CLOSING THE GAP BETWEEN EXTRACTABLES AND LEACHABLES

PDA TRAINING COURSE
EXTRACTABLES – LEACHABLES
ROME
01 – 02 MARCH, 2018

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The more we know,
the more we know we don't know!

Anonymous
Rome, 2018

Extractables / Leachables Testing: a Relatively New Science!

- ✓ Regulatory Requirements are becoming more and more Stringent.
- ✓ This leads to more and more Testing.
- ✓ More Testing increases the Understanding of the Interaction of the Materials with the Drug Products
- ✓ In order to have a proper "*Risk Mitigation*" a good Understanding of what can happen is of premordial importance!

2. LEACHABLES: A SUBSET OF EXTRACTABLES?

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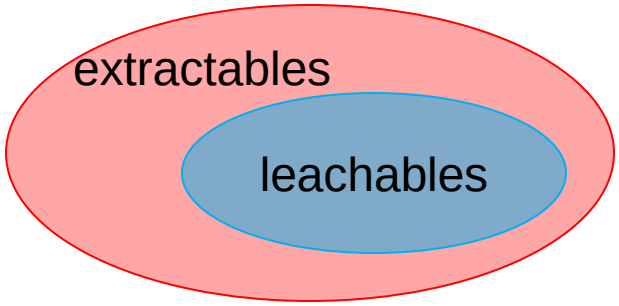
➔ THEORY:



In early stages of E/L research (5 – 10 years ago):

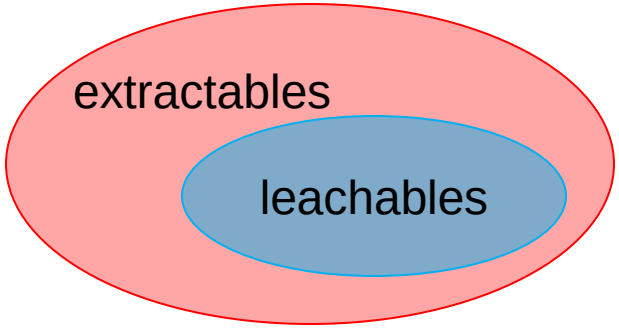
- *Consensus: Leachables are a subset of Extractables*
- *Extractable study should be designed to identify all potential leachables*

FDA and EMA also include this thinking in their Guidelines and Guidances

Migration studies may only be omitted if, based on the outcome of the extraction studies, the calculated maximum amount of individual leachable substance that may be present in the active substance/medicinal product leads to levels demonstrated to be toxicologically safe. When a migration study is not considered necessary and thus is not conducted, a justification should be provided.

2. LEACHABLES: A SUBSET OF EXTRACTABLES?

➔ THEORY:



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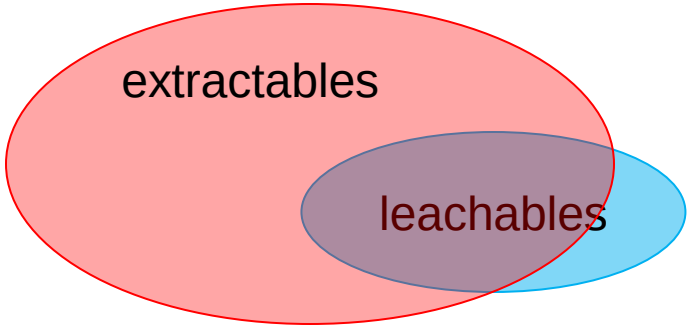


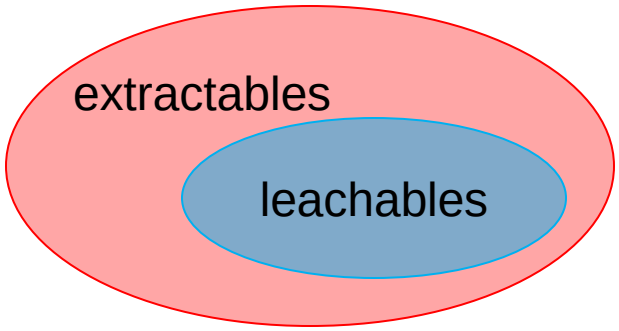
MIND THE GAP!

In the last 6-7 years, there is a growing consensus that – based upon experimental evidence – **Leachables are not always a subset of Extractables**!!

Yet, a lot of pharma companies adhere to the risk assessment of pharmaceutical containers and closures, solely based upon Extractables Data...

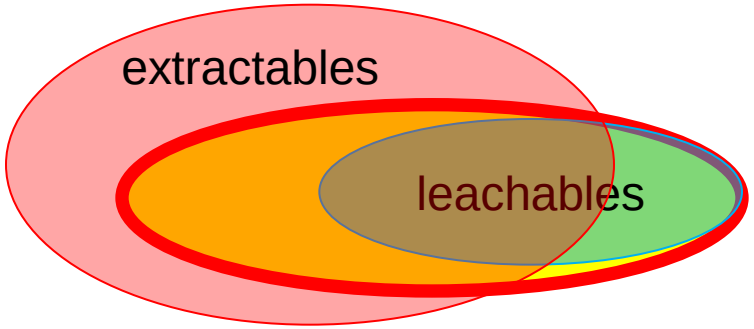
2. LEACHABLES: A SUBSET OF EXTRACTABLES?

➔ THEORY:



CLOSING THE GAP!!

➔ PRACTICE:

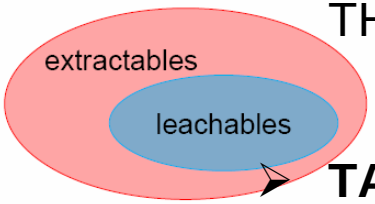


Additional Study Design

2. LEACHABLES: A SUBSET OF EXTRACTABLES?

TRADITIONAL STEPS IN THE SAFETY EVALUATION OF A PHARMACEUTICAL CONTAINER/CLOSURE

- A WELL DESIGNED **EXTRACTABLE STUDY** IS THE **FIRST STEP** IN THE SAFETY ASSESSMENT OF A CONTAINER CLOSURE SYSTEM



- **TARGET COMPOUNDS** FOR **LEACHABLE STUDIES** ARE SELECTED **BASED UPON THE RESULTS OF EXTRACTABLE STUDIES** ***(Remark: Pharmacopoeial tests are not equivalent to a well-designed extractable study!!)***

- **LEACHABLES CAN BE CONTROLLED/ASSESSED THROUGH EXTRACTABLES**

- USE **PLACEBO** AS AN EXTRACTION SIMULANT IN EXTRACTABLE STUDIES

3. CONSIDER THE STERILIZATION

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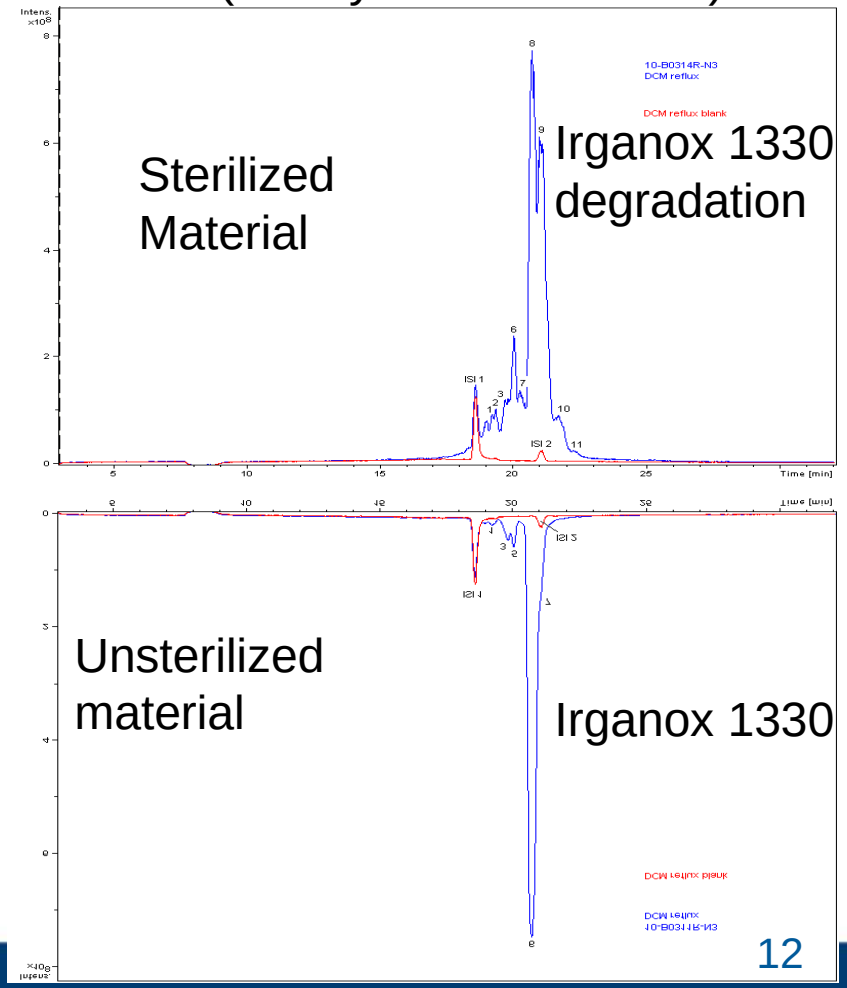
CASE STUDY

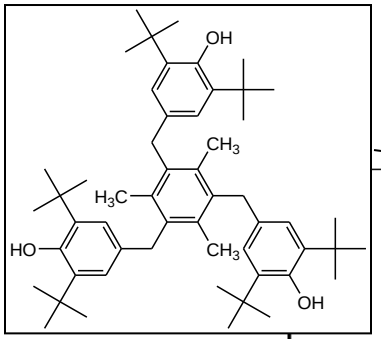
- Polypropylene Containers, Before and after sterilization (25kGy Beta irradiation)
- Extracted with Dichloromethane
- Ratio: 1 g/ 10 mL, reflux for 8h
- Analysis (presented): LC/MS (APCI-)

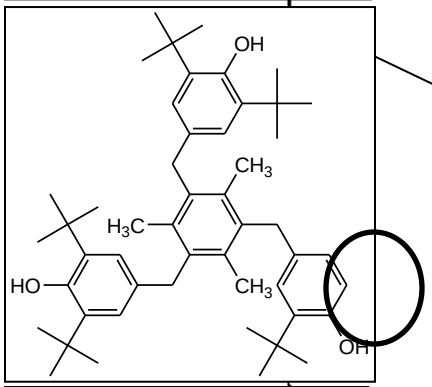


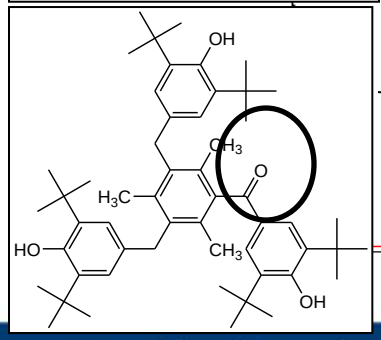
3. CONSIDER THE STERILIZATION

IRGANOX 1330

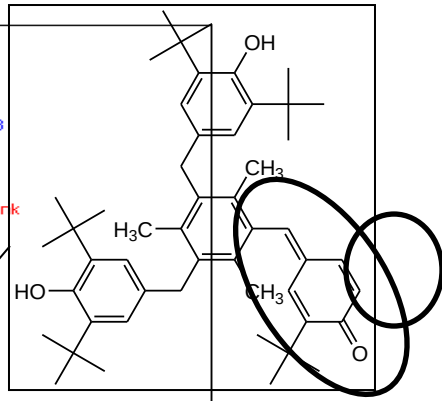
IRRADIATION STERILIZATION MAY LEAD TO DEGRADATION OF POLYMER ADDITIVES!!

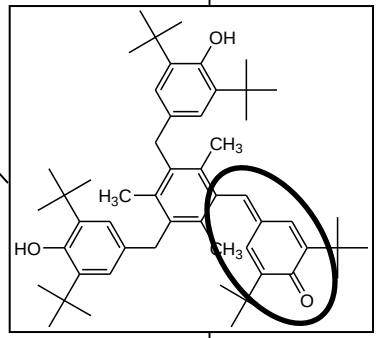




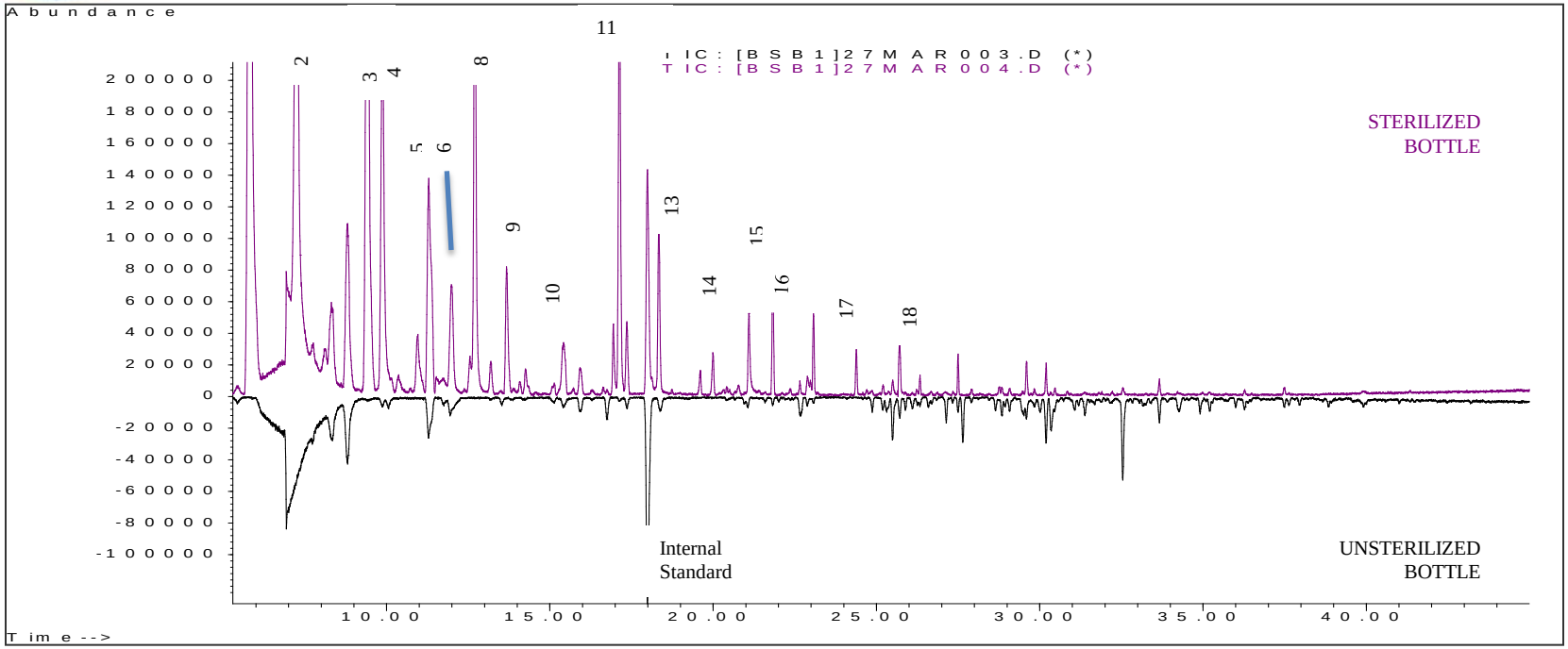


quinonemethide





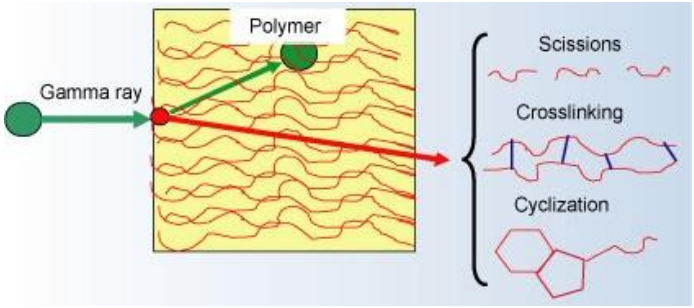
Sterilization of a Polyolefin: Polymer Degradation (Gamma Irratiation 50 kGy)



(1) TIC: n-butane
(2) IC: n-pentane
(3) IC: 3—methylpentane
(4) IC: n-hexane
(5) IC: butanal
(6)TIC: Hydrocarbon
(7) IC: cyclohexane
(8) IC: acetic acid
(9) IC: n-heptane
(10) IC: propanoic acid
(11) IC: 3-methylpentane
(12) TIC: Hydrocarbon
(13) IC: n-octane
(14) IC: 2-Hexanone
(15) IC: Butanoic acid
(16-18): TIC: HC

3. CONSIDER THE STERILIZATION

AGEING - STERILIZATION



POLYMER DEGRADATION (e.g. Scissions, Crosslinking, cyclization)

POLYMER ADDITIVE DEGRADATION (see example for Irganox 1330, but also the case study on biological reactivity (I168ox-diester)!)

CHANGES IN POLYMER CRYSTALLINITY

This will impact the: LEACHABLES SOLUBILITY

LEACHABLES MIGRATION

CONCLUSION: TEST FOR EXTRACTABLES AND LEACHABLES ON STERILIZED C/C SYSTEMS

4. CONSIDER THE WHOLE DEVICE / ADMINISTRATION PROCEDURE

4. CONSIDER THE WHOLE DEVICE

Typical Cases:

- Connectors, Tubing of Administration Set (tubing), Glue, Ports, Filters in I.V. Bag applications (not only film!)
- Silicone Oil, Glue extractables, Extractables from Barrel Manufacture
- Integrated Filter in Sterile Administrations (e.g. Ophthalmic)
- Reconstituting Solution (WFI, 0.9% NaCl), stored in Separate Vial / Syringe
  (Case study: see part E/L for Lyo Products)
- Cross Contamination during Sterilization (e.g. Autoclaving)
- ....

5. CONSIDER THE SECONDARY PACKAGING

Regulatory requirements

- FDA guidance document: ‘Container Closure systems for Packaging Human Drugs and Biologics’, 1999:

  “*if the packaging system is relatively permeable, the possibility increases that the dosage form could be contaminated by the migration of an ink or adhesive component…In such case the secondary packaging component should be considered a potential source of contamination and the safety of its materials of construction should be taken into consideration…”*

- EMA: ‘Guideline on Plastic Immediate Packaging Materials’, 2005*:*

  “it should be scientifically demonstrated that no components of ink or adhesives, applied to the outer surface of the container closure system, will migrate into the medicinal product.”

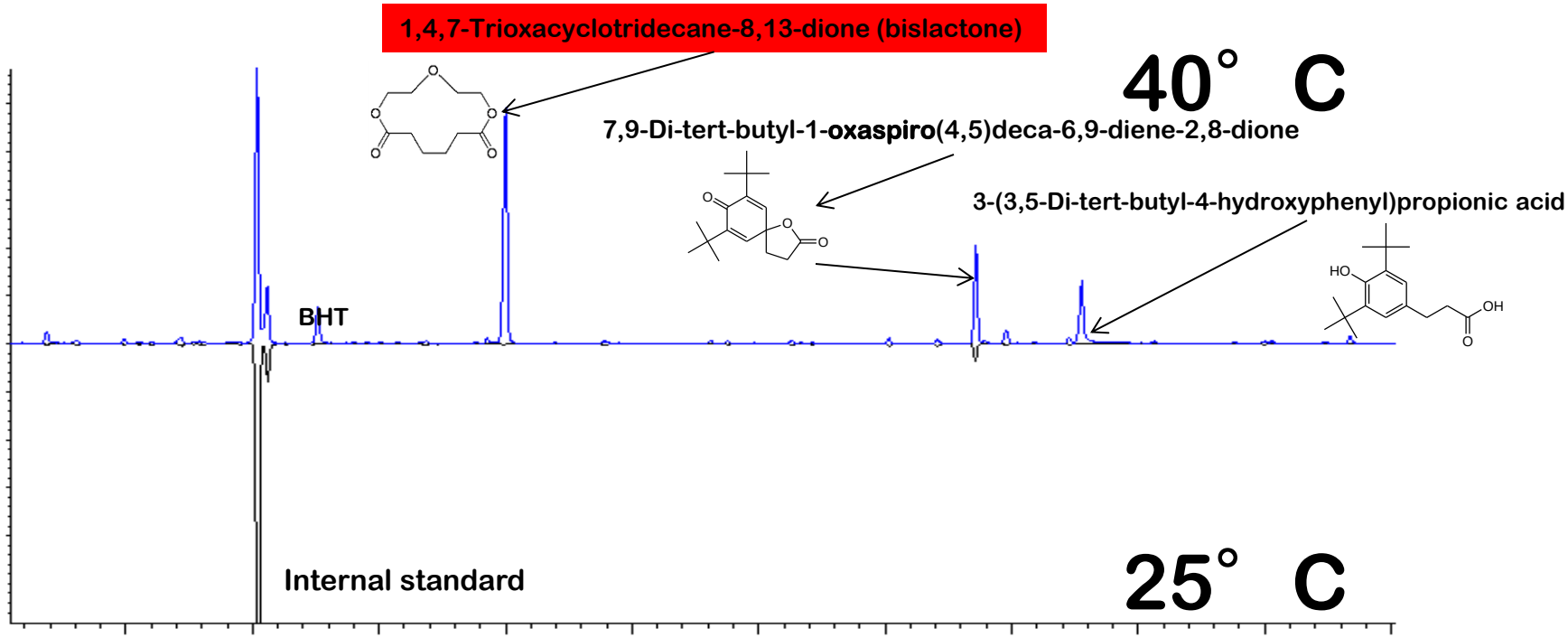
5. CONSIDER THE SECONDARY PACKAGING

Case study LEA: 100 mL flexible multi-layer bag containing a drug solution
ageing at 25° C and 40° C for 3 months
Results for S-VOC (Semi-Volatile Organic Compounds)

Conclusion:

1. MAIN Leachable: bislactone, from adhesive of ALUMINUM Multilayer overwrap!!

2. T increase leads to increased leaching behaviour of additives / degradation products



CASE STUDY 2

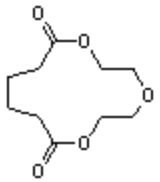
- Label
  - Adhesive
  - paper
  - Ink
  - Varnish
  - Typical extractable compounds:

    curing agents (e.g. Benzophenone, Irgacure 184), solvents (e.g.Toluene, acetone), residual monomers (e.g. Acrylates)

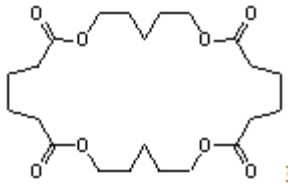
CASE STUDY 2

- Overpouch
  - Multilaminated foils often containing Aluminium layer
  - Typical extractable compounds:

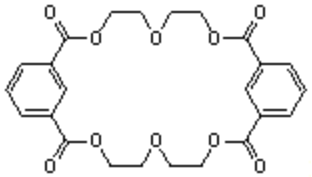
    Bislactone related compounds originating from polyurethane binding layers:



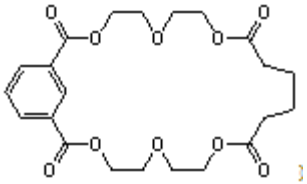
1,4,7-Trioxacyclotridecane-8,13-dione



Bislactone dimer



Heterodimer of Adipic acid di-glycol bis-lactone and Isophthalic acid di-glycol bis-lactone



Di-(Isophthalic acid di-glycol bis-lactone)

5. CONSIDER THE SECONDARY PACKAGING

Typical Cases:

- Overwrap (I.V.-Bags, Blow-Fill-Seal, ...)
- Label migration (Ophthalmic, I.V.-Bags, Polyolefin Containers)
- Ink Migration (I.V.-Bags, Blow-Fill-Seal)
- Needle Shield (Pre-Filled Syringe)

More delicate for Primary Packaging, made of materials with low barrier properties.

6. CONSIDER THE RIGHT EXTRACTION SOLVENT

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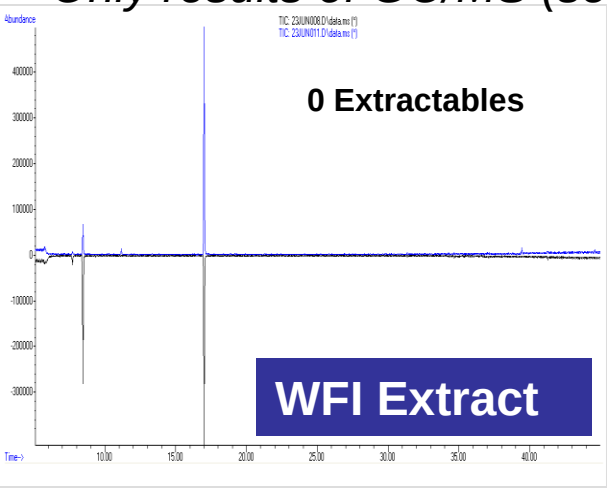
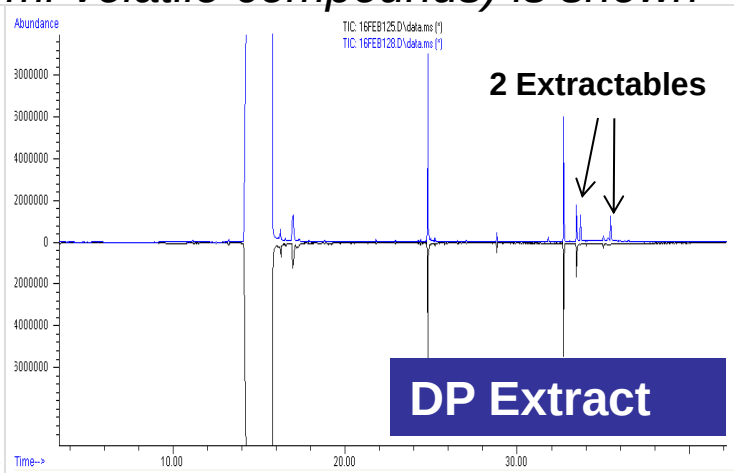
CASE STUDY: impact of contact solution on migration / extraction behavior

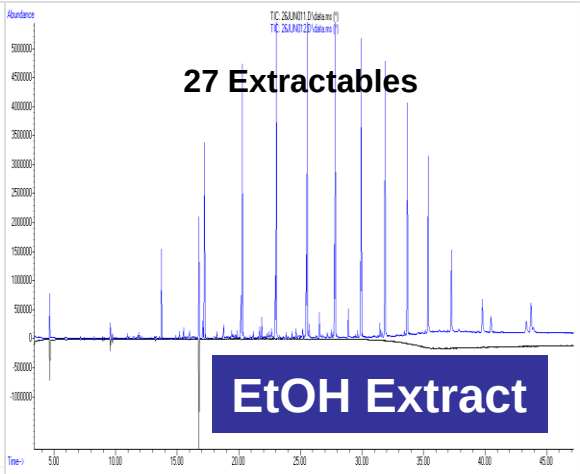
Extractable study of a POLYOLEFIN CONTAINER, using 3 solvents:

1. *Water for Injection (WFI)*
2. *Drug Product (containing 3% organic material)*
3. *Ethanol (96%)*

Identical extraction conditions for 3 experiments: refluxing for 8 h at 1 bottle/30mL ratio
Only results of GC/MS (semi-volatile compounds) is shown







Solubility of targets in WFI < Solubility of targets in DP << Solubility targets in EtOH

Interaction polymer-WFI < Interaction polymer-DP << Interaction polymer-EtOH

6. CONSIDER THE RIGHT EXTRACTION SOLVENT

CASE STUDY: PROVE OF EQUIVALENCY OF OLD VS NEW MATERIAL

SITUATION 1

PROOF OF EQUIVALENCY WITH **WFI**

WFI as extraction solvent

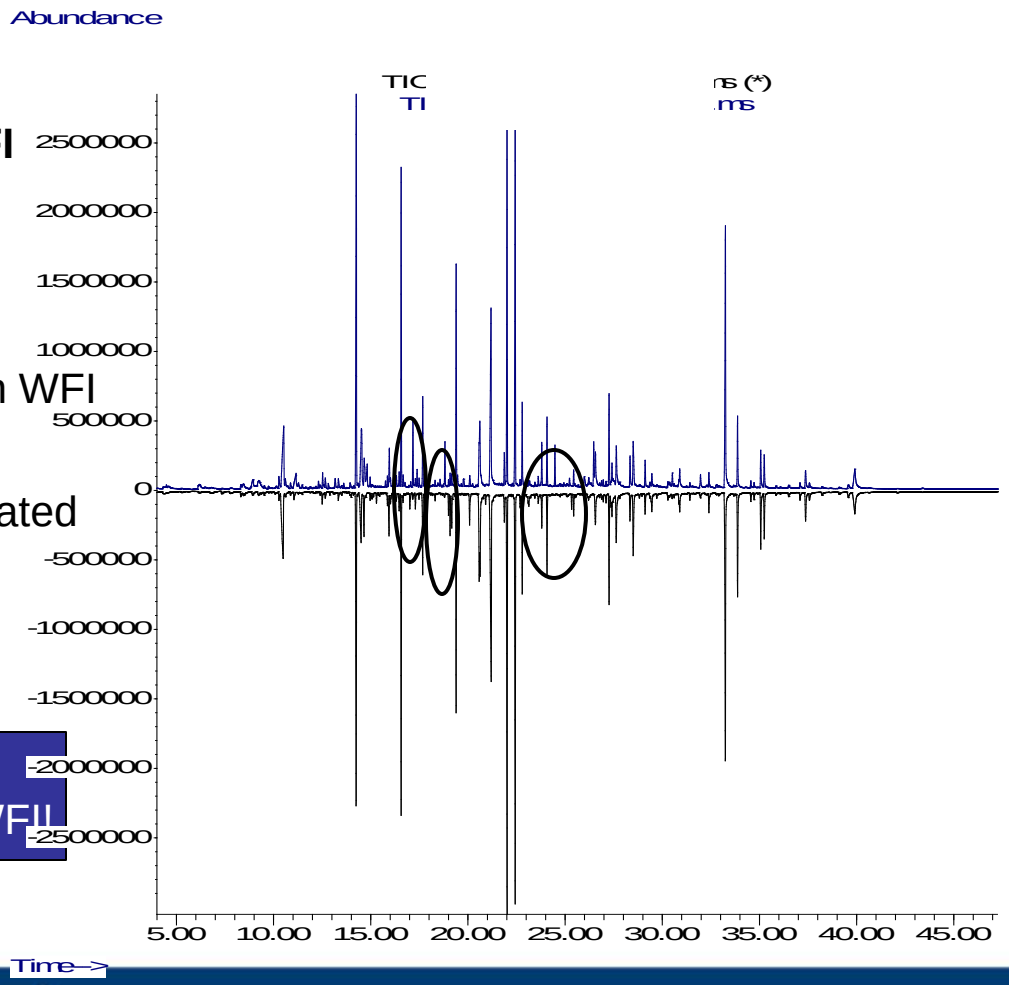
2 materials were refluxed for 8 hours in WFI

Extracted with DCM, subseq. concentrated

Analyzed with GC/MS (semi-volatiles)

Conclusion

almost the same extraction profile in WFI!



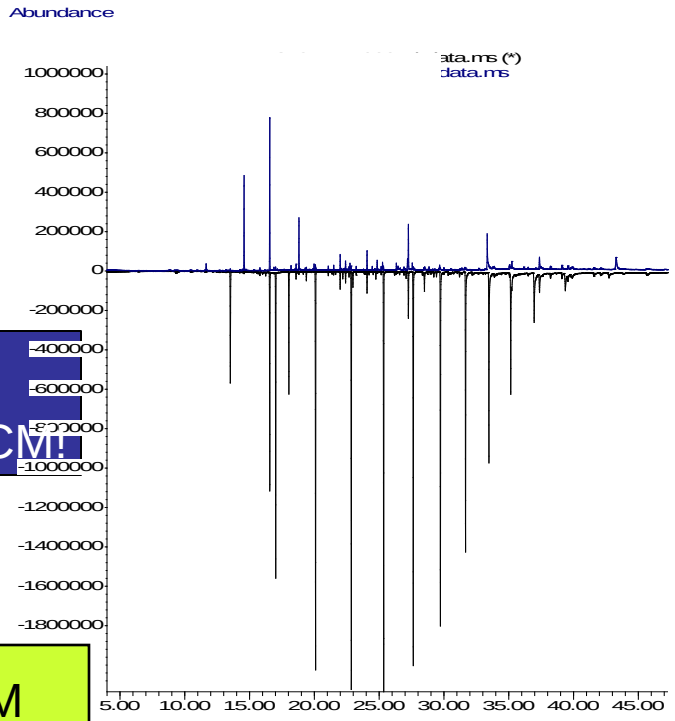
6. CONSIDER THE RIGHT EXTRACTION SOLVENT

SITUATION 2

DCM as extraction solvent

2 materials were refluxed for 8 hours in DCM

Analyzed with GC/MS (semi-volatiles)



Conclusion:
COMPLETELY DIFFERENT extraction profile in DCM!

MECHANISTIC CONSIDERATIONS

Solubility of targets in WFI << Solubility targets in DCM

Interaction polymer-WFI << Interaction polymer-DCM

ADVISE : Consider relevancy of adding additional solvent!

6. CONSIDER THE RIGHT EXTRACTION SOLVENT

THE CRITICALITY OF USING THE **DRUG PRODUCT (VEHICLE) (DP(V))** AS A SOLVENT

Perform E-study in Drug Product (Vehicle), suggested in:

FDA-Container/Closure Guidance (1999), (eg parenteral/Ophthalmic)

- If the extraction properties of the drug product vehicle may reasonably be expected to differ from that of water (e.g., due to high or low pH or due to a solubilizing excipient), then drug product should be used as the extracting medium.

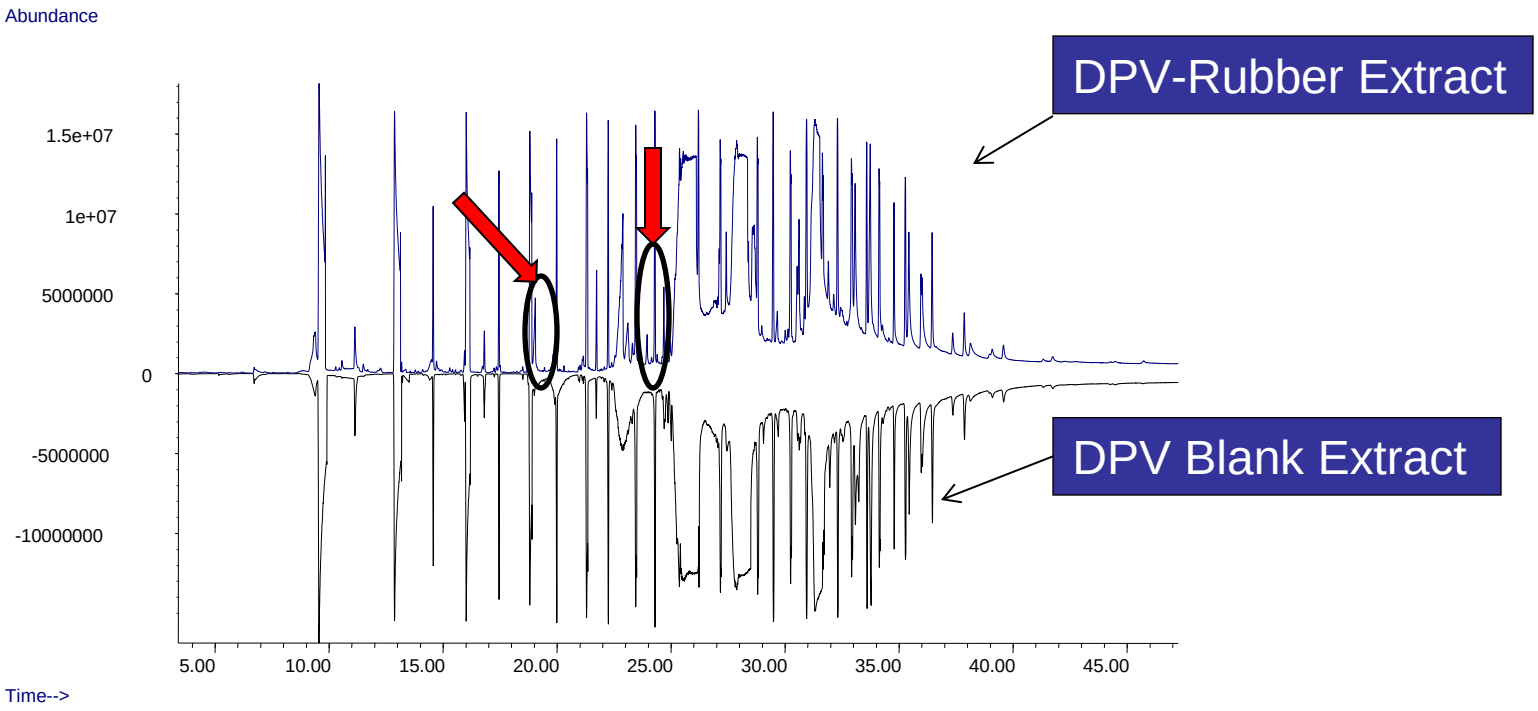
EMEA-Guideline - immediate packaging (2005)

stress conditions to increase the rate of extraction. The solvent used for extraction should have the same propensity to extract substances as the active substance/dosage form as appropriate. In the case of medicinal products the preferred solvent would be the medicinal product or placebo vehicle. The

6. CONSIDER THE RIGHT EXTRACTION SOLVENT

THE CRITICALITY OF USING THE **DP(V)** AS A SOLVENT

- Complex DPV: <u>COMPLEX INTERPRETATION OF E-STUDIES!!</u>



6. CONSIDER THE RIGHT EXTRACTION SOLVENT

THE CRITICALITY OF SELECTING **DP(V)** AS SOLVENT

Similar advantages/disadvantages as for WFI:

ADVANTAGE: simulation of extractables behaviour in DP(V): same extraction propensity!

DISADVANTAGE: Risk of missing the presence of compounds

- *Matrix interference of DP(V) (see previous slide)*

Risk of misinterpretation of analytical data

- *DP(V) Matrix degradant may be misinterpreted as extractable!*

Risk of underestimating the concentration of compounds

- *Extraction conditions – may potentially be to mild*
- *Difficult to select the right set of extraction conditions (e.g. extraction time, temperature!)*

EXAMPLE for DP(V) – does 8 hour reflux mimic a 3 year shelf life?

6. CONSIDER THE RIGHT EXTRACTION SOLVENT

THE CRITICALITY OF SELECTING **DP(V)** AS SOLVENT

ADVICE when selecting DP(V) as extraction solution:

1. Combine it with organic model solvent (e.g. IPA, DCM, Hexane)
    - *Minimize the risk of missing the presence of extractables*
2. If necessary: Use validated methods, developed for extraction study with DP(V) as solvent
    - *Eliminate matrix interference from DP(V) matrix*
    - *Assess DP(V) matrix degradation during extractable study*
3. Consider the right set of extraction conditions, relevant for the DP(V) contact
    - *Extraction time*
    - *Temperature*

7. CONSIDER THE PROCESSING STEPS

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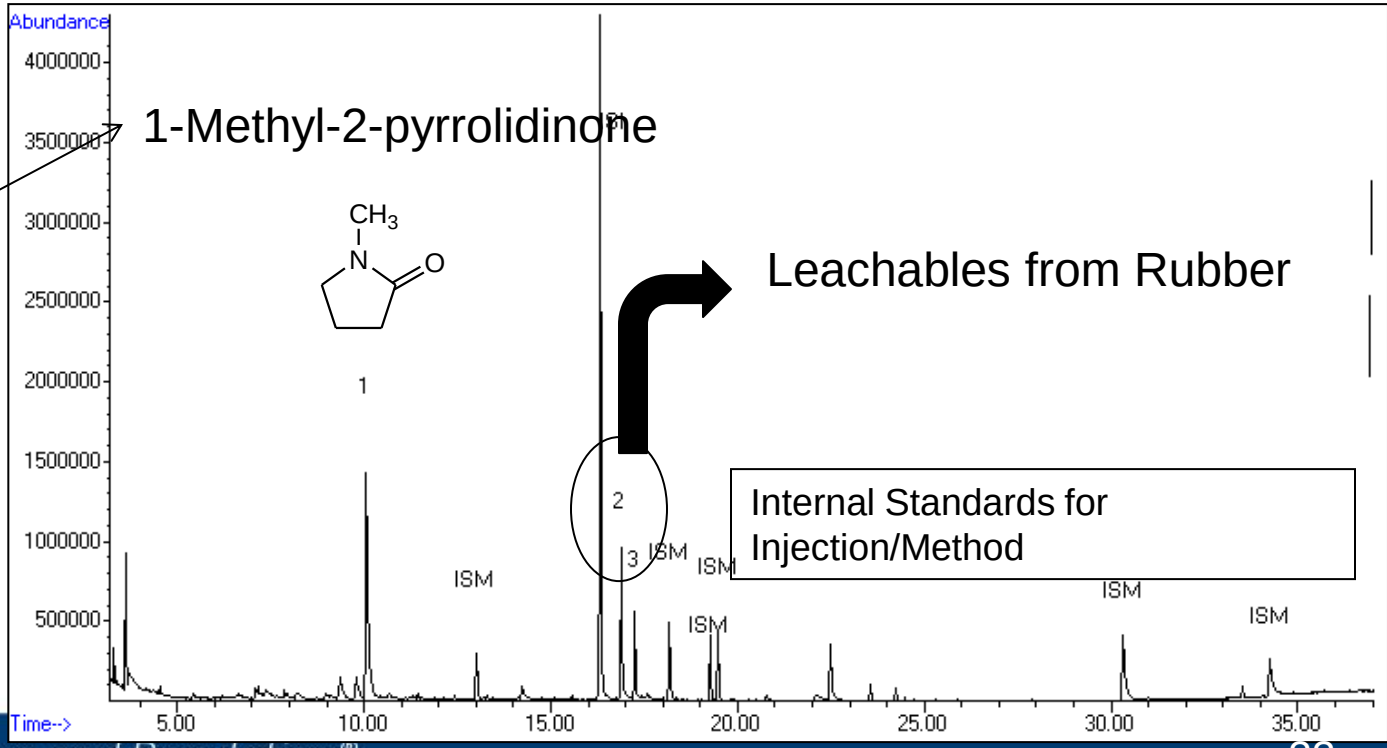
CASE STUDY: Leachable Study on a **vial system** (vial + rubber)
Using **Validated Methods** for Target Compounds, defined after Extractable Study + **Screening Method (**unexpected compounds)

RESULTS: 3 leachables were detected: 2 target compounds, 1 non-target compound (no increase in concentration over time)

Origin of non-target Compound:
Sterile Filtration prior to filling in the PFS!



7. CONSIDER THE PROCESSING STEPS

Typical Cases:

- Filtration
- Tubing for Filling
- Storage Containers of Excipients
- Intermediate Storage of API
- Lyophilization Equipment
- Cross Contamination during Sterilization (e.g. autoclaving)
- Inner/Outer layer cross contamination of Films.
- Diptubes in Storage Containers
- ....

8. EVEN THEN, THINGS CAN GO WONG!!

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The more we know,
the more we know we don't know!

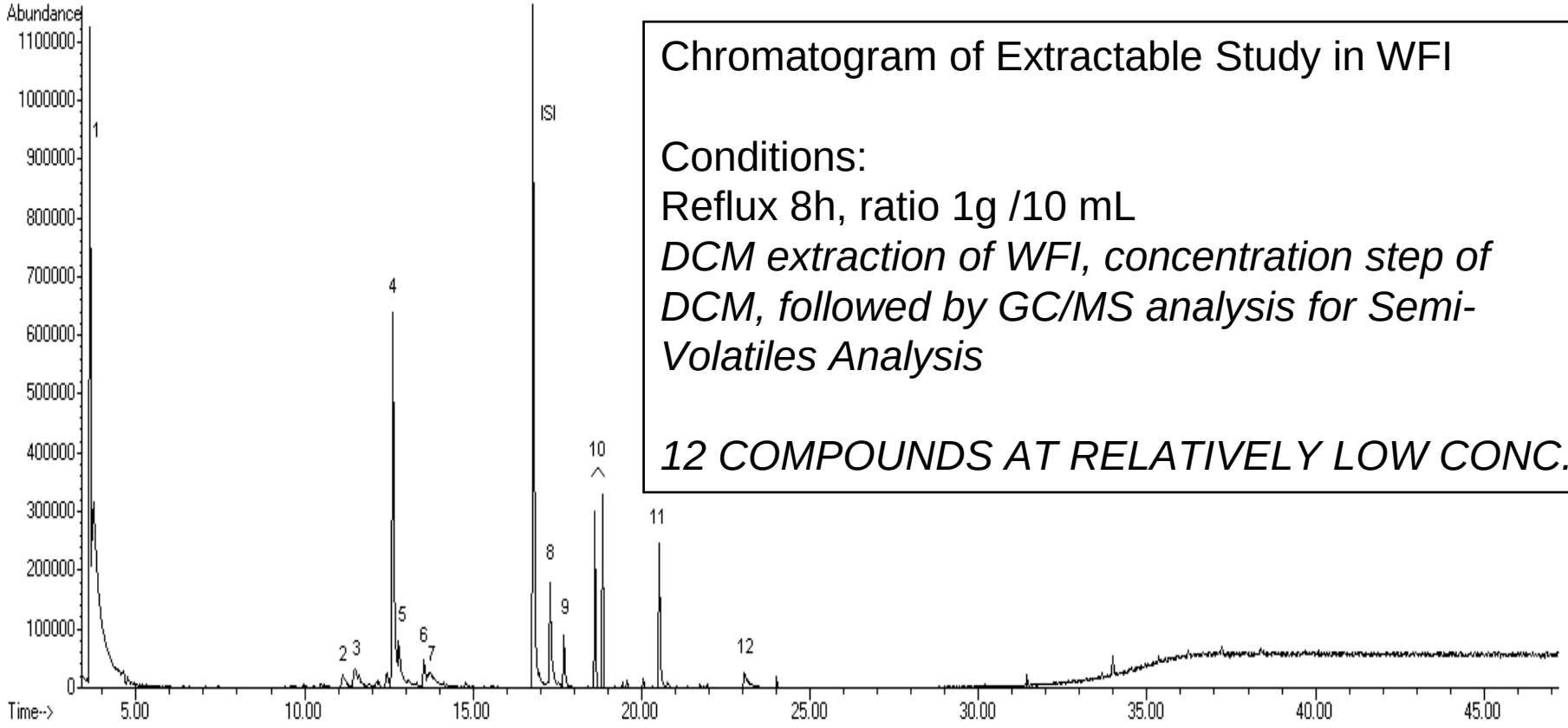
Anonymous
Rome, 2018

8. EVEN THEN, THINGS CAN GO WRONG

- Prefilled Glass Syringe
- Filled with WFI
- Stored for 3y at 25° C/60% R.H.
- Initial Extractables Study on Plunger (WFI, IPA)
- Leachables (Screening) Analyses after 3 years
  - Headspace GC/MS: Volatiles
  - DCM extraction + GC/MS: Semi-Volatiles
  - DCM extraction + LC/MS (APCI+/-): Non-Volatiles
- 6 different Combinations (Syringe/Plunger/Needle Shield) were tested.
- Results: for Semi-Volatiles, indicative for other groups of compounds

8. EVEN THEN, THINGS CAN GO WRONG

RESULT OF **WFI EXTRACTABLE STUDY** OF THE PLUNGER



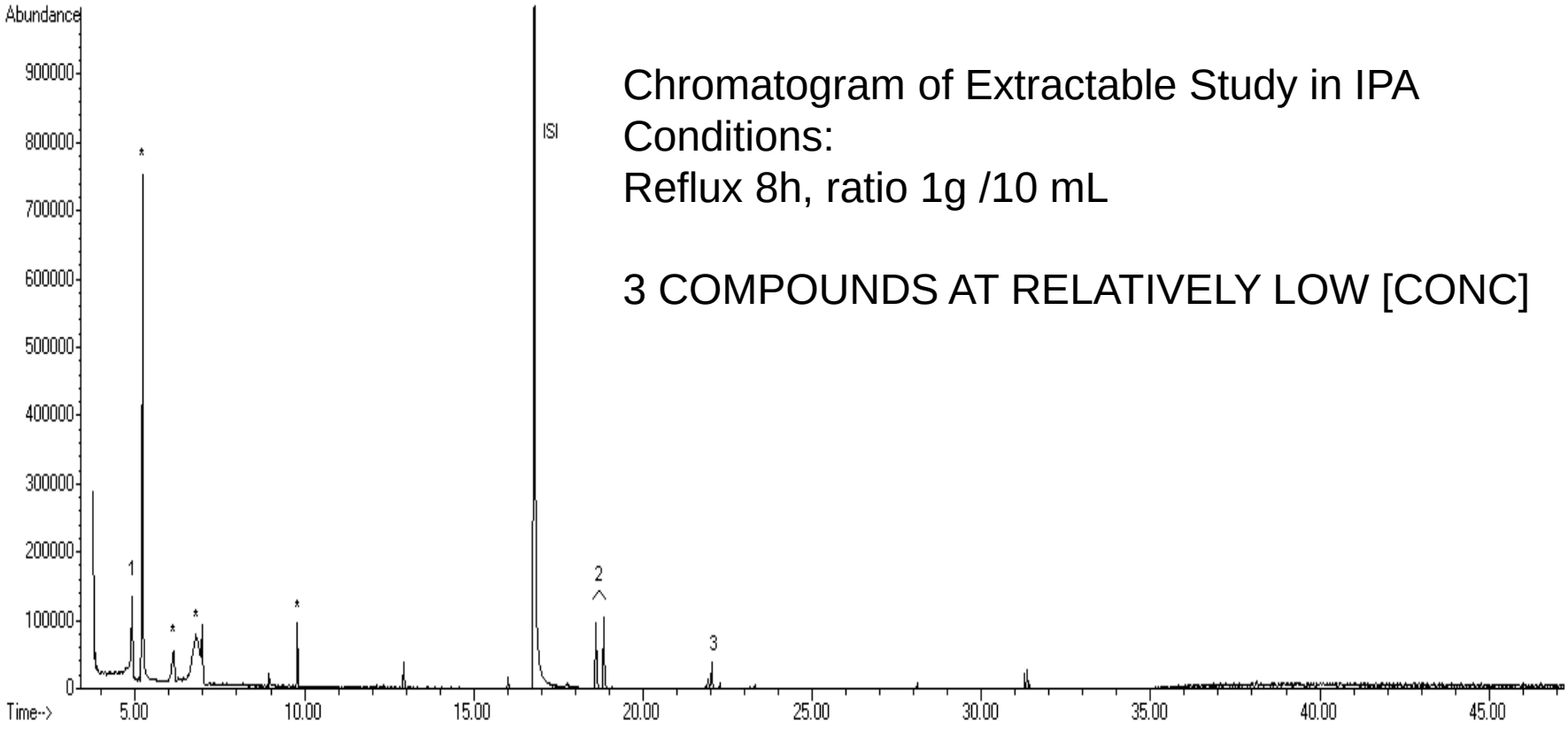
Chromatogram of Extractable Study in WFI

Conditions:
Reflux 8h, ratio 1g /10 mL
DCM extraction of WFI, concentration step of DCM, followed by GC/MS analysis for Semi-Volatiles Analysis

12 COMPOUNDS AT RELATIVELY LOW CONC.

8. EVEN THEN, THINGS CAN GO WRONG

RESULT OF **IPA EXTRACTABLE STUDY** OF THE PLUNGER

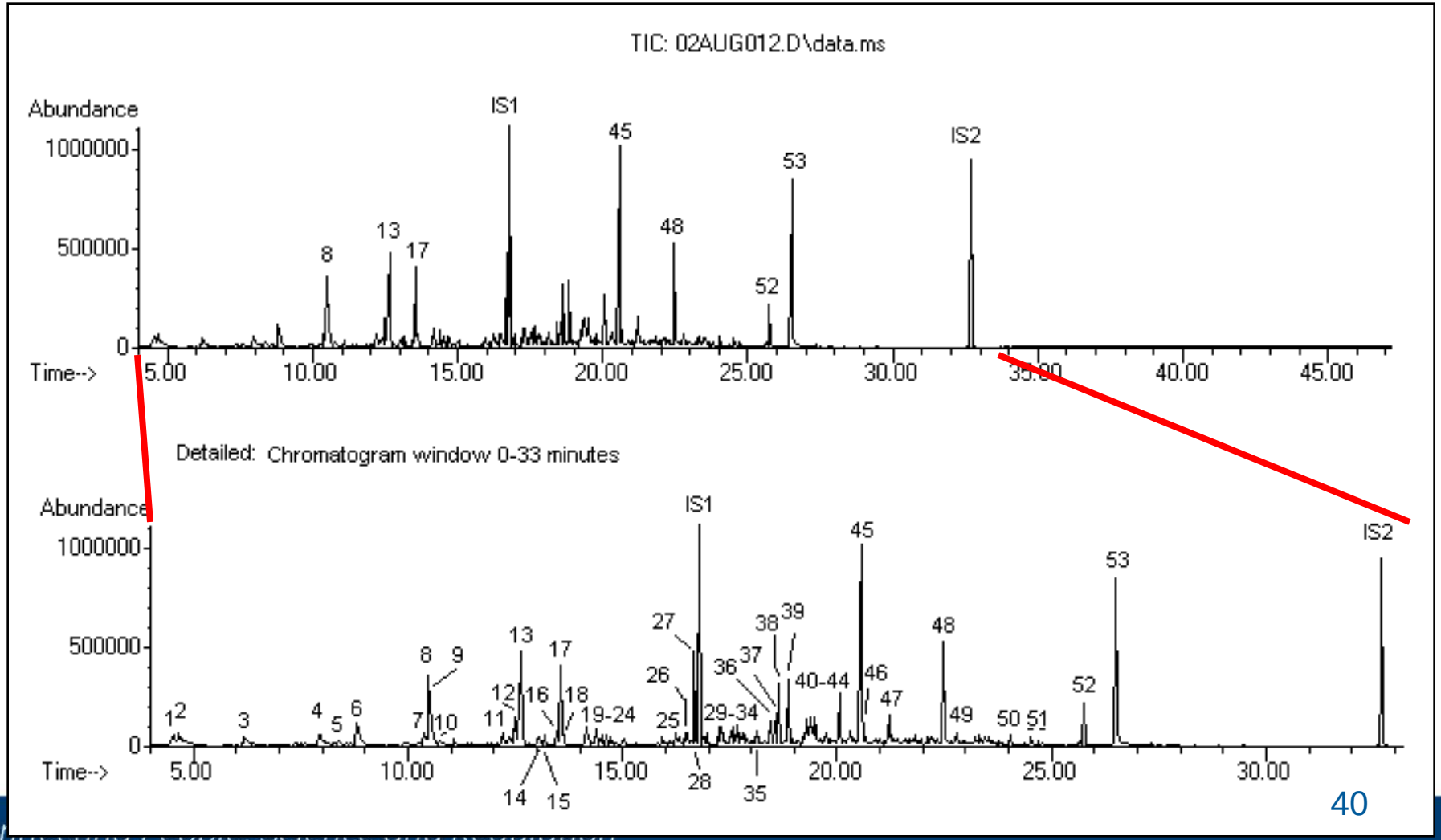


Chromatogram of Extractable Study in IPA
Conditions:
Reflux 8h, ratio 1g /10 mL

3 COMPOUNDS AT RELATIVELY LOW [CONC]

8. EVEN THEN, THINGS CAN GO WRONG

RESULT OF THE **LEACHABLE STUDY** OF THE **WFI- PREFILLED SYRINGE**
3 YEARS AT 25° C – 60% R.H.



8. EVEN THEN, THINGS CAN GO WRONG

LEACHABLES: compounds originating from:

1. **Rubber Plunger**
2. **Hydrolyzed** Compounds from Rubber Plunger
3. Compounds from **Needle Shield**
4. **Hydrolyzed/Oxidized** Compounds from Needle Shield
5. A lot of **“Unknown” Compounds**, both identity and origin is not clear
6. Results are **independent of Type of Rubber** / **Rubber Manufacturer** of the Rubber Plunger!!

Concentration range: from 10 µg/L to > 10 mg/L!

8. EVEN THEN, THINGS CAN GO WRONG

Observations when comparing the results of the Extractable Studies on the Rubber Plunger with the Leachable studies on the PFS system

- **Concentrations of Leachables was Higher** than the Extractables found with WFI as an Extraction Solvent
- Also for more **Aggressive solvents** (e.g. IPA), **not a good match** between Extractables and Leachables
- The observation was **independent of the type of rubber**

How can we **try** to explain these results?

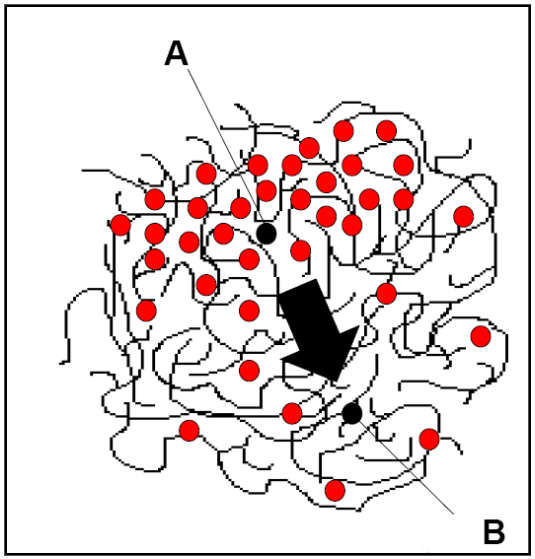
Extractable Studies: Temperature Dependence of Diffusion

By Heating up the material (boiling conditions), diffusion of extractables is increased

$$\frac{dC}{dt} = D \frac{d^2C}{dx^2}$$

With D = Diffusion coefficient

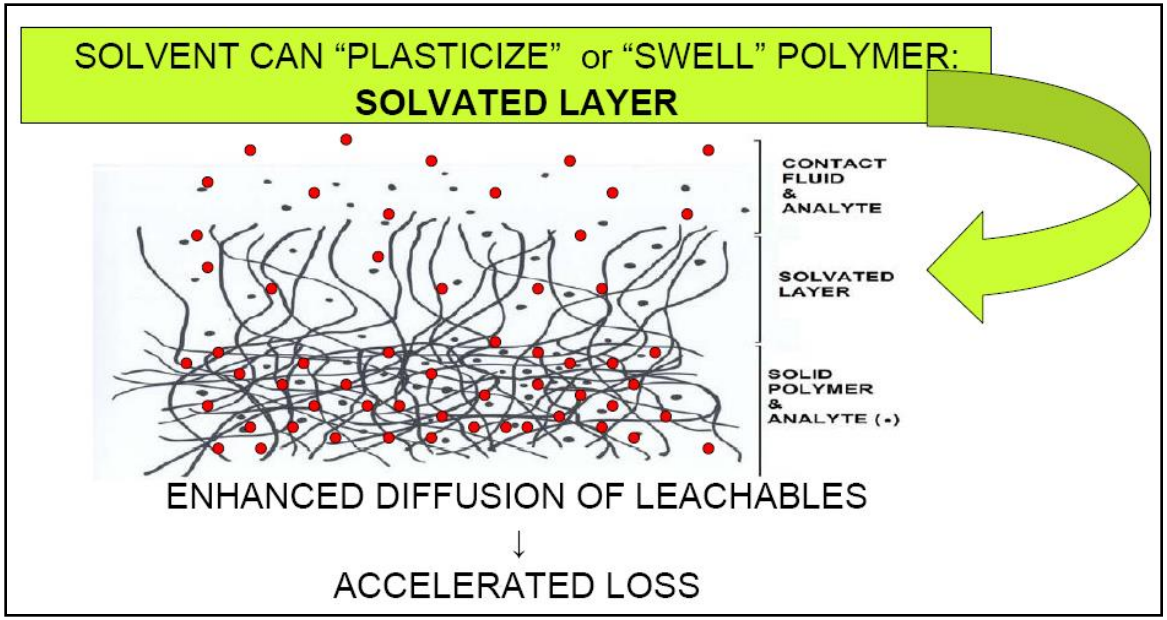
$$D = D_0 \exp(-E/RT)$$



This means that a temperature increase from Room Temperature to solvent boiling point will lead to an increase of D of approx. 2 orders of magnitude *(reference for typical D values: H. Zweifel, « Plastic Additives »)*

Or Reflux extraction of 8h will mimic approx. 800h (=33d of R.T. contact)

Extractable Studies: Interaction between Solvent - Material



For Rubbers: Hexane, DCM and IPA will show enhanced diffusion because of the solvent-material interaction

Completeness of extraction can be checked via Asymptotic Extraction Behaviour

Not to the same extent for WFI!

8. EVEN THEN, THINGS CAN GO WRONG

What is not investigated (sufficiently) in an extractable study?

8.1 MATERIAL DEGRADATION (ageing)

8.2 The **REACTION** (WFI: hydrolysis / O_2: oxidation) **of the leachables with the Drug Product** (solution)

8.1 MATERIAL DEGRADATION

What is not investigated (sufficiently) in an extractable study?

1. MATERIAL DEGRADATION – ASTM 1980 – 02:

Material Degradation: In general ASTM 1980 can be a "general" guidance

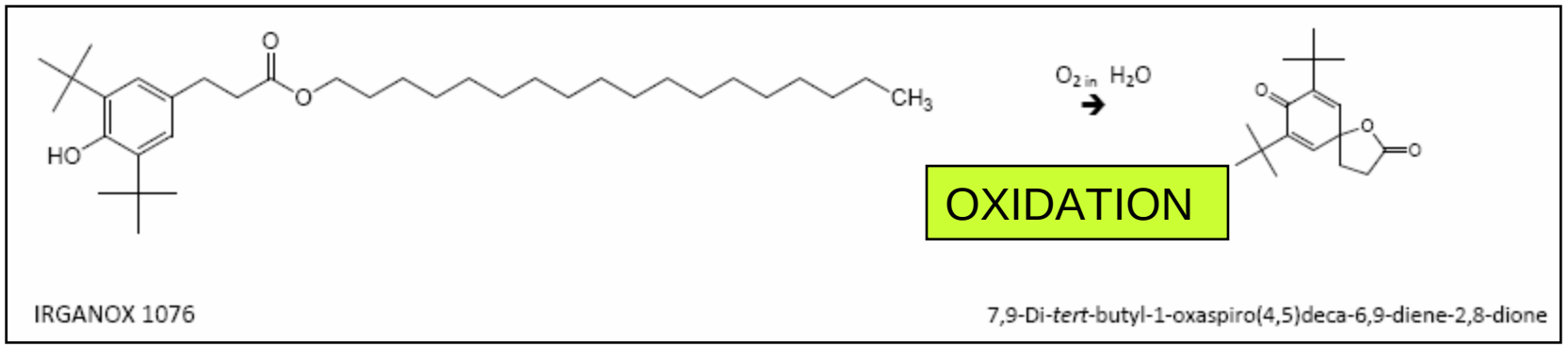
$$AAF = Q_{10}^{[(T_{AA} - T_{RT})/10]}$$

AAF: Accelerated Aging Factor
Q_{10}: Aging factor (10° C increase in T)
T_{AA}: Accelerated Aging Temperature
T_{RT}: Room temperature

8h at 100° C (eg. Refluxing in WFI) represents 1440h (60 days) of RT ageing
8h at 80° C (eg. Refluxing in IPA) represents 15 days of RT ageing

REMARK: Ageing of material is not always representative (Aqueous Environment versus Air (Oxygen!))

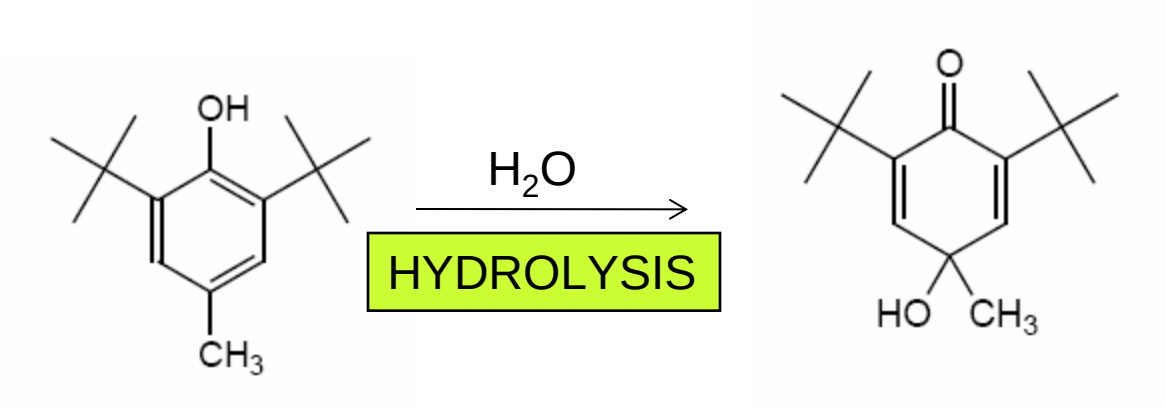
EXAMPLE N° 1 (Oxidation):



Dissolved Oxygen in WFI /DP(V) will Oxidize Irganox 1076 over time!

Occurrence of "oxaspiro" as a leachable is much more frequent than as an extractable!

EXAMPLE N° 2 (Hydrolysis):



BHT

BHT-OH

BHT-OH is seldom seen as an extractable, but it is regularly seen as a leachable!

EXAMPLE N° 3: Halogenated Rubber Oligomers – PART 1

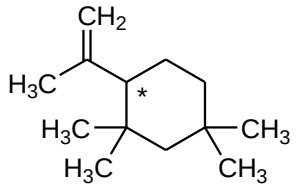
FORMATION OF THE HALOBUTYL ELASTOMERS
(for more details: see presentation "INJECTABLES")

$C_{13}H_{24}$ and $C_{21}H_{40}$ Oligomers

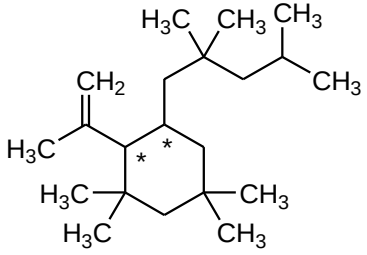
- Considered as
  - Cyclic aliphatic hydrobarbon compounds
  - One double bond

- No experimental data / Literature data is known about toxicity of these compounds

- Structure Activity Relationship Assessment (SAR): compound of low tox. risk.

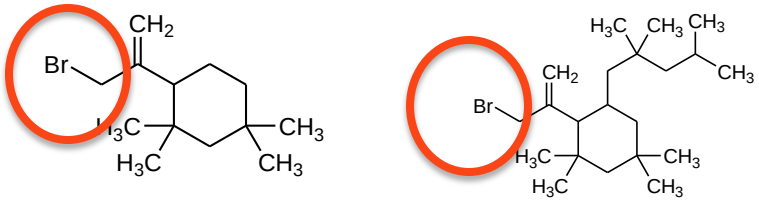


C13 oligomer



C21 oligomer

$C_{13}H_{23}Br$/ $C_{13}H_{23}Cl$ and $C_{21}H_{39}Br$/ $C_{21}H_{39}Cl$ Oligomers



- Considered as
  - **HALOGENATED** Cyclic Aliphatic Hydrobarbon compounds (Allyl Halide)
  - **Alkylating Agents**
  - One double bond

- Structure Activity Relationship (SAR) Assessment:

CARCINOGENICITY IN HUMANS IS PLAUSIBLE

- As no experimental data / Literature data is known about the toxicity of these compounds, a lot of Pharma companies:
  - Rely on the result of a SAR assessment to perform a tox evaluation
  - Conclude that these compounds are of High Concern

For potential Mutagenic/Carcinogenic compounds:

SCT: 0.15 µg/day (PQRI OINDP)

SCT/TTC: 1.5 µg/day (PQRI-PODP; EMA guideline on Genotoxic Impurities)

The low SCT/TTC levels for the Halogenated Oligomers mean:

- Low associated AET levels
- High level of method optimization to obtain these levels (certainly with LVP)
- e.g. SIM mode for GC/MS
- Can only be performed with appropriate analytical standards with known purity
  - *Method Selectivity*
  - *Accuracy*
  - *Sensitivity*
  - *Precision*
  - *...*

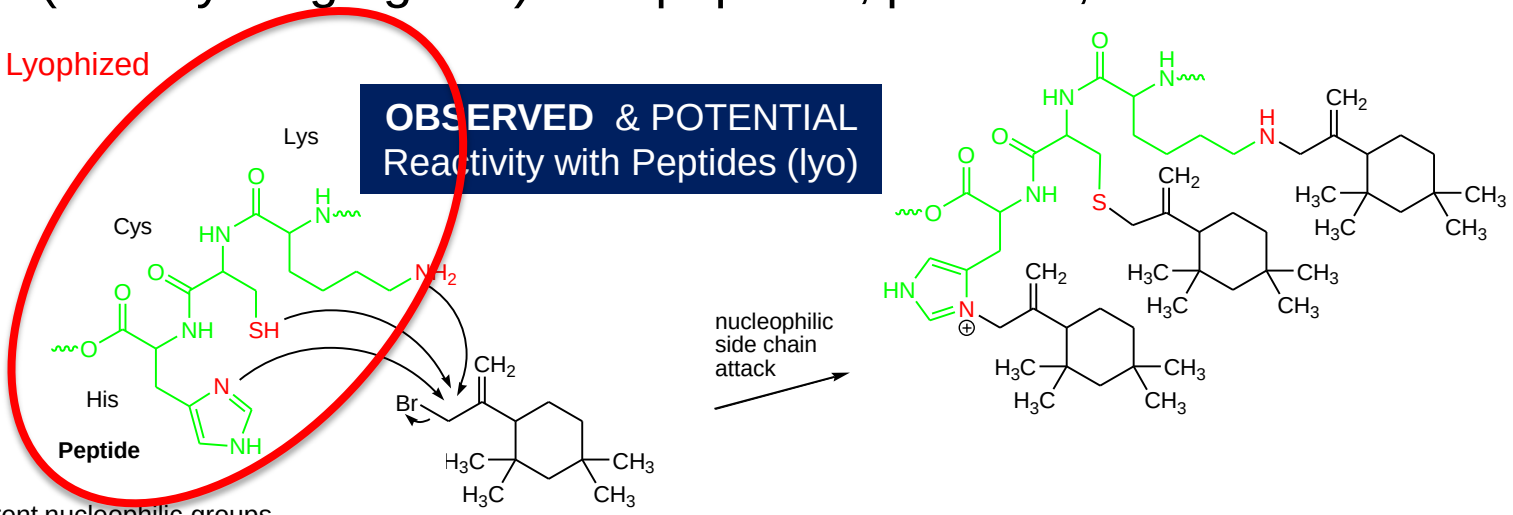
Ref. 4, 5 and 6

Observed Reactivity of $C_{13}H_{23}Br$ and $C_{21}H_{39}Br$

(as alkyating agents) with peptides, proteins, and nucleic acids

Example of Lyophized peptide

OBSERVED & POTENTIAL
Reactivity with Peptides (lyo)

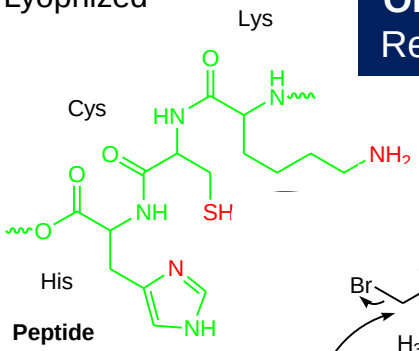


With different nucleophilic groups

Ref. 7

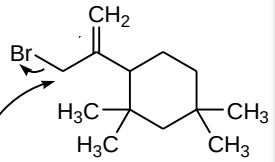
Observed Reactivity of $C_{13}H_{23}Br$ and $C_{21}H_{39}Br$ (as alkyating agents) with peptides, proteins, and nucleic acids

Example of Lyophized peptide

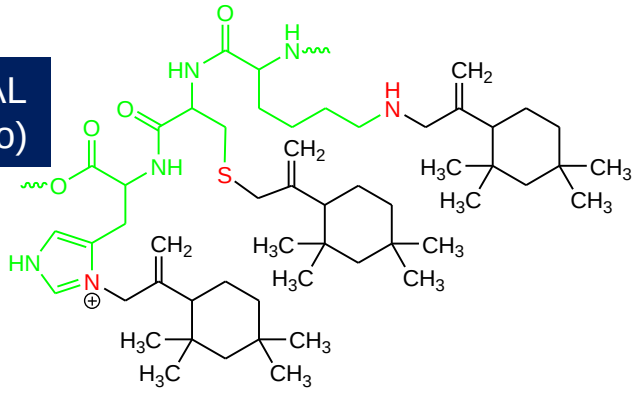


With different nucleophilic groups

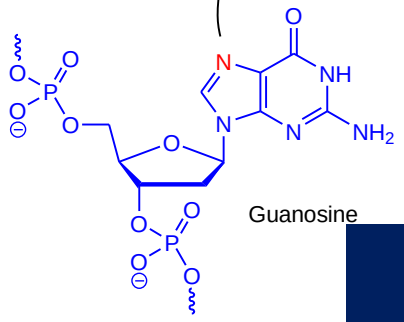
OBSERVED & POTENTIAL
Reactivity with Peptides (lyo)



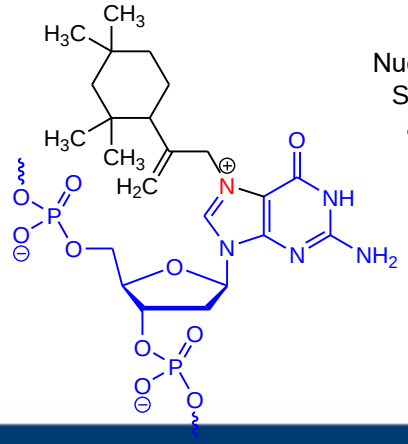
nucleophilic side chain attack



DNA



"N7" attack
Most nucleophilic site



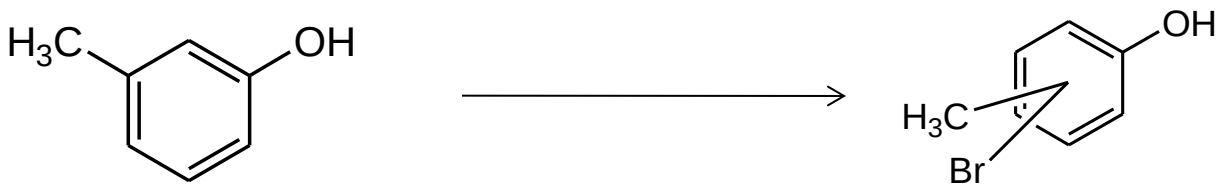
Nucleophilic “N7” attack is also SN2 reaction mechanism in anti-cancer drug Busulfan

POTENTIAL
Reactivity with DNA

Ref. 7

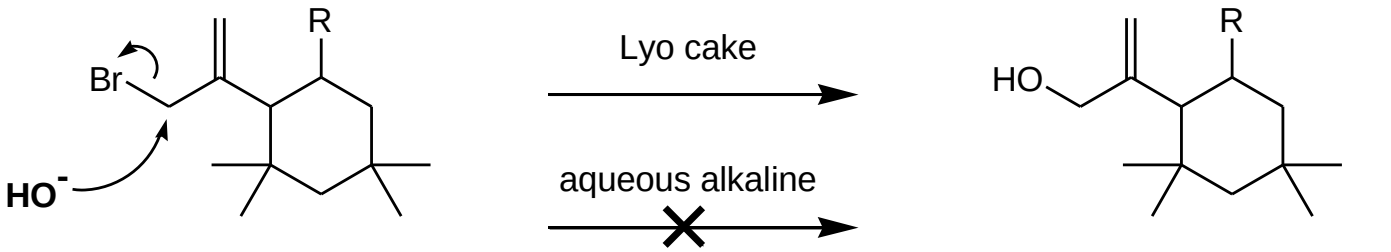
EXAMPLE N° 4: Halogenated Rubber Oligomers – PART 2

Cresol containing drug products, Bromocresol may be formed in the presence of Bromobutyl Stoppers (Mechanism is unknown)



EXAMPLE N° 5: Halogenated Rubber Oligomers – PART 3

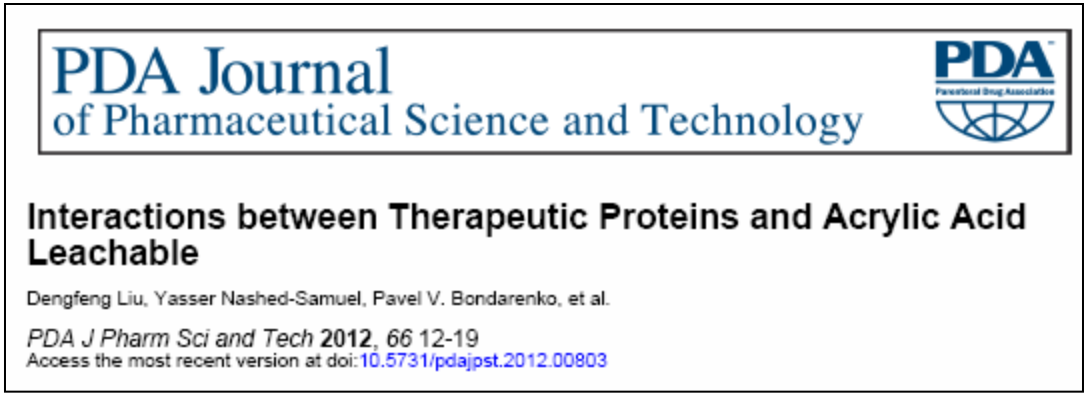
Formation of $C_{13}H_{23}OH$ out of $C_{13}H_{23}Br$ in Lyo Products



halogenated rubber oligomer

hydroxylated rubber oligomer

EXAMPLE N° 6: Acrylic Acid reaction with Proteins/Peptide



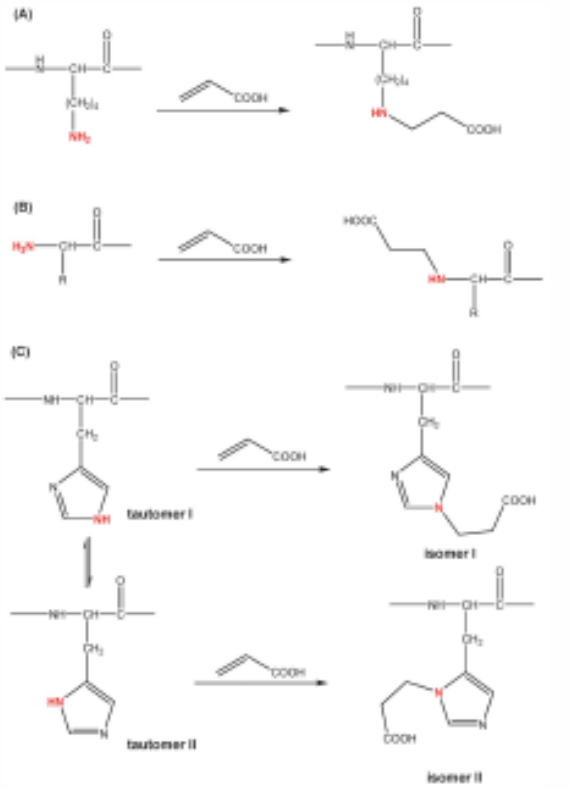
PDA Journal of Pharmaceutical Science and Technology

Interactions between Therapeutic Proteins and Acrylic Acid Leachable

Dengfeng Liu, Yasser Nashed-Samuel, Pavel V. Bondarenko, et al.

PDA J Pharm Sci and Tech **2012**, *66* 12-19
Access the most recent version at doi:10.5731/pdajpst.2012.00803

EXAMPLE N° 6: Acrylic Acid reaction with



Scheme 1

Proposed mechanisms for the interactions between acrylic acid and (A) lysine side chain, (B) N-terminus, and (C) histidine side chain.

Acrylic acid is a component of the acrylic adhesive used to attach the needle to the barrel of pre-filled glass syringes. Even though acrylic acid was not detected in the syringes currently used by Amgen, it was identified as a leachable (~5 µg/mL) in syringes from one of the potential vendors (X syringes). To investigate the potential interaction between the acrylic acid leachable and our protein drug products, a model IgG 2 antibody was filled into sterilized X syringes. After incubation, the antibody was digested and analyzed using an improved trypsin peptide mapping method (6).

Ten peptides were observed to be modified by acrylic acid (beside four peptides observed in prefilled syringes, another six new peptides were modified). Five peptides were modified through side chain of lysine, one peptide through N-terminus, and four peptides through side chain of histidine. The modification percentage was varied from 0.2% to 5.0%.

Those four modified peptides observed in prefilled syringes were confirmed by the spiking experiments. The selected ion chromatograph (SIC) for unmodified peptides (top) and the corresponding modified peptides (bottom) via spiking experiment is shown in Figure 1. Figure 2 shows MS/MS spectra of unmodified peptides (top) and the corresponding modified peptides (bottom) via spiking experiment.

Acrylic Acid:

- ✓ May be a leachable from the Needle Glue
- ✓ Potential Interaction between Acrylic Acid and Protein Drugs was investigated, with a IgG 2 antibody was used as model
- ✓ 10 peptides were observed to be modified
- ✓ 5 peptides were modified through side chain of Lysine
- ✓ 1 Peptide was modified through N-terminus
- ✓ 4 Peptides were modified through side chain of Histidine
- ✓ Confirmed via spiking experiments

EXAMPLE N° 7: Biological Reactivity of I168ox-diester

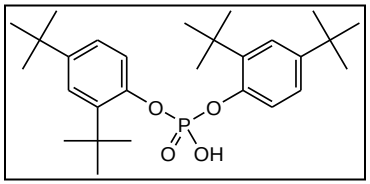
PDA Journal
of Pharmaceutical Science and Technology

Identification of a Leachable Compound Detrimental to Cell Growth in Single-Use Bioprocess Containers

Matthew Hammond, Heather Nunn, Gary Rogers, et al.

PDA J Pharm Sci and Tech **2013**, *67* 123-134
Access the most recent version at doi:10.5731/pdajpst.2013.00905

EXAMPLE N° 7: Biological Reactivity of I168ox-diester

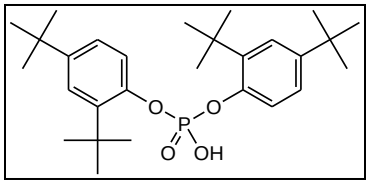


bDtBPP
or
I168ox-diester

Out of a large array of extracted compounds from polymer films used in presterilized, disposable biomanufacturing systems (e.g., BPCs), one compound, bDtBPP, can be shown to be highly detrimental to growth of a range of CHO cell lines, even at concentrations as low as 0.1 mg/L. The effect of bDtBPP on cells is rapid, quickly leading to a decrease in mitochondrial membrane potential. Studies of a film that contains significant quantities of extractable bDtBPP showed an exponential dependence of extracted bDtBPP on extraction temperature, and extracted bDtBPP also increased as a function of incubation time, with significant amounts of bDtBPP continuing to be extracted even after weeks of incubation. Experiments performed to understand the mechanism by which bDtBPP is generated suggest that exposure of oxidized Irgafos 168 (compound 8) to ionizing radiation is the primary pathway to bDtBPP formation, suggesting that manufacturers of single-use biomanufacturing components may have a variety of options to pursue in order to minimize the amount of bDtBPP that could leach from their products and adversely affect cell culture processes.

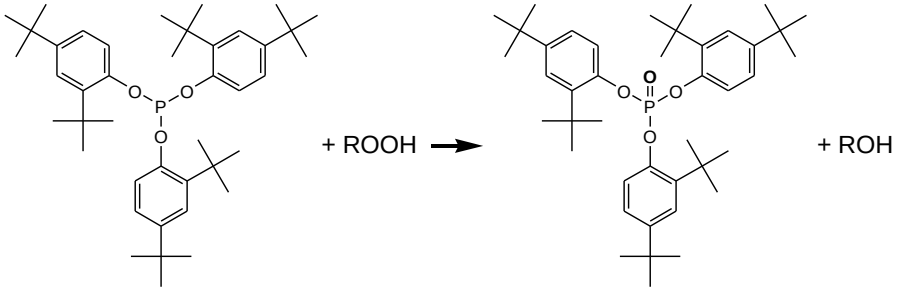
- ✓ A Range of Extracted Compounds were Investigated on their Impact on Cell Growth
- ✓ bDtBPP showed to be highly DETRIMENTAL to Cell Growth
- ✓ Even at < 0.1 mg/L!
- ✓ The effect is rapid, leading to a decrease in mitochondrial potential
- ✓ The Mechanism of Formation: see next slide

EXAMPLE N° 7: Biological Reactivity of I168ox-diester



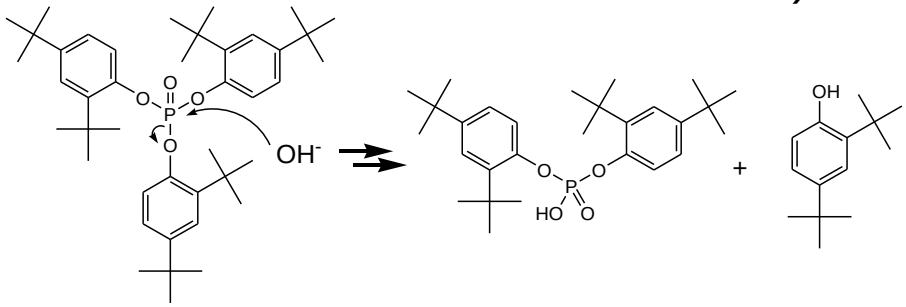
bDtBPP formation:

Step 1: Anti-oxidant I168 is oxidized to I168ox



Irgafos 168 Irgafos 168 Ox

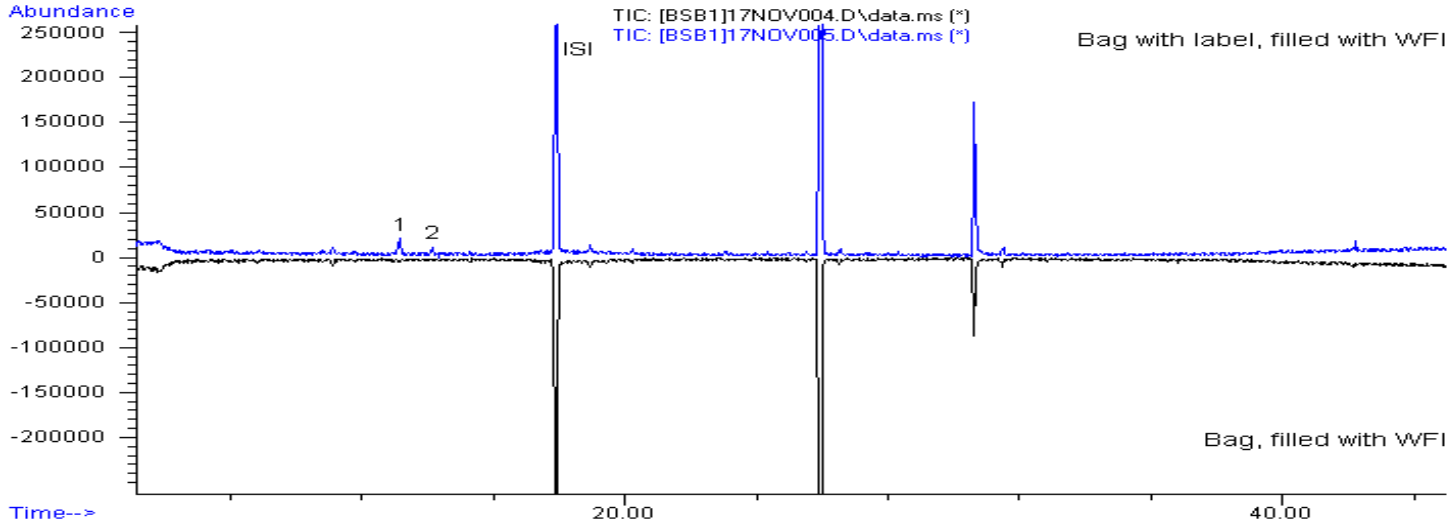
Step 2: During γ-Irradiation: I168ox degrades to I168ox-diester (POTENTIAL DEGRADATION PATHWAY)



Irgafos 168 Ox bDtBPP

EXAMPLE N° 8: Benzene formation/migration – Label/Ink

STUDY : Check the Migration of the Adhesive/Ink of the Label through the PVC layer of the Bag (results shown for *Headspace GC/MS*)



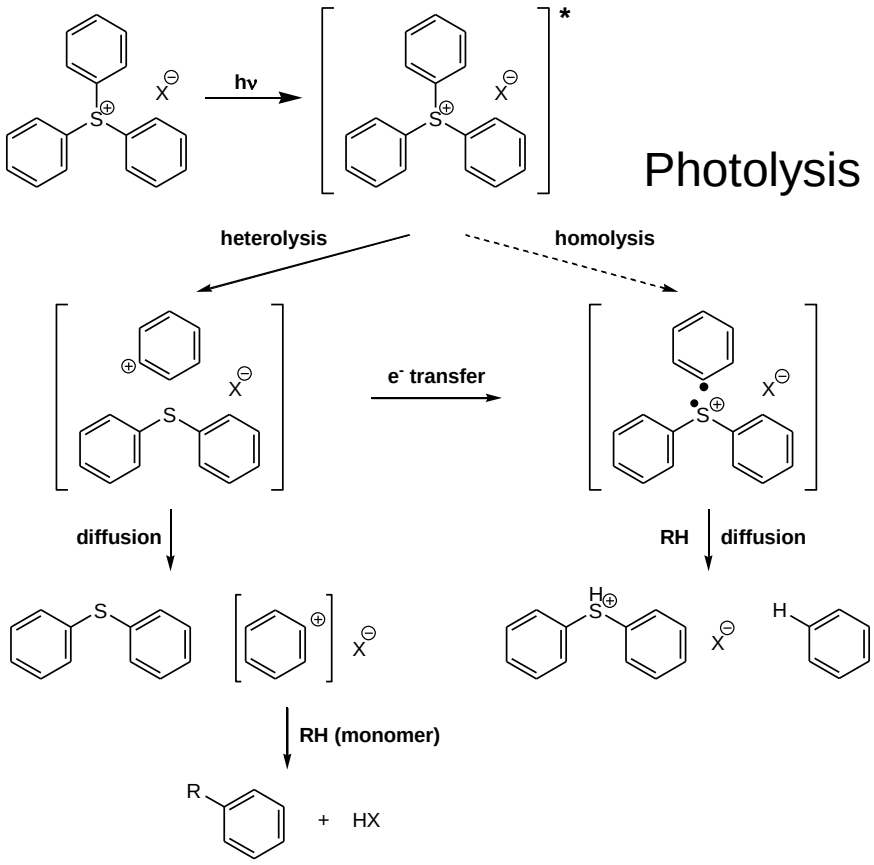
- **LABELED BAG vs. UNLABELED BAG – HS GC/MS**

  (1) IC: Benzene (5-10 µg/L)

  (2) IC: 1-butanol

EXAMPLE N° 8: Benzene Formation/Migration – Label/Ink

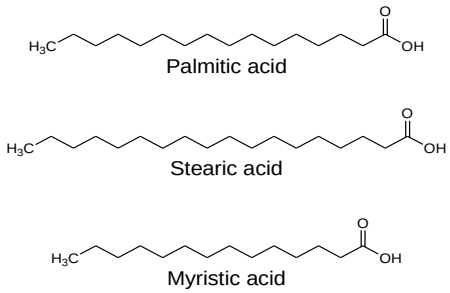
Triaryl sulfonium salts are photoinitiators for printing Inks

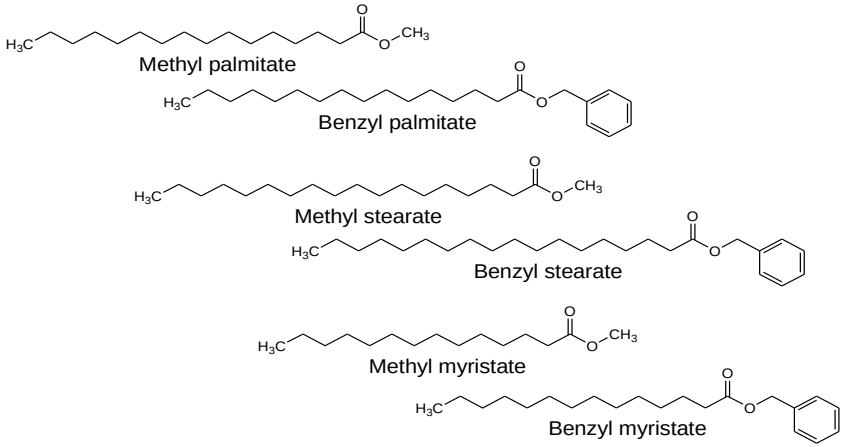


BENZENE FORMATION

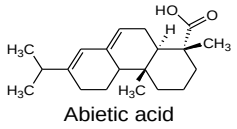
Source: Presentation of A. Hauk at ECA E/L Seminar, 2011

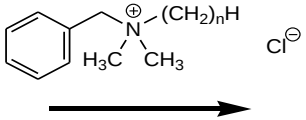
EXAMPLE N° 9: Benzalkonium Chloride Reactivity



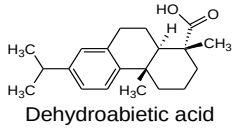


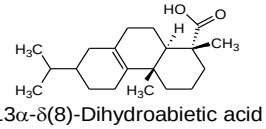
Acid Scavenger for PE/PP

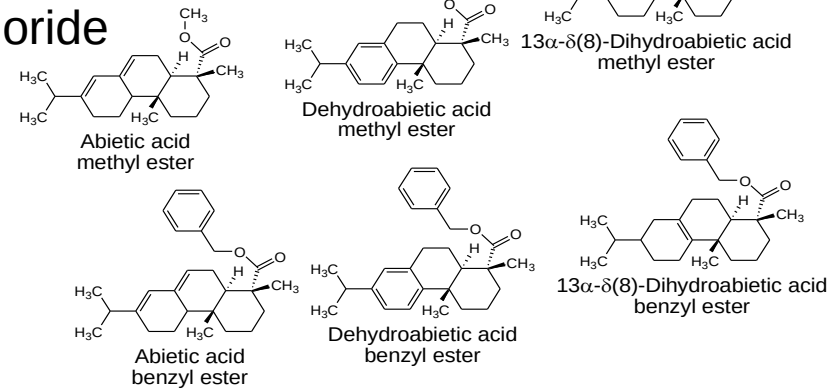




Benzalkonium Chloride



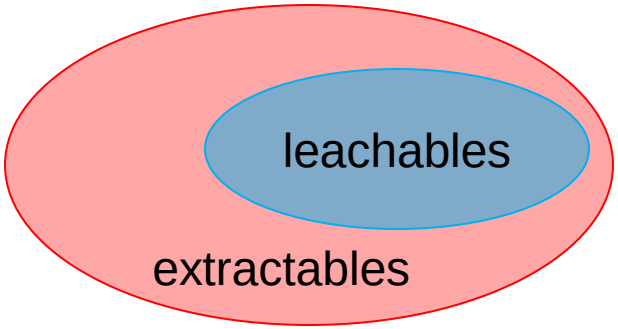




Label Migration Impurities

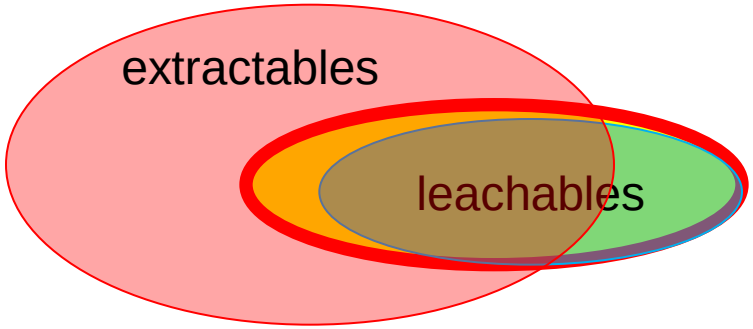
8.2 **REACTIVITY** OF LEACHABLES - DRUG PRODUCTS

➔ THEORY:



CLOSING THE GAP!!

➔ PRACTICE:



Additional Study Design

8. EVEN THEN, THINGS CAN GO WRONG

KINETICS OF	Extraction	Extraction	Accelerated Leachable St.	Real time/temp Leachable St.
	H_2O e.g. 8h reflux	DCM or IPA e.g. 8h reflux	e.g. 6 Mo, 40° C	e.g. 3 y at 25° C
EXTRACTION	SLOW – Incomplete no swelling/enhanced diffusion	FAST – complete Enhanced Diffusion Almost Asymptotic	Enhanced Diffusion controlled leaching is T-dependent $D = D_0 \exp(-E/RT)$	SLOW, but long term contact!
MATERIAL DEGRADATION	Slightly enhanced ASTM 1980: reflux at 100° C/8h: 60d at RT Even if they will be formed, will they come out?	Very Slightly enhanced ASTM 1980: (IPA) reflux at 80° C/8h: 15d at RT	Enhanced ASTM 1980: 6 Mo ageing at 40° C ≡ 17 Mo at 25° C	SLOW, but evaluated over LONG period! (e.g. 3y)
REACTION KINETICS • Dissolved O_2 in H_2O • Hydrolysis (H_2O) • Reaction with DP and leachates/materials • …	Slightly enhanced Low $[extr]_{init}$ will limit the formation of reaction comp. (i.e. for slow reactions)	Not relevant!	Enhanced, $k = k_0 \exp(-\mathbf{E_a}/RT)$ E_a: Activation Energy, reaction dependent (Pseudo) first order kinetics	SLOW, but evaluated over LONG period! (e.g. 3y)

9. LESSONS LEARNED

9. LESSONS LEARNED

1. Consider All Components of the **Pre-Filled Syringe**

2. Consider the Secondary Packaging (Needle Shield), the Processing Conditions, the right set of Conditions to perform the Extractable Study

3. <u>Do not rely solely on Extractable Studies</u> to perform a risk assessment of your Containers/Closures
   Even if the Guidelines themselves suggest that this could be sufficient

FDA

Table 4
Information That Typically Should Be Submitted for Injectable or Ophthalmic Drug Products

Description	Overall general description of container closure system, plus: <u>For Each Packaging Component</u>: • Name, product code, manufacturer, physical description • Materials of construction (for each: name, manufacturer and product code) • Description of any additional treatments (e.g., procedures for sterilizing and depyrogenating packaging components)
Suitability	<u>Protection</u>: (By each component and/or the container closure system, as appropriate) • Light exposure, when appropriate • Reactive gases (e.g., oxygen) • Moisture permeation (powders) • Solvent loss (liquid-based dosage forms) • Sterility (container integrity) or increased bioburden • Seal integrity or leak testing of tubes (ophthalmics) <u>Safety</u>: (for each material of construction, as appropriate) • Chemical composition of all plastics, elastomers, adhesives, etc.[a] • For elastomeric closures: USP Elastomeric Closures for Injections testing • For glass components: USP Containers: Chemical Resistance — Glass Containers • For plastic components and coatings for metal tubes: USP Biological Reactivity Tests • If the extraction properties of the drug product vehicle may reasonably be expected to differ from that of water (e.g., due to high or low pH or due to a solubilizing excipient), then drug product should be used as the extracting medium. • If the total weight of extracts significantly exceeds the amount obtained from water extraction, then an extraction profile should be obtained. • For plastic or elastomeric components undergoing heat sterilization, it is current practice to request that the extraction profile be obtained at 121 °C/1 hour using an appropriate solvent.

EMEA

Migration studies may only be omitted if, based on the outcome of the extraction studies, the calculated maximum amount of individual leachable substance that may be present in the active substance/medicinal product leads to levels demonstrated to be toxicologically safe. When a migration study is not considered necessary and thus is not conducted, a justification should be provided.

9. LESSONS LEARNED

3. If Safety Assessment is made on Extractables Results: check off with Leachable Studies!
   This will account for "unaccounted" leachables, such as polymer degradation, polymer additive degradants, process leachables, secondary packaging, or other extractables missed because of an ill designed study set-up

4. Consider – if possible – an additional **Accelerated Leachable study** (e.g. with screening methods) to verify the presence of "unexpected leachables" *(as a step in between extractable studies and full leachable studies)*

5. If the above is not possible: add a **screening step in the full leachable study**

Consider – if possible – an additional accelerated Leachable study (e.g. with screening methods) to verify the presence of "unexpected leachables"

Kinetics of	Extraction	Extraction	Accelerated Leachable St.	Real time/temp Leachable St.
	H_2O e.g. 8h reflux	DCM or IPA e.g. 8h reflux	e.g. 6 Mo, 40° C	e.g. 3 y at 25° C
EXTRACTION	SLOW – Incomplete no swelling/enhanced diffusion	FAST – complete Enhanced Diffusion Almost Asymptotic	Enhanced Diffusion controlled leaching is T-dependent $D = D_0 \exp(-E/RT)$	SLOW, but long term contact!
MATERIAL DEGRADATION	Slightly enhanced ASTM 1980: reflux at 100° C/8h: 60d at RT Even if they will be formed, will they come out?	Very Slightly enhanced ASTM 1980: (IPA) reflux at 80° C/8h: 15d at RT	Slightly enhanced ASTM 1980: 6 Mo ageing at 40° C ≡ 17 Mo at 25° C	SLOW, but evaluated over LONG period! (e.g. 3y)
REACTION KINETICS • Dissolved O_2 in H_2O • Hydrolysis (H_2O) • Reaction with DP and leachates/materials • …	Slightly enhanced Low $[extr]_{init}$ will limit the formation of reaction comp. (i.e. for slow reactions)	Not relevant!	Enhanced, $k = k_0 \exp(-\mathbf{E_a}/RT)$ E_a: Activation Energy, reaction dependent (Pseudo) first order kinetics	SLOW, but evaluated over LONG period! (e.g. 3y)

ANY QUESTIONS?

For further questions, please contact:

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http://www.toxikon.be/extractables-leachables-parenteral-injectables.html