

# PDA Training Course Extractables & Leachables

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## Analytical techniques used in E&L studies

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# Overview


- Analysis of extractables & leachables is a challenge!
- Analytical techniques for organic compounds
- Analytical techniques for inorganic compounds
- Screening methods vs validated methods
- Structural elucidation

# The diverse world of extractables

Why the analysis of E&L is a challenge...

# Diversity in CCS

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)

# Pharmaceutical CCS

## 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*
- ...

# Materials of construction for CCS

- 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
- ...

# Suppliers for a given material

## **Polyethylene** - produced by:

- *Borealis*
- *LyondellBasell*
- *SABIC*
- *Dupont*
- *Enichem*
- *INEOS*
- *TOTAL*
- ...

## **Pharmaceutical Rubbers** – main Global Suppliers:

- *Datwyler*
- *West Pharmaceutical*
- *Stelmi*

**Each supplier has different (health care) grades!**

# Each supplier: different grades

## **Polyethylene (PE)** - 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*



# Impurity profile of 1 grade

## INTENTIONALLY ADDED

- Pigments / colorants
- Clarifying agents
- Catalysts and Curing Agents
- Fillers
- Anti-oxidants
- Plasticizers
- Photostabilizers
- Slip agents
- Acid scavengers
- ...

## NON-INTENTIONALLY ADDED

- Related to the Polymer
  - Polymer Degradation Compounds
- Related to the Polymerization Process
  - Solvent residues
  - Monomers
  - Catalysts
  - Oligomers
- Related to the additives
  - Additive degradation compounds
- Related to secondary packaging
  - Glue, Labels, Carton/Paper
- Processing Impurities
  - Lubricants, surfactants, solvents
- ...

# Conclusion: diverse chemistry!

## PHYSICO-CHEMICAL PROPERTIES OF EXTRACTABLES

- Organic ↔ Inorganic
- Polar ↔ Non-polar
- Volatile ↔ Non-volatile
- Inert ↔ Reactive
- Small ↔ Large
- Charged ↔ Not charged

**UNIVERSE OF EXTRACTABLES:** 10.000 – 100.000 compounds

- Analytical method: identification and quantification

## COMBINATION OF ANALYTICAL TECHNIQUES REQUIRED

- For routine screening: labs need to be cost-effective
- Only possible with extensive material knowledge & databases

# Sample Preparation

The most important & most underestimated activity in the lab

# Trace analysis is a challenge

- Have **very experienced people** in sample preparation team
- Very **intensive training** for new staff in sample prep team
- **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?
- **UPW 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!

**KEEP YOUR  
WORK AREA  
CLEAN**  
IT IS PART OF YOUR JOB



# Organic compounds

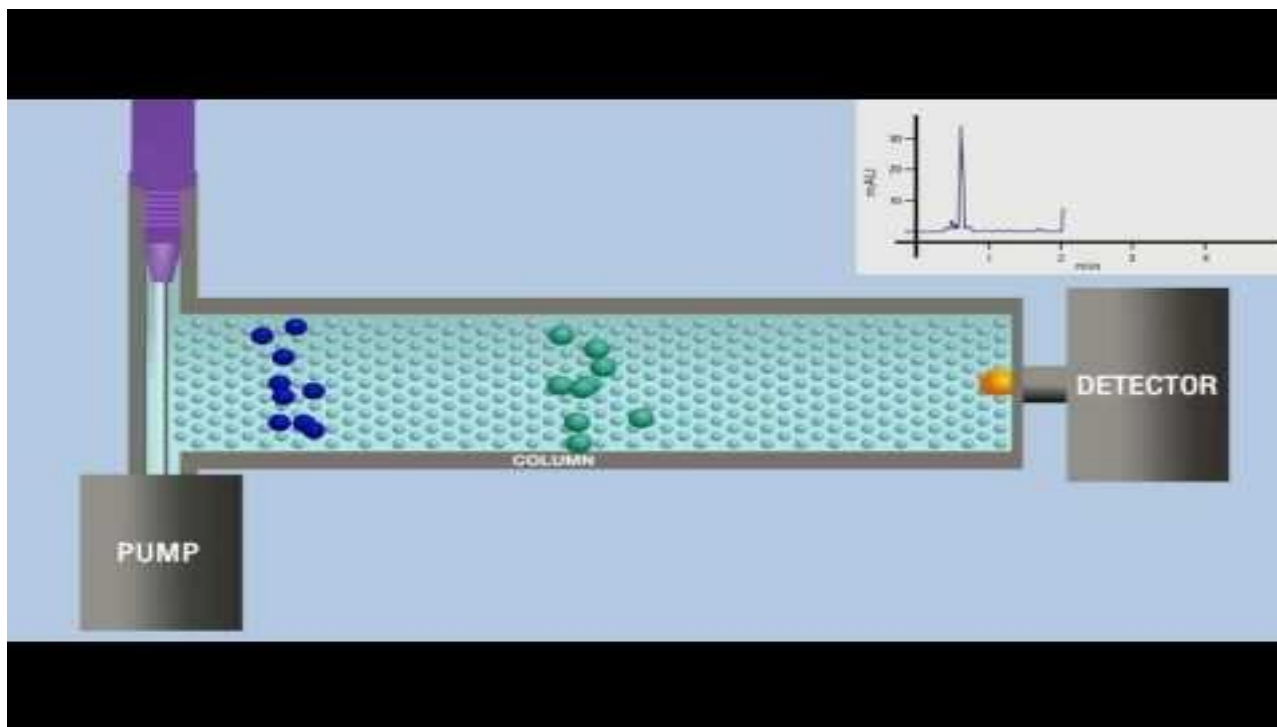
Chromatography – Mass Spectrometry

# Chromatography – Mass Spectrometry

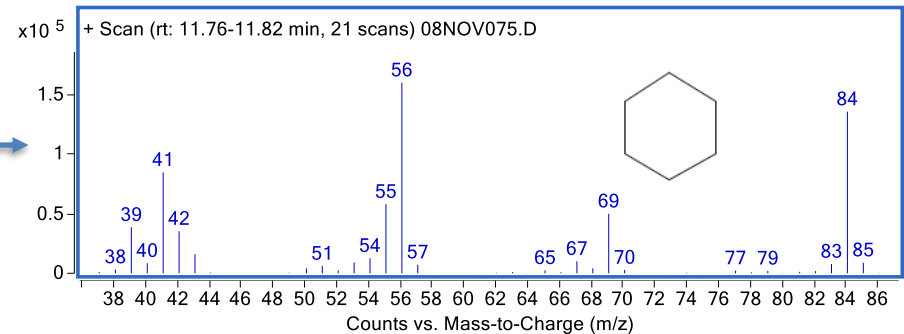
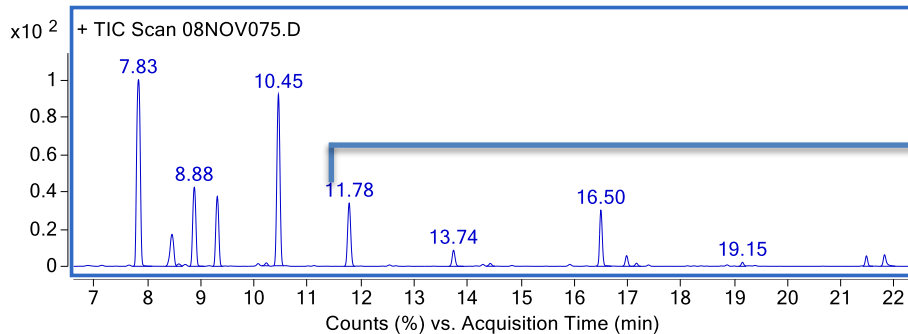
- Complex mixture of compounds!
- Analysis is 2-step process:
  - Separation
  - Detection (+ structural information of detected compound)
- Chromatography:
  - Separation technique
  - Involves 2 ‘phases’: stationary phase + mobile phase
- Mass Spectrometry:
  - Detection technique hyphenated to the chromatography system
  - Mass information of detected compounds

# Chromatography – Mass Spectrometry

Video animation on chromatography separation principle



# Chromatography – MS output



## Chromatogram

- Analytical output from chromatography system
- Detector signal intensity *in function of* analysis time
- Compound separation
- Retention time → depends on compound properties
- Peak area → measure of **quantity**

## Mass spectrum

- Analytical output from mass spectrometer
- Compound detection, but does more!
- Mass (fragment) information for each peak in chromatogram
- Very powerful tool for **identification**



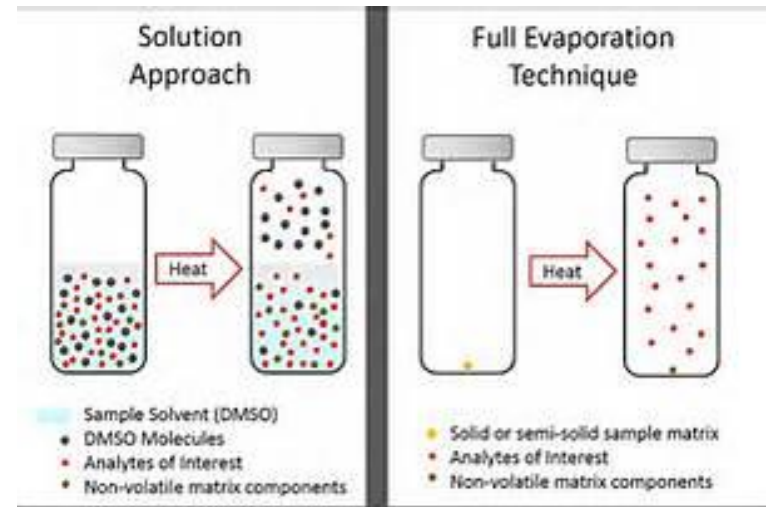


# Volatile Organic Compounds (VOC)

## Headspace – Gas chromatography – Mass Spectrometry (HS-GC/MS)



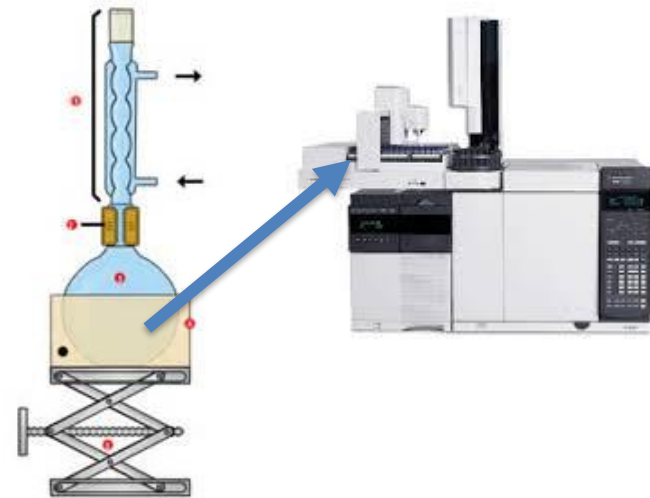
- Monomer residues
- Solvent residues from production steps
- Residues from polymer treatments
- Small polymer degradation products



# Semi-Volatile Organic Compounds (SVOC)

## Gas chromatography – Mass Spectrometry (GC/MS)

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



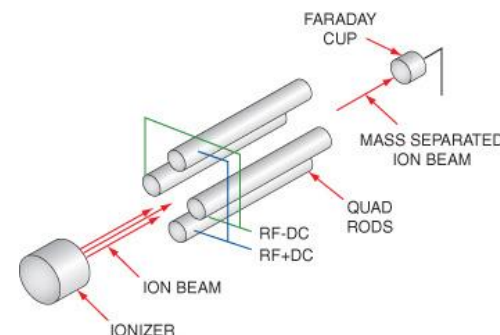
# GC

## SEPARATION of (semi-)volatile organic compounds (Mw < 650 Da)

- **Gas phase** separation technique using **narrow open tubular columns** coated with a **film of stationary phase**, mounted in temperature-programmable oven
- Separation of compounds based on **boiling point** and **polarity** owing to variations in **affinity with the stationary phase**
- A higher **film thickness** of stationary phase increases retention:
  - VOCs: high film thickness ( $\geq 1 \mu\text{m}$ )
  - SVOCs: low film thickness ( $\leq 0.25 \mu\text{m}$ )
- **Length** of capillary column increases resolution (but increases analysis time as well)
- Not well suited for polar compounds like acids, amines, diols... Where specific conditions may need to be applied

# MS (coupled to GC)

## DETECTION & MASS-BASED SEPARATION

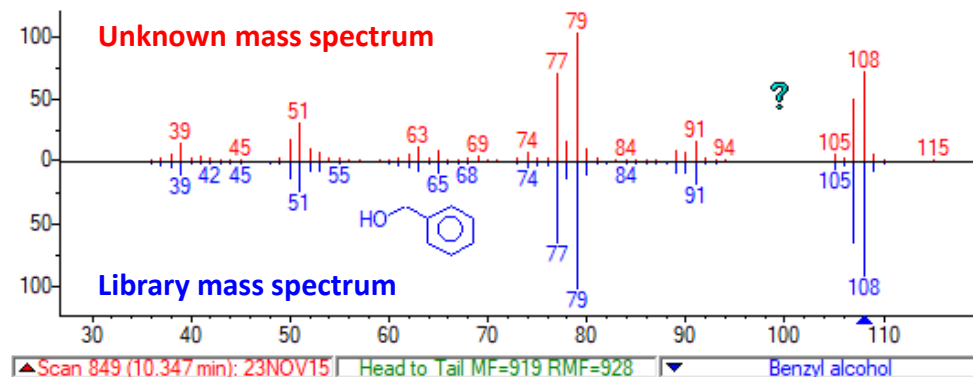


- 3 events: ionization / mass separation / detection – all happening under high **vacuum**
- Ionization: electron ionization (70 eV) → convert molecule into ion and induce further **fragmentation**
- **Quadrupole** mass analyzer:
  - Scanning mass filter → only 1 mass can pass through a given electric 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 (~ 3 scans/second)

# GC/MS spectrum

## GC/MS spectra are “standardized”

- Most GC/MS instruments for routine use make use of electron ionization – single quad technology
- Electron ionization (and associated molecule fragmentation) is a **very reproducible event**
  - ➔ Reproducible mass spectra are obtained across different instruments across the world
- Obtained mass spectra can be compared to commercial databases or in-house databases
  - ➔ In case of a good match may lead to identification of the compound



# Non-Volatile Organic Compounds (NVOC)

## Ultra Performance Liquid chromatography – Mass Spectrometry (UPLC/MS)

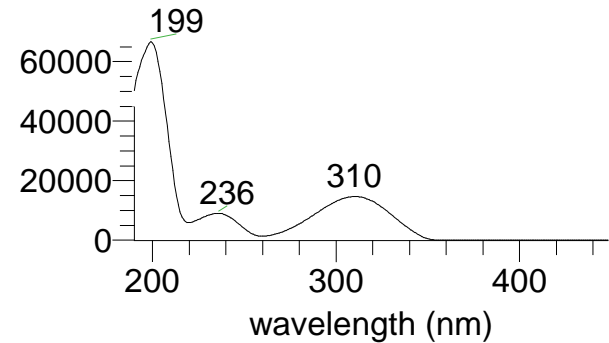
- Fillers
- Plasticizers
- Antioxidants
- Anti-slip agents
- Oligomers



# UPLC

- **Separation** technique suited for non-volatile organic compounds (NVOCs)
- **Liquid-based** separation technique using columns **packed** with stationary phase
- Using **high pressure** to pump sample – dissolved in mobile phase – through packed column
- Separation of compounds based on **affinity for the stationary phase**
  - Polar stationary phases: straight phase chromatography
  - Apolar stationary phases: reversed phase chromatography (most used)
- Optimizing separation by
  - Selection of chromatographic column (length, polarity of stationary phase)
  - Selection of mobile phase (water, methanol, acetonitrile)
- **Detection:**
  - Diode Array Detection (DAD – using UV spectrum)
  - (high resolution – accurate mass) Mass Spectrometry (primary choice)

# DAD/UV detector



## Advantages:

- Standard equipment in analytical lab
- Low cost
- UV detection simultaneous with MS detection: can be used as add-on detector

## Disadvantages:

- Not universal / generic (chromophore needed for detection)
- Limited sensitivity, depending on chromophore(s)
- Poor specificity, even for Diode Array Detectors (scanning UV)
  - Information about detected molecule is limited (e.g. link with API?)



# MS (coupled to LC)

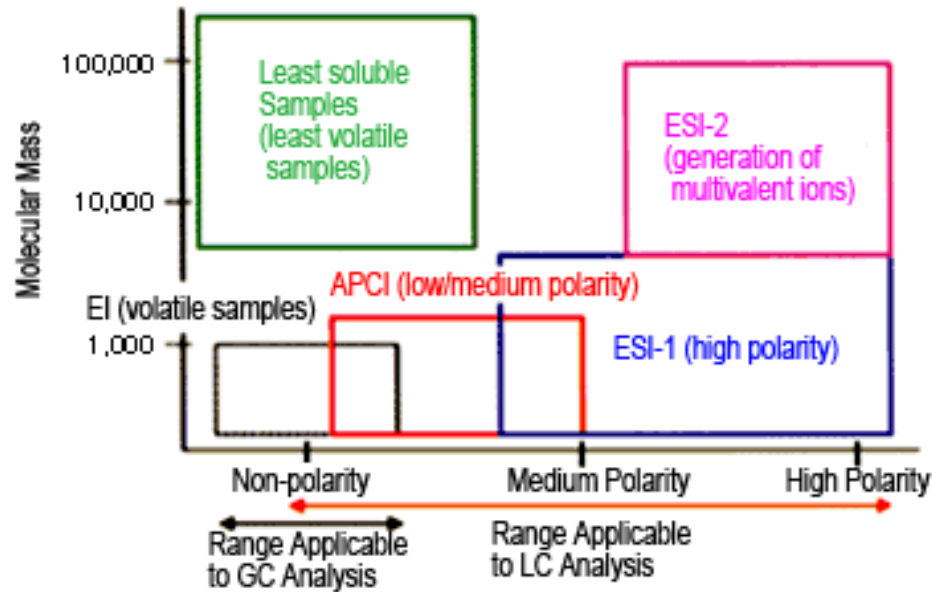
## Advantages:

- Increased specificity: (exact) mass
- Increased sensitivity
- Mass spectra may reveal more information about the identity of the compound
- Allows for building (in-house) mass spectral databases

## Disadvantages:

- Higher cost
- Contrary to GC/MS: no universal spectra (depends on ion source design, mobile phase, MS settings, ...) → no universal libraries!
- Need for multiple ionization methods to allow a broader range of target compounds for UPLC/MS

# Ionization vs Compound Range



- **Electron Ionization:** only works in gas phase under vacuum → not LC compatible
  - **Atmospheric Pressure Chemical Ionization:** LC up to medium polarity
  - **ElectroSpray Ionization:** LC medium polarity – high polarity
- Nowadays: more and more both APCI & ESI in E&L study design

# Modern LC/MS instrumentation

Older systems:

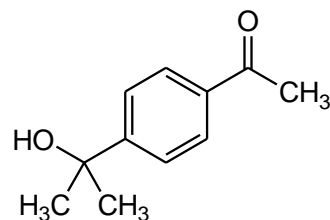
- **Quadrupole** or **ion trap** (cf. GC/MS)
- Low resolution: unit mass e.g. m/z 220 can be distinguished from m/z 221

Nowadays:

- **Q-TOF** or **Orbitrap** technology
- **High resolution & mass accuracy (HRAM)** e.g. m/z 220.000 can be distinguished from m/z 220.002
- High accuracy may allow determination of elemental formula when molecular ion is detected
- Extremely powerful technique in combination with UPLC when developing **in-house high resolution MS databases** in combination with retention time of reference compounds
- Contrary to GC/MS, UPLC/HRAM-MS is used in “first-pass” screening to compensate for the lack of mass spectral fingerprinting and availability of commercial databases like in GC/MS

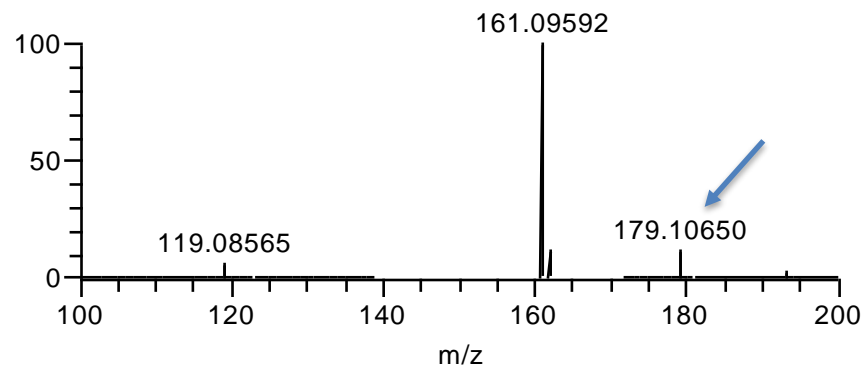
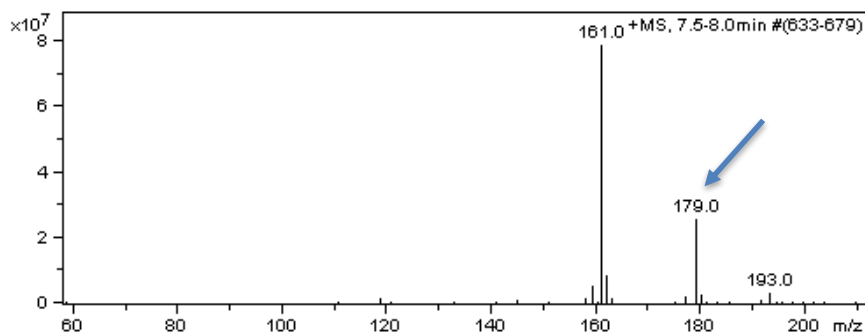
# Modern LC/MS instrumentation

**LC-QUADRUPOLE  
(LOW RESOLUTION)**



Peroxide curative related compound from EPDM rubber  
**Exact mass: 179.10666**

**LC-ORBITRAP  
(HRAM)**



**Mass error: 0.16 mDa or 1 ppm**

# Inorganic Compounds

Analytical Techniques

# Elements

## Inductively Coupled Plasma / Optical Emission Spectroscopy or MS



### Origin of elements

- Metals from glass
- Metals from rubbers
- Catalysts, used during polymerization process
- Fillers, added to polymer materials
- Acid scavengers
- Activators for rubber polymerization

### Technique

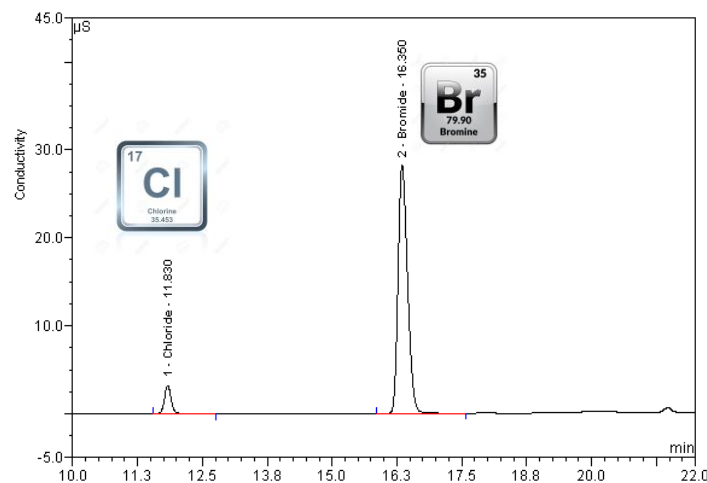
- ICP to produce **excited atoms**
- Excited atoms recombine, giving off electromagnetic radiation at **wavelengths characteristic for each element**
- Emission wavelengths detected by the spectrophotometer
- Or ions detected by mass spectrometry
- Intensity correlates to concentration → **quantitative** technique

# Anions

## Ion Chromatography (IC)

### Origin of anions

- Polyolefins: formate / acetate as oxidation products
- Halobutyl rubbers: bromide, chloride, fluoride
- Fluoropolymers: fluoride
- Trace impurities: nitrite, nitrate, phosphate, sulfate



*Example: UPW extract of a halobutyl rubber*

### Technique

- Special liquid chromatography technique
- Designed for separation and detection of ions
- Detection: conductivity or amperometry

# Other specific analytical methods

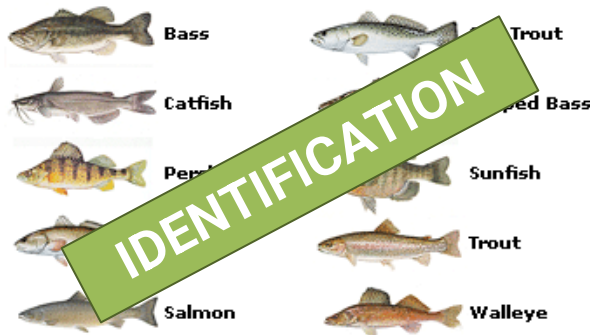
- **GF-AAS** for silicone oil detection and quantification
- **HPLC-UV** for **TMPTMA** (glue residue)
- HPLC-UV for **S<sub>8</sub>** (cross-linker)
- **pH** (release of acidic/alkalinic agents in UPW)
- **Conductivity** (release of salts in UPW)
- **Non-Volatile Residue** (gravimetric residue after evaporation of extract)
- **FTIR** – characterization of NVR
- **Total Organic Carbon**: *reconciliation with concentration of organic compounds from chromatographic techniques*
- ...



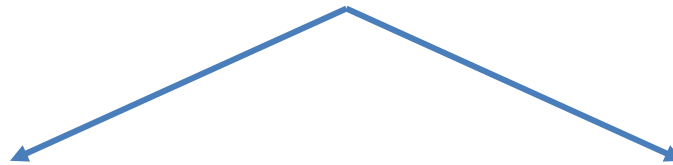
# Screening | Discovery

Identification & Quantification

# Different fishing techniques



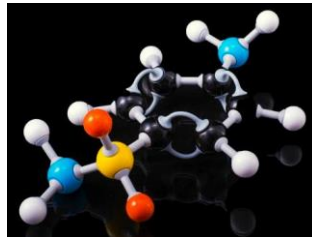
# Different analytical techniques



**IDENTIFICATION**

**QUANTIFICATION**

CAS No XXXXXXXX-YY-Z  
2 to 7 digits  
2 digits  
1 check digit

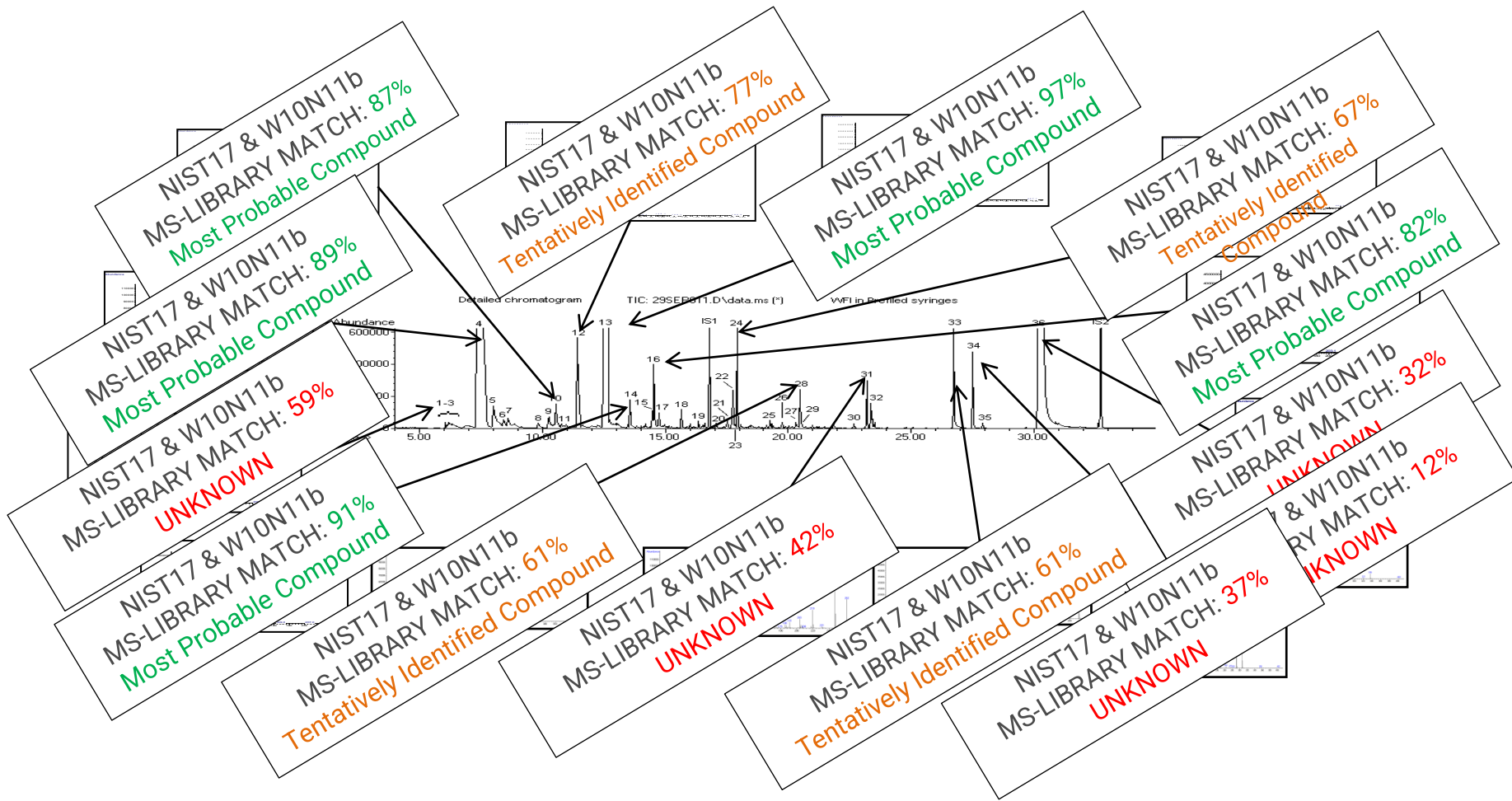


**Concentration**  
 $\mu\text{g/L}$   
 $\mu\text{g/unit}$        $\mu\text{g/g}$

# Concept of “screening” or “discovery”

- **Untargeted** analysis mode used in extractables studies (organic comp)
- 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 **estimated** or **semi-quantitative**: estimation of concentration
- Useful for follow-up in a leachables study

# Concept of “screening” or “discovery”



# Quantification in screening

Screening is untargeted → no prior knowledge about extractables / leachables profile

In case many extractables reported → accurate quantification for all is not practically feasible

## Estimated quantification

- Internal standard (I.S.) compound spiked to each (final) extract
- Assumption that response of analyte = response of I.S. (response factor = 1)
- Accounts for instrument variation
- Does not account for different response vs I.S. or liquid/liquid recovery

## Semi-quantitative quantification

- Internal standard (I.S.) compound spiked to each (final) extract
- Record analytical response of standard vs response of I.S. → relative response factor (RRF)
- Correct concentrations of confirmed ID's with RRF
- Accounts for instrument variation + response variation of analyte vs I.S.

# Validated Methods

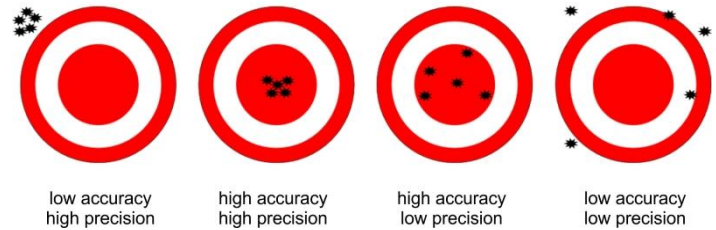
For accurate quantification

# Validated methods

- Chromatography – Mass Spectrometry instrumentation more or less the same
- Except: triple quadrupole (QqQ) instead of single quadrupole (selectivity + sensitivity)
- Validated methods are targeted → **leachables** to be quantified are a priori known
- Methods are **specifically developed and optimized** for the target leachables

## Validated quantification

- **Specific internal standard for each target leachable**
- **Quantitative performance of method is validated:**
  - **Selectivity / Specificity** → no interference from blank signal, drug matrix, other leachables...
  - **Limit of detection / Limit of quantification** → lowest concentration level for accurate quant
  - **Linear range** → concentration range validated for accurate quantification
  - **Precision** → variability of analytical method
  - **Accuracy** → closeness to true value





# Structural Elucidation

High-end Mass Spectrometry

# Structural elucidation - Introduction

- **Unknown / Partially identified compounds > AET** in 1<sup>st</sup> pass screening
  - Unknowns are treated as carcinogenic/mutagenic
  - To allow de-risking by tox assessment, a **structure is needed!**
  
- Request to **further increase ID level** (e.g. low margin of safety)
  - Tentative to Confident
  - Confident to Confirmed (standard should be available or synthesized)
  
- Goal of identification studies: generate / collect comprehensive set of **supporting data to increase the identification level** of a target compound

# Structural elucidation - Instrumentation



## Liquid Chromatography

- Orbitrap
- FT-Ion Cyclotron Resonance

## Requirements

- High-end mass spectrometers
- (Very) high resolution
- High mass accuracy
- Multiple ionization methods
- Tandem mass spectrometry

## Gas Chromatography

- Q-TOF
- Orbitrap



# Structural elucidation - HRAM

Element	Nominal Mass	Exact Mass
Hydrogen (H)	1	1.0078
Carbon (C)	12	12.0000
Nitrogen (N)	14	14.0031
Oxygen (O)	16	15.9949



Nitrogen gas: N<sub>2</sub>

Nominal mass: 28 Da  
Exact mass: 28.0062 Da



Carbon monoxide: CO

Nominal mass: 28 Da  
Exact mass: 27.9949 Da

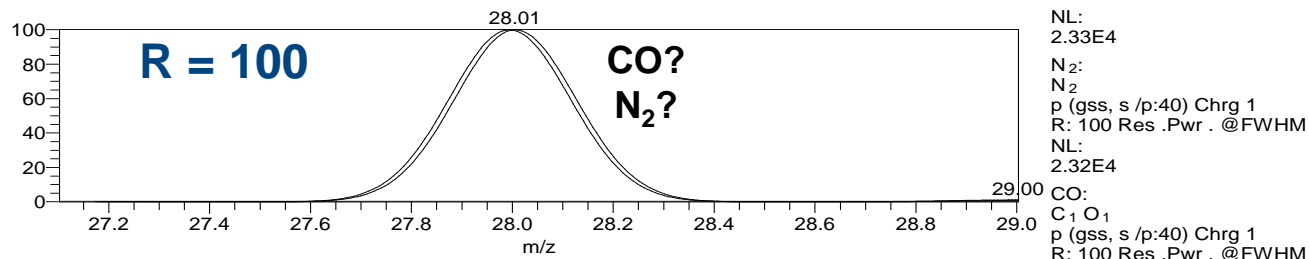


**Difference: 0.0113 Da**

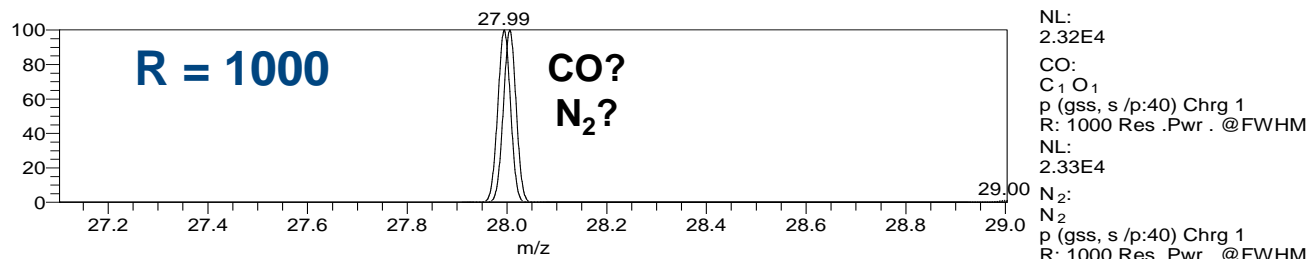
# Structural elucidation - HRAM

Misidentification of a compound with a mass of 28 Da can be fatal... how to be sure?

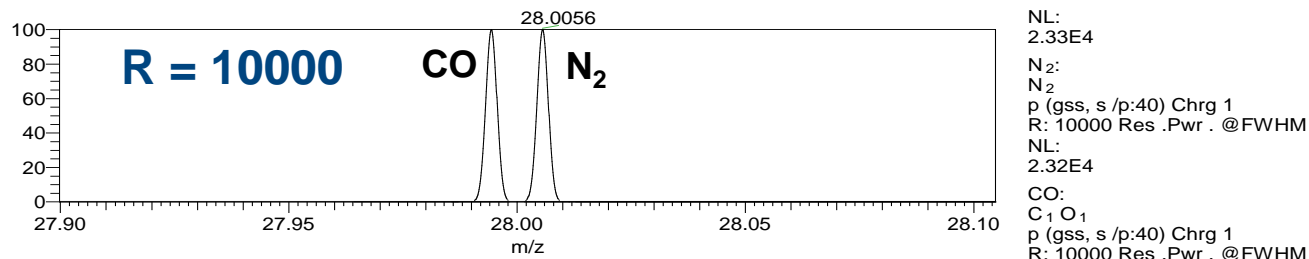
**Not separated**



**Not separated**



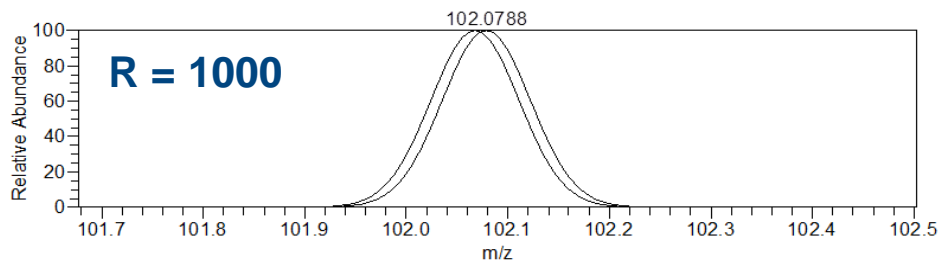
**Separated**



# Structural elucidation - HRAM

E&L example: 2 compounds where both have nominal mass 102...

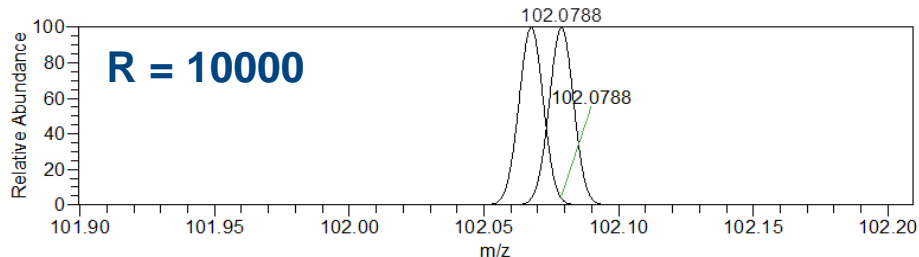
Not separated



NL:  
2.22E4  
C<sub>4</sub>H<sub>10</sub>N<sub>2</sub>O:  
C<sub>4</sub>H<sub>10</sub>N<sub>2</sub>O<sub>1</sub>  
p (gss, s /p:40) Chrg 1  
R: 1000 Res .Pwr . @FWHM

NL:  
2.21E4  
C<sub>5</sub>H<sub>10</sub>O<sub>2</sub>:  
C<sub>5</sub>H<sub>10</sub>O<sub>2</sub>  
p (gss, s /p:40) Chrg 1  
R: 1000 Res .Pwr . @FWHM

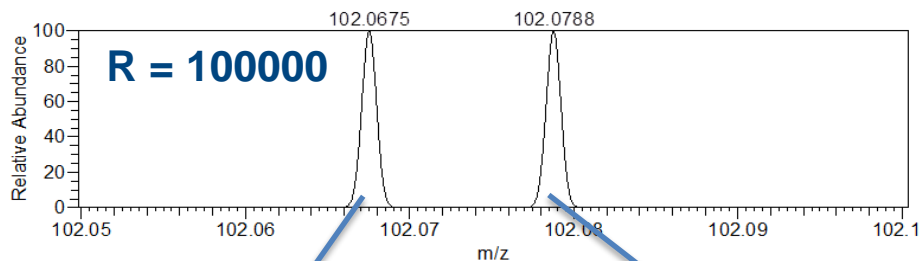
Close...



NL:  
2.22E4  
C<sub>4</sub>H<sub>10</sub>N<sub>2</sub>O:  
C<sub>4</sub>H<sub>10</sub>N<sub>2</sub>O<sub>1</sub>  
p (gss, s /p:40) Chrg 1  
R: 10000 Res .Pwr . @FWHM

NL:  
2.21E4  
C<sub>5</sub>H<sub>10</sub>O<sub>2</sub>:  
C<sub>5</sub>H<sub>10</sub>O<sub>2</sub>  
p (gss, s /p:40) Chrg 1  
R: 10000 Res .Pwr . @FWHM

Separated



NL:  
2.22E4  
C<sub>4</sub>H<sub>10</sub>N<sub>2</sub>O:  
C<sub>4</sub>H<sub>10</sub>N<sub>2</sub>O<sub>1</sub>  
p (gss, s /p:40) Chrg 1  
R: 100000 Res .Pwr . @FWHM

NL:  
2.21E4  
C<sub>5</sub>H<sub>10</sub>O<sub>2</sub>:  
C<sub>5</sub>H<sub>10</sub>O<sub>2</sub>  
p (gss, s /p:40) Chrg 1  
R: 100000 Res .Pwr . @FWHM

C<sub>5</sub>H<sub>10</sub>O<sub>2</sub> - isopropyl acetate

C<sub>4</sub>H<sub>10</sub>N<sub>2</sub>O - N-nitrosodiethylamine

# HRAM – Important take-aways

accurate mass alone **does not deliver a structure...**

... but delivers **the elemental formula** of the molecule and fragments of the molecule

high resolution **does not deliver a structure...**

... but enables to **separate molecules** with the same nominal mass but different elemental formulas

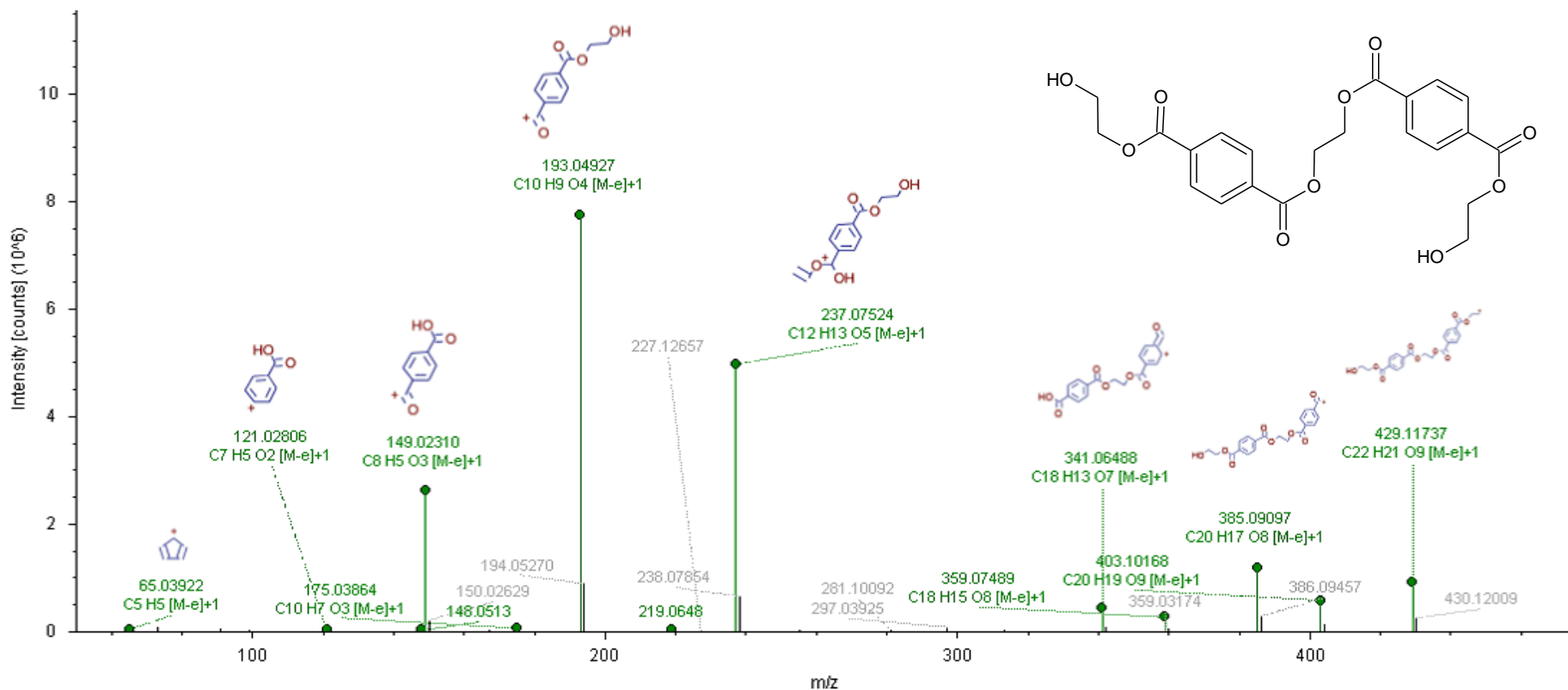
...but assists in confirming the elemental formula using isotope matching

**Mass spectral interpretation skills and expertise are required**



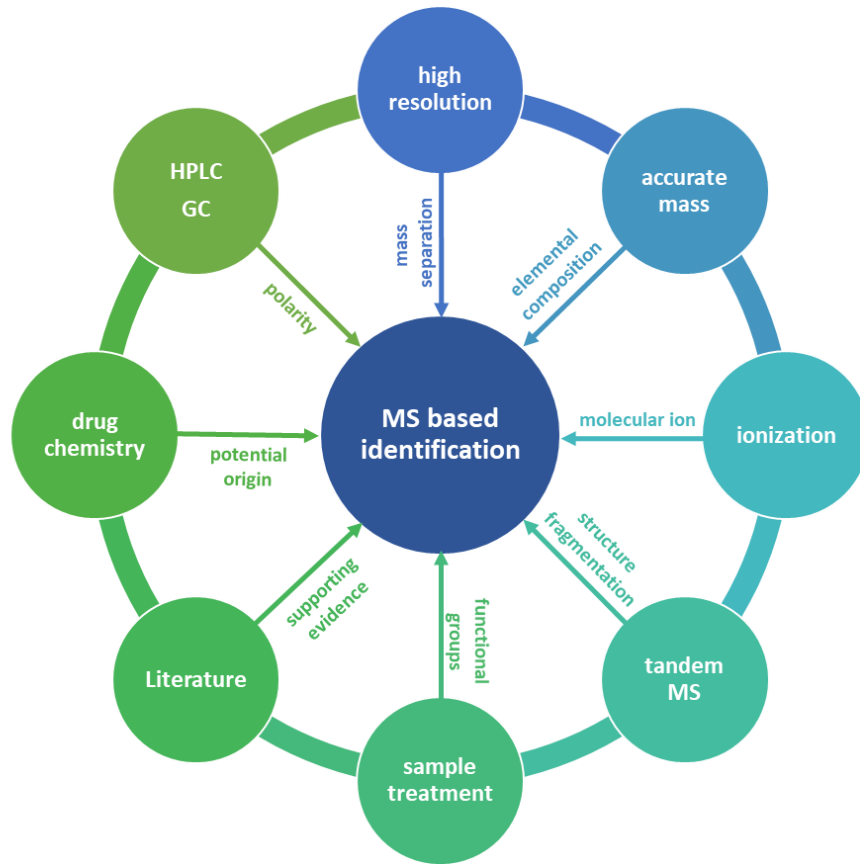
# Tandem Mass Spectrometry (MS<sup>n</sup>)

1. Select molecular ion & induce fragmentation
2. Measure all molecule fragments with HRAM





# Multi-angle approach required



**TEAM effort!**

Mass spec expert(s)

Drug chemistry expert

Material engineer

