Connecting People, Science and Regulation

ANALYTICAL TECHNIQUES, USED IN EXTRACTABLES / LEACHABLES TESTING

PDA WORKSHOP
EXTRACTABLES – LEACHABLES
Brussels
13-14 March, 2014

Dr. Piet Christiaens

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

(Non Limitative) List of types of Pharmaceutical Containers

INHALATION

- *Metered Dose Inhaler Components e.g.:*
  - Gaskets
  - Stem
  - Body
  - Metering Chamber
  - Protection Ring
  - Actuator
  - Cannister
- *Dry Powder Inhaler Components*
- *Nasal Spray Systems*
- *Nasal Dropper Systems*
- *...*

PARENTERAL

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

OPHTHALMIC

- *Eye Dropper Systems*
- *Tubes*
- *...*

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
- Bromobutyl Rubbers
- 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
- C-Flex
- Polycarbonate
- Teflon
- PEEK
- Glass
- 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)
- Catalysts
- Polymer/Rubber Additives
    - Antioxidants
    - Photostabilizers
    - Plasticizers
    - Lubricants
    - Acid Scavangers
    - Pigments/Colorants
    - Carifying/Nucleating Agents
    - Cross Linking Agents (Rubbers)
    - Initiators (Rubbers)
    - Accelerators (Rubbers)
- Polymer Additive Degradation Products
- Polymer Degradation Compounds
- Adhesives
- ...

Challenges in E/L-Testing

EXAMPLE FOR 1 GRADE OF POLYOLEFIN

POLYMER EXTRACTABLES: SUM OF

1. **INITIAL INGREDIENTS**

2. **IMPURITIES** *(e.g. monomers, polymer oligomers, solvent residues, glue residues, catalyst residues…)*

3. **REACTION/DEGRADATION PRODUCTS** OF POLYMER ADDITIVES

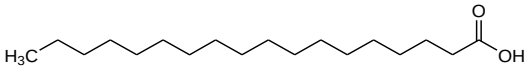
4. **DEGRADATION PRODUCTS** OF POLYMER

Challenges in E/L-Testing

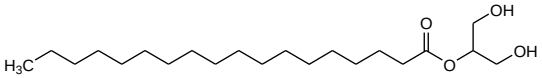
EXAMPLE FOR POLYOLEFINS

POLYMER EXTRACTABLES: SUM OF

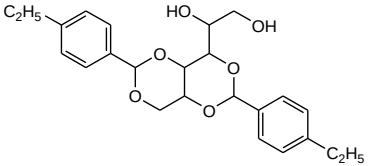
1. **INITIAL INGREDIENTS**



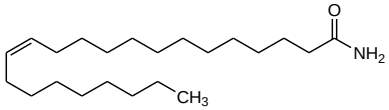
Stearic acid/stearate



Glycerol monostearate

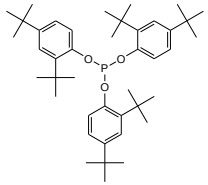


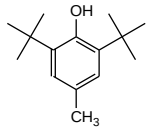
NC-4/
Millad

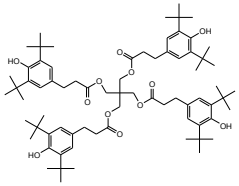


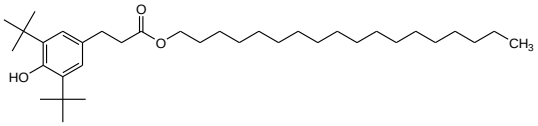
erucamide

antioxidants









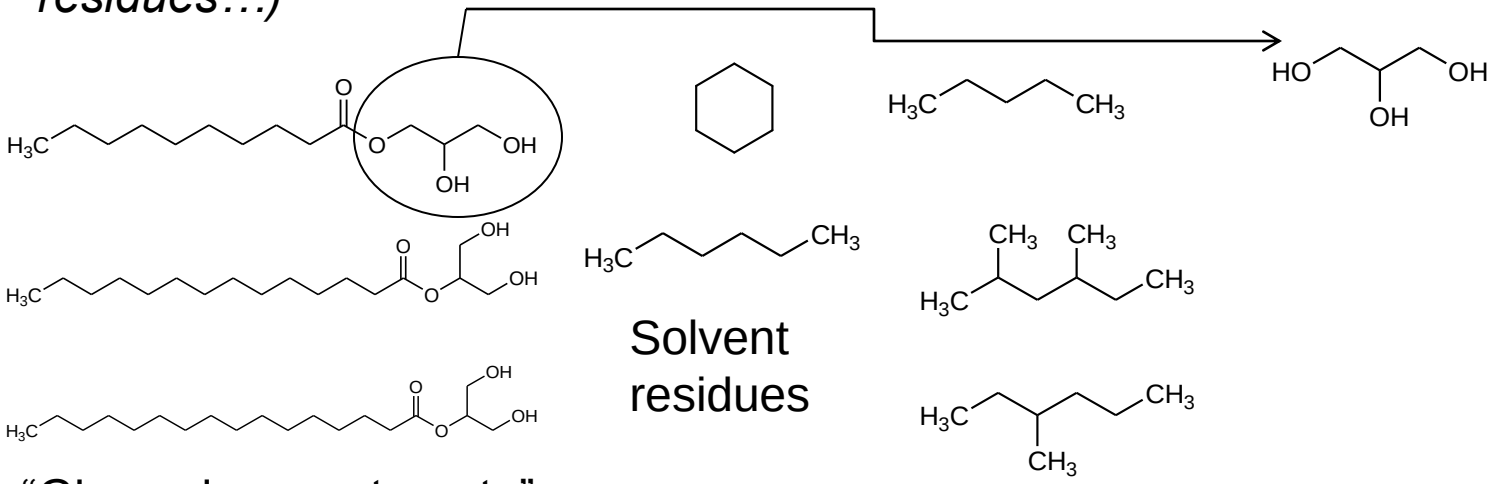
And many others...

Challenges in E/L-Testing

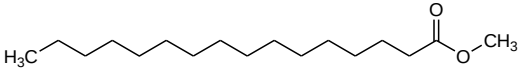
EXAMPLE FOR POLYOLEFINS

POLYMER EXTRACTABLES: SUM OF

2. **<u>IMPURITIES</u>** *(e.g. monomers, polymer oligomers, solvent residues, catalyst residues…)*



"Glycerol monostearate" impurities



Palmitic acid/palmitate

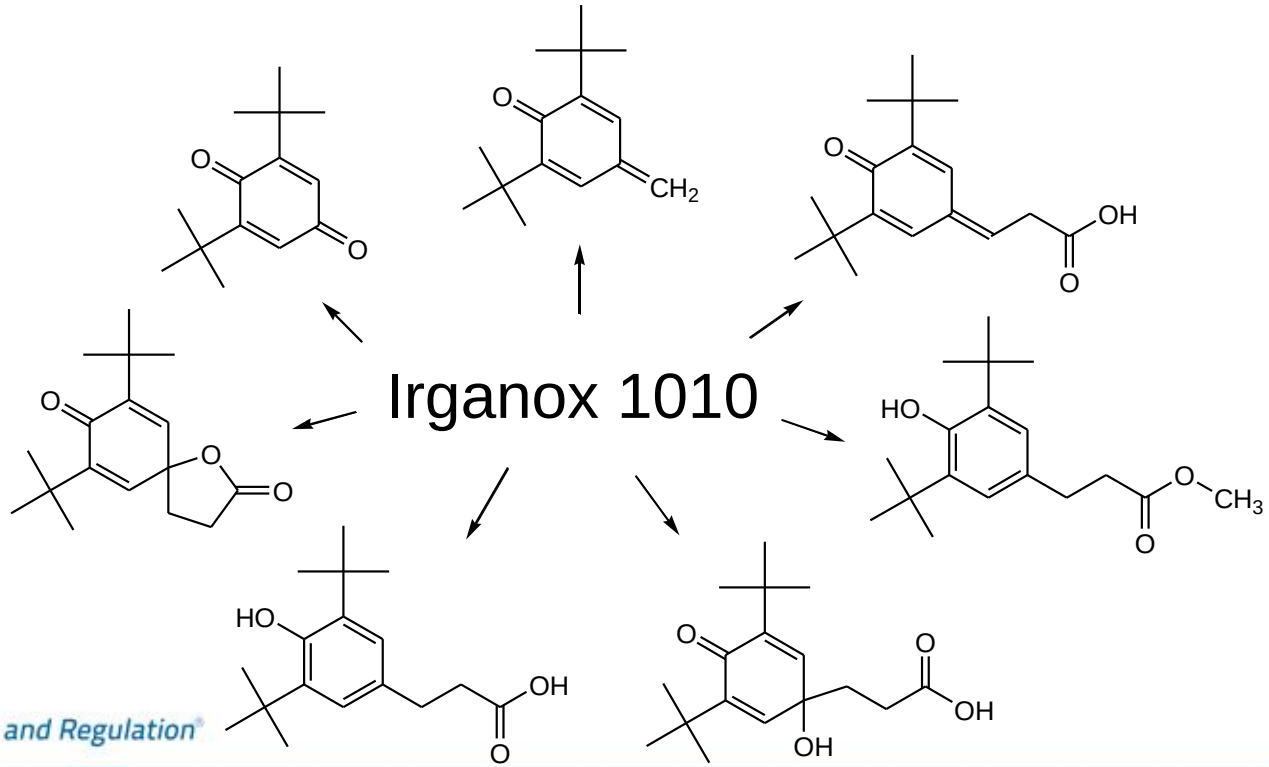
And many others...

Challenges in E/L-Testing

EXAMPLE FOR POLYOLEFINS

POLYMER EXTRACTABLES: SUM OF

3. **<u>REACTION/DEGRADATION PRODUCTS</u>** OF POLYMER ADDITIVES

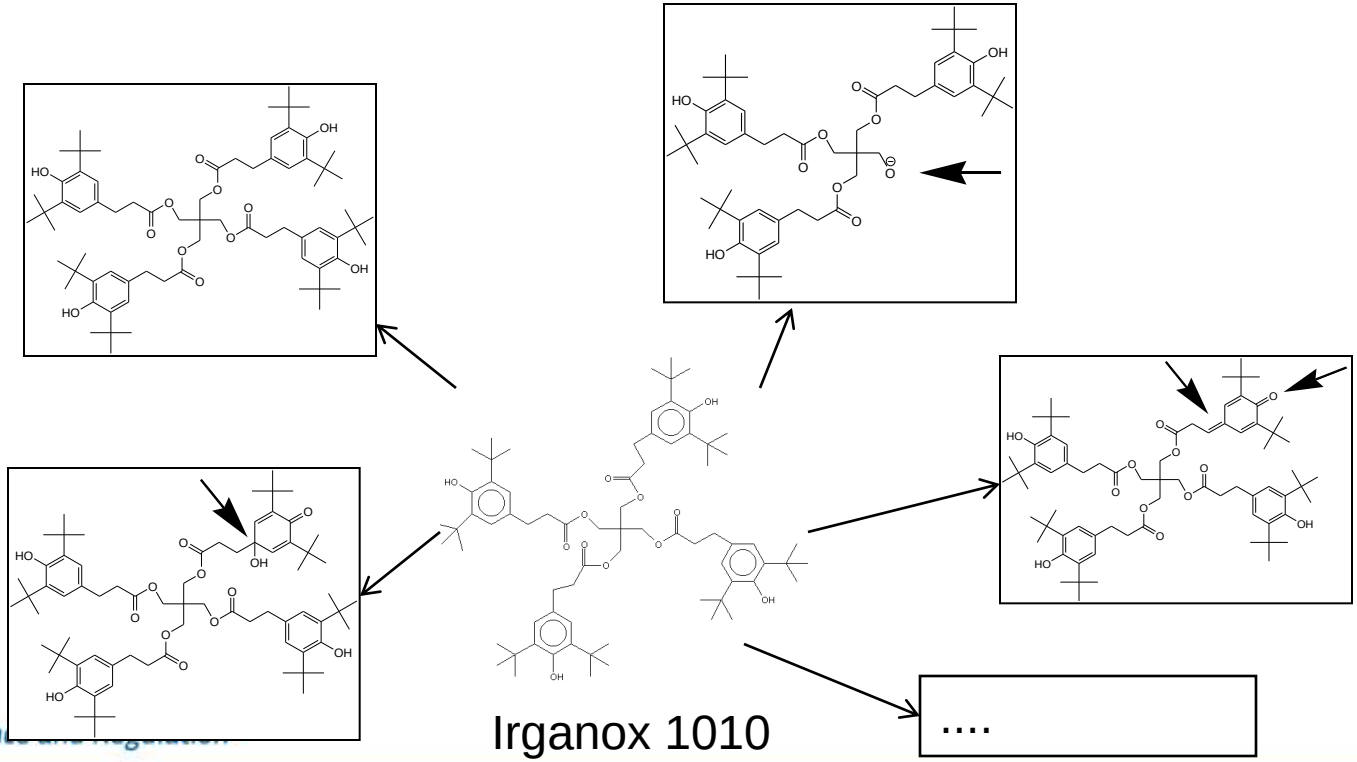


Challenges in E/L-Testing

EXAMPLE FOR POLYOLEFINS

POLYMER EXTRACTABLES: SUM OF

3. **<u>REACTION/DEGRADATION PRODUCTS</u>** OF POLYMER ADDITIVES

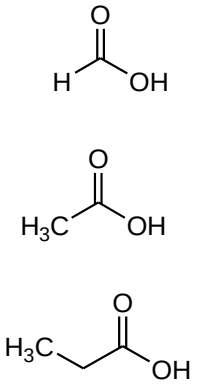


Challenges in E/L-Testing

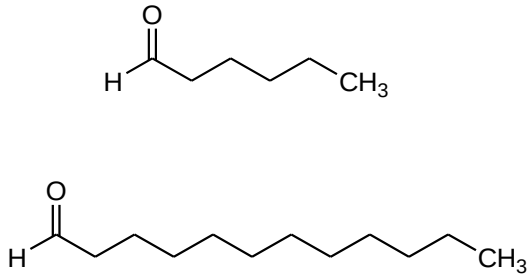
EXAMPLE FOR POLYOLEFINS

POLYMER EXTRACTABLES: SUM OF

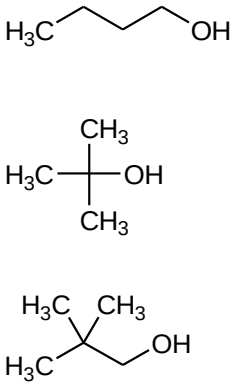
4. <u>DEGRADATION PRODUCTS</u> OF POLYMER



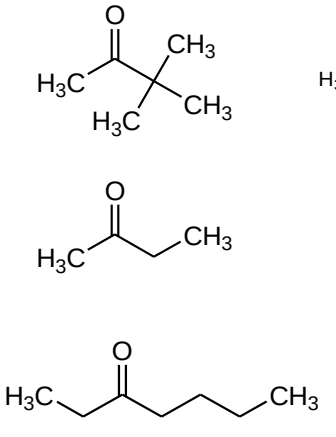
acids



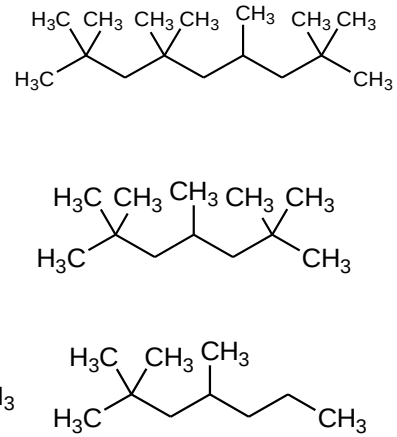
aldehydes



alcohols



ketones



Olefin fragments / oligomers

And many others...

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.
   Toxikon: **TOX-RAY** development

ANALYTICAL TECHNIQUES USED FOR EXTRACTABLES TESTING

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!

SAMPLE PREPARATION

- How to deal with human source contaminants (limonene, squalene, parabens, palmitic/stearic acid...)
- Headspace GC/MS: WFI should be completely SEPARATED
  - Sample prep
  - Storage of sample/extract
  - Filling into storage containers
  - Instruments
  - Holding times for HS-GC/MS are shorter!!
  - Avoid cross contamination from other solvents, regularly used in the lab (DCM, Hexane, IPA, Toluene, Chloroform...)
- Internal standards
  - Holding times of Internal Standards
  - Syringes: should be calibrated at least yearly
  - Have a cleaning procedure for syringes
  - Compatibility of Internal Standards with solvents

EXTRACTABLE STUDIES

IDENTIFICATION

IDENTIFICATION

IDENTIFICATION

IDENTIFICATION

ANALYTICAL TECHNIQUES

- INCREASE THE **KNOWLEDGE** ABOUT THE COMPOSITION OF THE POLYMER
- FOCUS: IDENTIFICATION OF EXTRACTABLES
- ADDS TO INFORMATION PROVIDED BY RAW MATERIAL SUPPLIERS OR C/C MANUFACTURERS
- EXTRACTABLES LIST: FOCUS FOR LEACHABLE STUDY
- IN SOME CASES: QUANTITATIVE EXTRACTABLES STUDIES (e.g. inhalation)

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
   - Self-Developed Databases (e.g. **TOX-RAY**)
   - **PROBLEM for LC/MS**: no Commercial Databases Available!

ANALYTICAL TECHNIQUES – CHROMATOGRAPHY

Sample → Chromato-graph → Ion Source → Mass analyser → Detector → Data analysis

Sample

Homogeneous but complex mixture

Liquid sample
Gas sample
Solid sample

Chromato-graph

Gas Chromatography or Liquid Chromatography

Mass analyser

Quadrupole (Q)
Orbi-Trap
Time of Flight (Tof)
Tandem mass spectrometer: Triple Quadrupole, Tof-Tof
Hybrid systems: Q-ToF
...

ANALYTICAL TECHNIQUES – GC/MS

Gass 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!!

ANALYTICAL TECHNIQUES – GC/MS

Sample

The most common method in GC is using an **automatic liquid sampler**. A liquid sample is introduced into a vaporising inlet where the sample is rapidly heated to the gas phase and subsequently transferred to the column inlet.

Other sample introduction systems:

Headspace GC-MS
PTV-GC-MS

ANALYTICAL TECHNIQUES – GC/MS

Chromatograph

Gas Chromatography (GC) is a separation technique were

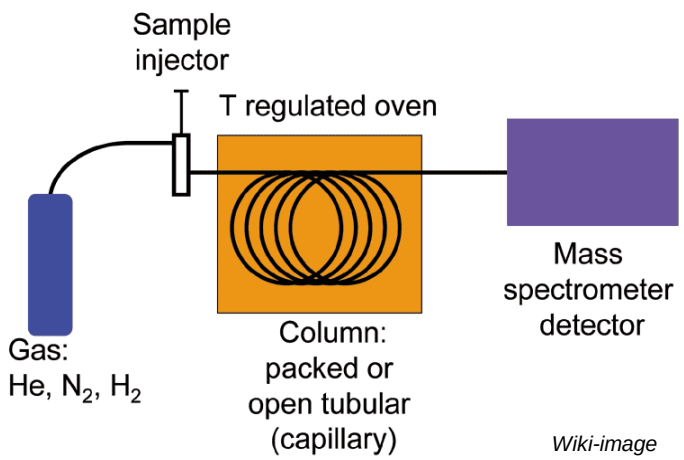
- **Mobile phase: gas**
- **Stationary phase: polymeric liquid** (polysiloxane) immobilised onto the inner wall of a long capillary column.

Retention of Analytes is determined by

Temperature of the GC-Oven/Flow rate of Gas/Column Length

vapour pressure of the analyte

chemical affinity of the analyte for the stationary phase.



Wiki-image

- Samples must be volatile
- Most analytes < 500 Da, Toxikon: up to 700Da
- Higly polar analytes may have a low recovery

"Standard" GC/MS: Quadrupole M.S.

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

Mass analyser

A mass spectrometer is an instrument that measures the masses of individual molecules that have been converted into ions.

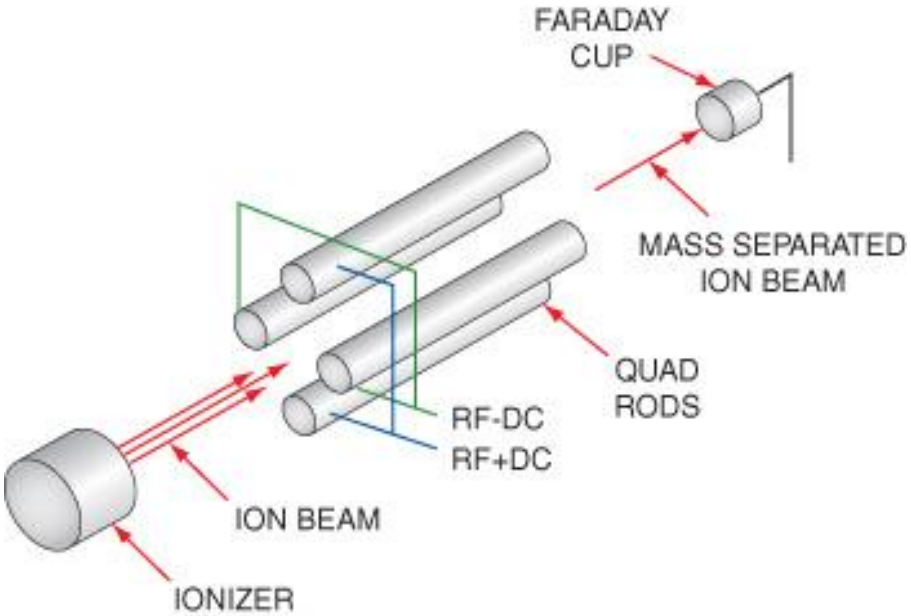
The resulting charged species are focussed and accelerated into a mass analysing device, typically a Quadrupole mass analyser. The mass analyser selectively allows ions of a certain mass to charge ratio to pass through the analyser to the electron multiplier where a signal is generated. The mass filter can be adjusted to allow selected ions to pass (Selected Ion Monitoring (SIM) mode) or to scan over a range of m/z values (Scan mode)

"Standard" GC/MS: Quadrupole M.S.

General Sequence of Things in a Mass Spectrometer (GC):

- High Vacuum
- Convert Molecules to Ions (Tungsten Filament)
- A Moving Ion (= charge) in a Magnetic Field gets deflected
- Only the right "m/z" can reach the detector and give a (charge) signal
- The charge signal is "strengthened" by a photomultiplier
- The Mass Filter (e.g. Quadrupole) scans a predefined mass range in milliseconds!
- This way, a complete mass spectrum can be obtained in a few milliseconds!

Standard GC/MS: Quadrupole M.S.



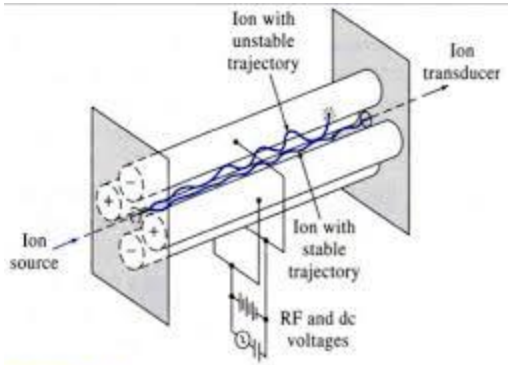


FIGURE 11-6 A quadrupole mass spectrometer.

Standard GC/MS: Quadrupole M.S.

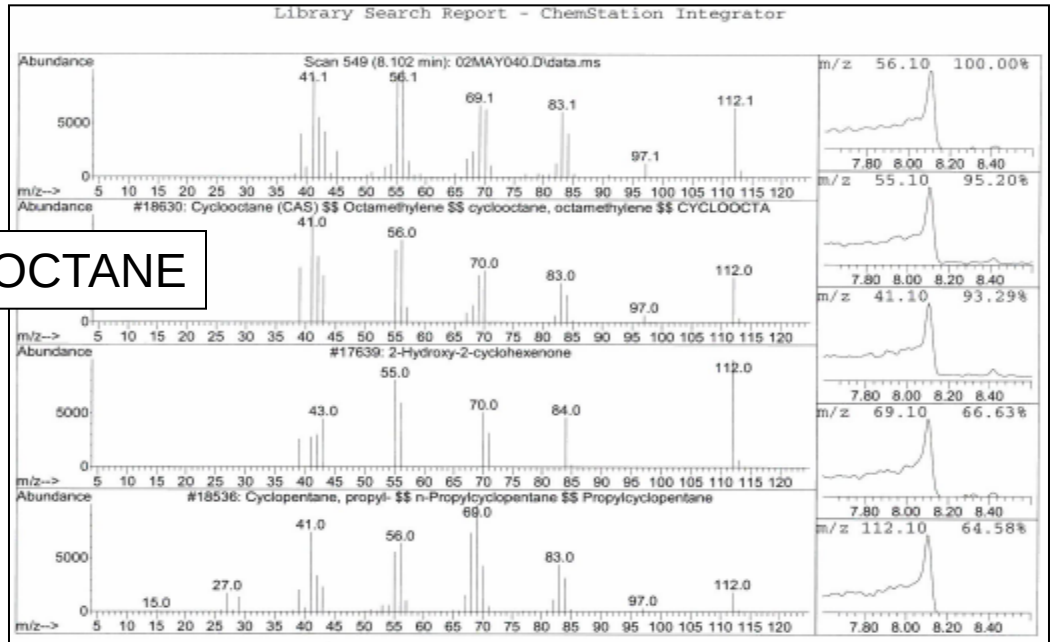
Standard GC/MS: Quadrupole M.S.

- 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 TOX-RAY)
  5. Can lead to Identification of Compound

Standard GC/MS: Quadrupole M.S.

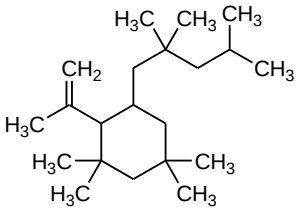
Example of FIT of an UNKNOWN MS with NIST/WILEY

CYCLOOCTANE

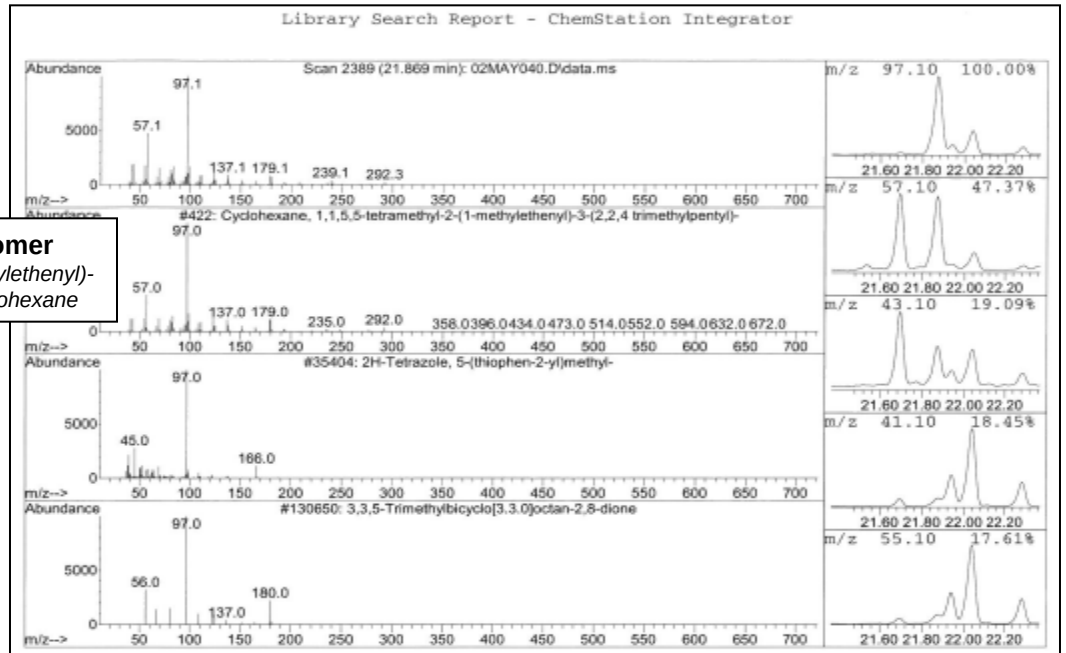


Standard GC/MS: Quadrupole M.S.

Example of FIT of an UNKNOWN MS with NIST/WILEY



$C_{21}H_{40}$ Rubber Oligomer
1,1,5,5-Tetramethyl-2-(1-methylethenyl)-3-(2,2,4-trimethylpentyl)-cyclohexane




```
Data File: T:\Lab Systems\GCMS\LSE2166\DATA\2013\1305\1305011\02MAY040.D
Sample    : 13-B1106-N2

Peak Number:        at    21.869 min  Area:        1  Area % 1.00

 The 3 best hits from each library.          Ref\#    CAS\#      Qual
-----------------------------------------------------------------------
    T:\Lab Systems\GCMS\MS Libraries\SVOC.L
     1 Cyclohexane, 1,1,5,5-tetramethyl...        422 900046-00-0   98
     2 T*-Chlorinated Hydrocarbon C21H3...        590 900063-00-0   42
     3 T*-Brominated Hydrocarbon C21H39...        424 900048-00-0   42

    T:\Lab Systems\GCMS\MS Libraries\NIST11.L
     1 2H-Tetrazole, 5-(thiophen-2-yl)m...      35404 1000315-87-7   53
     2 Cyclopentane, 1,1'-ethylidenebis-        35189 004413-21-2   50
     3 2,2,3,3-Tetramethylcyclopropanec...      92406 1000221-97-5   50

    T:\Lab Systems\GCMS\MS Libraries\W9N08.L
     1 3,3,5-Trimethylbicyclo[3.3.0]oct...     130650 999130-65-0   53
     2 2H-Tetrazole, 5-(thiophen-2-yl)m...      97068 999097-06-8   53
     3 Hept-2-ene, 2,4,4,6-tetramethyl-...      76719 103982-58-7   50
```

Compound **PRESENT** in **TOX-RAY** DB

Compound **NOT PRESENT** in NIST/WILEY

“Standard” GC/MS: Quadrupole M.S.

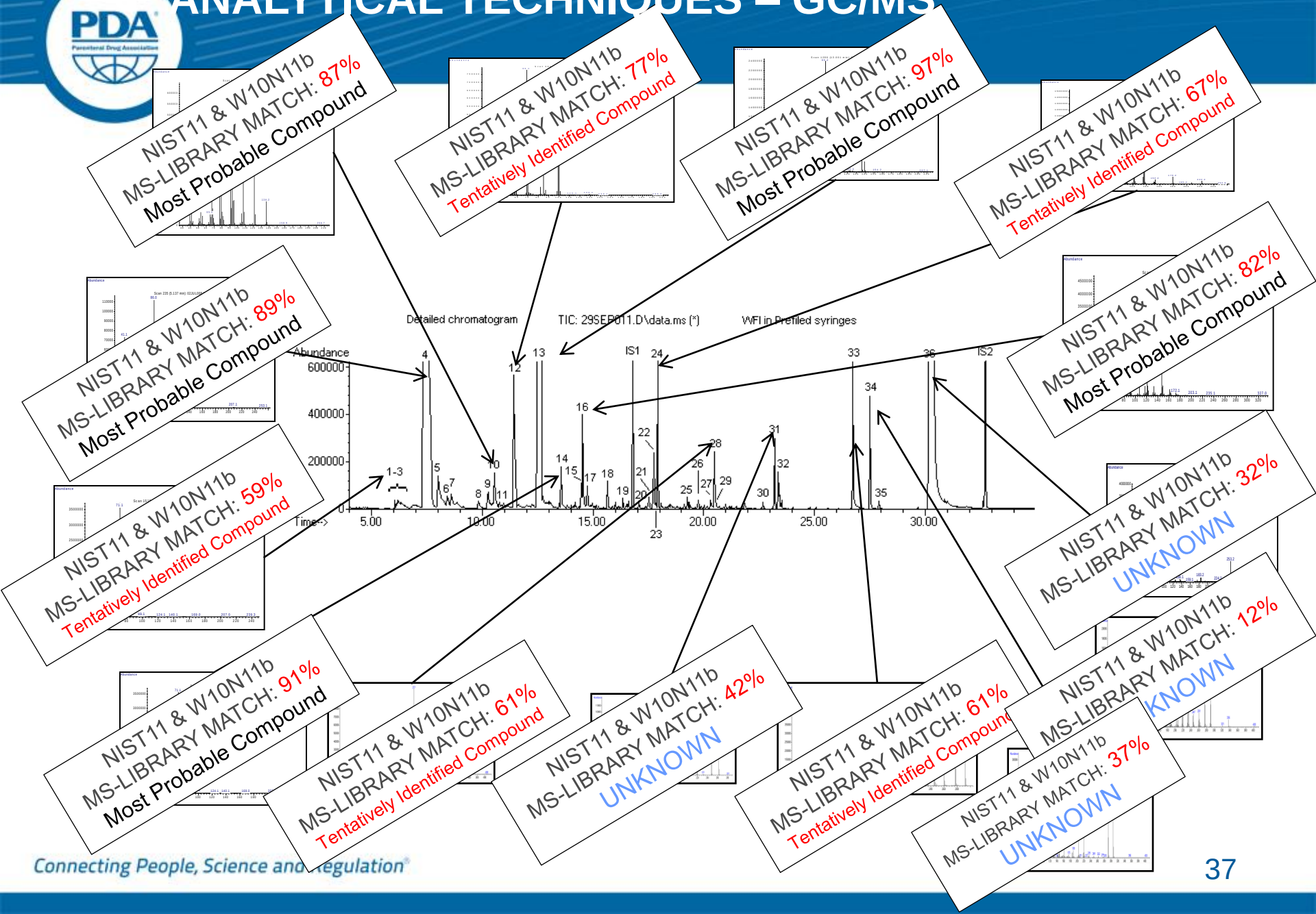
WHAT IS “SCREENING”?

- Trying to identify every single peak in a chromatogram
- Above a certain threshold

  - either Analytical (reporting threshold)
  - or Toxicological (e.g. AET)

Example: see next slide

ANALYTICAL TECHNIQUES – GC/MS



HEADSPACE GC/MS FOR VOLATILE COMPOUNDS

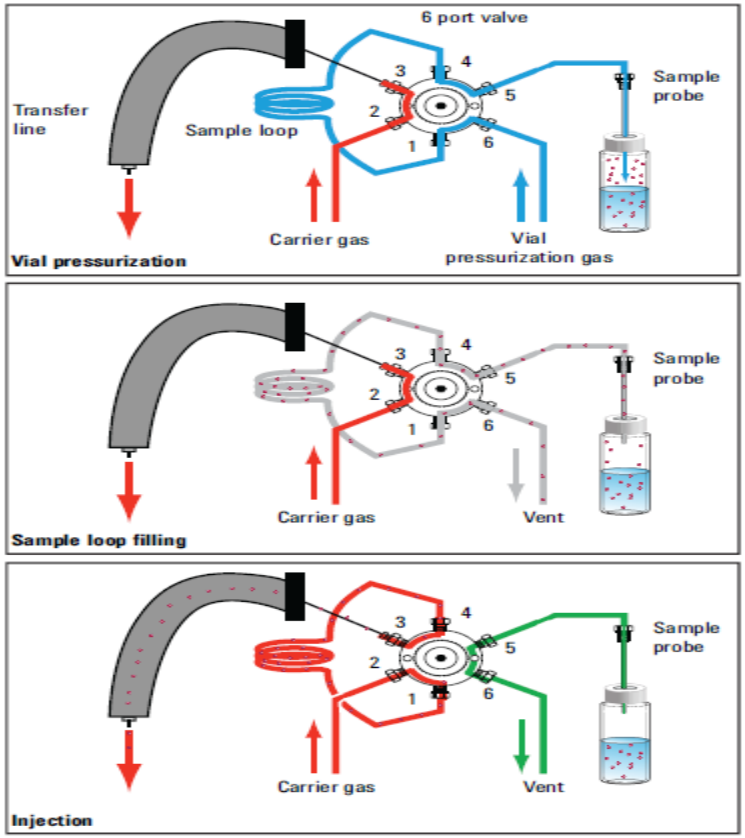
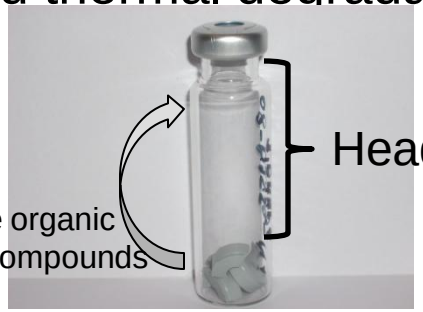


Figure 3 Valve and loop system sampling and injection stages

HEADSPACE GC/MS: VOLATILE COMPOUNDS

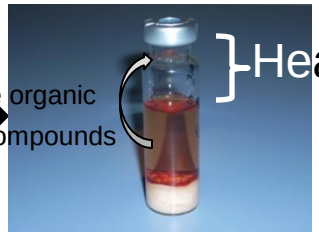
- EXTRACTABLE STUDIES: Heat the polymer material in a closed vial to a “polymer process temperature” – avoid thermal degradation

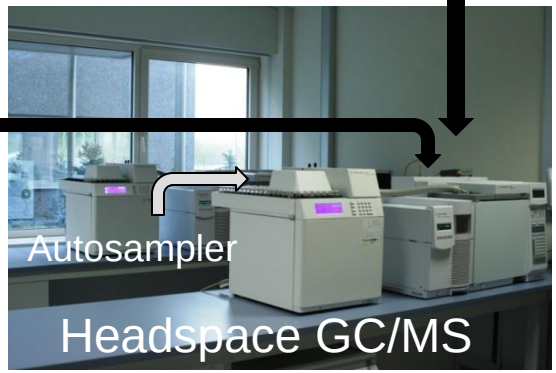
T ↗



- Desorption of the volatile “impurities” – potential leachables
- Inject the headspace of the vial into a GC/MS
- Similar for solutions:

T ↗





HEADSPACE GC/MS

AROMATIC COMPOUNDS: e.g. Benzene, Toluene, Ethylbenzene, Xylenes (POLYSTYRENE)
1,3-Bis-(1,1-dimethylethyl)benzene (POLYPROPYLENE)

ALDEHYDES, e.g. Butanal, Pentanal, Hexanal (LDPE, EVOH)
2-Ethylhexanal (PVC)
2-Methyl-2-propanal (PP)

ALCOHOLS: e.g. 2-Ethyl-1-hexanol, 1-Octanol (PVC)
Ethanol (EVOH)
2-Methyl-2-propanol (HDPE, PP)

KETONES: e.g. Acetone, 2-Butanone, 2-Hexanone, 3-Hexanone (PP, LDPE)
Methylethylketone (EVOH)

ACIDS: e.g. Acetic, Propionic, Butanoic, Pentanoic Acid (PP, LDPE)

ESTERS: e.g. Acetic Acid, 2-ethyl ester (PVC, EVA)

ALKANES (PE, PP)

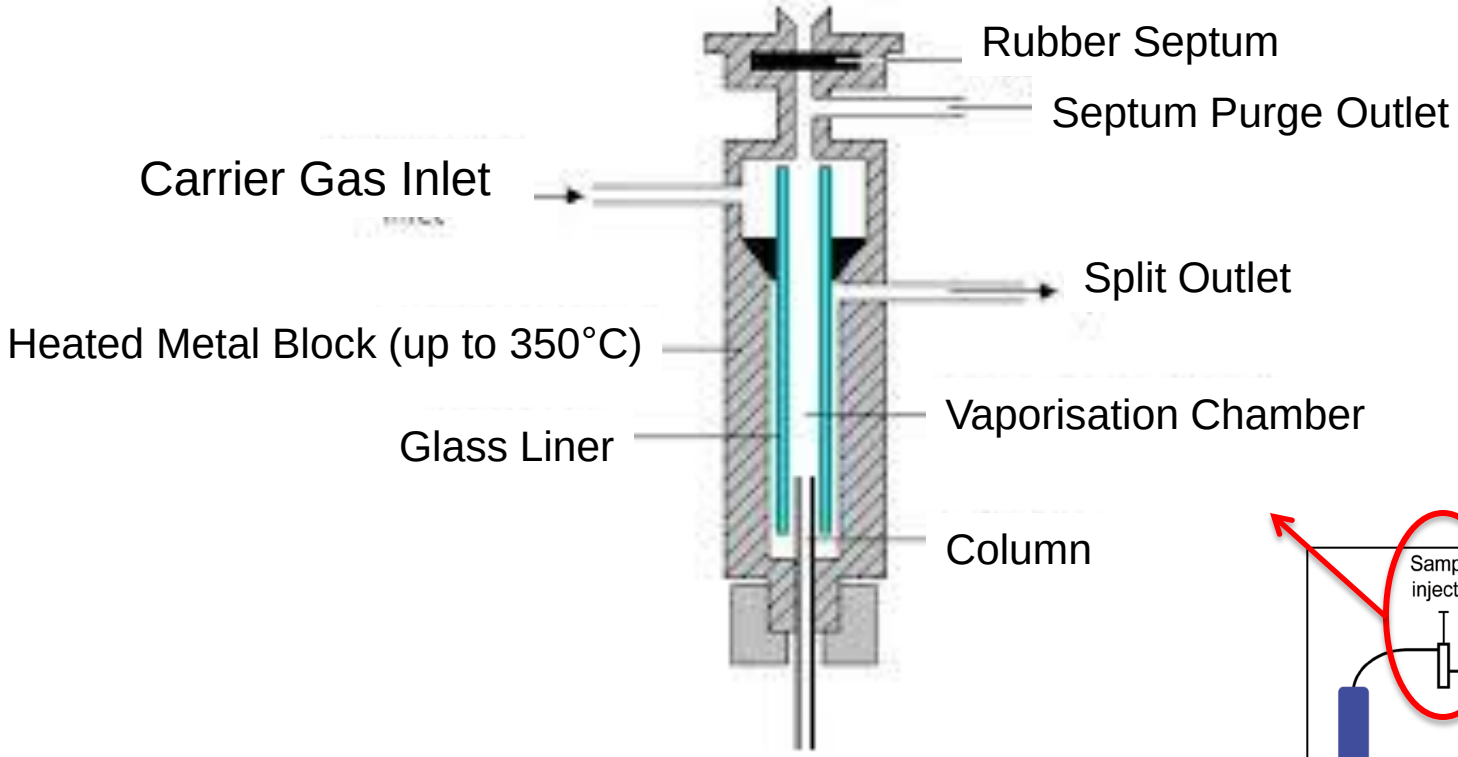
DIRECT INJECTION GC/MS FOR SEMI-VOLATILE COMPOUNDS

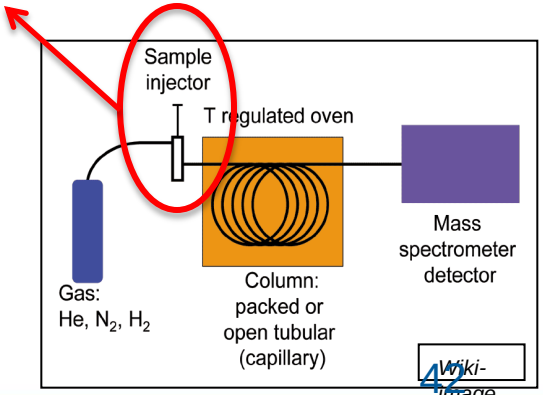
Possible Inlet Systems

- Split/Splitless Inlet (SSL): universal, mostly used
- Cool On-Column inlet (COC): minimal discrimination and compound breakdown
- Multimode Inlet (MMI) or Programmed Temperature Vaporizer (PTV): most versatile inlet

SPLIT/SPLITLESS INJECTOR

(only used in SPLITLESS Mode for Trace Analysis)

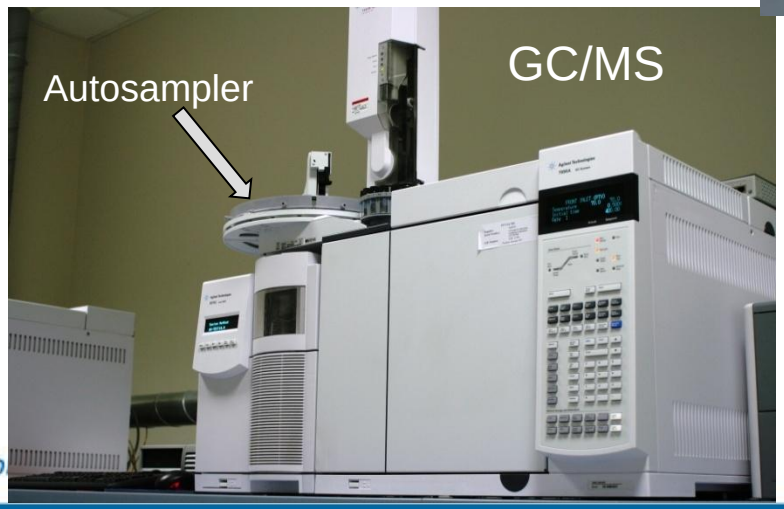




Wiki-Image

EXTRACTION GC/MS

- Extraction in an appropriate solvent (e.g.DCM, IPA, Hexane, WFI...)
- Release of semi-volatile “impurities” – potential leachables
- Inject the extract into a GC/MS



ANALYTICAL TECHNIQUES – D.I.-GC/MS

POLYMER ADDITIVES: e.g. Butylated Hydroxytoluene
Phthalates
Oleamide
Erucamide
Irgafos 168
Irganox 1076

DEGRADATION PRODUCTS: e.g. 2,6-di-*tert*-butyl-p-benzoquinone
1,3-di-*tert*-butylbenzene
Ethylbenzaldehyde
2,4-di-*tert*-butylphenol
3,5-di-*tert*-4-hydroxybenzaldehyde

OTHER IMPURITIES: e.g. Crown-ethers, alkylparabens, styrene , tricyclic sesquiterpenes , diterpenes, 1.4-Isopropanol acetophenone

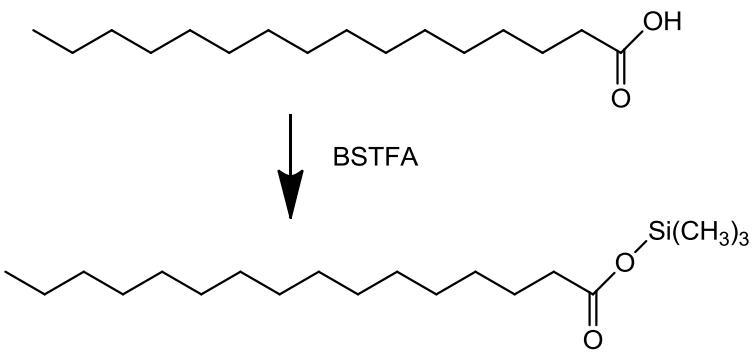
MONOMERS &OLIGOMERS: PBT-dimer,
Rubber oligomers,
Polyoxymethylene oligomers,
Acrylonitrile-Butadiene-Styrene oligomers,
Polyester Oligomers

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).*

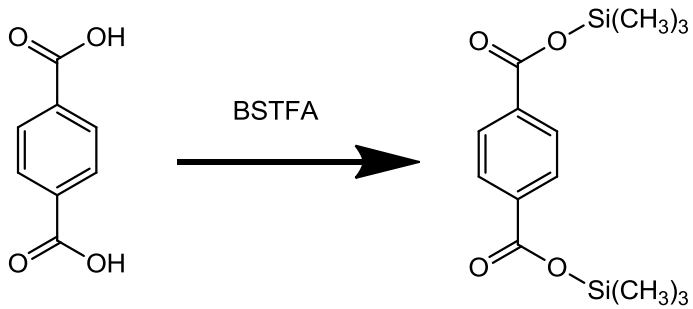
DERIVATISATION GC/MS: EXAMPLES

Peak 1: Palmitic acid



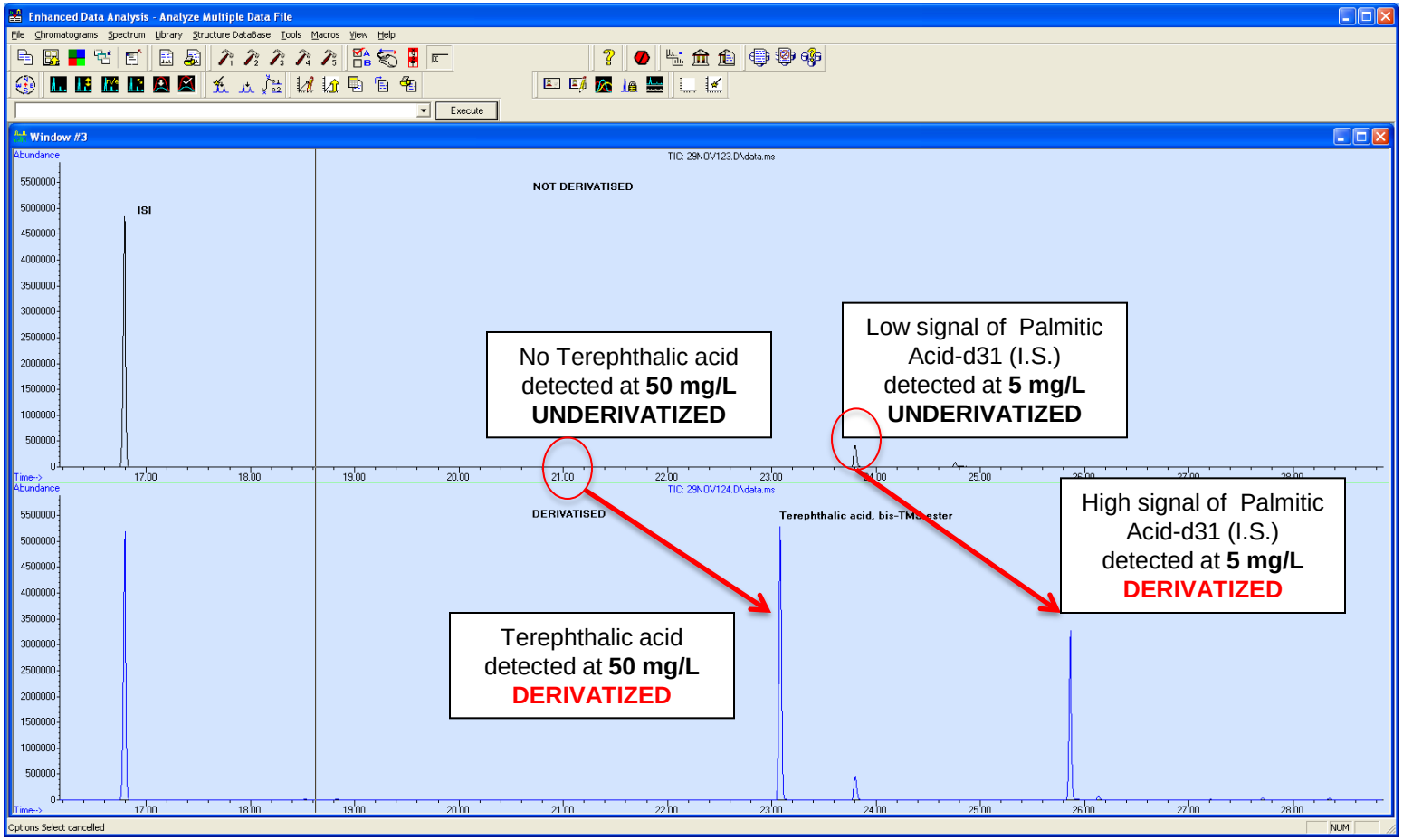
Trimethylsilyl ester of Palmitic Acid

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
- **GC-QQQ** or GC-”Triple Quad” Mass Spectrometer
- **GC-ToF** or GC-”Time-of-Flight” Mass Spectrometer

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

Other GC/MS Techniques

- **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-ToF or GC-"Time-of-Flight" Mass Spectrometer

- Accurate Mass Measurements: what does it bring?

- Principle: Every Atom has a specific Atomic Weight
  - C= 12,00000
  - H = 1,00794
  - O = 15,9994
  - N = 14,0067
  - ...
- Look for the best combination of Atoms which will fit the Accurate Mass the best, Measured with GC-ToF.

GC-TOF Accurate Mass Measurements

Example: a Compound - Accurate Molecular Mass of 136.05243 - was detected.

What could be the Elemental Formula? Using a CALCULATOR

Specify the mass

Accurate mass experimental result: 136.052430

Results:

	MF	Monoisotopic mass	PPM	mDa	unsaturation
1	$C_8H_8O_2$	136.0524295014	0.004	0	5
2	$C_3H_7FN_3O_2$	136.0522296921	1.472	-0.2	1.5
3	$C_5H_{11}ClNO$	136.0529166949	3.577	0.487	0.5
4	CH_8N_6S	136.0531149801	5.035	0.685	1
5	$C_3H_9ClN_4$	136.0515740244	6.292	-0.856	1

Most Probably, the Elemental Formula of this molecule is $C_8H_8O_2$

Cross Examining results of other Analytical results, revealed that this compound is **4-methylbenzoic acid**

However, this conclusion cannot be drawn, based solely on accurate mass!

Other GC/MS Techniques

- **GC-ToF** or GC-”Time-of-Flight” Mass Spectrometer

➢ For extracts with a lot of “Unknown” compounds, the extracts are analyzed with GC-ToF (in E.I. and C.I. Mode) in order to determine the

1. **Molecular Ion and hence the Elemental Composition (CI and/or EI)**
2. **Fragment information (EI)**
3. In combination with existing data, determine more about the **Structure and Source** of the compound
4. In some cases, in combination with **Derivatization Procedure**
5. In some cases, a **full identification** of the compound

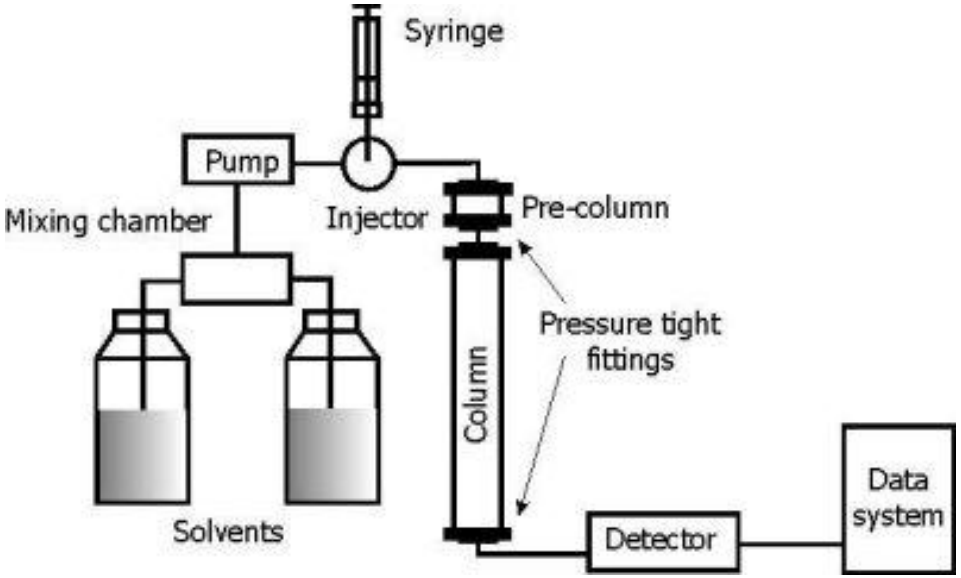
ANALYTICAL TECHNIQUES – LC/MS (UPLC-HRAM)

Chromatograph

High Pressure Liquid Chromatography (HPLC) is a separation technique
- mobile phase: liquid
- stationary phase: polymeric liquid immobilised on silica gel/particles packed in a column.
- Retention of analyte: determined by:
  - dimensions of the analyte
  - pore size
  - the chemical affinity of the analyte for the stationary phase.

Most common:
Reversed-phase chromatography (e.g. C18) with gradient elution

Principle:
Hydrophobic molecules in the polar mobile phase tend to adsorb to the hydrophobic stationary phase, and hydrophilic molecules in the mobile phase will pass through the column and are eluted first.
By decreasing the polarity of the mobile phase, gradually more hydrophobic molecules can be eluted from the column



ANALYTICAL TECHNIQUES – LC/MS (UPLC-HRAM)

However: Overlap with compounds from GC/MS *(Volatile & Semi-Volatile Compounds)*

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

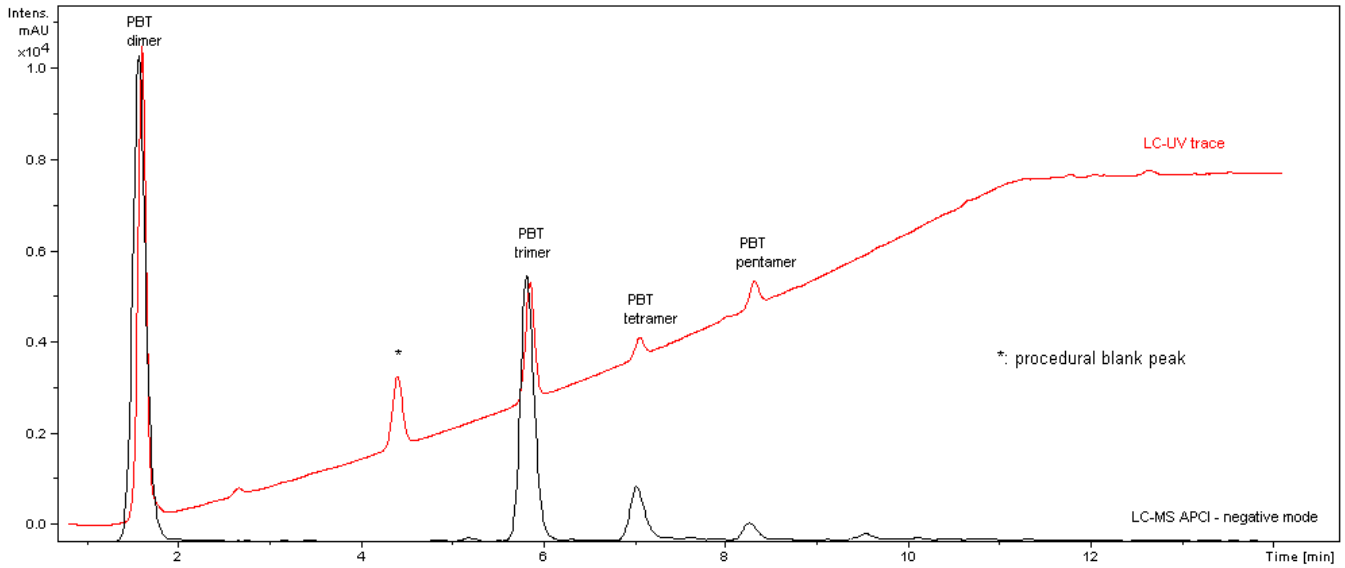
- Standard Equipment in a Lab
- Cost
- UV-Detector can be a *nice addition* to other Detectors, e.g. MS

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?

ANALYTICAL TECHNIQUES – LC/MS (UPLC-HRAM)

- Red trace: UV Chromatogram : detection yes, identification no
- Black trace: LC/MS chromatogram: Identification



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

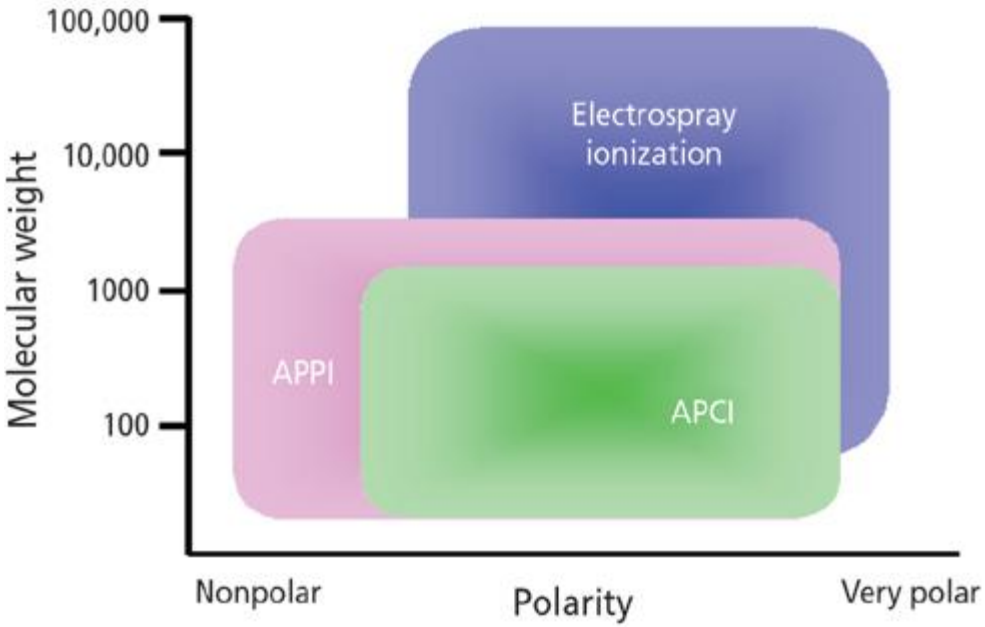
- Cost
- Not a Universal Detector (Target Molecules need to Ionize)
- However, different Ionisation Modes allow a broader detection of Compounds (APCI+/-; ESI+/-)

ANALYTICAL TECHNIQUES – LC/MS (UPLC-HRAM)

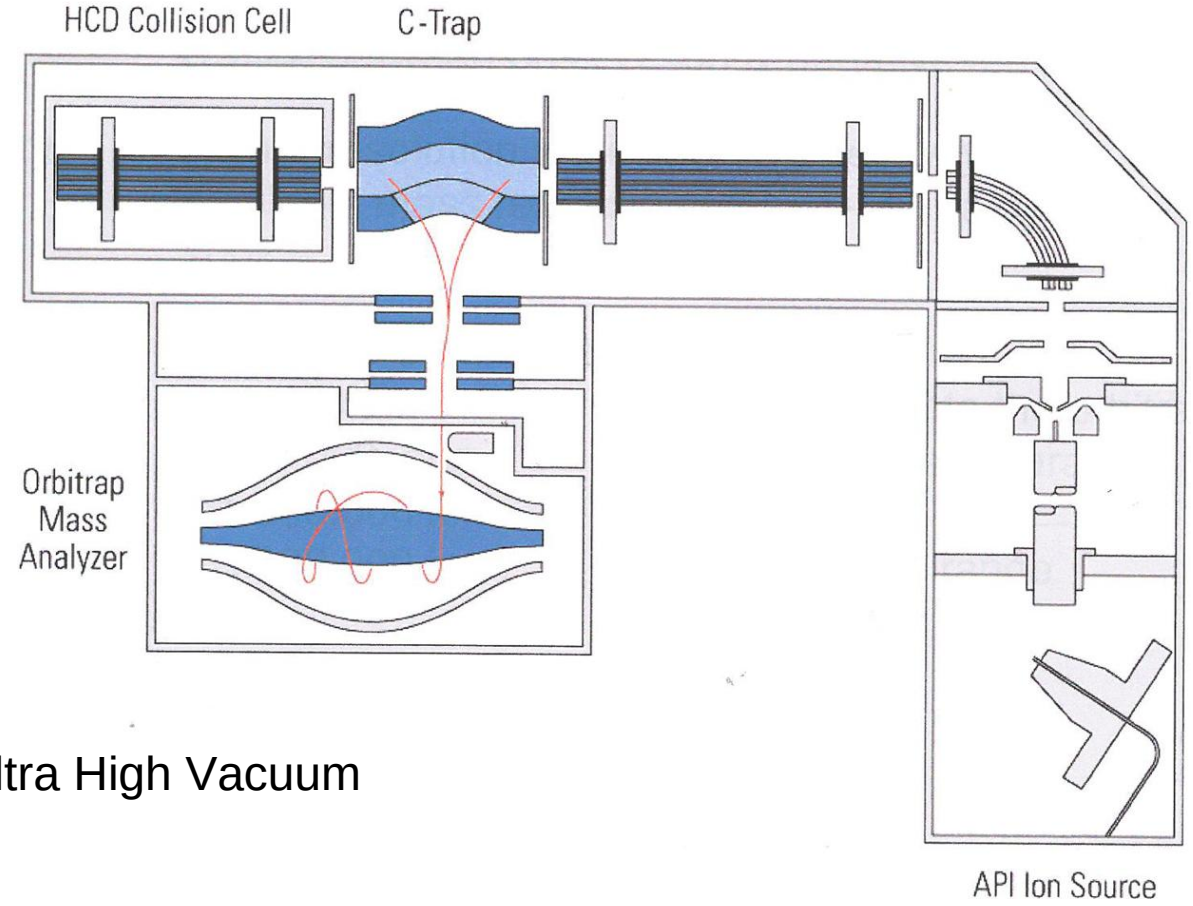
IONIZATION MODES
For Extractables and Leachables testing:

Focus is on Atomic Pressure Chemical Ionization : **small molecules and wide polarity range**

Complement with Electrospray Ionization for detection of very polar compounds



ANALYTICAL TECHNIQUES – LC/MS (UPLC-HRAM)



- MS detection:
  - APCI probe
  - Pre – Vacuum - - - → Ultra High Vacuum
  - Ion optics
  - **Orbitrap = analyzer + detector**
  - **HCD (*all ions* Higher-energy Collision-induced Dissociation)**

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
Orbitrap/Time-of-Flight (ToF)
Accuracy of Mass Detection - Orbitrap:

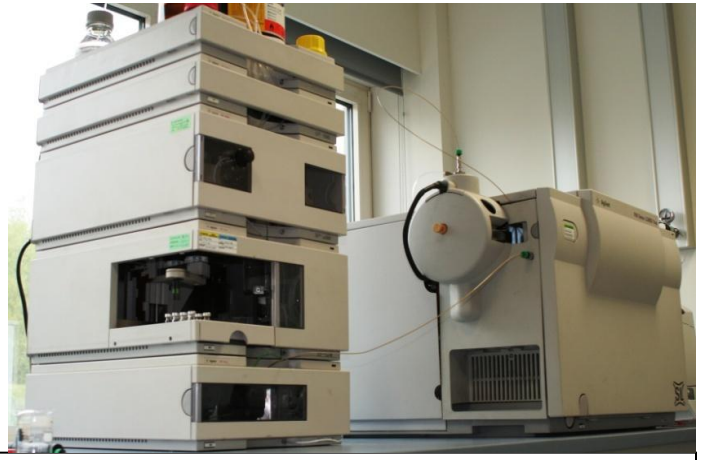
Mass error : sub ppm
m/z 220,2456 can be distinguished from m/z 220,2457

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!

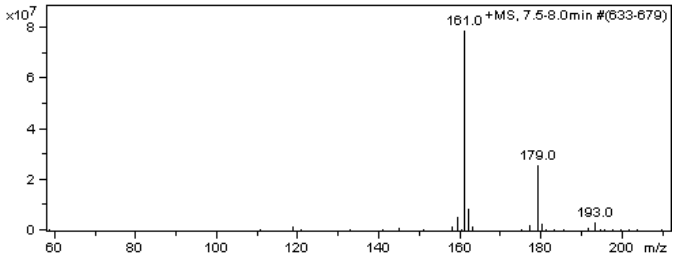
ANALYTICAL TECHNIQUES – LC/MS (UPLC-HRAM)

LC-ION TRAP
(LOW MASS ACCURACY)



LC-ORBITRAP
(HIGH MASS ACCURACY)

LOW RESOLUTION MASS



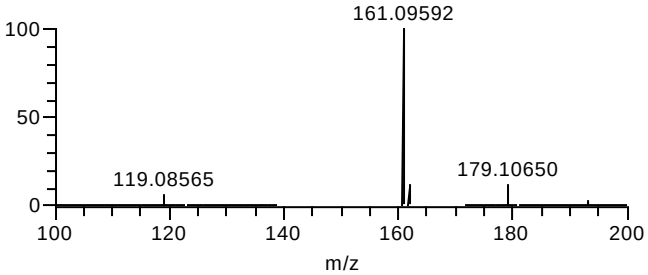
No information

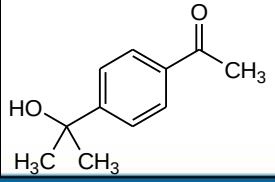
HIGH RESOLUTION ACCURATE MASS

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

Mass error:

1 ppm

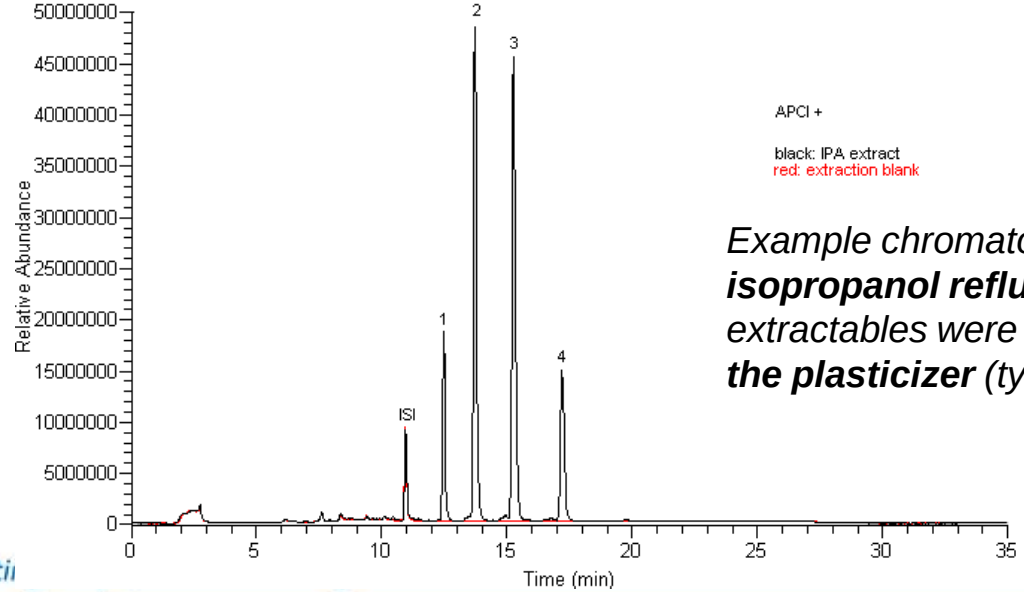




Peroxide curative related compound from EPDM rubber

LC-MS (Non-Volatile Compounds)

- Extraction in an appropriate solvent (DCM,IPA,Hexane,WFI...)
- Release of non-volatile “impurities” – potential leachables
- Inject the extract into a LC/MS



*Example chromatogram in APCI positive mode of an **isopropanol reflux extract of a chlorobutyl rubber**: all extractables were identified as **trimellitate esters from the plasticizer** (typical targets detected by LC/MS)*

ANALYTICAL TECHNIQUES – LC/MS (UPLC-HRAM)

POLYMER ADDITIVES: e.g. BHT
Irganox 1010
Irganox 245
Irganox 1330
Irganox 1076
Irganox 3114
Hostanox 03
Oleamide
Erucamide
EBS
Irgafos 168
Irgafos 126
A.O. 2246

OLIGOMERS: e.g. PBT dimer
PBT trimer
PBT tetramer
PBT pentamer
Caprolactam oligomers
ABS oligomers

DEGRADATION PRODUCTS: e.g. oxidized Irgafos 168, Irganox 245 degradation products, Irganox 1010 degradation products

NUCLEATING AGENTS: e.g. Millad 3988, NC-4

FATTY ACIDS: Myristic acid, Palmitic acid, Oleic acid, Stearic acid

PLASTICIZERS: Trioctyl trimellitate

…

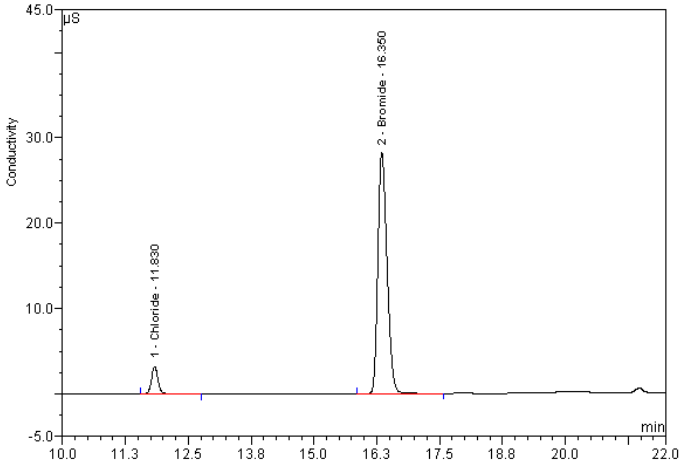
OTHER TECHNIQUES

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

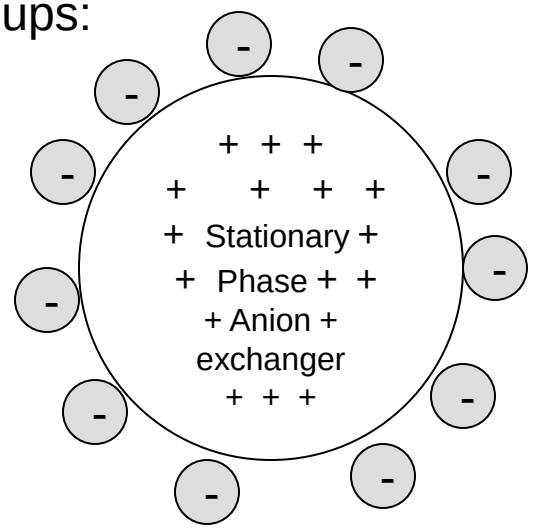
Sample: reflux extract with WFI (water for injection) of a halobutyl rubber

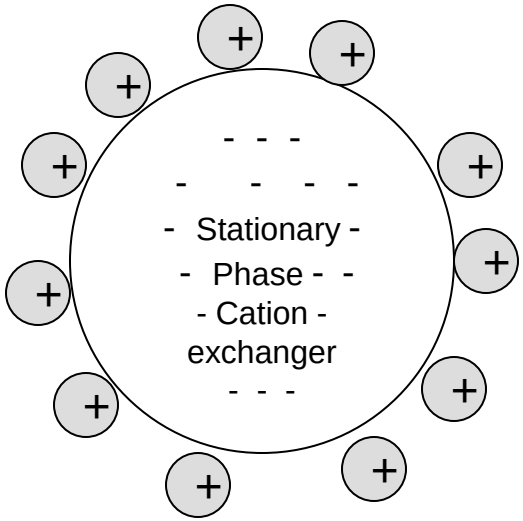


Ion Chromatography

Ion chromatography (or *ion exchange chromatography*) is a chromatographic separation technique based on the charge properties of molecules. Separation depends upon the reversible adsorption of charged solute molecules to immobilized ion exchange groups of opposite charge.

Anion exchange chromatography retains anions using positively charged functional groups:





Applying a salt gradient, ions can be gradually displaced from the stationary phase, eluted and detected (often by a conductivity detector).

ICP-OES

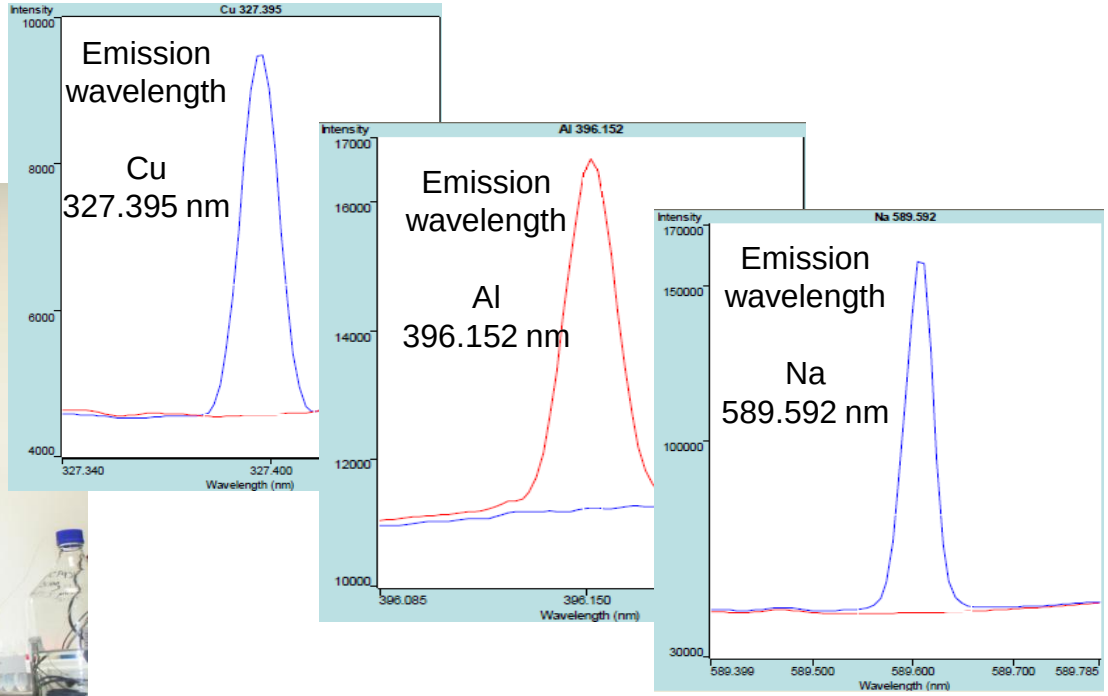
ICP-OES or ICP-MS:

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

ICP-OES

Inductively coupled plasma optical emission spectroscopy (ICP-OES), also referred to as inductively coupled plasma atomic emission spectrometry (ICP-AES), is a spectroscopic technique used for the detection of trace metals. By means of an inductively coupled plasma, atoms and ions are excited and emit electromagnetic radiation at wavelengths specific for each element. The intensity of the emission is a measure for the concentration of an element.

Sample: reflux extract with acidified WFI or acid digestion of the neat polymer



OTHER SPECIFIC METHODS

- ✓ GF-AAS For Silicone Oil Detection
- ✓ 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
- ✓ FTIR – characterization of NVR
- ✓ Total Organic Carbon: *reconsiliation with concentration of organic compounds from chromatographic techniques*
- ✓ ...

ANALYTICAL TECHNIQUES USED FOR LEACHABLES TESTING

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?

For further questions, please contact:
piet.christiaens@toxikon.be

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