How to set up extractables and leachables studies for single use systems used in (bio)production

PDA TRAINING WEBINAR

Karen Pieters, Ir.

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 sterilization filter

What are SUS?

- Materials or assemblies used in the production of a drug substance or drug product that are discareded as waste after one or a few uses.
- More popular in bioproduction, but also used for small molecule drug products in less extent (mainly bulk storage and filling line)
- Single-Use-Systems (SUS)
- Examples:

Filter capsules

bioreactor

Disposable chromatography columns

Single-use assemblies

Storage bags for bulk solution

Storage containers for buffers or intermediates

Filter cartridges

Disposable centrifuges

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1. REGULATORY REQUIREMENTS FOR SUS

- Polymeric single-use system (SUS) components offer significant advantages over conventional (i.e. reusable) stainless steel components in terms of flexibility, speed and efficiency of operation
- Use of SUS components in biopharmaceutical manufacturing has increased rapidly in recent years
- BUT, concerns regarding the potential leaching of compounds from the polymeric SUS component(s) into the process stream, resulting in a potential negative impact on product quality and/or process performance

➔ Regulatory guidelines and regulations for leachables of SUS

1. REGULATORY REQUIREMENTS FOR SUS

PRODUCTION COMPONENTS/MATERIALS

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 absorptive 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. The parts of the production equipment that come into contact with the product must not be reactive, additive... That it will affect the Quality of the Product..."*

1. REGULATORY REQUIREMENTS FOR SUS

OBSERVATIONS

- The CFR 211.65 and GMP's do ***not only*** refer to the ***impact on Safety***, but also on:
  - Quality (stability, activity,...) of the DP
  - 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 SUS

- **United States Pharmacopeia <665>:**

  Plastic materials, components, and systems used in the manufacturing of pharmaceutical drug products and biopharmaceutical drug substance and products

- **United States Pharmacopeia <1665>:**

  Characterization of plastic materials, components, and systems used in the manufacturing of pharmaceutical drug products and biopharmaceutical drug substance and products

Published ***IN DRAFT*** in Pharmacopeial Forum (PF) 45(2) (March/April 2019)

A new draft will be published in the Pharmacopeial Forum to address current comments (date unknown)

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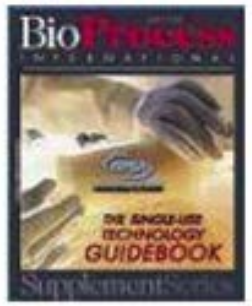
2. INTEREST GROUPS ON STANDARDIZATION

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)

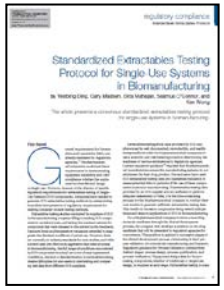


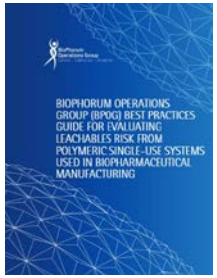
- Available at www.bpsalliance.org

2. INTEREST GROUPS ON STANDARDIZATION

BioPhorum Operations Group
Connect · Collaborate · Accelerate

- Global association of biopharmaceutical manufacturers (end users)
- Publications:
  - "Standardized Extractables Testing Protocol for Single-Use Systems in Biomanufacturing", issued in Nov 2014
  - "Best Practices Guide for Evaluating Leachables Risk from Polymeric Single-Use Systems used in Biopharmaceutical Manufacturing", issued in March 2017
  - "BioPhorum Best Practices Guide for Extractables Testing of Polymeric Single-Use Components used in Biopharmaceutical Manufacturing", issued in April 2020
- Available at www.biophorum.com





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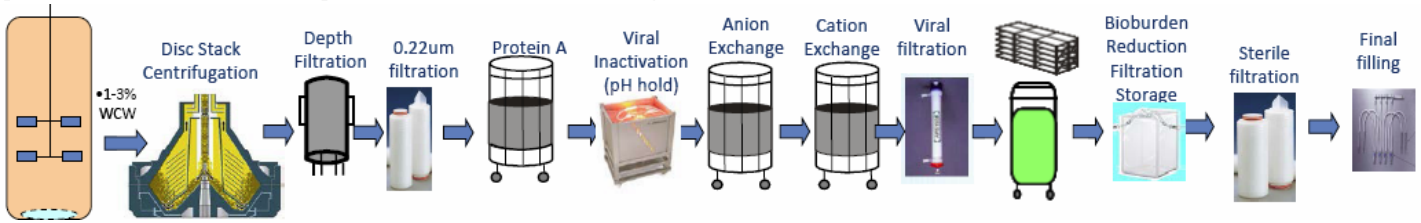
Why perform a risk assessment?

- 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

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?

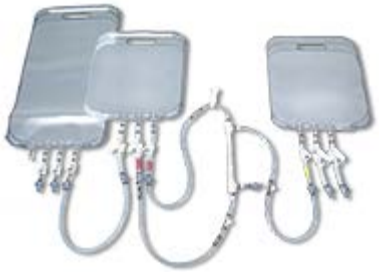
  Select single-use components with greatest potential for objectable levels of leachables with regard to **safety and quality** of the final product, and with regard to **process performance**

- When?

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

Create a list of "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,…


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

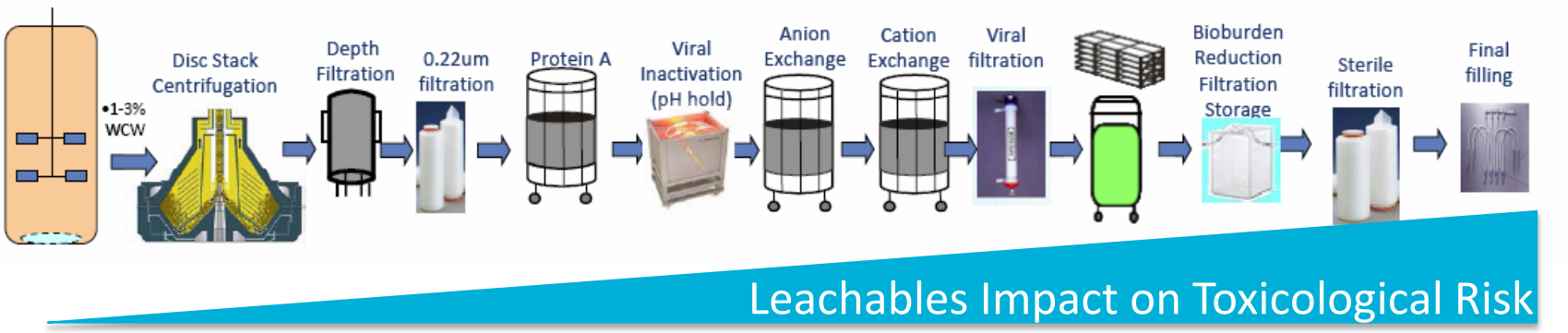
1. Material compatibility
2. Proximity to final DP / distance along production stream
3. Composition of contact solution
4. Surface area to Volume ratio
5. Contact temperature and contact time
6. Pretreatment steps
7. Process performance

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
- First, 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

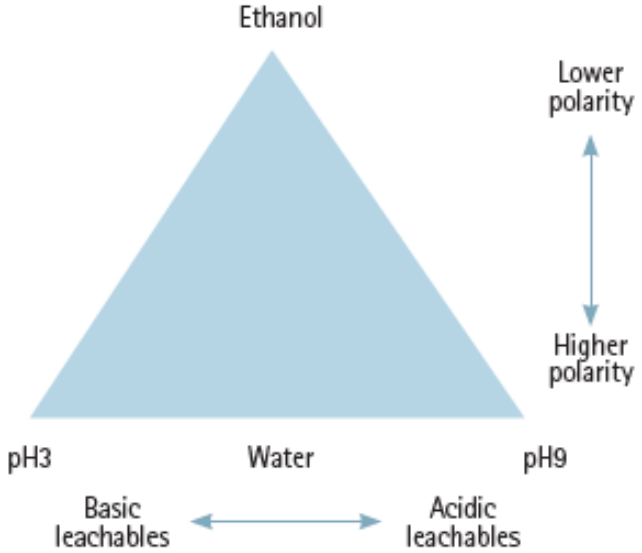
RISK FACTOR 2: Proximity to Final Product

- 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



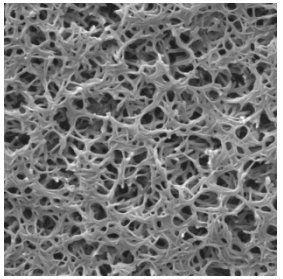
RISK FACTOR 3: Composition of the contact solution

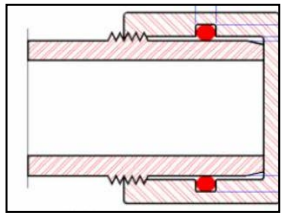
- Higher regulatory and safety concern for leachables in case of contact solutions with:
  - Low or high pH-values
  - High organic contents
  - Surfactants



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 usually result in higher surface-to-volume ratios





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

RISK FACTOR 6: Pretreatment steps

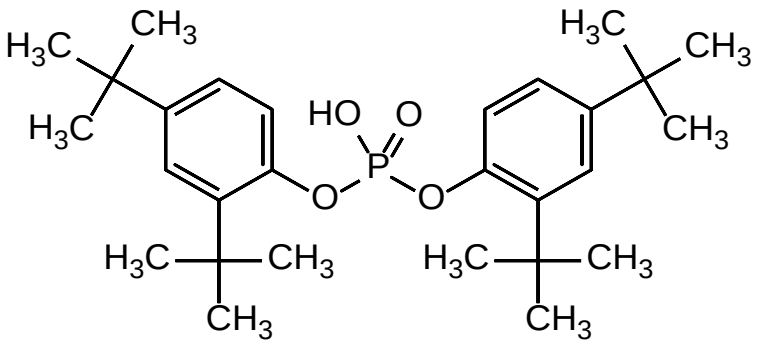
- STERILIZATION tends to change, and possibly increase leachables
  - Steam sterilization
  - Gamma irradiation
  - Ethylene oxide (EtO) sterilization

- 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!

RISK FACTOR 7: Process performance

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

e.g. bDtBPP (cell growth inhibition)



How to perform a risk assessment?

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

Risk factors

Risk levels with rating

Weight factor

BPOG E/L Risk Assessment
Example of Proposed Risk Assessment

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	

(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.

PharmaEd
9/15/2015

3.1 RISK ASSESSMENT

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

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

Consideration		Ratings [1]	Weight [2]
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	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	

USP<1665> draft: 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
- E.g. Sterilization filter:
  Step 1: 1233 ➔ 3321 (sequence to be given in order of decreasing digit values)

Table A-1. Dimensions Relevant to Risk Level

Risk Dimension	Duration	Temperature[a]	Solvent	Material Reactivity
Level 1	<24 h	Frozen (<−10°)	Aqueous (<5% organic v/v; pH ≥3 and pH ≤9)	Inert
Level 2	1−7 days	Refrigerated (2°−8°) Ambient (15°−25°)	Somewhat organic (5%−40% v/v)	Intermediate
Level 3	>7 days	Elevated (>30°)	Highly organic (>40% v/v) or extreme pH (pH <3 or pH >9)	Reactive

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

USP<1665> draft: Example of a risk evaluation matrix

- E.g. Sterilization filter:

  Step 1: Establish numerical risk sequence ➔ 3321

  Step 2: Link numerical risk sequence with a level of characterization

Table A-2. Linking the Numerical Risk Sequence with a Level of Characterization

If...	And...	Then the Characterization Level is...
Four dimension scores are Level 3	There is no additional qualifier (3333)	Level C (High Risk)
Three 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 dimension scores are Level 3	The other two dimension scores are both Level 2 (3322)	Level C
	One dimension score of Level 2 (3321)	Level B (Moderate Risk) or C (Low Risk)[a,b]
	The other two dimension scores are Level 1 (3311)	Level A or B[b,c]
One dimension score 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
No dimension score 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

[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 on risk than do material considerations.
[c] If one of the Level 1 scores is in the material considerations dimension, then Level A; otherwise, Level B.

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

USP<1665> draft: Example of a risk evaluation matrix

- E.g. Sterilization filter:

  Step 1: Establish numerical risk sequence ➔ 3321

  Step 2: Link numerical risk sequence with a level of characterization

  ➔ Level C (High risk)

  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?

    ➔ “Duration < 7 days” and “dialy dose < 10 mL” (factor = 1)

  ➔ Level C (High risk) testing is reduced to Level B (Moderate risk)

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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 upon the set-up

- Increasing demand for standardized extractables protocol for extractables testing performed by the supplier
  - Cover the 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

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- BPOG extractables protocol (2020):

	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			

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3.2 GATHERING EXTRACTABLES DATA

- BPOG extractables protocol (2020):

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

3.2 GATHERING EXTRACTABLES DATA

- USP <665> (draft): Standard Extractables Protocol (SEP)

Table 3. Standard Extraction Protocol for Components or Systems That are Designated as High Risk by Application of the Risk Evaluation Matrix

	Extraction Duration (days)		(40 °C)
Components	**1**	**7**	**21**
Storage container	–	–	X
Mixing bag	X	–	–
Bioreactor bag	–	–	X
Tubing connector and disconnector	–	–	X
Aseptic/sterile connector and disconnector	–	X	–
Sensor/valve	X	–	–
Molded parts of mixers	X	–	–
Polymer pump surfaces	X	–	–
Tubing	–	–	X
Gasket, O-ring	X	–	–
Sterilizing filter	X	–	–
Process filter	–	X	–
Tangential flow filtration	X	–	–
Chromatographic column	X	–	–
Filling needle	X	–	–
Stir bar	–	X	–

Solution C1: pH 3 (HCl/KCl)
Solution C2: pH 10 (PO4 buffer)
Solution C3: 50% EtOH in UPW

PF 45(2): March / April 2019

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.

- Single-use systems used for **specific application**
  - Simulated extractables study might be considered
  - Simulation solvent: pH, polarity, organic content
  - Worst case contact temperature and time versus real use
  - Pretreatment steps: sterilization

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 sterilization filter

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 the 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

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

  - o (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
  - o safety concern thresholds dependent on the administration route of the final product

3.3 EVALUATION OF EXTRACTABLES DATA

- **Parenteral Drug Products (PDPs) – *to be published***

Tox endpoint	General tox.	Sensitizer & irritant	Carcinogen
Class	Class I	Class II	Class III
Threshold level (µg/day)	50	5	1.5 (PDP-SCT)

ANALYTICAL EVALUATION THRESHOLD (AET)

➔ **Translating the SCT into Analytical Thresholds for Extractables studies**

$$\text{AET}\left(\frac{\mu g}{test\ item}\right) = \frac{\text{SCT}\left(\frac{\mu g}{\text{day}}\right)}{\text{number of doses/day}} \text{ x } \frac{\text{number of doses}}{\text{test item}}$$

$$\text{AET}\left(\frac{\mu g}{test\ item}\right) = \frac{1.5\ \frac{\mu g}{\text{day}}}{1\ \text{dose/day}} \text{ x } \frac{1000\ \text{doses}}{\text{filter}} = 1500\ \frac{\mu g}{\text{filter}}$$

OVERVIEW

1. Regulatory requirements for SUS
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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 sterilization filter

3.4 LEACHABLES STUDY

- Monitor compounds of concern with regard to
  - Safety
  - Quality
  - Efficacy
  - Process performance

- Quantitative determination of target leachables
  - LOQ should be at or below the AET level of the corresponding threshold level/PDE
  - Combined with screening analyses to screen for unexpected leachables

3.4 LEACHABLES STUDY

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

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 sterilization filter**

STEP 1: EXT / SIM STUDY – SET-UP

Sponsor info:

- Capsule filter: PES membrane & PP housing
- Filter used for sterilization of solution in formulation step
- Composition contact solution:
  - Biological product composed of 10% organic content, PS80 and Phosphate buffer
- Contact time & temperature:
  - 2 h at room temperature (< 25 ºC)
- Pretreatment:
  - Filter is flushed with contact solution before use in process

STEP 1: EXT / SIM STUDY – SET-UP

Extractables study / simulation study set-up:

- Preflush of the filter (sponsor instructions)
- Dynamic extraction by circulation (see next slide)
- 3 h at 30 ºC (sponsor request) (worst case for “2 h at room temperature”)
- Simulation solvents:
  - 50% Isopropanol (IPA) in Ultrapure water (UPW)
  - UPW
- Analytical techniques:
  - HS-GC/MS screening → VOC
  - GC/MS screening → SVOC
  - HRAM-UPLC/MS screening → NVOC
  - ICP/OES → elements
  - ICP/MS → Hg
  - IC → Acetate / formate / sulphate anions

STEP 1: EXT / SIM STUDY – SET-UP

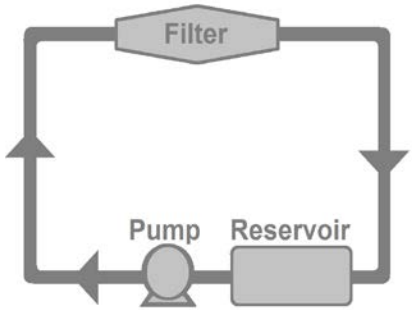
Extractables study / simulation study set-up:

Safety Concern Threshold (SCT)	1.5 µg/day
Maximum daily dose (sponsor info)	0.25 mL/day
Minimum batch volume used for 1 filter	5L
Estimated Analytical Evaluation Threshold (AET) (1.5 µg/day / 0.25 mL/day) (1.5 µg/day / 0.25 mL/day) x 5L / filter	 6000 µg/L 30000 µg/filter
Final AET (taking into account a 50% Uncertainty Factor for screening methods (PQRI))	3000 µg/L 15000 µg/filter

Reporting limit set at 3000 µg/L (~15000 µg/filter) or lower

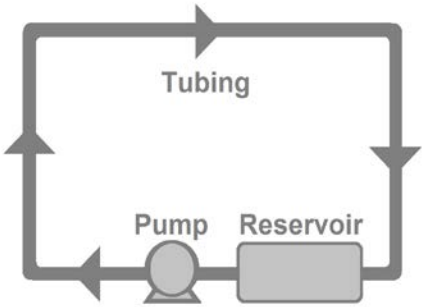
Dynamic extraction by recirculation

- Filter extraction:
  - o Simulation solvent (5 L) in glass bottle is put in water bath (30 ºC)
  - o Solvent is circulated by peristaltic pumping through Silicone tubing and filter for 3 h



Filter extract

- Blank circulation:
  - o Simulation solvent (5 L) in glass bottle is put in water bath (30 ºC)
  - o Solvent is circulated by peristaltic pumping through Silicone tubing for 3h without any contact to the filter



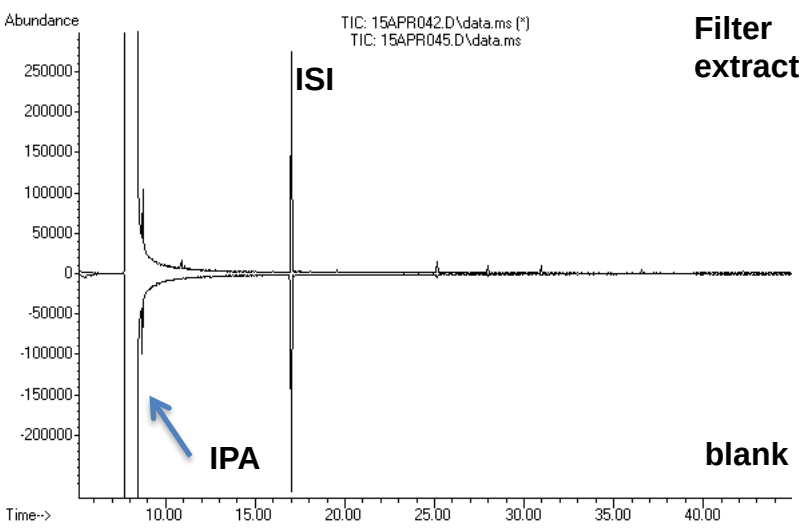
Blank circulation

STEP 1: EXT / SIM STUDY – SET-UP

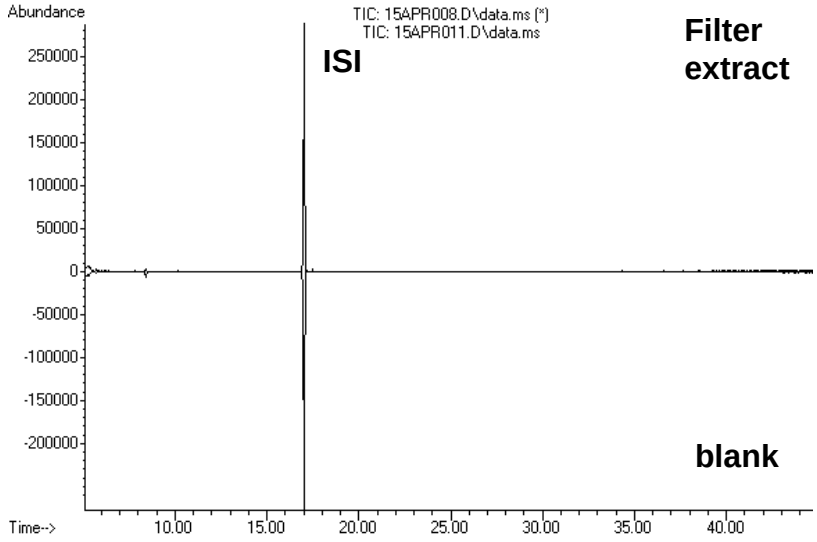
HS-GC/MS screening analysis:

- 50% IPA : no compounds > RL of 330 µg/filter
- UPW: no compounds > RL of 25µg/filter

50% IPA



UPW

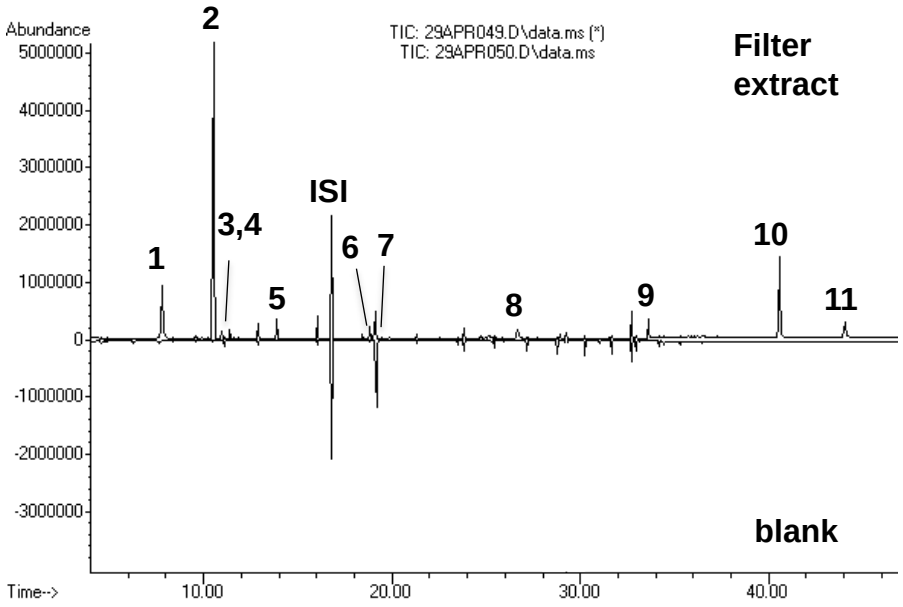


STEP 1: EXT / SIM STUDY – SET-UP

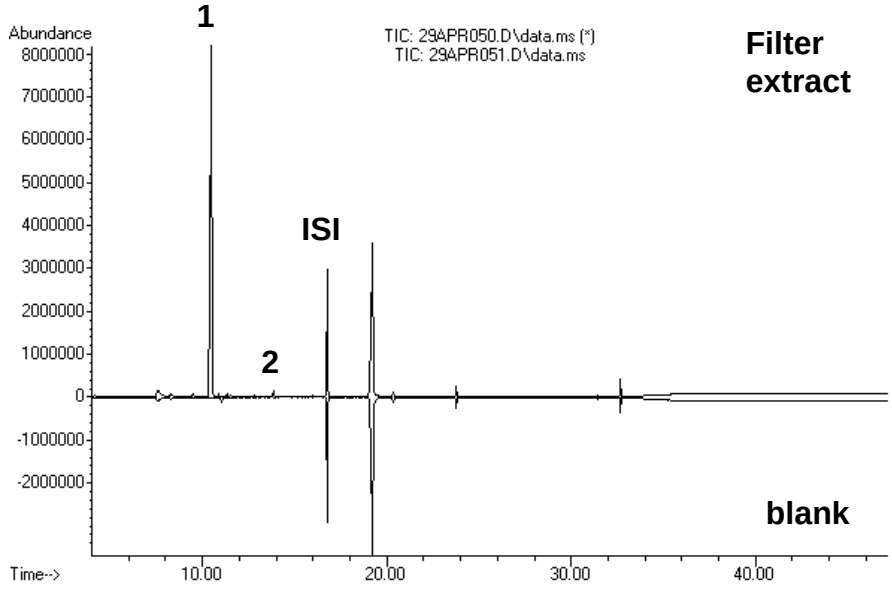
GC/MS screening analysis:

- 50% IPA: 11 compounds > RL of 130 µg/filter
- UPW: 2 compounds > RL of 25 µg/filter

50% IPA



UPW

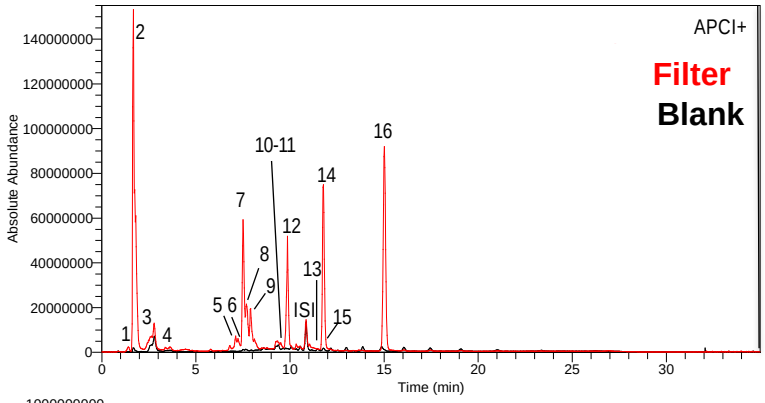


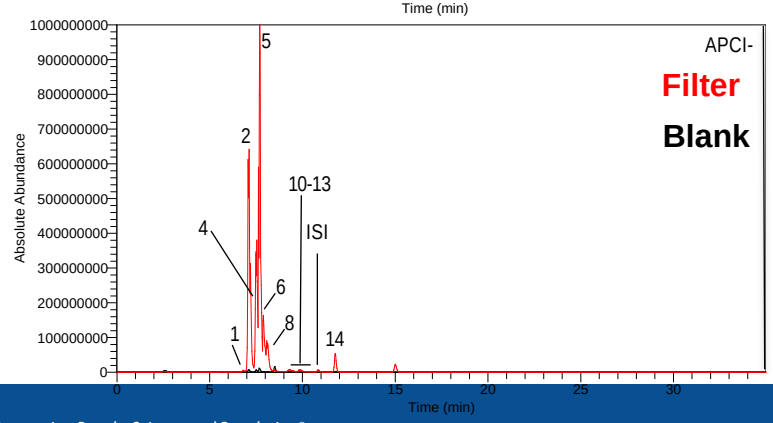
STEP 1: EXT / SIM STUDY – SET-UP

HRAM-UPLC/MS screening analysis

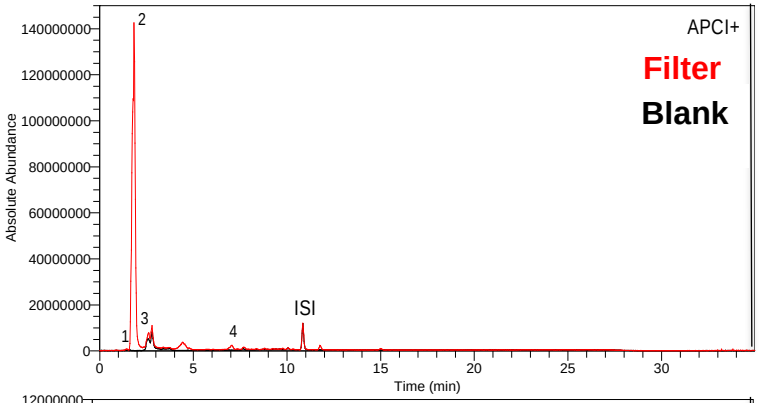
- 50% IPA: 16 compounds > RL of 130 µg/filter
- UPW: 4 compounds > RL of 25 µg/filter

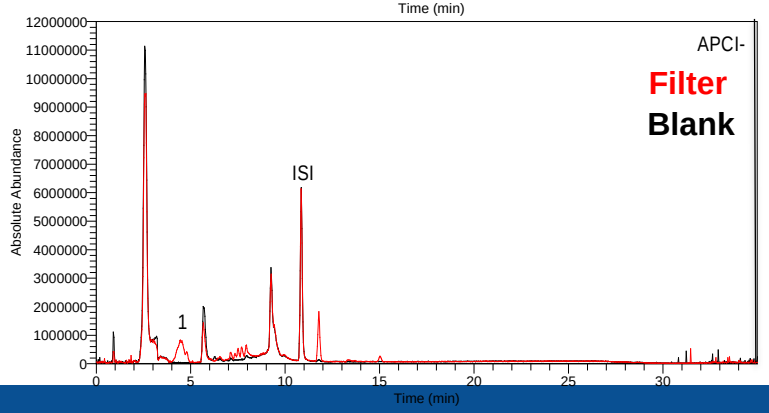
50% IPA





UPW





STEP 1: EXT / SIM STUDY – SET-UP

Results 50% IPA extract

GC/MS

N°	ID Level	Organic Compounds	CAS-Number	t_R (min)	Test result (µg/filter)
50% IPA extract of the filter Reporting limit: 130 µg/filter					
1	IC	2-Methylpentane-2,4-diol	107-41-5	7.80	2800
2	IC	1-Methyl-2-pyrrolidinone	872-50-4	10.54	12000
3	TIC	Compound with formula $C_6H_{11}NO$	-	10.97	220
4	TIC	Compound with formula $C_6H_{11}NO$	-	11.38	270
5	IC	Tetrahydrothiophene 1,1-dioxide	126-33-0	13.90	480
6	IC	1-Dodecanol	112-53-8	18.44	150
7	MPC	3,6,9,12-Tetraoxatetradecan-1-ol	5650-20-4	19.83	140
8	IC	1-Octadecanol	112-92-5	26.65	900
9	IC	Erucamide	112-84-5	33.60	540
10	IC	Irgafos 168	31570-04-4	40.57	3000
11	IC	Irgafos 168 Oxidized	95906-11-9	44.04	930

IC: Identified Compound; MPC: Most Probable Compound; TIC: Tentatively Identified Compound; t_R: retention time.

HRAM-UPLC/MS

N°	ID Level	Organic Compounds	CAS-Number	Extracted ion	t_R (min)	Test result (µg/filter)
50% IPA extract of the filter APCI(+) mode Reporting limit: 130 µg/filter						
1	IC	Tetrahydrothiophene 1,1-dioxide	126-33-0	153.058	1.41	2800
2	IC	1-Methyl-2-pyrrolidinone	872-50-4	100.076	1.68	17000
3	IC	1-Methyl-2-piperidinone	931-20-4	114.091	2.65	2500
4	TIC	$C_7H_{13}NO$	-	128.107	3.62	140
5	U	-	Mass spectrum	729.090	7.11	130
6	U	-	Mass spectrum	743.106	7.23	170
7	U	-	Mass spectrum	961.109	7.51	1100
8	U	-	Mass spectrum	821.116	7.69	500
9	U	-	Mass spectrum	1021.109	7.91	470
10	U	-	Mass spectrum	485.358	9.79	130
11	IC	Irganox 3114	27676-62-6	219.174	9.81	190
12	IC	Erucamide	112-84-5	338.341	9.86	1700
13	U	-	Mass spectrum	440.409	11.16	310
14	IC	Irgafos 168 oxidized	95906-11-9	663.453	11.78	2200
15	U	-	Mass spectrum	468.440	11.85	220
16	IC	Irgafos 168	31570-04-4	647.458	15.02	3700
APCI(-) mode						
1	U	-	Mass spectrum	509.073	6.80	260
2	U	-	Mass spectrum	695.051	7.07	53000
3	TIC	$C_{31}H_{38}O_3N_2$	-	485.282	7.48	200
4	U	-	Mass spectrum	927.070	7.54	18000
5	U	-	Mass spectrum	787.078	7.70	51000
6	U	-	Mass spectrum	1019.096	7.90	5400
7	U	-	Mass spectrum	499.008	8.02	560
8	U	-	Mass spectrum	879.104	8.12	4200
9	U	-	Mass spectrum	1111.122	8.23	330
10	IC	Palmitic acid	57-10-3	255.233	9.33	5900
11	IC	Irganox 3114	27676-62-6	564.344	9.81	270
12	IC	Erucamide	112-84-5	336.327	9.86	1600
13	IC	Stearic acid	57-11-4	283.264	9.91	4000
14	IC	Irgafos 168 oxidized	95906-11-9	473.283	11.77	1700
15	IC	Irganox 1076	2082-79-3	529.463	13.66	180
16	IC	Irgafos 168	31570-04-4	205.160	15.01	2700

IC: Identified Compound; TIC: Tentatively Identified Compound; U: Unidentified compound; t_R: retention time.

- Selection of targets for 'leachables study'
  - 5 targets detected by both techniques
  - 8 targets only detected by 1 technique
    - → 2 targets covered by 'marker compound'
  - Unidentified compounds detected above the final AET that require attention during LEA study

Results UPW extract

GC/MS

N°	ID Level	Organic Compounds	CAS-Number	t_R (min)	Test result (µg/filter)
UPW extract of the filter Reporting limit: 25 µg/filter					
1	IC	1-Methyl-2-pyrrolidinone	872-50-4	10.50	3400
2	IC	Tetrahydrothiophene 1,1-dioxide	126-33-0	13.85	28

IC: Identified Compound; MPC: Most Probable Compound; TIC: Tentatively Identified Compound; t_R: retention time.

- Additional target compounds?
  - 1 unique compound compared to 50% IPA, but in low concentration

HRAM-UPLC/MS

N°	ID Level	Organic Compounds	CAS-Number	Extracted ion	t_R (min)	Test result (µg/filter)
UPW extract of the filter APCI(+) mode Reporting limit: 25 µg/filter						
1	IC	Tetrahydrothiophene 1,1-dioxide	126-33-0	153.058	1.44	210
2	IC	1-Methyl-2-pyrrolidinone	872-50-4	100.076	1.83	4500
3	IC	1-Methyl-2-piperidinone	931-20-4	114.091	2.64	200
4	TIC	$C_{18}H_{33}O_5N$	-	344.242	7.07	37
APCI(-) mode						
1	TIC	Polyethoxylated compound	-	287.186	4.56	29

IC: Identified Compound; TIC: Tentatively Identified Compound; t_R: retention time.

IC

ANION	Results (µg/filter)		Limits (µg/filter)	
	Blank	Filter extract	LOD	LOQ
Formate	<300	<300	300	1000
Acetate	<300	<300	300	1000
Sulfate	<300	<300	300	1000

LOD: Limit of Detection; LOQ: Limit of Quantification.

- No Acetate/formate/sulphate detected

ICP/MS

Sample	Results	Reporting limit
	µg/filter	µg/filter
UPW blank extract	<3	3
UPW filter extract	<3	3

- No Mercury detected

Results UPW extract (2)

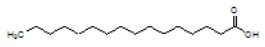
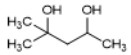
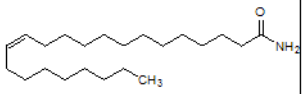
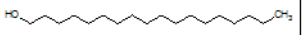
ICP/OES

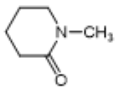
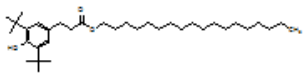
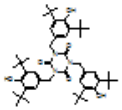
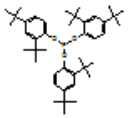
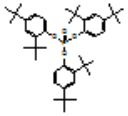
ELEMENT	Results (µg/filter)		Limits (µg/filter)		ELEMENT	Results (µg/filter)		Limits (µg/filter)	
	Blank	Filter extract	LOD	LOQ		Blank	Filter extract	LOD	LOQ
Aluminum (Al)	<20	<20	20	30	Palladium (Pd)	<100	<100	100	300
Antimony (Sb)	<10	<10	10	30	Platinum (Pt)	<20	<20	20	50
Arsenic (As)	<30	<30	30	50	Selenium (Se)	<50	<50	50	130
Barium (Ba)	<5	<5	5	10	Silicon (Si)	<100	**600**	100	300
Boron (B)	<10	<10	10	30	Silver (Ag)	<5	<5	5	15
Cadmium (Cd)	<5	<5	5	10	Strontium (Sr)	<5	<5	5	10
Calcium (Ca)	[20]	[30]	20	50	Sulfur (S)	<100	<100	100	300
Chromium (Cr)	<5	<5	5	10	Thallium (Tl)	<30	<30	30	50
Cobalt (Co)	<3	<3	3	5	Tin (Sn)	<50	<50	50	100
Copper (Cu)	<10	<10	10	30	Titanium (Ti)	<5	<5	5	10
Iron (Fe)	<10	<10	10	30	Vanadium (V)	<10	<10	10	30
Lead (Pb)	<20	<20	20	30	Zinc (Zn)	<5	<5	5	10
Lithium (Li)	<3	<3	3	5	Gold (Au)	<50	<50	50	100
Magnesium (Mg)	<20	<20	20	30	Iridium (Ir)	<50	<50	50	100
Manganese (Mn)	<3	<3	3	5	Osmium (Os)	<10	<10	10	30
Molybdenum (Mo)	<10	<10	10	30	Rhodium (Rh)	<10	<10	10	30
Nickel (Ni)	<10	<10	10	30	Ruthenium (Ru)	<10	<10	10	30

LOD: Limit of Detection; LOQ: Limit of Quantification; [values between square brackets are detected below the quantification limit (indicative)]; **Values in bold are detected above the quantification limit.**

- Additional target element → Silicon

Overview selected organic target compounds

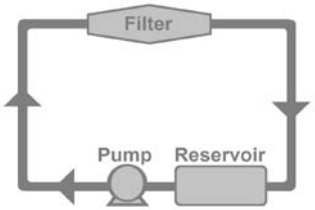
Chemical name; [CAS No.] formula	Structure	Origin
Hexadecanoic acid; Palmitic acid; (also marker for Stearic acid) [57-10-3] $C_{16}H_{32}O_2$		Processing aids in activators, dispersing agents, plasticizers, acid scavengers, mold release agents, and lubricants in polymer processing.
2-Methylpentane-2,4-diol; Hexylene glycol [107-41-5] $C_6H_{14}O_2$		-
Erucamide; (*Z*)-13-Docosenamide; Atmer SA1753; Eur. Pharm. Ref.: Add 21 [112-84-5] $C_{22}H_{43}NO$		slip agent, anti-fogging or lubricant
1-Octadecanol; Stearyl alcohol; Octadecyl alcohol (also used as marker for 1-Dodecanol) [112-92-5] $C_{18}H_{38}O$		associated to Irganox 1076
Tetrahydrothiophene 1,1-dioxide; Sulfolane [126-33-0] $C_4H_8O_2S$		-
1-Methyl-2-pyrrolidinone; *N*-Methyl-2-pyrrolidone; 1-Methyl-2-pyrrolidone; NMP [872-50-4] C_5H_9NO		Solvent in production of Polyethersulfone

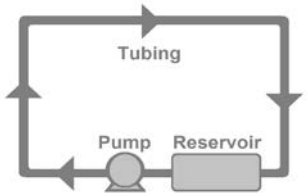
Chemical name; [CAS No.] formula	Structure	Origin
1-Methyl-2-piperidinone [931-20-4] $C_6H_{11}NO$		-
Irganox 1076; Octadecyl-3(3,5-di-*tert*-butyl-4-hydroxyphenyl) propionate; Eur. Pharm. Ref.: Add 11 [2082-79-3] $C_{35}H_{62}O_3$		widely used stabilizer (primary antioxidant) for polymers
Irganox 3114; 1,3,5-Tris(3,5-di-*tert*-butyl-4-hydroxybenzyl)-1,3,5-triazine-2,4,6(1H,3H,5H)-trione; Eur. Pharm. Ref.: Add 13 [27676-62-6] $C_{48}H_{69}N_3O_6$		multi-functional antioxidant used in ABS resin, polyester, Nylon, PE, PS, PVC, PU, cellulose plastic and rubber
Irgafos 168; Tris(2,4-di-*tert*-butylphenyl) phosphite; Eur. Pharm. Ref.: Add 12 [31570-04-4] $C_{42}H_{63}O_3P$		widely used stabilizer (secondary antioxidant) for polymer
Irgafos 168 Oxide Tris(2,4-di-*tert*-butylphenyl) phosphate; [95906-11-9] $C_{42}H_{63}O_4P$		oxidation product of Irgafos 168

→ Used as targets in Method Suitability Test

STEP 3: LEACHABLES STUDY - RESULTS

- Dynamic extraction by recirculation
  - Filter extraction:
    - Pre-flush (8 L) of filter with Drug product (DP)
    - DP (5L) in glass bottle is put in water bath (25 ºC)
    - DP is circulated by peristaltic pumping through tubing and filter for 2 h
  - Blank circulation
    - DP in glass bottle is put in water bath (25 ºC)
    - Solvent is circulated by peristaltic pumping through tubing for 2h without any contact to the filter



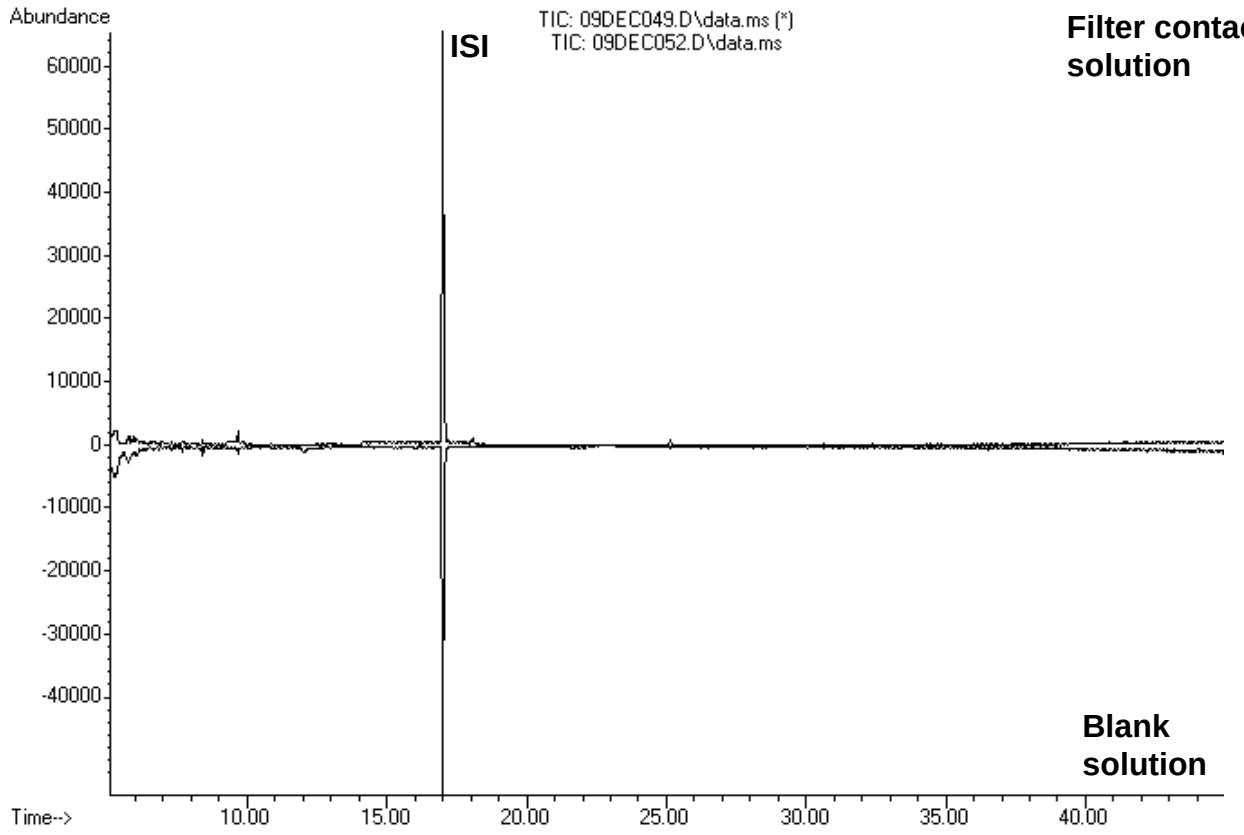


→ "Worst case leachables study" (compared to real-use conditions as performed by sponsor)

- Final AET: 3000 µg/L or lower (cf. Extractables study)

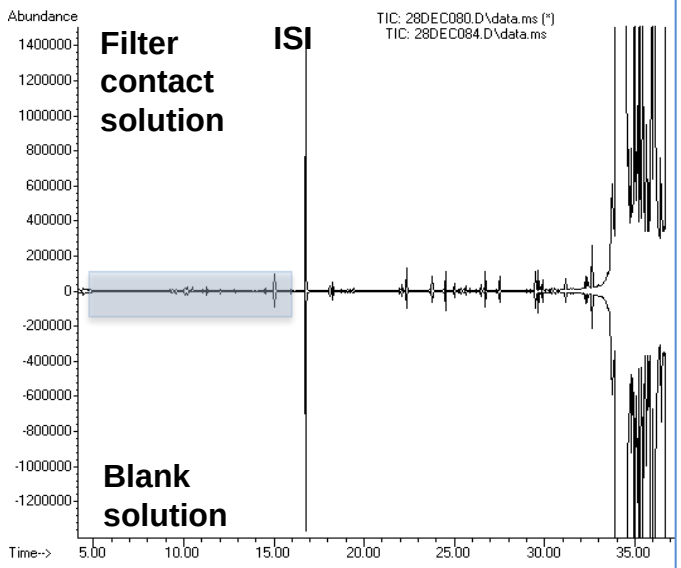
HS-GC/MS

- No compounds detected > 65 µg/L (Final AET: 3000 µg/L)

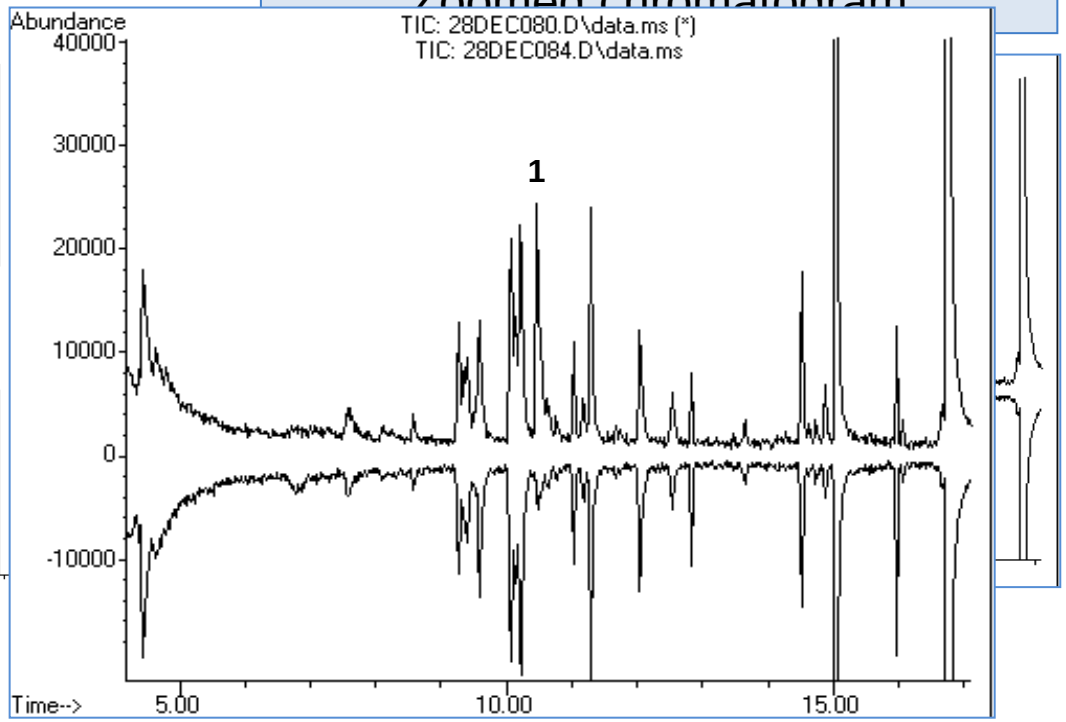


STEP 3: LEACHABLES STUDY - RESULTS

GC/MS



Zoomed chromatogram



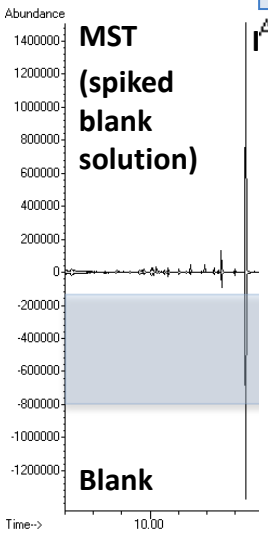
no.	ID Level	ORGANIC COMPOUND	CAS-No	t_R (min)	Result (µg/L)
1	IC	1-Methyl-2-pyrrolidinone	872-50-4	10.46	1000
IC: Identified Compound; reporting limit: 500 µg/L					

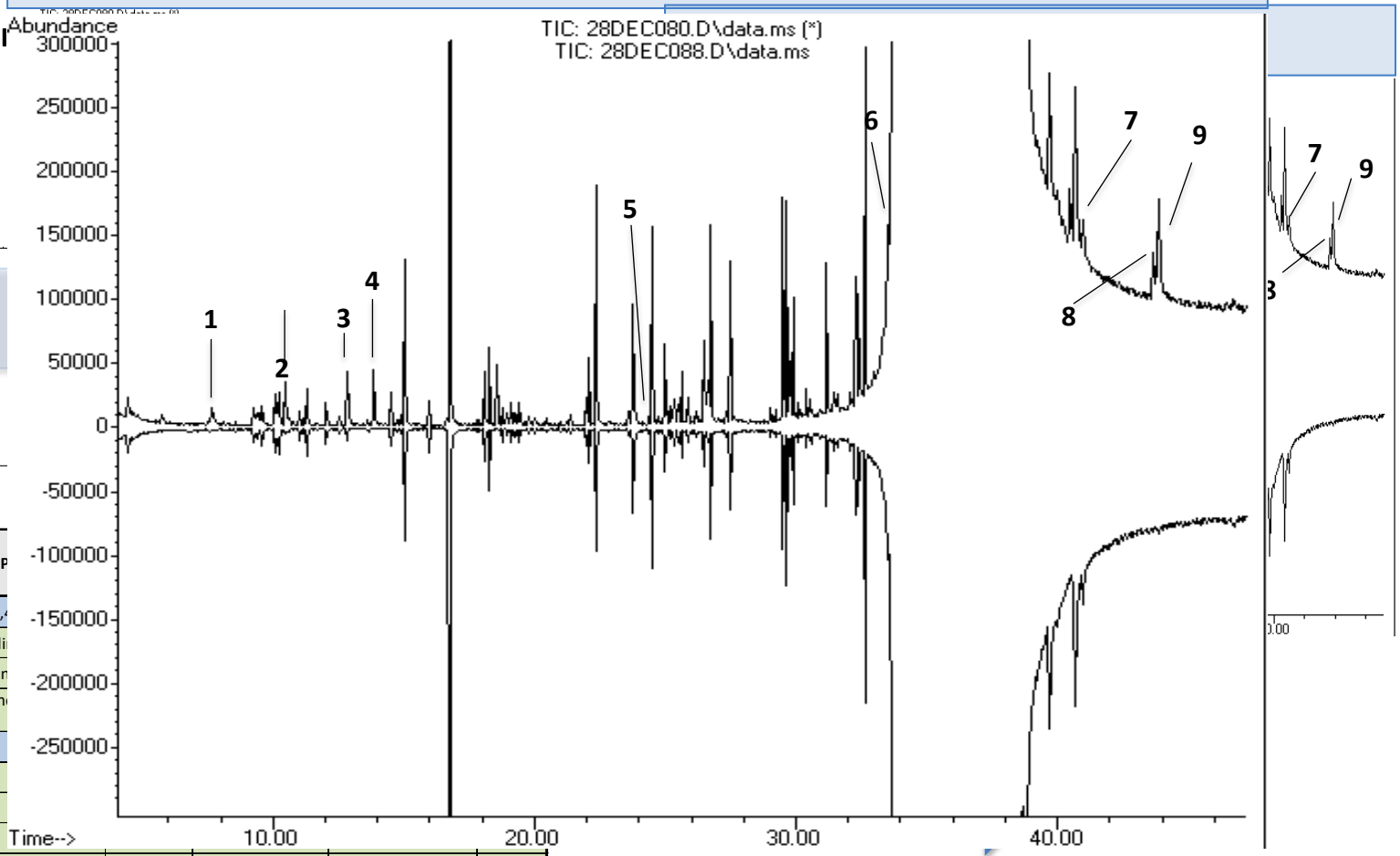
➡ Only 1 target compound detected, but < Final AET (3000 µg/L)

STEP 3: LEACHABLES STUDY - RESULTS

GC/MS – MST results

Zoomed chromatogram



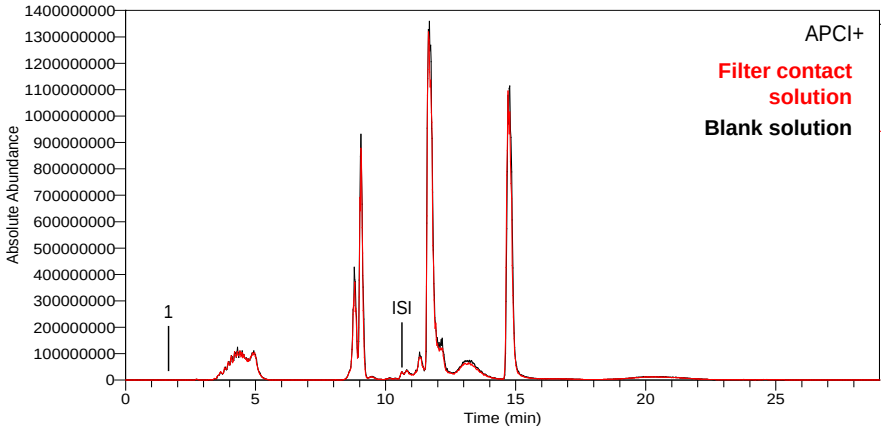


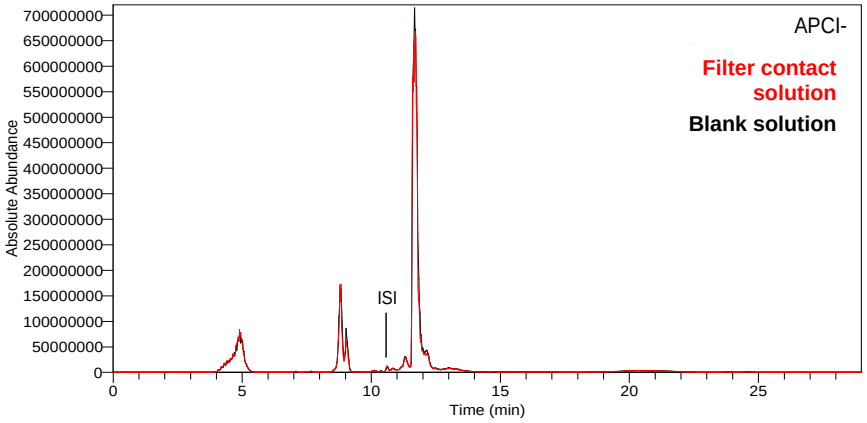
N°	TARGET COMP				
1	2-Methylpentane-2,4				
2	1-Methyl-2-pyrrolidi				
3	1-Methyl-2-piperidin				
4	Tetrahydrothiophen dioxide				
5	1-Octadecanol				
6	Erucamide				
7	Irgafos 168				
8	Irganox 1076				
9	Irgafos 168 oxidized	43.87	5730	5300	93

Remark: Spiked concentrations were rounded to 3 significant figures; measured concentrations and the calculated ratio were rounded to 2 significant figures.

STEP 3: LEACHABLES STUDY - RESULTS

HRAM-UPLC/MS





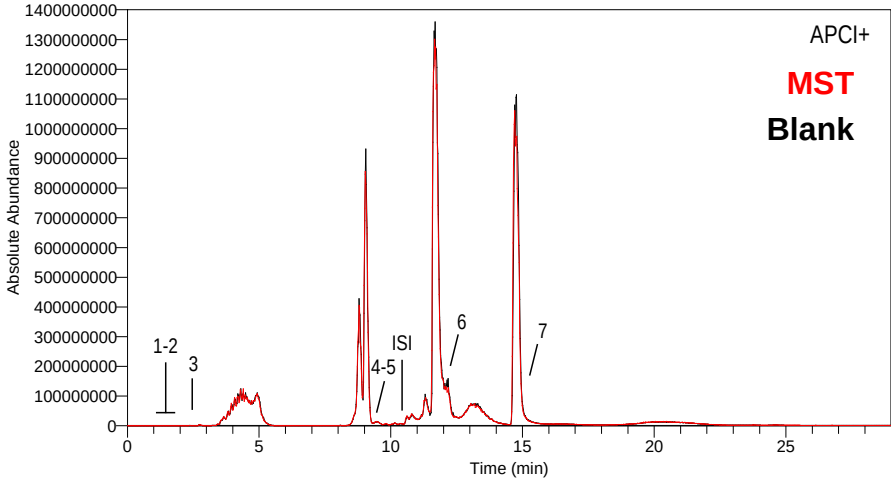
➡ Evaluated using “Extracted ion chromatograms”

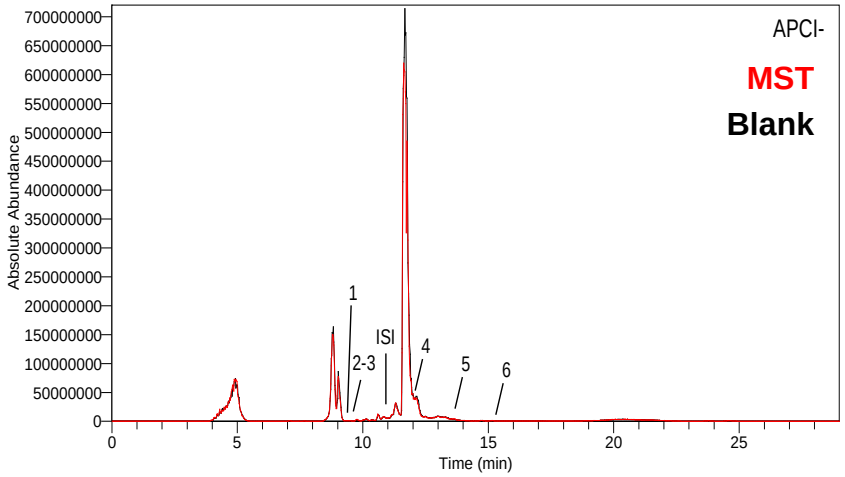
No.	ID	NON-VOLATILE COMPOUND	CAS-No	EI (m/z)	t_R (min)	Results (µg/L)
POSITIVE IONIZATION MODE (APCI+): -N20						
1	IC	1-Methyl-2-pyrrolidinone	872-50-4	100.076	1.78	1500
NEGATIVE IONIZATION MODE (APCI-): -N21						
No differential Non-Volatile Organic Compounds detected above the reporting limit of 1500 µg/L.						
reporting limit: 1500 µg/L.						

➡ Only 1 target compound detected, but < Final AET (3000 µg/L)

STEP 3: LEACHABLES STUDY - RESULTS

HRAM-UPLC/MS – MST results





N°	TARGET COMPOUND	t_R (min)	Spiked concentration (µg/L)	Measured concentration (µg/L)	Ratio (%)
POSITIVE IONIZATION MODE (APCI+)					
1	Tetrahydrothiophene-1,1-dioxide	1.39	5940	6800	115
2	1-Methyl-2-pyrrolidinone	1.75	5980	3200	54
3	1-Methyl-2-piperidinone	2.36	5930	8200	140
4	Irganox 3114	9.76	5880	5300	90
5	Erucamide	9.84	5980	5400	90
6	Irgafos 168 oxidized	11.81	5730	5700	100
7	Irgafos 168	15.14	5930	4800	81

N°	TARGET COMPOUND	t_R (min)	Spiked concentration (µg/L)	Measured concentration (µg/L)	Ratio (%)
NEGATIVE IONIZATION MODE (APCI-)					
1	Palmitic acid	9.39	5870	4000*	69*
2	Irganox 3114	9.75	5880	6500	110
3	Erucamide	9.83	5980	6000	100
4	Irgafos 168 oxidized	11.80	5730	5900	100
5	Irganox 1076	13.64	5960	9400	160
6	Irgafos 168	15.16	5930	3900	66

* Corrected for the concentration in the blank solution (16-B7028-N20/N21);
Remark: Spiked concentrations were rounded to 3 significant figures; measured concentrations and the calculated ratio were rounded to 2 significant figures.

- Spiked at AET level: 6000 µg/L
- Detected level in MST: 3200 µg/L
- Detected result in sample: 1500 µg/L

→ OK!

Thank you for your attention!

For further questions, please do not hesitate to contact:
kpieters@nelsonlabs.com