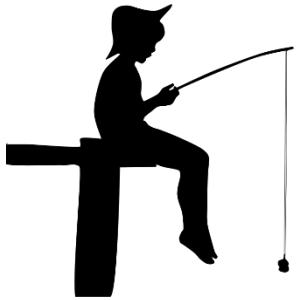
Identification of E&L compounds

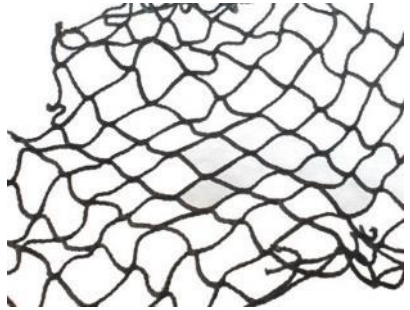
The need for (high-end) analytical techniques

WARD D'AUTRY
25 MAR 2021

What is screening? The lake fishing metaphor

USE ARRAY OF FISHING TECHNIQUES





SPECIES?

IDENTIFICATION

AMOUNT PER SORT?

QUANTITATION

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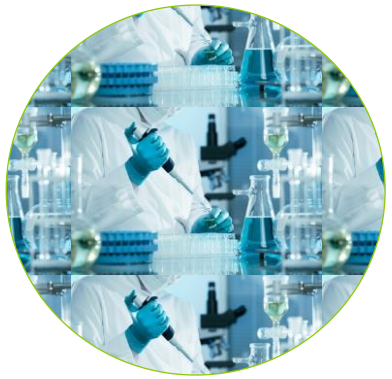
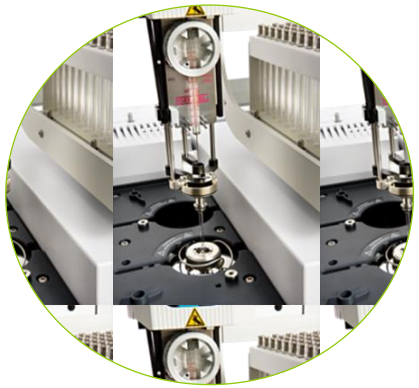
What is screening? This time in the E&L lab

DP or extract



Sample preparation
(emptying, liquid/liquid…)

Prep of analytical sample
(spike I.S. to final extract)



Instrument analysis
(HS-GC/MS, GC/MS, UPLC/MS…)

WHAT?

HOW MUCH?

IDENTIFICATION

Data evaluation
Data interpretation
Data review
Reporting

QUANTIFICATION

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Identification

- Process of **securing the identity** of an extractable or leachable
- Detected with a **screening** method (HS-GC/MS, GC/MS or LC/MS)
- **Above the reporting threshold** (AET)
- **Unambiguous**: besides name, use structure and/or CAS number

CHEMICAL STRUCTURE

CHEMICAL UNIQUE IDENTIFIER NUMBER

CAS No XXXXXXX-YY-Z

- 2 to 7 digits
- 2 digits
- 1 check digit

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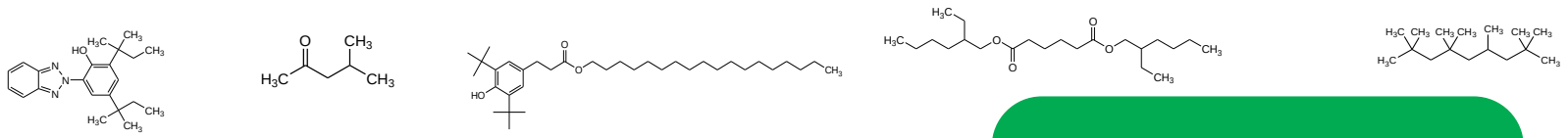
Why is screening such a challenge for LVP? Part I



Different **SUPPLIERS**

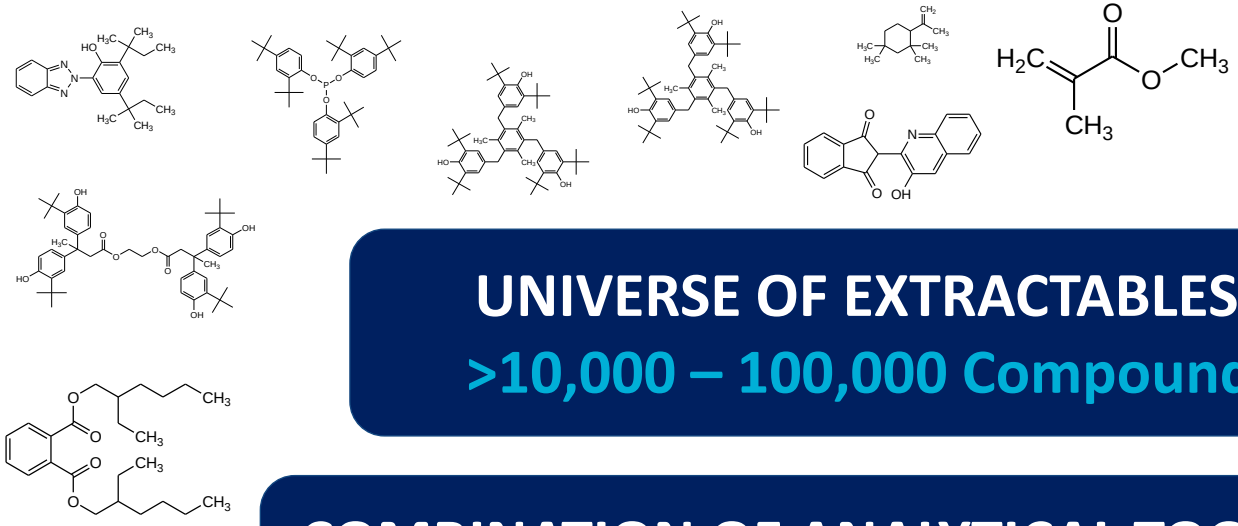
Each with different **GRADES & FORMULATIONS**

Different **STERILIZATION & PROCESSING** Modes and Conditions

- Polymer Additives
- Processing Aids
- Oligomers
- Curing Agents
- Impurities in Polymer Additives
- Monomer Residues
- Filter Residues
- Degradation Compounds of Oligomers
- Anti-oxidants
- Acid Scavengers

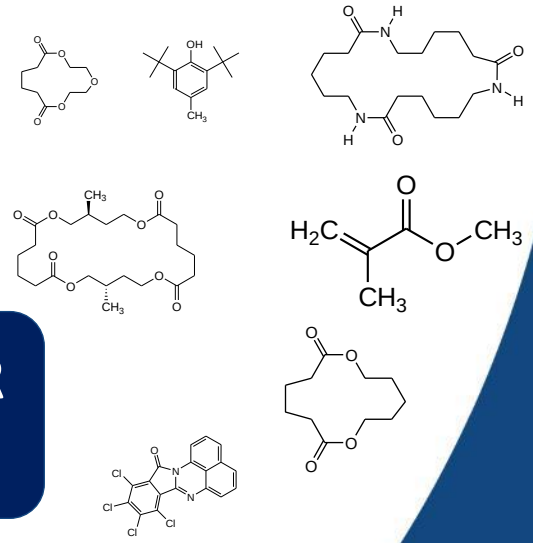
A lot of **MATERIALS**

A lot of **LVP Systems**



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

COMBINATION OF ANALYTICAL TOOLS FOR DISCOVERY & IDENTIFICATION



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Why is screening such a challenge for LVP? Part II

High maximum daily doses (100 mL → liter scale)

↓

Low qualification thresholds / AET (1 ppb or even less)

↓

Close to **detection limits** of analytical methods

↓

Often **long lists of detected extractables or leachables** > AET to report

↓

Every reported compound > AET needs to be carefully identified…

↓

…to allow accurate and comprehensive tox assessment

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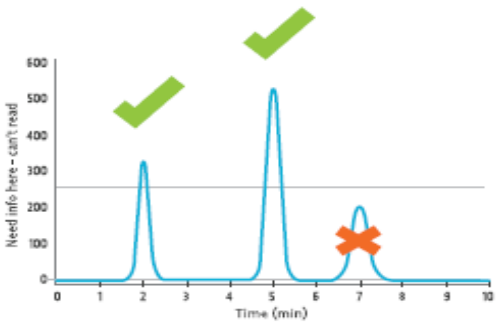
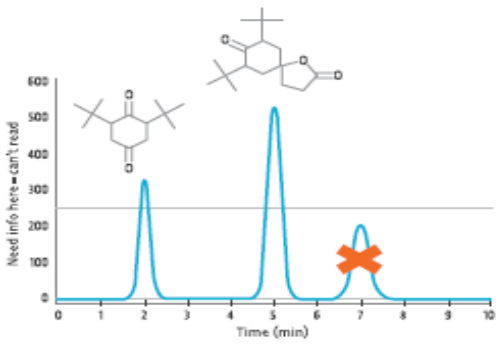
Consequence of incorrect identifications



AET

ANALYTICAL CHEMISTRY

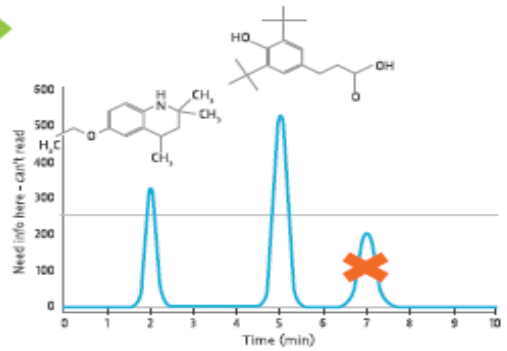


RIGHT ID

PDE, TI, ...
Accute Systemic tox
Carcinogenic tox
Sensitizer, Irritant
Reprotox

V

TOXICOLOGY



WRONG ID

FATAL

PDE, TI, ...
Accute Systemic tox
Carcinogenic tox
Sensitizer, Irritant
Reprotox

X

Identification: small mistakes, large consequences

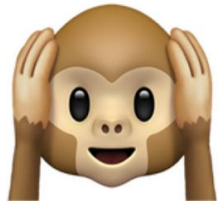
Error of omissio

No dete found

sessment

afety compromised

FATAL ERROR



Error of in-exact or wron ation

Wrong structu elationship

assessment

afety compromised

FATAL ERROR

GOOD IDENTIFICATION PRACTICES ARE KEY!

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First Pass Identification

SCREENING METHODS

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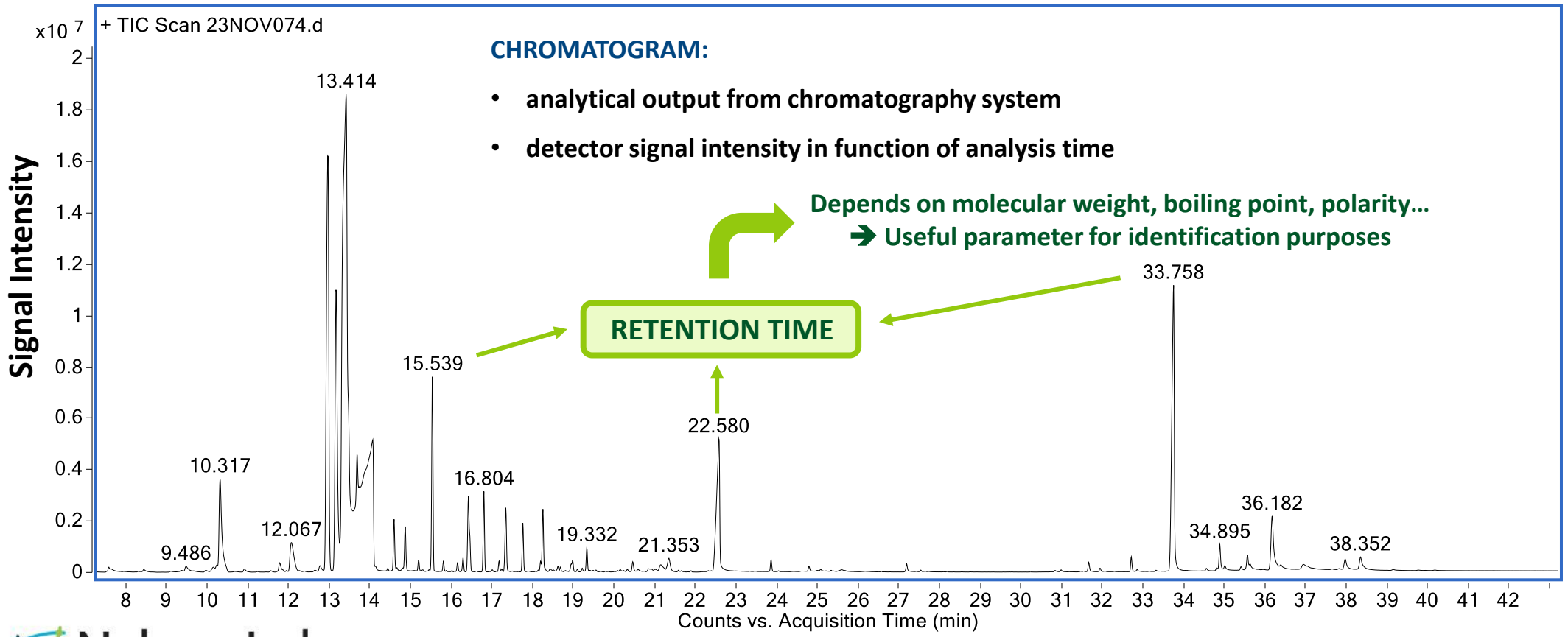
Chromatography – Mass Spectrometry Screening Analysis

Chromatograph: separation of extractables or leachables

Mass spectrometer: detection of extractables or leachables *(but does more than that!)*

Chromatography / Mass Spectrometry software: aids in identification & quantification of detected compounds



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Chromatography – Mass Spectrometry Screening Analysis

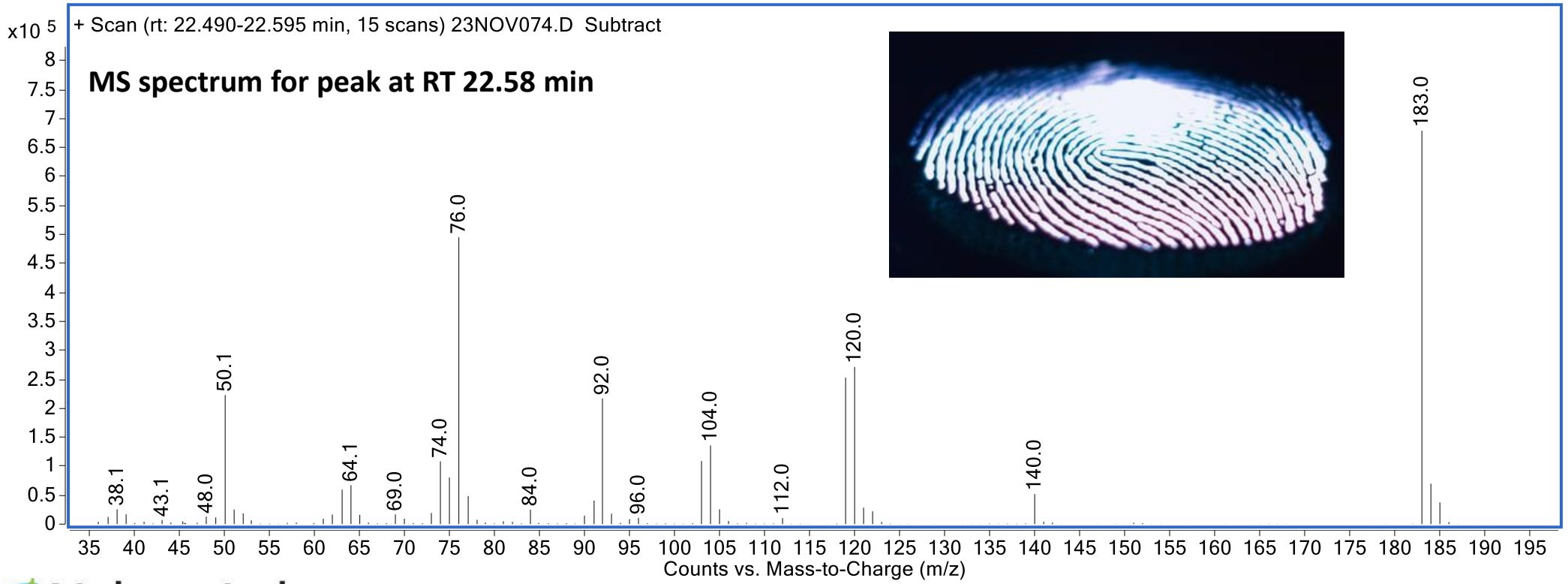
Mass spectrometers do not only serve as a detector, but provide mass spectra for each detected compound

MASS SPECTRUM:

- **analytical output from mass spectrometer**
- **mass (fragment) information for each detected peak**

VERY POWERFUL TOOL FOR IDENTIFICATION!

(if you know how to interpret the data…)



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Identification levels (USP 1663)

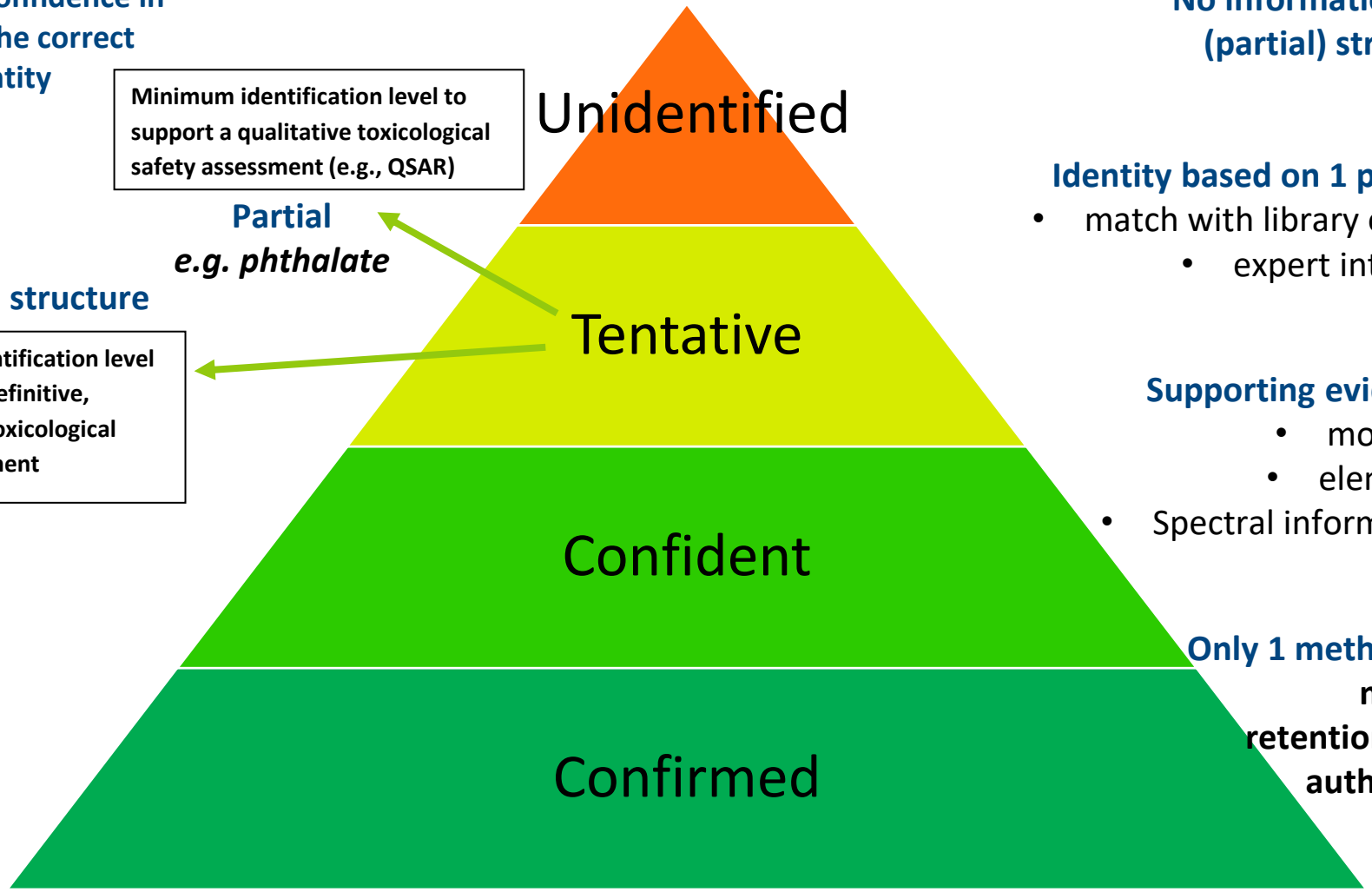
Increasing confidence in securing the correct identity

Minimum identification level to support a qualitative toxicological safety assessment (e.g., QSAR)

Partial
e.g. phthalate

Full structure

Minimum identification level to support a definitive, quantitative toxicological safety assessment



No information about (partial) structure

Identity based on 1 piece of information:
- match with library or literature spectrum
- expert interpretation

Supporting evidence for tentative ID:
- molecular weight
- elemental formula
- Spectral information from other method

Only 1 method to secure an ID as confirmed:
mass spectral match +
retention time match with analysis of
authentic reference standard

Decreasing certainty for correct identification

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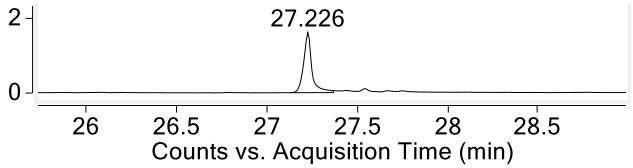
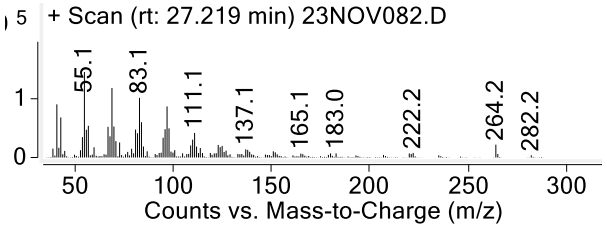
The confirmed ID level

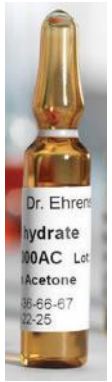
Full match with authentic standard?

Need for **INTERNAL compound database!**



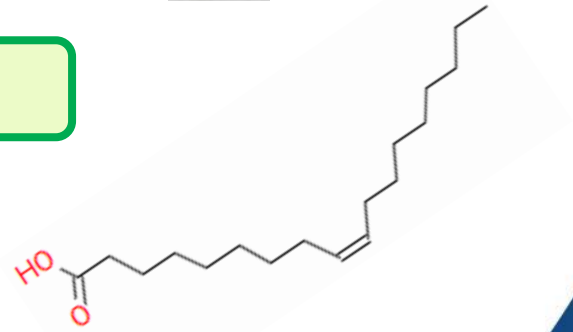
Measure standards with **own methods**





Capture RT & MS spectrum in database

Name: Oleic acid
Formula: $C_{18}H_{34}O_2$
MW: 282 Exact Mass: 282.25588 CAS#: 112-80-1 ID#: 142 DB: svoc_mh.hp
Other DBs: None
Comment: (Z)-9-Octadecenoic acid |RI:1635|
187 m/z Values and Intensities:



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Nelson Labs Database: 6000 compounds (VOC, SVOC, NVOC)

Excerpt of the NELSON LABS Discovery and Screener Database for Semi-Volatile Organic Compounds Characterized by Gas Chromatography/Mass Spectrometry (GC/MS), RT 19 – 19.2 min.

RT (min)	Compound Name	CAS Number	RRF	Target Mass	Q1	Q1 ratio	Q2	Q2 ratio	Q3	Q3 ratio
18.97	Bis(2-ethylhexyl) ether	10143-60-9	1.13	57	71	86	43	7.8	41	36
19.01	4-Hydroxy-3-methylacetophenone	876-02-8	0.413	135	150	39.4			107	18.7
19.03	Cyclopentyl phenyl ketone	5422-88-8	0.758	105	77				133	15.2
19.05	2,4-Di-tert-butylphenol	96-76-4	0.984	191					57	14
19.07	2-(Decyloxy)ethanol	23238-40-6	0.352					9.9	43	65
19.08	Tridecanal	10486-19-8	0.2				2	81.7	43	81.1
19.08	1,4-Isopropanol acetophenone	54549-72-2					121	15.7	164	11.1
19.08	1-Naphthol	90-				87.8	116	41.9	145	11.3
19.08	2-(2-Phenoxyethoxy)ethanol				94	76.5	77	52.4	182	26.3
19.12	Triisobutyl phosphate			99	57	19.9	155	14.8	41	12.4
19.13	BHT		4	205	220	25.6	206	15.5	57	11.5
19.13	Dimethyl isophthalate		0.557	163	194	24.2	135	23.7	76	11
19.15	N,N-Di-n-butyl-2-chloroaceta	-59-1	0.59	86	120	77.5	156	52	162	34.7
19.17	Cyclododecanone	830-13-7	0.697	55	41	79.8	71	73.3	98	63.1
19.2	2-Phenylphenol	90-43-7	0.676	170	169	75.1	141	33.1	115	23.6

CONFIRMED

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LC/MS: no universal libraries

- 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

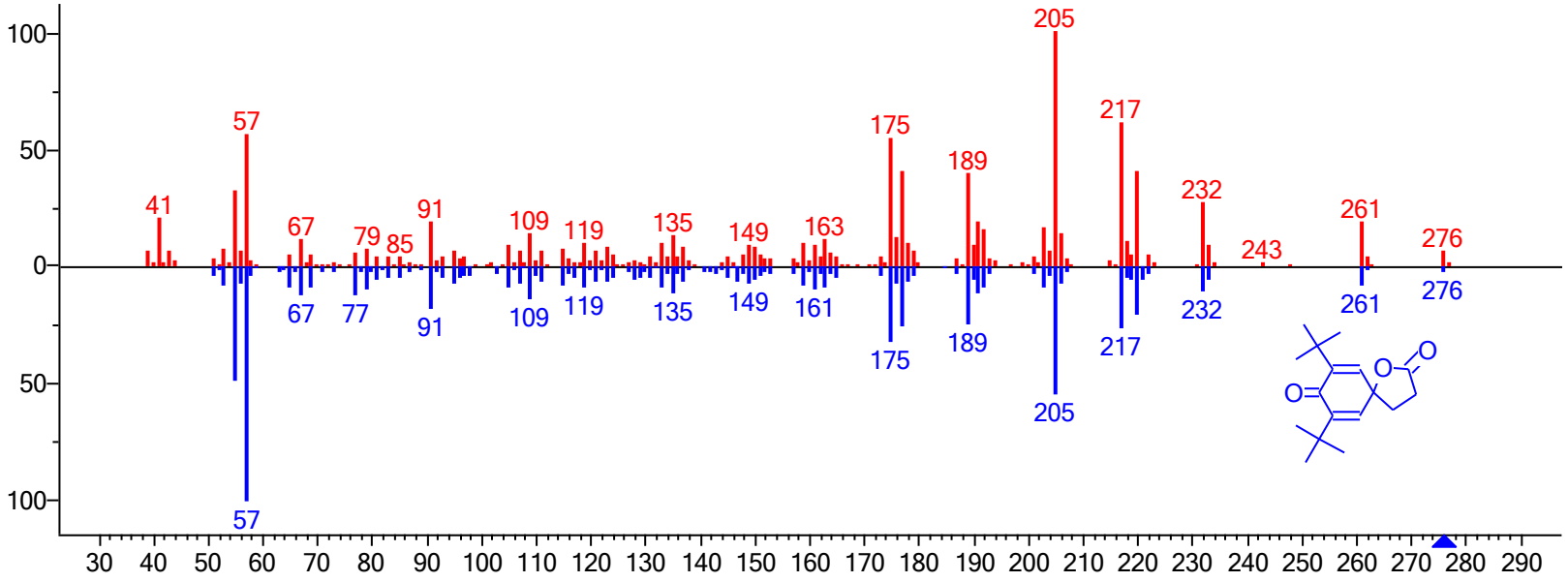
Hexane extract of a PU component

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

Tentative identifications

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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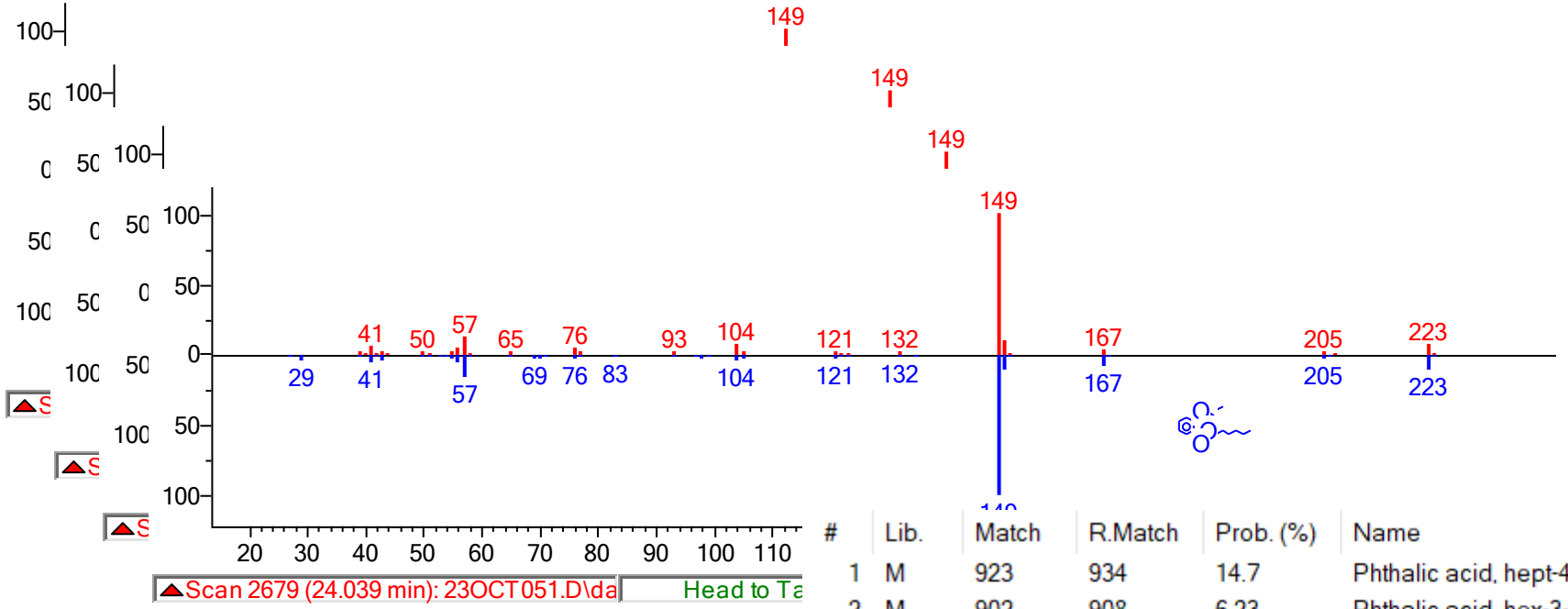
Tentative identifications

When you have a wealth of compounds with similar mass spectra…



What to report?

Phthalate

= partial TIC

#	Lib.	Match	R.Match	Prob. (%)	Name
1	M	923	934	14.7	Phthalic acid, hept-4-yl isobutyl ester
2	M	902	908	6.23	Phthalic acid, hex-3-yl isobutyl ester
3	M	901	909	5.99	Phthalic acid, hept-3-yl isobutyl ester
4	M	892	897	4.34	Phthalic acid, hept-2-yl isobutyl ester
5	M	891	898	4.18	Phthalic acid, isobutyl 2-propylpentyl ester
6	M	887	892	3.53	Phthalic acid, 2-ethylbutyl isobutyl ester
7	M	881	884	2.77	Phthalic acid, isobutyl 2-methylpent-3-yl ester
8	M	879	885	2.56	Phthalic acid, butyl isohexyl ester
9	M	878	885	2.46	Phthalic acid, butyl 2-ethylbutyl ester
1..	M	876	878	2.27	Phthalic acid, 2,4-dimethylpent-3-yl isobutyl ester

All possible candidates!

Hit 1 is not automatically the compound to report!!

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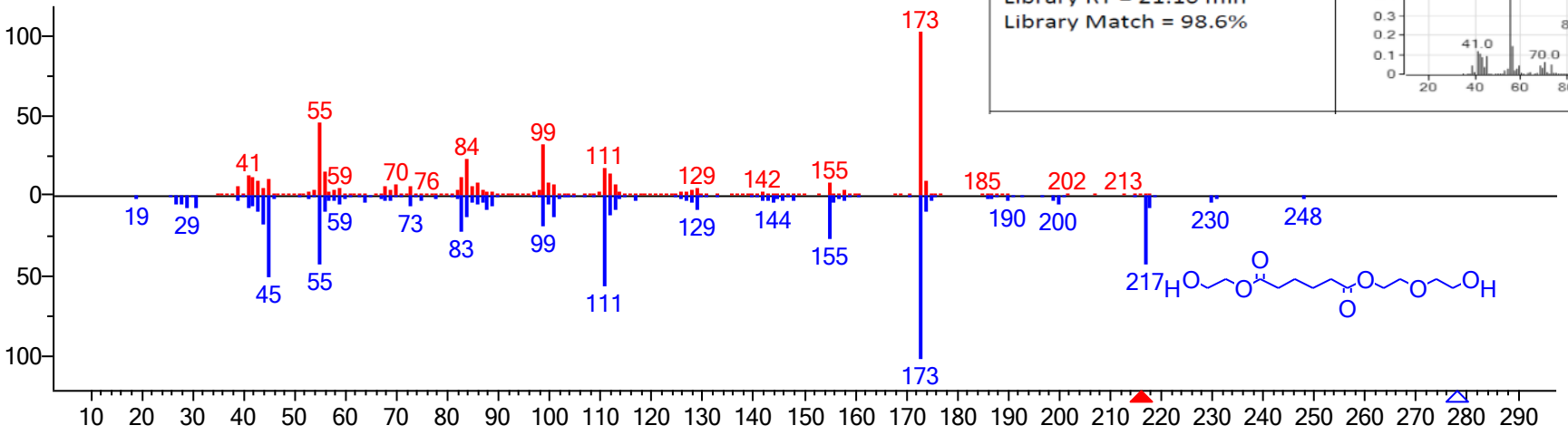
Tentative identifications

When the mass spectrum is not in the database…

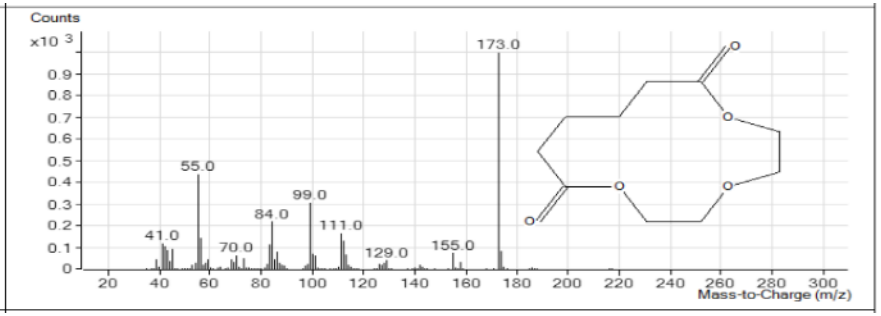


#	Lib.	Match	R.Match	Prob. (%)	Name
1	M	691	693	36.7	Ethylene glycol - Adipate - Diethylene glycol
2	M	681	685	25.9	Diethylene glycol adipate
3	M	637	659	5.61	Succinic acid, 2-(2-chlorophenoxy)ethyl ethyl ester
4	M	630	632	4.30	Butanamide, N-methyl-4-(methylthio)-2-(2,2-dimethylpr
5	M	621	623	3.12	Diethylene glycol - Adipate - Diethylene glycol
6	M	619	640	2.88	1-Dimethylthexylsilyloxyheptane

Best hit Nelson Labs DB

EXACT IDENTIFICATION

1,4,7-Trioxacyclotridecane-8,13-dione
Library RT = 21.10 min
Library Match = 98.6%



The "Home Court" Advantage

There is no compound with a high match factor

→ Compound not in database

→ Unidentified or Tentative identification by expert interpretation

Again: reporting hit 1 would be a wrong identification

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Confident identifications

- **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)

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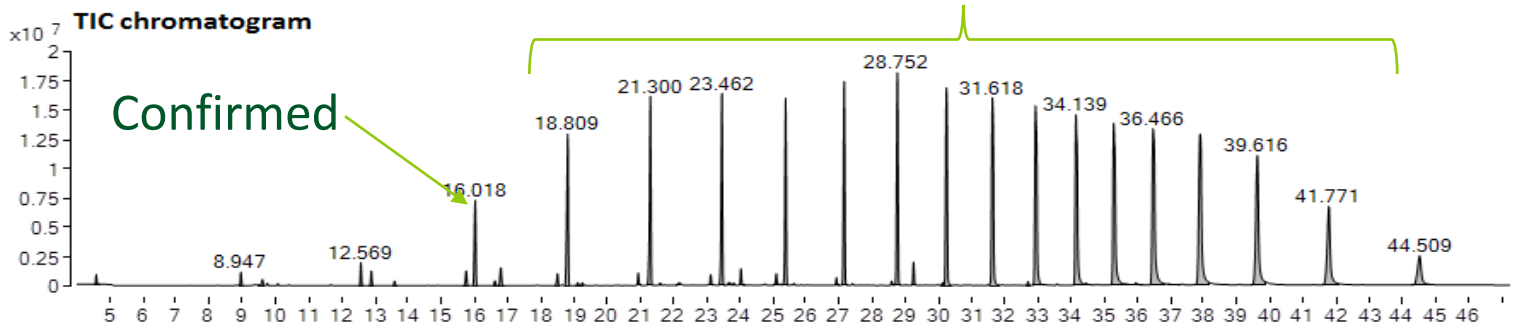
Confident identifications

Case: homologue series

Tentative/Confident

Confirmed



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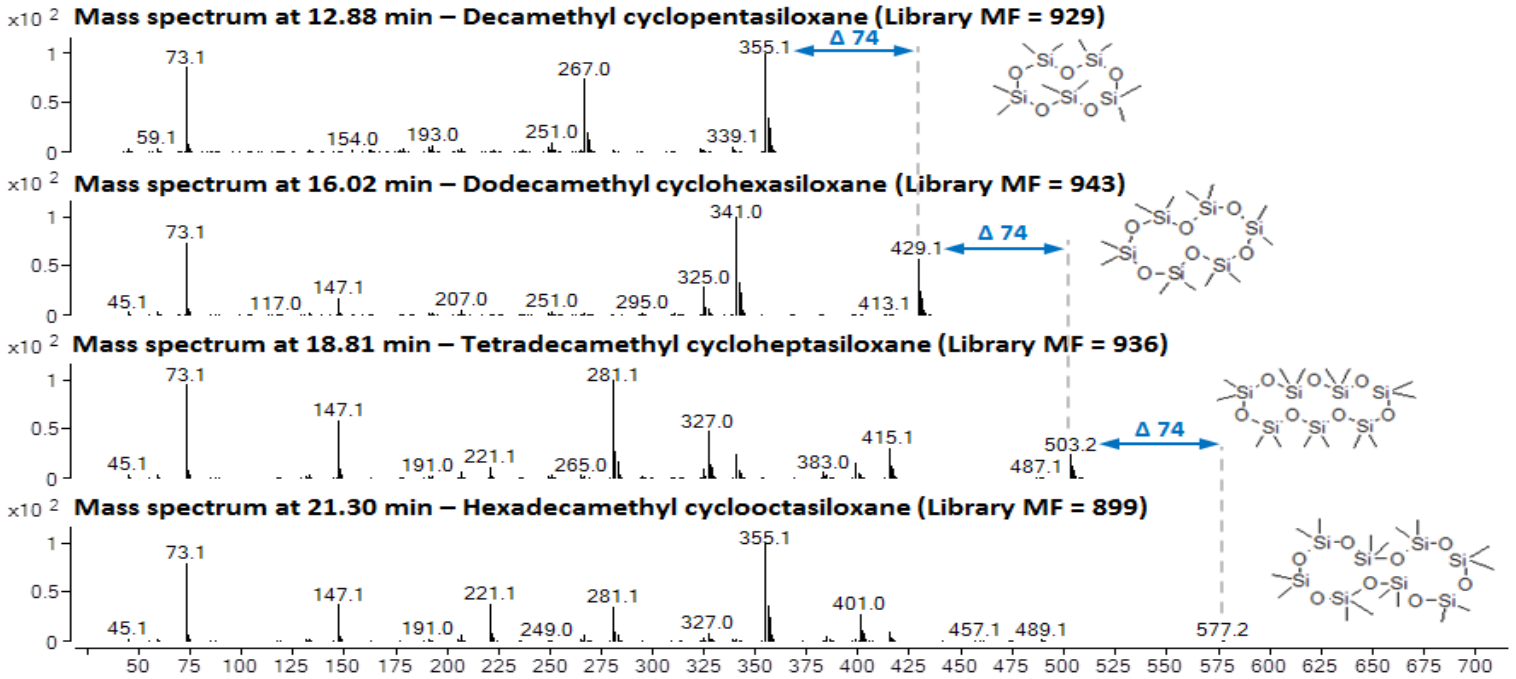

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Second Pass Identification

INCREASE IDENTIFICATION LEVEL

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When second pass comes into play?

- **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)
  - TIC 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**

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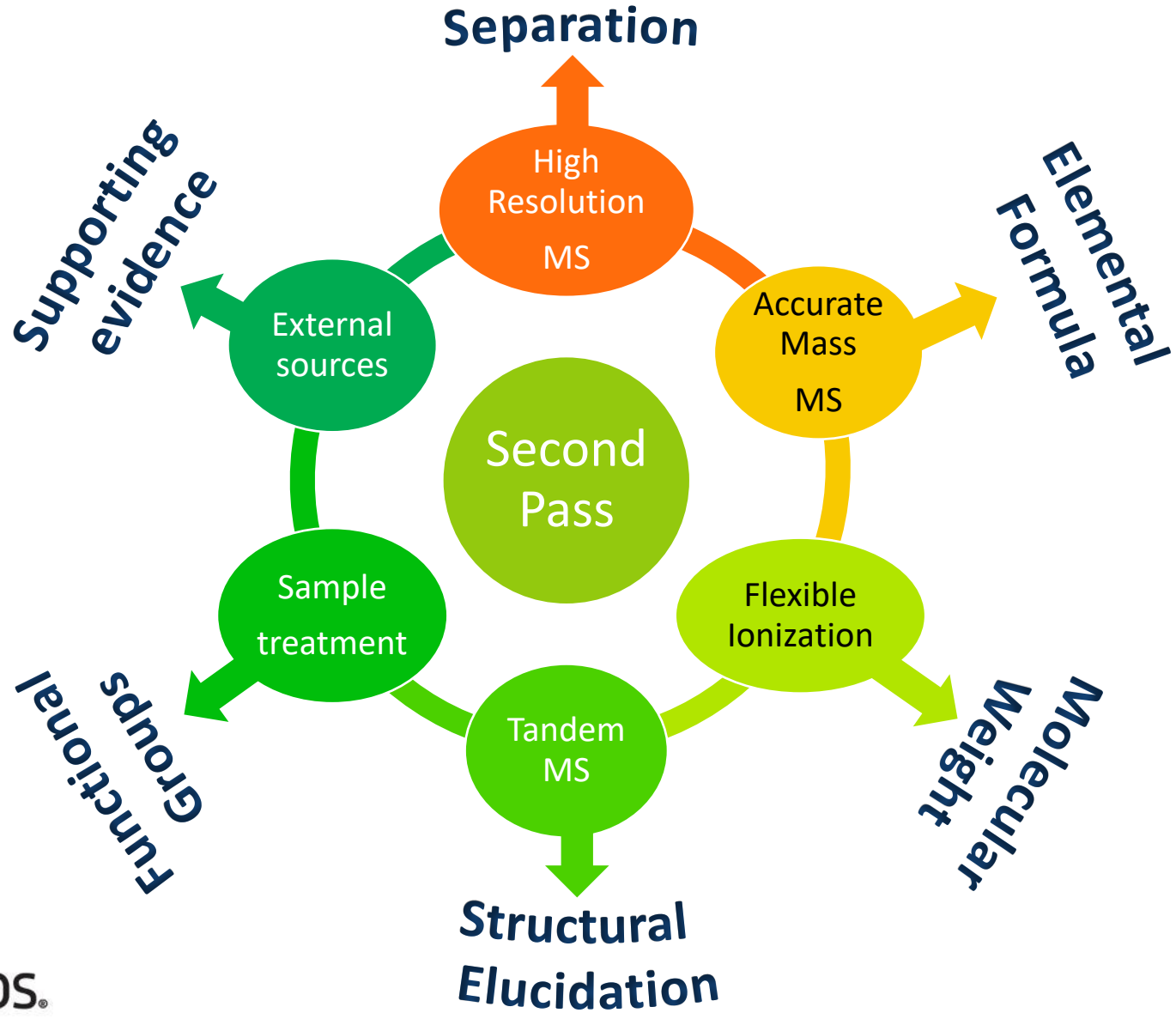
What is second pass testing?



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Why need for High Resolution / Accurate Mass Spectrometry?

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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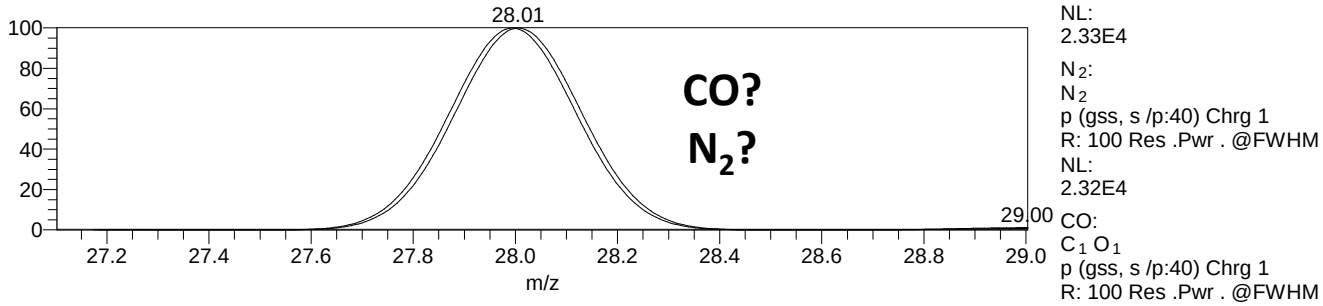
High Resolution / Accurate Mass Spectrometry

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

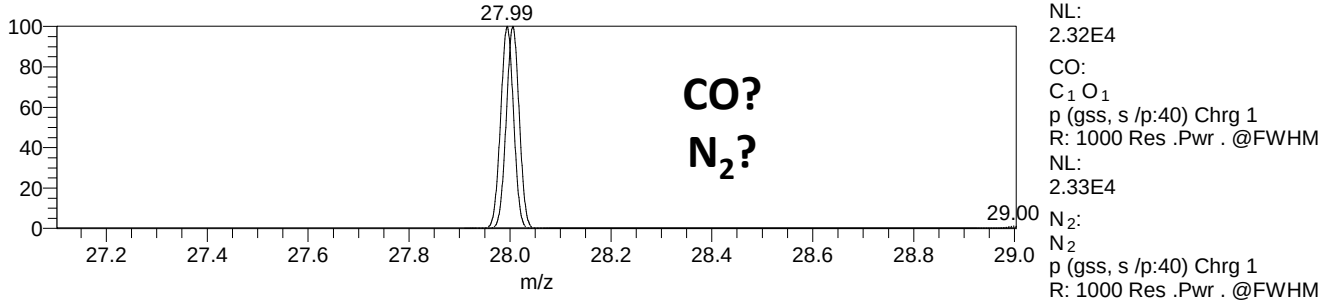
Not separated **R = 100**

GC/MS Quadrupole



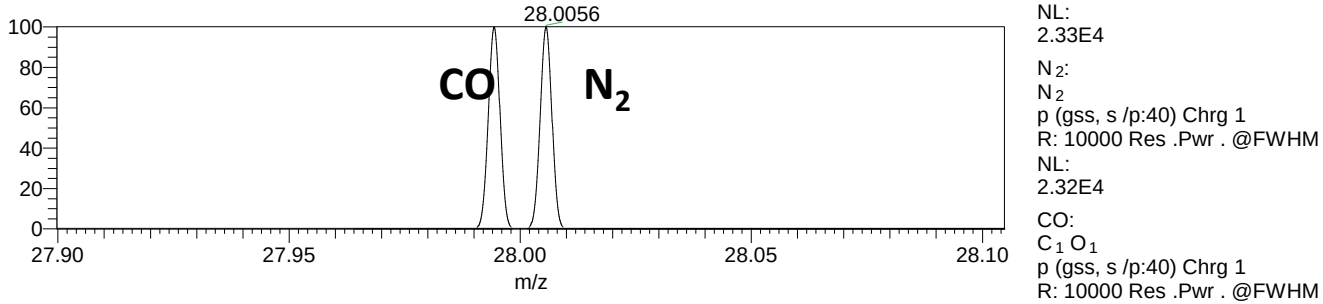
Not separated **R = 1000**

GC/MS Q-TOF



Separated **R = 10000**

GC/MS Orbitrap



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Organic example: 2 compounds where both have nominal mass 102…

Not separated

LC/MS Quadrupole

R = 1000

LC/MS TOF

Close…

R = 10000

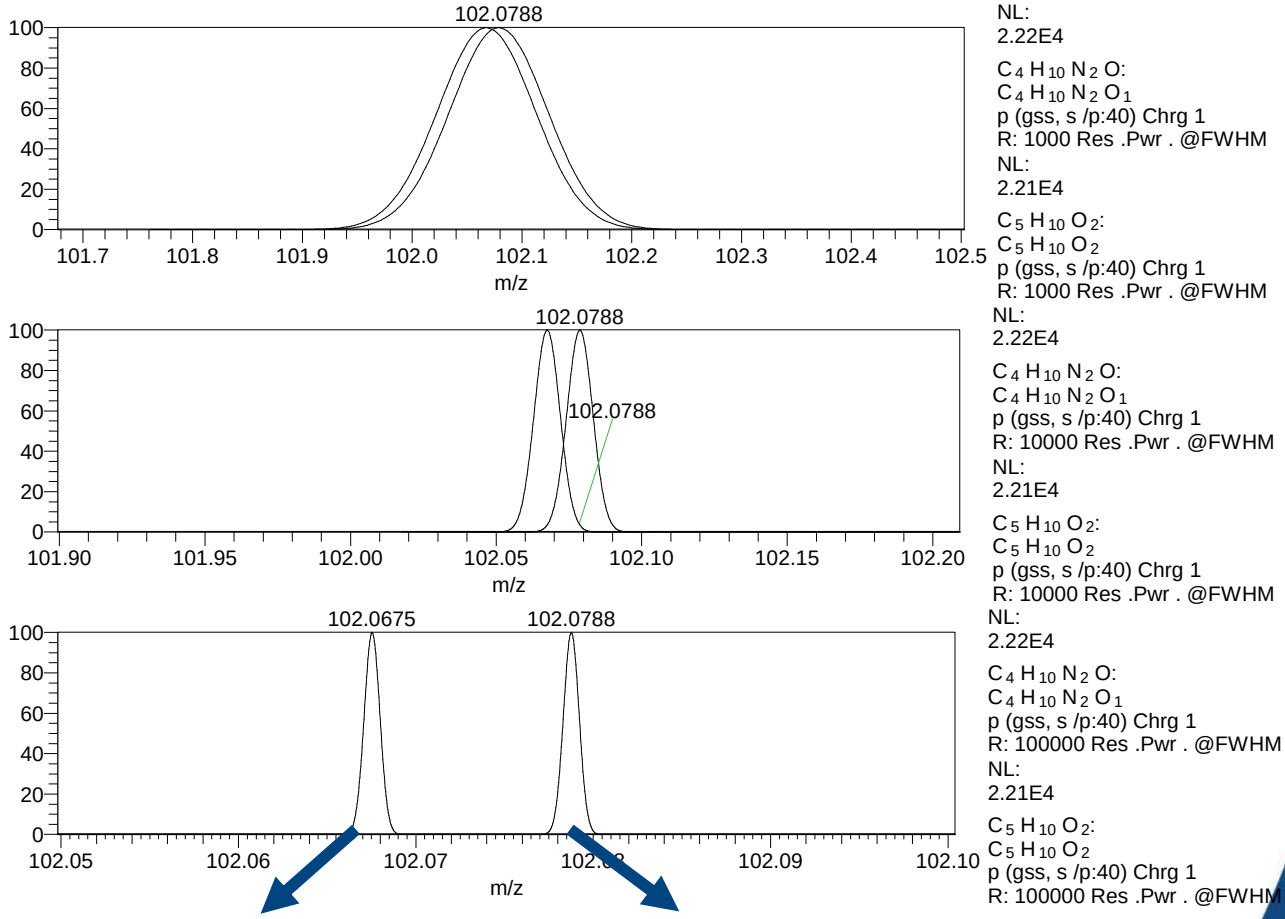
LC/MS Q-TOF

LC/MS Orbitrap

Separated

R = 100000

LC/MS Orbitrap



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

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Sometimes very high resolution needed to separate compounds with same nominal mass!

High Resolution / Accurate Mass Spectrometry

Important take aways around HRAM MS:

accurate mass alone does not deliver a structure…

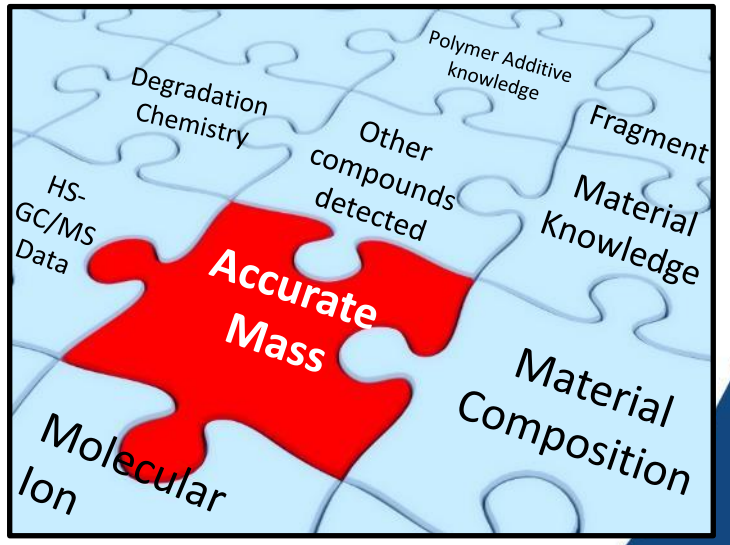
… but delivers **the elemental formula** of the molecule and fragments of the molecule

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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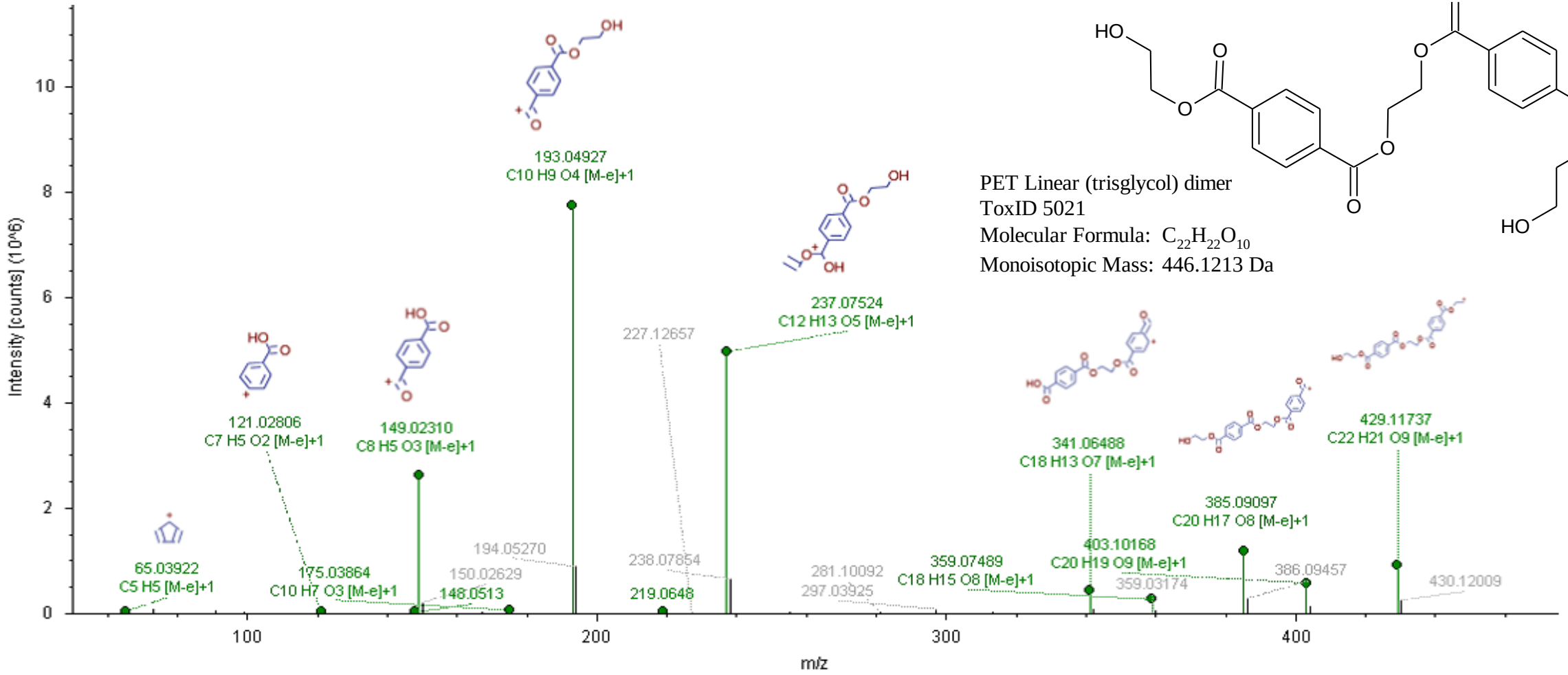
High Resolution / Accurate Mass Spectrometry

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



PET Linear (trisglycol) dimer
ToxID 5021
Molecular Formula: $C_{22}H_{22}O_{10}$
Monoisotopic Mass: 446.1213 Da

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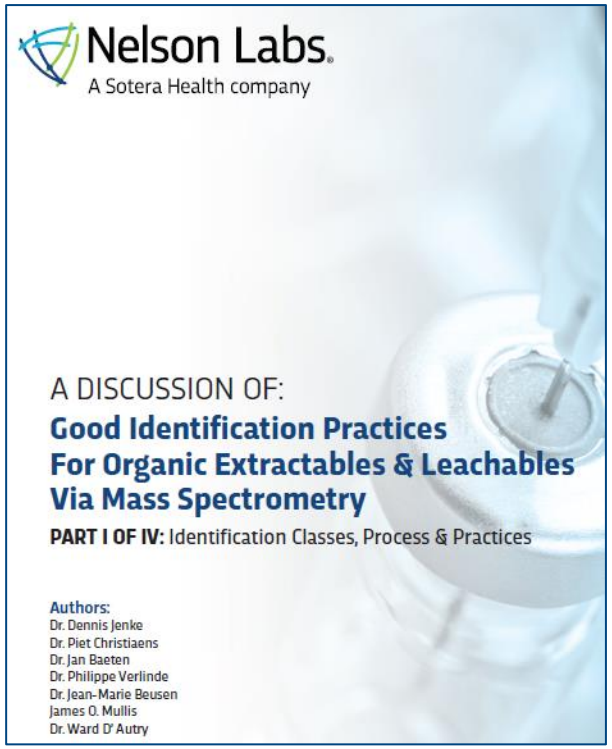
Read more…

NELSON LABS White Paper Series
Part 1: Introduction and Description of the ***"Identification Classes & Processes"***



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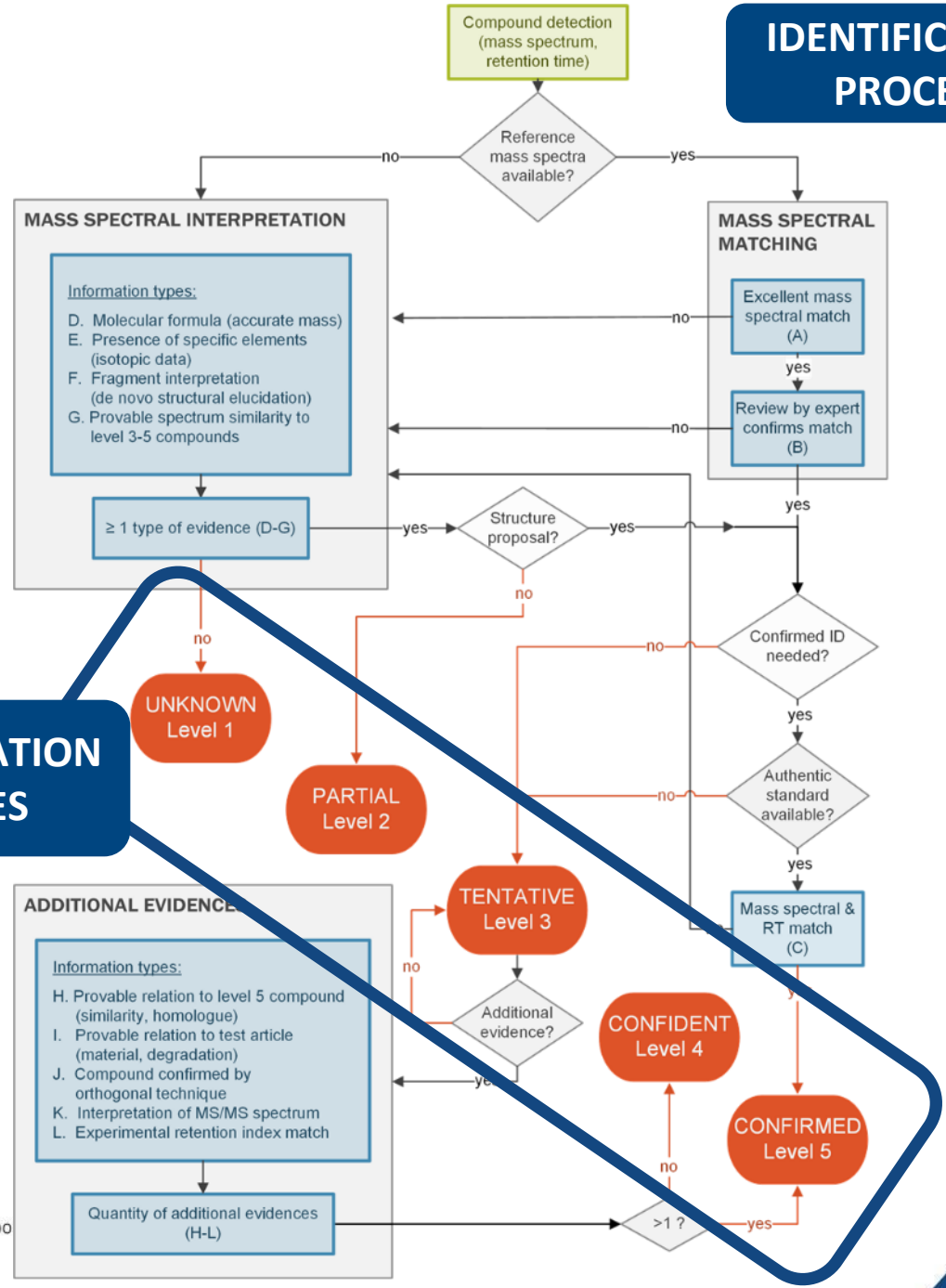
IDENTIFICATION PROCESS



IDENTIFICATION CLASSES

Read more…

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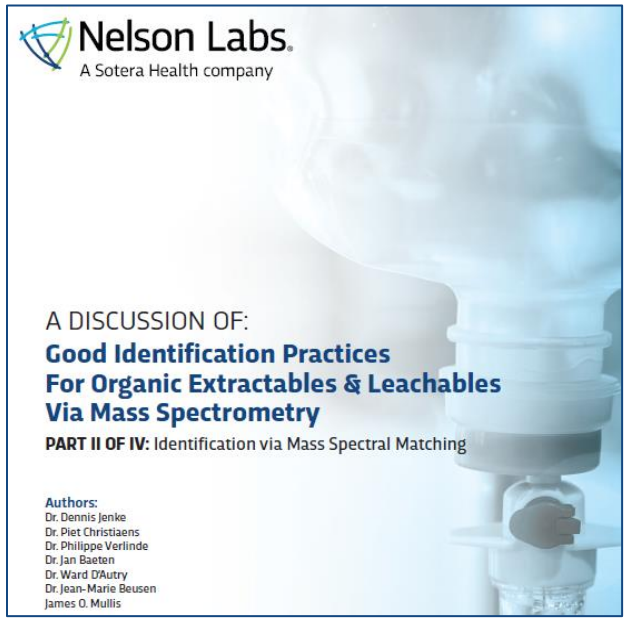
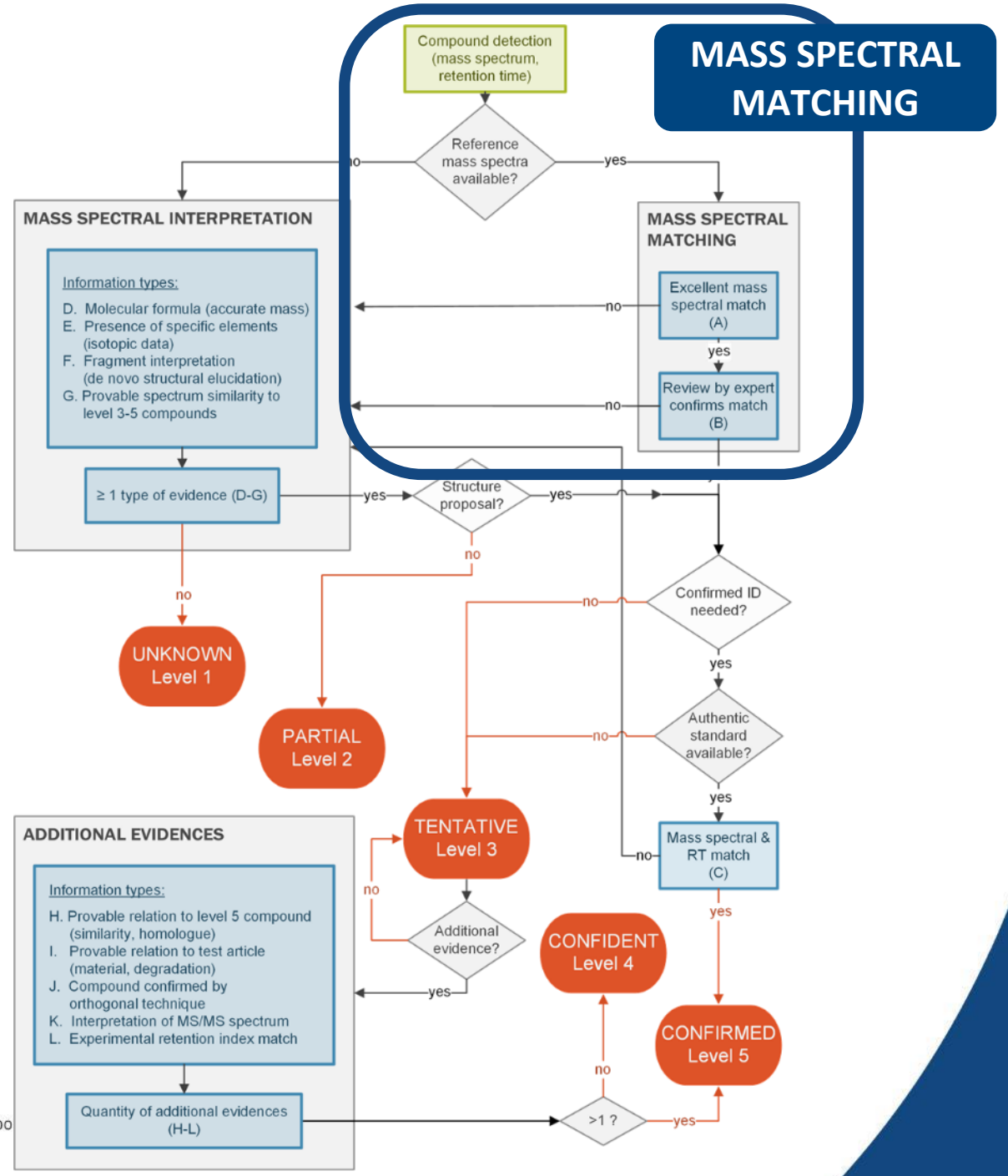
Part 2: ***"Mass Spectral Matching"***





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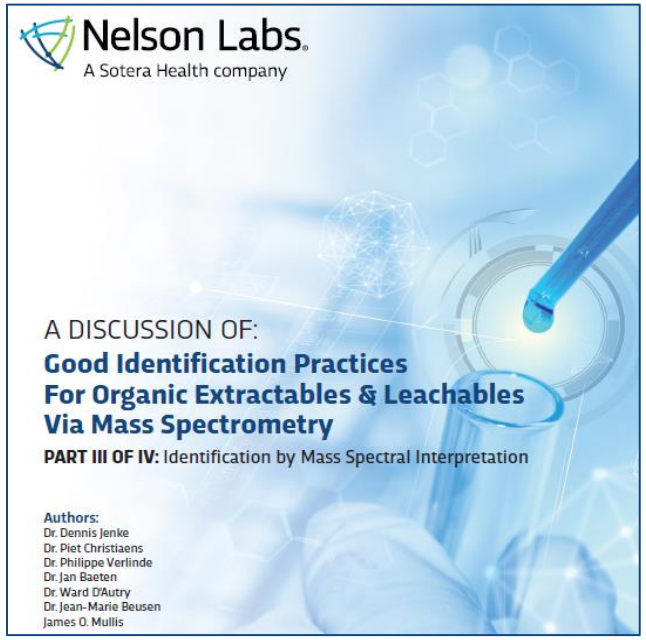
Part 3: ***"Mass Spectral Interpretation"***



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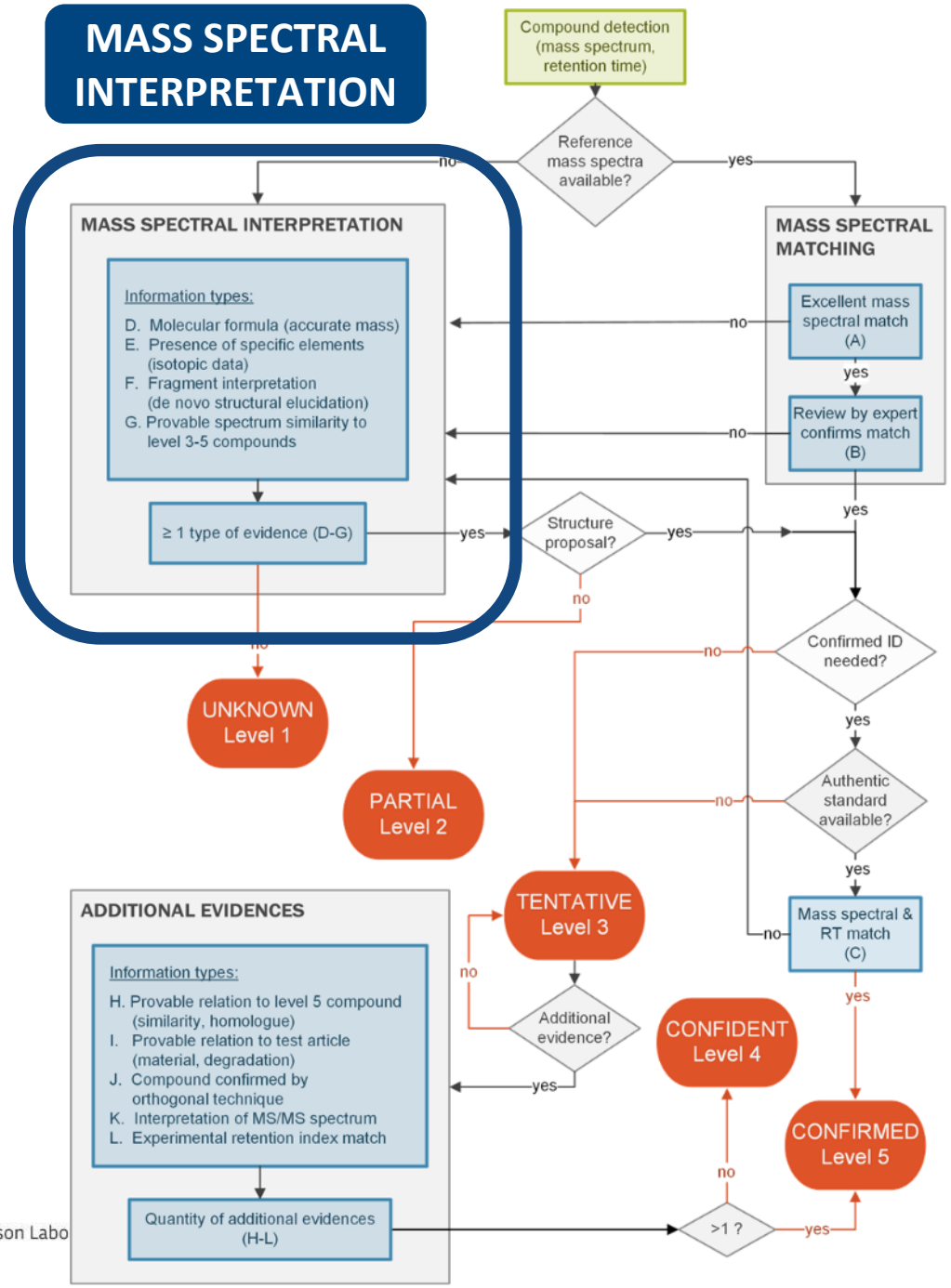

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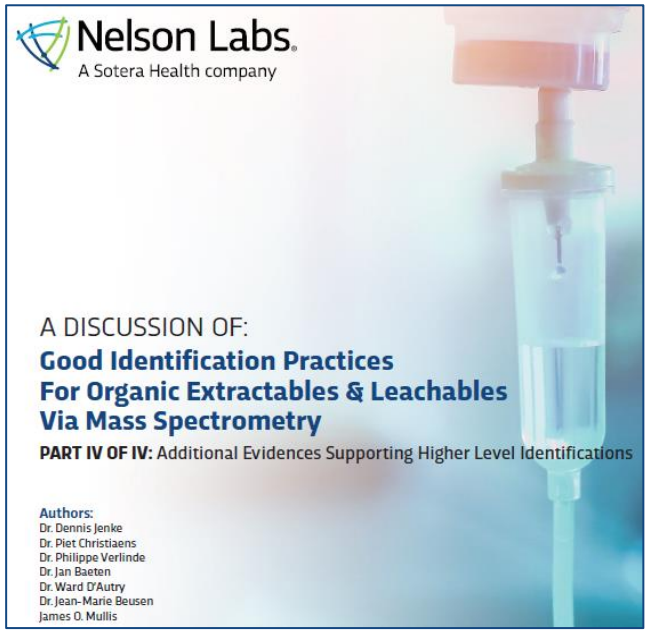
Part 4: ***"Additional Evidences*** *supporting* ***Higher Level Identifications"***



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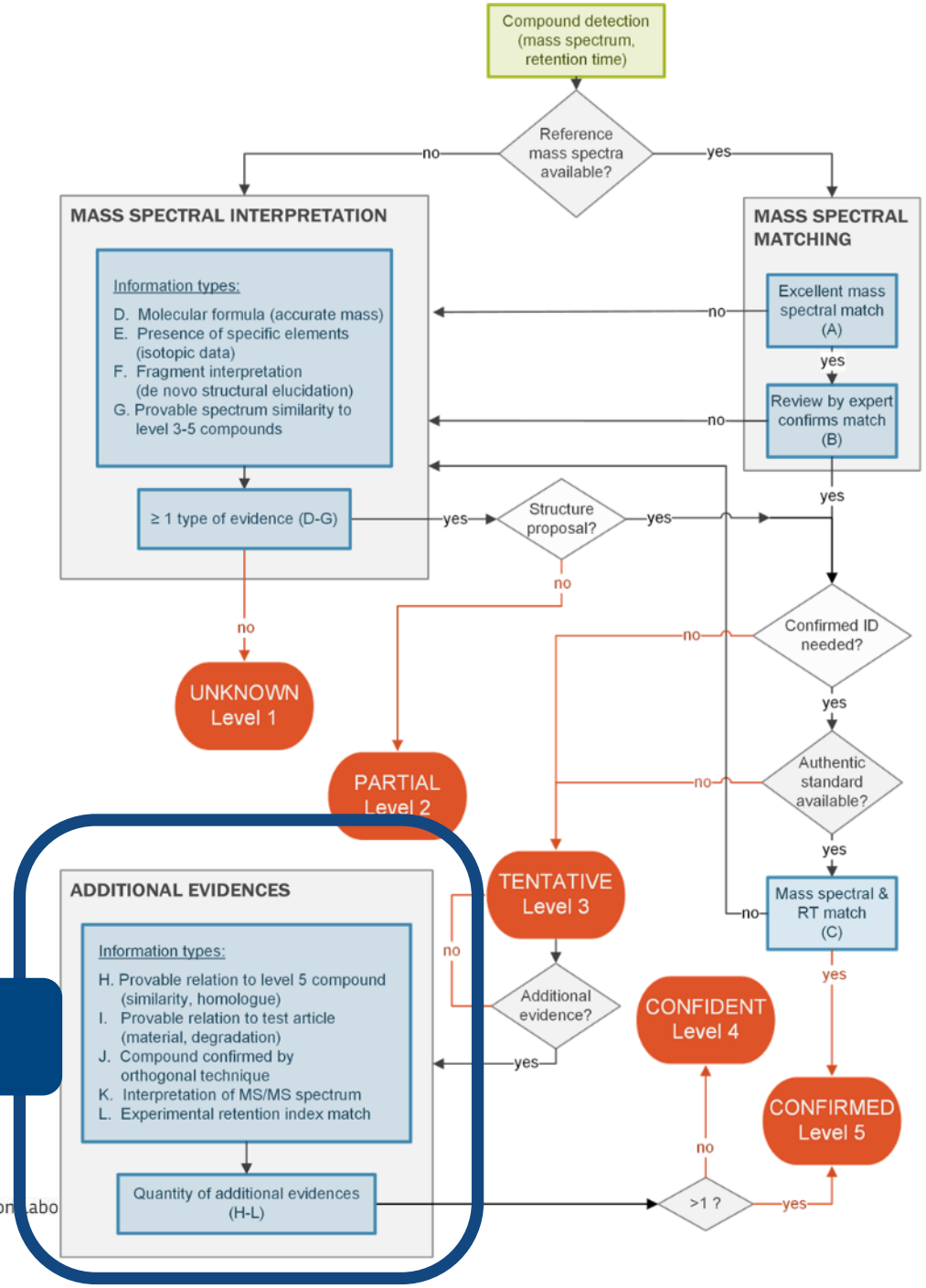
ADDITIONAL EVIDENCES



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