Current and Novel Approaches to Quantitation in Non-Targeted Screening Analysis Applied to Extractables and Leachables from Medical Devices

Dennis Jenke; Principal Consultant, Nelson Labs, Europe

Nelson Labs Europe
Medical Device Workshop,
April 26, 2023

AGENDA

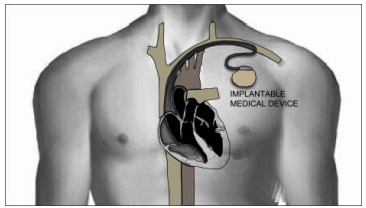
- **Non-Targeted Analysis in Extractables and Leachables**
- **When to Quantitate, The Analytical Evaluation Threshold, AET**
  - The “Too Many Peaks” Challenge in E&L NTA
  - Definition and Application of the AET
  - Complications in Applying the AET
    - Response Factor Variation
    - AET versus Method Sensitivity
  - Adjustments to the AET; The Uncertainty Factor (UF)
    - Calculation of the UF
    - Application of the UF
  - Final Concentration-based Check of AET Status

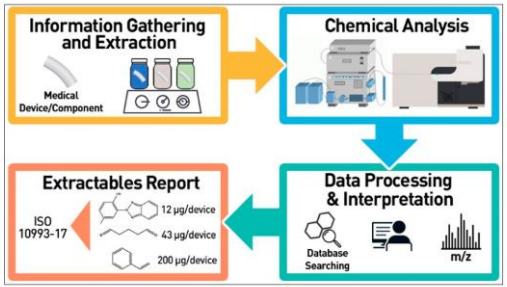
- **How to Quantitate, Semi-quantitative Analysis**
  - Quantitation Categories
  - Quantitation Challenges
  - Quantitation Approaches

Nelson Labs®
A Sotera Health company

Non-Targeted Analysis in Extractables and Leachables

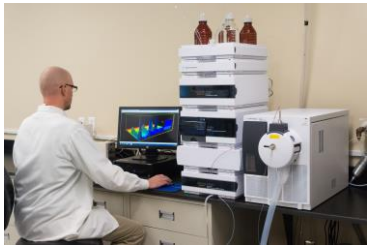
- During the clinical use of a medical device, **the device will contact the patient**, either directly or indirectly and either for the short term or the long term.
- During contact **the medical device and the patient will chemically interact**.
- One such interaction is the transfer **(leaching) of chemicals** on or in the medical device (leachables) to the patient.
- These leached chemicals are important as **they may adversely affect patient health** and well-being. Thus, leachables are a patient safety risk.
- In order to establish the safety risk, leachables must be **discovered**, correctly **identified** and accurately **quantified**.

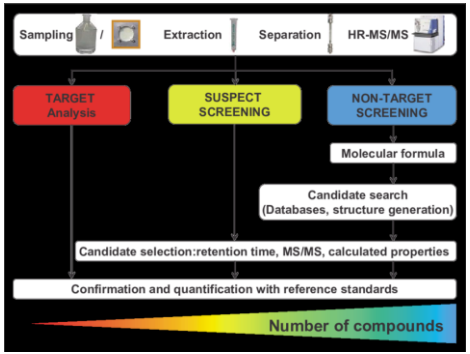




Nelson Labs.
A Sotera Health company

Non-Targeted Analysis in Extractables and Leachables





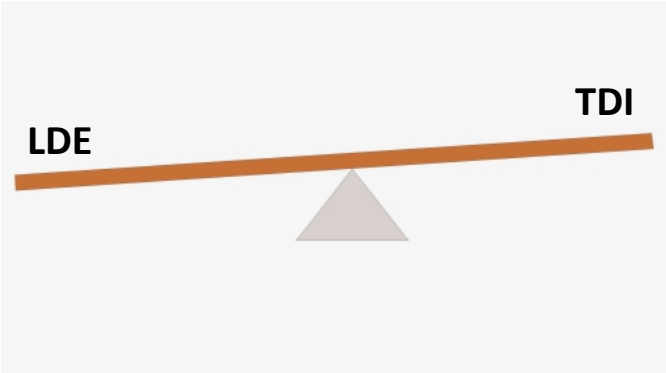
- As it is challenging, both practically and ethically, to measure device-related leachables directly in the body, device **leachables are approximated by device extractables**.
- Device extractables are ascertained by **subjecting the medical device to laboratory extraction** conditions and **analytically characterizing the** resulting **extract**.
- For organic extractables, the analytical process **involves multiple orthogonal and complementary chromatographic methods coupled to** information-rich detectors such as **mass spectrometers**.
- Because the leachables cannot be specified up-front, **the analytical process involves screening**, otherwise known as **Non-target analysis (NTA)**.

Nelson Labs.
A Sotera Health company

Establishing the Safety Risk Associated with Leachables

- The safety risk posed by leachables is established by a process called **toxicological safety risk assessment (TSRA)**.
- In essence, the TSRA involves comparing the **patient's daily exposure to leachables (LDE)** and the **leachable's tolerable daily intake (TDI)**.
- In order to establish the LDE, the concentration of an extractable (as a potential leachable) in the extract must be determined (**quantitation**).
- In order to establish the TDI, the extractable's identity must be established (**identification**).

The Essence of TSRA



Two Sides of the Leachables Coin

Identification **Quantitation**

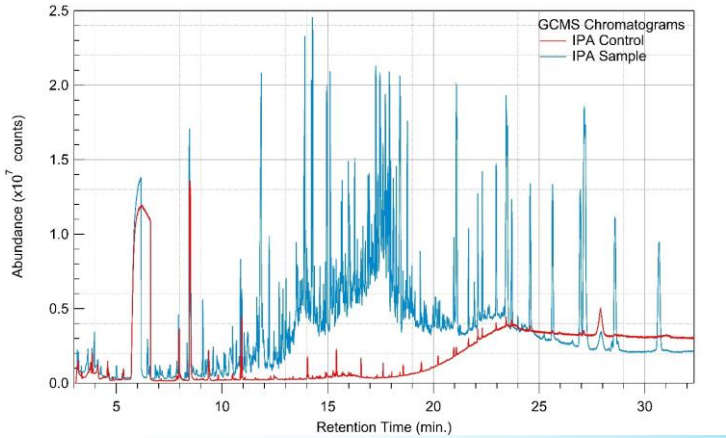
When to Quantitate, The Analytical Evaluation Threshold, AET

Quantitation: process of assigning a concentration to an analyte present in a sample [ISO 10993:18(2020)]

Quantitation:
How many marbles are in this jar?

The "Too Many Peaks" Challenge in E&L NTA

It is often the case that extracts of a medical device contain a large number of extractables, exacerbated by exhaustive and aggressive extractions and by expectations that extractables be measured at very low levels. Thus, a chromatogram generated by testing an extract might contain **so many peaks that quantifying and assessing each and every peak becomes a challenge**.



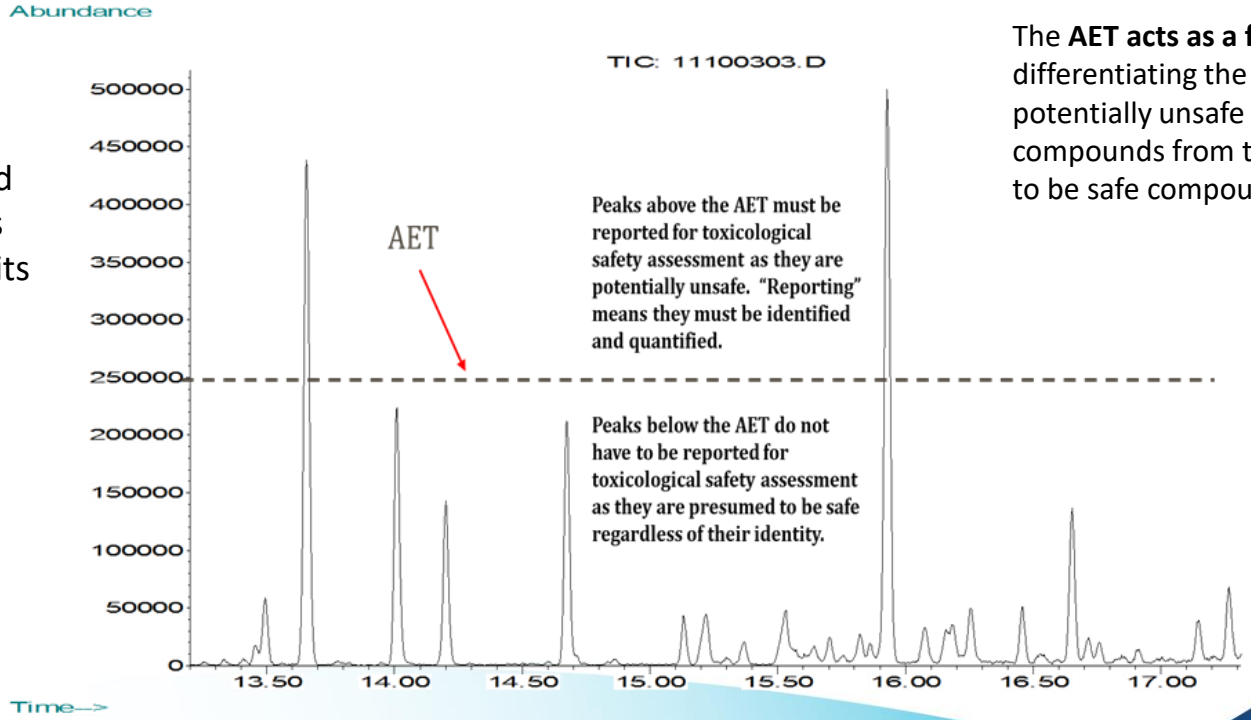
Nelson Labs.
A Sotera Health company

Definition and Application of The Analytical Evaluation Threshold, AET

The **Analytical Evaluation Threshold (AET)**: that concentration of an extractable or leachable below which **the compound does not have to be reported for safety assessment** as its adverse effect on safety is negligible.

Toxicological Basis:

There is a dose of a compound below which the compound is likely to be safe regardless of its identity.

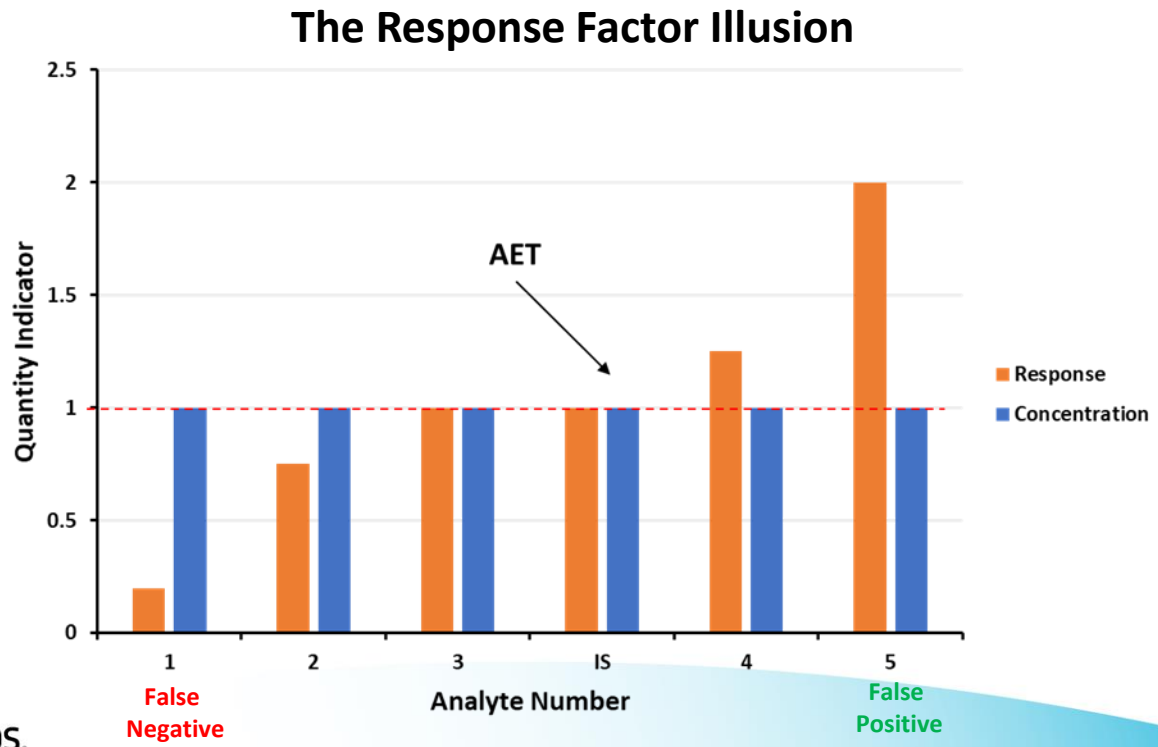


The **AET acts as a filter**, differentiating the potentially unsafe compounds from the likely to be safe compounds

Nelson Labs.
A Sotera Health company

Complications in Applying the AET, Response Factor Variation

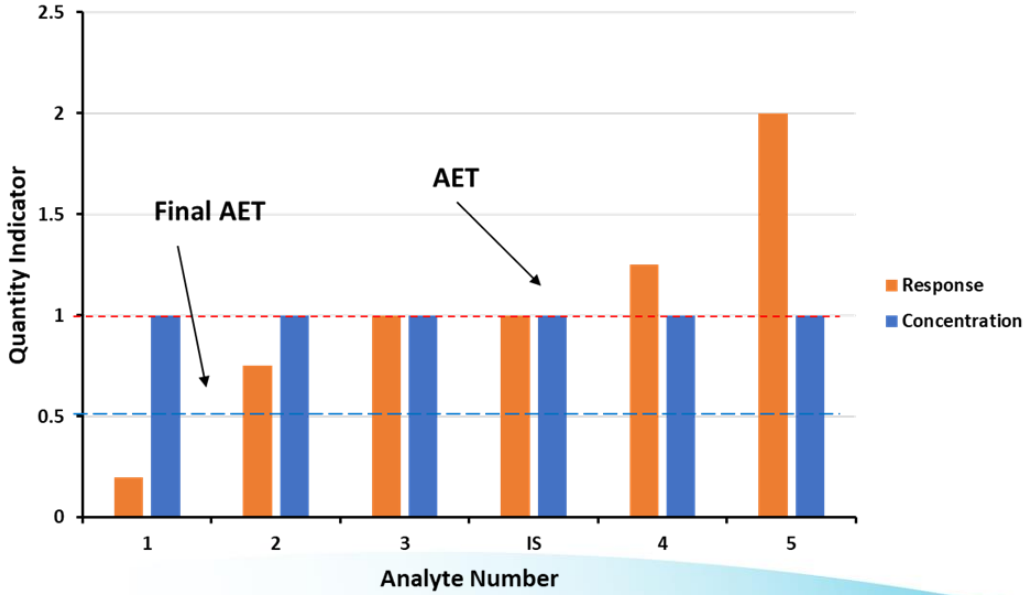
The AET concept works well when all analyte's have the same response factor. However, GC/MS and LC/MS **response factor's vary quite substantially** from compound to compound.



Adjusting the AET for Response Factor Variation, The Uncertainty Factor

The AET can be "adjusted" to account for response factor variation by establishing the magnitude of the variation (the so-called **Uncertainty Factor", UF**) and reducing the AET using the UF to produce the **"Final" AET**.

Final AET = AET/UF



Calculating the Uncertainty Factor from a Database of Response Factors

Calculating RSD From a RRF Database

RT	Relative Response Factor (RRF)	Compound	CAS N°
17.67	0.754	Methyl undecanoate	1731-86-8
17.69	0.158	Triethanolamine	102-71-6
17.7	1.13	2-tert-Butyl-4-ethylphenol	96-70-8
17.72	0.82	2-Propenyl ester of Cyclohexanepropanoic acid	2705-87-5
17.72	0.662	N-Methylphthalimide	550-44-7
17.75	0.718	Cinnamyl alcohol, trimethylsilyl ether	900580-00-0
17.78	0.506	2-Ethylhexyl thioglycolate	7659-86-1
17.78	1.445	1,3,5-Tri-tert-butylbenzene	1460-02-2
17.78	0.702	alpha-Ionone	127-41-3
17.78	0.577	4-Butylbenzyl alcohol	60834-63-1
17.8	0.505	2,4,6-Trimethylbenzoic acid	480-63-7
17.8	0.617	4-(Methylthio)benzaldehyde	3446-89-7
17.82	0.426	4-Isopropylbenzoic acid	536-66-3
17.82	0.479	4-Hydroxy-3-methylbenzaldehyde	15174-69-3
17.83	0.341	2-Azacyclononanone	935-30-8
17.83	0.712	cis-6,10-Dimethyl-5,9-undecadien-2-one	3879-26-3
17.87	0.299	trans-Cinnamic acid	140-10-3
Mean	0.638		
% RSD	48.0%		

$$UF = \frac{1}{1 - RSD}$$

where RSD is the relative standard deviation of the response factors in the RF database

UF Values Calculated Using Nelson Labs' RRF Database

Technique	NELSON SOP	Mean	RSD	Calculated Uncertainty Factor (UF)	Rounded* Uncertainty Factor (UF)
Headspace GC/MS	**SOP0451**	**-**	**-**	-	**10**
GC/MS	**SOP0487**	**0.603**	**45.9%**	1.85	**2**
LC/MS (APCI+)	**SOP0264**	**1.036**	**81.3%**	5.34	**5**
LC/MS (APCI-)	**SOP0264**	**0.885**	**80.0%**	4.99	**5**
LC/MS (ESI+)	**SOP0268**	**0.753**	**81.1%**	5.28	**5**
LC/MS (ESI-)	**SOP0268**	**1.041**	**76.6%**	4.29	**5**

Nelson Labs
A Sotera Health company

Recommended AET Practice: AET Check After Quantitation

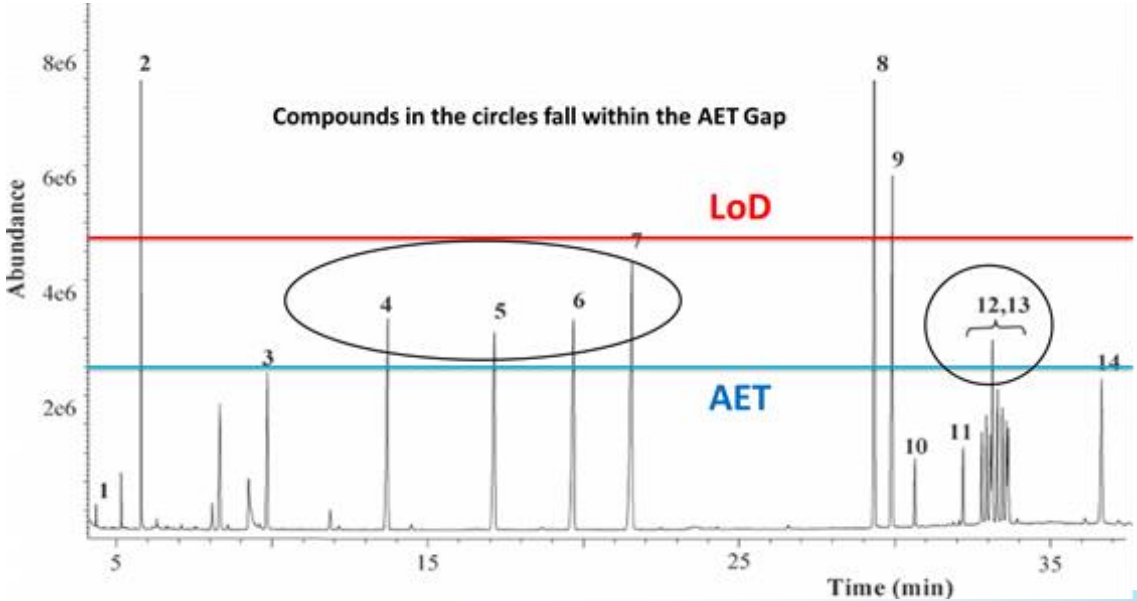
After extractables have been quantified, the reportable concentrations should be checked against the AET to further manage false positives and negatives.

Analyte	Response Assessment			Concentration Assessment			Comment
	Response*	Final AET Expressed as a Response*	≥ AET?	Reportable Conc, µg/mL	Initial AET, µg/mL	≥ AET?	
1	8	10	No	12	5	Yes	Very low responder
2	10	10	Yes	6	5	Yes	Low responder
3	10	10	Yes	5	5	Yes	Equal responder#
4	20	10	Yes	10	5	Yes	High responder
5	20	10	Yes	4	5	No	Very high responder

Only those extractables whose reportable concentrations are equal to or greater than the (initial) AET should be reported for TSRA.

Complications in Applying the AET, Insufficient Sensitivity

- Because all compounds at or above the AET must be quantified, the test method's **Limit of Quantitation (LoQ) must be less than or equal to the Final AET**.
- Because a compound must be detected before it can be quantified, the test method's **Limit of Detection (LoD) must be less than or equal to the Final AET**.



If the LoD > than the Final AET, then you cannot even find all the compounds you might need to report.

What to do if you are in the “AET Gap”?

- **Switch careers.**
- **Retire.**
- **Pretend it didn’t happen and hope everybody forgets it over the weekend.**
- **Report it in the “small print” and hope nobody reads it.**
- **Document and report the steps you took to lower the LoD/LoQ.**
- **Account for the potential toxicity of compounds in the “AET gap” via supporting data and the “preponderance of data”.**

What to do if you are in the “AET Gap”?

The “Preponderance of Evidence” Approach

A leachable “in the Gap” is less likely to be unsafe if the item it leached from consists of materials that :

1. Are approved for food contact application.
2. Are compliant with compendial standards.
3. Are biocompatible.
4. Conform to compositional regulations.
5. Are used in approved products.
6. Are GRAS.

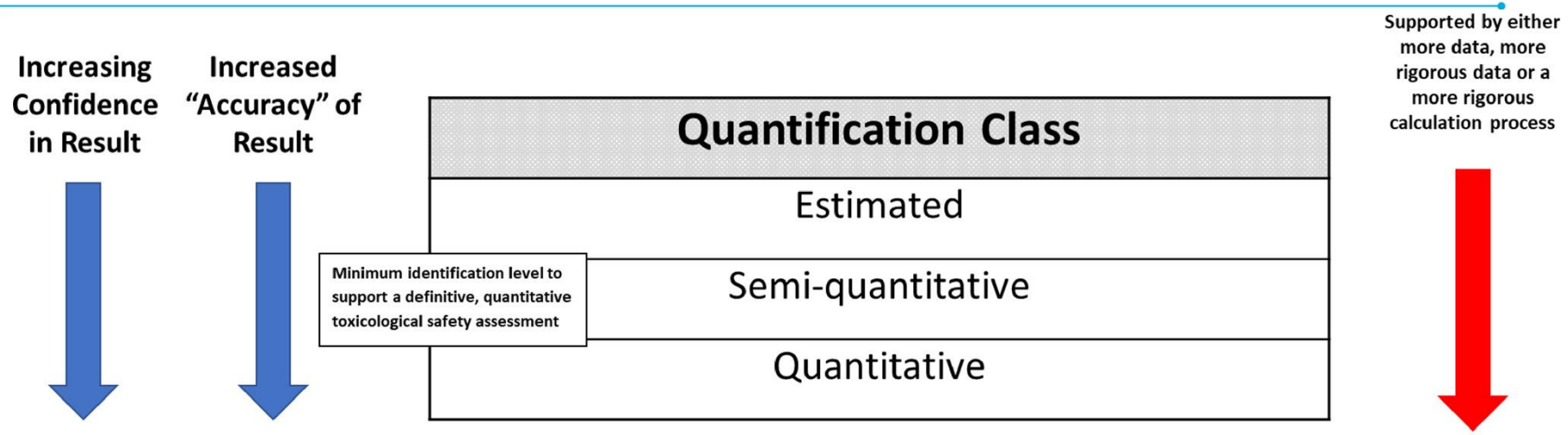
Quantitation of Extractables and Leachables

- There are multiple means by which extractables and leachables can be quantitated.
- It is generally accepted that extractables and leachables should be quantified in a **semi-quantitative** manner.

From ISO 10993:10(2020): semi-quantitative analysis; analytical approach which provides an analyte's concentration by using the response from a surrogate substance (or substances), specifically accounting for the relative responses of the analyte and the surrogate

This **definition focuses on the approach** by which semi-quantitation is accomplished and not the performance characteristics that describe a semi-quantitative result. By focusing on the approach, this definition **excludes approaches that might be able to achieve the same performance characteristics** as the described approach.

Quantitation of Extractables and Leachables – Quantitation Categories



Accuracy Expectations for Quantitation:

Quantitative: 80 – 120% (achievable only via calibration curves)

Semi-quantitative: 50 – 200% (achievable via compound-specific response factors or potentially via linkage to an appropriate surrogate)

Estimated: < 50% or > 200% (single-compound surrogate)

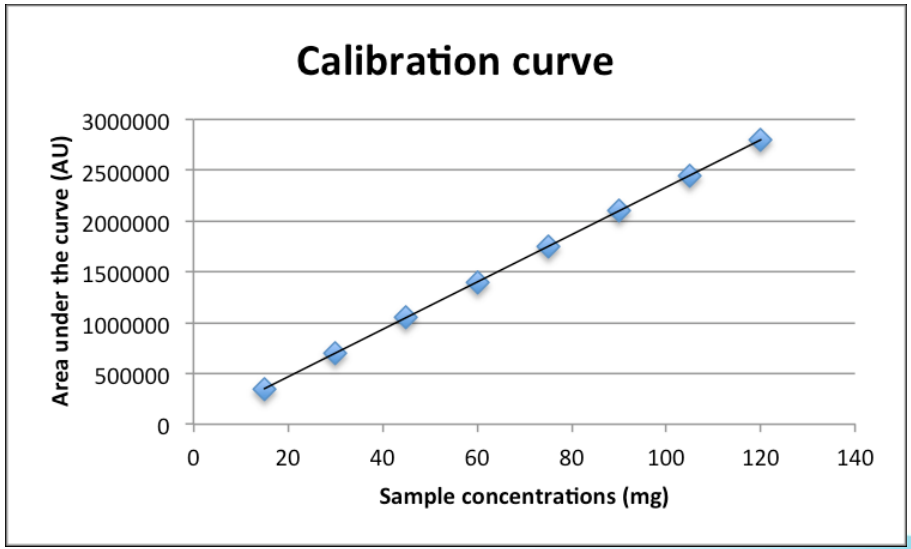
Nelson Labs
A Sotera Health company

Quantitation of Extractables and Leachables – Calibration Curves

The most accurate means of producing quantitative concentration data for extractables ism via the use of a calibration curve, specifically generated for the compound of interest by using the compound of interest.

The Gold Standard

The calibration curve is generated by analyzing standards and determining the curve's best fit equation.



The calibration curve is used by analyzing the sample and putting the analyte's response into the best fit equation.

Nelson Labs.
A Sotera Health company

Quantitation of Extractables and Leachables – Response Factors

Determination of Response Factors:

An analyte's **Response Factor (RF_A)** is determined by injecting a standard containing a known concentration of the analyte (C_A) and noting it's response (R_A):

$$RF_A = C_A/R_A$$

An analyte's **Relative Response Factor (RRF_A)** is determined by injecting a standard containing a known concentration of the analyte (C_A) and a surrogate (C_S) and noting their responses (R_A and R_S):

$$RRF_A = (C_A \times R_s)/(R_A \times C_S)$$

The advantage of the RRF is that if the surrogate standard is added to all analyzed samples, the R_A value "normalizes" the R_S value versus injection-to-injection imprecision.

Quantitation of Extractables and Leachables – Response Factors

Use of Response Factors:

Using the **Response Factor** (RF_A): the sample is analyzed and the response of the analyte (R_A) is used to calculate the analyte's concentration (C_A):

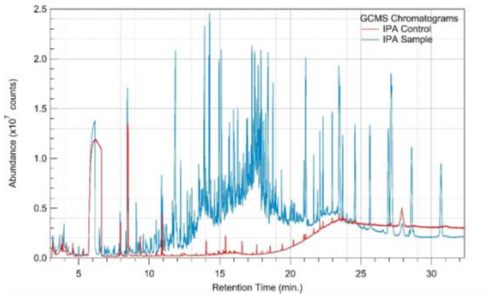
$$C_A = RF_A \times R_A$$

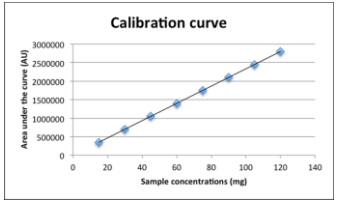
Using the **Relative Response Factor** (RRF_A): the sample, spiked to contain a known concentration of the surrogate standard (C_S), is analyzed and the responses of the analyte (R_A) and the surrogate standard (R_S) are used to calculate the analyte's concentration (C_A):

$$C_A = (R_A \times C_S)/(RRF_A \times R_S)$$

Quantitation of Extractables and Leachables – Quantitation Challenges

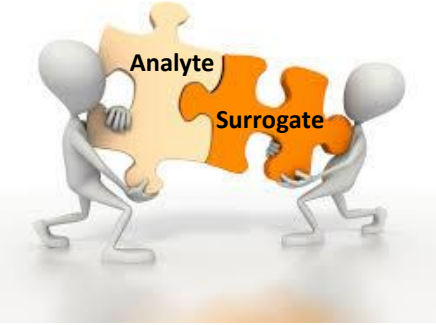
A curve for each peak???





- The population of potential extractables and leachables is so large **that it is a chore to generate calibration curves for all possible compounds** (otherwise organizations would develop a database of calibration curves).
- The number of compounds in a typical extractables profile is so large that **generating calibration curves "on the fly" is difficult.**
- Furthermore, generating calibration curves "on the fly" is a **second pass activity** (That is, compounds are identified as first pass and then quantified as second pass). Remember, we are talking NTA here.
- **Linear dynamic ranges may be relatively small**, reducing the utility of a calibration curve.

Quantitation of Extractables and Leachables – Quantitation Challenges



- Response Factor Variation dictates that quantitation be performed via calibrations that **use either the compound of interest or a closely matched response factor surrogate**.
- **A robust and correct means of linking compounds to a closely matched response factor surrogate has yet to be enumerated.** Thus, calibrations must be performed with the compound of interest.
  - Quantitation cannot be performed for compounds that are not identified.
  - Quantitation cannot be performed for identified compounds for which a reference standard cannot be procured.

Quantitation of Extractables and Leachables – Leveraging Identification Class

The quantitation approach can be different for confidentially identified compounds versus all other compounds.

Confirmed identification: identification secured by **matching the analyte's** experimental retention time and mass spectrum with the experimental retention time and mass spectrum of **a reference standard**.

Key point: The analysis of a reference material to obtain its retention time and mass spectrum also allows for the collection of the **compound's response factor**.

Thus, a compound whose identity has been confirmed can be quantitated based on its own response characteristics. Such an approach will produce a semi-quantitative result.

Quantitation for a Compound with a Confident or Tentative Identity

As it logical that a confidently or tentatively identified compound's identity would be confirmed if a reference standard could be procured, it is presumed that a reference standard cannot be secured and thus that the compound cannot be quantified using it own response.

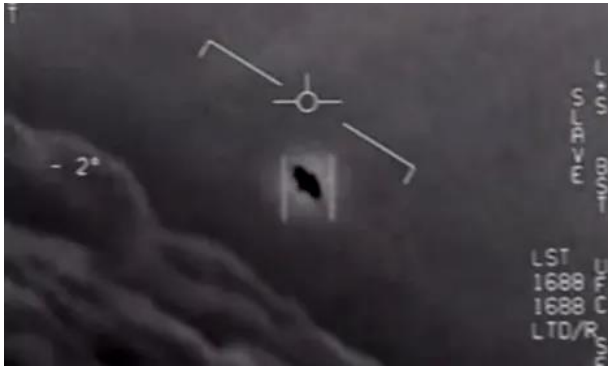
However, there may be sufficient information available for the confidently or tentatively identified compound that it can be **linked to a surrogate standard** whose response properties are known. Such an approach might produce a semi-quantitative result.

Quantitation for a Unidentified Compound

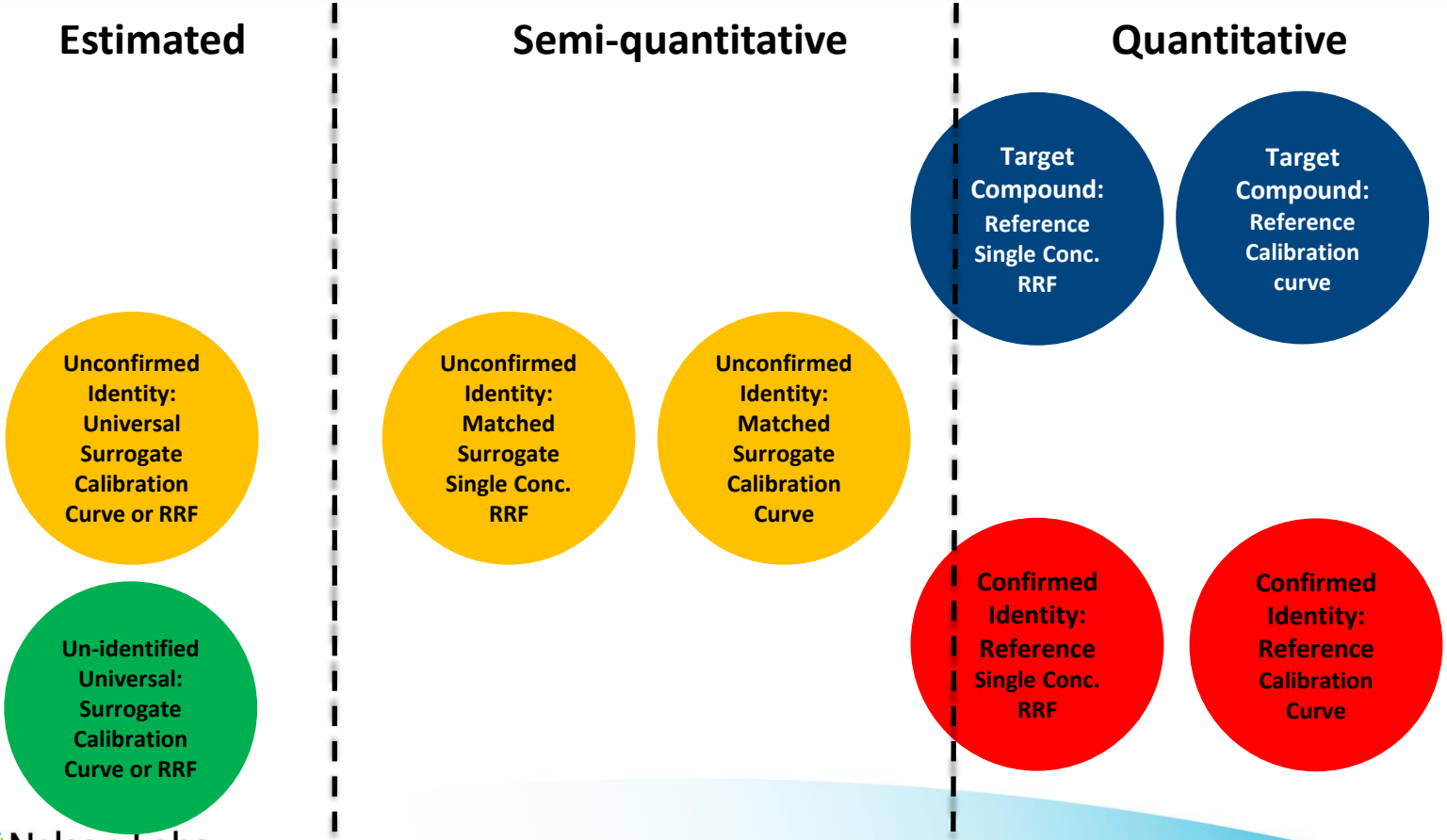
As there is **no basis for linking** an unidentified compound with a surrogate, quantitation for an unidentified compound must be based on a “universal” surrogate standard and a concentration thus obtained must be viewed as an estimate.

Surrogate

Analyte



Quantitation of Extractables and Leachables – Leveraging Identification Class



Nelson Labs.
A Sotera Health company

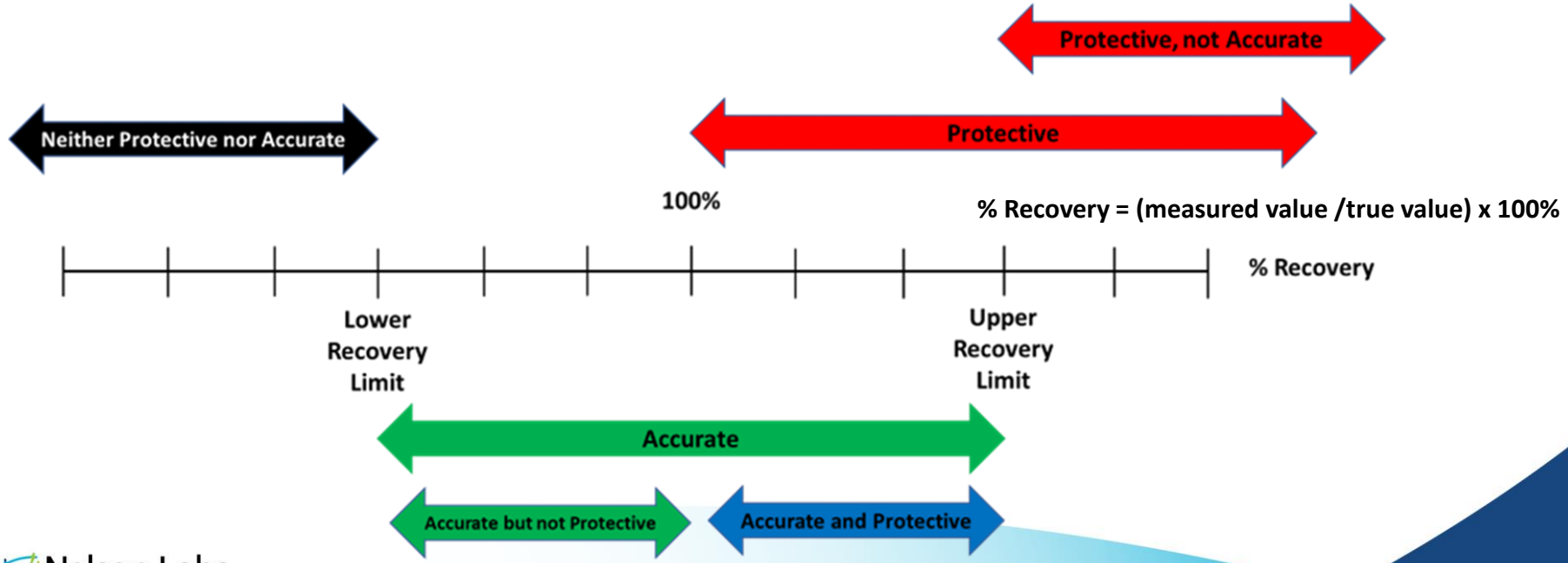
What is the objective of quantitation with respect to toxicological safety risk assessment?

- **To be accurate** (quantitative analysis)
- **To be protective*** (protective analysis)

* A concentration estimate is said to be protective if the estimate is greater than or equal to the analyte's true concentration. In the case of a protective concentration, it is impossible that patient exposure is underestimated.

Quantitation of Extractables and Leachables – Accurate vs Protective

- **Accurate:** The calculated concentration is the same as the true value (within a range of acceptable deviation)
- **Protective:** The reported concentration is not less than the true value. (In other words, the reported concentration is always equal to or greater than the true value)



Objective:

- To produce a semi-quantitative concentration estimate for as many compounds as possible.
- If a semi-quantitative concentration estimate cannot be obtained, the concentration estimate should be protective.

The Nelson Approach to Protective and Semi-quantitative Analysis

For Extractables with Confirmed Identities:

Use the compound-specific RRF to produce a **semi-quantitative** concentration estimate

Could this approach produce fully quantitative data?

(Yes, if the calibration curve is linear and the analyte response falls in the linear dynamic range.)

RRF is the slope of the calibration curve

Table 4. Comparison of the RRF Values and Slopes of Calibration Curves, GC/MS.

Compound		Calibration Range	RRF	Calibration	RRF versus
Name	CAS RN		Value	Curve Slope	Slope, %
2-Ethylhexanoic acid	149-57-5	500µg/L – 50mg/L	0.346	0.494±0.016	70
N,N-Dibenzylformamide	5464-77-7	500µg/L – 50mg/L	0.485	0.748±0.019	87
1-Chlorododecane	112-52-7	50µg/L – 50 mg/L	0.568	0.856±0.026	66
2-Ethyl-1-Hexanol	104-76-7	50µg/L – 50 mg/L	0.515	0.518±0.020	99
2-Undecanone	112-12-9	50µg/L – 50 mg/L	0.627	0.692±0.030	91
Diphenylamine	122-39-4	50µg/L – 50 mg/L	0.834	0.938±0.021	89
n-Heptacosane	593-49-7	50µg/L – 50 mg/L	0.995	1.149±0.038	87
BHT	128-37-0	50µg/L – 50 mg/L	1.010	0.923±0.010	109
DEHP	117-81-7	50µg/L – 50 mg/L	1.010	1.104±0.027	91
Irgafos 168	31570-04-4	50µg/L – 50 mg/L	1.298	0.964±0.016	135
Pyrene	129-00-0	50µg/L – 50 mg/L	1.377	1.193±0.017	115
				Mean RRF Accuracy, %	93 ± 22%

Nelson Labs. A Sotera Health company

The Nelson Approach to Protective and Semi-quantitative Analysis

For Unidentified Extractables and Extractables with Unconfirmed Identities:

Use a universal, single surrogate RRF, coupled with a UF, to produce a **semi-quantitative/protective** concentration estimate

Two important aspects of this approach:

- The single surrogate is chosen so that its RRF value is equal to the median value of a RRF database.
- The concentration estimates obtained by using the single surrogate RRF are adjusted upwards via application of the UF.

The Nelson Approach to Protective and Semi-quantitative Analysis

For Unidentified Extractables and Extractables with Unconfirmed Identities:

Protective:

- By use of the RRF_{median}, the approach is already protective for 50% of the compounds
- By adding the UF to the RRF_{median}, the approach becomes protective for a much larger set of compounds (for GC/MS, it is estimated the use of the RRF_{mean} plus UF = 2 is **protective for 84% of the compounds**).

Semi-quantitative:

- Whether a method is semi-quantitative or not depends on one's definition of acceptable accuracy for "semi"-quantitation applied to E&L.
- If one accepts accuracy of 50% - 200% (a factor of 2 either way) as being semi-quantitative, then using the UF and RRF_{mean} together produces a **semi-quantitative result for 78% of the compounds** (by GC/MS).

Concluding Summary

For the AET:

- After the AET is calculated considering the specifics of the application and the extraction, it is adjusted downward by the application of an Uncertainty Factor to account for response factor variation and produce the Final AET.
- A compound's status versus the AET is established by the compound's response relative to the Final AET.
- After compounds whose responses are equal to or above the Final AET have been quantified, their concentration should be checked against the (initial) AET to confirm their reporting status.

For the UF:

- The UF is calculated using the variation in RRF vales for a population of compounds whose RRF values have been established by analyzing authentic standards.
- The value of the UF will vary from method to method.

For quantitation:

- Compounds with confirmed identifies are quantified semi-quantitatively by using their individual RRF.
- Other compounds are quantified semi-quantitatively and/or protectively by using an individual surrogate standard with RRF_{mean} and then multiplying the result by the UF.

Time for Questions

Thank you!

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

Nelson Labs.
A Sotera Health company