Nelson Labs®
A Sotera Health company

Analytical techniques for E&L studies

IDENTIFICATION | QUANTIFICATION | STRUCTURAL ELUCIDATION

31 March 2022

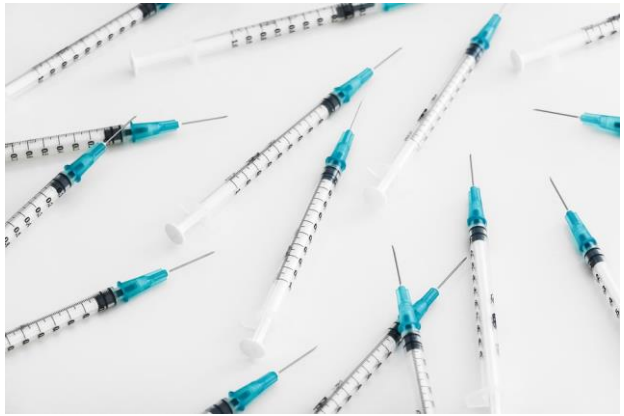
Intro - The broad spectrum of extractables and leachables

Barrel
Plunger
Stopper

CONTAINER COMPONENTS

Vial
Syringe

CONTAINER TYPE



MATERIAL SUPPLIER

Supplier A
Supplier B
…

Autoclave
Irradiation

STERILIZATION

MATERIAL FORMULATION

Medical grade
Healthcare grade
Ultrapure grade
Inert grade
Additive-free grade
…

Chlorobutyl rubber
Bromobutyl rubber
Nitrile rubber
COC / COP

MATERIAL OF CONSTRUCTION

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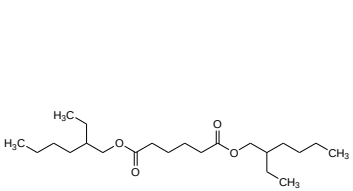
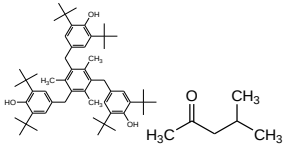
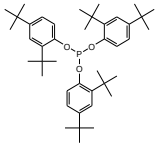
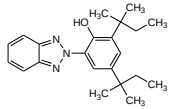
Intro - The broad spectrum of extractables and leachables

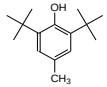




INORGANIC



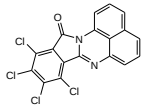


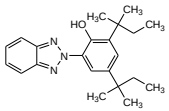


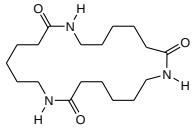
POLARITY

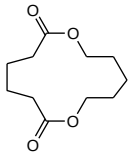
UNIVERSE OF EXTRACTABLES:
>10,000 – 100,000 Compounds

SIZE





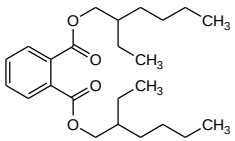


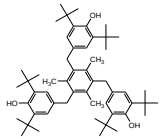


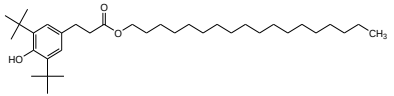
VOLATILITY

COMBINATION OF ANALYTICAL TOOLS FOR
DISCOVERY & IDENTIFICATION

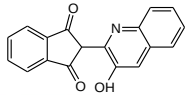
REACTIVITY

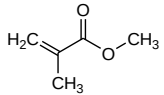


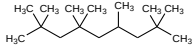


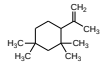


IONIC









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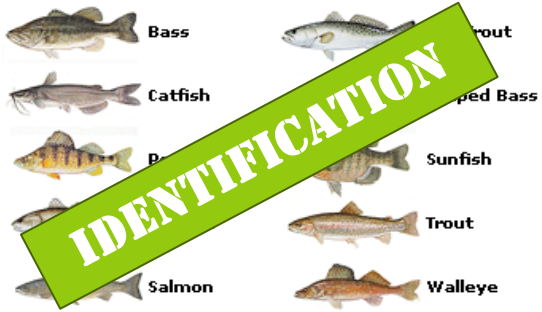
Intro - Screening

DIFFERENT FISHING TECHNIQUES

SPECIES?



WEIGHT

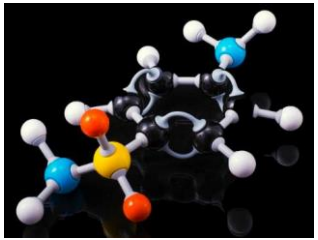
Intro - Screening

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**

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Analytical techniques – Sample Preparation

THE MOST IMPORTANT & MOST UNDERESTIMATED ACTIVITY IN THE LAB

- 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!

The quality of an analysis largely depends on the quality of the prep

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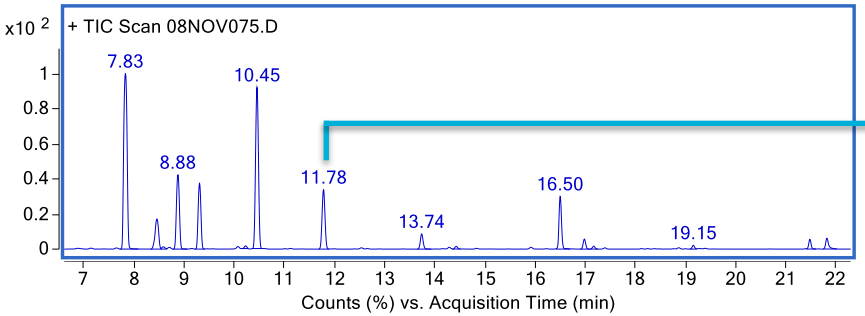
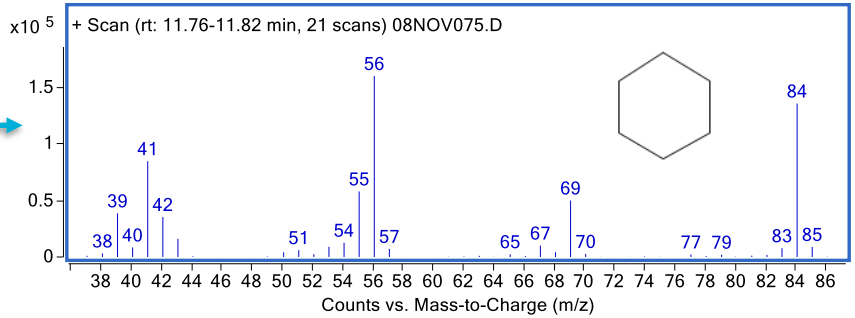
Analytical techniques – Organic compounds

CHROMATOGRAPHY – MASS SPECTROMETRY





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**

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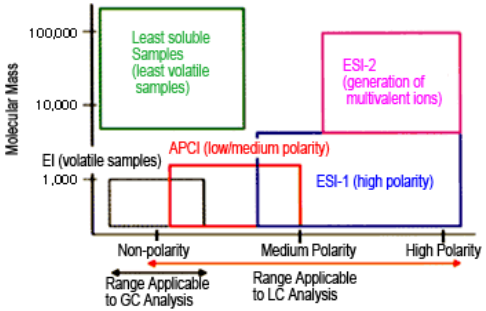
Analytical techniques – Organic compounds – Volatility & Polarity

CHROMATOGRAPHY – MASS SPECTROMETRY

(Headspace –) Gas chromatography – Mass Spectrometry

Liquid Chromatography – Mass Spectrometry



Volatile organic compounds (VOC)

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

Semi-Volatile organic compounds (SVOC)

Lubricants
Plasticizers
Antioxidants
Polymer degradation products
High boiling solvents

Non-volatile organic compounds (NVOC)

Fillers
Plasticizers
Antioxidants
Anti-slip agents
Oligomers

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Analytical techniques – Organic compounds – Analytical purpose

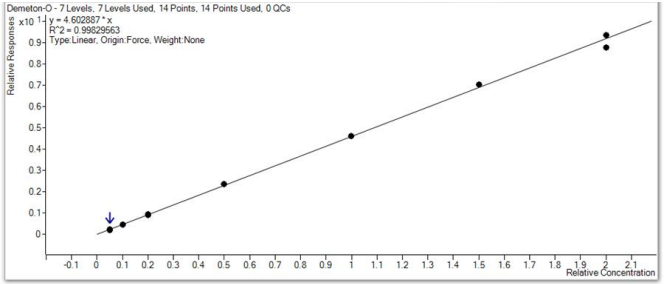
CHROMATOGRAPHY – MASS SPECTROMETRY

Screening method

Untargeted
Focus on **identification**
Estimated / semi-quantitative
Extractables / Screening leachables
Reporting limit ~ AET

Validated method

Targeted
Focus on **quantification**
Quantitative
Validated leachables studies



Analytical techniques – Elements

INDUCTIVELY COUPLED PLASMA / OPTICAL EMISSION SPECTROSCOPY or MASS SPECTROMETRY

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

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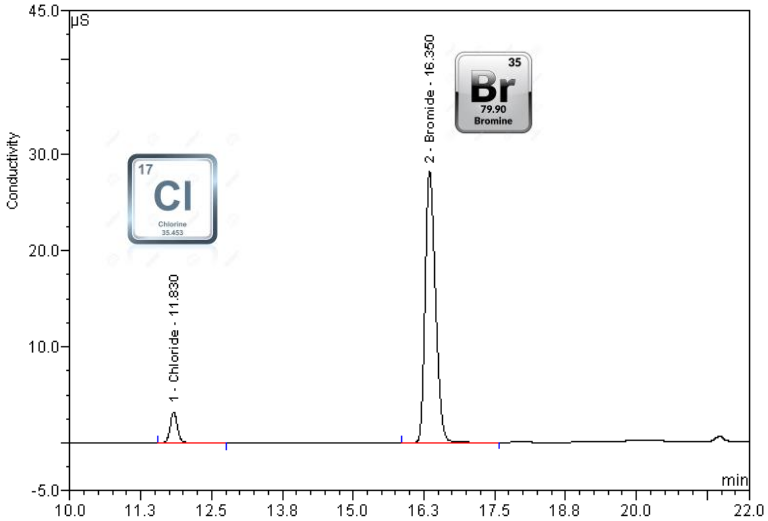
Analytical techniques – Anions

ION CHROMATOGRAPHY

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

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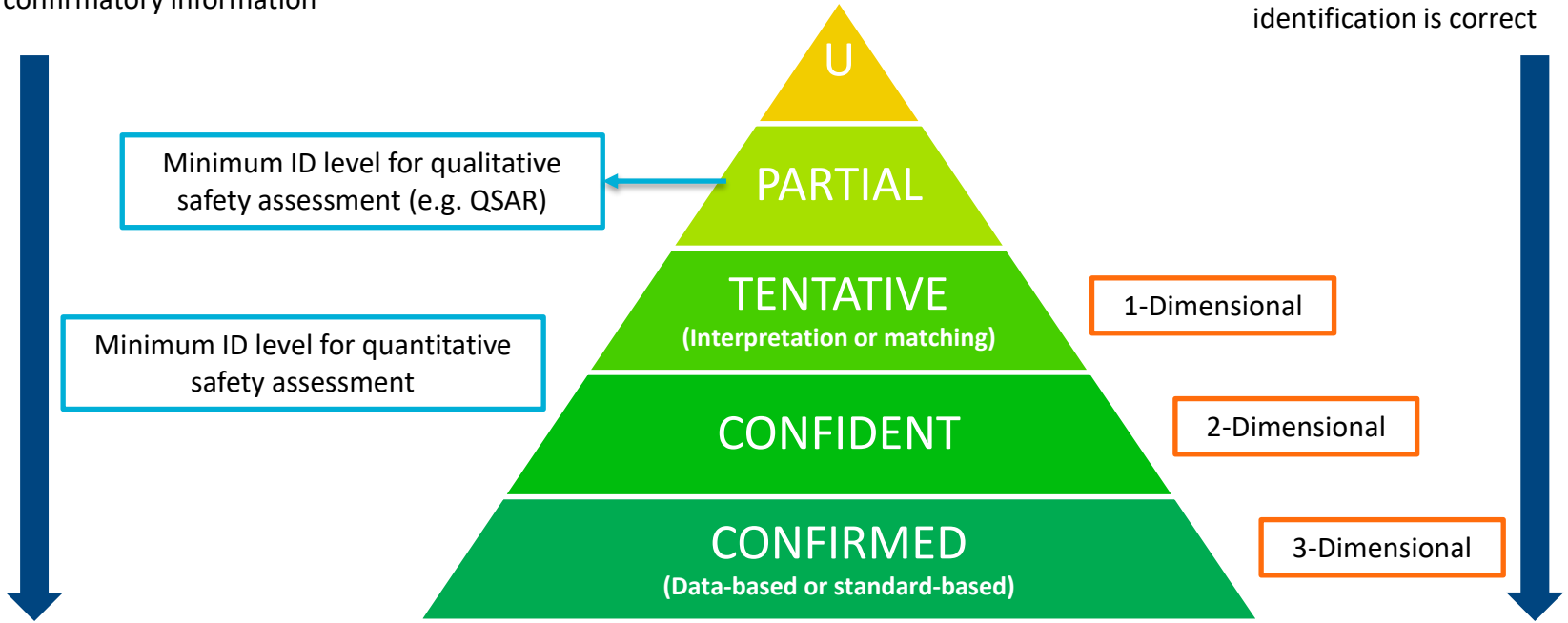
Analytical techniques – Identification (screening) – Identification levels

***References**: USP 1663 | Nelson Labs e-Book Good Identification Practices*

Increasing amount or rigor of confirmatory information

Increasing certainty that the identification is correct



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Analytical techniques – Identification (screening) – Confirmed ID level

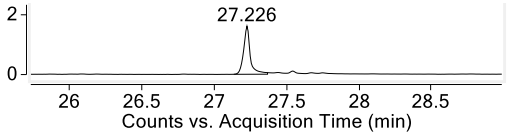
Measure standards with own screening methods

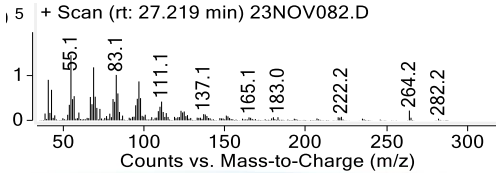
Capture RT & MS spectrum in database

Match with standard in database = confirmed

3-Dimensional

1. Retention time | **2. Mass spectrum** | **3. Certificate of Analysis**





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Analytical techniques – Identification (screening) – Confirmed ID level

- GC/MS spectra are 'standardized' → universal libraries possible (NIST, Wiley, EPA…)
- LC/MS spectra depend on vendor, instrument settings… → no universal libraries

  → **the internal database is the only database!**

The "Home Court" Advantage

NO DATABASE

Hexane extract of a PU component

no.	ID Level	ORGANIC COMPOUND	CAS-No./ ToxID	EI (m/z)	t_R (min)	Result (µg/cm²)
POSITIVE IONIZATION MODE (APCI+)						
1	U	-	-	173.080	3.50	0.17
2	U	-	-	251.211	7.17	0.15
3	U	-	-	219.185	7.55	0.49
4	U	-	-	145.122	8.02	0.16
5	U	-	-	353.242	7.72-8.32	1.5
6	U	-	-	145.122	8.18	0.31
7	U	-	-	145.122	8.33	0.25
8	U	-	-	145.122	8.69	0.12
9	U	-	-	145.122	9.19	0.16
10	U	-	-	527.298	9.41	0.12
11	U	-	-	145.122	9.47	0.10
12	U	-	-	338.340	9.71	0.14
13	U	-	-	731.412	10.87	170
14	U	-	-	559.517	11.11	0.15
15	U	-	-	585.533	11.39	0.23
16	U	-	-	535.518	11.47	0.51

ID LEVEL: 1 = Confirmed; 2 = Confident; 3 = Tentative , 4 = Partial, U = unknown

WITH DATABASE

Hexane extract of a PU component

no.	ID Level	ORGANIC COMPOUND	CAS-No./ ToxID	EI (m/z)	t_R (min)	Result (µg/cm²)
POSITIVE IONIZATION MODE (APCI+)						
1	1	1,4,7-Trioxacyclotridecane-8,13-dione	6607-34-7	173.080	3.50	0.17
2	U	-	-	251.211	7.17	0.15
3	U	-	-	219.185	7.55	0.49
4	1	35-Crown-7	66055-34-3	145.122	8.02	0.16
5	3	Hump of butoxylated hydrogenated MDI	-	353.242	7.72-8.32	1.5
6	2	40-Crown-8	ToxID 6005	145.122	8.18	0.31
7	2	45-Crown-9	ToxID 6006	145.122	8.33	0.25
8	2	50-Crown-10	ToxID 6007	145.122	8.69	0.12
9	2	55-Crown-11	ToxID 6008	145.122	9.19	0.16
10	3	Irganox 1010 degradation product	ToxID 5005	527.298	9.41	0.12
11	2	60-Crown-12	ToxID 6009	145.122	9.47	0.10
12	1	Erucamide	112.84-5	338.340	9.71	0.14
13	1	Irganox 1010	6683-19-8	731.412	10.87	170
14	U		-	559.517	11.11	0.15
15	2	Ethylene bis(linoleamide)	14614-46-1	585.533	11.39	0.23
16	2	N,N'-Ethylene myristyl oleyl diamide	ToxID 5888	535.518	11.47	0.51

ID LEVEL: 1 = Confirmed; 2 = Confident; 3 = Tentative , 4 = Partial, U = unknown

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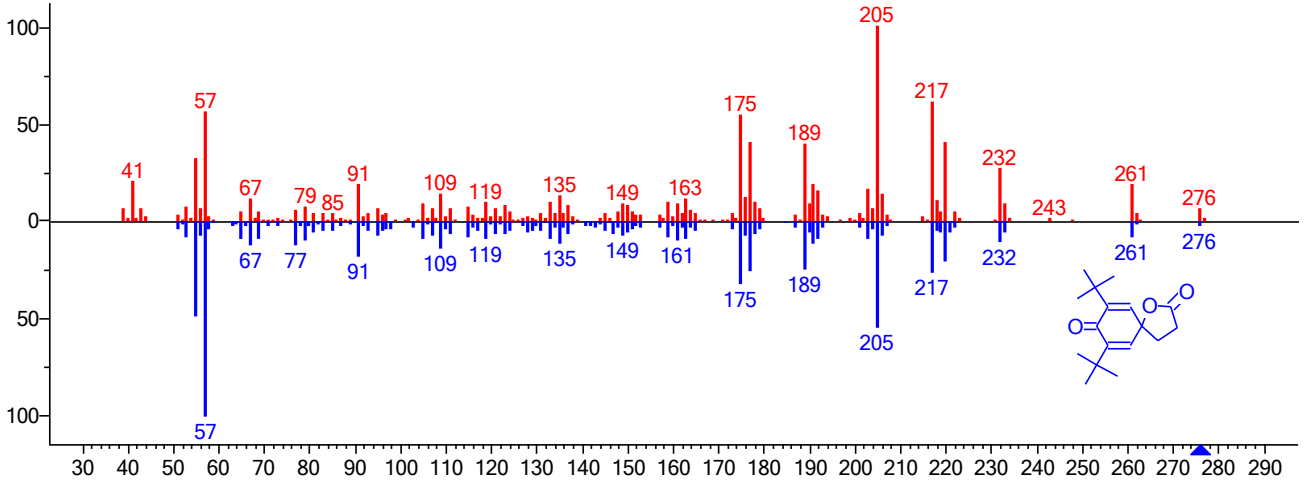
Analytical techniques – Identification (screening) – Tentative ID level

Commonly achieved through computerized database matching algorithms (e.g. NIST, Wiley…)



#	Lib.	Match	R.Match	Prob. (%)	Name
⊞ 1	M	877	895	90.4	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione
⊞ 2	M	626	666	0.90	2,5-di-tert-Butyl-1,4-benzoquinone
3	M	613	654	0.58	p-Benzoquinone, 2,5-bis(1,1,3,3-tetramethylbutyl)-
4	M	607	679	0.45	4H-1-Benzopyran-4-one, 5-hydroxy-2-methyl-7-(2-propenyloxy)-
5	M	604	679	0.40	Benzo[b]dihydropyran, 6-hydroxy-4,4,5,7,8-pentamethyl-

Never trust the algorithm!

Every 'hit' to be carefully evaluated by a trained mass spectrometrist:

- Visual inspection
- Match factors
- Probability
- "InLibrary" score

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Analytical techniques – Identification (screening) – Confident ID level

- **Additional evidence** for tentative identification
  - **Molecular weight** confirmation (chemical ionization, *vide infra*)
  - **Elemental formula** confirmation (accurate mass, *vide infra*)
  - Confirmed identification in **orthogonal technique**
  - **Material/processing knowledge** (e.g. expected anti-oxidant degradation after gamma-sterilization)
  - **Homologue series** with provable relationship to confirmed compound
  - Compound within **expected retention index window** (based on **experimental** RI data from **same GC column phase** in NIST)

Analytical techniques – Identification (screening) – Errors

Error of omission

No detection / not fo[illegible]

FATAL ERROR

N[illegible]essment

Safety compromised

Error of in-exact or wrong identification

Wrong structure activity rel[illegible]ship

FATAL ERROR

Wr[illegible]ssessment

Safety compromised

GOOD IDENTIFICATION PRACTICES ARE KEY!

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Analytical techniques – Quantification - 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 – Quantification - Screening

In screening analysis, there is inherent uncertainty:

- **Only 1 batch of test item (extractables) | batch to batch variability**
- **Only 1 analysis of extract | analytical procedure variability**
- **Compounds with unknown → confident ID level | analytical response variability vs I.S.**
- **Liquid/liquid extraction | variability of extraction recovery**
- **…**

Use of uncertainty factors for chromatography – mass spectrometry screening methods

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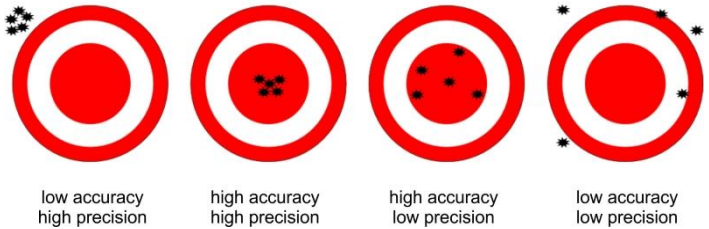
Analytical techniques – Quantification – Validated methods

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



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SECOND PASS IDENTIFICATION | STRUCTURAL ELUCIDATION

High-end Mass Spectrometry

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Second pass identification - Intro

- **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 second pass studies: generate / collect comprehensive set of **supporting data to increase the identification level** of a target compound

Second pass identification - 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

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Second pass identification – High Resolution Accurate Mass

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_2

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

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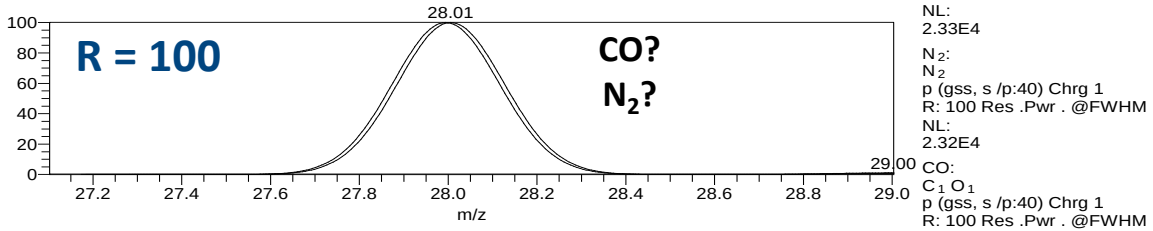
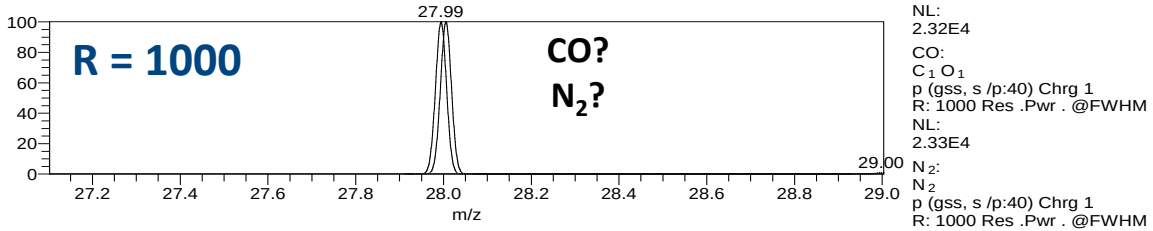
Second pass identification – High Resolution Accurate Mass

Assigning the wrong identification for a compound with a mass of 28 Da can be fatal… how to be sure?

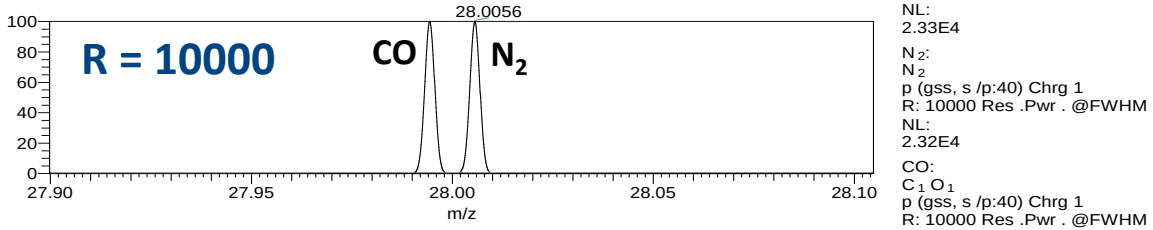
Not separated



Not separated



Separated



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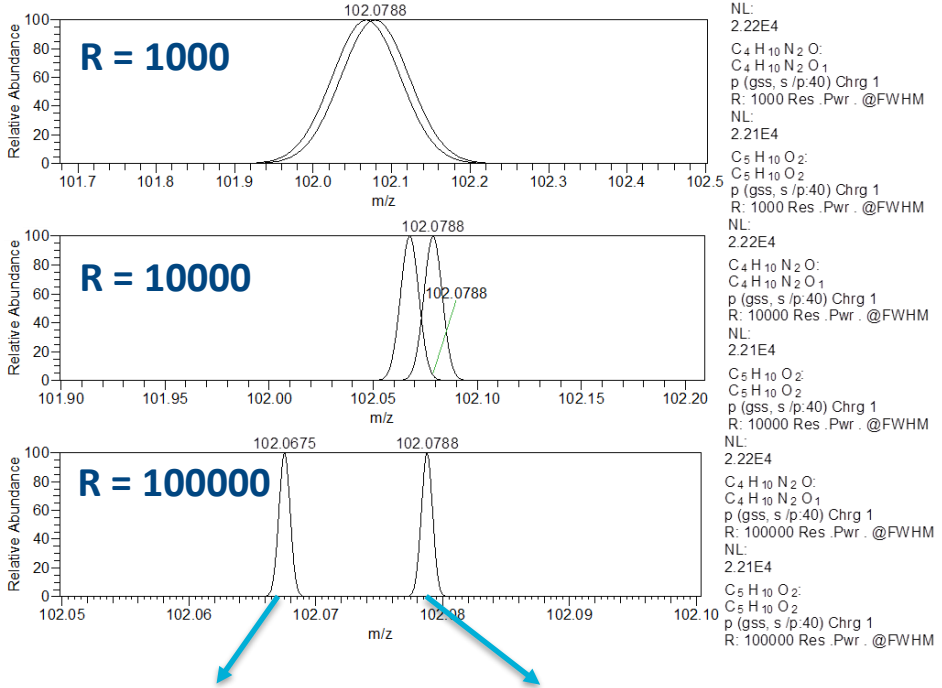
Second pass identification – High Resolution Accurate Mass

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**

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Second pass identification – High Resolution Accurate Mass

Important take aways around HRAM MS:

accurate mass alone does not deliver a structure…

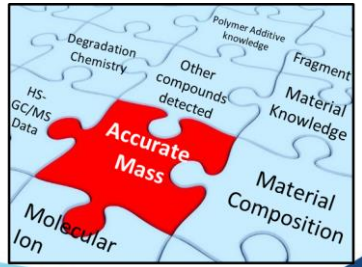
… but delivers **the elemental formula** of the molecule and fragments of the molecule

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



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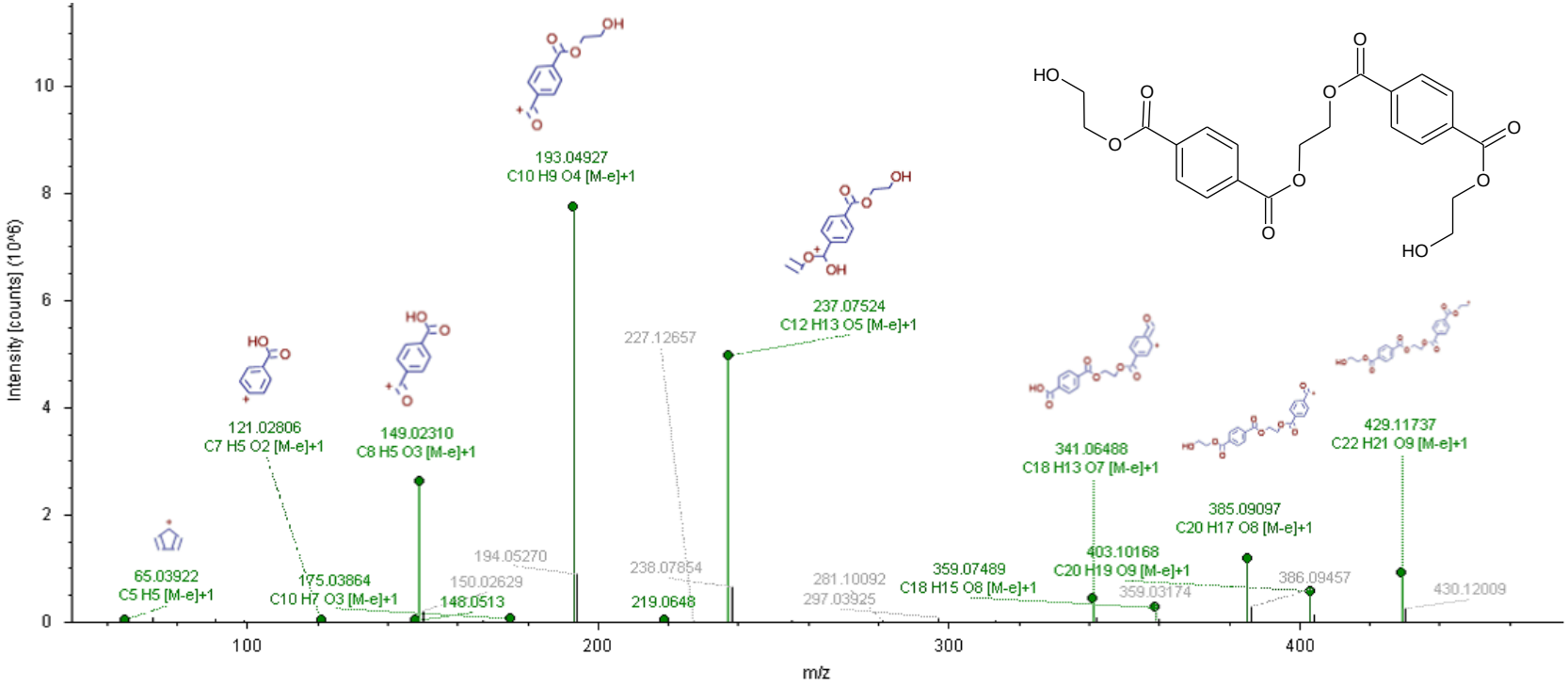
Second pass identification – Tandem Mass Spectrometry

Case: “de novo” structural elucidation and interpretation of HRAM MS/MS fragmentation spectra



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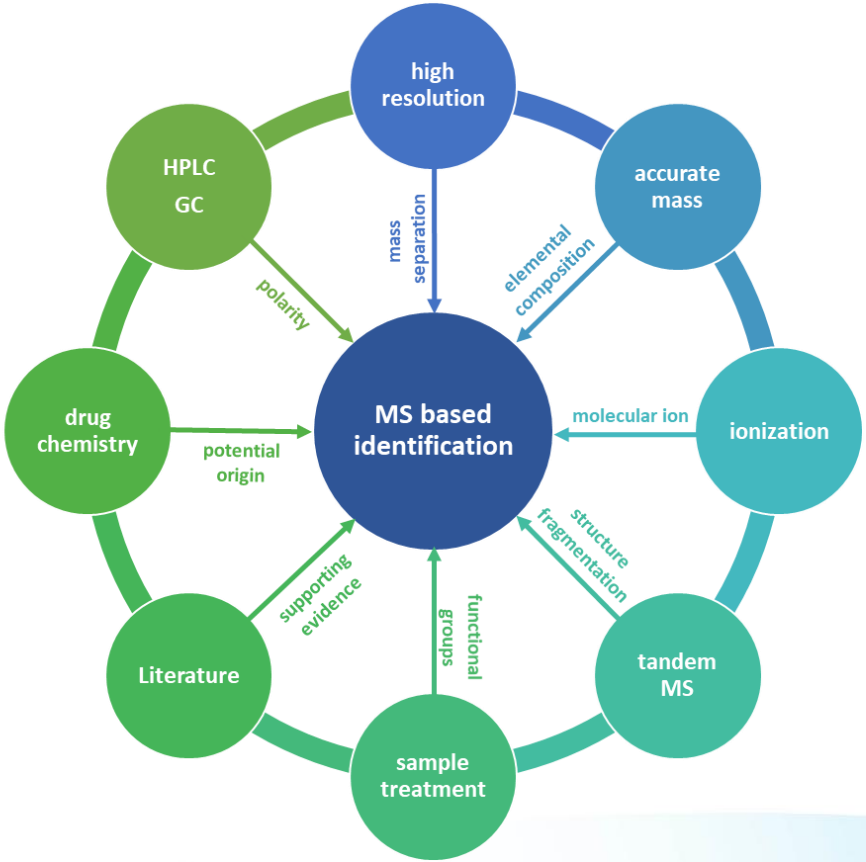
The beautiful art of identifying an unknown



TEAM effort!

Mass spec expert(s)

Drug chemistry expert

Material engineer

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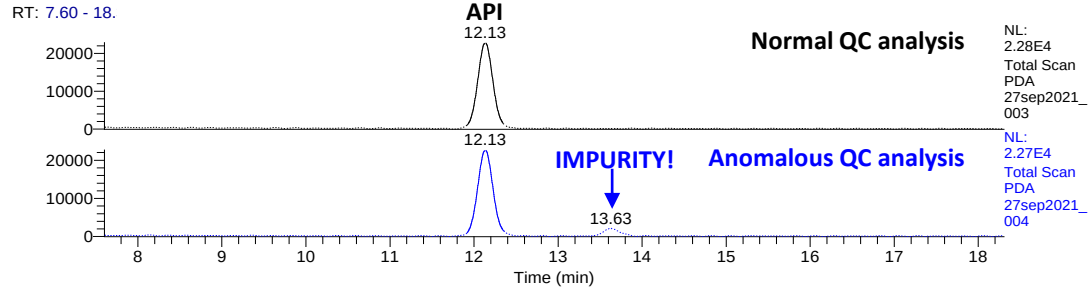
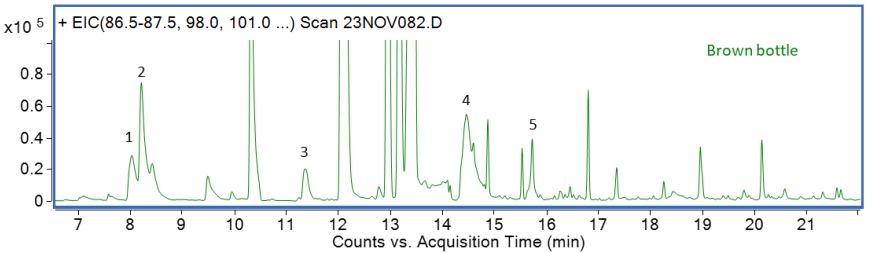
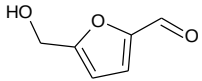
Other identification projects

DP/DS IMPURITIES



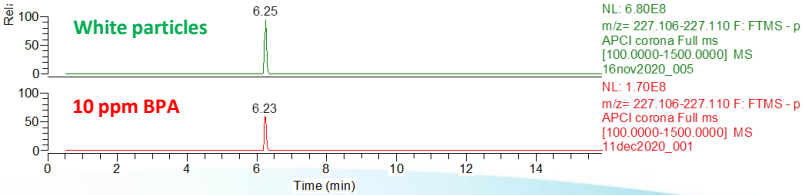
DP/DS DISCOLORATION

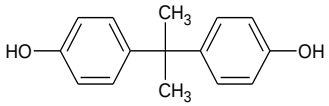




5-Hydroxymethyl-2-furancarboxaldehyde
CAS: 67-47-0
Molecular Formula: $C_6H_6O_3$
Monoisotopic Mass: 126.032 Da

PARTICLES





Bisphenol A
CAS: 80-05-7
Molecular Formula: $C_{15}H_{16}O_2$
Monoisotopic Mass: 228.115 Da

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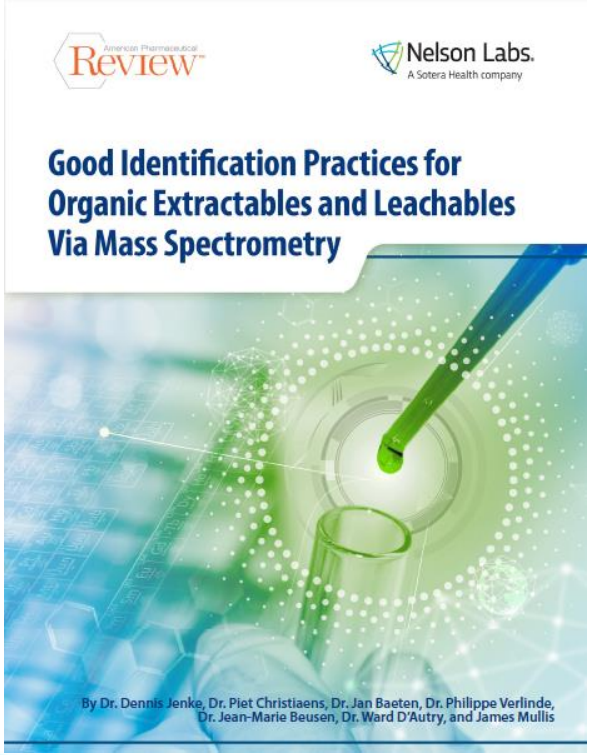
Further reading

E-Book Good Identification Practices:

https://www.nelsonlabs.com/good-identification-practices-for-organic-extractables-and-leachables-via-mass-spectrometry/



PDA article series about identification and mitigating errors in screening for E&L:

- PDA Journal of Pharmaceutical Science and Technology January 2020, 74 (1) 90-107
- PDA Journal of Pharmaceutical Science and Technology January 2020, 74 (1) 108-133
- PDA Journal of Pharmaceutical Science and Technology January 2020, 74 (1) 134-146

Thank you

Questions?

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