ANALYTICAL TECHNIQUES, USED IN EXTRACTABLES TESTING

PDA TRAINING COURSE
EXTRACTABLES – LEACHABLES
BASEL
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CHALLENGES IN E/L TESTING

Challenges in E/L-Testing

Diversity of not-API Related Compounds in E/L research is Tremendous!!

Broad spectrum of:

- Types of Containers
- Types of Materials used in the Manufacture of Containers
- Number of Suppliers per Material
- Number of Grades (per supplier) for each type of Material
- Type of Sterilization (impact on material impurity profile)

Challenges in E/L-Testing

Incomplete List of types of Pharmaceutical Containers and Components

INHALATION

- *Metered Dose Inhaler Components*
  e.g.:
  - Gaskets
  - Stem
  - Body
  - Metering Chamber
  - Protection Ring
  - Actuator
  - Canister
- *Dry Powder Inhaler Components*
- *Nasal Spray Systems*
- *Nasal Dropper Systems*
- *Blow-Fill Seal containers*
- *Nebulizers*
- *...*

OPHTHALMIC

- *Eye Dropper Systems*
- *Tubes*
- *Blow-Fill-Seal containers*
- *...*

PARENTERAL

- *Bottles*
- *Vials*
- *(Pre-Filled) Syringes*
- *Cartridges*
- *(Rubber) Stoppers*
- *Rubber Plungers*
- *Sealing Discs*
- *Needle Shields*
- *Tip Caps*
- *I.V. Bags*
- *Administration Sets*
- *...*

DERMAL/TOPICAL

- *Spray Systems*
- *Tube systems*
- *...*

SINGLE USE SYSTEMS

- *(Multilayer) Bags*
- *Tubings*
- *Connectors*
- *Ports*
- *Filters (+ Housing)*
- *Chromatographic Columns*
- *Lyo trays*
- *...*

SECONDARY PACKAGING

- *Labels*
- *Adhesive/Glue (e.g. on labels)*
- *Ink*
- *Overwrap foils*
- *Blisters*
- *Cardboard packaging*
- *...*

Challenges in E/L-Testing

Pharmaceutical Containers can be made of different Materials

- Low Density Polyethylene
- High Density Polyethylene
- Polypropylene
- Rubbers
- Butyl Rubbers
- Chlorobutyl Rubbers w/o Coating
- Bromobutyl Rubbers w/o Coating
- EPDM Rubbers
- Isoprene Rubbers
- Nitrile Rubbers
- Latex Rubbers
- Other Rubbers
- Multi-layer Films and Foils
- Polyurethane (PU)
- Ethylvinyl Acetate (EVA)
- Ethylvinyl Alcohol (EVOH)
- Polyamide (Nylon-6, Nylon-66)
- Cyclic Olefin Copolymers (COC)
- Cyclic Olefin Polymers (COP)
- Polyethylene Terephthalate (PET, PETG)
- Polybutylene Terephthalate (PBT)
- Polyacetal (POM)
- Polymethylmethacrylate (PMMA)
- Acrylonitrile Butadiene Styrene (ABS)
- Silicone
- Thermo Plastic Elastomers (TPE's)
- Polycarbonate
- PTFE
- PEEK
- Glass w/o Coating
- Metals
- …

Challenges in E/L-Testing

Each Material has different Suppliers

EXAMPLES

Polyethylene - produced by:
- *Borealis*
- *LyondellBasell*
- *SABIC*
- *Dupont*
- *Enichem*
- *INEOS*
- *TOTAL*
- *...*

Pharmaceutical Rubbers - main Global Suppliers:
- *Datwyler*
- *West Pharmaceutical*
- *Stelmi*

Each Supplier has different Different Grades!

Challenges in E/L-Testing

Each Supplier has different Different Grades

EXAMPLES

PolyEthylene - produced by:

- *Borealis: over 30 different Medical Grades*
- *LyondellBasell: over 30 different Medical Grades*
- *SABIC: over 30 different Medical Grades*
- *Dupont: different grades*
- *Enichem: different grades*
- *INEOS: different grades*
- *TOTAL: different grades*
- *...*

Pharmaceutical Rubbers - main Global Suppliers:

- *Datwyler: over 100 different commercial rubber formulations*
- *West Pharmaceutical: over 100 different commercial rubber formulations*
- *Stelmi: also, a broad range of commercial rubber formulations*

Challenges in E/L-Testing

Per Material, Supplier and Grade:

what makes up the Impurities Profile?

- Solvent residues (e.g. of Polymerization)
- Polymer residues (e.g. Monomers, Oligomers)
- Catalyst residues
- Polymer/Rubber Additives
  - Antioxidants
  - Photostabilizers
  - Plasticizers
  - Lubricants
  - Acid Scavengers
  - Antistatic agents
  - Pigments/Colorants
  - Carifying/Nucleating Agents
  - Cross Linking Agents (Rubbers)
  - Initiators (Rubbers)
  - Accelerators (Rubbers)
  - UV curing agents
- Polymer Additive Degradation & Reaction Products
- Polymer Degradation Compounds
- Adhesives
- ...

Challenges in E/L-Testing

Conclusion:

1. The broad diversity of pharma containers, materials, suppliers and grades, leads to a extremely long list of potential impurities (leachables), introduced into the drug product

2. The compounds cannot be investigated with 1 analytical technique. Typically, at least 3 to 5 analytical techniques will need to be combined.

3. Compound Identification is of high importance, therefore the detection needs to be compound specific (e.g. MS-detection)
    - *Headspace GC/MS – Volatile Organic Compounds*
    - *GC/MS – Semi-Volatile Organic Compounds*
    - *LC/MS – Non-Volatile Organic Compounds*
    - *ICP – Metals*
    - *IC – Anions*

Challenges in E/L-Testing

Conclusion:

4. For Companies / Labs, only performing E/L-testing, every E/L-project could turn out into a high level research project (with the need for high level analytical techniques) because of the lack of materials knowledge

5. For Labs, performing E/L-studies on a routine basis, excessive analytical costs (associated with high-end analytical procedures) should be avoided in FIRST PASS testing.
   eg NELSON's propriatery MS/RT Database, built from authentic standards (5000)

SAMPLE PREPARATION:

THE

MOST IMPORTANT &

THE MOST UNDERESTIMATED

ACTIVITY IN THE LAB!!!

SAMPLE PREPARATION – CHALLENGES IN TRACE ANALYSIS

- Have **very experienced people** in Sample Preparation
- Very **Intensive Training** for new staff in Sample Prep
- **QC on solvents** used – select batches of clean solvents with suppliers
- **QC on extraction equipment**
- **Separate glassware**
- Precleaning of glassware – **validation of Cleaning** Procedures
- **Sampling of test articles** – how to handle Test Articles?
- **WFI sample prep** should be **separated** from solvent sample prep
- Correction for **absorbed solvents**?
- How to **concentrate extracts** – while avoiding cross contaminations
- **Storage of extracts** under controlled conditions
- **Holding times** of extracts
- Selection of **type of containers for storage** of extracts
- How to keep **DEHP** out of the Lab!

EXTRACTABLE STUDIES

IDENTIFICATION

IDENTIFICATION

IDENTIFICATION

CORRECT IDENTIFICATION

EXTRACTABLE STUDIES

A **Broad Identification** in “First Pass” Extractable Studies Requires:

1. A Compound Specific Detector: **Mass Spectrometry**

2. A **Database** to allow Identification based upon Mass Spectra
   - Commercial Databases for GC/MS: NIST, WILEY
   - **PROBLEM for LC/MS**: no Commercial Databases Available!
   - Self-Developed Databases (e.g. NELSONS proprietary DB)

ANALYTICAL TECHNIQUES USED FOR EXTRACTABLES TESTING

ANALYTICAL TECHNIQUES – GC/MS

Gas Chromatography / Mass Spectrometry (GC/MS)

Headspace GC/MS
(neat and after sample prep)

for Volatile Compounds

Direct Injection GC/MS
(after sample prep)

for Semi-Volatile Compounds

However, the GC/MS part of the Instrumentation is the same for the two techniques!!

"Standard" GC/MS

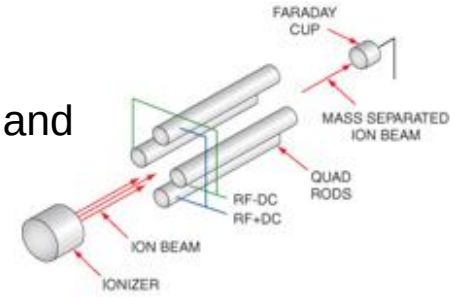
Gas Chromatography: Separation of Organic Molecules based on:

- Polarity – Interaction/Affinity with the Stationary Phase
- Boiling Point – GC-Oven temperature
- Film Thickness of the Chromatographic Capillary Column
  - *Volatile Compounds: high film thickness (>1 µm)*
  - *Semi-Volatile Compounds: low film thickness (≤0.25 µm)*
- Length of the Chromatographic Capillary Column
  - *Volatile Compounds: 30 m to 60 m*
  - *Semi-Volatile Compounds: 30 m*
- Polar Organic Compounds may need more specific conditions
  - *Acids, Amines, Alcohols....*

ANALYTICAL TECHNIQUES – GC/MS

"Standard" GC/MS: Quadrupole M.S

- 3 events: ionization / mass separation / detection – all happening under high **vacuum**
- Ionization: electrion ionization (70 eV) → convert molecule into ion and induce further **fragmentation**
- Quadrupole mass analyzer:
  - mass filter → only 1 mass can pass through a given electric field
    → other masses are removed
  - By rapidly sweeping the electric field → scanning of a mass range
  - Scanning goes extremely fast: milliseconds
  - Ions that reach the detector induce a signal that is measured
  - Mass spectrum: bar-graph plot of signal intensity vs. mass (unit)
  - Multiple mass spectra are recorded each second of the analysis



ANALYTICAL TECHNIQUES – GC/MS

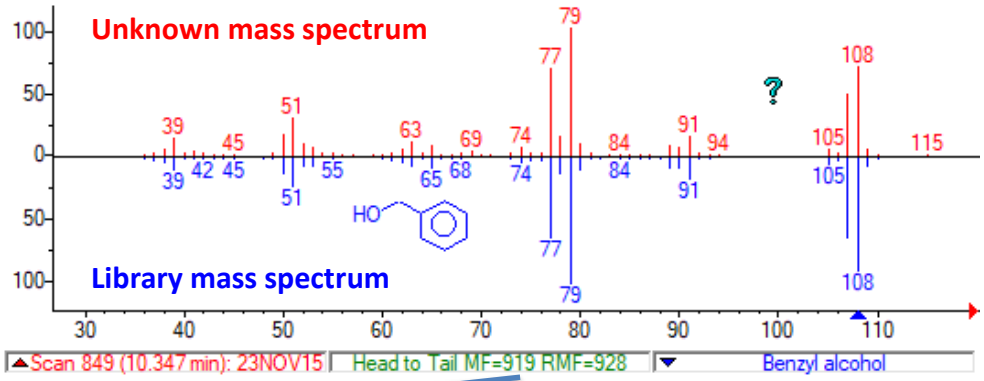
Mass spectra obtained by GC/MS are “standardized”

- A GC/MS “Mass Spectrometer” is ***Standardized***:
  1. Quadrupole (or Ion Trap)
  2. Ionisation: Electron Impact Ionisation of 70 eV
  3. Gives Reproducible Mass Fragmentation:

     Reproducible Mass Spectrum
  4. Mass Spectrum can be compared to commercially available Databases, such as NIST or WILEY – or self-developed MS-Databases (eg Nelson Labs Unique compound screener database)
  5. Can lead to Identification of Compound

ANALYTICAL TECHNIQUES – GC/MS

Example of a mass spectral search against the NIST library:



Match factor

→ Degree of similarity

Hit list

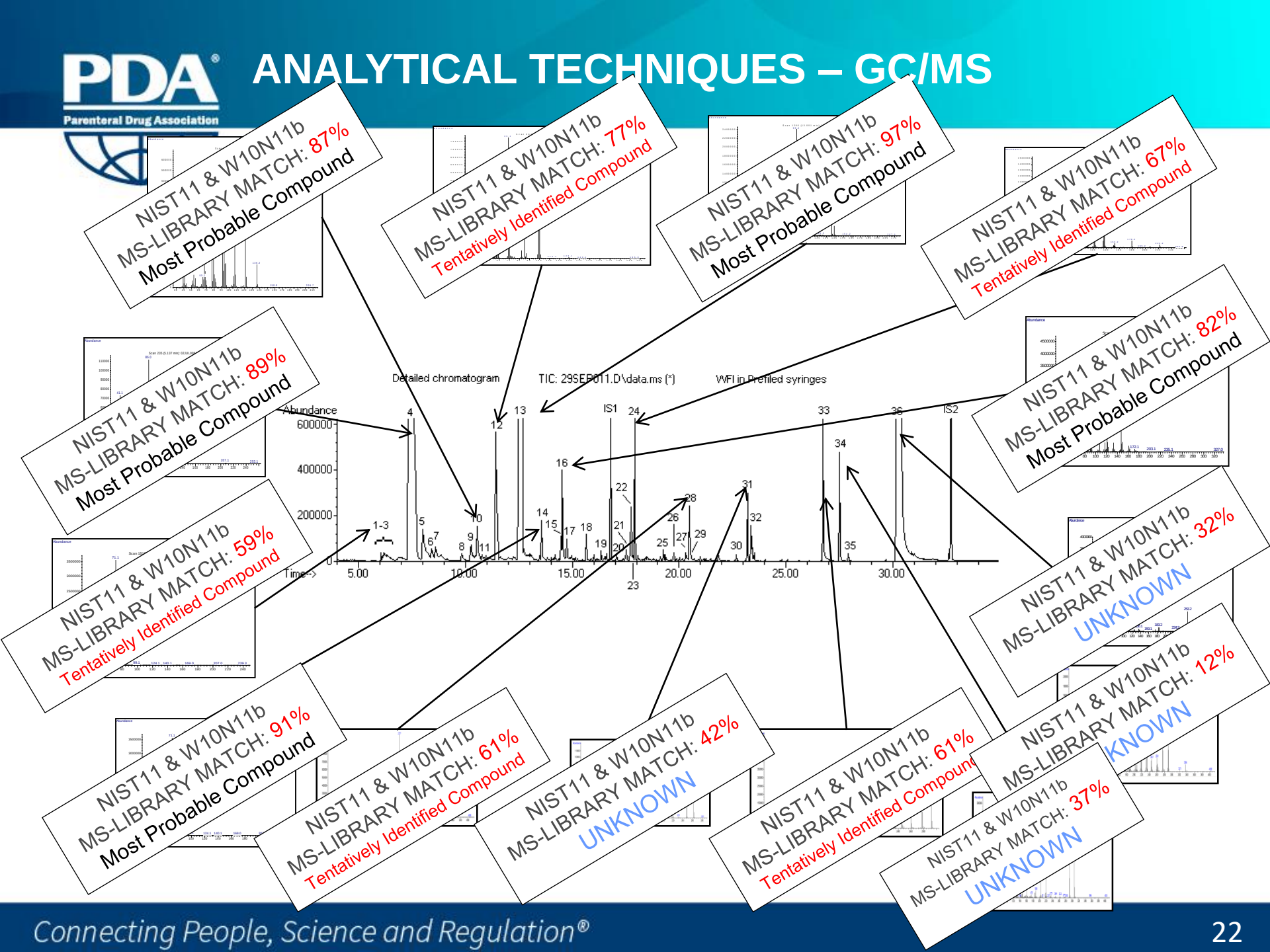
#	Library	Match	R.Match	Prob. (%)	Name
⊞ 1	svoc_...	919	928	50.8	Benzyl alcohol
⊞ 2	mainlib	912	923	50.8	Benzyl alcohol
⊞ 3	voc_...	910	915	50.8	Benzyl alcohol
4	mainlib	851	854	7.71	N-α,N-ω-Di-cbz-L-arginine
5	mainlib	845	872	6.06	N-Cbz-glycylglycine p-nitrophenyl ester
⊞ 6	mainlib	839	845	4.76	Phenol, 3-methyl-
⊞ 7	svoc_...	839	842	4.76	2-Methylphenol
⊞ 8	svoc_...	835	837	4.76	3-Methylphenol

Library compound

WHAT IS “SCREENING”?

- Trying to identify every peak in a chromatogram...
- ... above a certain threshold:
  - Either based on analytical feasibility (reporting threshold)
  - Or based on toxicological threshold (e.g. AET)
- Generate a list of extractables from the tested material with focus on **identification**
- Screening is **semi-quantitative**: estimation of concentration
- Useful for follow-up in a leachables study

ANALYTICAL TECHNIQUES – GC/MS



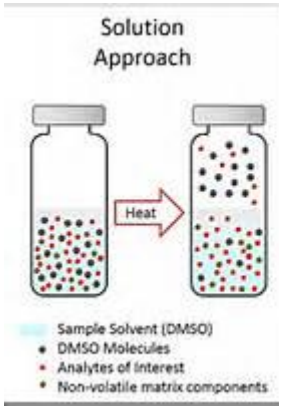
5. ANALYTICAL TECHNIQUES TO PERFORM E/L STUDIES

VOC

HS-GC/MS
Screening

Volatile Organic Compounds (typically MW < 200)

- **Monomer Residues**
- **Solvent Residues from Production steps**
- **Residues from polymer treatments (e.g. Washing)**
- **Small Polymer Breakdown products**



5. ANALYTICAL TECHNIQUES TO PERFORM E/L STUDIES

VOC

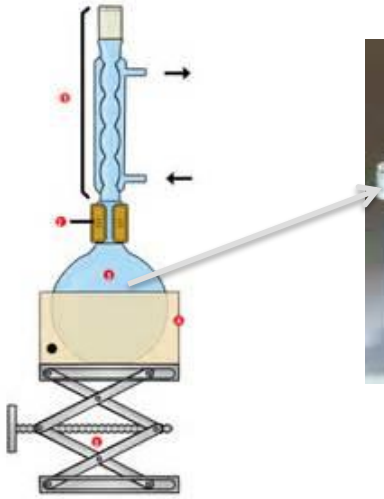
HS-GC/MS **Screening**

SVOC

GC/MS **Screening**

Semi-Volatile Organic Compounds (MW < 650)

- **Lubricants**
- **Plasticizers**
- **Antioxidants**
- **Polymer degradation products**
- **Solvents with an elevated boiling point**

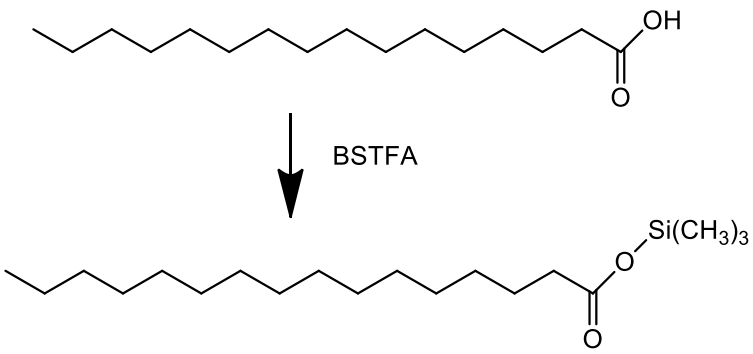


Derivatisation GC/MS

- A combined Headspace-GC/MS, GC/MS and LC/MS approach is suited for a broad list of organic compounds.
- However, compounds containing functional groups such as: ***Organic acids, amines, alcohols, polyols, aldehydes, ketones...*** may not always be very sensitive in regular GC/MS analysis!!
- A Derivatisation Method is using **BSTFA** as derivatisation agent *(conversion to more volatile, less polar trimethylsilyl esters).*

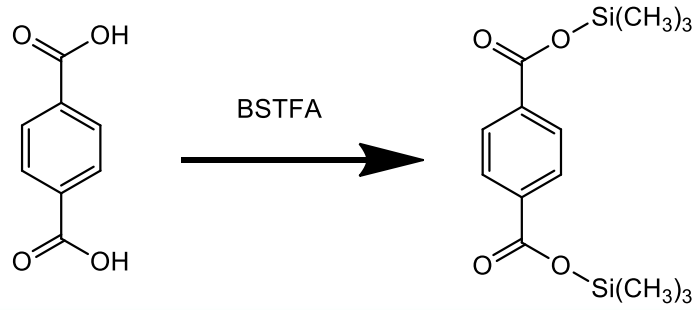
DERIVATISATION GC/MS: EXAMPLES

Peak 1: Palmitic acid



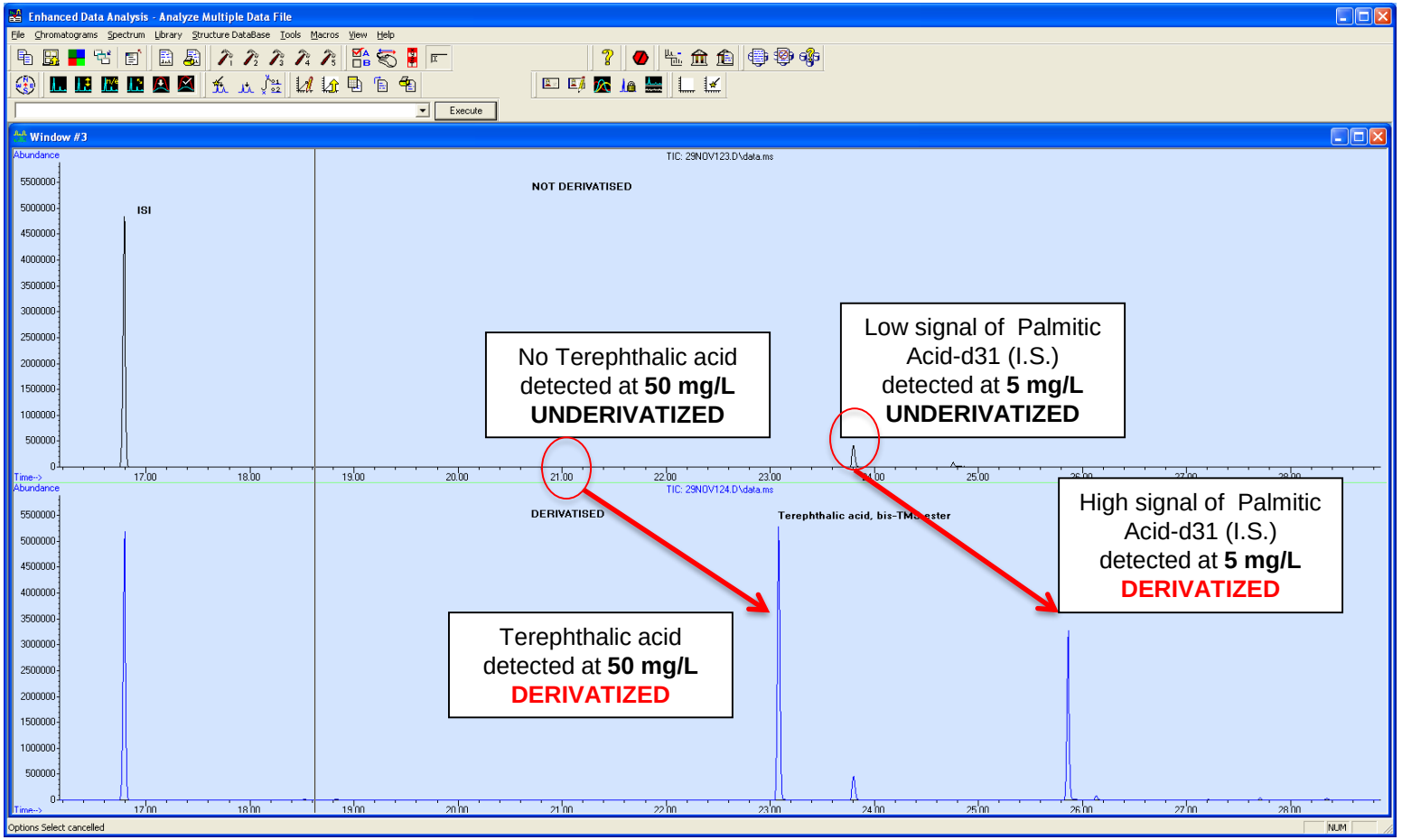
Trimethylsilyl ester of Palmitic Acid

Peak 2: Terephthalic acid



Trimethylsilyl ester of Terephthalic Acid

DERIVATISATION GC/MS: RESULTS



Other GC/MS Techniques *(High-End GC/MS)*

GC-MS (C.I.): Chemical Ionisation GC/MS

- "Soft Ionization" Compared to Electron Impact (E.I. 70eV)
- The molecule is less Fragmented
- Detection of Molecular Ion
- Allows to determine the Molecular Mass (i.e. With GC-ToF)
- Can be used for "Second Pass" Identifications

GC-QQQ or GC-"Triple Quad" Mass Spectrometer

- **Targeted** analysis in complex matrices
- Very low Detection Limits in complex matrices due to elimination of matrix interferences

Other GC/MS Techniques

GC-(Q)-ToF or GC-"Time-of-Flight" Mass Spectrometer (High resolution Accurate Mass MS)

- Allows for mass determination with an accuracy up to 3 decimals
- Principle of deduction of elemental formula: using the exact atomic masses
  - C = 12.000
  - H = 1.008
  - O = 15.995
  - N = 14.003
- Search for the best combination of atoms for a given exact mass that was measured with GC-(Q)TOF

GC-TOF Accurate Mass Measurements: example

- Accurate mass experimental result: 108.058
- Free elemental formula calculators available on the internet

	MF	Monoisotopic mass	PPM	mDa	unsaturation
1	C_7H_8O	108,058	5,415	-0,585	4
2	$C_5H_6N_3$	108,056	17,841	-1,928	4,5
3	$C_2H_8N_2O_3$	108,053	42,644	-4,608	0
4	$H_{12}O_6$	108,063	48,935	5,288	-5
5	$H_6N_5O_2$	108,052	55,071	-5,951	0,5
6	$CH_8N_4O_2$	108,065	61,311	6,626	0
7	$C_3H_{10}NO_3$	108,066	73,734	7,968	-0,5
8	$C_6H_8N_2$	108,069	98,532	10,648	4

- Most likely: the elemental formula of the unknown compound is **C_7H_8O**
- This way the elemental formula is found, but not (yet) the structure!

5. ANALYTICAL TECHNIQUES TO PERFORM E/L STUDIES

VOC

HS-GC/MS
Screening

SVOC

GC/MS
Screening

NVOC

UPLC/MS
Screening

Non-Volatile Organic Compounds

- **Fillers**
- **Plasticizers**
- **Antioxidants**
- **Anti-slip agents**
- **...**

ANALYTICAL TECHNIQUES – LC/MS (UPLC-HRAM)

The principle of HPLC

- High Pressure
- Separation, mostly reverse phase chromatography
- Optimizing separations by
  - Selection of Chromatographic Column (Polarity, Length...)
  - Selection of the Elution Solution (WFI, MeOH, ACN...)
- Detection of the Compounds (UV: DAD; Mass Detection)

HPLC - UV

Advantages

- Standard Equipment in a Lab
- Low Cost
- UV-Detector can be a *nice addition* to other Detectors, e.g. MS

Disadvantages

- Not a Universal Detector (Target Molecules need Chromophores)
- Non specific
- Not very Sensitive
- Information about the Detected Molecule is limited
  - E.g. Is the molecule linked to the API?

LC-MS

Advantages

- Specificity
- Sensitivity
- More can be said about the Identity of the Compound
- Quality of Information HRAM > Low Resolution
- Allows to build Databases for Identification

Disadvantages

- Cost
- Not a Universal Detector (Target Molecules need to Ionize)
- Different Ionisation Modes allow a broader detection of Compounds (APCI+/-; ESI+/-)

ANALYTICAL TECHNIQUES – LC/MS (UPLC-HRAM)

LC-MS

Older systems:
LOW Resolution Mass spectrometer
Ion Trap/Single Quad

Accuracy of Mass Detection is poor: 1 Dalton

m/z 220 can be distinguished from 221

HIGH Resolution LC-MS (LC-HRAM)
Orbitrap/Time-of-Flight (ToF)
Accuracy of Mass Detection - Orbitrap:

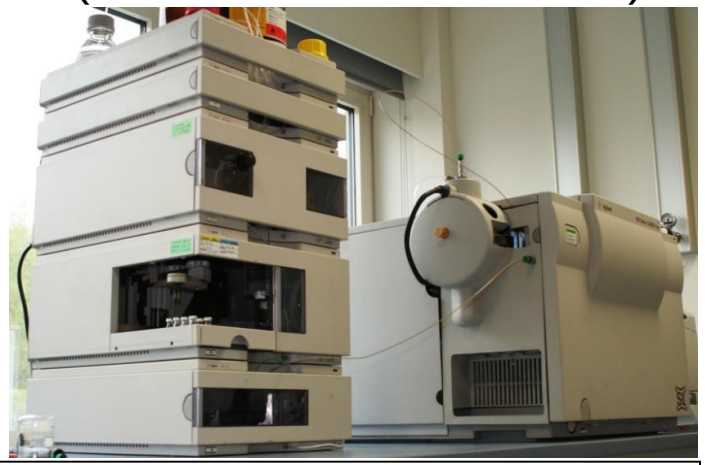
Mass error : sub ppm
m/z 220,2456 can be distinguished from m/z 220,2457

MAJOR ADVANTAGES!

- **Robust:** accurate mass is independent of the system
- High Accuracy in mass detection allows **elemental composition** analysis of an unknown analyte
- Extremely **powerfull if coupled to a UPLC**
- Building specificity into your databases based on mass accuracy and retention time!

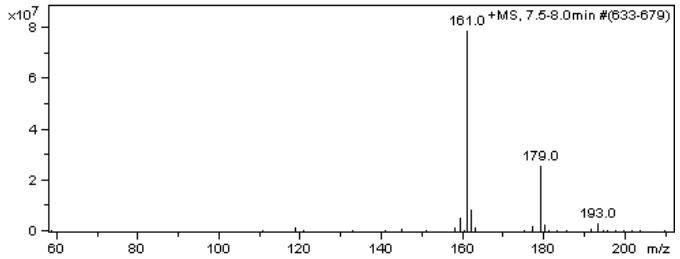
ANALYTICAL TECHNIQUES – LC/MS (UPLC-HRAM)

LC-ION TRAP
(LOW MASS ACCURACY)



LC-ORBITRAP
(HIGH MASS ACCURACY)

LOW RESOLUTION MASS



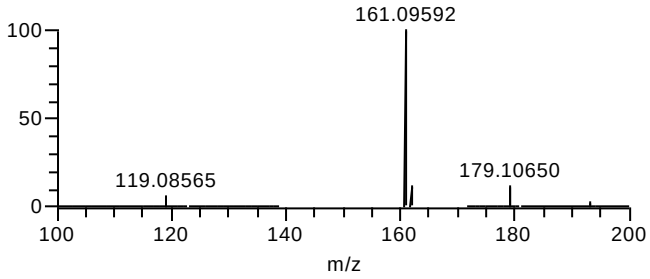
No information

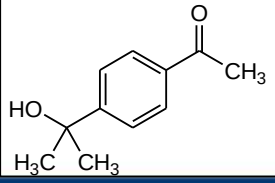
HIGH RESOLUTION ACCURATE MASS

$C_{11}H_{14}O_2$ exact monoisotopic mass: 179.10666

Mass error:

1 ppm





Peroxide curative related compound from EPDM rubber

5. ANALYTICAL TECHNIQUES TO PERFORM E/L STUDIES

VOC

HS-GC/MS
Screening

SVOC

GC/MS
Screening

NVOC

UPLC/MS
Screening

ICP/OES

ELEMENTS

- **Elements**
- **Heavy metals**
- **Quantitative**

ICP-OES

ICP-OES or ICP-MS:

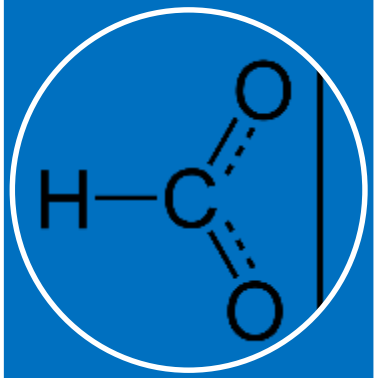
- Metals from Glass
- Metals from Rubbers
- Catalysts, used in the polymerization
- Fillers, added to Polymers
- Acid Scavengers
- Activator systems for Rubbers
- ...

ICP-MS

ICP-OES

5. ANALYTICAL TECHNIQUES TO PERFORM E/L STUDIES

VOC	SVOC	NVOC		
HS-GC/MS **Screening**	GC/MS **Screening**	UPLC/MS **Screening**	ICP/OES	IC



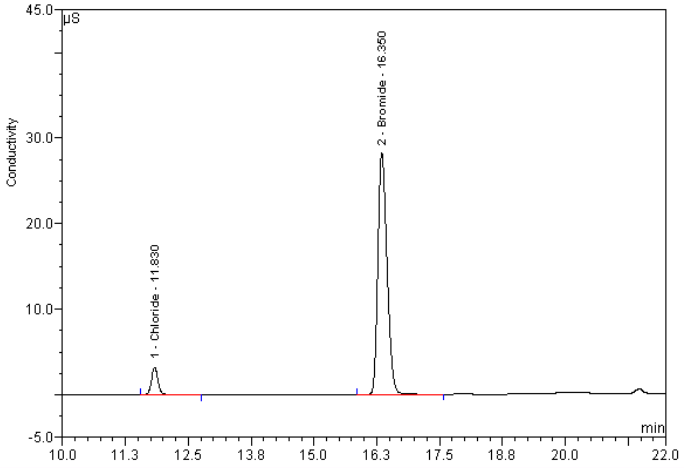
OTHER TECHNIQUES

Ion Chromatography:

- PolyOlefins (e.g. After Irradiation/Ageing): Acetate & Formate
- Halobutyl Rubbers: Bromide, Chloride, Fluoride
- Other trace impurities: Nitrite, Nitrate, Phosphate, Sulphate

- Example: Halobutyl rubbers may contain traces of bromide or chloride ions, either from side-products generated during the halogenation step, or rubber degradation products, or impurities. Additionally, fluoride may be released from fluoropolymer coatings

Sample: reflux extract with WFI (water for injection) of a halobutyl rubber



OTHER (SPECIFIC) METHODS

- ✓ **GF-AAS** For Silicone Oil Detection
- ✓ **ESI**-UPLC-HRAM (**Electron Spray**: BPOG Method)
- ✓ **HPLC-UV** for TMPTMA (glue residue)
- ✓ HPLC-UV for S_8 (Cross Linker)
- ✓ **pH** (release of acidic/alkalinic agents in UPW)
- ✓ **Conductivity** (release of salts in UPW)
- ✓ **Non-Volatile Residue** (gravimetric residue)
- ✓ **FTIR** – characterization of NVR
- ✓ **Total Organic Carbon**: *reconsiliation with concentration of organic compounds from chromatographic techniques*
- ✓ ...

ANALYTICAL TECHNIQUES USED FOR LEACHABLES TESTING

LEACHABLES STUDIES

TECHNIQUES USED IN LEACHABLE STUDIES

- ✓ Headspace GC/MS: Volatile Compounds
- ✓ Direct Injection GC/MS: Semi-Volatile Compounds
- ✓ D.I. GC-QQQ: Semi-Volatile Compounds
- ✓ LC-QQQ: Non-Volatile Compounds
- ✓ Ion Chormatography: (An)Ions
- ✓ ICP-OES or ICP-MS: Metals

Specific Analysis/Techniques for specific target analyses...

(See further presentation *“Leachable Studies”*)

ANY QUESTIONS?