PDA Training Course Extractables & Leachables

31 May 2022

Identifying and Mitigating Errors in Organic Extractables / Leachables Screening

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PDA Parenteral Drug Association

Nelson Labs A Sotera Health company

CONNECTING PEOPLE SCIENCE AND REGULATION®

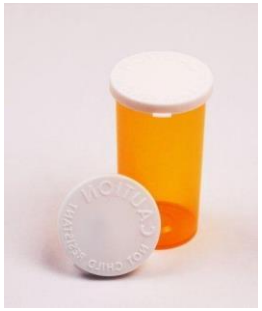
Overview

1. Identify and consider **three errors** which can occur during the activity of screening samples for organic extractables and leachables

2. Discuss how an **internally-developed database** of analytical data can be used to identify, mitigate and correct these errors

3. Consider how such a database provides a means for
   (a) evaluating testing laboratories on the basis of good scientific practices
   (b) optimizing information assessment and management.

The essence of packaging – product compatibility



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The way we wish things happened:

Packaging has:
- Raw polymer(s)

Drug Product has:
- Active ingredient

Packaged Drug Product has:
- Active ingredient
- Inert packaging

The way things often happen:

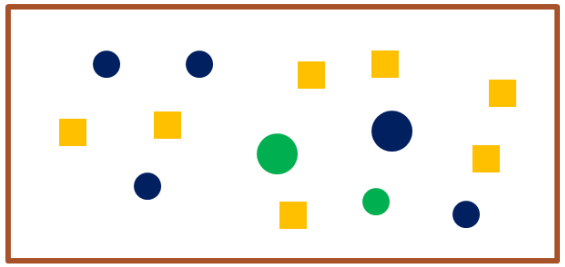
Packaging has
- Raw polymers
- Additives
- Extractables

Drug Product has:
- Active
- Excipients
- Additives
- Impurities

Packaged Drug Product has:
- Active
- Excipients
- Additives
- Impurities
- Leachables

Screening vs Targeting (1)

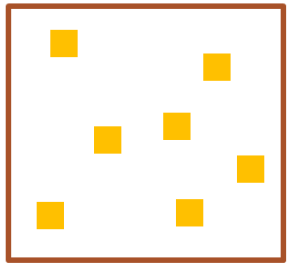
Sample

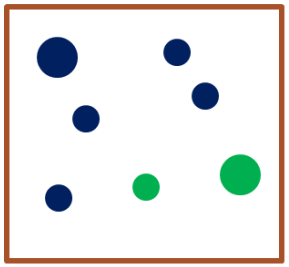


Circles represent leachables.
Squares represent drug product's intrinsic Components (ingredients and impurities)

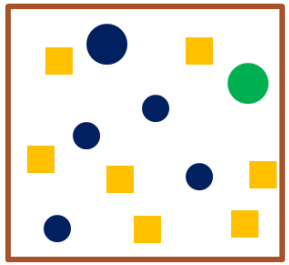
Separation Process

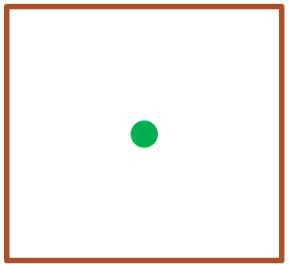
Screening:
Separate Circles from Squares





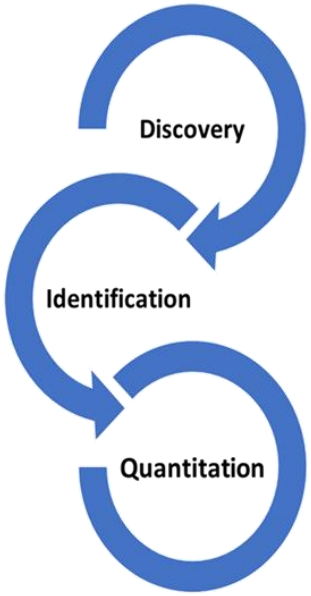
Targeting:
Separate Green Circles with a Diameter of 0.5 cm from Everything Else





Screening vs Targeting (2)

Screening



1. Are there substances unique to the sample (versus an appropriate blank) that are present in the sample above a certain concentration threshold?
2. If yes, what are they identities of those substances?
3. If yes, what are the concentrations of those substances?

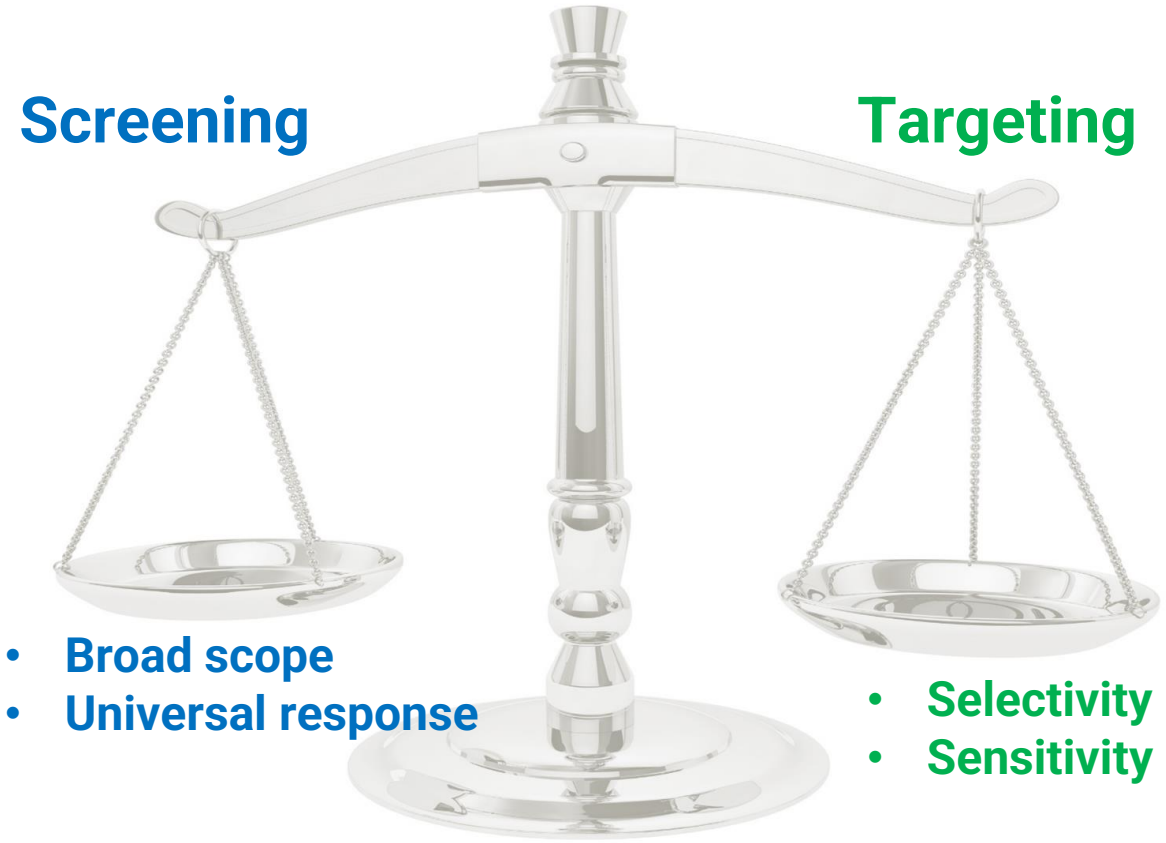
NET-FISHING

Targeting

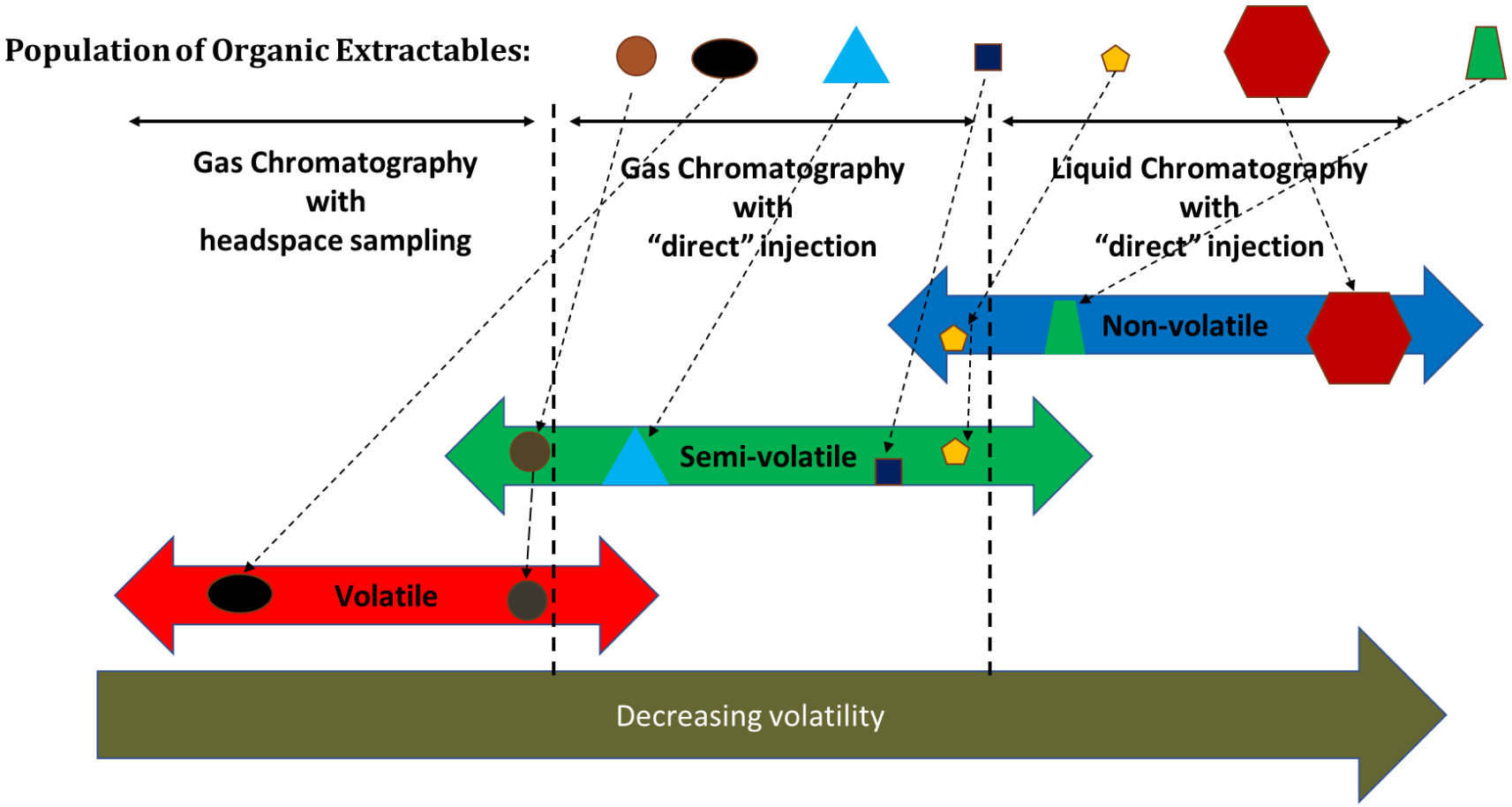
1. Is a specified substance present in the sample in reportable quantities?
2. If yes, what is the concentration of the specified substance?

FLY-FISHING

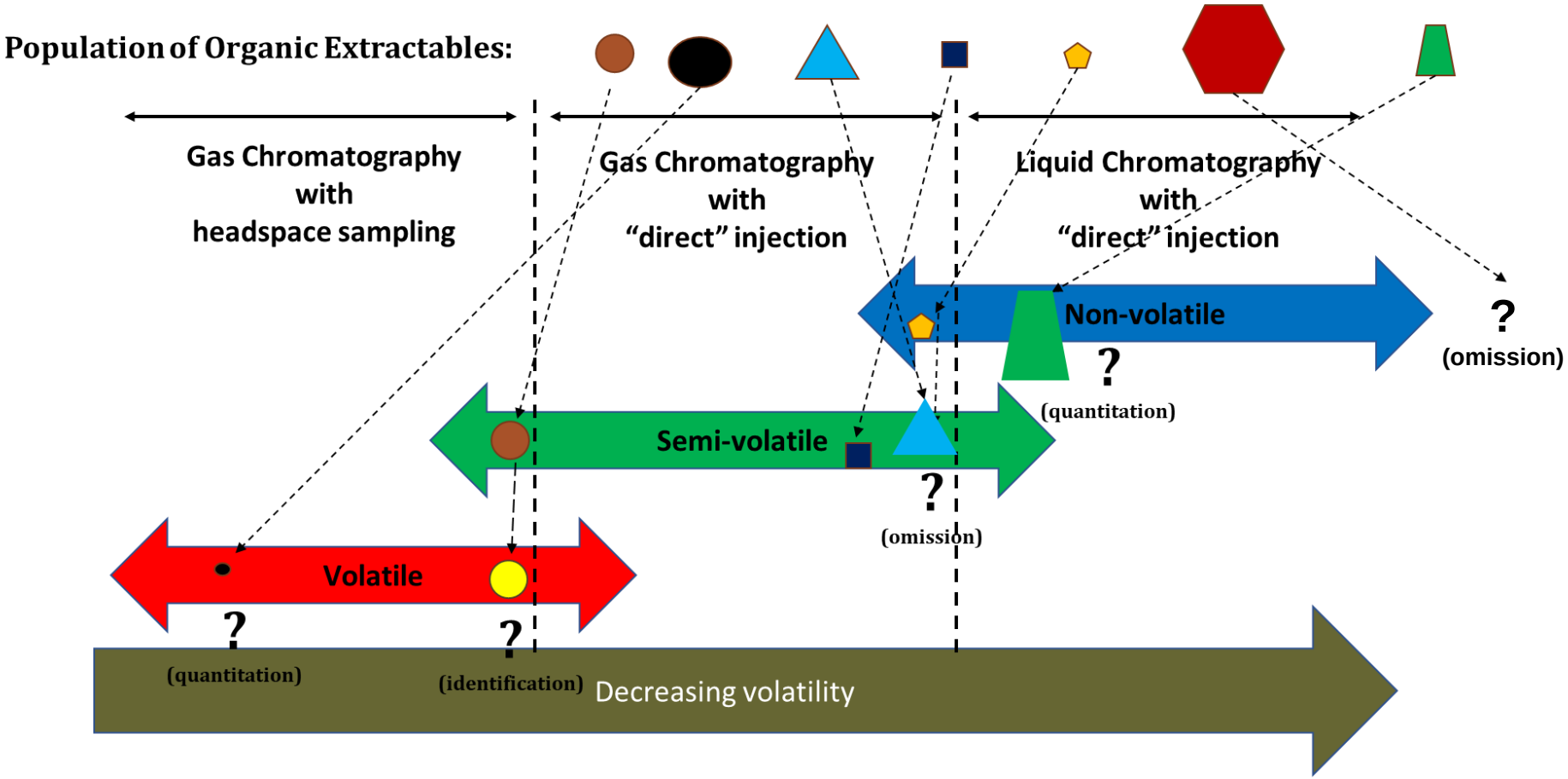
Screening vs Targeting (3)



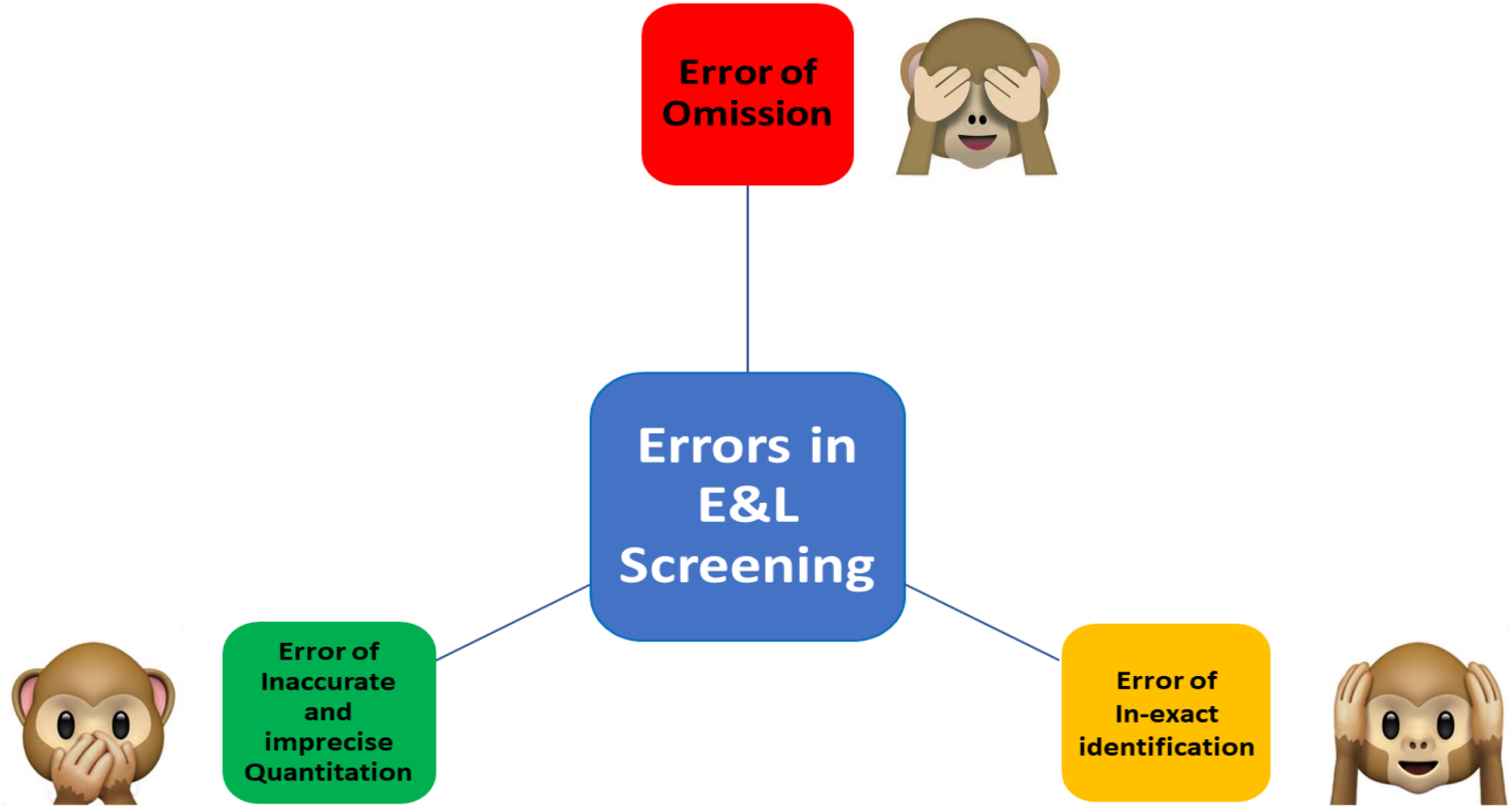
Organic Screening Methods - IDEAL



Organic Screening Methods - REAL



Three Errors in Organic E&L Screening



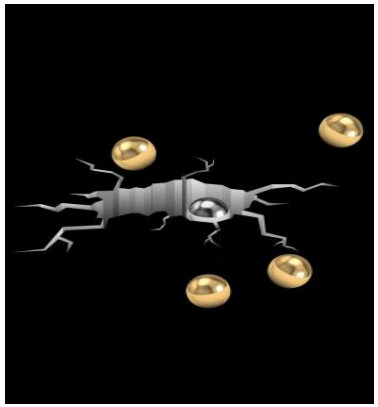
The error of omission

An error of omission occurs when the analytical screening process **fails to account for all extractables and leachables** present in a sample at a level above an established evaluation threshold.

Commission of an error of omission is a **fatal error** as the assessment of the extractables or leachables profile is irreversibly compromised by committing the error. An extractable or leachable which is not accounted for by the analytical process is an extractable or leachable that cannot and **will not be toxicologically assessed**.

Types of omission errors

Falling through the cracks



Failing to see a tree in the forest

Falling through the cracks (1)

1. It never made it to the analytical column in the first place.

Problem	Omission risk	Mitigation
VOCs: water-soluble compounds poorly partition in headspace	Small acids, alcohols, amines, thiols	Neat headspace; Complementary analyses
Water-soluble compounds with poor liquid/liquid recovery	Caprolactam, pentaeythritol, …	Direct injection if possible; Include ESI
Incompatibility extraction solvent with analytical method	Peak splitting, no retention or retention time shift	Gain knowledge/expertise on incompatibilities; use complementary analyses

Falling Through the Cracks (2)

2. Something wacky happens while it's in the instrument

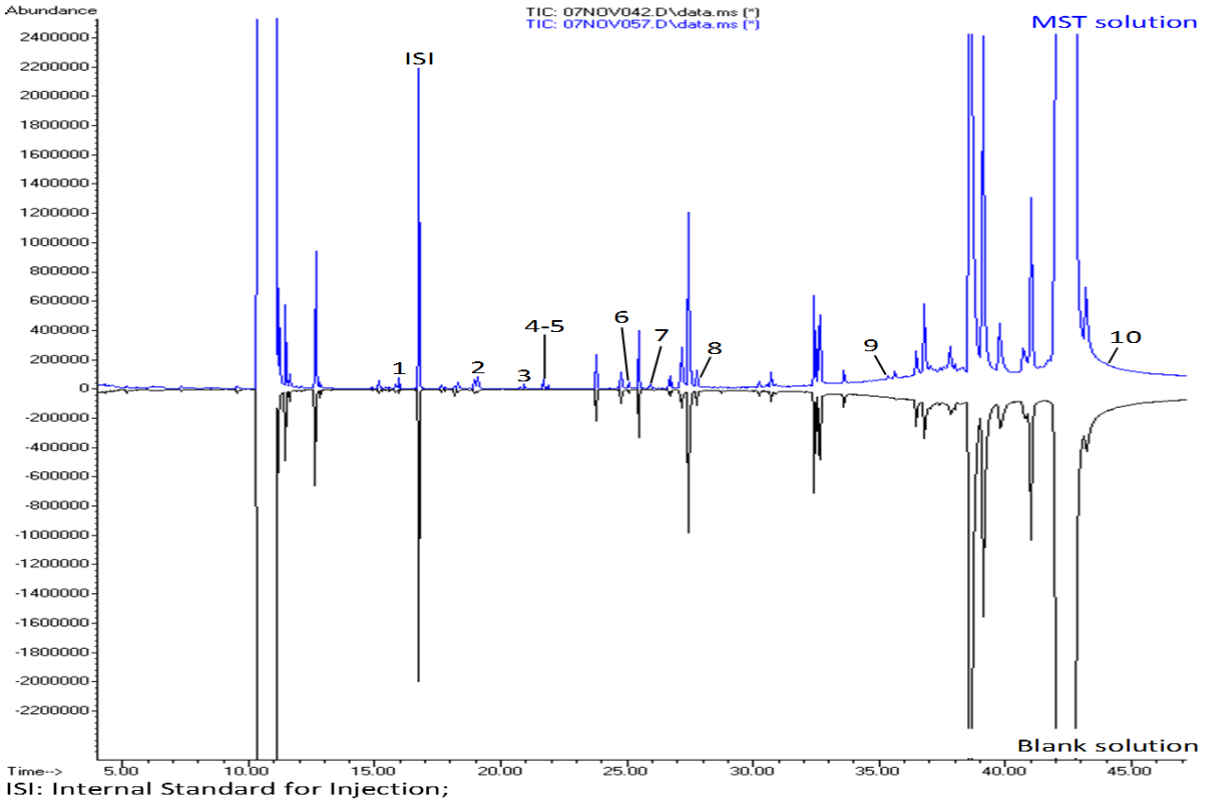
Problem	Omission risk	Mitigation
Compounds co-eluting with large solvent peak	Small hydrocarbons, ethyl formate, CS_2, acetonitrile	Neat headspace; Software enabling deconvolution
Humps of compounds present in large quantities e.g. hydrocarbon mixtures	Anti-oxidants, BADGE	Software enabling deconvolution Complementary analyses (e.g. LC/MS does not 'see' hydrocarbons)
Compounds sticking to the column or other surfaces along the sample path	Acidic or alkaline compounds, polymeric additives (e.g. Tinuvin 622)	Optimize analytical procedure (pH of extract / mobile phase) Complementary analyses

Falling Through the Cracks (3)

3. It does not come off the column
4. Something wacky happens in the detector

Problem	Omission risk	Mitigation
Compounds below the scanned mass range of the MS	Formaldehyde, methanol…	Specific analytical methods
Compound exceeding the scanned mass range of the MS	Irganox 1010, tetrabromobisphenol A	Set scan range wide enough
Poor ionization in APCI or ESI	PFAS, PAHs, polar compounds	Use both APCI & ESI in study design Complementary techniques

Failing to see a tree in the forest



Bottom chromatogram:

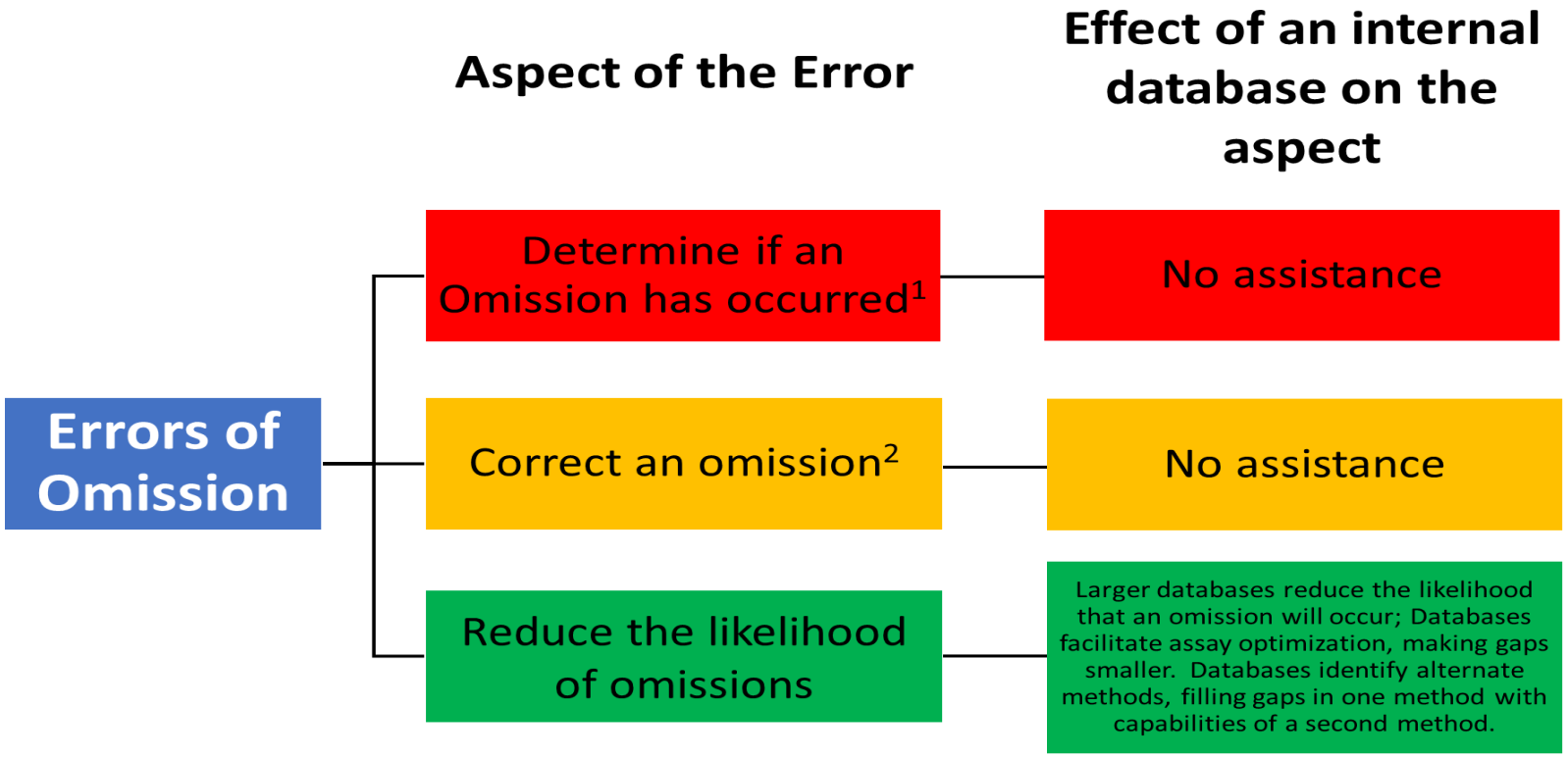
- Blank drug product matrix

Top chromatogram:

- Blank drug product spiked with 10 leachables at AET concentration level

Matrix peaks may obscure the leachable compounds or even make them not visible, while you know they must be there!

An in-house developed E&L analytical database



(1) No direct help of the database, but is may give insights in compounds at risk for omission
(2) The correction itself usually requires a complementary technique

An in-house developed E&L analytical database

Excerpt of the *NELSON LABS Discovery and Screener Database* for Semi-Volatile Organic Compounds Characterized by Gas Chromatography/Mass Spectrometry (GC/MS).

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	37.8	41	36
19.01	4-Hydroxy-3-methylacetophenone	876-02-8	0.413	135	150	39.4	77	27.2	107	18.7
19.03	Cyclopentyl phenyl ketone	5422-88-8	0.758	105	77	36.2	174	24.5	133	15.2
19.05	2,4-Di-tert-butylphenol	96-76-4	0.984	191	206	16.3	192	14.3	57	14
19.07	2-(Decyloxy)ethanol	23238-40-6	0.352	57	85	75.5	71	69.9	43	65
19.08	Tridecanal	10486-19-8	0.281	57	41	86.1	82	81.7	43	81.1
19.08	1,4-Isopropanol acetophenone	54549-72-3	0.557	163	43	66.2	121	15.7	164	11.1
19.08	1-Naphthol	90-15-3	0.53	144	115	87.8	116	41.9	145	11.3
19.08	2-(2-Phenoxyethoxy)ethanol	104-68-7	0.912	45	94	76.5	77	52.4	182	26.3
19.12	Triisobutyl phosphate	126-71-6	0.539	99	57	19.9	155	14.8	41	12.4
19.13	BHT	128-37-0	0.884	205	220	25.6	206	15.5	57	11.5
19.13	Dimethyl isophthalate	1459-93-4	0.557	163	194	24.2	135	23.7	76	11
19.15	N,N-Di-n-butyl-2-chloroacetamide	2567-59-1	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

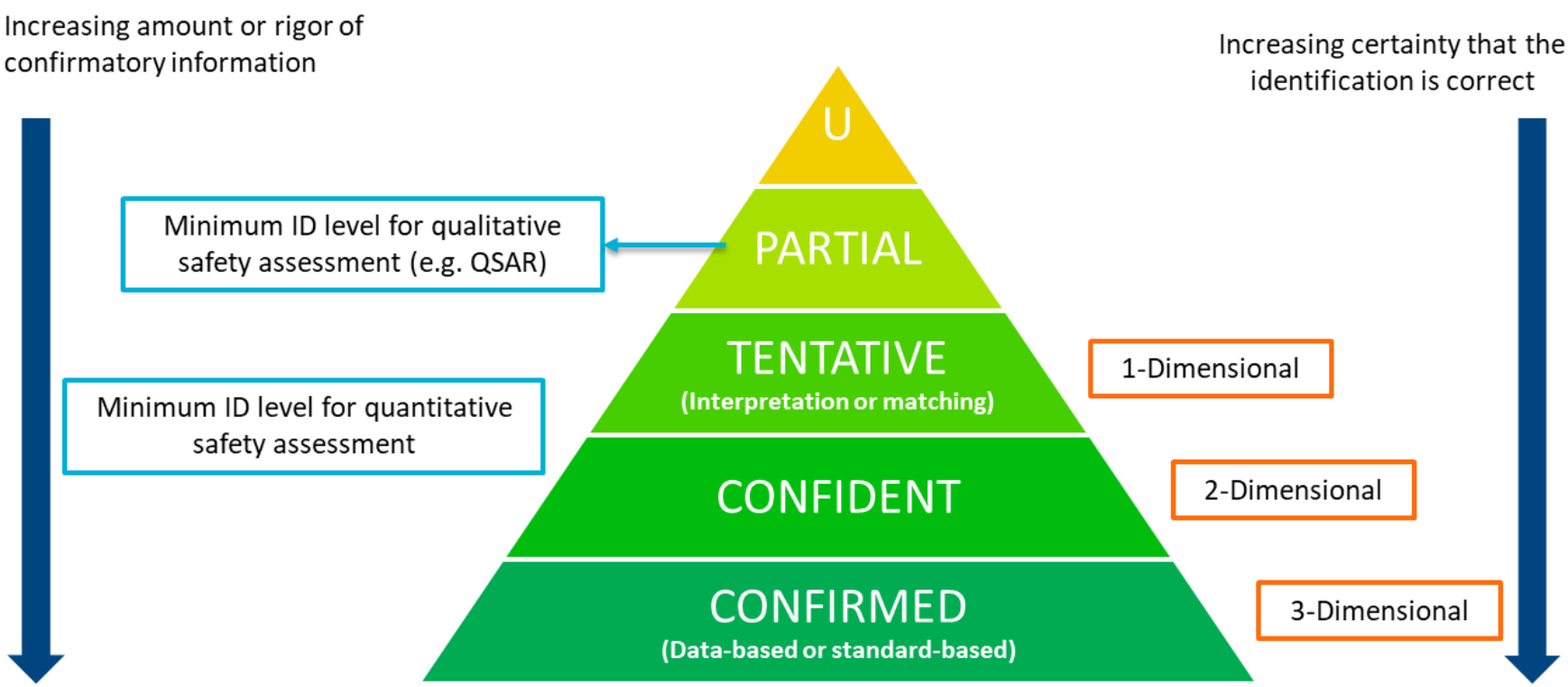
The error of in-exact identification

Once all the extractables or leachables at levels above a justified reporting threshold have been accounted for, the **identities** of the individual extractables or leachables must be established as **it is the identity that links an extractable or leachable to the toxicological data which enables its assessment**.

Commission of an error of in-exact identification is a **fatal error** because such an error precludes a proper assessment. An extractable or leachable which is not properly identified will be **incorrectly assessed**.

Various levels of identification

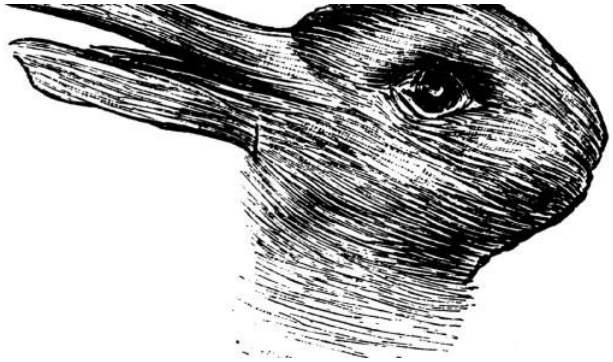
***References**: USP 1663 | Nelson Labs e-Book Good Identification Practices*



Types of identification errors

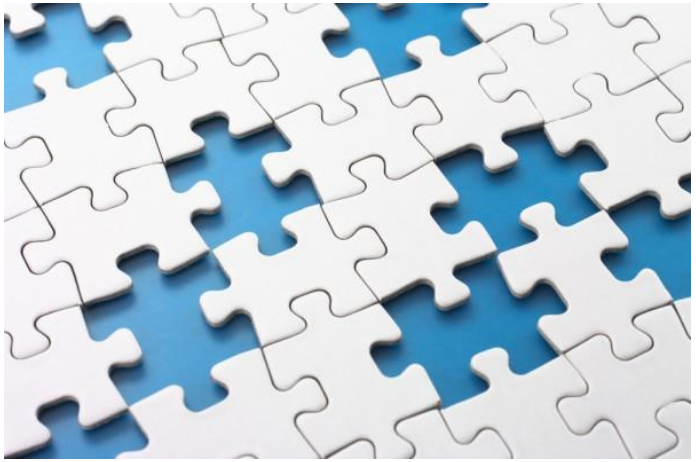
No Identity:

Incorrect Identity:

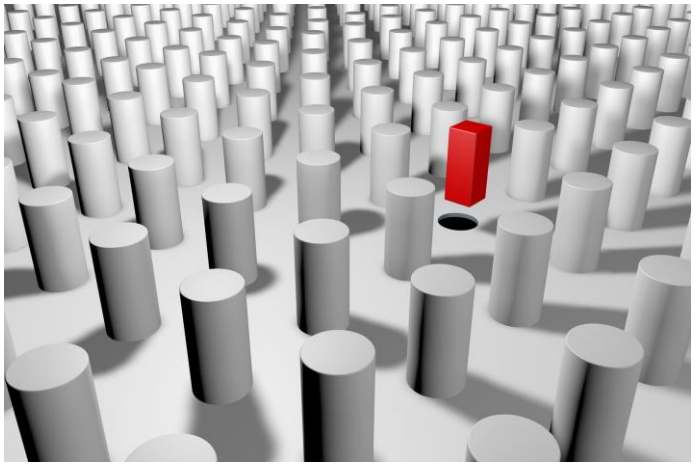


No identity

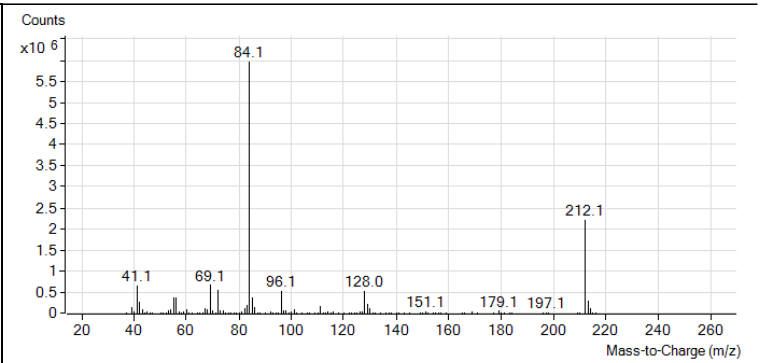
Substance produces insufficient or inconclusive analytical data to support the identification process



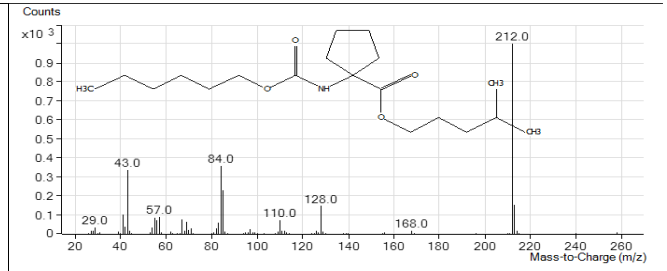
No match is obtained in searched resources, mostly spectal databases

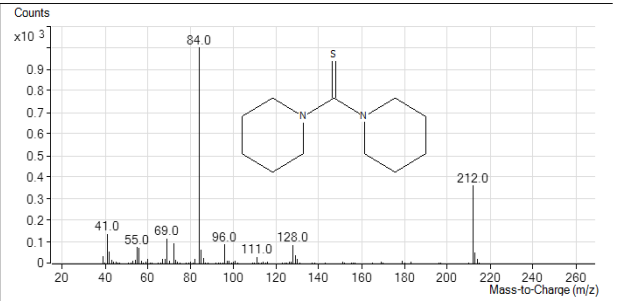


Wrong identity (using NIST as black box)

Detected Compound	
Acquired RT = 24.61 min	

*** or even worse,
"Any match score wins!"

Best hit from NIST/Wiley	
INEXACT IDENTIFICATION	
1-aminocyclopentane-carboxylic acid, N-hexyloxy-carbonyl, isohexyl ester	
Library RT not available	
Library Match = 80.7%	

Best hit Nelson Labs DB	
EXACT IDENTIFICATION	
1,1'-Carbonothioyl-bispiperidine	
Library RT = 24.58 min	
Library Match = 98.4%	

The "Home Court" Advantage

Error mitigation using in-house E&L database

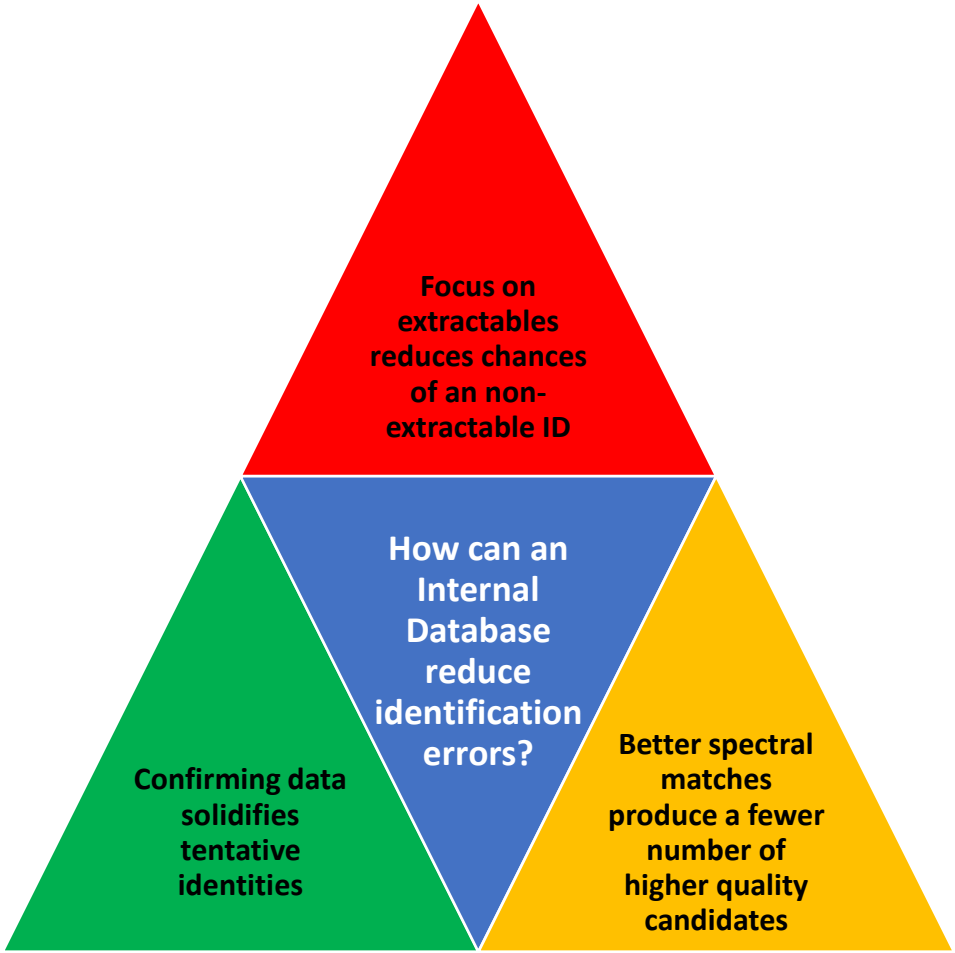
1. As the size of the database increases, the probability that the compound of interest is in the database increases.

2. Because the entries are all extractables, securing a false identity as a “non-extractable” is less likely.

3. Because the match information (e.g. mass spectrum) for the compound of interest and the compounds in the database is obtained on the same analytical systems using the same analysis conditions, there are **less sources of variation that could lead to poorer matches**.

4. Internal databases can contain **secondary supporting information** (e.g., retention time).

Error mitigation using in-house E&L database



The error of inaccurate quantitation

An error of inaccurate quantitation occurs when the concentration estimate provided by the screening method is **inaccurate**.

Commission of an error of omission is a **critical error** effecting the correctness of the impact assessment. However, it is not a fatal error because even an inexact impact assessment could lead to the correctly assessed risk.

Occurrence of quantitation errors

1. There are few, if any, detection methods that are **universal** in the sense that the detector's response is equivalent across all analytes. Thus, accurate quantitation requires response calibration with authentic standards for each potential analyte.

2. In any given situation, the population of potential analytes is large and consists of chemically and structurally diverse substances. This makes response calibration with authentic standards for each potential analyte **practically prohibitive**.

3. As a compromise between accuracy and practicality, either a single internal standard or a small set of internal standards is used to calibrate response to concentration. The **response factor (RF)** obtained for the internal standard is used to produce a **concentration estimate** for each analyte. When the internal standard and the analyte do not have the same response factor, a quantitation error occurs.

4. **Concentration mis-matches** between the internal standard and the analytes of interest may further exacerbate quantitation errors.

RF variation GC/MS vs LC/MS

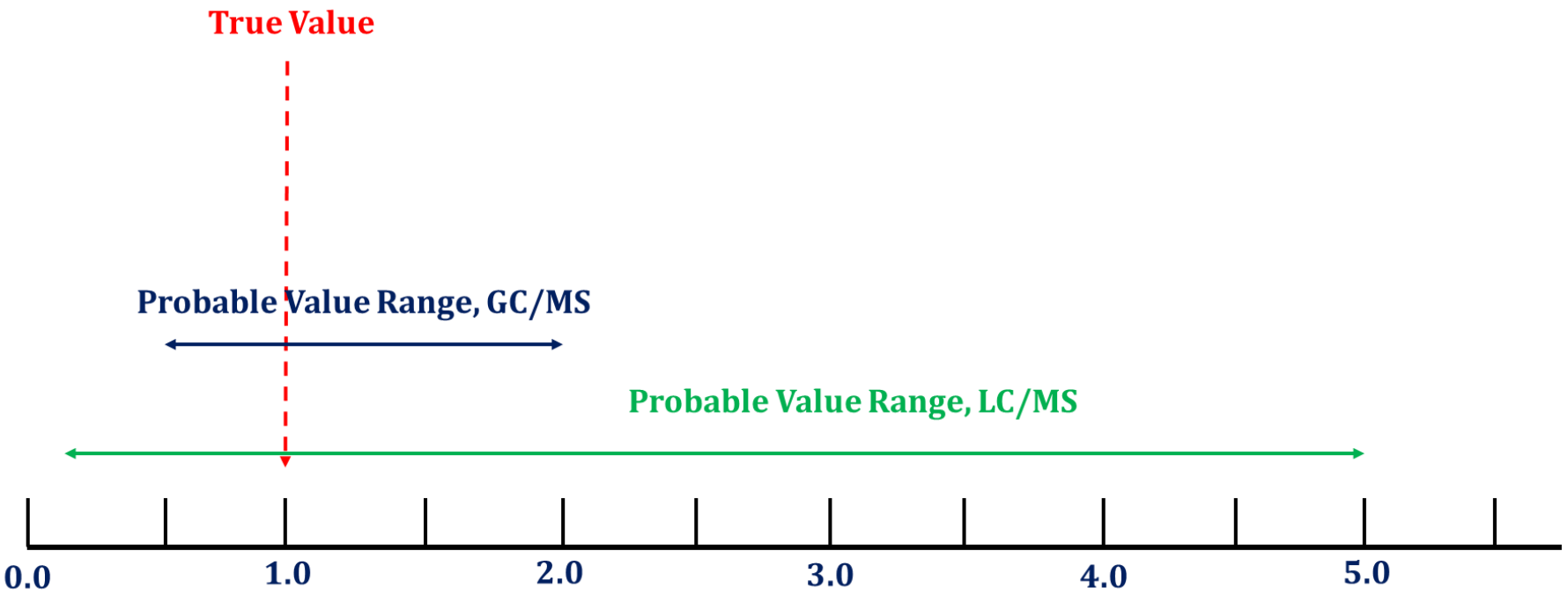
For Semi-volatile Substances by “Direct Injection” GC/MS:

- For many of the most commonly encountered extractables and leachables, the established range in response factor is a factor of 4.
- This means that if the response factor of an internal standard is assigned a value of 1, the absolute response factors for extractables and leachables will vary from 0.5 to 2.0.
- There are many cases where extractables have absolute response factors well outside the range of 0.5 to 2.0.

For Non-volatile Substances by “Direct Injection” LC/MS:

- For many of the most commonly encountered extractables and leachables, the established range in response factor is a factor of 25.
- This means that if the response factor of an internal standard is assigned a value of 1, the absolute response factors for extractables and leachables will vary from 0.2 to 5.0.
- There are many cases (more than for GC) where extractables have absolute response factors well outside the range of 0.2 to 5.0.

RF variation GC/MS vs LC/MS



Relative Response Factors (RRF)

- An RRF accounts for the difference in response of an extractable/leachable and an internal standard (I.S.)

Procedure:

- Prepare standard solution with known amounts of authentic reference standard (R.S.) & internal standard (I.S.)
- Record analytical response of R.S. vs response of I.S. → calculate relative response factor (RRF)
- Capture MS spectrum, retention time & RRF in internal database

Screening analysis :

- I.S. spiked to each (final) extract
- Correct concentrations of database hits with RRF

$$RRF = \frac{R_{RS}}{R_{IS}} \times \frac{C_{IS}}{C_{RS}}$$

$$C_{sample} = \frac{R_{compound}}{RRF_{compound}} \times \frac{C_{IS}}{R_{IS}}$$

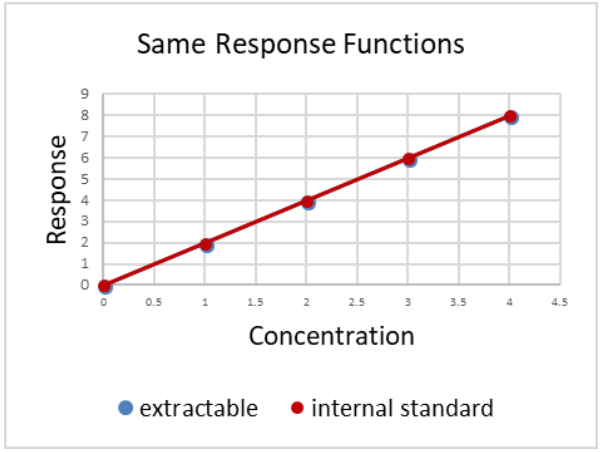
RRF: advantages and limits

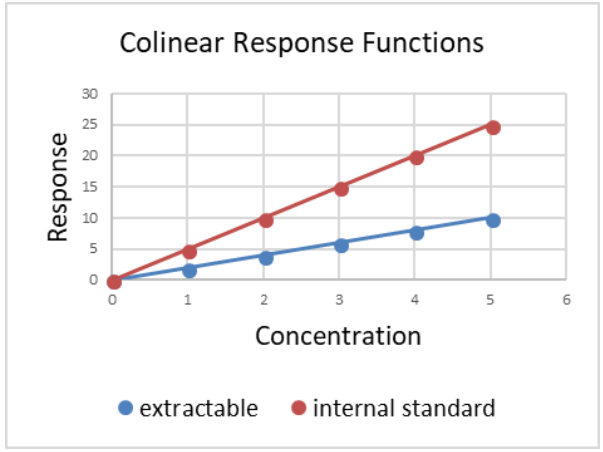
Relative response factors work well when either:

- The concentration of the internal standard and analyte are similar
- The response function for the internal standard and analyte are similar.

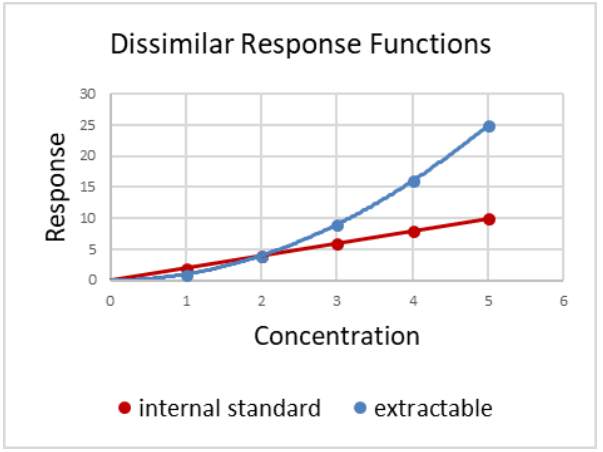
It does not work well in case of different response functions!

Works well





Does not work well



RRF: Identify the optimal analytical method

CAS	Compound Name	Technique Specific RRF - values		
		HS-GC-MS	GC-MS	LC-MS
Complementing GC-MS & LC-MS RRF Entries				
1568-83-8	Bisphenol A dimethyl ether	*n.d*	**1.630**	0.101
2943-75-1	Triethoxyethyl-n-octylsilane	*n.d*	**1.210**	0.013
80-46-6	4-tert-Pentylphenol	*n.d*	**1.110**	0.100
101-02-0	Triphenyl phosphite	*n.d*	**0.922**	0.279
80-07-9	Bis(4-Chlorophenyl) sulfone	*n.d*	**0.893**	0.050
149-30-4	2-Mercaptobenzothiazole	*n.d*	0.112	**0.459**
619-21-6	3-Formylbenzoic acid	*n.d*	0.078	**1.081**
1212-29-9	1,3-Dicyclohexythiourea	*n.d*	0.062	**1.043**
2306-33-4	Monoethyl phthalate	*n.d*	0.041	**0.410**
4559-70-0	Diphenylphosphine oxide	*n.d*	0.024	**0.936**

Bolded entries reflect the method that would give the most accurate and reportable concentration estimate

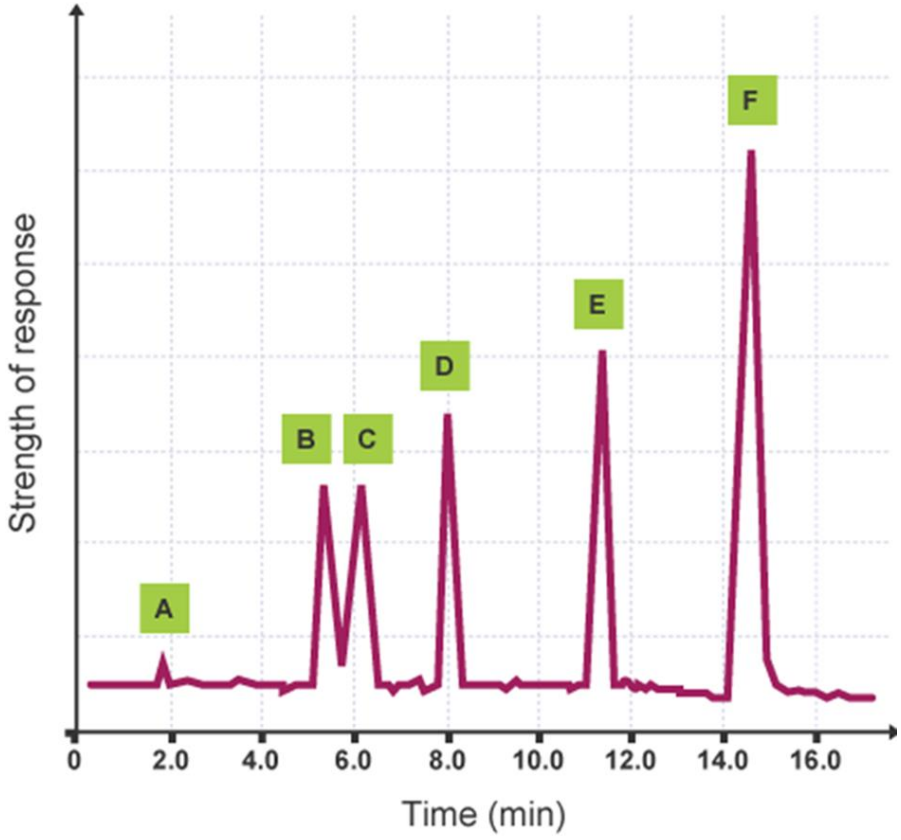
System Suitability Testing (SST)

- Assures that the analytical method is **able to perform the task(s) where it was designed and qualified for**
- Detects situations where the analytical method produces data of **insufficient quality** to be useful or credible
- **How**: using a SST mixture of compounds, representative for E&L population of compounds and **challenging the analytical method on its performance**.
- Typically a number of substances **different in chemical nature**
- Set **specifications on SST performances** e.g. resolution, sensitivity, peak tailing… When out of spec: measurements in the sequence are not valid!

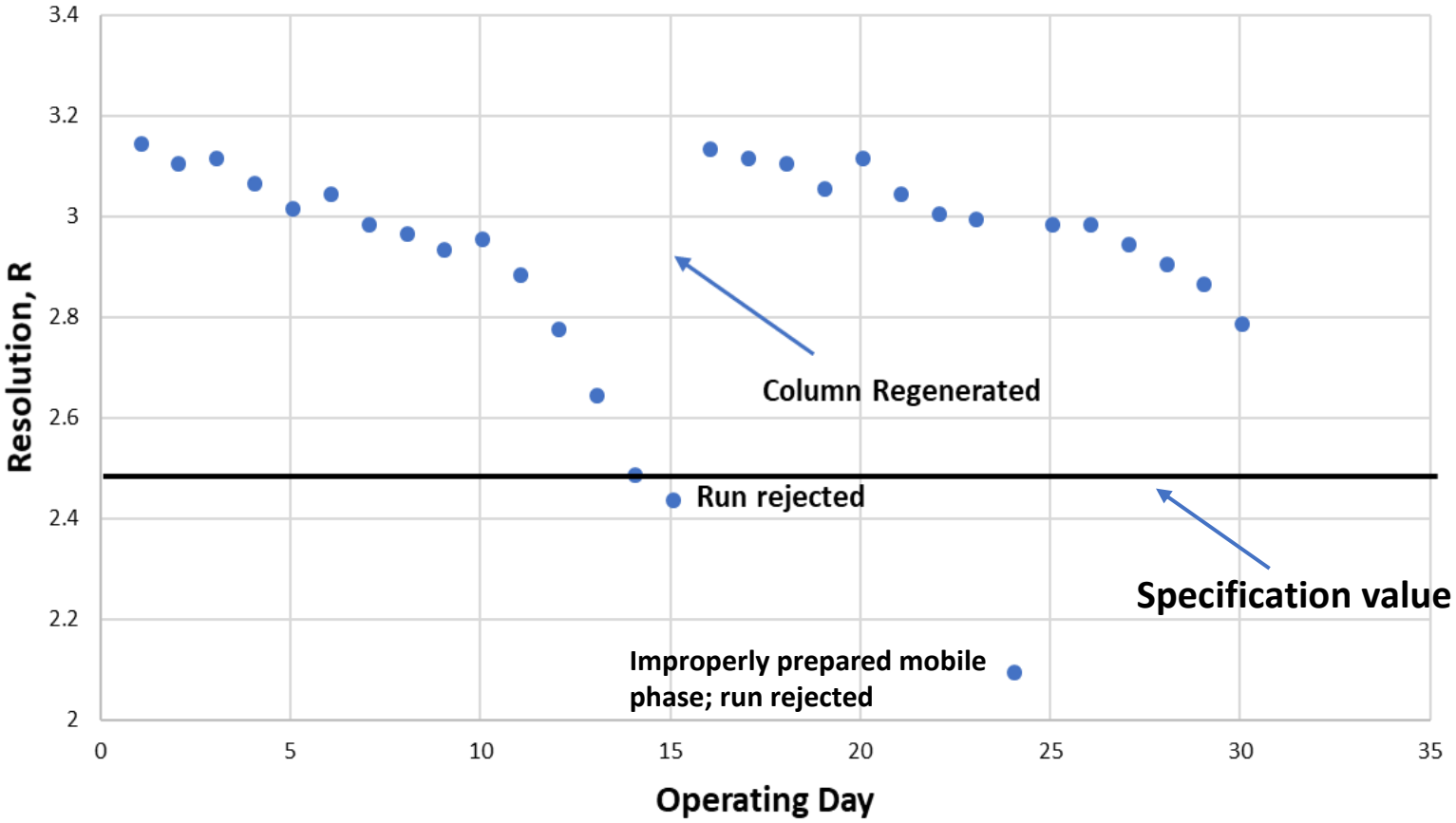
System Suitability Testing (SST)

Chromatogram for a System Suitability Mixture containing six members.

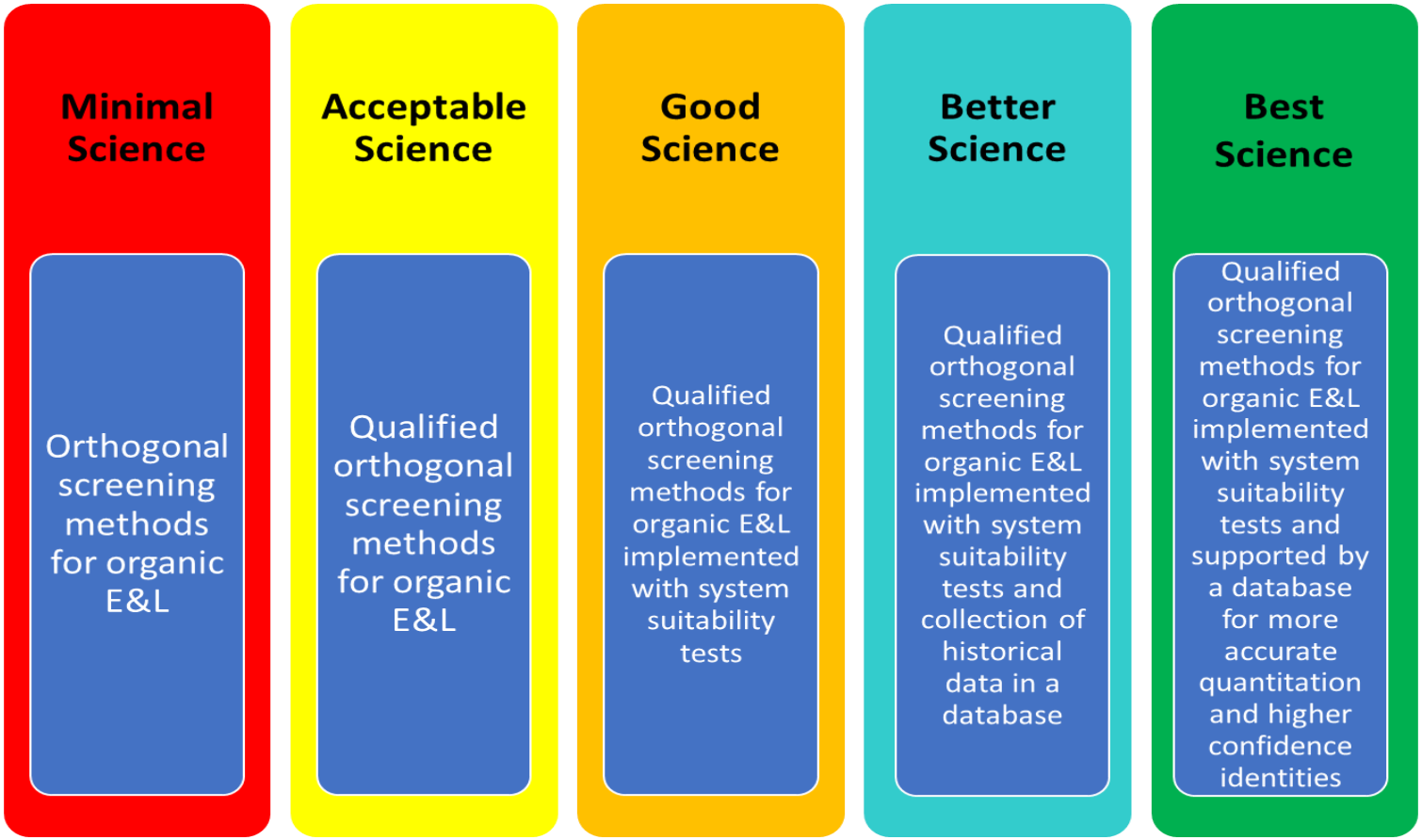
- The substances associated with peaks A and F are the **anchor substances**, confirming the breadth of the method.
- Substances associated with peaks B and C represent the **critical pair**, whose resolution establishes that the chromatographic efficiency is adequate.
- Substances associated with peaks D and E address method sensitivity (quantitation) and the ability to produce an intepretatable mass spectrum (identification).



System Suitability Testing (SST)



The internal database as differentiating factor



The internal database as differentiating factor

Current screening process for establishing an extractables profile:

1. Responses are collected.
2. Responses are individually processed to obtain tentative identities. If more rigorous identities are required, further processing is necessary. If tentative identities cannot be obtained, further processing is required.
3. Responses are individually processed to obtain concentration estimates.

Future targeted process (supported by a database) for establishing an extractables profile:

1. Responses are collected and “automatically” processed to obtain confirmed identities and accurate concentrations.
2. Responses that do not produce a “hit” in the database are further processed.

What if the database is really information-rich…

- What if the database were to contain toxicological safety data, such as QSAR alerts for mutagenicity and sensitization? Such a database would provide alerts to potentially hazardous substances.
- What if the database contained permissible daily exposure (PDE) data? The database could calculate margins of safety (MoS), based on inputted clinical use information, thereby "automating" certain aspects of toxicological safety assessments.
- What if the database contained reactivity alerts such as "this compound has been known to cause proteins to precipitate" or "at high pH, this compound can react with alkaline earths in a drug formulation to form precipitates"? Now you have a database that alerts to potential product quality issues.
- What if the database contained information on "extractables to extractables associations" or "extractables to sources" associations. Now the database can lead one to examine the extractables profile and ask questions such as "if I saw this extractable, why didn't I see this other related extractable?" or "Does my tentative ID make sense in terms of what I know about this material?"

Conclusion

1. **Three errors** can occur during the activity of screening samples for organic extractables and leachables:
   a) Error of Omission
   b) Error of Inexact Identification
   c) Error of Inaccurate and Imprecise Quantitation

2. These errors can be identified, mitigated and corrected via a robust, well-populated and information rich **internally-developed database** of analytical data.

3. Moreover, such a database provides a means for
   a) Evaluating testing laboratories on the basis of good scientific practices
   b) Optimizing information assessment and management.

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