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A Sotera Health company

Good Identification Practices

STRUCTURE IS KEY FOR TOXICOLOGICAL RISK ASSESSMENT

26 APRIL 2023

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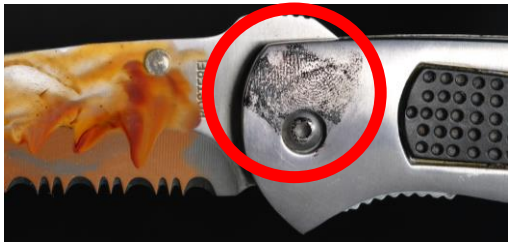
5 years later…

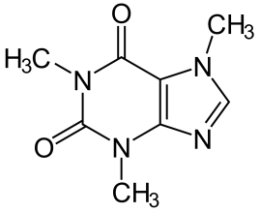


DIFFERENT ANALYTICAL TECHNIQUES

IDENTIFICATION

CAS No XXXXXXX-YY-Z

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



58-08-2

QUANTIFICATION

Concentration

µg/L

µg/unit **µg/g**

Screening | Analytical techniques

CHROMATOGRAPHY – MASS SPECTROMETRY

(Headspace –) Gas chromatography – Mass Spectrometry

Liquid Chromatography – Mass Spectrometry

Volatile organic compounds

VOC

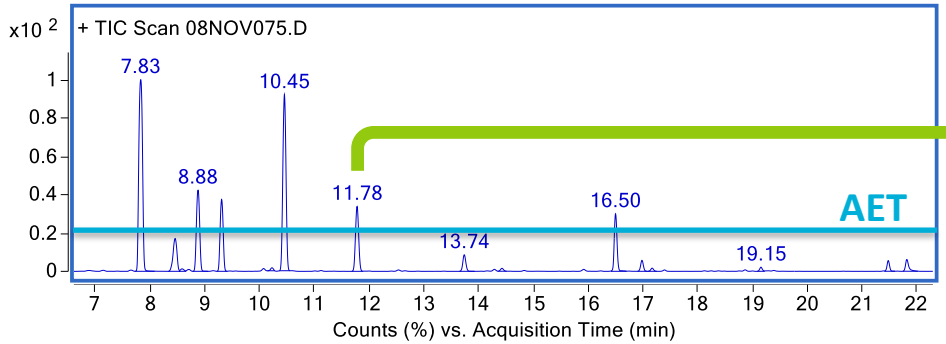
Semi-Volatile organic compounds

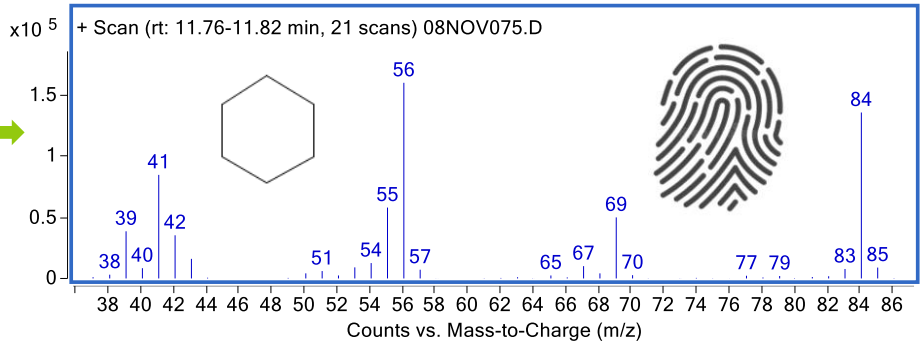
SVOC

Non-volatile organic compounds

NVOC

CHROMATOGRAPHY – MASS SPECTROMETRY





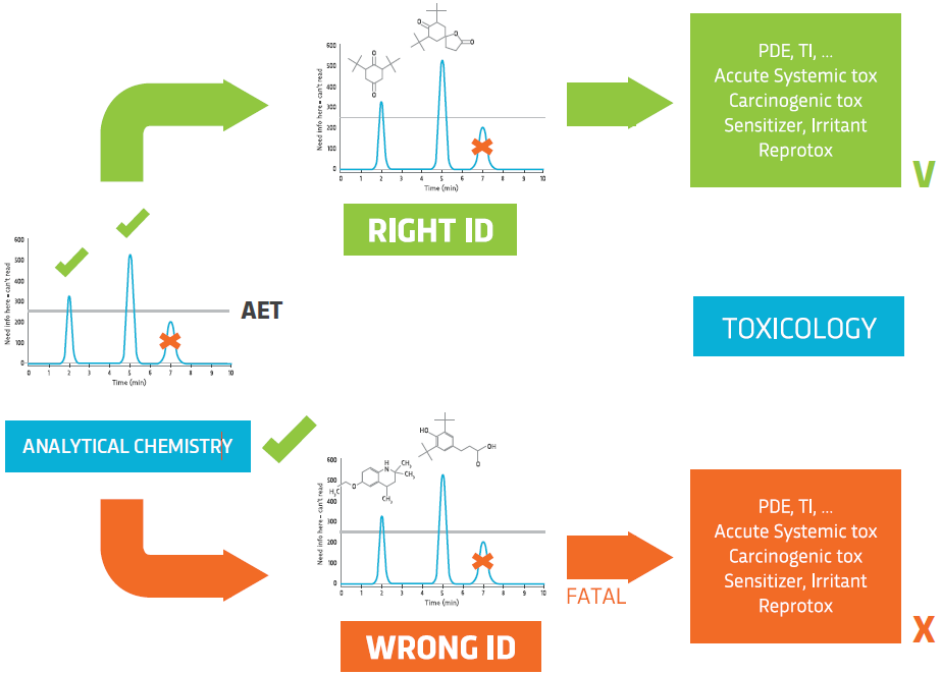
Chromatogram

- Analytical output from chromatography system
- Detector signal intensity *in function of* analysis time
- Compound separation
- Retention time → discriminator for **identification**
- 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**

Screening | Identification | Importance of correct identification



Error of omission

No detection / not discovered

↓

No tox assessment

↓

Safety compromised

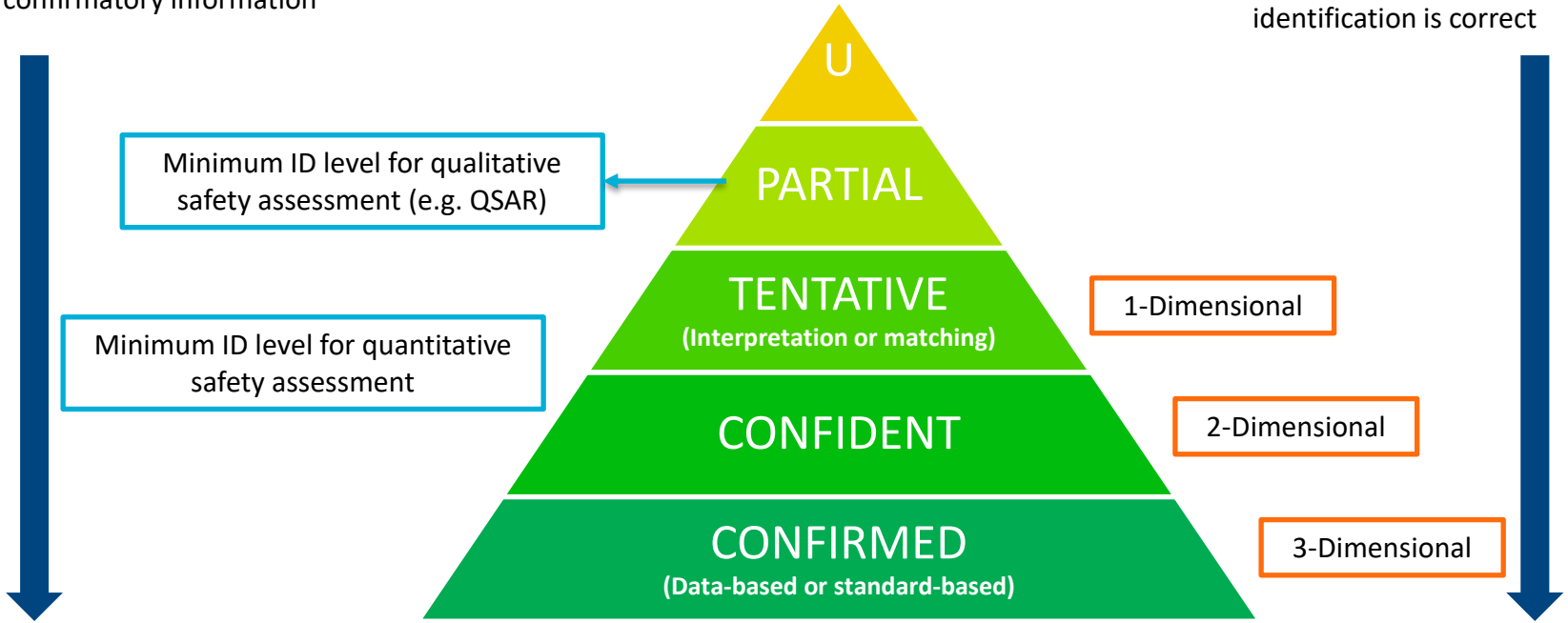
GOOD IDENTIFICATION PRACTICES ARE KEY!

Identification | Identification levels

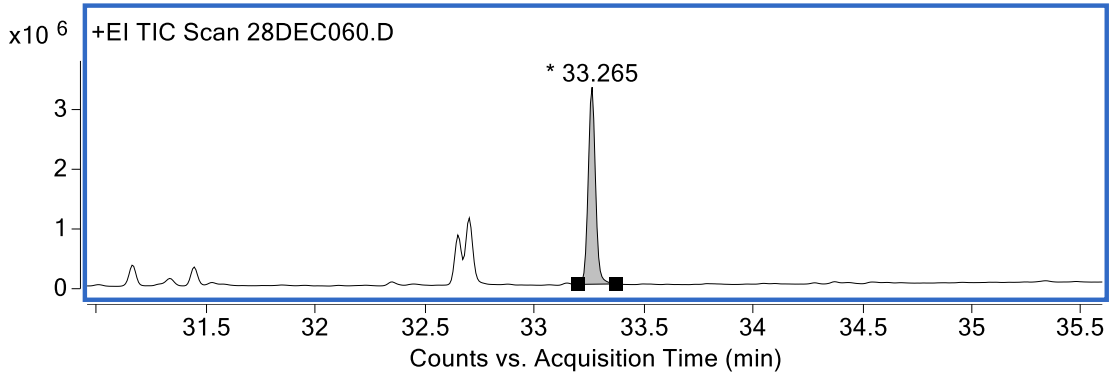
***References**: USP 1663 | Nelson Labs e-Book Good Identification Practices*

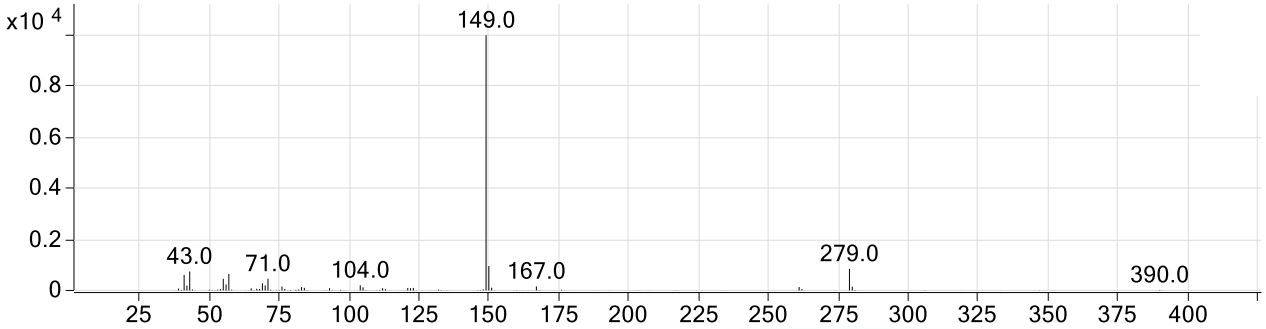
Increasing amount or rigor of confirmatory information

Increasing certainty that the identification is correct

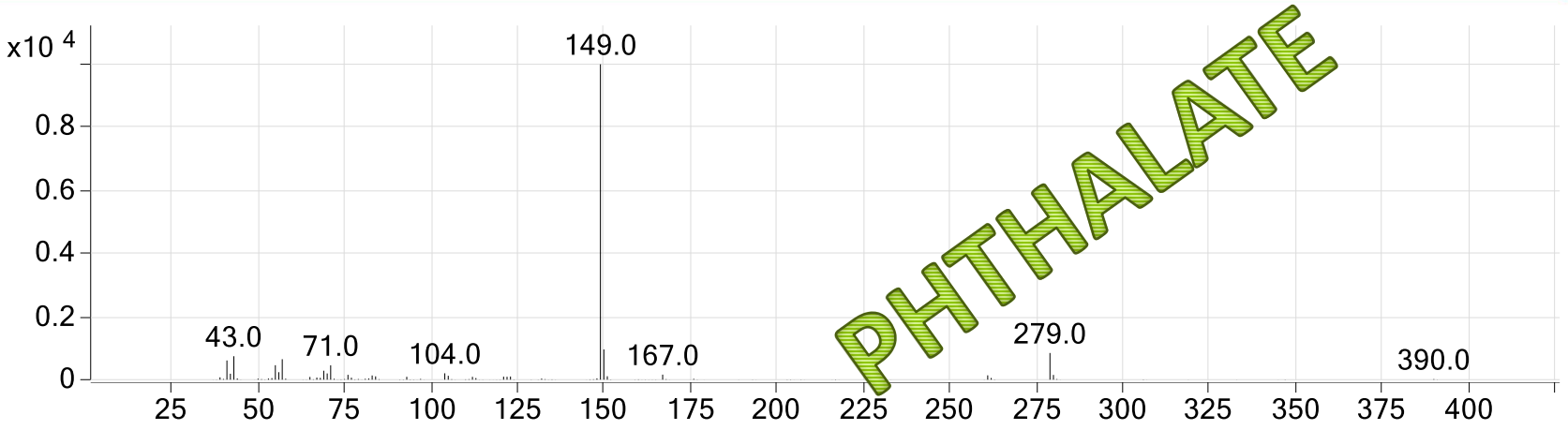


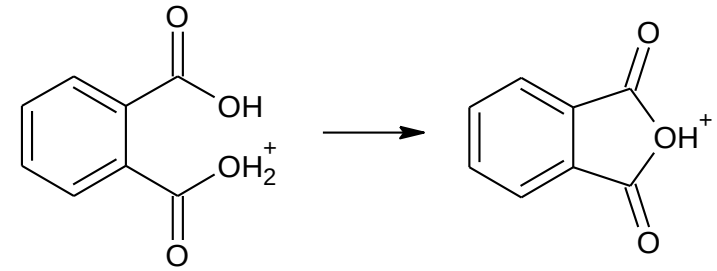
Identification | Identification levels | Unidentified compound





Identification | Identification levels | Partial Identification



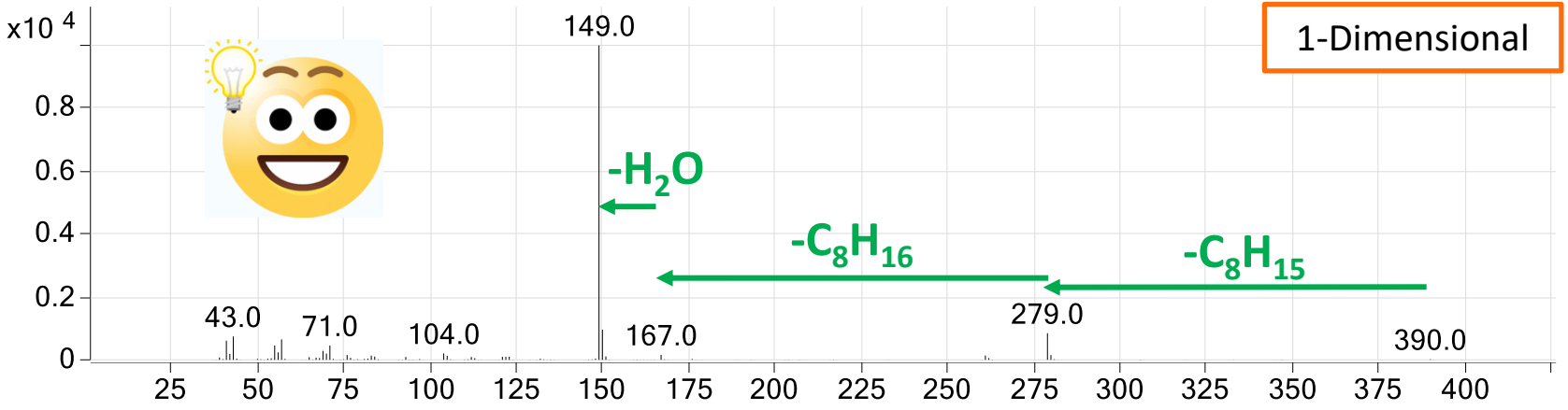


Nominal Mass: 167 Da

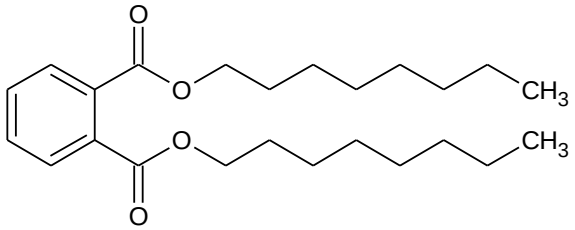
Nominal Mass: 149 Da

Diagnostic pair of ions for phthalate esters

Identification | Identification levels | Tentative ID - Interpretation

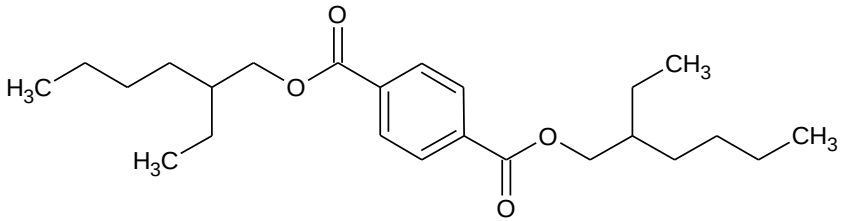


1-Dimensional



Molecular Formula: $C_{24}H_{38}O_4$

Nominal Mass: 390 Da



Molecular Formula: $C_{24}H_{38}O_4$

Nominal Mass: 390 Da

Identification | Identification levels | Tentative ID – Matching (1)

1-Dimensional

National Institute of Standards and Technology

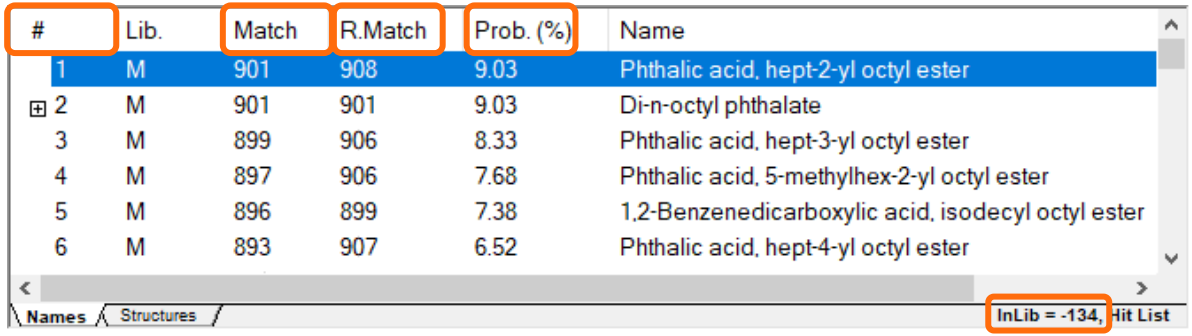
> 300 000 GC/MS spectra

WILEY

> 840 000 GC/MS spectra

Identification | Identification levels | Tentative ID – Matching (2)

1-Dimensional



#	Lib.	Match	R.Match	Prob. (%)	Name
1	M	901	908	9.03	Phthalic acid, hept-2-yl octyl ester
2	M	901	901	9.03	Di-n-octyl phthalate
3	M	899	906	8.33	Phthalic acid, hept-3-yl octyl ester
4	M	897	906	7.68	Phthalic acid, 5-methylhex-2-yl octyl ester
5	M	896	899	7.38	1,2-Benzenedicarboxylic acid, isodecyl octyl ester
6	M	893	907	6.52	Phthalic acid, hept-4-yl octyl ester

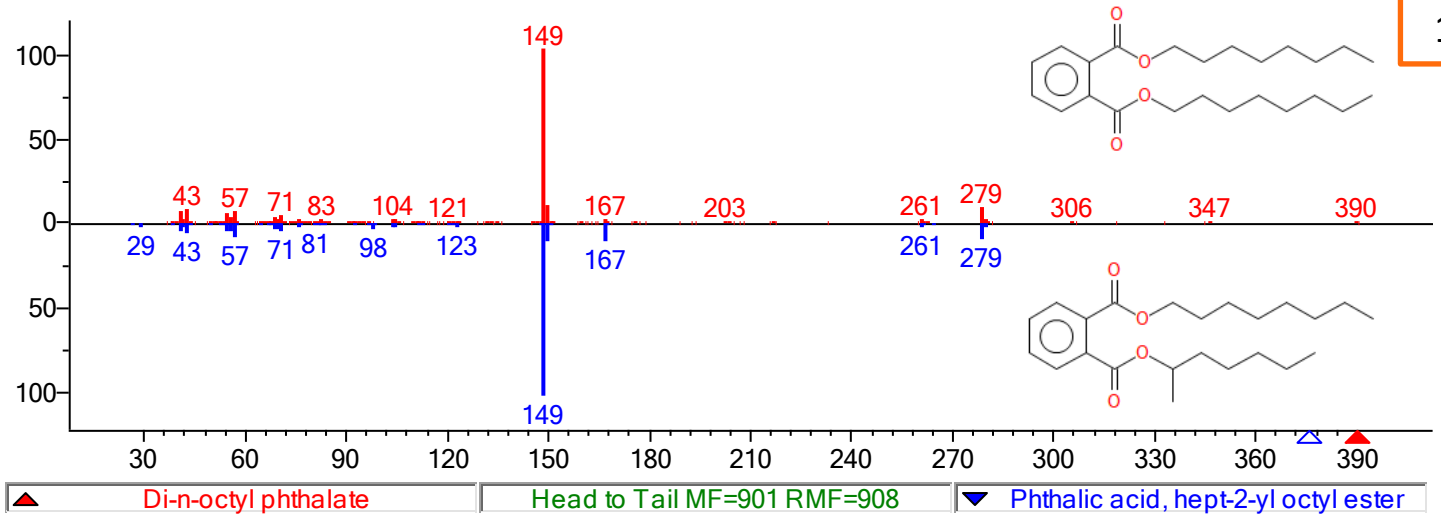
Ranking
Match Factor → how good is the fit of the unknown spectrum with the reference spectrum?
Reverse Match Factor → how good is the fit of the reference spectrum with the unknown spectrum?
Probability (%) → is that the probability that the ID is correct?
InLib score → the probability that the compound is actually in the searched library. Wait a minute…

When do we have a "good match"?

Can I always safely report the top hit from the NIST/Wiley database?

Identification | Identification levels | Tentative ID – Matching (3)

1-Dimensional

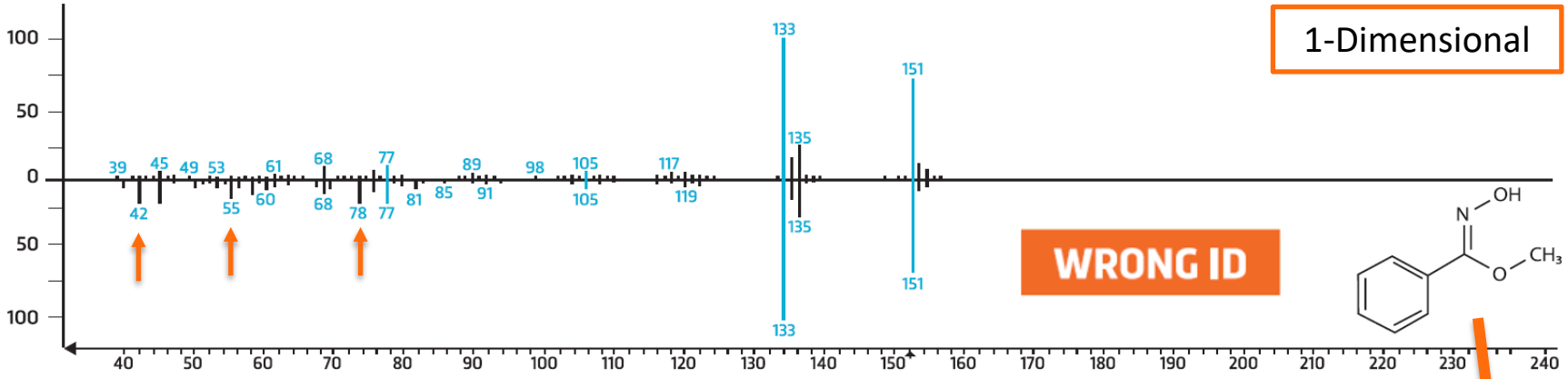


The top hit is a wrong identification! Even with a (reversed) match factor > 900 !! 😮

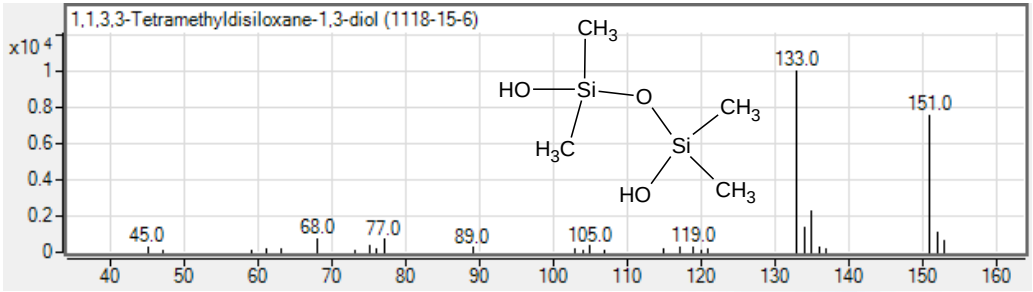
A GC/MS spectrum (EI) does not always show the molecular ion → key piece of info missing!

There can be many similar spectra in an extremely large database → increased risk of false positive hits

Identification | Identification levels | Tentative ID – Matching (4)



Correct ID in Nelson Labs database:



Small differences, big consequences

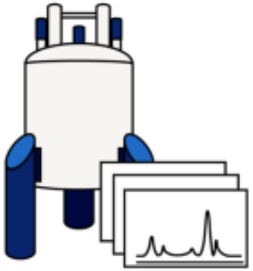
1-Dimensional

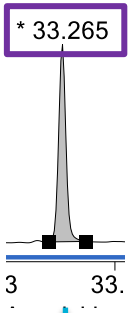
Careful review by mass spectrometrist strongly recommended

Identification | Identification levels | Confident ID (1)

How to add a second dimension to upgrade the ID level?

2-Dimensional





33.265 min → RI = 2700

Retention Index (RI)

NIST RI database

Experimental RI median±deviation (#data)
Semi-standard non-polar: 2690±10 (4)
Standard non-polar: 2686±3 (6)

Estimated non-polar retention index (n-alkane scale):
Value: 2832 iu
Confidence interval (Esters): 47(50%) 201(95%) iu

AI predicted non-polar retention index (n-alkane scale):
Value: 2737 iu
Confidence interval : 13(50%) 69(95%) iu

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Identification | Identification levels | Confident ID (2)

How to add a second dimension to increase confidence?

2-Dimensional

Ingredients	Concentrations (phr)
PVC	100
Organotin stabilizer	2
PE wax	1
Ester lubricant	3
Stearic acid	0.3
$CaCO_3$	14
ACR	7
DOP	2

Composition of the materials of construction

ACR, acrylic resin; DOP, dioctyl phthalate; PE, polyethylene; phr, parts by weight per hundred parts of resin; PVC, poly(vinyl chloride).

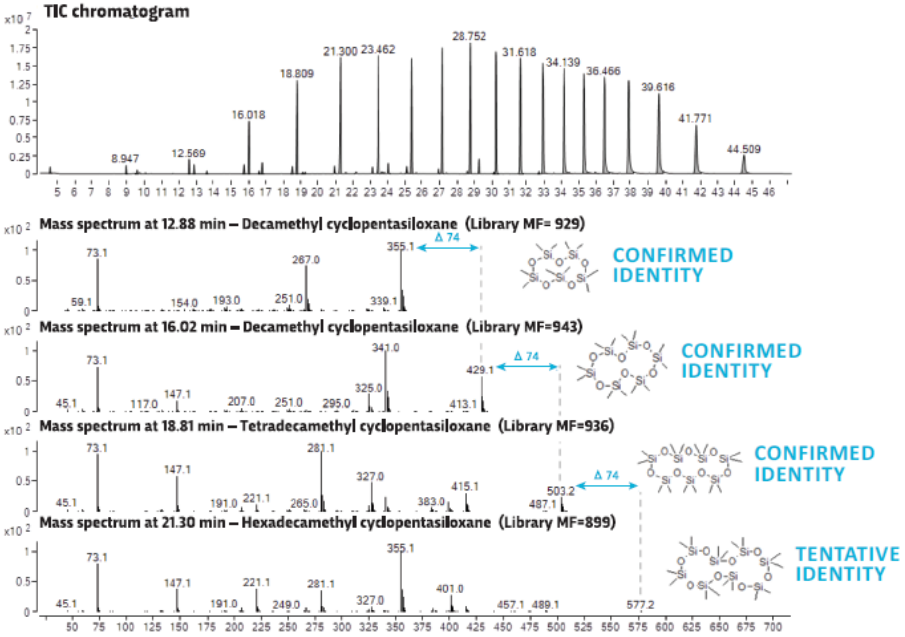
Wang et al.; Journal of Vinyl and Additive Technology, 22 (3), 2014

Identification | Identification levels | Confident ID (3)

How to add a second dimension to increase confidence?

2-Dimensional

Homologue series



ID level can be increased to **confident**

Homologues confirmed in same sample

Spectrum linked to series of confirmed compounds

Identification | Identification levels | Confirmed ID – Standard based

Measure standards with own screening methods

↓

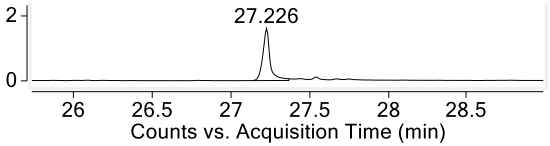
Capture RT & MS spectrum in database

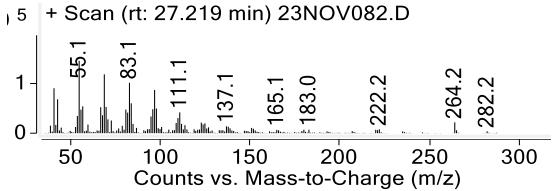
↓

Match with standard in database = confirmed

3-Dimensional

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





Identification | Identification levels | Confirmed ID – Data-based

Alternative pathways allowed

1. Spectral Matching	**2. Retention Index**	**3. Material composition**
1. Interpretation	**2. NMR**	**3. Provable relation to confirmed compound**
…		

Identification | LC/MS

What about LC/MS?

Same principles! But identification flow can be somewhat different from GC/MS

- LC/MS spectra less universal → Less commercial LC/MS libraries (but they are evolving!)
- Use of high-resolution accurate mass (HRAM) instrumentation for screening
  → extra dimension for identification
- Use of data-dependent tandem MS → "on the fly" collection of MS/MS data of compounds
  → MS/MS spectrum can be used as extra dimension for identification

Identification | Home-court advantage of in-house LC/MS database

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

Without database, data interpretation is huge effort to identify compounds and/or increase the ID level

Matching with in-house LC/MS database is fast and effective road to many confirmed ID's

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SECOND PASS IDENTIFICATION | STRUCTURE ELUCIDATION

High-end Mass Spectrometry

Second pass identification | Introduction

- **Unidentified / Partially identified compounds > AET** in 1st pass screening
  - Unidentified compounds → considered carcinogenic/mutagenic
  - To allow de-risking by tox assessment, a **structure is required**!

- Request to **further increase ID level (e.g. low margin of safety)**
  - Tentative to Confident
  - Confident to Confirmed

- Goal of second pass studies: generate / collect **supporting data (analytical and other) to increase the identification level**

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

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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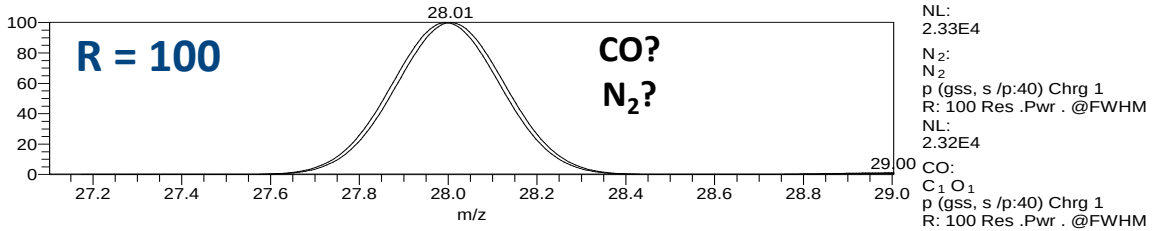
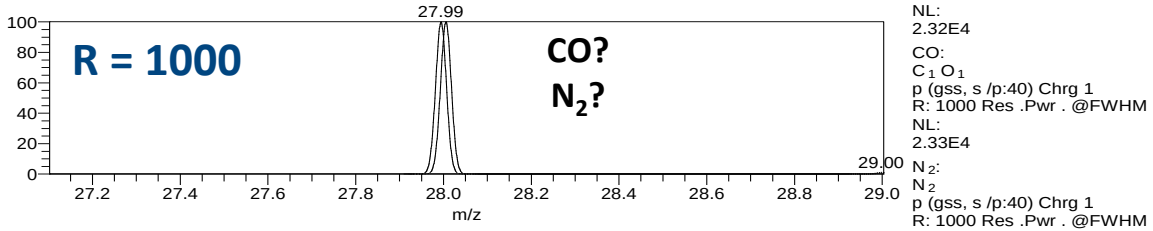
Second pass identification | High Resolution Accurate Mass

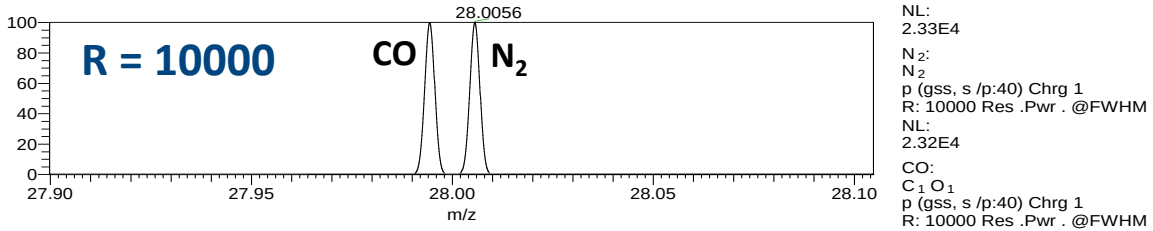
Not separated



Not separated



Separated



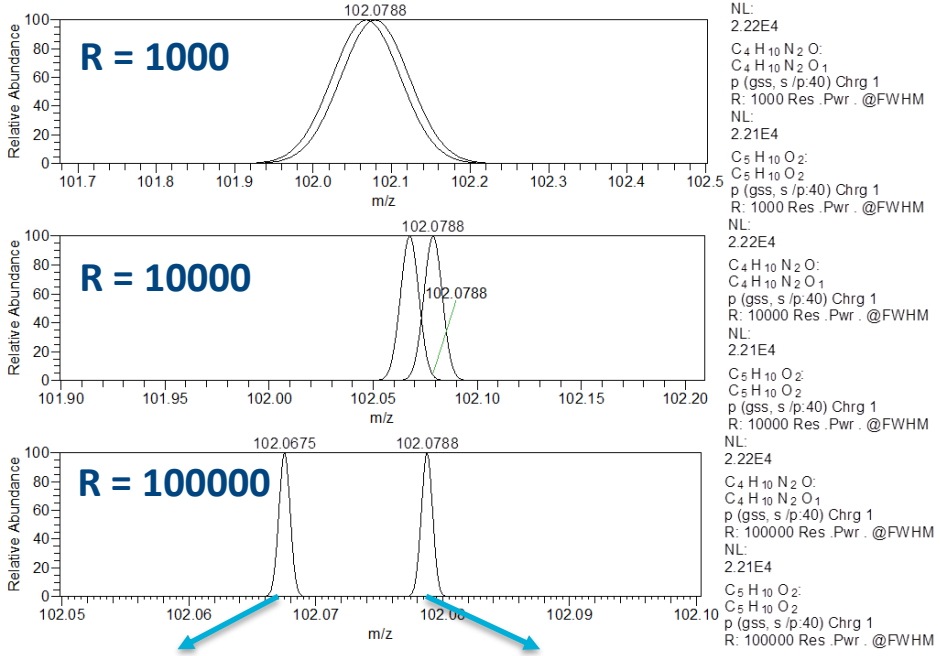
Second pass identification | High Resolution Accurate Mass

E&L example: 2 compounds both with nominal mass 102

Not separated

Close…

Separated



$C_5H_{10}O_2$ - isopropyl acetate

m/z 102.0675

$C_4H_{10}N_2O$ - N-nitrosodiethylamine

m/z 102.0788

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Second pass identification – High Resolution Accurate Mass

Key take aways concerning HRAM MS:

accurate mass alone does not deliver a structure…

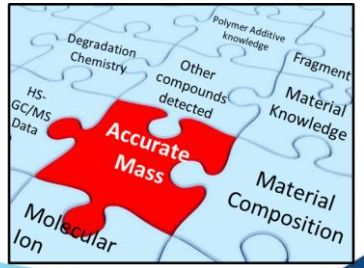
… but delivers the **elemental formula** of the molecule and fragments of the molecule

high resolution does not deliver a structure…

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

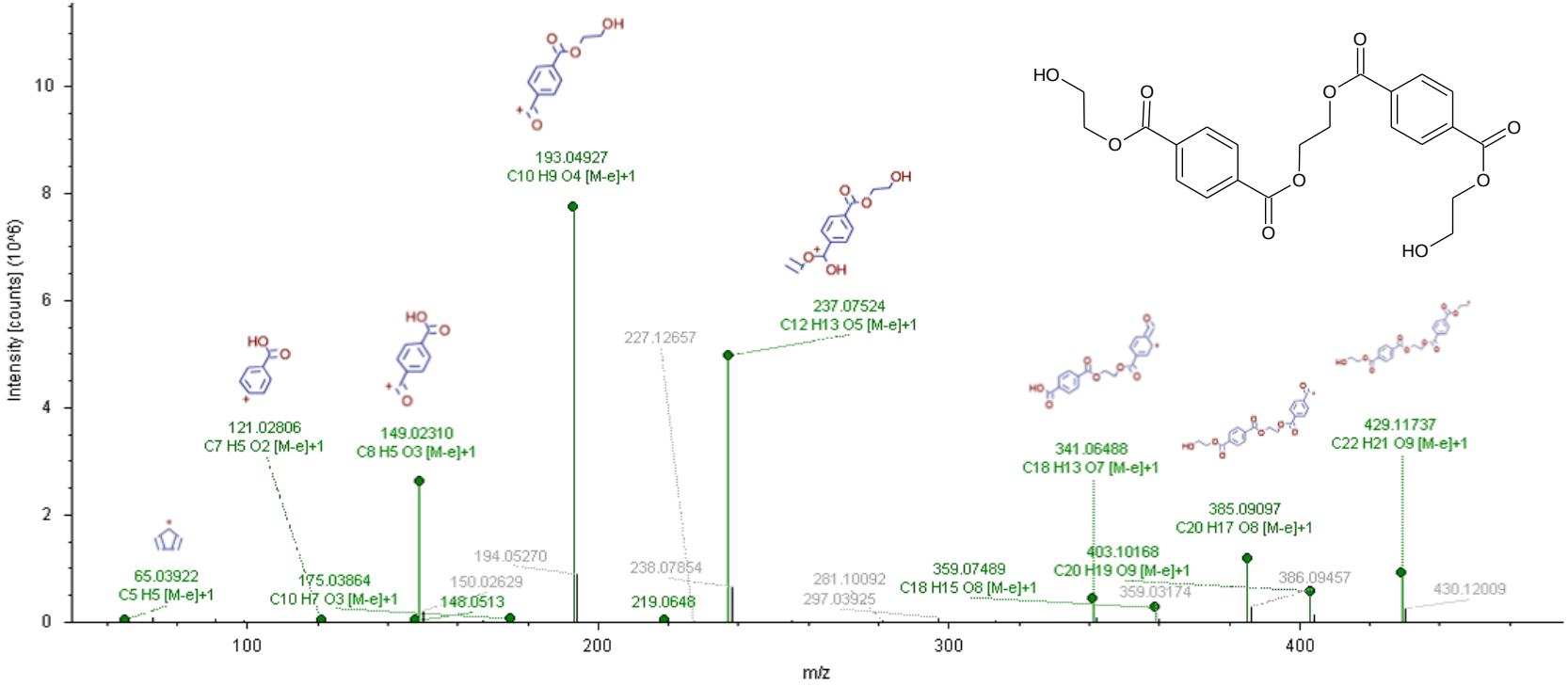
…but assists in confirming the elemental formula using **isotope matching**

It delivers key pieces of the puzzle for structure elucidation

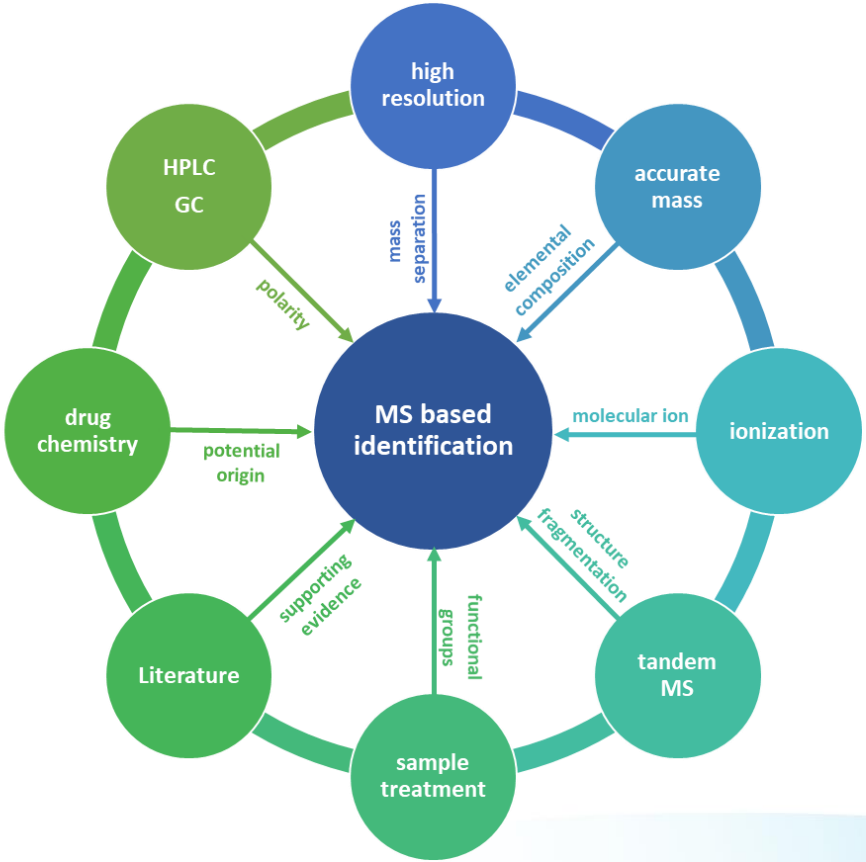


Second pass identification – Tandem Mass Spectrometry

Case: “de novo” structure elucidation of PET related oligomer using MS/MS spectrum



The beautiful art of structure elucidation



TEAM effort!

Mass spec expert(s)

Drug chemistry expert

Material engineer

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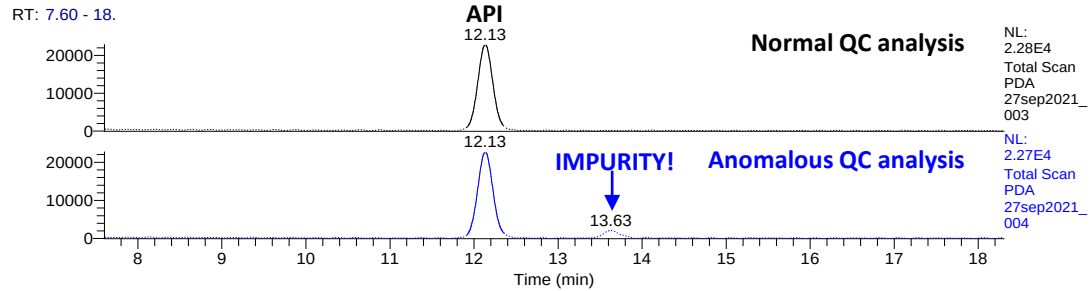
Wrap-up: good identification practices

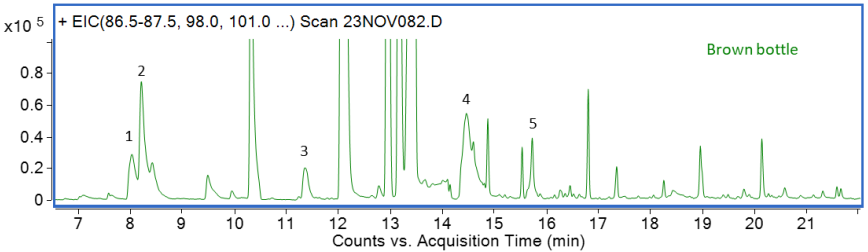
PRACTICE	REASON
Use complementary analytical techniques	Reduces risk of omission
Report appropriate identification levels	Toxicologist defines MoS based on ID level
Review database hits obtained by matching	Mitigates risk of reporting wrong ID
Collect supporting information	Extra dimension(s) to increase ID level
Develop and maintain in-house database	1. Most efficient path to confirmed ID's 2. Mitigates risk of reporting wrong ID

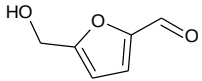
Other structure elucidation projects

DP/DS IMPURITIES



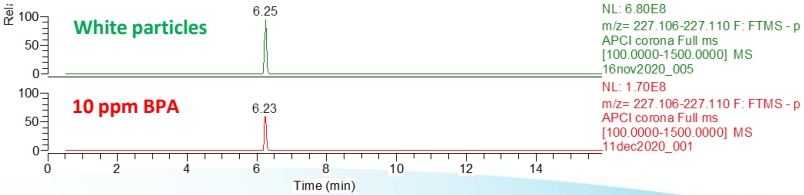
DP/DS DISCOLORATION

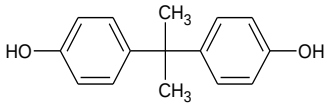




5-Hydroxymethyl-2-furancarboxaldehyde
CAS: 67-47-0
Molecular Formula: $C_6H_6O_3$
Monoisotopic Mass: 126.032 Da

PARTICLES





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

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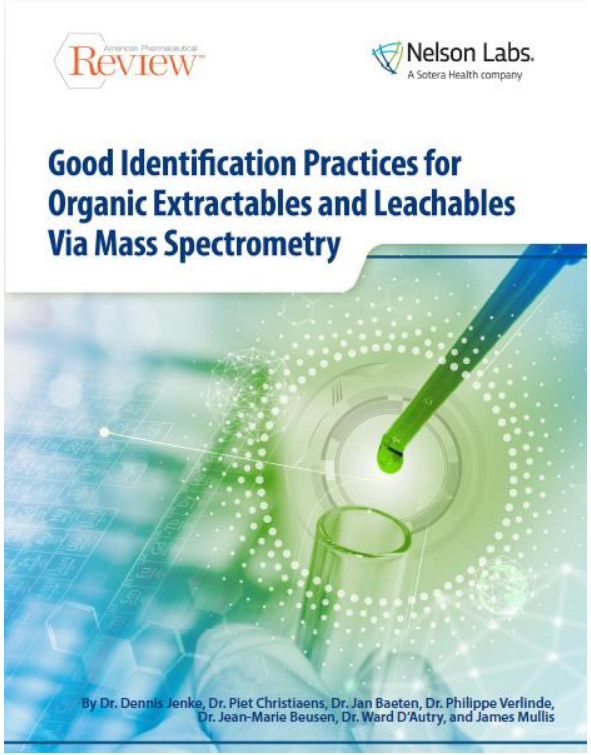
Further reading

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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