Analytical Techniques and Methodologies, used in Extractables and Leachables testing

Dr. Dries Cardoen

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Sample Preparation – Challenges in Trace Analysis

The Most Important and Most Underesetimated Lab Activity!!

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!

Analytical techniques

Different
STERILIZATION & PROCESSING
Modes and Conditions

Each with different
GRADES & FORMULATIONS

Different
SUPPLIERS

A lot of
MATERIALS

A lot of
DEVICES and CONTAINERS

- Processing Aids
- Polymer Additives
- Oligomers
- Curing Agets
- Impurities in Polymer Additives
- Monomer Residues
- Polymer Degradation Compounds
- Glue/Adhesive residues
- Filter Residues
- Degradation Compounds of Oligomers
- Anti-oxidants
- Acid Scavengers
- Slip-Agents
- Polymer Additive Degradation Compnds

UNIVERSE OF EXTRACTABLES:
>10,000 – 100,000 Compounds

NECISSITY TO HAVE
COMBINED Analytical Techniques, Methodologies and **Supporting Tools** to
DISCOVER, IDENTIFY and SEMI-**QUANTIFY**
This broad diversity of chemicals!

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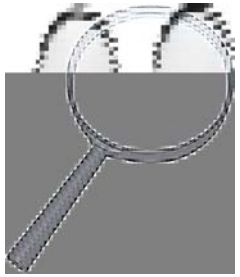
CHROMATOGRAPHIC SCREENING IN EXTRACTABLES & LEACHABLES

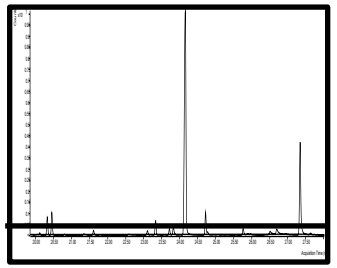
DISCOVER ALL COMPOUNDS

ABOVE A TOX THRESHOLD (AET)

IDENTIFY ALL COMPOUNDS

(SEMI-)QUANTIFY ALL COMPOUNDS





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Extractables Studies:

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

1. A Compound Specific – **INFORMATION RICH** - Detector: **Mass Spectrometry**
2. A **Database** to allow Identification based upon Mass Spectra
   - » Commercial Databases for GC/MS: NIST, WILEY: **Be Careful!**
   - » **PROBLEM for LC/MS**: no Commercial Databases Available!
   - » **Self-Developed Databases** (e.g. NELSON LABS proprietary DB)

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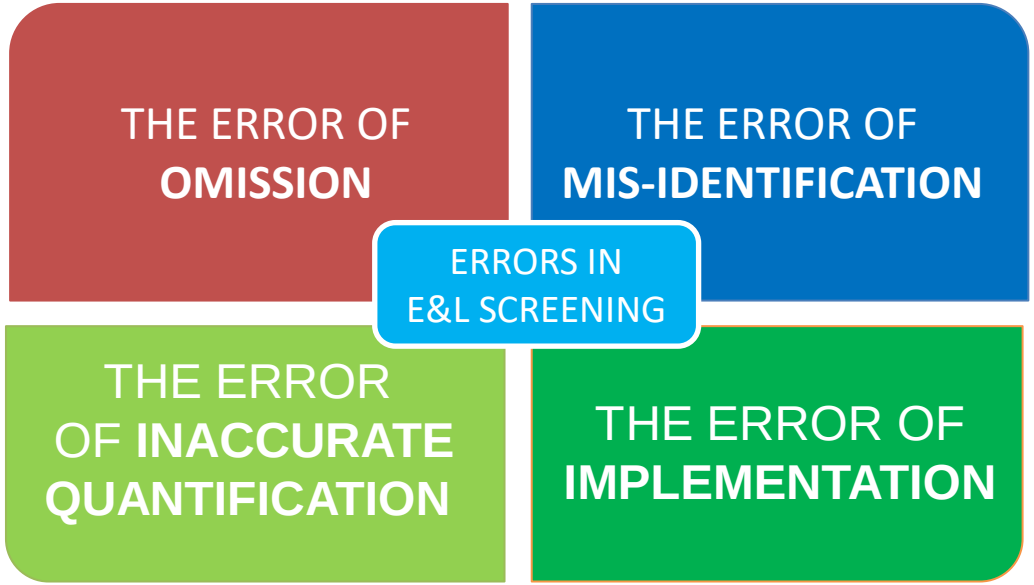
Errors in Organic Extractables Screening



PDA Journal
of Pharmaceutical Science and Technology

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Gas Chromatography with Mass Spectral detection (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!!

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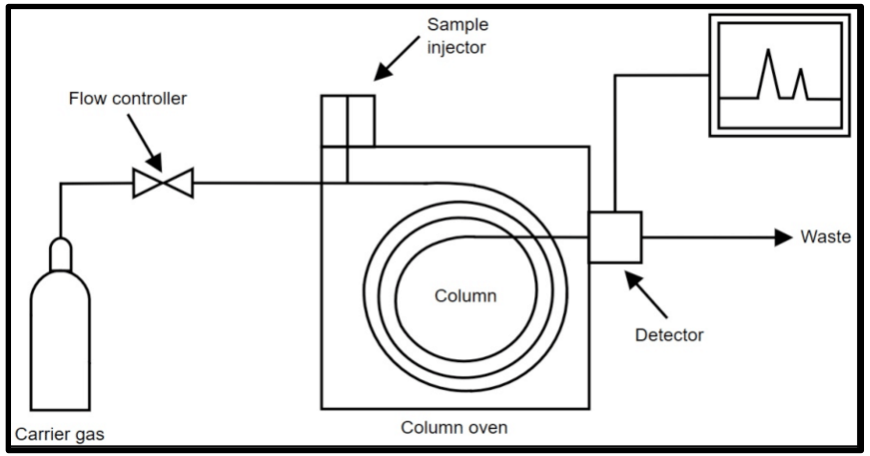
Gas Chromatography with Mass Spectral detection (GC/MS)

"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)*
- **Capillary Column 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....*



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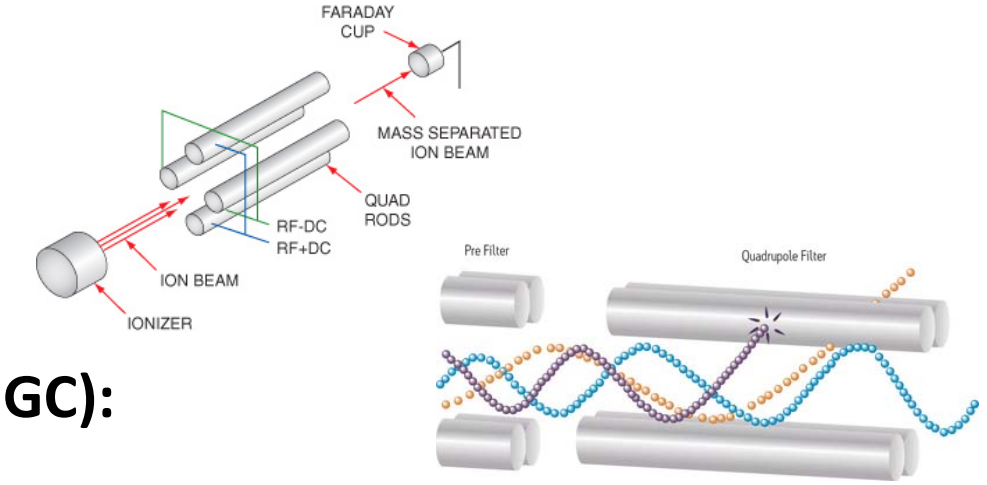
Gas Chromatography with Mass Spectral detection (GC/MS)

"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**!

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Gas Chromatography with Mass Spectral detection (GC/MS)

Standard GC/MS: Quadrupole M.S.

- A GC/MS "Mass Spectrometer" is ***Standardized***:
  1. Quadrupole (or Ion Trap)
  2. **Ionisation: ALWAYS 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

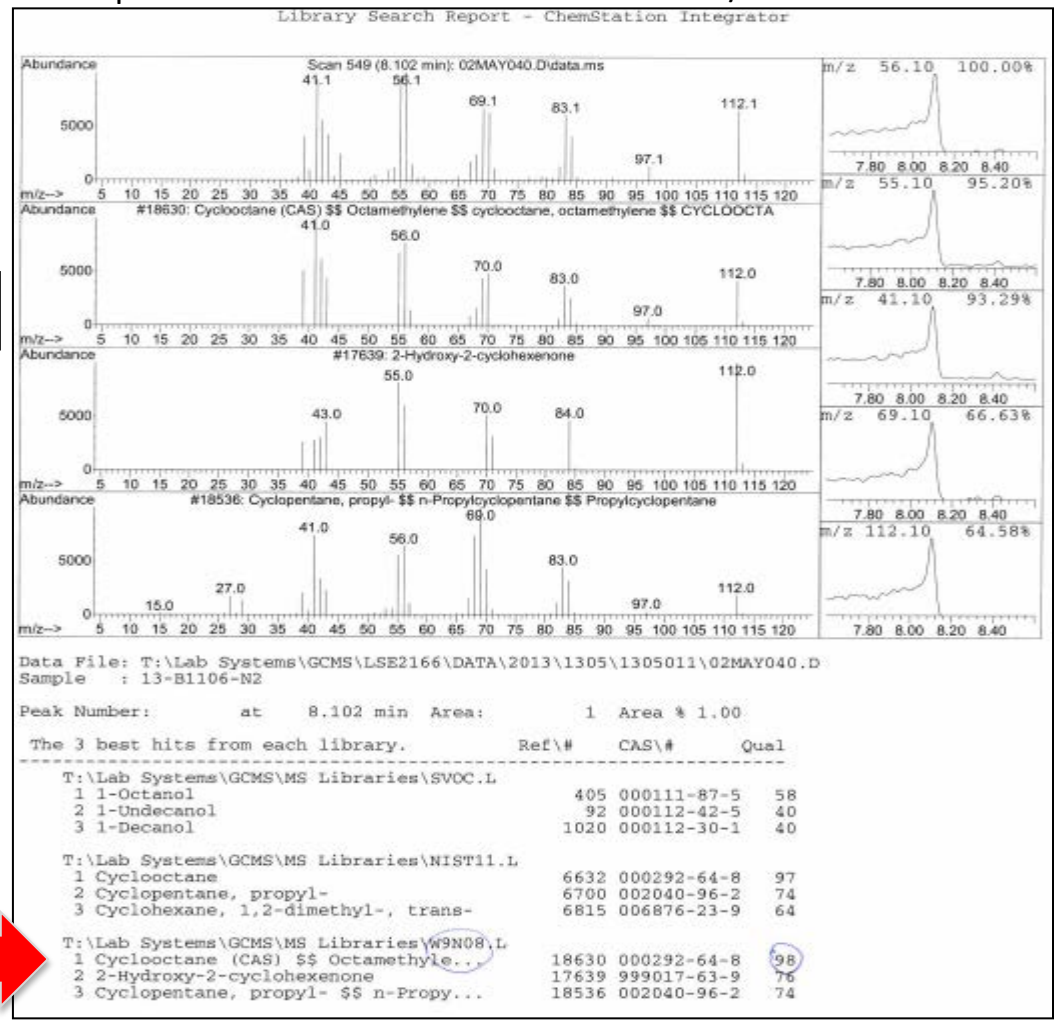
     => Be careful with automated NIST reporting based upon "mass spectal matching"

Gas Chromatography with Mass Spectral detection (GC/MS)

Standard GC/MS: Quadrupole M.S.

Example of FIT of an UNKNOWN MS with NIST/WILEY

CYCLOOCTANE



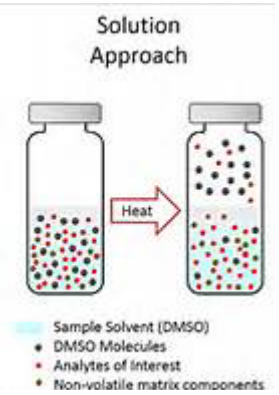
Gas Chromatography with Mass Spectral detection (GC/MS)

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



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Gas Chromatography with Mass Spectral detection (GC/MS)

VOC

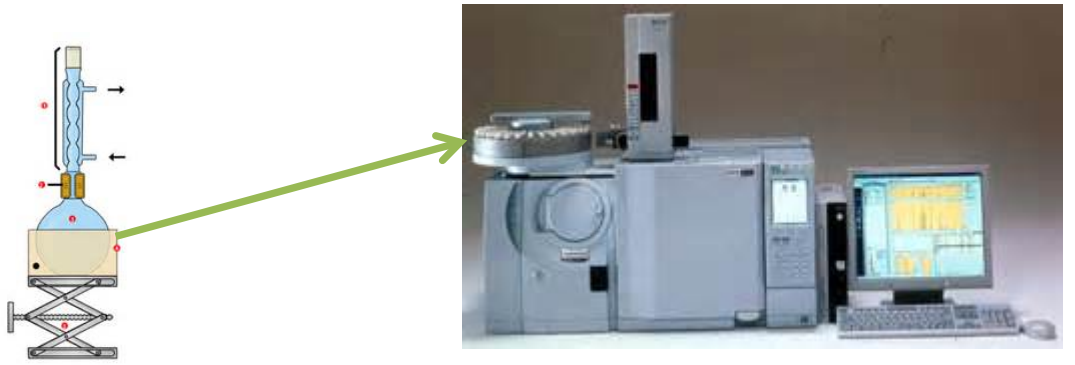
HS-GC/MS
Screening

SVOC

GC/MS
Screening

Semi-Volatile Organic Compounds (MW < 650)

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



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Gas Chromatography with Mass Spectral detection (GC/MS)

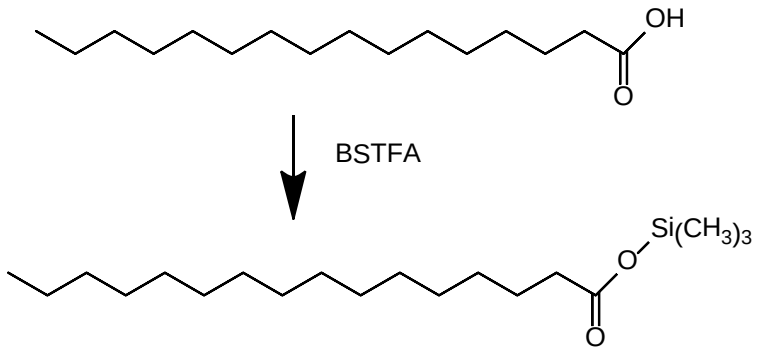
*Derivatisation GC/MS - **POLAR ORGANICS***

- 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!!
- ONE of the solutions is a Derivatisation Method is using **BSTFA** as derivatisation agent *(conversion to more volatile, less polar trimethylsilyl esters).*
- *Another Solution for POLAR Organic Compounds is the use of **ESI+/-** as an ionziation technique for **LC/MS***

Gas Chromatography with Mass Spectral detection (GC/MS)

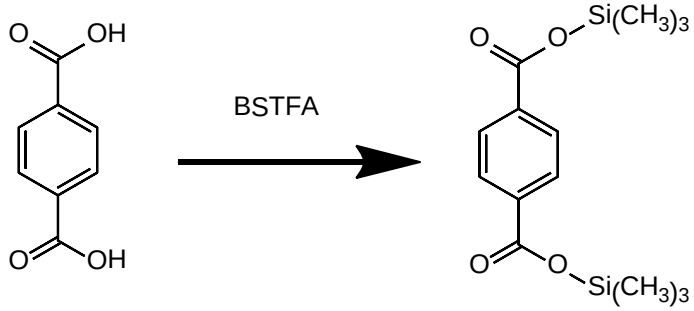
DERIVATISATION GC/MS: EXAMPLES

Peak 1: Palmitic acid



Trimethylsilyl ester of Palmitic Acid

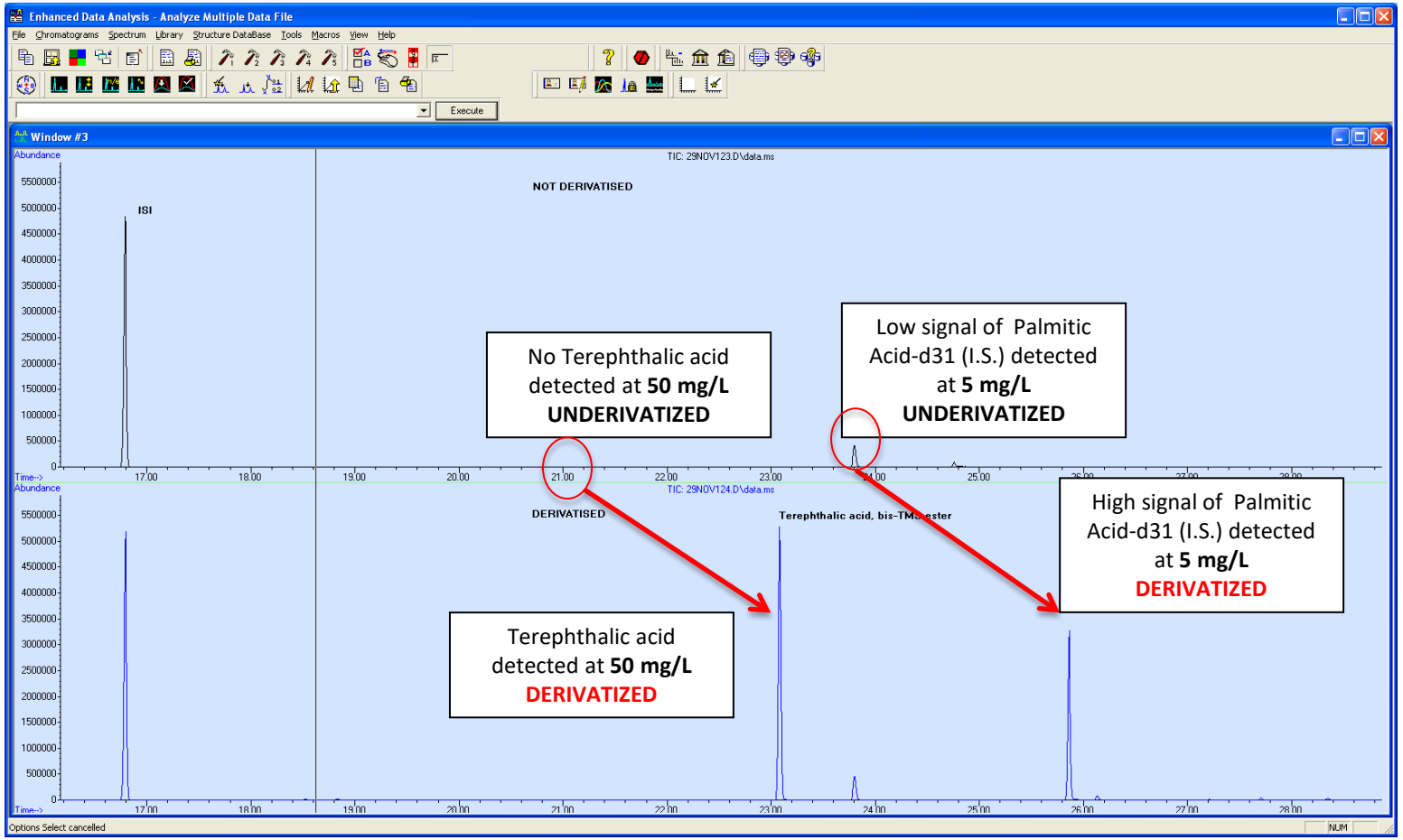
Peak 2: Terephthalic acid



Trimethylsilyl ester of Terephthalic Acid

Gas Chromatography with Mass Spectral detection (GC/MS)

DERIVATISATION GC/MS: RESULTS



Gas Chromatography with Mass Spectral detection (GC/MS)

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

Gas Chromatography with Mass Spectral detection (GC/MS)

Other GC/MS Techniques

GC-(Q)-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-(Q)TOF - Accurate Mass Measurements

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

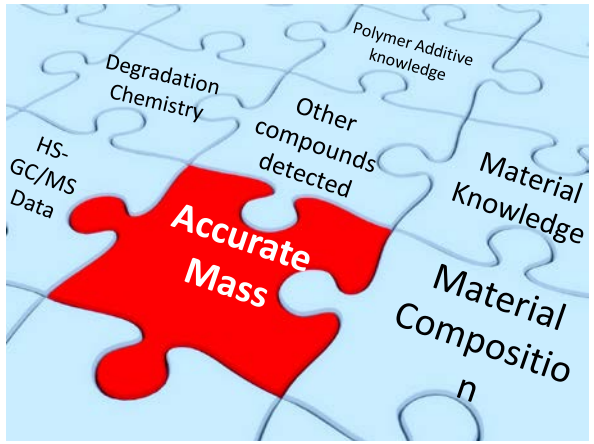
What could be the Elemental Formula? Using a CALCULATOR

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 solely based on accurate mass information!

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Gas Chromatography with Mass Spectral detection (GC/MS)

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

- For ***extracts with a lot of "Unknown" compounds***, the extracts are analyzed with GC-(Q) 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*

Liquid Chromatography with Mass Spectral detection

VOC	SVOC	NVOC
HS-GC/MS **Screening**	GC/MS **Screening**	UPLC/MS **Screening**

Non-Volatile Organic Compounds

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

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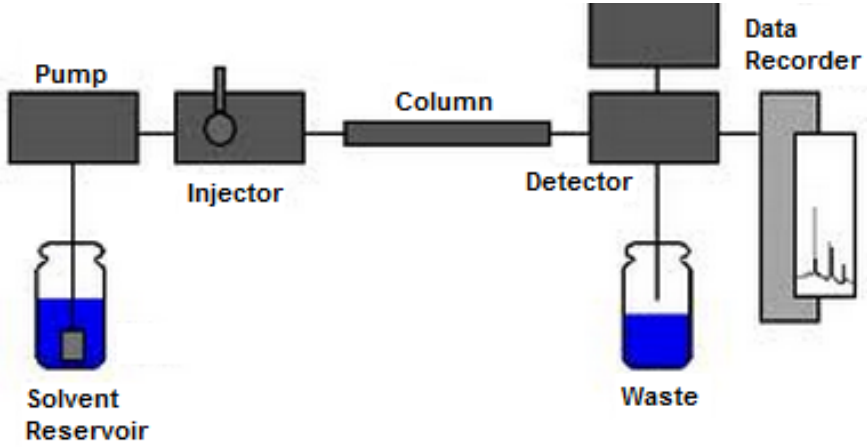
Liquid Chromatography with Mass Spectral detection

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, Diode Array Detector*
  - *CAD detector*
  - *RI detector*
  - *Unit Mass Spectrometer*
  - *Accurate Mass Spectrometer*



Principle of HPLC

Liquid Chromatography with Mass Spectral detection

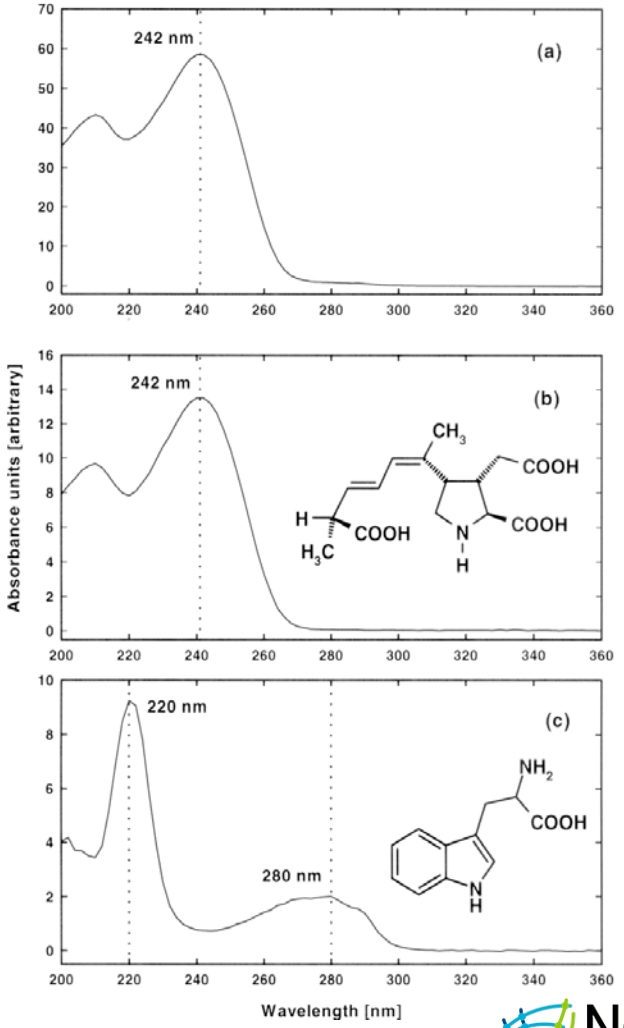
HPLC – UV or Diode Array Detection

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



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Liquid Chromatography with Mass Spectral detection

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)
  However, different Ionisation Modes allow a broader detection of Compounds (APCI+/- and ESI+/-)

Liquid Chromatography with Mass Spectral detection

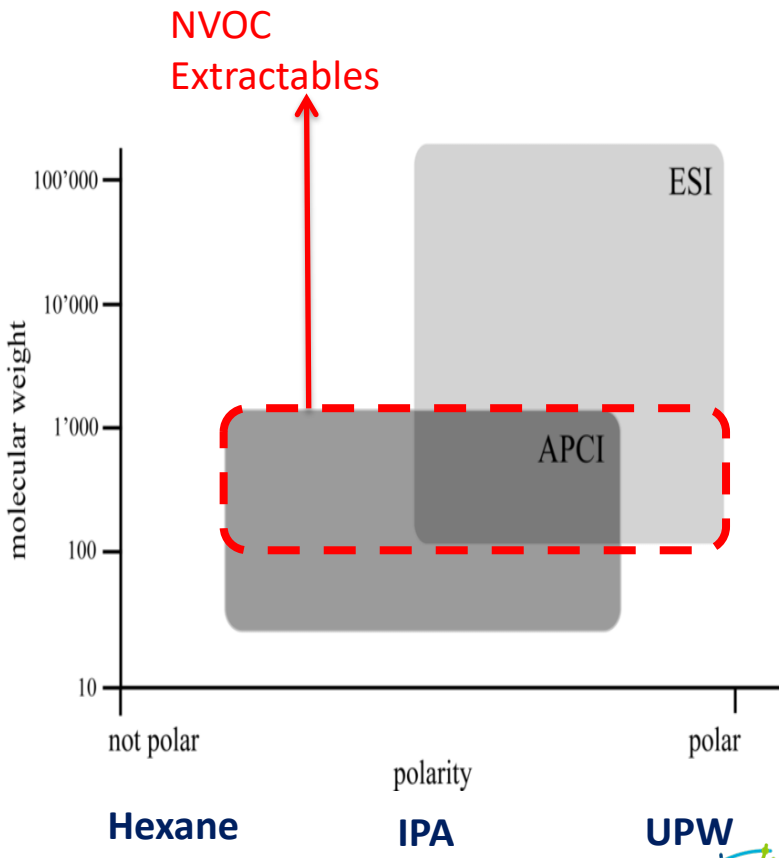
DETECTOR SELECTION FOR LC/MS

- Discussion about the selection of the right detector for LC/MS (ESI vs APCI)

ESI +/-	APCI +/-
Very Polar NVOCs Ionized NVOCs	Low Polar NVOCs Medium Polar NVOCs

Like Dissolves Like

UPW	IPA & Hexane



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Liquid Chromatography with Mass Spectral detection

LC-MS

LOW Resolution LC/MS

Accuracy of Mass Detection: 1 Da

m/z 179 can be distinguished from 180

High Resolution Accurate Mass (HRAM) LC/MS

Accuracy of Mass Detection (Orbitrap): 5 ppm

m/z 179.1066 can be distinguished from m/z 179.1067

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**

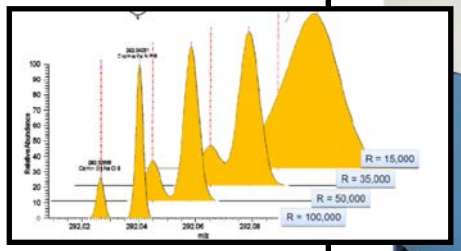
Building specificity into your databases based on mass accuracy and retention time!

Liquid Chromatography with Mass Spectral detection

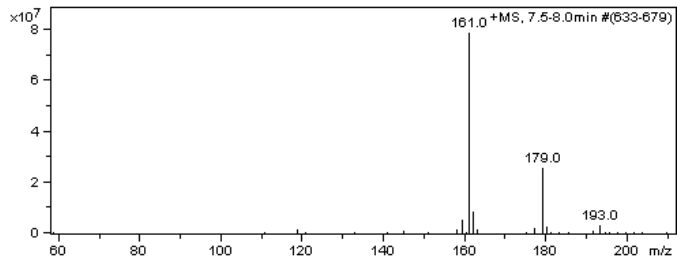
LC- SINGLE QUAD or ION TRAP
(LOW MASS ACCURACY)

LC-ORBITRAP or LC-(Q)ToF
(HIGH MASS ACCURACY)

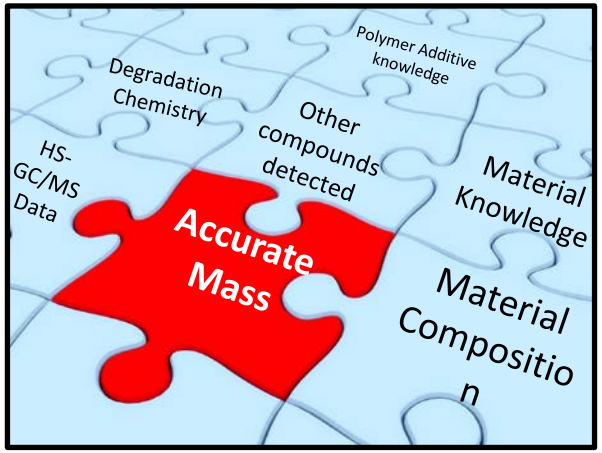
High Mass Resolution



LOW RESOLUTION MASS

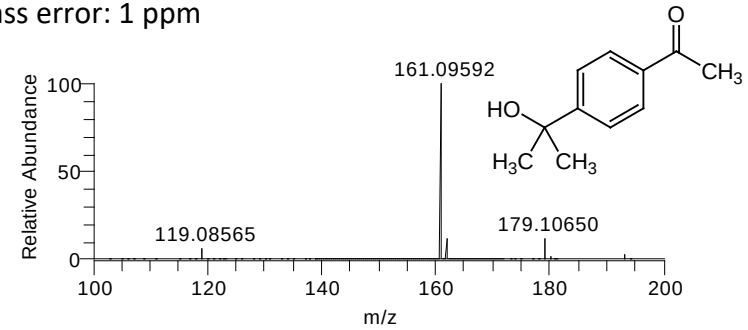


No information



HIGH RESOLUTION ACCURATE MASS

$C_{11}H_{14}O_2$ exact monoisotopic mass: 179.10666
Mass error: 1 ppm



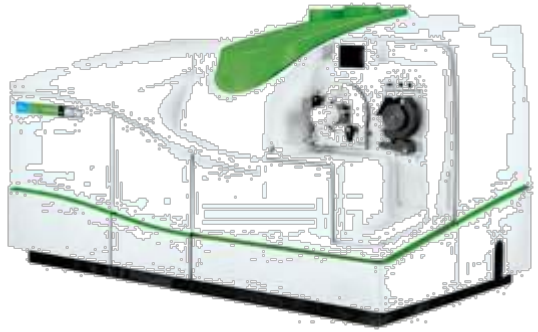
Peroxide curative related compound from EPDM rubber
(based on corroborating information together with HRAM data)

Inductively Coupled Plasma

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

ELEMENTS

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



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Inductively Coupled Plasma

ICP-OES or ICP-MS:

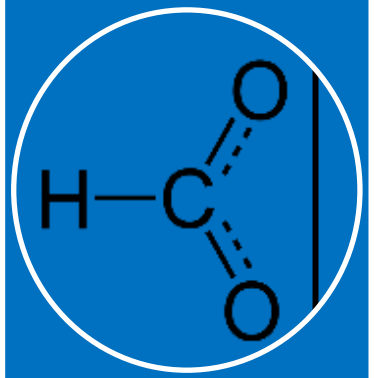
- Metals from Glass
- Metals from Rubbers
- Catalysts, used in the polymerization
- Fillers, added to Polymers
- Acid Scavengers
- Activator systems for Rubbers
- ...

ICP-MS

ICP-OES

Ion Chromatography

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



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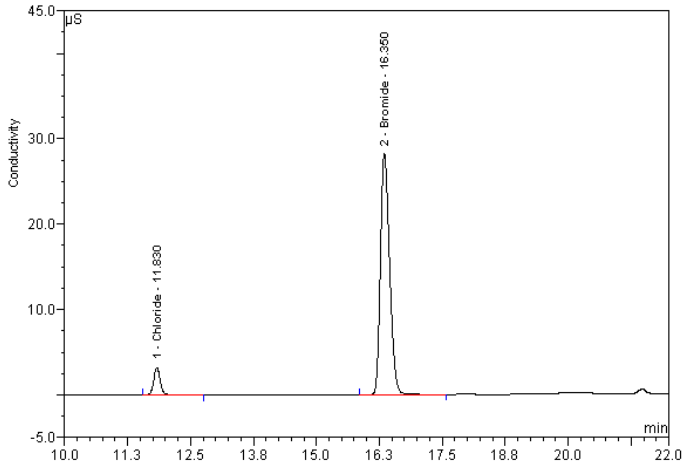
Ion Chromatography

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



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** (gravimetric residue)
- ✓ **FTIR** – characterization of NVR
- ✓ **Total Organic Carbon**: *reconsiliation with concentration of organic compounds from chromatographic techniques*
- ✓ …

Analytical technieques used for leachables testing

Analytical technieques used for leachables testing

- ✓ 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...

Questions?

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