PDA Training Course Extractables & Leachables

01 June 2022

E&L TESTING OF SINGLE-USE SYSTEMS FOR PRODUCTION

Koen Smets, Senior E&L expert

PDA Parenteral Drug Association

Nelson Labs. A Sotera Health company

CONNECTING PEOPLE SCIENCE AND REGULATION®

Overview

1. Regulatory requirements for SUS
2. Interest groups on standardization
3. How to set up extractables and leachables studies for SUS?
    - 3.1 Risk assessment of the materials used in the production process
    - 3.2 Gather extractables data
    - 3.3 Evaluation of extractables data
    - 3.4 Leachables study
4. Case study: E&L testing of a PET bottle

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1. REGULATORY REQUIREMENTS FOR SINGLE-USE SYSTEMS

U.S.

Title 21 of the Code of Federal Regulations (CFR) 211.65 (1)

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

EUROPE

ICH Q7 – GMP Practice Guide

"...Equipment should not be constructed so that *surfaces that contact raw materials, intermediates or API's* ***do not alter the quality of the intermediates and API's*** *beyond the official or other established specifications..."*

EU – GOOD MANUFACTURING PRACTICES

"...Production Equipment ***should not present any hazard*** *to the products. Parts of production equipment that come into contact with the product must not be reactive, additive or absorptive to such an extent that it will affect the quality of the product and thus present any hazard"*

1. REGULATORY REQUIREMENTS FOR SINGLE-USE SYSTEMS

OBSERVATIONS

- The CFR 211.65 and GMP's do ***not only*** refer to the ***impact on Safety***, but also on:
  - Quality
  - Purity
  - Strength (e.g. adsorptive behavior)
  - Reactive behavior
  - Additive behavior

- Reasoning of Regulators
  - Know your process
  - Know the impact of SUS on the quality of the product
  - Prove that you have made an assessment

1. REGULATORY REQUIREMENTS FOR SINGLE-USE SYSTEMS

United States Pharmacopeia <665>:

Plastic components and systems used to manufacture pharmaceutical drug products and biopharmaceutical drug substances and products

United States Pharmacopeia <1665>:

Characterization and qualification of plastic components and systems used to manufacture pharmaceutical drug products and biopharmaceutical drug substances and products

Published ***IN DRAFT*** in Pharmacopeial Forum (PF) 43(3) [May – Jun. 2017]
Published ***UPDATED DRAFT*** in Pharmacopeial Forum (PF) 45(2) [Mar. – Apr. 2019]
Published ***2nd UPDATED DRAFT*** in Pharmacopeial Forum (PF) 46(5) [Sep. – Oct. 2020]
Published in USP: ***targeted offical date: 01 May 2026*** ***(see next slide)***

1. REGULATORY REQUIREMENTS FOR SINGLE-USE SYSTEMS

STATUS UPDATE USP <665>

(taken from USP website on 02 May 2022)

<665>Plastic Components and Systems Used to Manufacture Pharmaceutical Drug Products and Biopharmaceutical Drug Substances and Products

Type of Posting: Notice of Intent to Revise
Posting Date: 25–Feb–2022
Targeted Official Date: 01–May–2026, Revision Bulletin
Expert Committee: Packaging and Distribution Expert Committee

In accordance with the Rules and Procedures of the Council of Experts, this is to provide notice that the General Chapters–Packaging and Distribution Expert Committee intends to revise ⟨665⟩ *Plastic Components and Systems Used to Manufacture Pharmaceutical Drug Products and Biopharmaceutical Drug Substances and Products.*

As currently published, there are no requirements that are mandatory for compendial compliance purposes in this chapter. General Notices 3.10, *Applicability of Standards* states that a chapter below ⟨1000⟩ does not become an applicable general chapter unless referenced as such in General Notices, a monograph, or another applicable general chapter numbered below ⟨1000⟩. As none of these situations currently applies to ⟨665⟩, it is not an applicable general chapter. However, there have been inquiries around the applicability of the chapter and the current official date of May 1, 2022. To address these inquires and to give USP time to engage stakeholders regarding the advisability of making ⟨665⟩ an applicable general chapter and track the ICH Q3E development effort, USP intends to extend the official date for ⟨665⟩ to May 1, 2026.

It is anticipated that the revision will be posted as a Revision Bulletin April 29, 2022.

Should you have any questions, please contact Desmond G. Hunt, Scientific Liaison to the General Chapters–Packaging and Distribution Expert Committee (301–816–8341 or dgh@usp.org).

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2. INTEREST GROUPS ON STANDARDIZATION

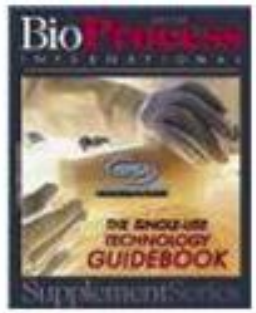
BPSA

BIO-PROCESS SYSTEMS ALLIANCE
Advancing Single-Use Worldwide

- Trade association of suppliers and users of single-use bioprocess technologies

- Publications:
  - Recommendations for Extractables and Leachables Testing (2008)
  - Recommendations for Testing and Evaluation of Extractables from Single-use Process Equipment (2010)

- Available at www.bpsalliance.org



2. INTEREST GROUPS ON STANDARDIZATION

BPOG (BioPhorum Operations Group)

- Global association of Biopharmaceutical manufacturers (end users)
- Publications:
  - Standardized Extractables Testing Protocol for Single-Use Systems in Biomanufacturing (Nov 2014)
  - Best Practices Guide for Evaluating Leachables Risk from Polymeric Single-Use Systems used in Biopharmaceutical Manufacturing (Mar 2017)

BioPhorum

- Global association of end users and suppliers
- Publications:
  - BioPhorum Best Practices Guide for Extractables testing of Polymeric Single-Use Components used in BioPharmaceutical Manufacturing (Apr 2020)
  - A Comprehensive Review of BioPhorum Standardized Extractables Testing Data: A Deep-Dive into Similarities, Differences and Trends Across Extraction Solvents and Time Points (Sep 2020)

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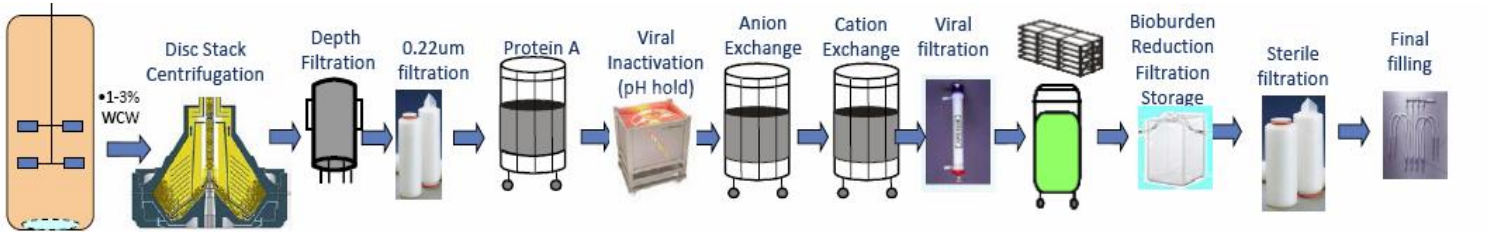
3.1 RISK ASSESSMENT

Why perform a risk assessment?

- Bioproduction process may contain a lot of different SUS



Bioproduction example from a slide from Presentation at IQPC Conference "Disposable Solutions", Munich, 18-20 FEB2014: "BPOG's Extractable Protocol Standardization Journey – Review 2013 Process ande Planning for 2014" Ken Wong (Sanofi-Pasteur), with permission of the Author.

- Many SUS are custom made
  - Bag from Vendor A
  - Tubing from Vendor B
  - Filter from Vendor C
  - Connectors from Vendor D

- Complete E/L assessment for each component can be a challenging task

3.1 RISK ASSESSMENT

Perform a risk assessment

- Instead of testing every SUS for extractables, a **risk-based approach** can be applied to focus on the materials with high impact

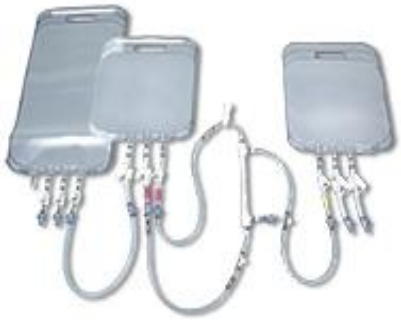
- GOAL?
  Select single-use components with greatest potential for objectable levels of leachables regarding **safety** and **quality** of the final product, and **process performance**

- When?
  Best **performed early in the process development** when changes are more easily addressed

3.1 RISK ASSESSMENT

Create a list a “product contact materials”

- Understand your manufacturing process from start to finish!
- List any material with potential to leach into the final product through “product contact” with starting materials, intermediates, final DP,...
- Can include:
  tubing, bags, filters, connectors, O-rings, tangential flow cassettes, chromatographic resins, final bulk storage vessels,…


3.1 RISK ASSESSMENT

"RISK FACTORS" to consider for E/L assessment of "product contact materials"

1. Material compatibility
2. Proximity to final DP / distance along production stream
3. Composition of contact solution
4. Surface area to Volume ratio
5. Contact temperature and contact time
6. Pretreatment steps
7. Process performance

3.1 RISK ASSESSMENT

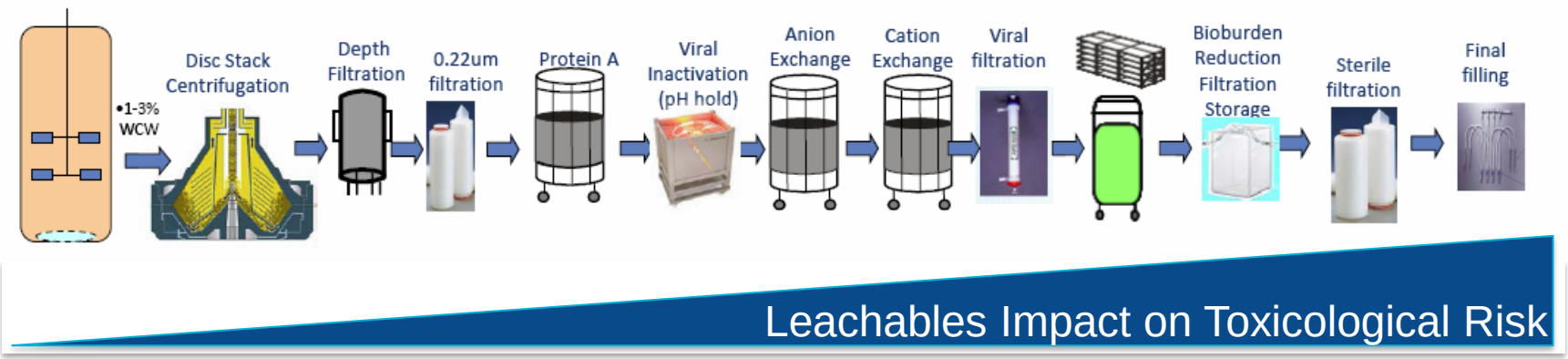
RISK FACTOR 1: Material compatibility

- Most formulations are **aqueous-based** and therefore compatible with most SUS components
- Most biopharmaceutical materials pass USP<87> and USP<88> testing
- Obtain **manufacturers recommended operating parameters** such as pH range, temperature, pressure…
  - Is material being used within these recommended operating parameters?
- Materials with great number and/or level of additives ➔ greater total pool of potential extractables
- E.g. USP<1665>:
  - **"low risk" component**: total level of plastic additives in component is **≤0.1%**
  - **"intermediate risk" component**: total level of plastic additives in component is **>0.1% and ≤1%**
  - **"high risk" component**: total level of plastic additives in component is **>1%**

3.1 RISK ASSESSMENT

RISK FACTOR 2: Proximity to Final Product

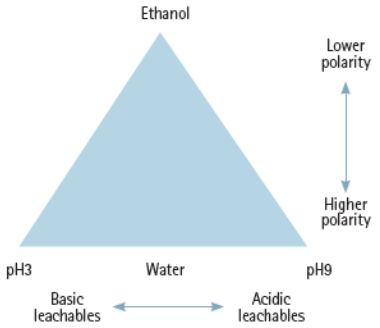
- Materials used in the **final filling line** have **direct risk** to the final product
- Locations **upstream** in the process **MAY** have **reduced risk** to the end-product
- TRUE in case of processing steps that can remove migrated compounds from the process
  - **Ultrafiltration / diafiltration** → removal of impurities?
  - Lyophilization → removal of volatiles?
  - Ideally, supporting data should be obtained



3.1 RISK ASSESSMENT

RISK FACTOR 3: Composition of the contact solution

- Higher regulatory and safety concern for leachables in case of contact solutions with:
  - Low or high pH-values
  - High organic contents
  - Surfactants



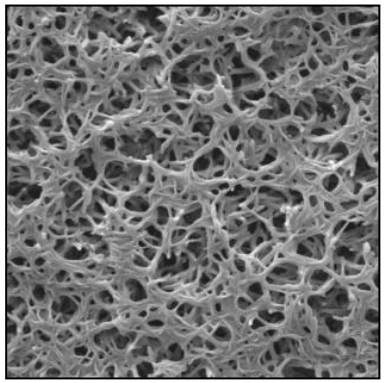
E.g. USP<1665>: the process stream is:

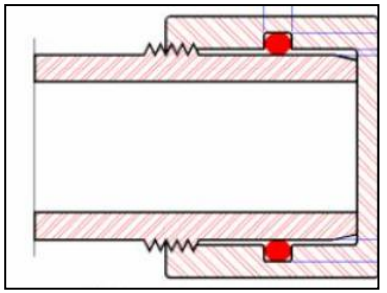
Process Streams Containing...	Definition of Process Stream		
	Aqueous	Somewhat Organic	Highly Organic
Organic solvents	<5% by volume	>5% and ≤40% by volume	>40% by volume
Surfactans (e.g. Polysorbate 80)	≤0.1% by weight	>0.1% and ≤0.5% by weight	>0.5% by weight
Blood or blood-derived substances (e.g. Albumin)	<1% by weight	≥1% and <25% by weight	≥25% by weight
Lipids and proteins	<1% by weight	≥1% and <5% by weight	≥5% by weight

3.1 RISK ASSESSMENT

RISK FACTOR 4: Surface-to-volume ratio

- The higher the ratio, the higher the risk!!
- High → Filters: porous structure leads to large internal surface area
- Low → O-ring seals
- Smaller process volumes are more critical





3.1 RISK ASSESSMENT

RISK FACTOR 5: Contact temperature and time

- Evidently, **higher risk** in case of
  - **higher temperatures** → more rapid migration

and/or

  - **longer times** → more time for migration

3.1 RISK ASSESSMENT

RISK FACTOR 6: Pretreatment steps

- **STERILIZATION** tends to increase leachables
  - Steam sterilization
  - Gamma irradiation
  - E-beam sterilization

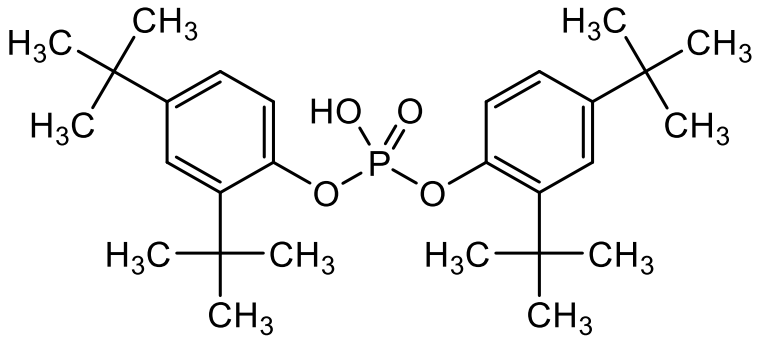
- **RINSING** prior to product contact tends to lower leachables
  - E.g. Preflushing filters with WFI
  - Flush solution has to be removed from the process stream!

3.1 RISK ASSESSMENT

RISK FACTOR 7: Process performance

- Do single-use systems have impact on the performance of the production process?

e.g. bDtBPP (cell growth inhibition)



3.1 RISK ASSESSMENT

How to perform a risk assessment?

- Assign numerical values to different **risk factors** and convert to final risk score
- Risk assessment should be clear and well argumented towards the authorities
- Different company-specific approaches might be used
- Risk assessment based on ICH Q9 Quality Risk Management

BPOG: Example of numerical values that indicate the risk level, including weight factors assigned to each risk factor

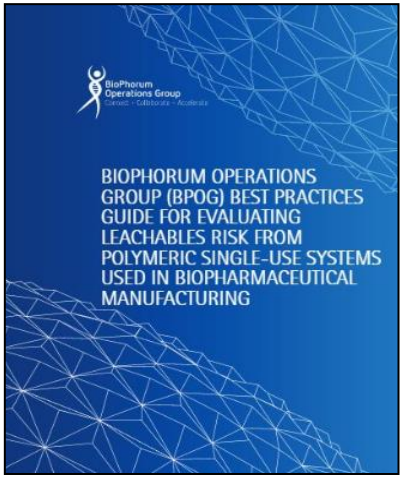
BPOG E/L Risk Assessment
Example of Proposed Risk Assessment

Risk factors

Risk levels with rating

Weight factor

Consideration	Ratings [1]		Weight [2]
Distance along production stream (DAS)	1	Synthesis: Vial thaw, Inoculum, Expansion, Production, Harvest, Plasma	0.40
	3	Purification: Affinity chromatography, Viral inactivation, Ion exchange chromatography, Viral filtration, UF/DF	
	5	Bulk Drug Substance: Filtration, BDS storage	
	9	Final Formulation, Fill / Finish Potency adjustment, Sterile filtration Filling, Lyophilization, FDP Storage	
Exposure Temperature (ET)	1	Frozen	0.15
	3	0 C to <10 C	
	5	10 C to <30 C	
	9	> 30 C	
Exposure duration (ED)	1	Transient (i.e. ≤ 60minutes)	0.15
	3	Short (i.e.≤ 24 hours)	
	5	Medium (i.e.≤ 7 days)	
	9	Long (i.e. > 1 week or more)	
Process Fluid Interaction (PFI)	1	Non-solvent/No penetration of polymeric component	0.15
	3	Low solvation power or low penetration of polymeric component	
	5	Medium solvation power or medium penetration of polymeric component	
	9	High solvation power or high penetration of polymeric component	
Dilution ratio (DR)	1	< 1.E-03 m^2/L e.g. fittings, connectors, gaskets	0.15
	3	1.E-02 - < 1.E-03 m^2/L e.g. short/high diameter tubing	
	5	1.E-01 - < 1.E-02 m^2/L e.g. long low diameter tubing	
	9	> 1.E-01 m^2/L e.g. filters, final container	



(2017)

(1): Parameter range definitions in this table represent an example. Individual companies should develop their specific range definitions according to their internal policies / SOPs.

(2): Weight levels used in the table represent an example. In this example, 0.40 is used for DAS rating and 0.15 is used for all other considerations. Individual companies may use equal weight distribution or may assign weight according to their internal policies.

Example: Sterilization filter

Risk rating (EPR) =

(9 x 0.40)
+
(5 x 0.15)
+
(3 x 0.15)
+
(5 x 0.15)
+
(9 x 0.15)
=
6.9

Consideration	Ratings [1]		Weight [2]
Distance along production stream (DAS)	1	Synthesis: Vial thaw, Inoculum, Expansion, Production, Harvest, Plasma	0.40
	3	Purification: Affinity chromatography, Viral inactivation, Ion exchange chromatography, Viral filtration, UF/DF	
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	9	Final Formulation, Fill / Finish Potency adjustment, Sterile filtration Filling, Lyophilization, FDP Storage	
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Dilution ratio (DR)	1	< 1.E-03 m^2/L e.g. fittings, connectors, gaskets	0.15
	3	1.E-02 - < 1.E-03 m^2/L e.g. short/high diameter tubing	
	5	1.E-01 - < 1.E-02 m^2/L e.g. long low diameter tubing	
	9	> 1.E-01 m^2/L e.g. filters, final container	

E / L Propensity Rating (EPR)	Calculated Risk Rating	Risk Category
	6.3 – 9.0	High
	3.7 – 6.2	Medium
	1.0 – 3.6	Low

Filter should be tested

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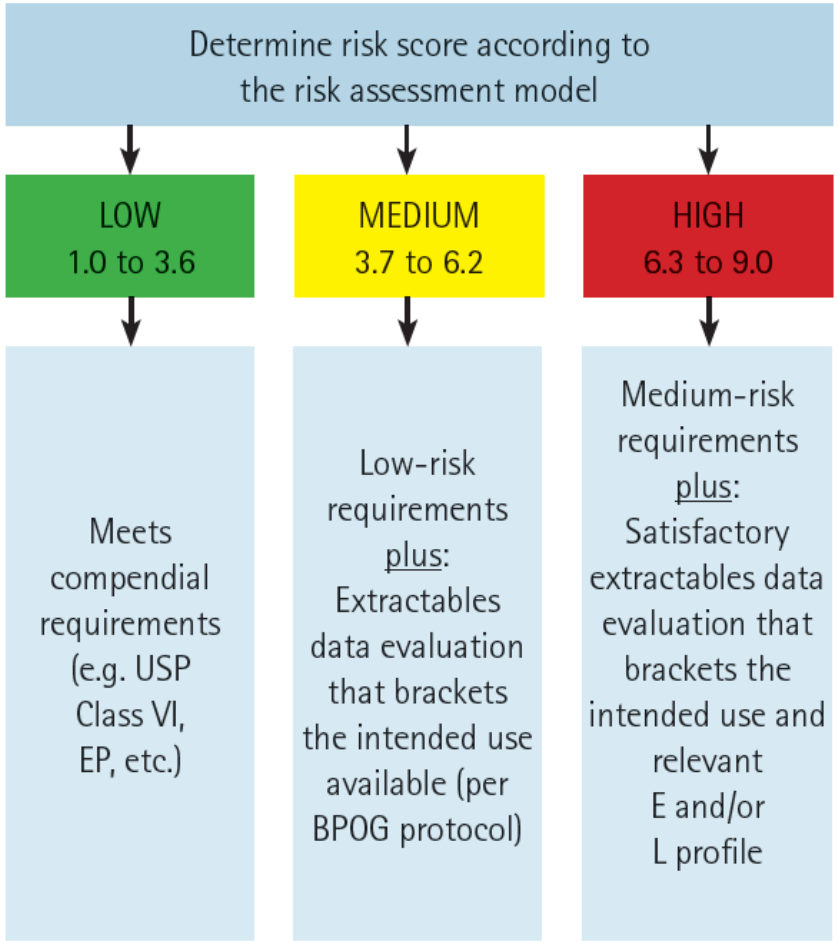
	6.3 – 9.0	High
	3.7 – 6.2	Medium
	1.0 – 3.6	Low

Filter should be tested



3.1 RISK ASSESSMENT

USP <1665>: Example of a risk evaluation matrix

- **Risk evaluation matrix uses a 3-step process**:
  Step 1: Establish values for each risk dimension
  Step 2: Link the numerical risk sequence with a level of characterization
  Step 3: Use mitigating factors to adjust the characterization level

Risk Dimension	Duration of contact	Temperature of contact[a]	Chemical Composition of the Process Stream	Chemical composition of the Component
Level 1	<24 h	Refrigerated (2 °C – 8 °C)	Aqueous (≤5% organic v/v; pH ≥3 and pH ≤ 9)	Low risk
Level 2	1-7 days	Ambient (15 °C – 25 °C)	Somewhat organic (>5% and ≤40% v/v)	Intermediate risk
Level 3	>7 days	Elevated (>30 °C)	Highly organic (>40% v/v) or aqueous, extreme pH (pH <3 or pH >9)	High risk

[a] The gaps in the temperature ranges reflect temperature ranges that are rarely experienced in manufacturing processes.

3.1 RISK ASSESSMENT

USP <1665>: Example of a risk evaluation matrix

- E.g. Sterilization filter:
  Step 1: Establish values for each risk dimension ➔ 3321
  Step 2: Link the numerical risk sequence with a level of characterization

If...	And...	Then the Characterization Level is...
Four of the dimension scores are Level 3	There is no additionanl qualifier (3333)	Level C (high risk)
Three of the dimension scores are Level 3	The other dimension score is Level 2 (3332)	Level C
	The other dimension score is Level 1 (3331)	Level C
Two of the dimension scores are Level 3	The other two dimension scores are both level 2 (3322)	Level C
	One dimension score is Level 2 (3321)	Level B (Moderate risk) or C[a,b]
	The other two dimension scores are Level 1 (3311)	Level A (Low risk) or B[b,c]
One of the dimension scores is Level 3	All of the other dimension scores are Level 2 (3222)	Level B
	One of the other dimension scores is Level 1 (3221)	Level B
	Two of the other dimension scores are Level 1 (3211)	Level A or B[b,c]
	All of the other dimension scores are Level 1 (3111)	Level A
None of the dimension scores is Level 3	All of the dimension scores are Level 2 (2222)	Level B
	Not all of the dimension scores are Level 2	Level A

Temperature is Level 2 score ➔ Level C (high risk)

[a] If the Level 2 score is in temperature, solvent, or duration dimensions, then Level C; otherwise, Level B.
[b] In these cases the temperature, solvent, or duration dimensions have a greater influence of risk than do component composition.
[d] If one of the Level 1 scores is in the component composition dimension, then Level A; otherwise, Level B.

3.1 RISK ASSESSMENT

USP <1665>: Example of a risk evaluation matrix

- E.g. Sterilization filter:

  Step 1: Establish values for each risk dimension ➔ 3321

  Step 2: Link the numerical risk sequence with a level of characterization ➔ Level C

  Step 3: Use mitigating factors to adjust the characterization level
  - Clearance after contact processing step? ➔ No (no mitigation factor)
  - Clinical use of the final DP?
    - Dosage form: solid oral, liquid oral or topical? ➔ No (no mitigation factor)
    - Duration <7 days ➔ No (no mitigation factor)
    - Dialy dose volume < 10 mL ➔ No (no mitigation factor)

  ➔ Level C testing is established

Note: the “clearance” mitigation and the “clinical use” mitigation factors are additive.

3.1 RISK ASSESSMENT

Risk Level	Extraction Solutions for Chemical Testing[a]	Chemical Testing of Extracts
Low (A)	*Solution C1*	• Non-volatile residue • UV absorbance
Moderate (B)	*Solution C1*	• Organic extractables profiling
High (C)	*Solution C1, Solution C2, and Solution C3*	• Organic extractables profiling • Extracted elements (as necessary and appropriate)[b]

[a] See *Extraction solutions*.
[b] The relevance of extractable elements testing should be considered by the component's potential user. Should such testing be deemed necessary, it is the user's responsibility to establish and justify the means by which testing is accomplished, taking into account extraction conditions, target elements, and reporting requirements.

C1: 50% EtOH in UPW
C2: UPW pH 3 (HCl/KCl)
C3: UPW pH 10 (phosphate buffer)

3.1 RISK ASSESSMENT

	Extraction solvent C1: Semi-polar	Extraction solvent C2: Polar, Low pH	Extraction solvent C3: Polar, High pH
Recommended choice	1/1 (v/) mixture of ethanol and water	0.05 M KCl solution pH adjusted to 3.0 with HCl	0.1 M disodium hydrogen phosphate buffer adjusted to pH 10.0
Alternate Choices (with justification)	Other analytically expedient semi-polar solvents	• 0.1 M Phosphoric acid • Solution containing other salt adjusted to pH 3.0	• 0.5 N NaOH • Solutions containing other buffers adjusted to pH 10 or higher

Alternate aqueous solutions prepared at pH values outside these pH values are allowed when they reflect the pH of the process stream

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3.2 GATHERING EXTRACTABLES DATA

- Extractables data from the supplier:
  Is the data **suitable for the intended application(s)**?
  - Composition of extraction solvents: organic content, pH, polarity
  - Extraction conditions: time and temperature
  - Pretreatments steps: sterilization
  - Analytical techniques: screening, combination of different techniques
- Can extractables data generated by different suppliers be compared?
  - Outcome of extractables study is highly dependent on the set-up
- Increasing demand for **standardized extractables protocol** for **extractables testing performed by the supplier**
  - Cover majority of the biopharmaceutical applications
  - Easily compare data from different suppliers

3.2 GATHERING EXTRACTABLES DATA

BPOG extractables protocol (2014)

	SOLVENTS						TIME				
	50% Ethanol	1% PS-80	5M NaCl	0.5N NaOH	0.1M Phosphoric acid	WFI[a]	Time 0 (≤ 30 min)	24 hrs	7 days	21 days	70 days
							Temperature				
							25°C	40°C			
Storage, Mixing, and Bioreactor Bags	X	X	X	X	X	X	X	X		X	X[b]
Tubing	X	X	X	X	X	X	X	X		X	X[b,c]
Tubing Connectors & Disconnectors	X	X	X	X	X	X	X	X		X	
Aseptic Connectors & Disconnectors	X	X	X	X	X	X	X	X	X		
Sterilizing-Grade / Process Filters	X	X	X	X	X	X	X	X	X		
TFF Cassettes	X	X	X	X	X	X	X	X		X	
Sensors and Valves	X	X	X	X	X	X	X	X		X[d]	
Molded Part of Mixers	X	X	X	X	X	X	X	X		X	
Chrom. Columns; Elastomer Parts; Wetted Polymeric Surfaces of Positive Displacement Pumps	X	X	X	X	X	X	X	X			
Filling Needles	X	X	X	X	X	X	X	X			

[a] If WFI is not available, use deionized water [b] Necessary to support 3-year storage time at 0°C [c] Tubing is integrated with bag during storage
[d] The 21-day time-point only applies to sensors used with bioreactor (e.g., DO and pH)

Reference: Presentation at 'Bioproduction 2015', Dublin, 14 Oct 2015, presented by D. Buckley and A.Sexton

Rationale for updating BPOG protocol -> cf. BioPhorum Best Practices Guide for Extractables Testing of Polymeric Single-Use Components (2020)

3.2 GATHERING EXTRACTABLES DATA

BioPhorum extractables protocol (2020)

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

Reference: BioPhorum Best Practices Guide for Extractables Testing of Polymeric Single-Use Components (2020)

3.2 GATHERING EXTRACTABLES DATA

USP <665> Standard Extractables Protocol (2022)

Components	Extraction duration (days)		
	1 day (24 ± 1 h)	7 days (168 ± 4 h)	21 days (504 ± 8 h)
Chromatography column housing	X	-	-
Connectors, disconnectors, fittings, overmolded junctions for tubing[a]	X	-	-
Containers (bags, bottles, carboys) not intended for storage (such as mixing bags or bioreactors)[b]	X	-	-
Filling needles	X	-	-
Filters (process, sterilizing, and virus)	X	-	-
Filtration cassettes (tangential flow)	X	-	-
Impellers and molded parts for bio-reactors and mixers[b]	X	-	-
Ports on containers not intended for storage (such as mixing bags or bioreactors)	X	-	-
Small components (O-rings, gaskets, check valves, diaphragms, septa, polymer pump surfaces, sensors)	X	-	-
Tubing attached to containers not intended for storage	X	-	-
Aseptic connectors and disconnectors[a]	-	X	-
Closures (e.g., molded stoppers) for storage containers	-	-	X
Containers (bags, bottles, carboys) intended for storage	-	-	X
Ports on containers intended for storage	-	-	X
Tangential flow modules for perfusion or continuous processing	-	-	X
Tubing attached to containers intended for storage	-	-	X
Tubing for fluid transport[c]	-	-	X

[a] The duration of use for connectors and disconnectors is generally longer when aseptic connectors and disconnectors are used to maintain process sterility versus when unsterilized components are used in non-sterile process operations. This generalization is reflected in the different extraction durations for aseptic versus nonsterile components.

[b] These items can be used in several different manufacturing situations and circumstances. The duration of extraction should be consistent with the circumstances of use during manufacturing. If warranted and justified, longer extraction durations of 7 or 21 days can be used.

[c] Tubing for fluid transport can be used in several different manufacturing situations and circumstances. The duration of extraction should be consistent with the circumstances of use during manufacturing. If warranted and justified, shorter extraction durations of 1 or 7 days can be used.

3.2 GATHERING EXTRACTABLES DATA

- What if no supplier data are available or suitable?
  - ➔ It is the **responsibility of the end user** to demonstrate that the single-use system is **suitable** for his end application and that it does not alter the quality or safety of his end-product

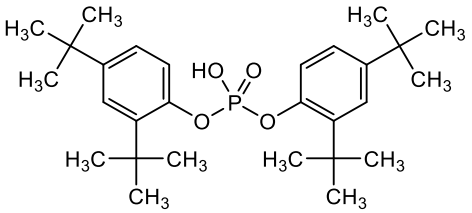
- USP <665> speaks to qualification procedures and acceptance criteria
  - ➔ alternative approaches for qualification may be appropriate in justified circumstances, but are subject to agreement by an appropriate regulatory authority

Overview

1. Regulatory requirements for SUS
2. Interest groups on standardization
3. **How to set up extractables and leachables studies for SUS?**
    - 3.1 Risk assessment of the materials used in the production process
    - 3.2 Gather extractables data
    - **3.3 Evaluation of extractables data**
    - 3.4 Leachables study
4. Case study: E&L testing of a PET bottle

3.3 EVALUATION OF EXTRACTABLES DATA

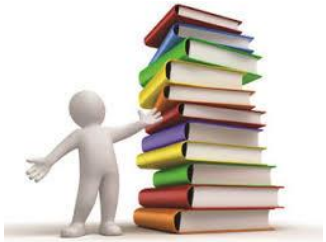
- Impact on **process performance:**
  - e.g. Bis(2,4-di-tert-butylphenyl)hydrogen phosphate (bDtBPP) causing cell growth inhibition



- Impact on the **final product**:
  - **Safety impact**: related to the toxicity of extractables (potential leachables)
    - Is there a safety risk towards the patient?
    - e.g. Mutagenic compounds ending up in the final product administered to the patient
  - **Quality impact**:
    - e.g. Compounds promoting the formation of protein aggregates
  - **Efficacy impact**:
    - e.g. Compounds altering the tertiary structure of the protein causing loss of activity

3.3 EVALUATION OF EXTRACTABLES DATA

- Safety evaluation based on the toxicity of the compound



- Literature data often very limited or non-existent:
  - *polymer oligomers*
  - *polymer degradation compounds*
  - *polymer additive degradation compounds*
  - *reaction products*

- (Q)SAR ((Quantitative) Structure Activity Relationship)
  software packages might assist in assessing the safety risk of extractables
  E.g. Derek Nexus, Sarah Nexus, MultiCase, Leadscope

- PQRI: Product Quality Research Institute
  - safety concern thresholds dependent on the administration route of the final product

Overview

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    - **3.4 Leachables study**
4. Case study: E&L testing of a PET bottle

3.4 LEACHABLES STUDY: GENERAL CONSIDERATIONS

MOST CASES:

- Concentration extractable compounds << final AET
  → no leachable study required

When to perform a subsequent leachable study:

- Extractable compounds > final AET
- Filling line
  (Worst-case final AET approximation: all potential filling line leachables end up in the first CCS)
- Storage applications (e.g. storage bag for DS)

3.4 LEACHABLES STUDY: GENERAL CONSIDERATIONS

Set-up:

- **Before and after the process step**
- **Integrated in the container leachables study**
  - Blank reference should not have been in contact with the process materials
  - Sometimes not possible to generate a true blank, since the DS is manufactured in single-use
  - Use placebo solution as a blank, but cause differential peaks originating from the DS

Final leachables results to be subjected to thorough **toxicological assessment** to classify the SUS as safe for use in the bioproduction process

Overview

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    - 3.1 Risk assessment of the materials used in the production process
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    - 3.3 Evaluation of extractables data
    - 3.4 Leachables study
4. **Case study: E&L testing of a PET bottle**

STEP 1: EXTRACTABLES / SIMULATION STUDY – SET-UP

Sponsor info:

- 5-L PET bottle with HDPE cap (filling volume = 4 L)
- Used for storage of drug substance
- Composition contact solution/drug substance:
  - Blood protein (2.4%),
  - buffer (contains Na^+, K^+, phosphate) (pH 3.0-4.0)
- Contact time & temperature: 12 months at 2- 8 ℃

STEP 1: EXTRACTABLES / SIMULATION STUDY – SET-UP

Extractables study set-up (USP<665>):

- Filling and shaking incubation (inverted) of 125-mL bottles (filling volume = 100 mL)
- 21 days at 40 °C
- Extraction solvents:
  - 50% ethanol in UPW (C1)
  - UPW pH 3 (KCl/ HCl) (C2)
  - UPW pH 10 (phosphate buffer) (C3)
- Analytical techniques:
  - HS-GC/MS screening → VOC
  - GC/MS screening → SVOC
  - HRAM-UPLC/MS screening → NVOC
  - ICP/OES → elements
  - ICP/MS → Hg

STEP 1: EXTRACTABLES / SIMULATION STUDY – SET-UP

AET calculation:

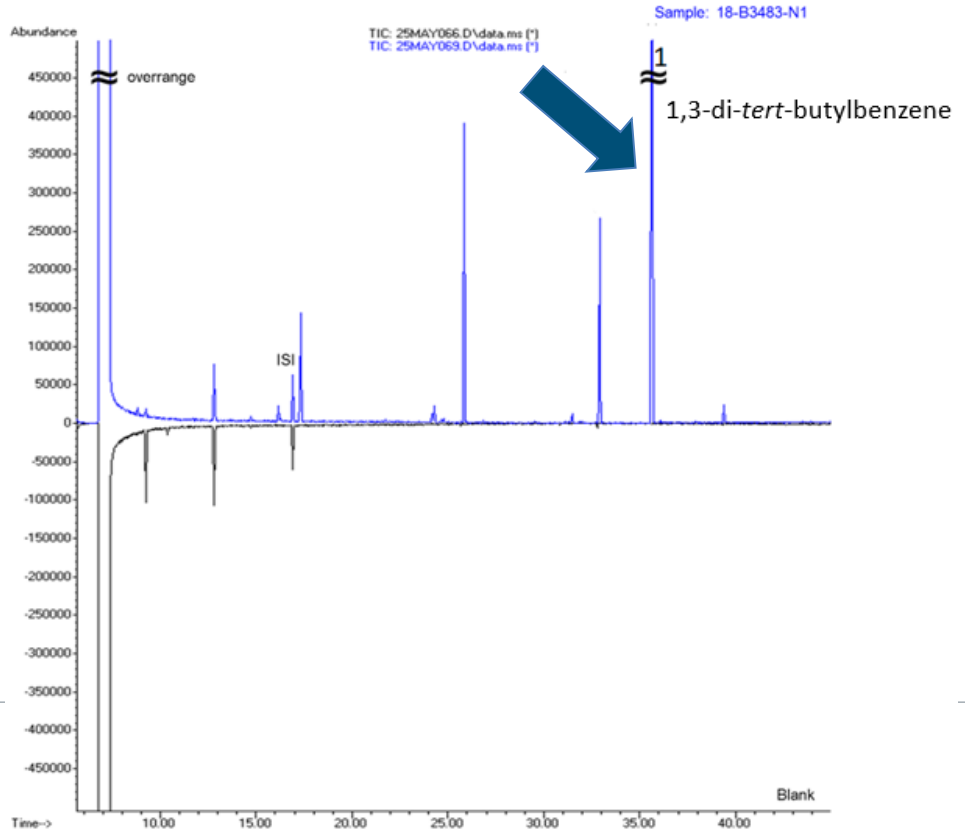
Safety concern threshold (PQRI)	1.5 µg/day
Safety concern threshold (non-chronically (ICH M7, PQRI))	**5 µg/day**

Safety concern threshold (non-chronically (ICH M7, PQRI))	**5 µg/day**
Max. daily dose (drug substance)	0.078 L/day
AET in drug substance (5 µg/day/0.078 L/day)	64 µg/L
Surface area of 5 L PET bottle	0.1842 m^2
AET in µg/m^2 (64 µg/L * 4 L/0.1842 m^2)	1400 µg/m^2
Final AET in µg/m^2 (incl. 50% uncertainty for screening analysis)	**690 µg/m^2**

STEP 1: EXTRACTABLES / SIMULATION STUDY – RESULTS

HS-GC/MS screening analysis:

- UPW pH 3: **no compounds** > final AET of 690 µg/m^2
- UPW pH 10: **no compounds** > final AET of 690 µg/m^2
- 50% ethanol extract: **1 compound** > final AET of 690 µg/m^2



50% ethanol

STEP 1: EXTRACTABLES / SIMULATION STUDY – RESULTS

GC/MS screening analysis:

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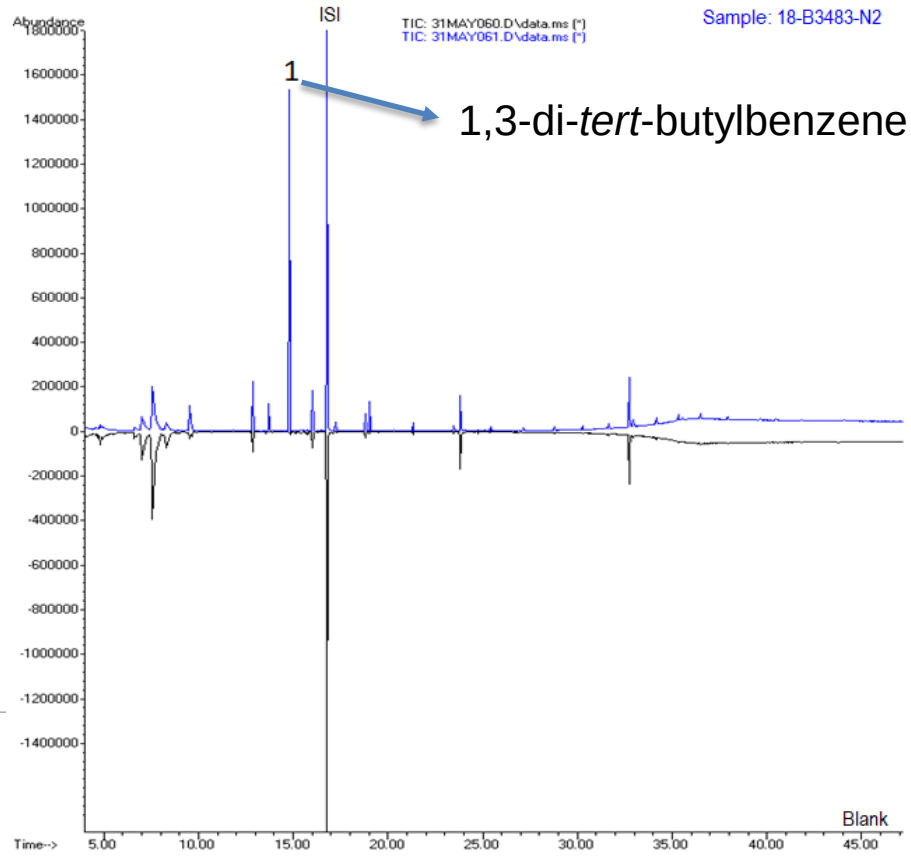


50% ethanol

STEP 1: EXTRACTABLES / SIMULATION STUDY – RESULTS

HRAM-UPLC/MS screening analysis:

- UPW pH 3: **no compounds** > final AET of 690 µg/m^2
- UPW pH 10: **no compounds** > final AET of 690 µg/m^2
- 50% ethanol extract: **no compound** > final AET of 690 µg/m^2

STEP 1: EXTRACTABLES / SIMULATION STUDY – RESULTS

Results for ICP/OES

Elements	Results (µg/m²)	
	Blank	**UPW, pH 3**
Aluminum (Al)	<10	<10
Antimony (Sb)	[10]*	<10
Arsenic (As)	<30	<30
Barium (Ba)	<5	<5
Beryllium (Be)	<2	<2
Boron (B)	<10	<10
Cadmium (Cd)	<5	<5
Calcium (Ca)	[20]	**60**
Chromium (Cr)	<5	<5
Cobalt (Co)	<2	<2
Copper (Cu)	<10	<10
Indium (In)	<20	<20
Iron (Fe)	<10	<10
Lead (Pb)	<10	<10
Lithium (Li)	<2	<2
Magnesium (Mg)	<10	<10

Elements	Results (µg/m²)	
	Blank	**UPW, pH 3**
Manganese (Mn)	<2	<2
Molybdenum (Mo)	<10	<10
Nickel (Ni)	<10	<10
Palladium (Pd)	<100	<100
Platinum (Pt)	<20	<20
Potassium (K)	N/A	N/A
Selenium (Se)	<40	<40
Silicon (Si)	<100	<100
Silver (Ag)	<5	<5
Sodium (Na)	**100**	**190**
Strontium (Sr)	<5	<5
Sulfur (S)	<100	<100
Thallium (Tl)	<20	<20
Tin (Sn)	<40	<40
Titanium (Ti)	<5	<5
Vanadium (V)	<10	<10
Zinc (Zn)	<5	<5

No further follow-up required in leachable study

STEP 1: EXTRACTABLES / SIMULATION STUDY – RESULTS

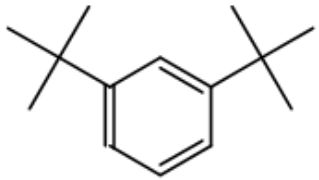
Results for ICP/MS

Element	Results (µg/ m^2)		Reporting Limit (µg/m^2)
	Blank	**UPW, pH 3**	
Mercury (Hg)	<2	<2	2

No Mercury detected

STEP 2: EVALUATION OF EXTRACTABLES DATA

Selected target compound

Chemical name; synonyms [CAS No./ToxID] formula mol. wt.	Structure
1,3-Di-*tert*-butylbenzene [1014-60-4] $C_{14}H_{22}$ 190.32	

→ Used as target in Method Suitability Test (HS-GC/MS and GC/MS)

STEP 3: LEACHABLES STUDY – SET-UP

Storage under real conditions

- Contact sample:
  - 125 mL bottles filled with 100 mL drug substance (DS)
  - Storage under inverted conditions at 5 °C

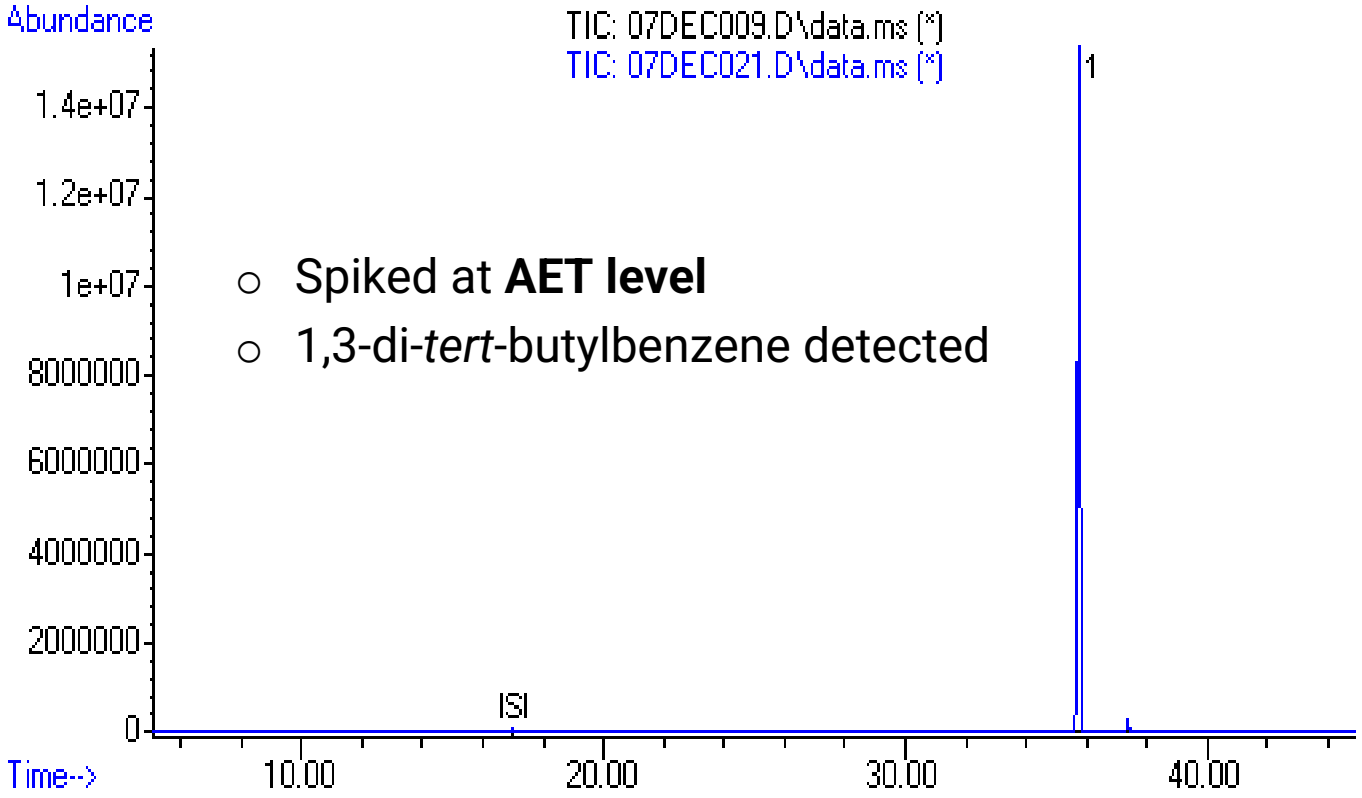
- Blank solution:
  - DS in inert glass botte stored at 5 °C

T0 & T12 months

Final AET: 690 µg/m² or lower (cf. Extractables study)

STEP 3: LEACHABLES STUDY– RESULTS

HS-GC/MS – MST result for 1,3-di-tert-buylbenzene:

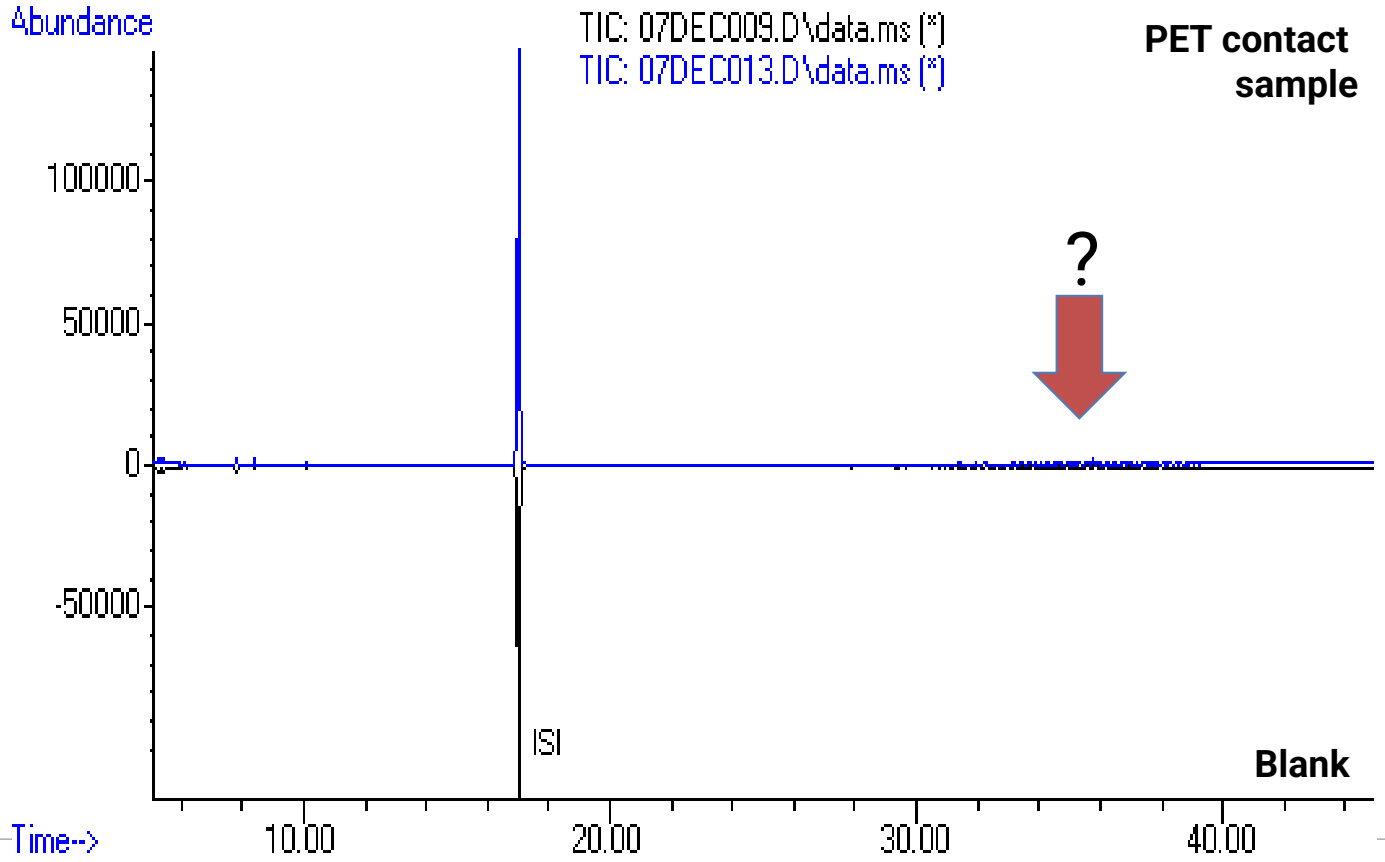


- Spiked at **AET level**
- 1,3-di-*tert*-butylbenzene detected

STEP 3: LEACHABLES STUDY– RESULTS

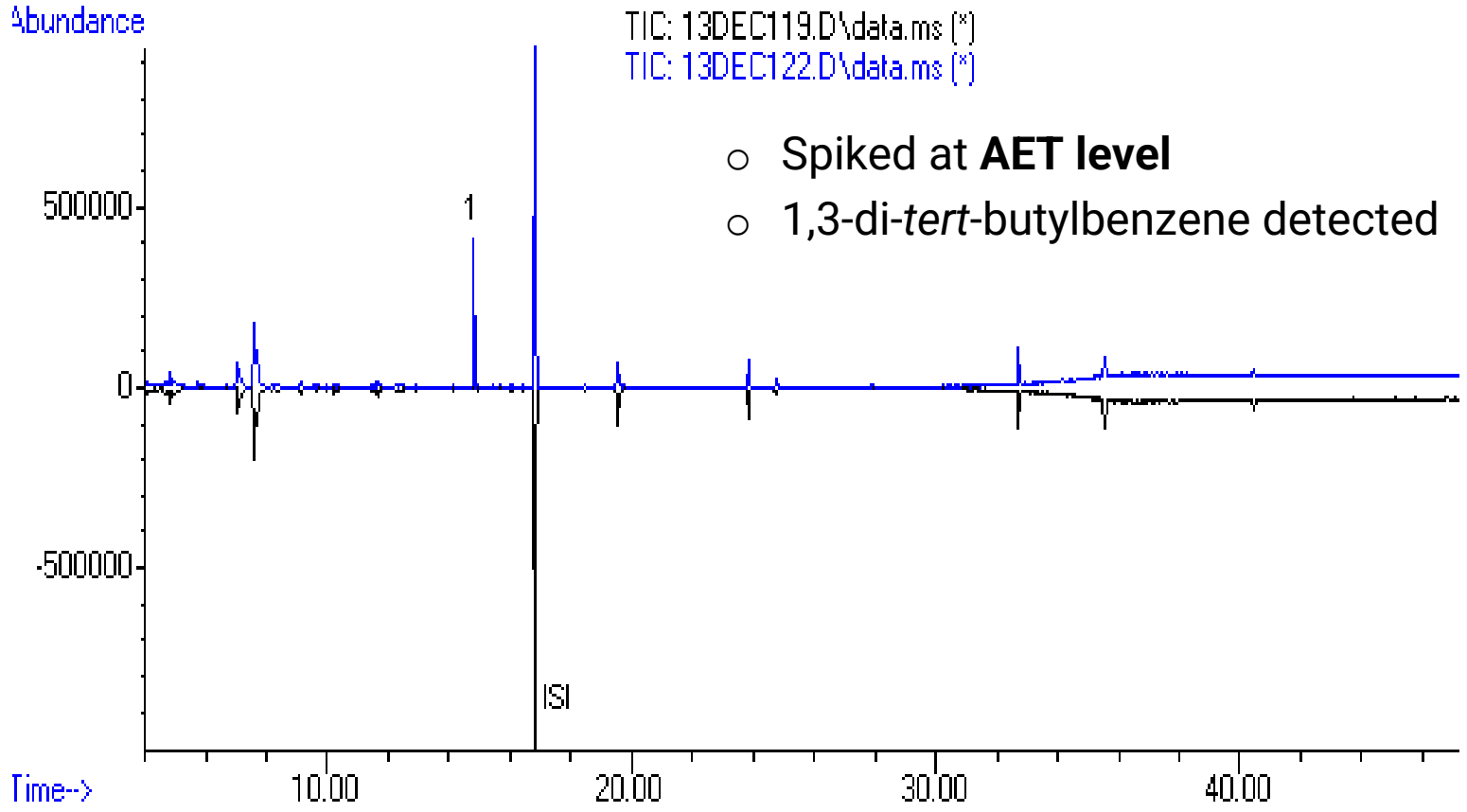
HS-GC/MS contact sample:

- No compounds detected > final AET of 690 µg/m² for T0 & T12M



STEP 3: LEACHABLES STUDY– RESULTS

GC/MS – MST result for 1,3-di-tert-buylbenzene:

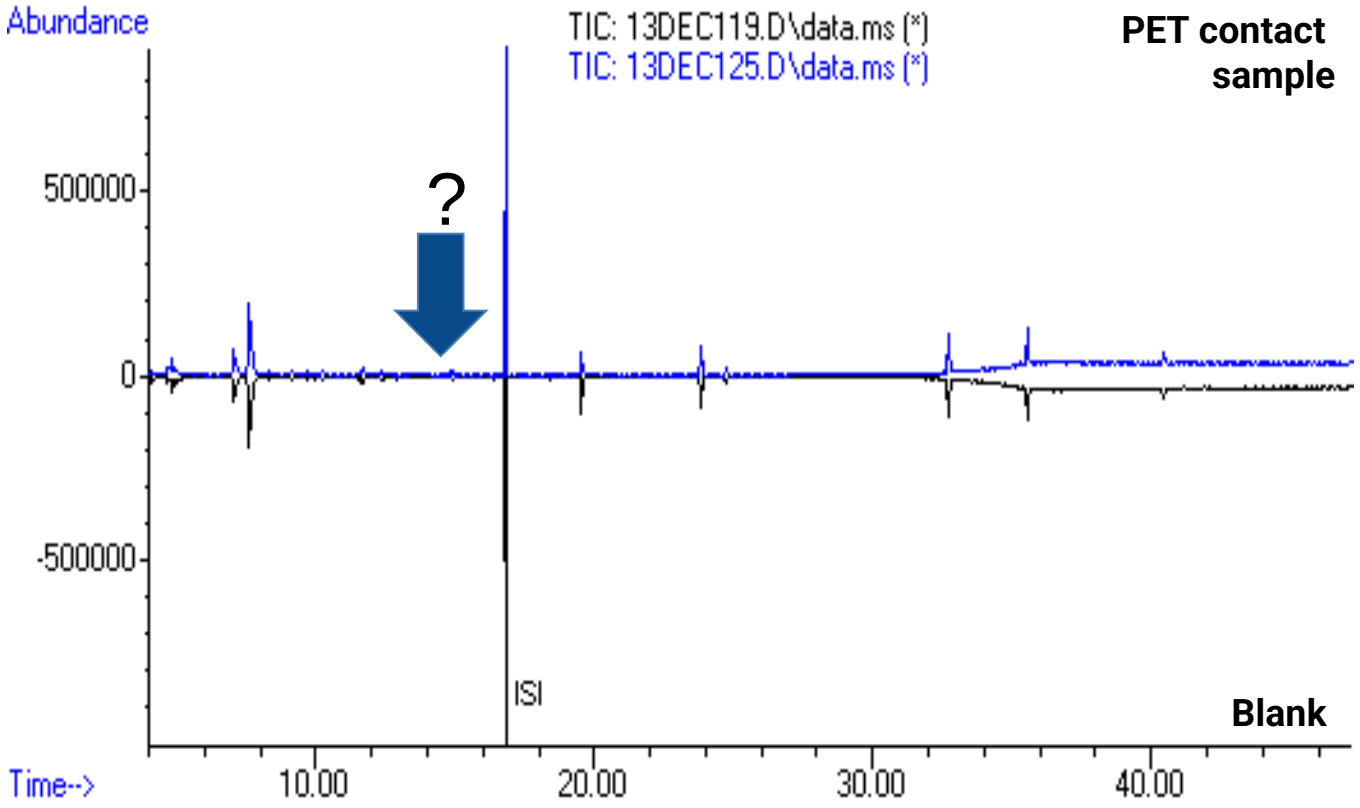


- Spiked at **AET level**
- 1,3-di-*tert*-butylbenzene detected

STEP 3: LEACHABLES STUDY– RESULTS

GC/MS contact sample:

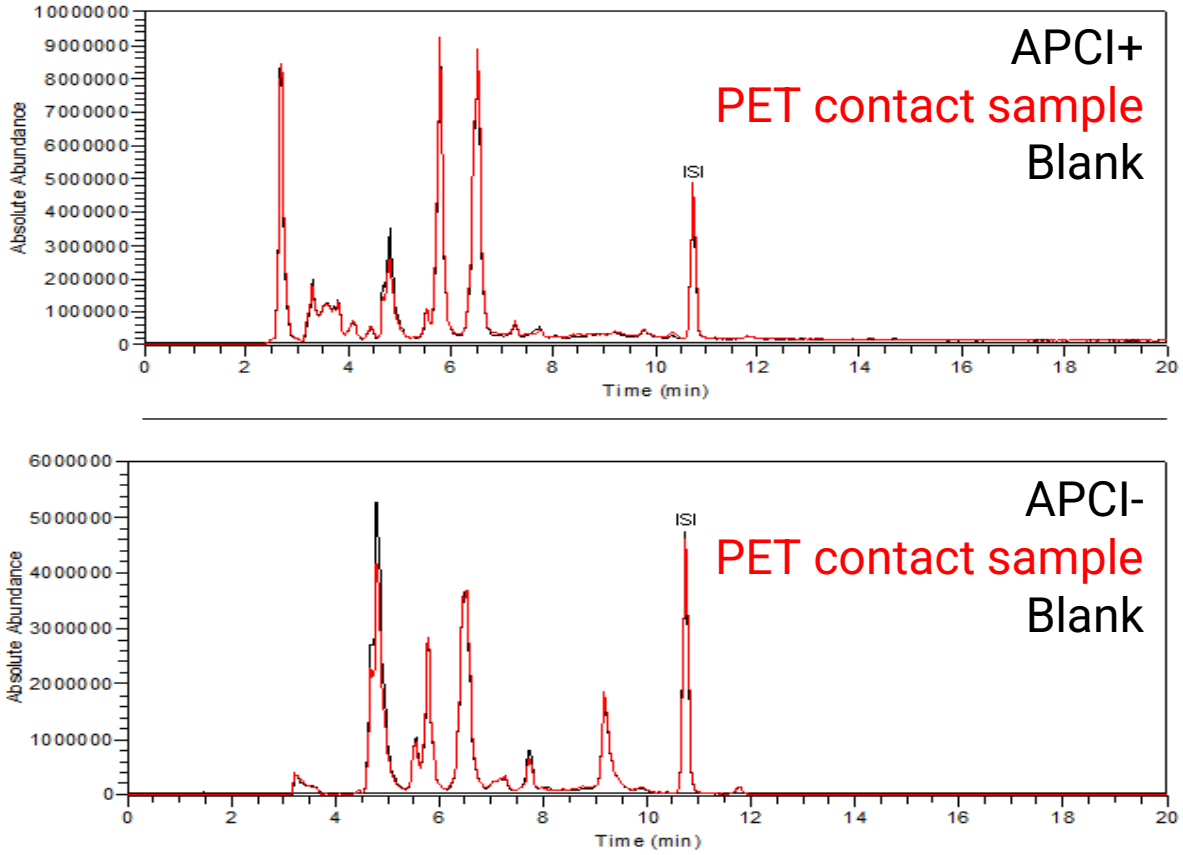
- No compounds detected > final AET of 690 µg/m² for T0 & T12M



STEP 3: LEACHABLES STUDY– RESULTS

HRAM-UPLC/MS contact sample:

- No compounds detected > final AET of 690 µg/m² for T0 & T12M



QUESTIONS?

REFERENCES

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- ICH Q7: Good Manufacturing Practice for Active Pharmaceutical Ingredients (November 1, 2000).
- EU GMP EudraLex – Volume 4 – Part I – Basic Requirements for Medicinal Products – Chapter 3 – Premise and Equipment (March 1, 2015).
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- USP <1665>: Characterization and qualification of plastic components and systems used to manufacture pharmaceutical drug products and biopharmaceutical drug substances and products (2022).
- “Recommendations for Extractables and Leachables Testing” by the Extractables and Leachables Subcommittee of the Bio-Process System Alliance (2008)
- “Recommendations for Testing and Evaluation of Extractables from Single-Use Process Equipment” Bio-Process Systems Alliance (2010)
- “Standardized Extractables Testing Protocol for Single-Use Systems in Biomanufacturing” by BPOG (Nov 2014)
- “Best Practices Guide for Evaluating Leachables Risk from Polymeric Single-Use Systems used in Biopharmaceutical Manufacturing” by BPOG (Mar 2017)

REFERENCES

- "BioPhorum Best Practices Guide for Extractables testing of Polymeric Single-Use Components used in BioPharmaceutical Manufacturing" by BioPhorum (Apr 2020)
- "A Comprehensive Review of BioPhorum Standardized Extractables Testing Data: A Deep-Dive into Similarities, Differences and Trends Across Extraction Solvents and Time Points" by BioPhorum (Sep 2020)