kleenpak capsule filters, thermoplastic tubing, Kleenpak sterile connectors and Allegro biocontainers. The model system was designed with three parts – a fluid supply system, a filter manifold and a biocontainer manifold.

Figure 4a

Fluid Supply System and Filter Manifold

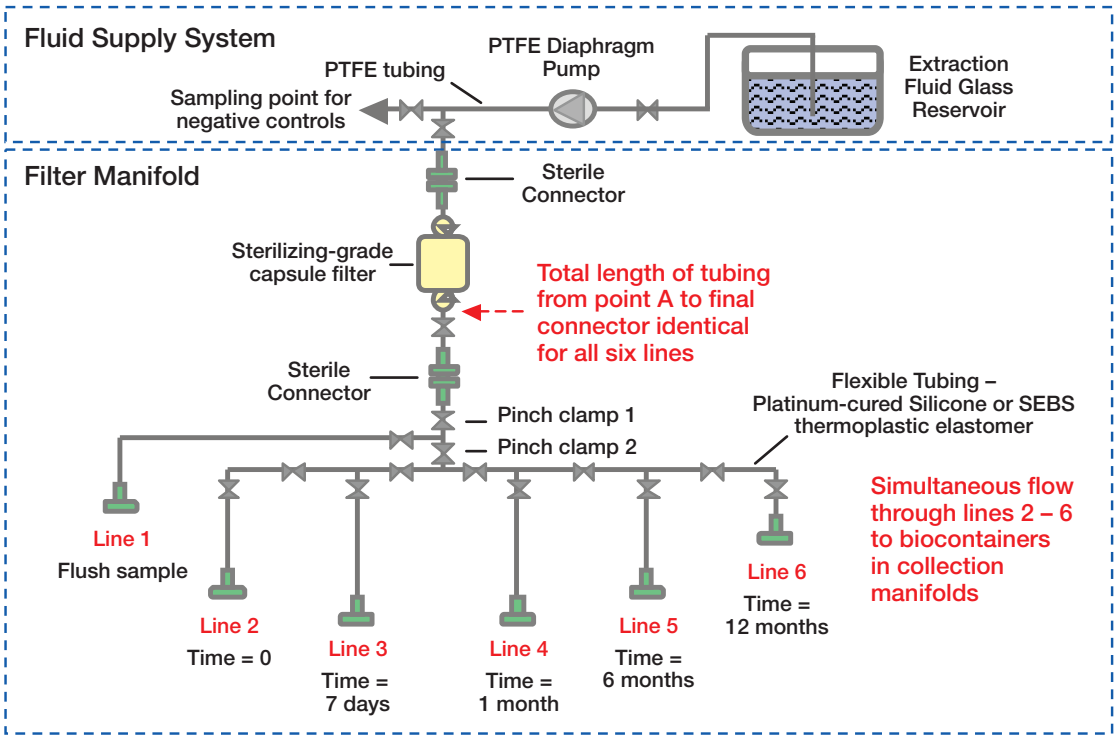
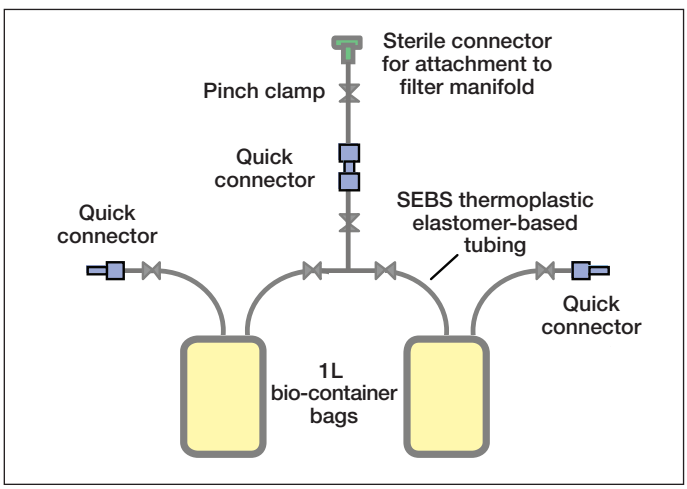


Figure 4b

Biocontainer Manifold



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This model system enabled us to study the effects of conditions such as pre-flushing and storage time in biocontainers for up to one year. The extraction procedure was also performed under conditions more typical of process conditions whereas studies on the individual components were performed under worst case conditions using procedures specific to each component.

Using this model approach, we were able to determine whether the extractables profiles, both qualitatively and quantitatively, were in agreement with the profiles already established for the individual components.

Conclusions drawn from these studies showed that:

- It is feasible to perform extractable studies on complete single-use systems.
- A short system flush can remove a substantial proportion of extractable material.
- Water extractables for organic compounds were substantially lower than ethanol extractables.
- In most cases, no significant increase in extractables was obtained after storage for up to 12 months.
- The compounds identified were also found in previous studies on the individual components.
- All compounds could be traced to the materials of construction of single-use components.

5.3. Analytical Methods

Extracts from the various studies were subjected to detailed physico-chemical analyses using thirteen different methods, as detailed in Table 2. Advanced analytical techniques within our own resources enable us to identify and characterize organic and inorganic compounds at levels down to parts-per-billion (ppb) and for some compounds down to parts-per-trillion (ppt) levels.

Table 2

Analytical Methods

	Water Extracts	Ethanol Extracts
TOC (Total Organic Carbon)	•	n/a*
pH	•	n/a*
Conductivity	•	n/a*
IC (Ion Chromatography)	•	n/a*
Nonvolatile Residue	•	•
FTIR (Fourier Transform Infrared) Spectroscopy	•	•
UV (Ultraviolet) Spectroscopy	•	•
Headspace GC/MS (MS: Mass Spectrometry)	•	•
Direct Injection GC/MS (Gas Chromatography MS)	•	•
Derivatization GC/MS	•	•
LC/UV (Liquid Chromatography / Ultraviolet)	•	•
LC/MS and LC/MS/MS	•	•
ICP/MS (Inductively Coupled Plasma MS)	•	•

** Test not applicable for ethanol extracts*

The results of all extractables studies are stored in a comprehensive data library, and are a vital part of the validation support that is available to users of Pall single-use systems. An example of some of the GC/MS data obtained from ethanol extracts in the model system is shown in Table 3.

Table 3

Example of GC/MS Results in Pall Data Library for Extractables

Method	Flush Sample	One-year Sample
Headspace GC/MS	3-Methylpentane, Hexane, Methylcyclopentane, Acetal, 3-Methylheptane, Octane (All below 3 ppm)	3-Methylpentane, Hexane, Methylcyclopentane, Acetal, 3-Methylheptane, Octane 2-Methylpentane, Cyclohexane, 1-Octene (All below 5 ppm)
Direct Injection GC/MS	Decamethylcyclopentasiloxane, Dodecamethylcyclohexasiloxane, A series of siloxanes, 1,3-di-tert-butylbenzene (1,3-DTBB), 2,4-di-tert-butylphenol (2,4-DTBP), 1-dodecanol, Lauryl acrylate and hydrocarbon isomer (All below 1 ppm)	Decamethylcyclopentasiloxane, Dodecamethylcyclohexasiloxane, A series of siloxanes, 1,3-DTBB, 2,4-DTBP, 1-dodecanol, Lauryl acrylate, Hydrocarbon isomers, 1-tridecanol, 1-pentadecanol, 1-octadecanol, A series of aliphatic hydrocarbons, C_{12} to C_{26} (All below 3 ppm)
Derivatization GC/MS	Oxalic acid, Malonic acid, Lauric acid, Succinic acid, Palmitic acid, Stearic acid (All below 0.3 ppm)	Succinic acid, Palmitic acid, Stearic acid (All below 0.3 ppm)

A series of articles by Pall published in *BioProcess International* and the *PDA Journal of Pharmaceutical Science and Technology* give more detailed information on these extractables studies (as well as other subjects on single-use technology) and can be obtained from our website or from your local Pall office or from the PDA [9 – 11, 13].

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6. User-specific Process Validation

Supplier's generic data can help to qualify the use of disposables in the process and, in some cases, the data may alone be sufficient without the need for user-specific validation. It is therefore important before planning any process-specific studies to establish the answers to three basic questions:

1. Is the supplier's data based on properly designed tests?
2. Are the analytical methods qualified and validated?
3. Do the test conditions bracket your process conditions and represent a worst case?

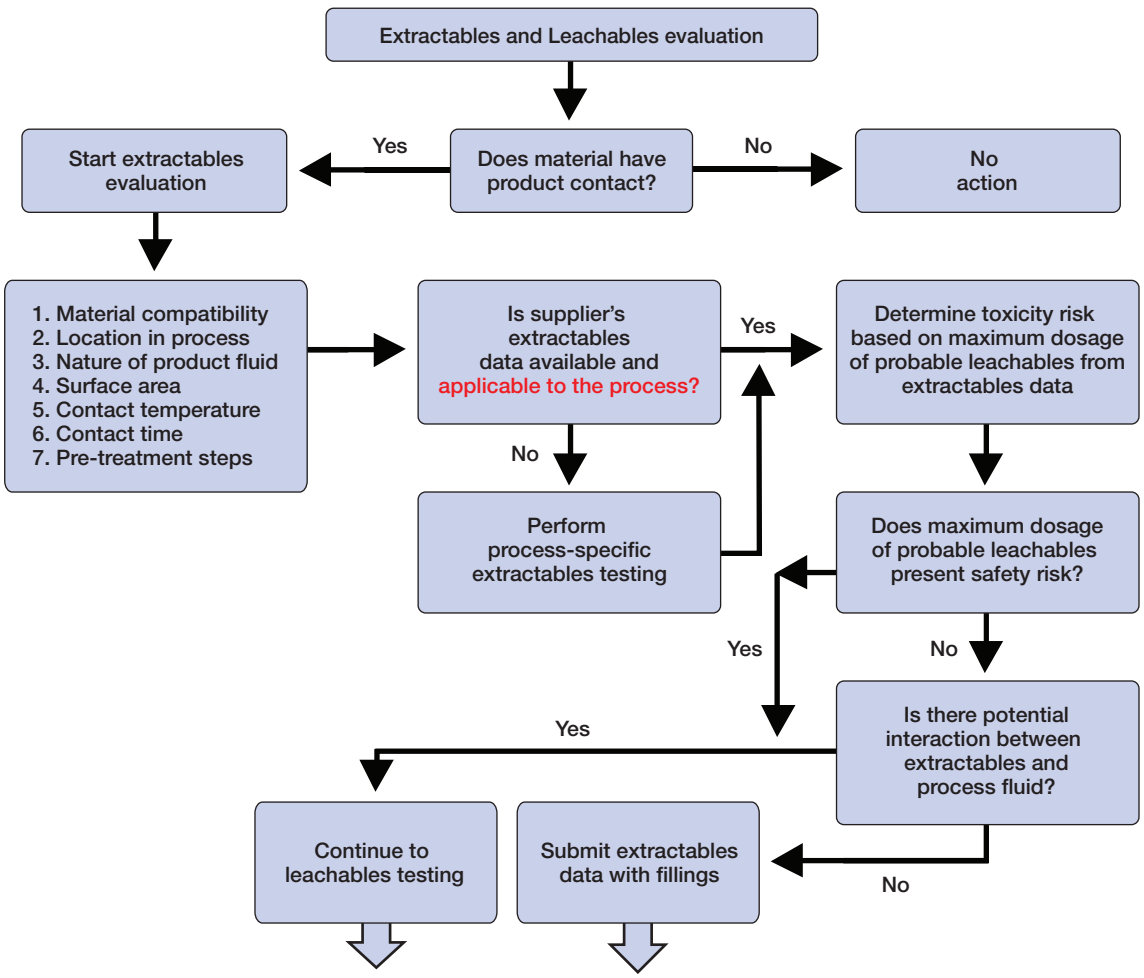
If all three answers are positive, then the data can be used for the process validation. Otherwise, a product- and process-specific extractables study should be performed to avoid the possibility of non-conformance during regulatory inspections.

The next key question is: 'How can users set about the task of qualifying extractables and leachables for so many different processes?' The best approach is to perform a risk assessment using a decision tree such as that shown in Figure 5. If, for example, the component does not have product contact, then generally no extractables information is required. On the other hand, if it has product contact but is not compatible with the fluid, then the component is not suitable for the process.

In the next stage of evaluation, one factor to consider is the proximity in the process to the final filled product – the closer to the final product, the higher the risk. Other factors that can influence extractables and leachables include the nature of the product fluid such as aqueous or organic; the contact time and temperature which affect the kinetics and thermodynamics of leaching of soluble compounds; the surface area of the component in fluid contact, and any pre-treatment such as gamma irradiation or steam sterilization.

Figure 5

Risk Assessment Decision Tree



As discussed previously, product- and process-specific extractables testing may be unnecessary if supplier's data is available and applicable to the process. Further decisions are based on toxicity and safety risks, and possible interactions between extractables and the process fluid. Depending on the course of this risk assessment, product- and process-specific extractables and/or leachables testing may be required.

7. Practical Approach to Performing User-specific Studies

7.1. Extractables

If the risk assessment determines that a user-specific extractables study is required, this procedure can be successfully completed by following some basic steps:

7.1.1. Select a Reasonable Worst Case Model Solvent

The solvent must be based on the composition of the product. In some cases, water may be the simple choice but for some product formulations, the choice may not be so obvious. Pall can assist in the decision process using our well-established Model Solvent Approach[SM]. Our strategy is based on sound chemical science and our wide experience in performing user-specific extractables studies.

7.1.2. Select Reasonable Worst Case Conditions

As discussed previously, conditions should represent 'reasonable' worst case and not 'extreme' worst case to ensure that the data will be relevant. The purpose is to identify and quantify extractables that are probable leachables in the specific process.

7.1.3. Select a Suitable Model System

Ideally the full scale single-use system should be used but if it is too large to be set up in the laboratory, then a scaled down version can be tested. If so, then the ratio of surface area to fluid volume must be maintained to represent a worst case.

7.2. Leachables

User-specific leachables studies require a different protocol to ensure that the important relationship to extractables data is achieved and that an accurate and meaningful interpretation of the results is possible. The main requirements of the protocol are as follows:

- The process fluid, not a model solvent, must be used for extraction.
- Actual, or slightly worse, process conditions should be used – not exaggerated worst case.
- As with extractables, the full scale system or a scaled down version can be used.

Due to possible interference of the process fluid with analytical tests, it may be necessary to remove interfering compounds from samples of extract by liquid/liquid or solid phase extraction prior to GC and LC analysis.

8. Case Study on User-specific Extractables and Leachables Test

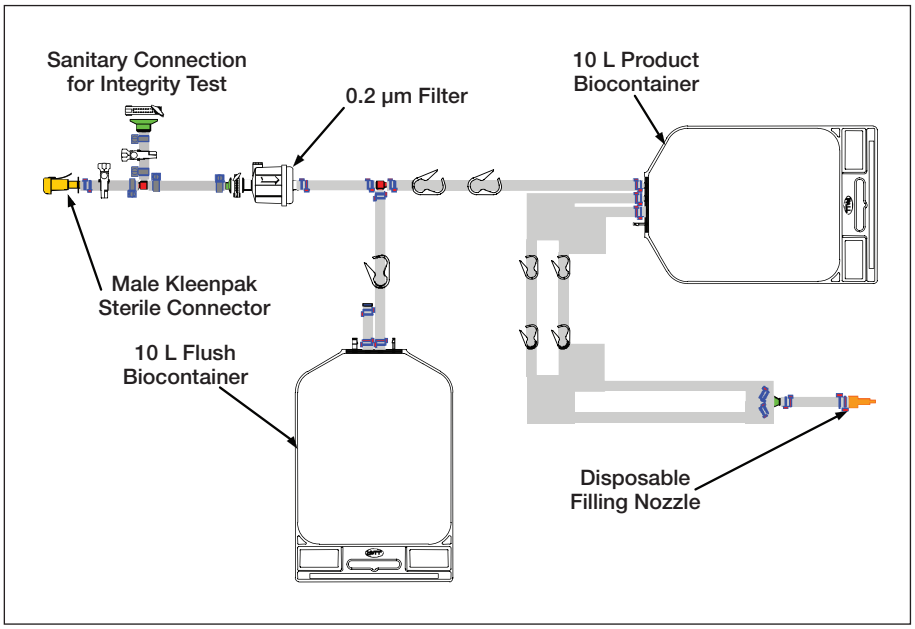
The general strategy and practical approach to user-specific extractables and leachables testing can be demonstrated conveniently by describing one of Pall's case studies.

The first task was to review and fully understand the user's single-use system.

Process fluid. The process fluid was a relatively simple formulation containing the active pharmaceutical ingredient (API), a surfactant and water.

Process system. The single-use system, shown in Figure 6, is used for final product sterilization prior to a filling and vial closure machine.

Figure 6
Single-use System



The system is based on a single manifold pre-sterilized by gamma irradiation and connected to the product supply by a sterile connector (Pall Kleenpak connector). The fluid passes through flexible tubing to a 0.2 µm sterilizing grade capsule filter (Pall Mini Kleenpak capsule) and is then collected in a 10 L flexible biocontainer (Pall Allegro biocontainer) before transfer through flexible tubing and a disposable nozzle to the filling machine. A T-piece downstream of the filter links to another 10 L biocontainer for collection of water used for system flushing and wetting of the filter for pre-use integrity testing. A sanitary connection upstream of the filter provides a connection for the integrity test instrument.

8.1. Risk Assessment

If a risk assessment shows that the supplier's data on extractables is not sufficient or applicable, it is usual to perform a user-specific extractables test. Using the data, a second risk assessment is completed to establish whether leachables testing is also required. As each test, extractables or leachables, takes approximately six months, a minimum period of 12 months should be allocated in the validation plan if extractables and leachables tests are done in series.

In this case study, the user had a planned filing date of less than one year and therefore decided as fail-safe measure after the risk assessment to perform both extractables and leachables testing in parallel.

8.2. Selection of Model Solvent

The properties of the model solvent should reflect the chemical and physical characteristics of the product fluid. An unsuitable selection could give erroneous and potentially misleading results. As an extreme example, if heptane was chosen as a model solvent for an aqueous formulation, then the large differences in polarity and chemistry would give a distorted extractables profile with little relevance to leachables.

When formulating a suitable model solvent, a good reference is Snyder's published data [5] which groups different types of solvents into Selectivity Classes, as shown in Table 4. The data also shows volatile solvents within each Class that are ideal for extractables studies as they can be easily removed from extracts or separated from extracted compounds during various analyses.

Table 4

Solvent Selectivity Classes

Group	Solvent Types	Volatile Examples
VIII	Cresols; Fluoralkanols; Water	Water
I	Aliphatic ethers; Some amides	None
II	Aliphatic alcohols	Ethanol; Methanol; IPA
III	Amides; Glycol ethers	Dimethylformamide (DMF)
IV	Glycols	Propylene glycol
V	Methylene & Ethylene chloride	Methylene chloride
VIa	Poly-ethers; Dioxane	2-ethoxyethanol
VIb	Aliphatic esters; Ketones	Ethyl acetate; Acetone; MEK
VII	Most aromatic solvents	Toluene; Phenol
None	Volatile pH adjusting agents	Ammonia; HCl; Acetic acid

By applying Pall's Model Solvent Approach℠ in this case study, a suitable model solvent mixture was prepared as detailed in Table 5. No addition of pH adjuster was required to maintain pH in the product's range of 6.2 – 7.2.

Table 5

Model Solvent Approach for Extractables Studies

Product Constituent		Assigned Model Solvent	
Type	**Concentration**	**Type**	**Proportion**
API	3.1% w/v	DMF	5% v/v
Surfactant	0.1% w/v	Ethanol	10% v/v
Water	Q.S.	Water	85% v/v

The full scale single-use system was used in the study. The extraction was performed by attaching a PTFE pump, PTFE tubing and glass reservoir to the system (similar to the fluid supply system shown in Figure 4a) and recirculating the solvent through the system, excluding the product biocontainer, which was filled with solvent and subjected to a soak test by agitation on an orbital shaker.

To simulate worst case conditions, test parameters shown in Table 6 were set.

Table 6

Extractables and Leachables Test Conditions

Parameter	Worst Case Test Conditions	Typical Process Conditions
Gamma irradiation	50 kGy	Minimum 25 kGy
Temperature	25 °C – 30 °C	15 °C – 25 °C
Test time – Filtration system	24 hours recirculation	16 hours single pass
Test time – Biocontainer contact	7 days with agitation	7 days static storage
System flush	No flush for Extractables; Flush for leachables	Flush

8.3. Analysis of Extracts

Due to the complex nature of compounds extracted from multi-component systems, a combination of analytical methods was used, both qualitative and quantitative, to target specific compounds or physico-chemical properties, as shown in Table 7.

Table 7

Analytical Methods and Their Target Compounds or Properties

Analytical Method	Symbol	Target Compounds or Properties
Non-volatile Residue	NVR	Total mass of extractables after evaporation of the model test solvent
Fourier Transform Infrared	FTIR	Qualitative analysis of NVR extracts (oligomers of polymeric materials)
Ultraviolet Spectroscopy	UV	Compounds with chromophores
Gas Chromatography / Mass Spectrometry – Direct injection	Direct injection GC/MS	Semi-volatile compounds
Gas Chromatography / Mass Spectrometry – Derivatization	Derivatization GC/MS	Organic acids (especially long chain fatty acids) by first derivatizing them into the corresponding esters
Gas Chromatography / Mass Spectrometry – Headspace	Headspace GC/MS	Volatile compounds
Liquid Chromatography / Ultraviolet / Mass Spectrometry	LC/UV/MS	Additives or their degradation products from the polymers
Inductively Coupled Plasma Spectroscopy	ICP/MS	Metal Ions

Quantitative non-volatile residue analysis is important to obtain the total mass of extractables after evaporation of the test solvent. Headspace GC/MS analysis will detect low molecular weight compounds such as those formed by the action of free radicals produced by gamma irradiation of organic polymeric components. ICP/MS analysis for metal ions is also important as some metal ions can cause oxidation and precipitation of proteins.

In this study, compounds and metal ions such as those shown in Table 8 were identified and quantified from both the extractables and leachables extracts, using authentic standards based on fully qualified and validated methods.

Table 8

Examples of Identified Compounds and Quantities

Compound or Metal Ion	Extractables from System	Leachables from System
2-Propanol	<4 mg	<3 mg
2,4-ditertbutylphenol	<1 mg	<1 mg
Stearic acid	<3 mg	<2 mg
Ca, Na and Al	<2 mg	<0.05 mg*

** Less than Limit of Quantification (LOQ)*

The omission of the system flush in the extractables test would be consistent with the increased quantities in the extracts compared to the levels of leachables from the flushed test system.

8.4. Interpretation of Data

How can we interpret this data with respect to product safety and risks to the patient?

In this case study, the leachables data, as it was available, was used for toxicity assessment. The method described in this report is one of the commons ways implemented in the industry.

Using 2-propanol as an example, we need to calculate the amount taken in by a patient per day based on the normal daily dosage for the drug product.

A. Total amount of 2-propanol leachables from system	<3 mg
B. Volume of final drug product in biocontainer (see Figure 6)	10 L
C. Concentration of 2-propanol in final product (A ÷ B)	<0.3 µg/mL
D. Patient daily dosage (2 x 5 mL; 5 mL/dose)	10 mL
E. Total intake of 2-propanol/patient/day (C x D)	<3 µg

From these calculations, the total daily intakes for other compounds can also be calculated, as shown in Table 9.

Table 9

Calculated Total Daily Intakes of Leachable Compounds

Compound or Metal Ion	Leachables from System	Total Daily Intake/Patient
2-propanol	<3 mg	<3 µg
2,4-ditertbutylphenol	<1 mg	<1 µg
Stearic acid	<2 mg	<2 µg
Ca, Na and Al	<0.05 mg	<0.05 µg

The next step is to determine whether this daily intake of each compound is acceptable with reference to safety and toxicity data.

To assist in this assessment, we can use Cramer's classification of compounds [6] which uses a decision tree for ranking chemicals into three classes (Class I, II, and III) according to their expected level of systemic toxicity, mainly on the basis of chemical structure and reactivity. Class I compounds have the lowest risk.

We can then carry out a Structure-Activity Relationship (SAR) using Deductive Estimation of Risk from Existing Knowledge (DEREK), which is widely used to evaluate chemical structures for the presence of alerts on genotoxicity and carcinogenicity [7]. Some additional classes IV to V relate to other higher risk compounds and genotoxicants.

Using this approach and other factors, the PQRI Toxicology Team [8] has recently proposed scientifically-justified thresholds for leachables in parenteral and ophthalmic products as a recommendation, as shown in Table 10.

Table 10

PQRI Toxicology Team Proposals for Safety Thresholds

	Class I	Class II	Class III	Class IV Sensitizer	Class IV Irritant	Class V Genotoxicant
Threshold Level/Day	150 µg	45 µg	7.5 µg	5 µg	5 µg	0.15 µg

The high risk genotoxicants have a recommended daily threshold of 0.15 µg. However all other classes have a threshold of at least 5 µg and as high as 150 µg for Class I compounds.

In the case study, none of the leachable compounds was a genotoxicant and therefore had threshold values from 5 µg to 150 µg. The calculated daily intake for 2-propanol was <3 µg day, which is below even the lowest threshold level of 5 µg/day. Similarly, daily intakes for all other compounds were below the thresholds for their Cramer classifications. It was concluded that the leachables did not present a safety concern.

9. Deliverables from Pall on User-specific Extractables and Leachables Studies

In previous sections, we have presented the background to extractables and leachables in single-use systems, the regulatory position, the practical approach to measurement using Pall's Model Solvent Approach and the assessment of toxicity.

In this section, we explain what Pall can offer to users in validating their single-use systems for extractables and leachables.

9.1. Expert Consultation

Introduction of single-use systems into a process can present new and demanding challenges especially to manufacturers with limited experience in this area. Single-use technology has many benefits but can also potentially create some major difficulties if the wrong approach is taken. Access to expertise in this subject can therefore help to ensure that systems are designed appropriately within a clear project plan, delivered on time and to budget, and meet regulatory approval. As discussed previously, extractables and leachables are particular areas of concern in which Pall's expertise and consultation process can set the project on the right course and ensure that suitable qualification procedures and documentation are produced.

9.2. Detailed Test Protocols

Pall can work closely with the user to generate test protocols that have a practical and sound science-based approach including:

- Justification of worse-case test conditions
- Extractables protocols with model solvents based on Pall's Model Solvent Approach.

9.3. High Quality Scientific Study Reports

Pall can deliver science-based reports on user-specific extractables and leachables studies and the all-important risk assessment. The reports include comprehensive analytical work using advanced methods such as GC/MS, LC/UV/MS and ICP/MS. Generic reports on extractables from Pall single-use components with standard solvents are also available to the user to supplement their validation documentation.

9.4. Validation Report Package

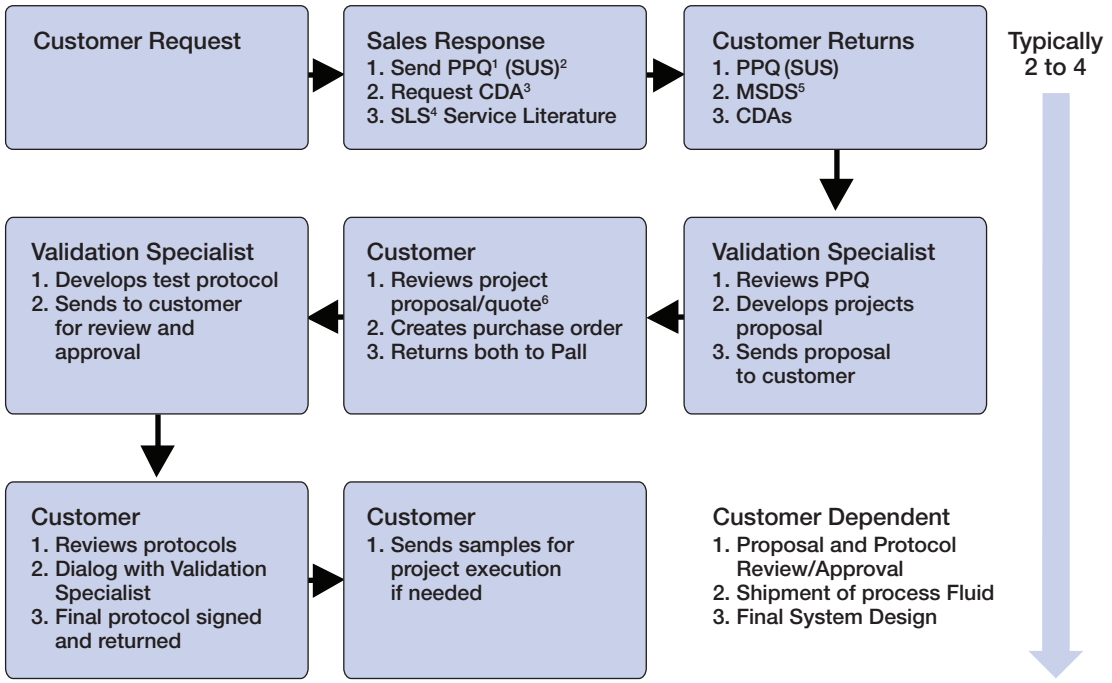
All aspects of the project are then consolidated into an extractables and leachables validation report package for the single-use system. This package can be directly incorporated into the user's process-specific validation documentation for regulatory filings. Our track record shows that all of these completed packages submitted to regulatory agencies have been successfully approved during regulatory reviews.

9.5. Validation Project Flow Chart

Due to the complexity of the single-use system, the extractables/leachables validation process consists of many steps, some of which need cooperation from the end users. The flow charts below depict the phase I and phase II of the project management (Figures 7 and 8).

Figure 7

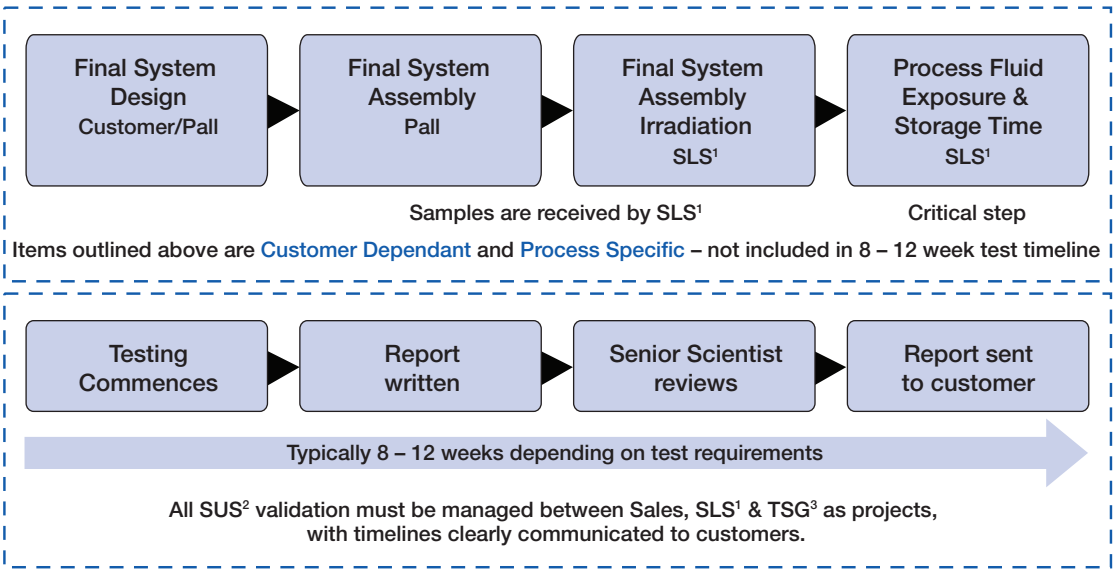
Single-use Systems Validation Flow Chart – Phase 1 Project Initiation



1. PPQ = Process and Product Questionnaire 2. SUS = Single-use System 3. CDA = Confidentiality Agreement
4. SLS = Scientific & Laboratory Services 5. MSDS = Material Safety Data Sheet
6. Quote must include Pall Allegro SUS Part Number and cost as separate line item.

Figure 8

Single-use Systems Validation Flow Chart – Phase 2 Project Execution



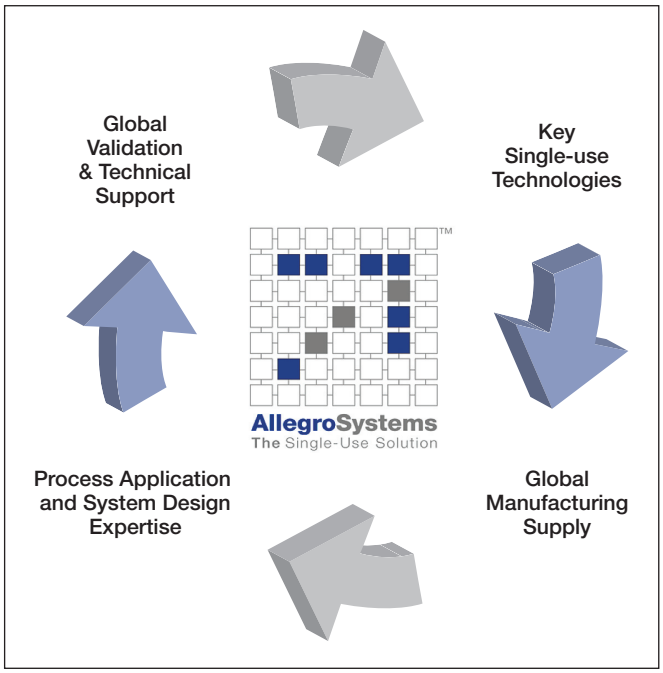
1. SLS = Scientific & Laboratory Services 2. SUS = Single-use System 3. TSG = Technical Support Group.

10. Pall Worldwide Support and Services for Single-use Systems

The Pall services described for extractables and leachables studies are part of a much wider capability of support and services offered by Pall for single-use systems. This capability is fundamental part of Pall's Allegro Systems program for single-use systems. The four components of this program are shown in Figure 9.

1. The foundation of the program is a portfolio of key single-use technologies including:
   - Mustang membrane chromatography
   - Stax capsule depth filters
   - Kleenpak TFF capsules
   - Kleenpak sterile connectors and disconnectors
   - Allegro single-use TFF systems
   - Allegro single-use filling needle
   - Allegro biocontainers
   - Kleenpak capsule filters
   - Allegro disposable mixers

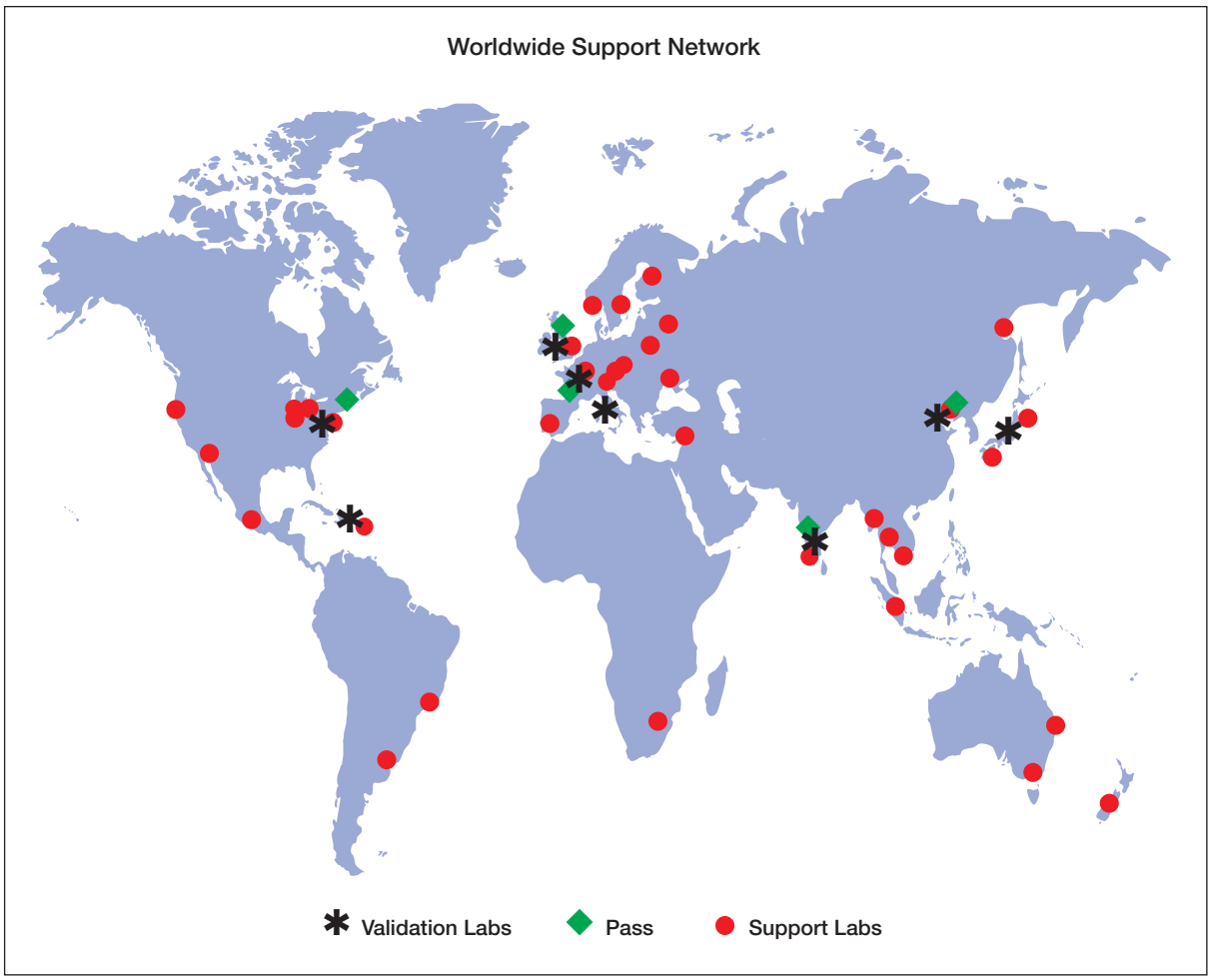
Figure 9
Pall Allegro Program for Single-use Systems



2. Through our global manufacturing and supply, we have dual manufacturing of our systems to offer short lead times around the world as well as double assurance of product supply. We bring together our products and appropriately validated third party technologies to offer a total system capability.
3. Our in-house process application and design expertise comes from our Pall Advanced Separations Systems Group (PASS) with engineering teams in five major centers around the world, as shown in Figure 10. They design customized single-use systems for upstream and downstream processing in accordance with Good Design Practice (GDP) and Good Engineering Practice (GEP).

4. We support these systems with validation expertise from our eight Global Validation Services laboratories, as shown in Figure 10, whose specialists are very familiar with the requirements of Good Manufacturing Practice (GMP) and regulatory issues. They can provide local validation services and support you in implementation of single-use systems.

Figure 10
Pall Validation, Engineering and Technical Support Services Worldwide



11. Why Consider Pall Services for Extractables and Leachables Studies?

The types of services described above may be familiar to you, so why should you consider specifically Pall services for extractables and leachables studies?

The information provided in this publication may have been of assistance in making that decision but the following considerations may also be of help to you:

11.1. Industry Leader

First of all, Pall is seen in the industry as a leader in this field. We pioneered work on extractables and leachables from filters in the past and now we are bringing that experience into single-use systems [9 – 11, 13]. In this context, we have made contributions to industry guides such as the BPSA Extractables Guide [12] which is co-authored by Dr Weibing Ding of Pall Scientific and Laboratory Services and Jerold Martin of Pall Life Sciences (and Chairman of BPSA). The BPSA is the lead trade organization addressing the challenges of implementing single-use technology into the pharmaceutical industry. We have also conducted seminars on this subject for US FDA and other pharmaceutical regulatory agencies as we are recognized globally for our specialized knowledge and practical experience in this critical area.

11.2. Expertise in Requirements for Single-use System Applications

Pall has many years of experience in dealing with users' processes. We can help them reach appropriate solutions by designing extractables and leachables protocols for critical fluid pathways using full or scaled down systems. Most importantly, our approach makes the data relevant to leachables in the user's process.

We also have the expertise and equipment to perform in-house chemical analyses by our Validation Services teams.

Our consulting services can then provide guidance on issues such as risk assessment and regulatory issues.

11.3. Published Articles

We have published many technical articles on single-use systems and some of these articles are referenced at the end of this publication. We have endeavored in all our publications to provide detailed technical content without a commercial bias so as to make the information relevant and of practical benefit to the industry. Acknowledgement of the value of this published material is best obtained from the reader's feedback such as the comment received to a Pall published article in 2009 [13]:

"While the article itself is well written and represents solid thinking, a solid effort and an important contribution, I am even more impressed by the high level of science that is evidenced in the data provided…"

12. Summary and Conclusions

1. In this report, we have shown why extractables and leachables from single-use systems must be evaluated with a science-based approach to determine their identity, quantity and toxicity.
2. We have detailed the Pall Model Solvent Approach and the range of chemical analytical methods necessary to obtain relevant data.
3. We have also shown the importance of a risk assessment and how that may be performed using a decision tree to determine the most suitable validation plan.
4. To assist users on the practical aspects, a Pall case study has been described for a single-use system in which the extractables and leachables validation data was generated. The related process validation filings have been accepted by the health authorities.

Our ultimate objective from this report is to help ensure that users can successfully and confidently develop and validate single-use processes using an approach that saves time and costs, and meets regulatory requirements.

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