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!
  - The Diverse World of Extractables
  - Sample Preparation - Extract
- Analytical techniques for Organic Compounds
  - Instrumentation
  - Screening & Discovery of Organic Compounds – FIRST PASS Approach
  - Structure elucidation – SECOND PASS Approach
- Analytical techniques for inorganic compounds
- Validated Methods

Analysis of extractables & leachables is a challenge!

The Diverse World of Extractables

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)

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

Analysis of extractables & leachables is a challenge!

Sample Preparation - Extract

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!

Analytical techniques for Organic Compounds

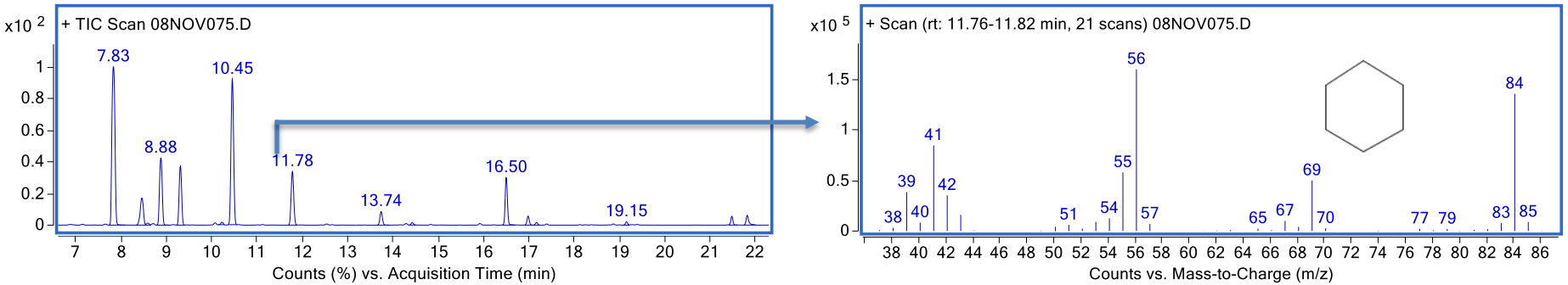
Instrumentation

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 – 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**

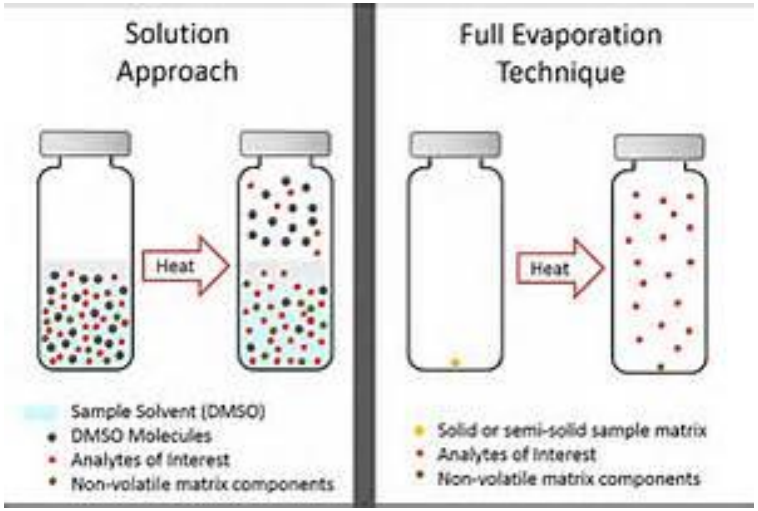
Mass spectrum

- 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)

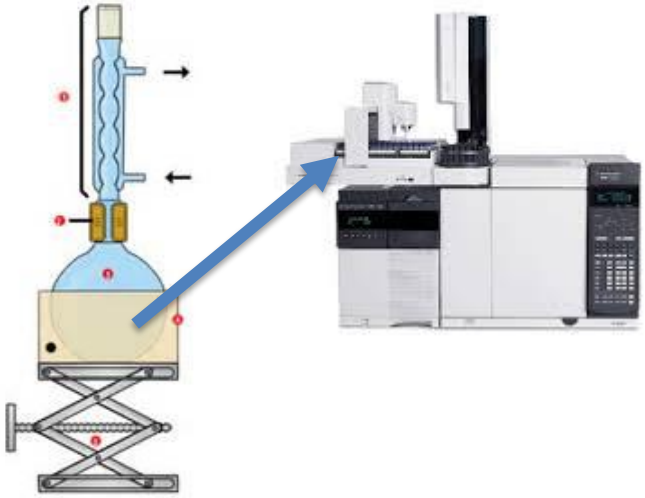
- Monomer residues
- Solvent residues from production steps
- Residues from polymer treatments
- Small polymer degradation products



Semi-Volatile Organic Compounds (SVOC)

Gas chromatography – Mass Spectrometry (GC/MS)

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



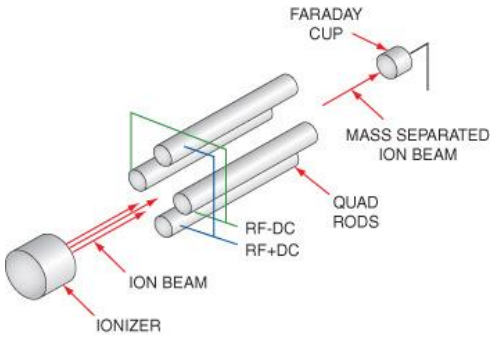
GC

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

- **Gas phase** separation technique using **narrow open tubular (capillary-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**
  - VOCs: 6% cyanopropyl/phenyl and 94% polydimethylsiloxane, USP phase G43 (or DB-624)
  - SVOCs: 5% phenyl and 95% polydimethylsiloxane, USP phase G27 (or DB-5)
- A higher **film thickness** of stationary phase increases retention:
  - VOCs: high film thickness (eg 1.4 µm): *more retention for smaller volatile compounds*
  - SVOCs: low film thickness (eg 0.25 µm)
- **Length** of capillary column increases resolution (but increases analysis time as well)
  - VOCs: eg 60m capillary column
  - SVOCs: eg 30m capillary column
- 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

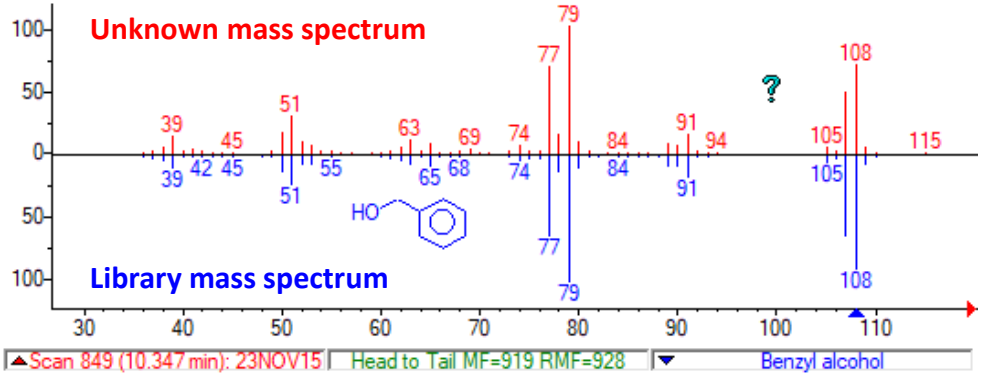


- 3 events: ionization / mass separation / detection – all happening under high **vacuum**
- Ionization: electron ionization (70 eV) → convert molecule into ion and induce further **fragmentation**
- **Quadrupole** mass analyzer:
  - Scanning mass filter → only 1 mass can pass through a given electric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



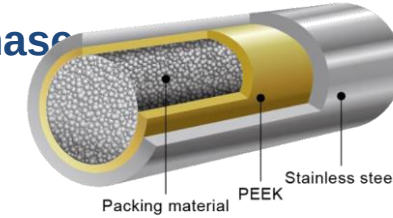
Non-Volatile Organic Compounds (NVOC)

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

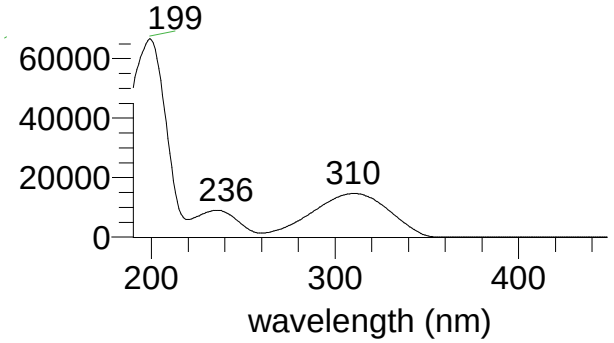
- Fillers
- Plasticizers
- Antioxidants
- Anti-slip agents
- Oligomers

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
    - hydrophilic interaction chromatography (HILIC) stationary phase
  - *Apolar stationary phases*: reversed phase chromatography: MOST COMMON
    - hydrophobic C18-bonded silica, USP phase L1
- **Optimizing separation** by
  - Selection of chromatographic column (length, polarity of stationary phase)
  - Selection of mobile phase (water, methanol, acetonitrile or mixtures or gradients)
    - Effective gradient: ends with strong mobile phase: purpose to elute strongly retained compounds (mitigating injection-to-injection carry-over)
- **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
- Broad dynamic range

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)

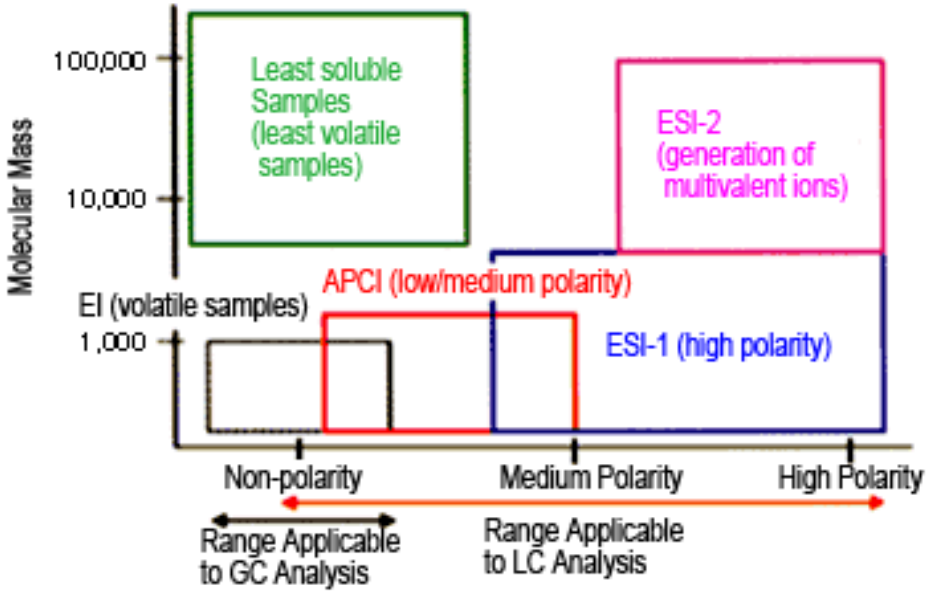
Advantages:

- 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



- **E**lectron **I**onization: only works in gas phase under vacuum → not LC compatible
- **A**tmospheric **P**ressure **C**hemical **I**onization (**APCI**): LC up to medium polarity
- **E**lectro**S**pray **I**onization (**ESI**): LC medium polarity – high polarity

Nowadays: more and more both APCI & ESI in E&L study design

Modern LC/MS instrumentation

Older systems:

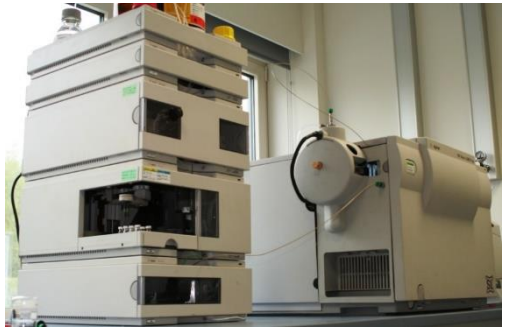
- **Quadrupole** or **ion trap** (cf. GC/MS)
- Low resolution: unit mass e.g. m/z 220 can be distinguished from m/z 221

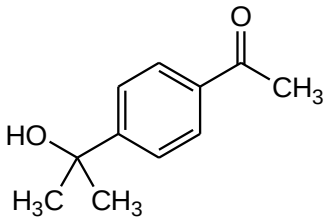
Nowadays:

- **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

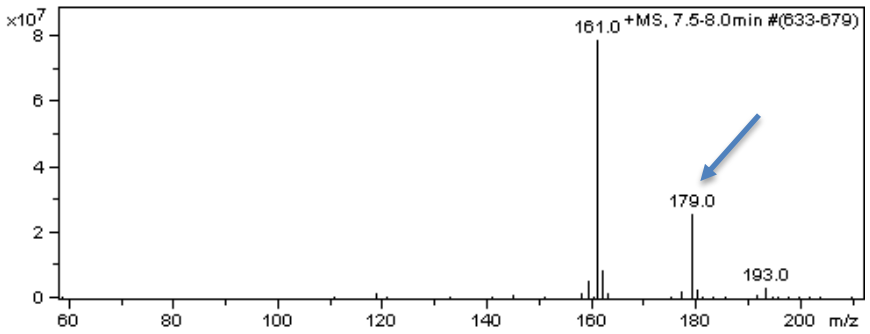
LC-QUADRUPOLE
(LOW RESOLUTION)

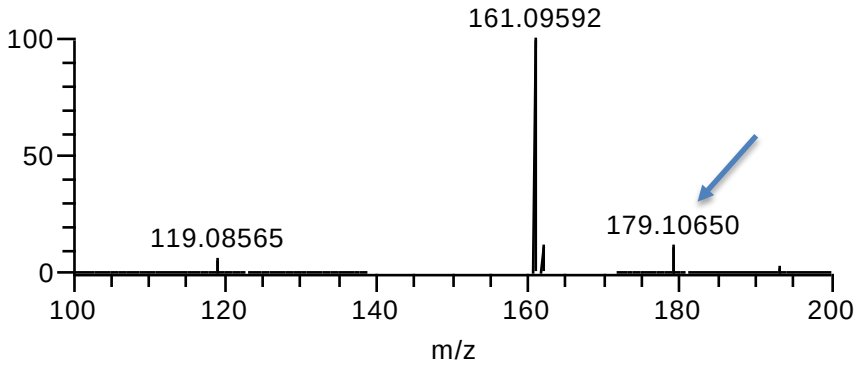




Peroxide curative related compound from EPDM rubber
Exact mass: 179.10666

LC-ORBITRAP
(HRAM)





Mass error: 0.16 mDa or 1 ppm

VALUE OF ACCURATE MASS: SEE LATER

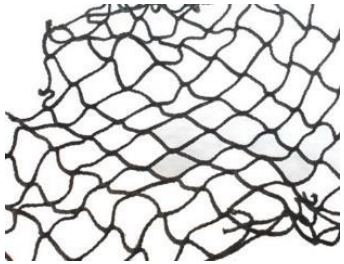
Analytical techniques for Organic Compounds

Screening & Discovery

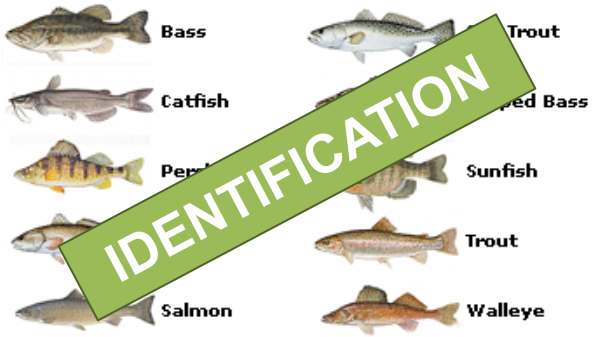
Chromatography – Mass Spectrometry

Different fishing techniques

TARGET ANALYSIS



NON-TARGET ANALYSIS (NTA)

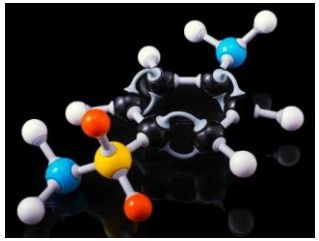


Different analytical techniques



IDENTIFICATION

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



QUANTIFICATION

Concentration

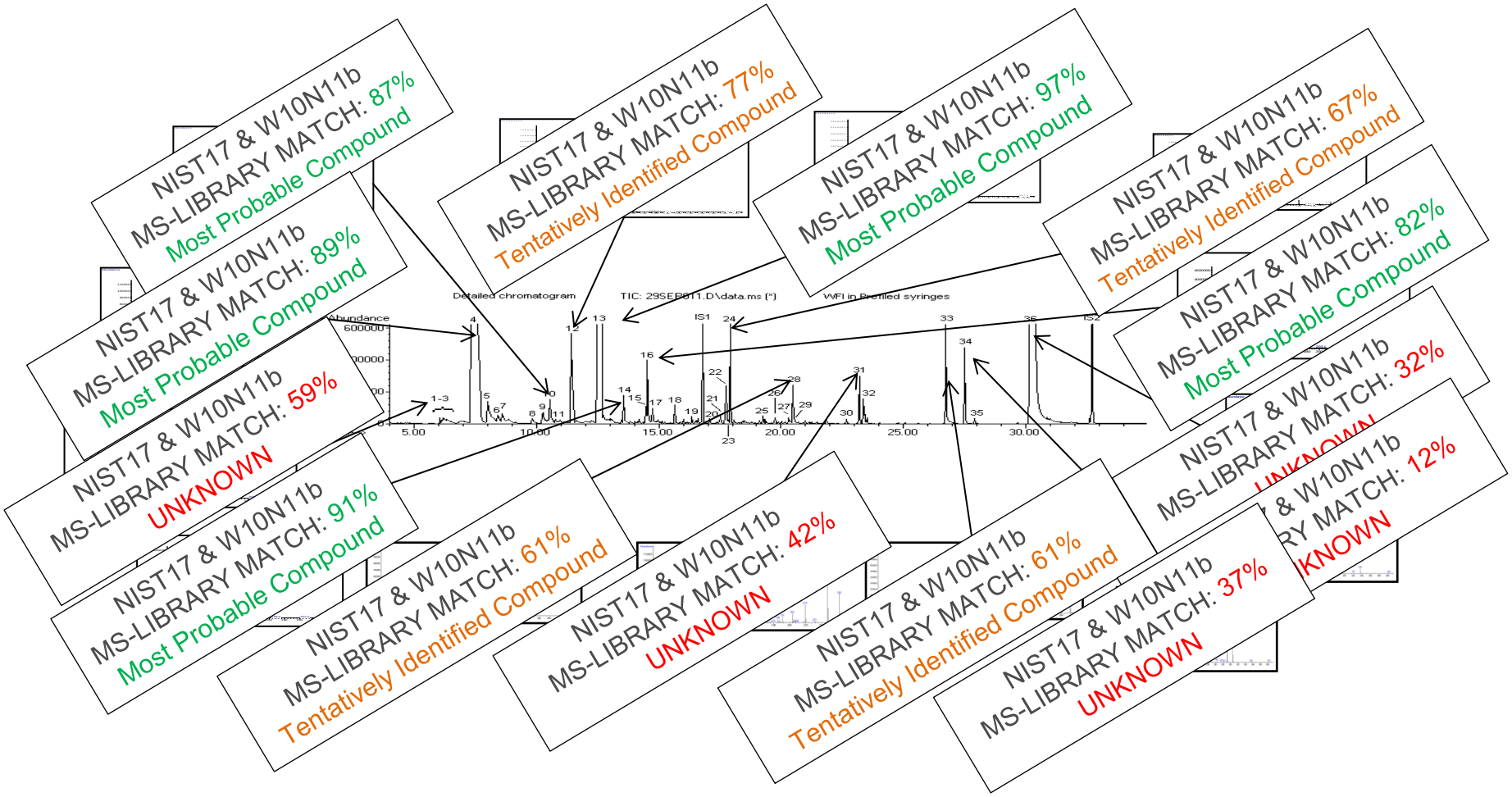
µg/L

µg/unit **µg/g**

Concept of “screening” or “discovery”

- **Non-targeted** analysis (NTA) 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.

Analytical techniques for Organic Compounds

SECOND PASS Approach (Structure Elucidation)

Structure elucidation - Introduction

- **Unknown / Partially identified compounds > AET** in 1st 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

Requirements

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

Liquid Chromatography

- Orbitrap
- FT-Ion Cyclotron Resonance

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
....		

Example for value of Accurate Mass:

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

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

(see next slide)

Structural elucidation - HRAM

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!

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

Isopropyl acetate

$C_5H_{10}O_2$

Nominal mass: 102 Da

Exact mass: 102.068079 Da

- N-nitrosodiethylamine

$C_4H_{10}N_2O$

Nominal mass: 102 Da

Exact mass: 102.07931 Da

Difference: 0.01123 Da

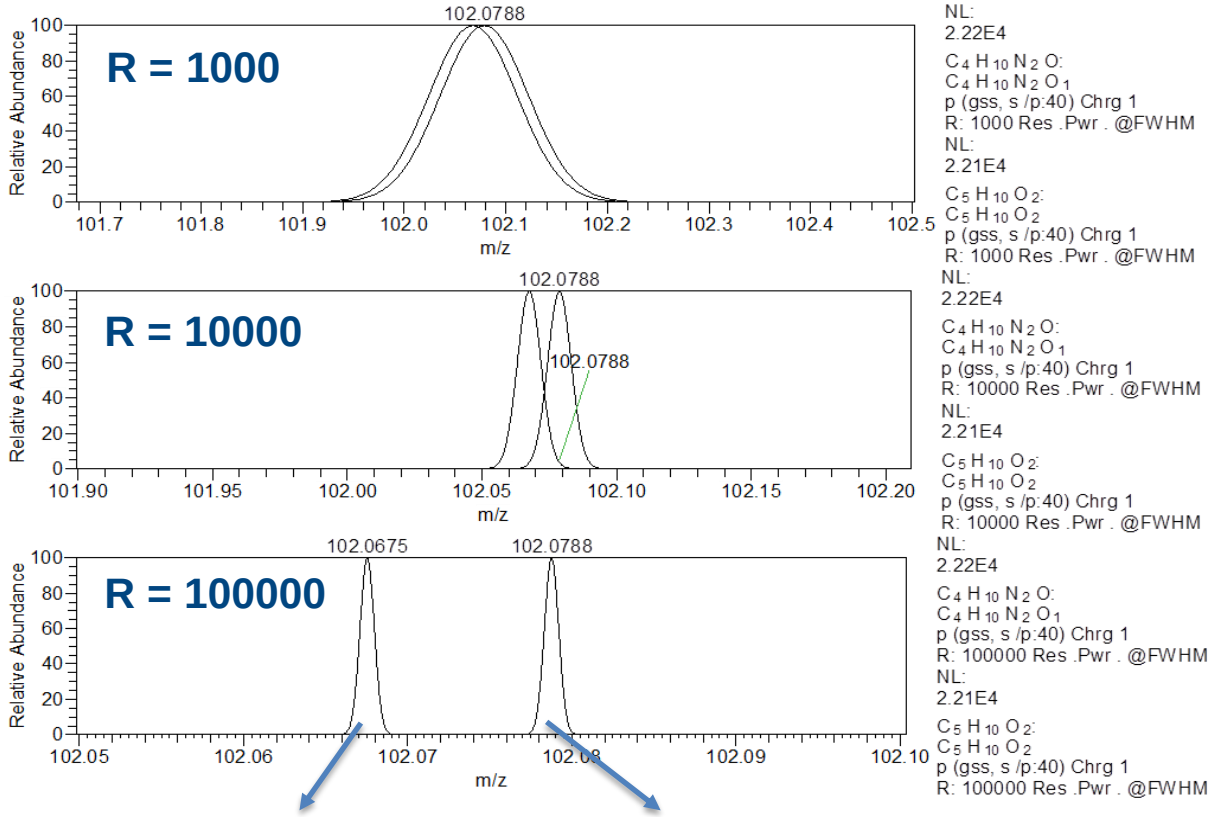
Structural elucidation - HRAM

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

Not separated

Close…

Separated



$C_5H_{10}O_2$ - isopropyl acetate $C_4H_{10}N_2O$ - N-nitrosodiethylamine

HRAM – Important take-aways

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

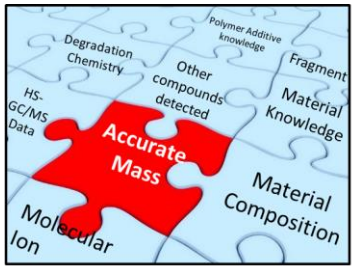
… but delivers **the elemental formula** of the molecule and fragments of the molecule

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



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

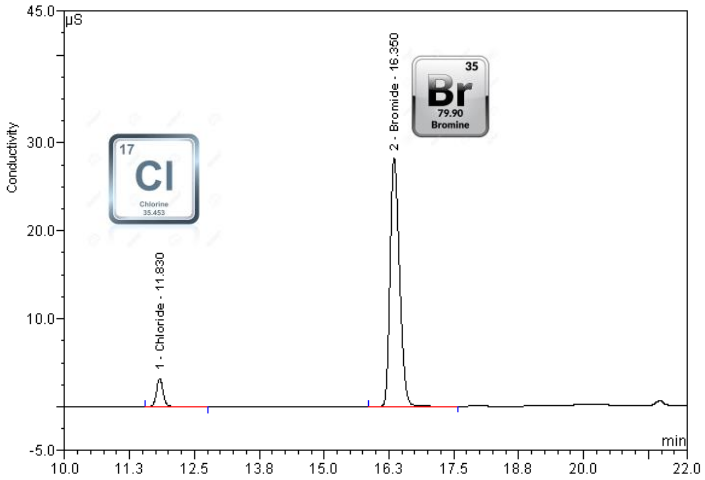
- ICP to produce **excited atoms**
- Excited atoms recombine, giving off electromagnetic radiation at **wavelengths characteristic for each element**
- Emission wavelengths detected by the spectrophotometer
- Or ions detected by mass spectrometry
- Intensity correlates to concentration → **quantitative**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

Other specific analytical methods

- **GF-AAS** for silicone oil detection and quantification
- **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 after evaporation of extract)
- **FTIR** – characterization of NVR
- **Total Organic Carbon**: *reconcilliation with concentration of organic compounds from chromatographic techniques*
- ...

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