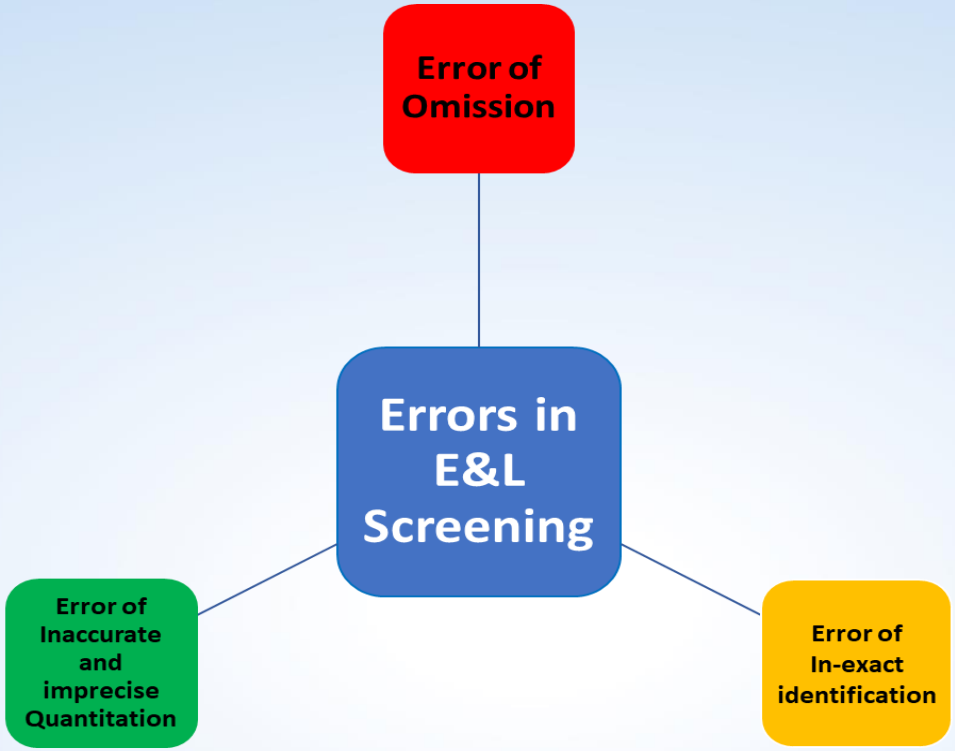
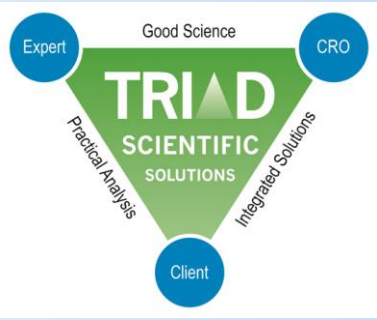




Identifying and Mitigating Errors in Organic Extractables/Leachables Screening

Dennis Jenke; Principal Consultant, Nelson Labs, Europe; Chief Executive Scientist, Triad Scientific Solutions, LLC

PDA Extractables & Leachables Training Course, BASEL, FEBRUARY 2020

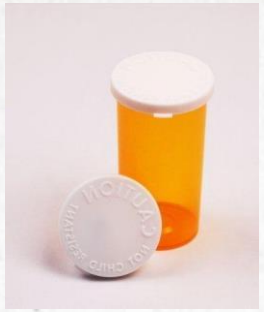
In this presentation we will:

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



+

=

The Way We Wish Things Happened:

Packaging has:
- Raw polymer(s)

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

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

The Way Things Often Happen:

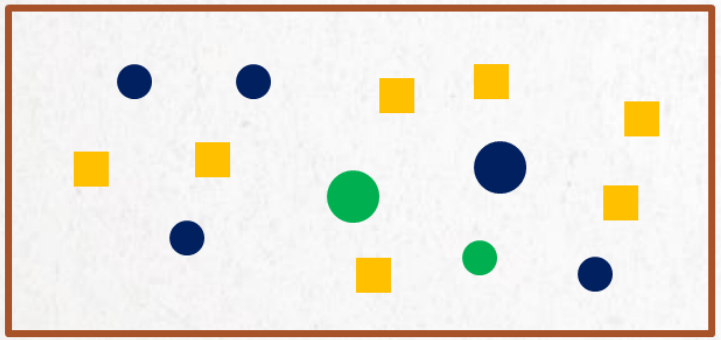
Packaging has
- Raw polymers
- Additives
- Extractables

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

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

Screening versus Targeting (1)

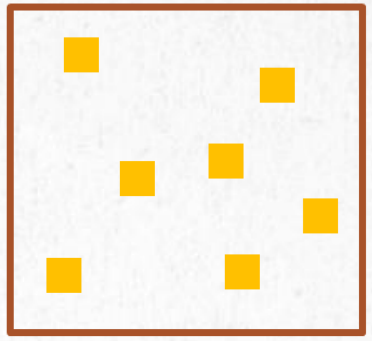
Sample

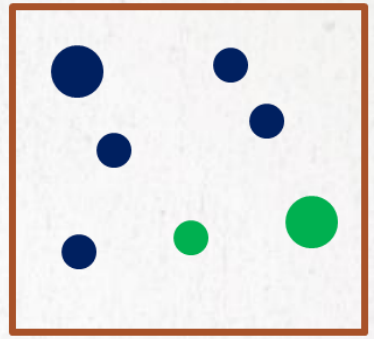


Circles represent leachables.
Squares represent drug product's intrinsic Components (ingredients and impurities)

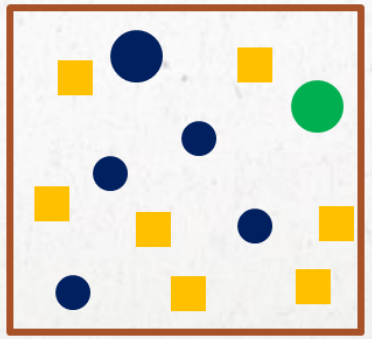
Separation Process

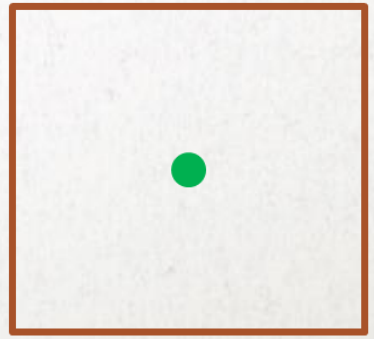
Screening:
Separate Circles from Squares





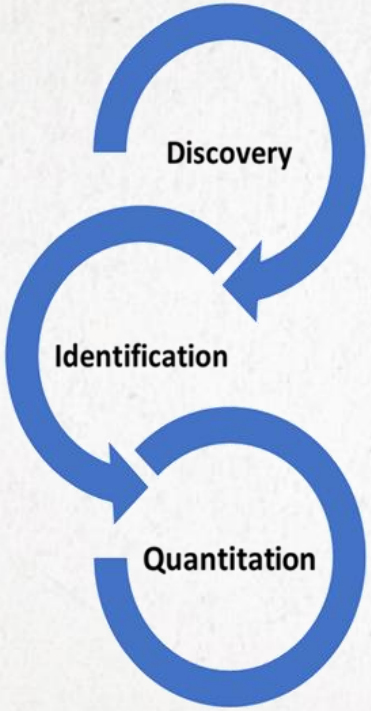
Targeting:
Separate Green Circles with a Diameter of 0.5 cm from Everything Else





Screening versus 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?

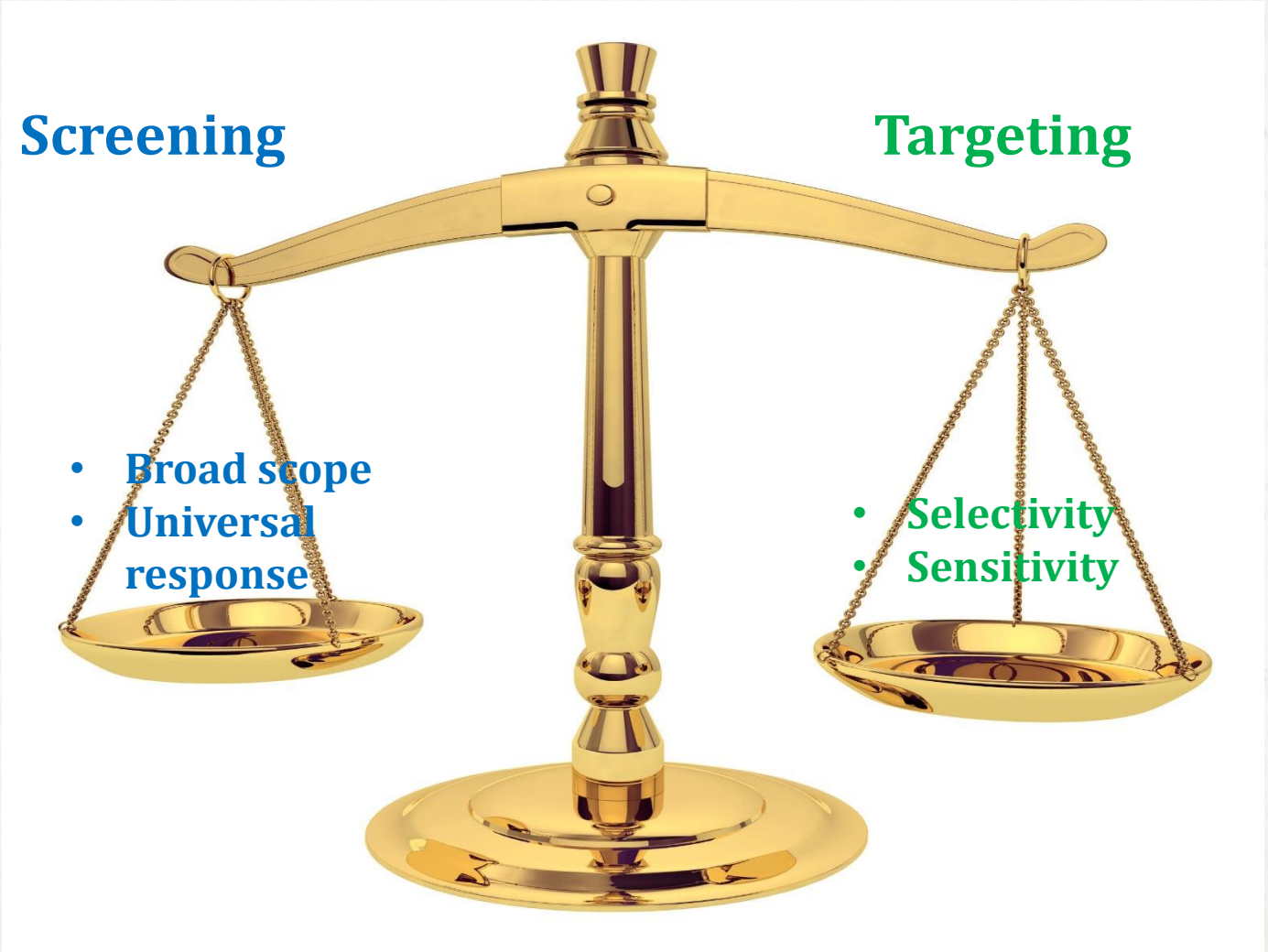
NET-FISHING

Targeting

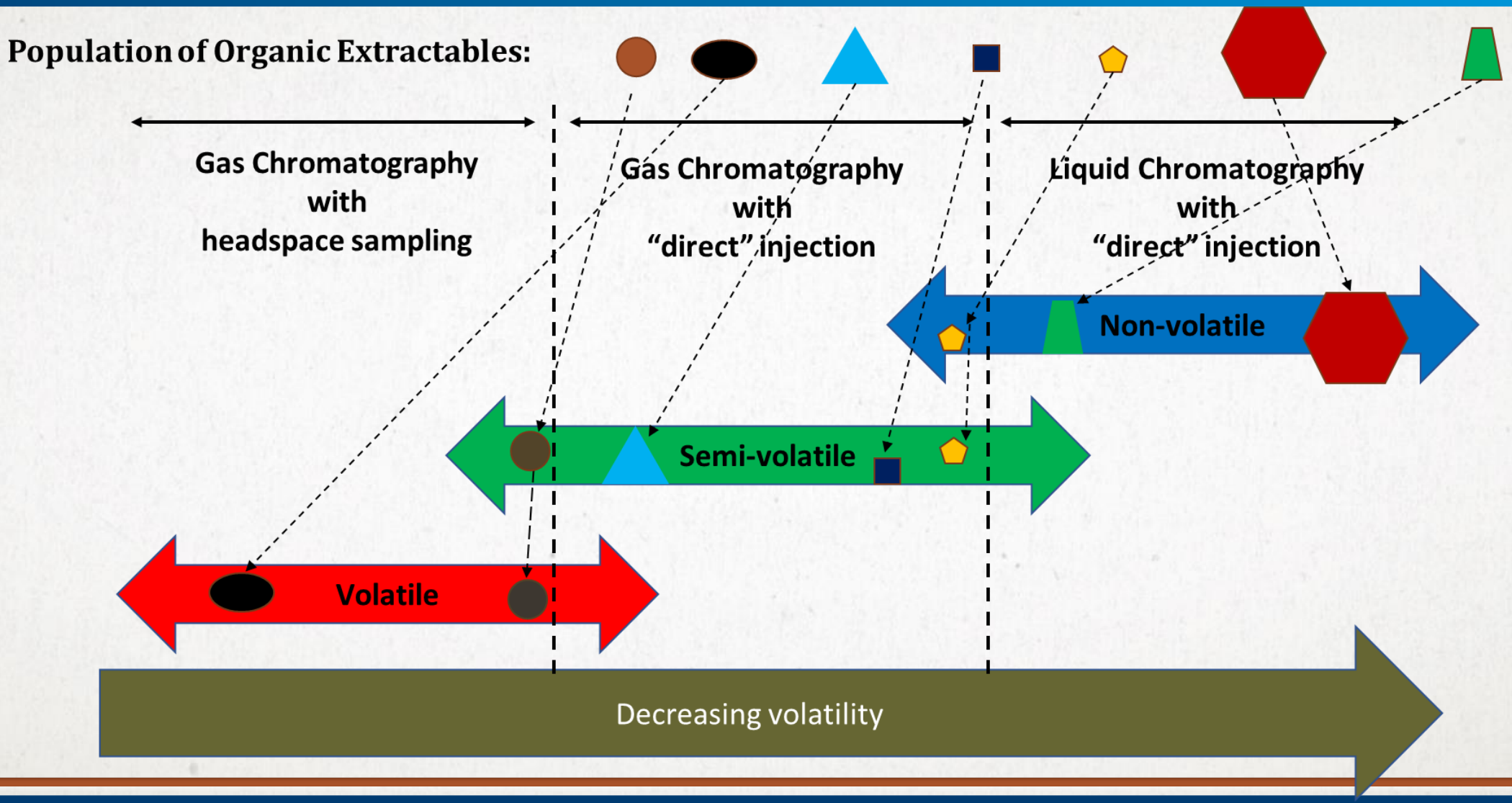
1. Is a specified substance present in the sample in reportable quantities?
2. If yes, what is the concentration of the specified substance?

FLY-FISHING

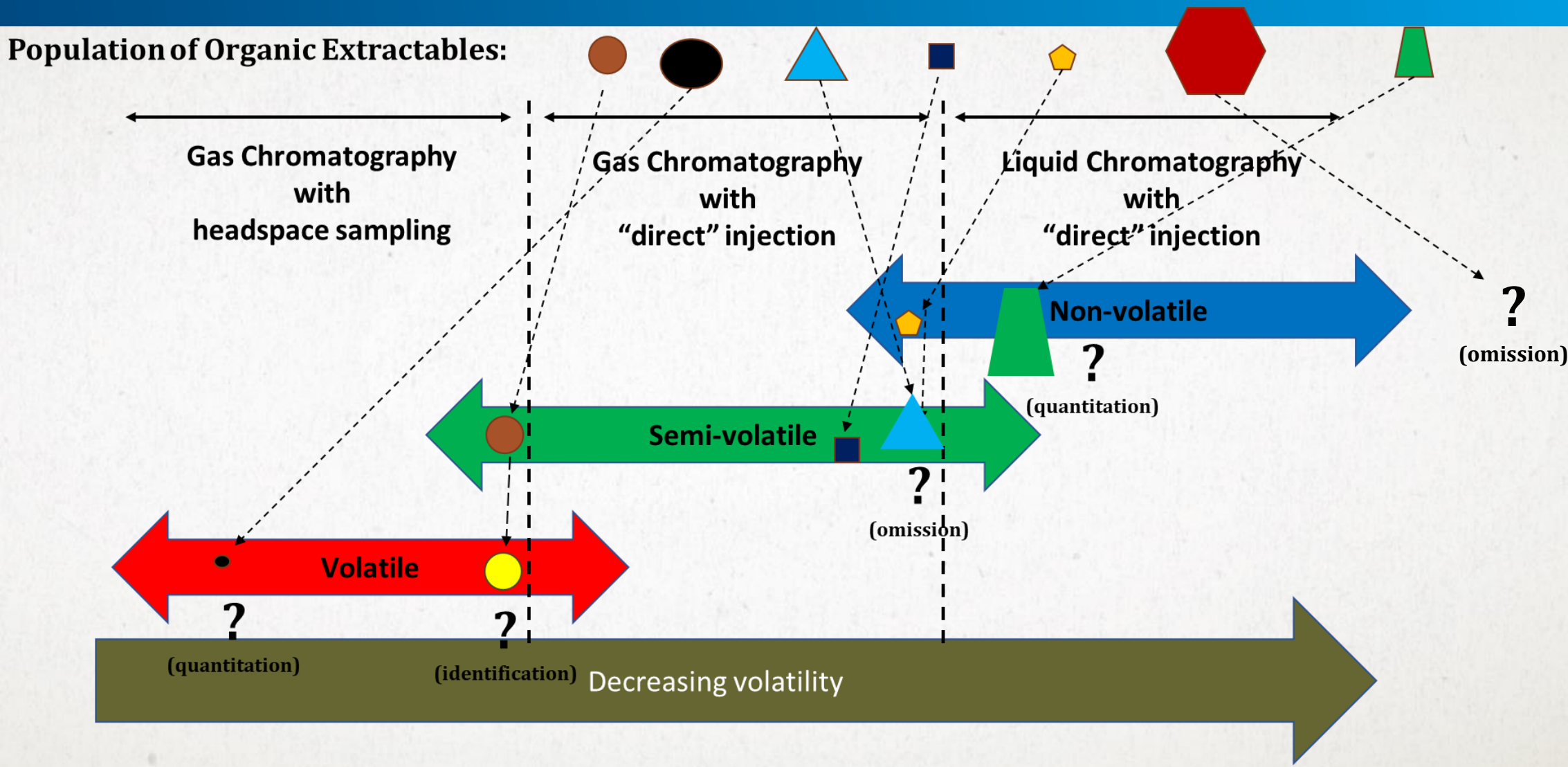
Screening versus Targeting (3)



Chromatographic Methods for Organics Screening; Ideal



Chromatographic Methods for Organics Screening; Real



Errors in Organics E&L Screening

Errors in E&L Screening

Error of Omission

Error of Inaccurate and imprecise Quantitation

Error of In-exact identification

Mitigating Errors in Organics E&L Screening –An Internally Developed Extractables/Leachables 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 Omission

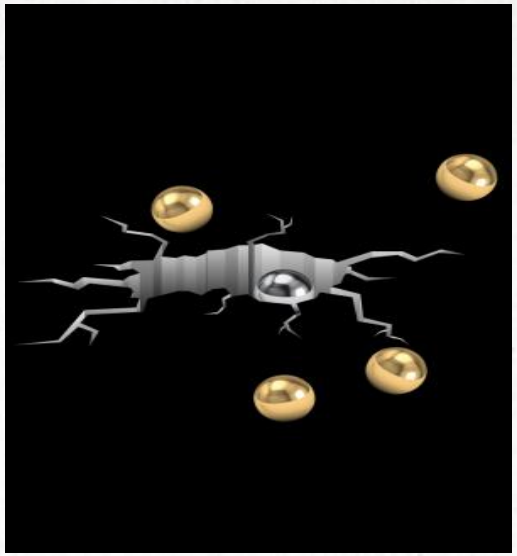
Error of Omission

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 assessed.

Types of Omission Errors

Falling Through the Cracks



Failing to see a Tree in the Forest

Types of Omission Errors; Falling Through the Cracks

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

Type of Problem	Description of Problem	Potential Omissions	How to avoid Omissions	
Sample preparation/ Sample introduction	Compounds with high water solubility will poorly partition into the headspace, reducing sensitivity	Low MW Acids, Alcohols, Amines, thiols, etc	Low MW Acids: Aqueous extracts via Ion Chromatography Amines & thiols: Use orthogonal analytical methods that show a sufficient response for these compounds (HPLC-ESI; derivatization GC/MS) Verify the results of the neat Headspace-GC/MS analyses	HEADSPACE GC/MS ON NEAT MATERIALS
Liquid/Liquid Extraction of Aqueous Extracts prior to analysis	Compounds with a low partitioning into the organic phase might not be extracted via a Liquid/Liquid Extraction	Caprolactam, Pentaerythritol, others	Verify the response of those compounds in other analytical screening methods (eg LC/MS APCI/ESI direct injection; derivatization GC/MS)	Direct Injection GC/MS of Organic Extracts
Sample Introduction	Extraction solvent's properties cause sub-optimal separations (peak splitting, band broadening, retention time shifting)		Optimize the analytical methodology to reduce issues. Verify the responses of compounds that may be affected in other Orthogonal Analytical Methods (e.g. Direct Injection GC/MS for hexane)	Direct Injection LC/MS (APCI+/-) of Extracts

Types of Omission Errors; Falling Through the Cracks

2. Something wacky happens while its in the instrument.

Type of Problem	Description of Problem	Potential Omissions	How to avoid Omissions
Chromatography	Compounds eluting in the solvent peak (when analysing WFI/Alcohol-mixes)	Isomers of pentene, pentane, diethyl ether, isomers of pentadiene, ethyl formate, carbon disulfide, acetonitrile…	Verify the results of the neat Headspace-GC/MS analyses Consider Mass Spectral Deconvolution options in combination with an Internally Developed Mass Spectral Database
Chromatography	Compounds eluting in the higher quantities causing a broad hump (e.g., Hydrocarbons for Rubbers) that could mask co-eluting compounds	Irgafos 168, BADGE related Compounds…	Screening the GC/MS chromatogram with an internal database could discover and identify these compounds (after deconvolution). Verify the response of those compounds in other analytical screening methods (eg LC/MS APCI/ESI direct injection; derivatization GC/MS)
Chromatography	Some compounds with specific functional groups will show sub-optimal chromatography or will not elute/ remain adsorbed on the chromatography column	Acidic compounds (pKa below the mobile phase pH), polar, ionic, or zwitterionic compounds), large polymeric additives (eg Tinuvin 622)	Optimize the analytical methodology to reduce issues. Verify the responses of compounds that may be affected in other Orthogonal Analytical Methods

HEADSPACE GC/MS ON NEAT MATERIALS

Direct Injection GC/MS of Organic Extracts

Direct Injection LC/MS (APCI+/-) of Extracts

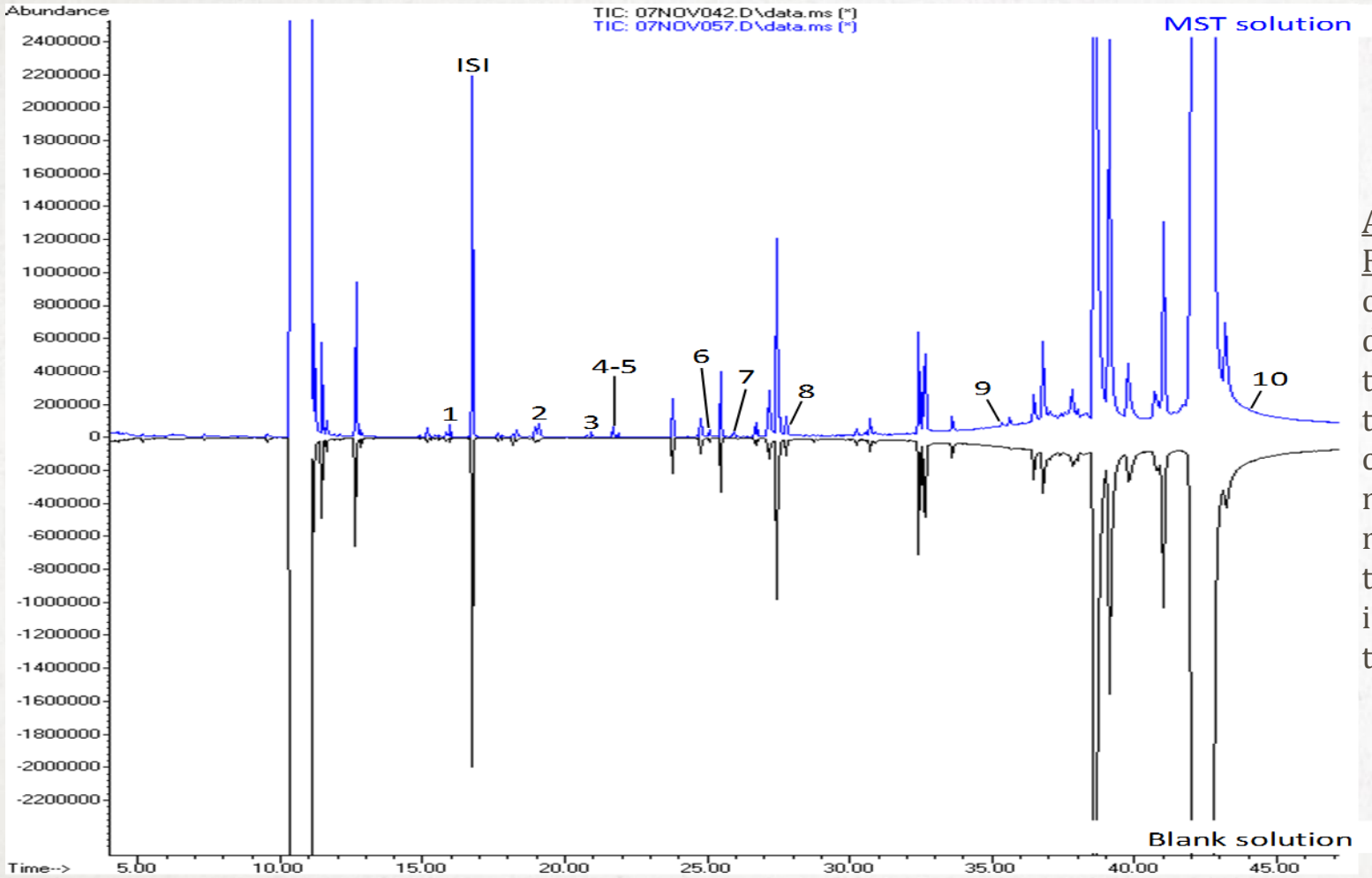
Types of Omission Errors; Falling Through the Cracks

3. It never makes it off the column.

4. Something wacky happens in the detector.

Type of Problem	Description of Problem	Potential Omissions	How to avoid Omissions	
Mass Spectrometry	Compounds with low MW fall outside of the scanned mass range	Formaldehyde, Methanol…	Methanol: consider GC/FID as alternative technique Formaldehyde: consider either GC/FID or specific method (e.g. LC/UV after derivatization)	HEADSPACE GC/MS ON NEAT MATERIALS
Mass Spectrometry	Compounds with higher MW may be missed because they fall outside the of the scanned mass range	Irganox 1076, Irgafos 168 Ox, tetrabromobisphenol A, etc	Experience with the analytical method will reveal compounds that may fall outside the MS detector range. Make the MS-scan range broader. Verify the response of those compounds in other analytical screening methods allow a proper safety assessment (e.g., LC/MS APCI/ESI; derivatization GC/MS)	Direct Injection GC/MS of Organic Extracts
Mass spectrometry: APCI and ESI	Compounds are poorly or not ionized by the ionization technique	Perfluorinated organic compounds in APCI (e.g. PFAS), polycyclic aromatic hydrocarbons, esters	Verify the compounds with the other Ionisation Mode (e.g., ESI for perfluorinated compounds). Consider an orthogonal detection technique e.g. UV/DAD to detect compounds with chromophoric groups.	Direct Injection LC/MS (APCI+/-) of Extracts

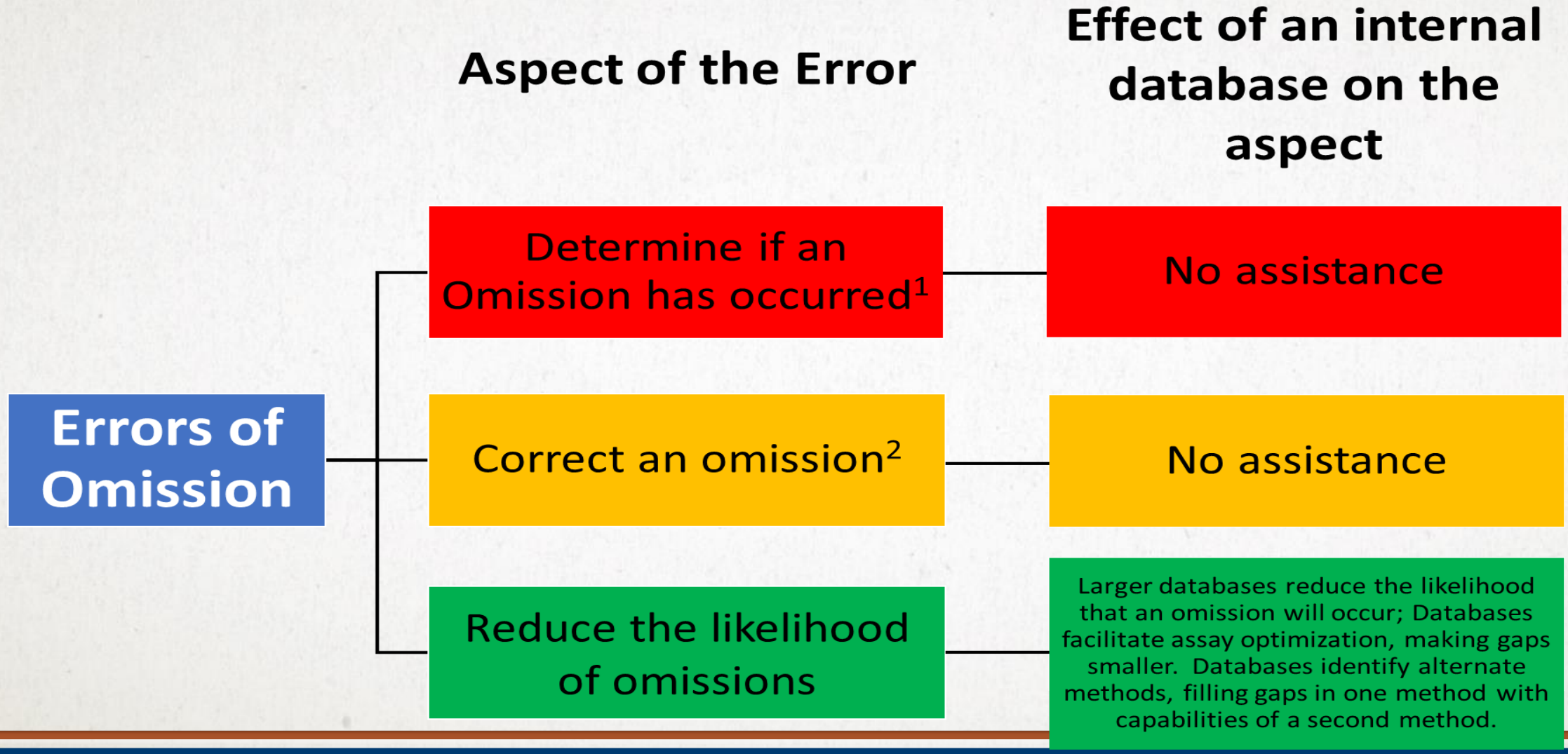
Types of Omission Errors: Failing to see a Tree in the Forest



ISI: Internal Standard for Injection;

An Example of the "Failing to See a Tree in the Forest" Type of Omission Error. The bottom chromatogram is the response to the injection of a drug product "blank" and the top chromatogram is the response to a drug product spiked to contain ten potential leachables. In the blank chromatogram that there are regions where the matrix interference are so great that leachable responses would be obscured. In fact, it is difficult to distinguish the responses for several of the ten intentionally added substances, even knowing that the drug product had been spiked with them.

Use of a Database to Address Errors of Omission



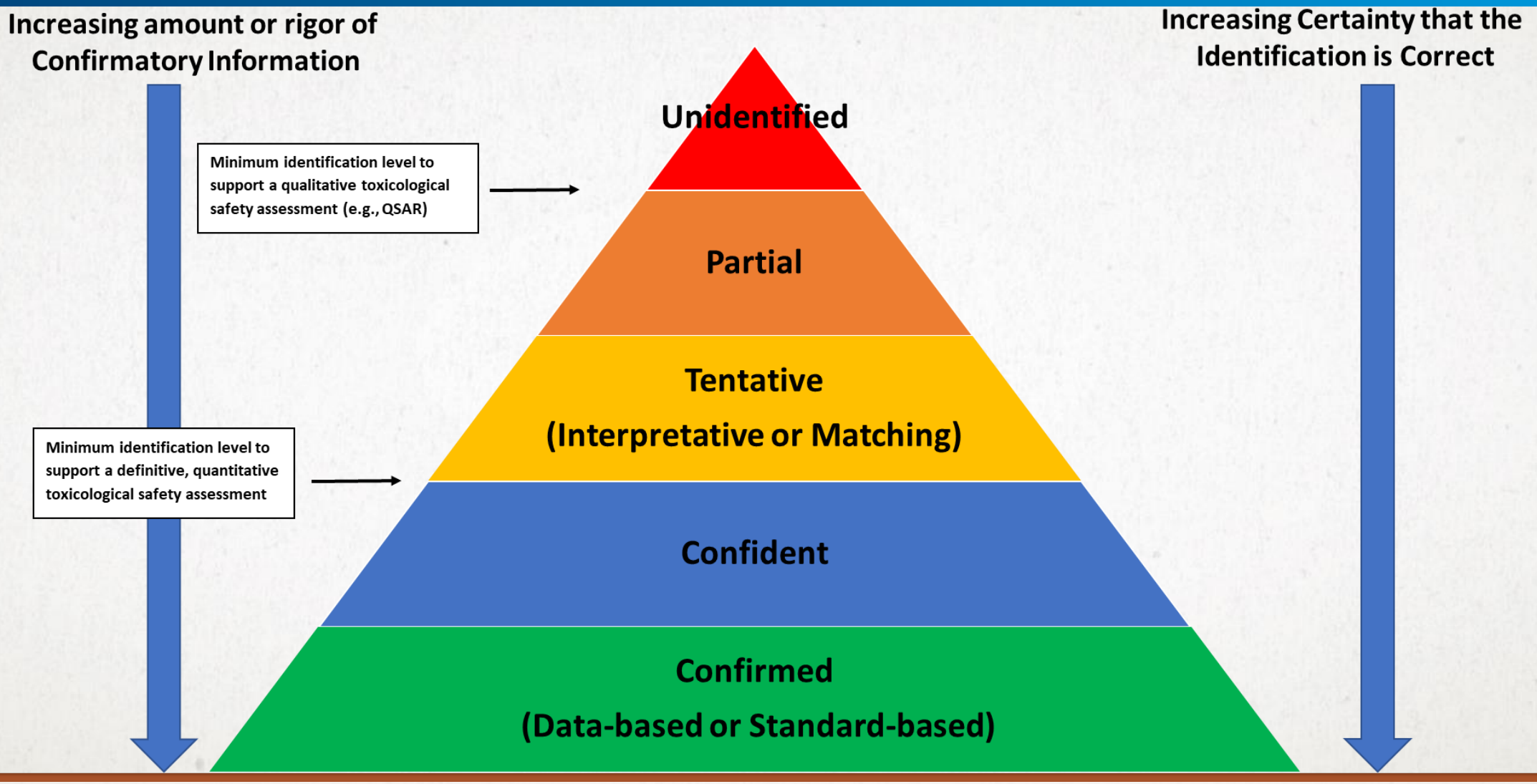
The Error of In-Exact Identification

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 that data which enables its assessment.

Commission of an error of <u>in-exact identification is a fatal error</u> because such an error precludes a proper assessment. An extractable or leachable which is not properly identified cannot be properly assessed.

The Various Levels of Identification



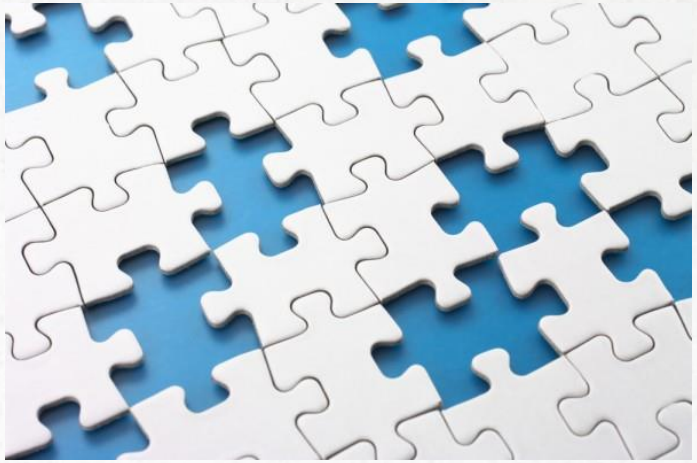
Types of Identification Errors

No Identity:

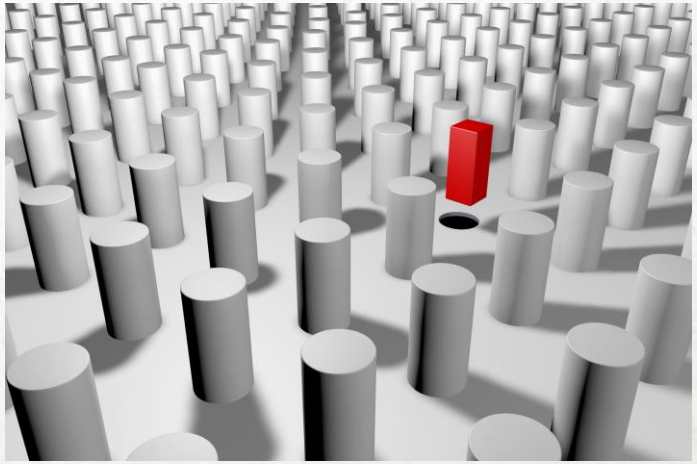
Incorrect Identity:

Types of Identification Errors: No Identity

Substance produces insufficient data to support the identification process



No match is obtained in searched resources



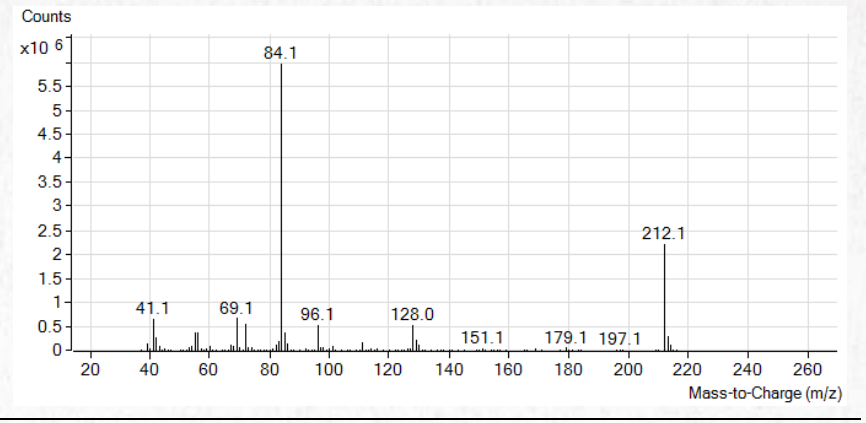
Types of Identification Errors: Wrong Identity

A "Simple" Identification: "The Highest Match Score Wins!"***

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

Detected Compound

Acquired RT = 24.61 min



The "Home Court" Advantage

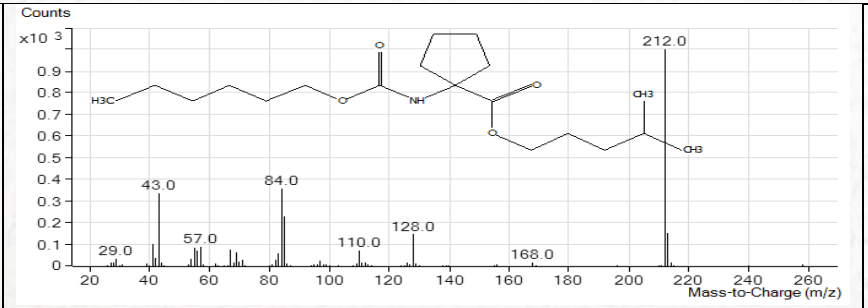
Best hit from NIST/Wiley

INEXACT IDENTIFICATION

1-aminocyclopentane-carboxylic acid, N-hexyloxy-carbonyl, isohexyl ester

Library RT not available

Library Match = 80.7%



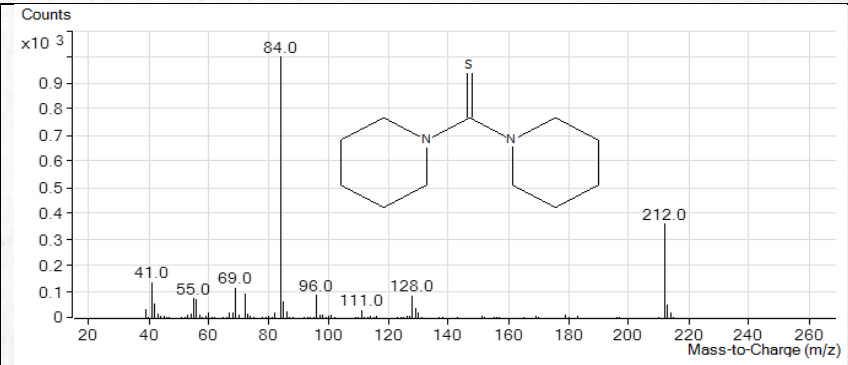
Best hit Nelson Labs DB

EXACT IDENTIFICATION

1,1'-Carbonothioyl-bispiperidine

Library RT = 24.58 min

Library Match = 98.4%



Errors of In-exact Identification; The 'Home Court' Advantage of an Internally Developed and Populated Database

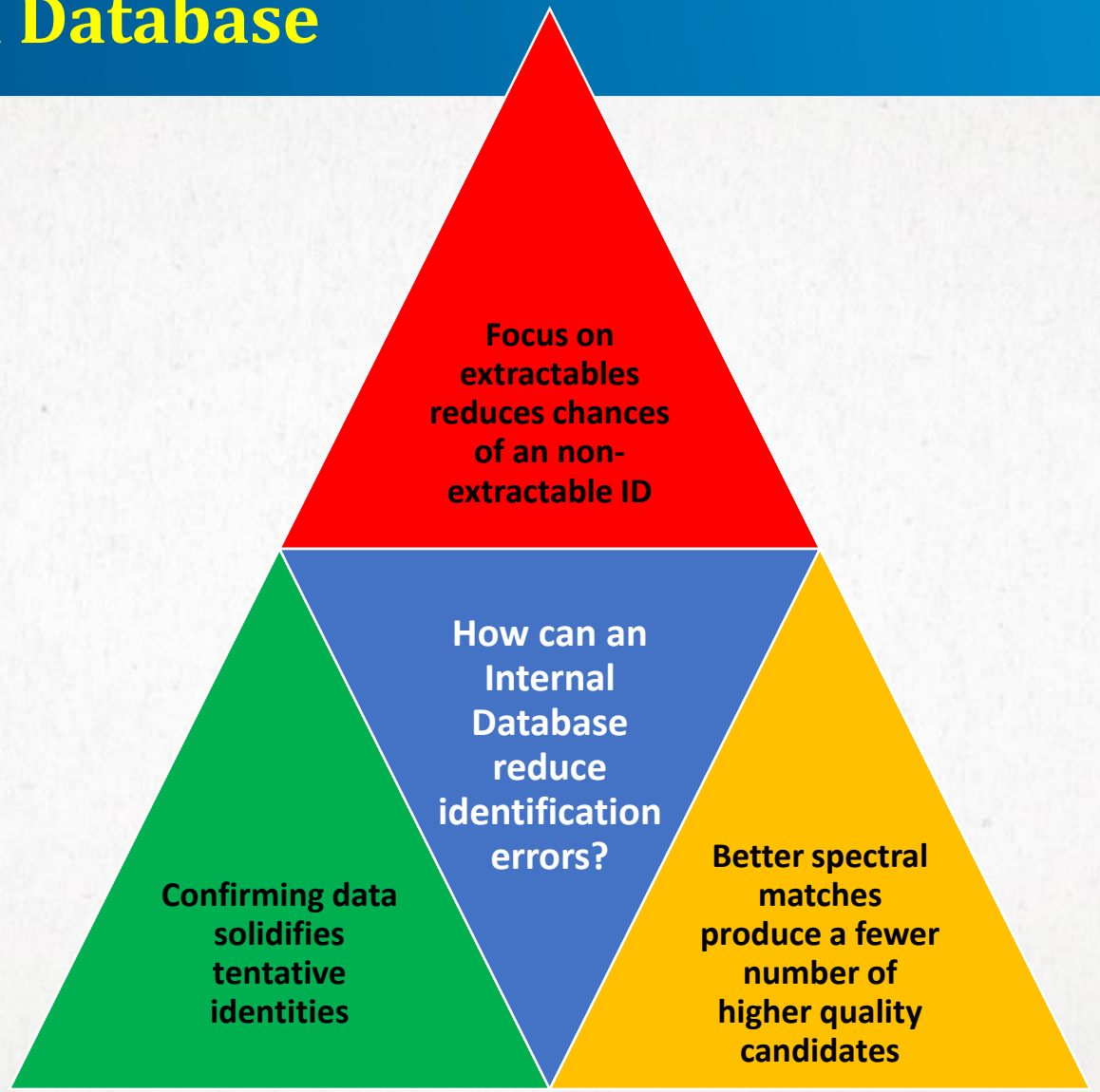
1. As the size of the database increases, the probability that the compound of interest is in the database increases.

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).

Errors of In-exact Identification; Resolution with an Internal Database



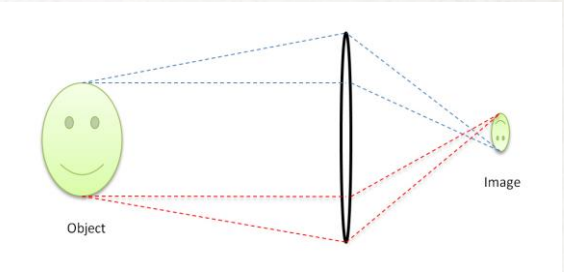
The Error of Inaccurate Quantitation

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 correct assessed risk.

Problem Statement, 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 require 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.

Quantitation Errors in Perspective

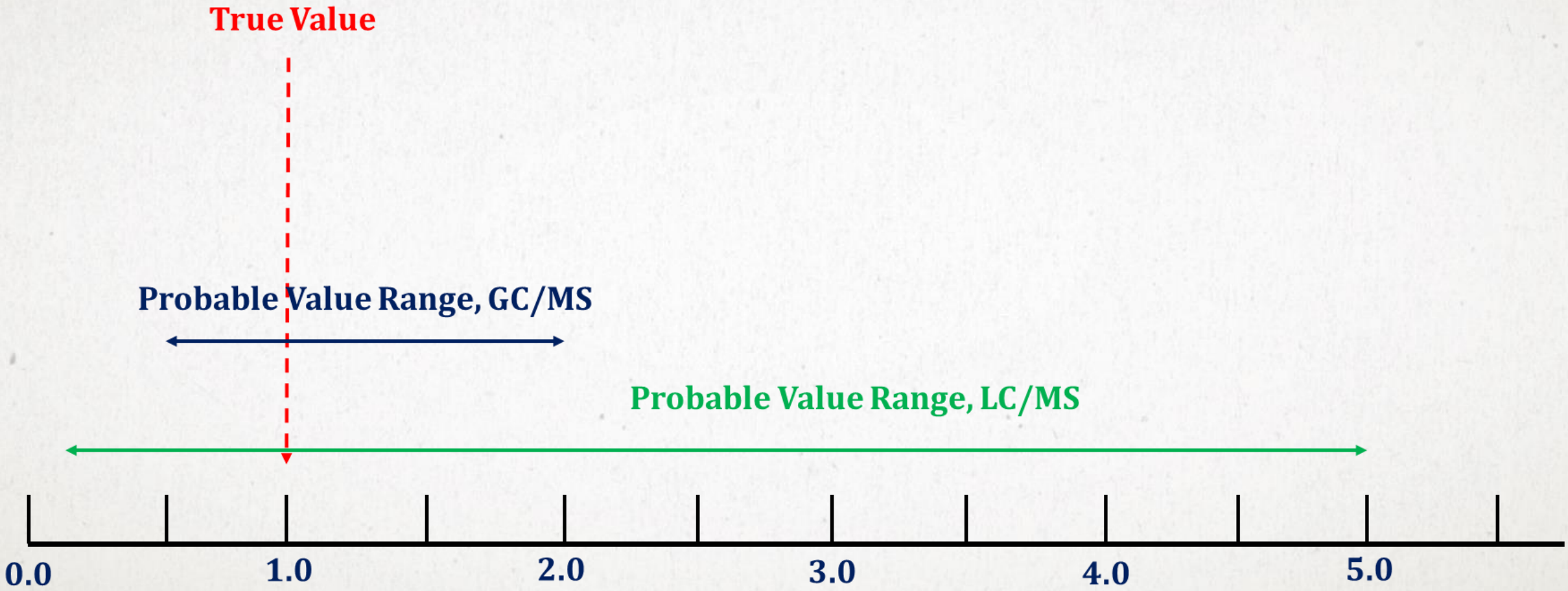
For Semi-volatile Substances by "Direct Injection" GC/MS:

- 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.

Quantitation Errors in Perspective



Quantitation Errors in Perspective

Distribution of RRFs for compounds in the Nelson Labs Screener Database for HS-GC-MS, GC-MS, derivatization GC-MS and LC-MS (APCI). Bracketed values correspond to the subset of "quantifiable" data with RRF values between 0.1 and 5.0.

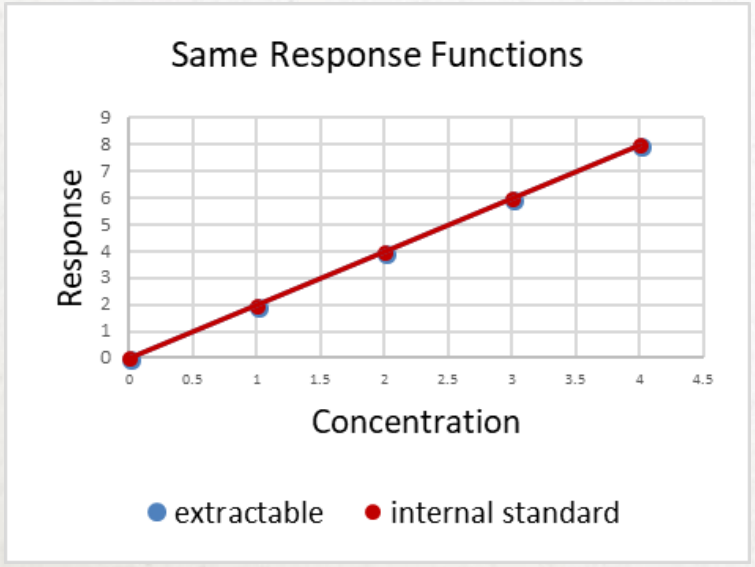
Parameter	HS-GC-MS	GC-MS	Deriv. GC-MS	LC-MS (APCI & ESI)
Total number of entries	987	3076	408	1985
Total number of entries with RRFs:	246	2247	408	1129
RRFs < 0.1:	22%	7%	3%	33%[2]
Median RRF	0.640 (0.909)	0.590 (0.628)	0.949 (0.960)	0.251 (0.541)
Mean RRF	0.799 (0.997)	0.645 (0.687)	0.989 (1.017)	0.543 (0.749)
Standard Deviation	0.811 (0.728)	0.425 (0.407)	0.530 (0.512)	0.921 (0.651)
Minimum RRF	< 0.001	<0.001	0.008	< 0.001
Maximum RRF	5.390	4.611	3.547	16.500

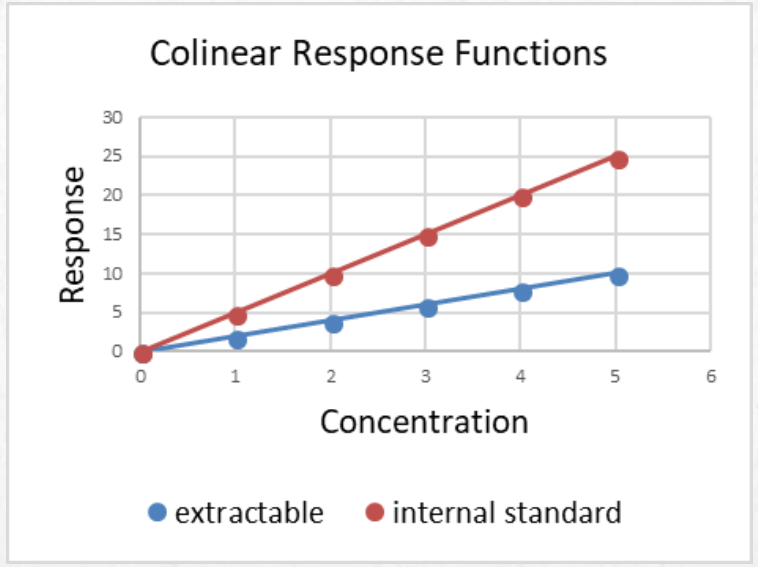
Quantitation Errors and Response Functions

Relative Response factors work well when either:

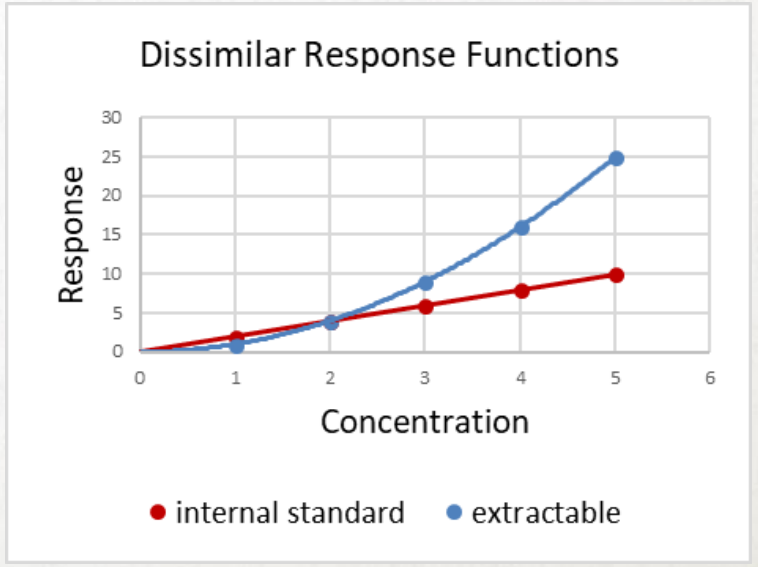
- The concentration of the internal standard and analyte are similar,
- The response function for the internal standard and analyte are similar.

Works Well





Does not Work Well



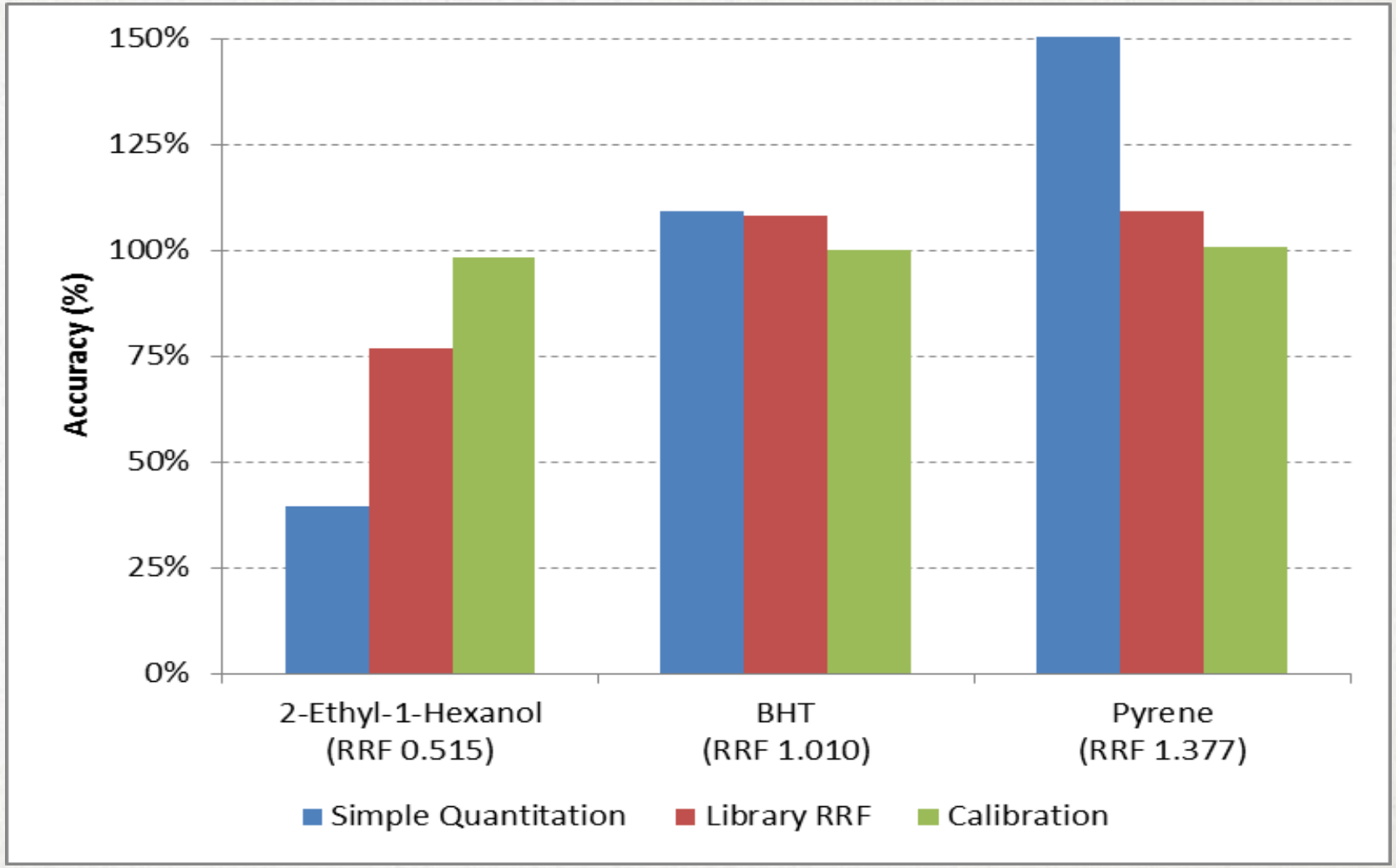
Use of RRF to Identify the Optimal Analytical Reporting Method

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

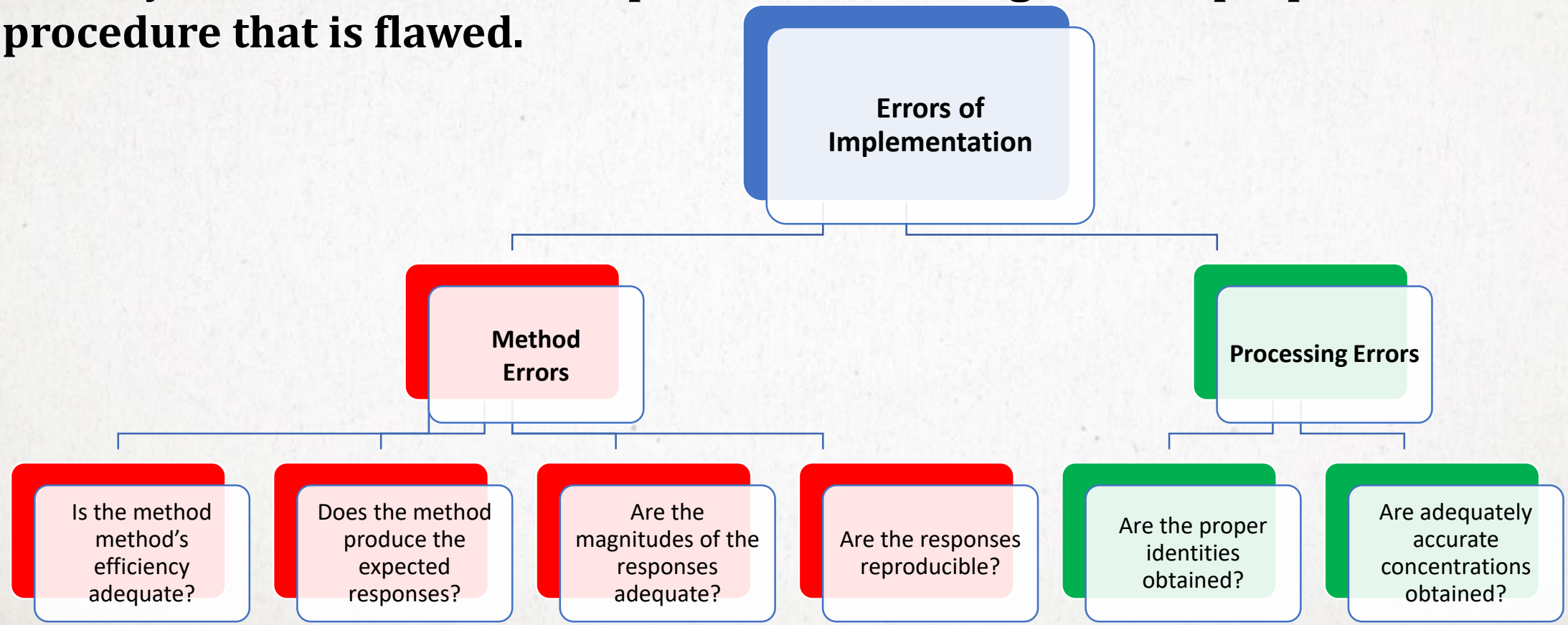
How Big are Quantitation Errors Anyway?

Comparison of different quantitation approaches for three compounds at 10 mg/L in an artificial extract using an internal standard at a concentration 10 mg/L.



Errors of Implementation and the Role of System Suitability

With an error of implementation, it is not the method or procedure itself that is faulty but rather it is the implementation of a good and proper method or procedure that is flawed.



The Function of System Suitability Testing

Emphasizing the positive, the purpose of system suitability testing is to establish that the screening method and its associated data processing procedures have been implemented in such a way that the method and procedure are able to perform the task(s) they were designed and qualified to perform.

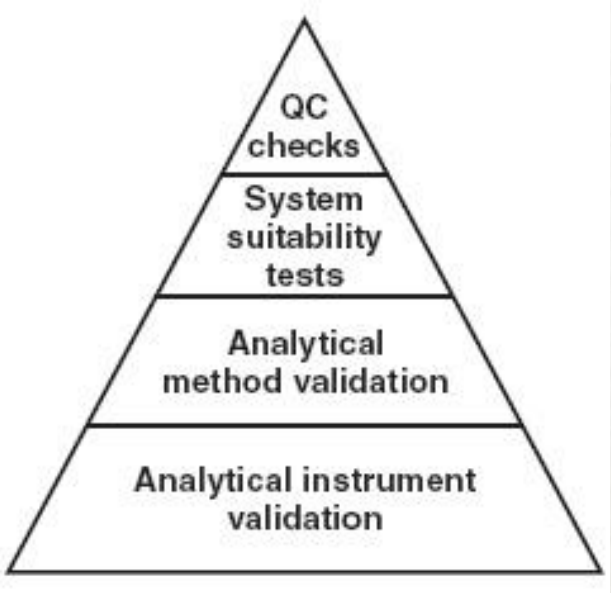
Considering the potential negative, system suitability testing can identify situations where the method or procedure cannot or has not produced data of sufficient quality to be either useful or credible.

The Essence of System Suitability Testing – The Suitability Mixture

System suitability is established using a group of substances, termed the system suitability mixture, that is representative of that portion of the population of extractables and leachables that the method is designed to address.

Key Attributes of the System Suitability Mixture:

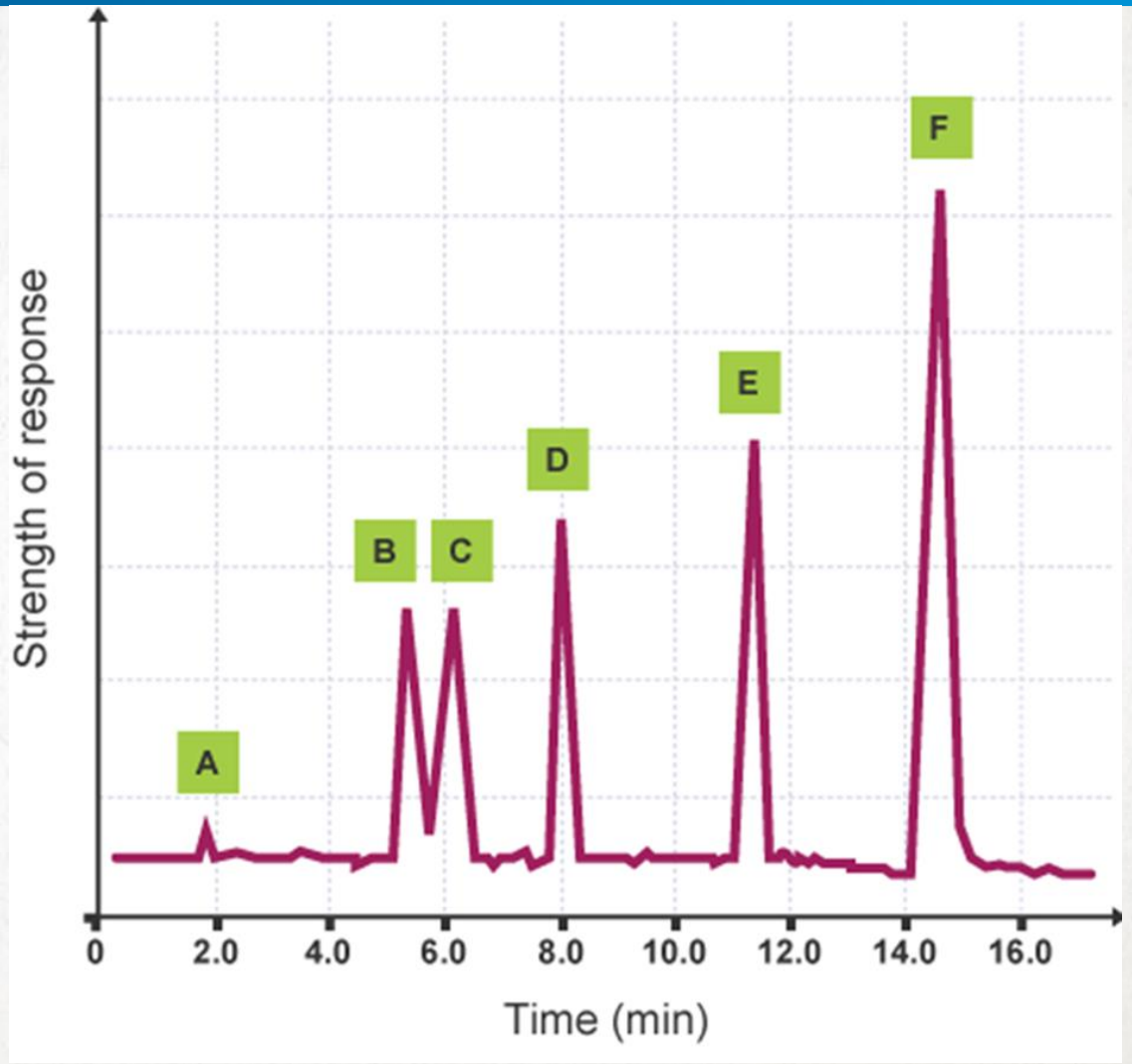
- Number of substances in the Mixture
- Chemical nature of substances in the mixture



The Proper System Suitability Mixture (?)

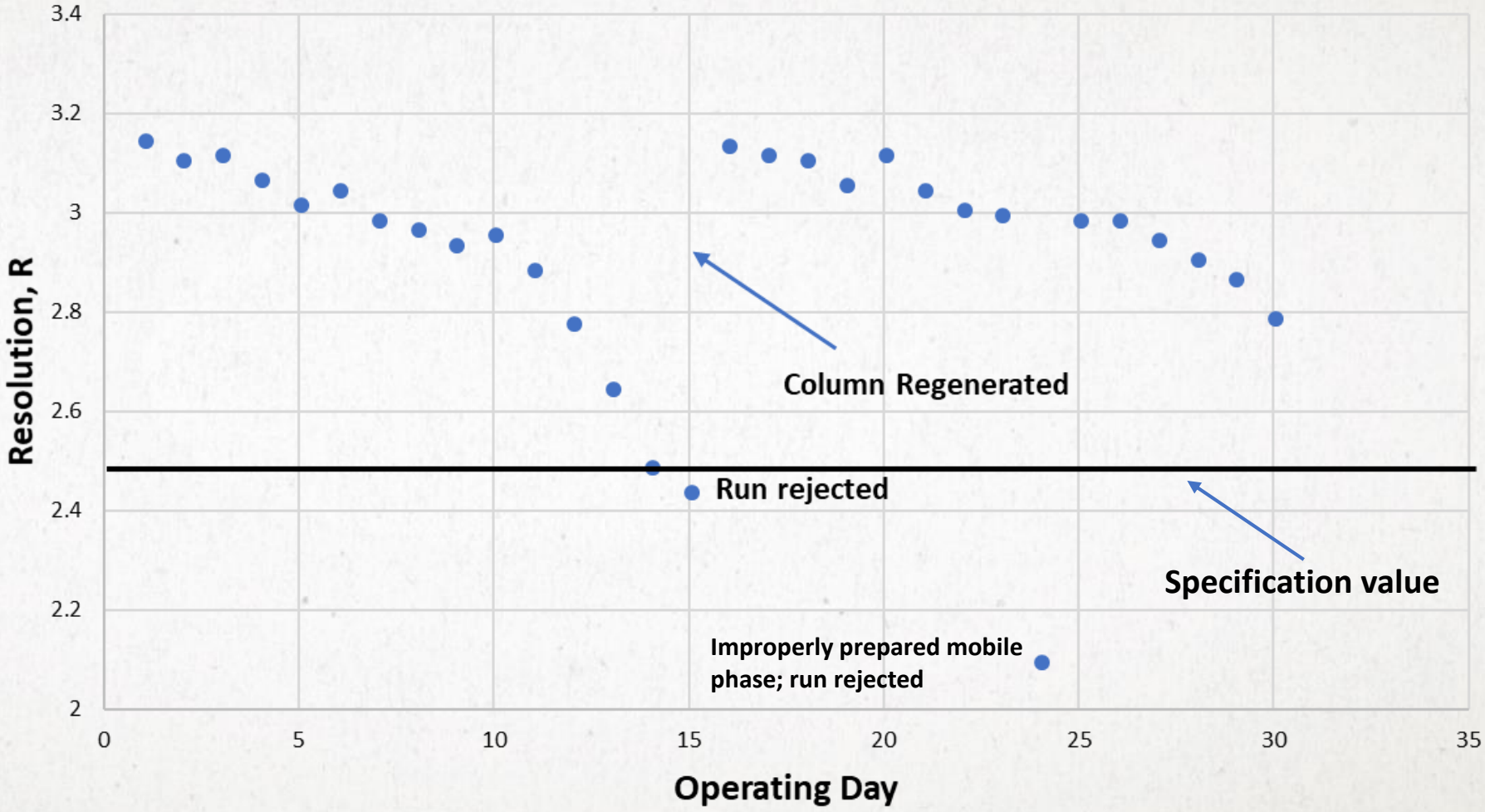
Chromatogram for a System Suitability Mixture Containing Six Members.

- **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).**

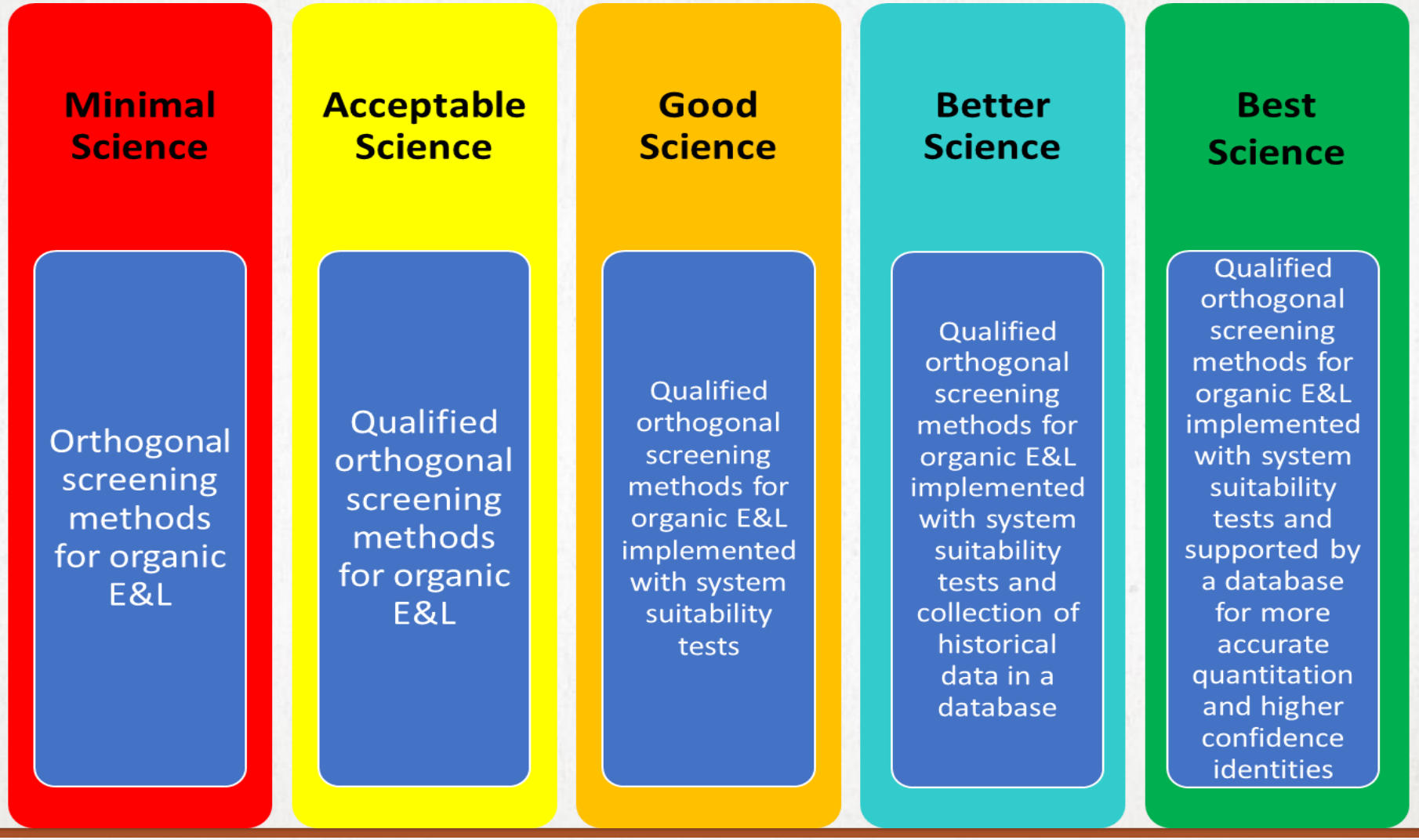


The System Suitability Database as a Diagnostic Tool

System Suitability Control Chart for Chromatographic Resolution for a Critical Pair. As more chromatographic runs are performed, column performance degrades, eventually resulting in an out of specification result and a rejected run. Regeneration of the column improves performance and acceptable runs are possible again. On day 24, the mobile phase was improperly prepared, resulting in a one-time performance excursion. Based on the first cycle, the operating duration of the system is roughly 14 days. Further charting of resolution data will define the optimal operating duration with greater accuracy.



The Database as a Differentiating Factor for the Level of Science



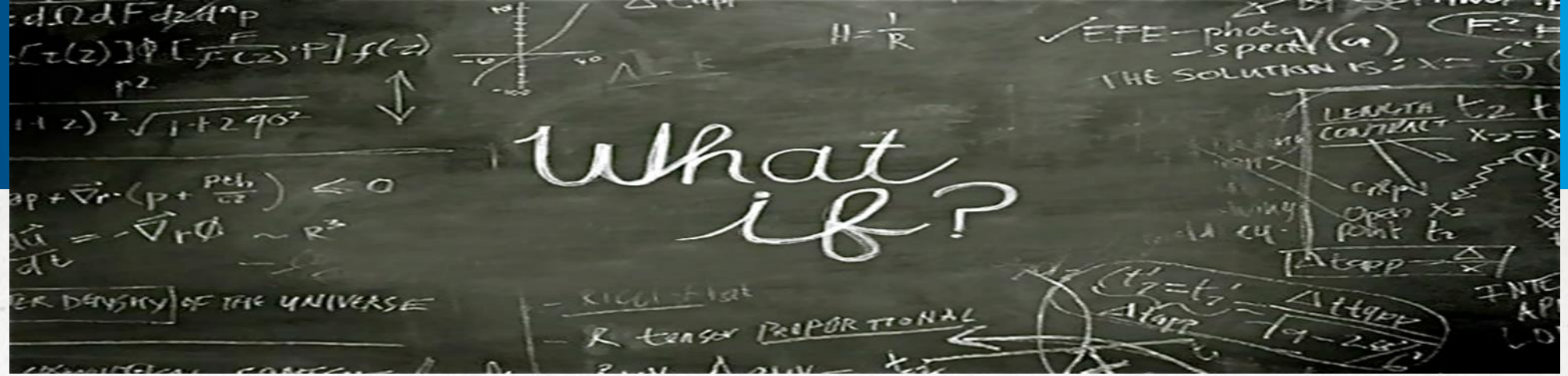
Changing the Game with an Extractables and Leachables Database

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 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?"

In 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

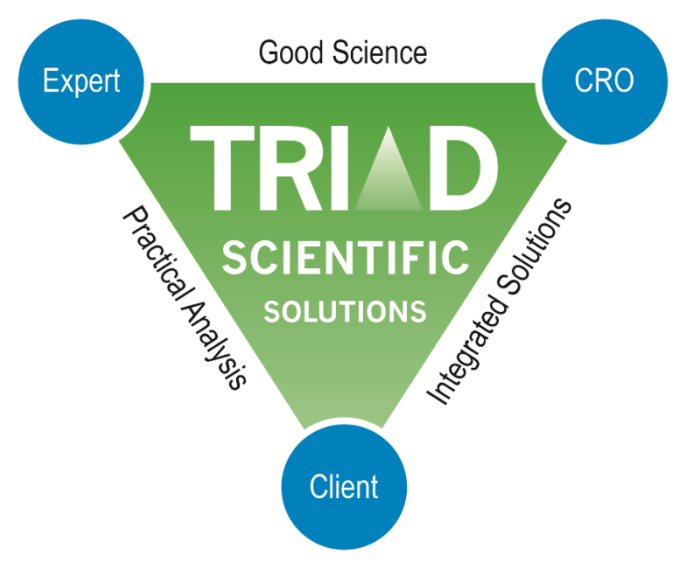
2. These errors can be identified, mitigated and corrected via a robust, well-populated and information rich internally-developed database of analytical data.

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

THANK YOU and TIME FOR QUESTIONS

Contact the presenter at:
dennisjenke@triadscientificsolutions.com
www.triadscientificsolutions.com

Acknowledgements



Nelson Labs™ EUROPE
FORMERLY TOXIKON EUROPE