Considerations for Reference Standard Selection and Coverage Map for E&L in Medical Devices

Dmitriy Pastarnak, M.S.
Edwards Lifesciences
Draper, UT

Disclaimer

The ideas expressed in this presentation are my own and do not necessarily reflect the views of Edwards Lifesciences.

Proposed Approach to Reference Standard Selection

Utilized For: Long-term implants to limited-use devices

Chemical Selection Guided by *a priori* Information:

Disclosed information (e.g., COA, SDS, Mfg Process, Patents) and Published literature

Historical data on our devices (e.g., feasibility, executed E&L studies)

Study Approach for Material/Component Analysis:

Individual material/component studies mimic ISO 10993-18 and FDA draft guidance design

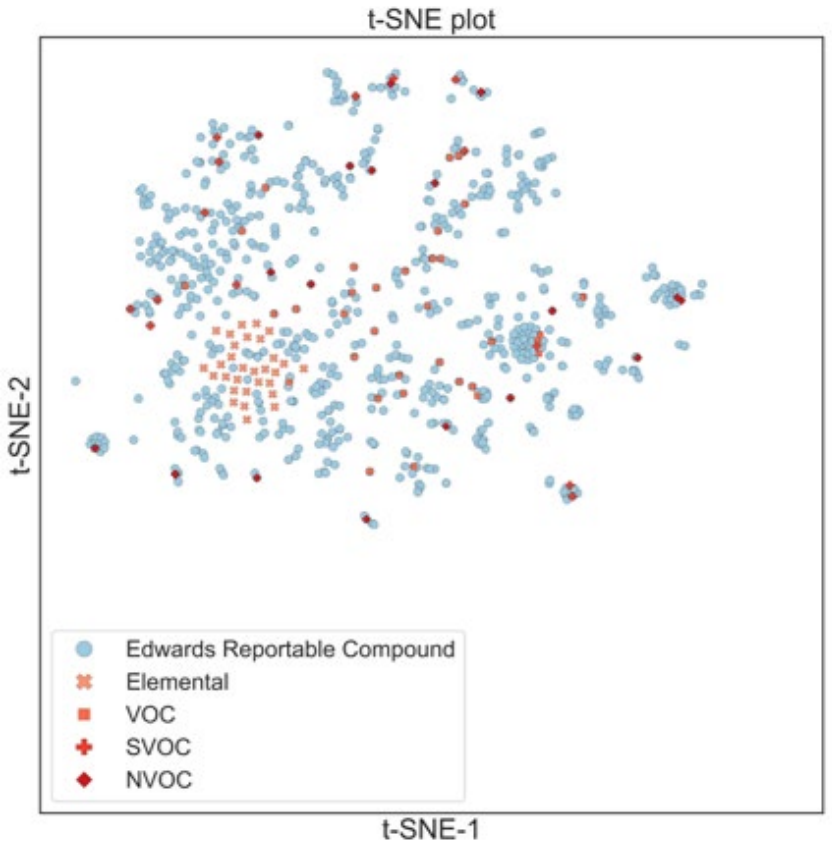
Reference Standard Selection:

Based on all available information to match our extractable chemical space

Re-run characterization studies with standards as new chemical spaces are identified

Reference Standard Utilization:

Uncertainty Factor, calibration, system suitability precision and accuracy, and spike and recovery of manipulated device extracts at or below the study AET, and quantitation



Empirical Formula	DBE	MW	BP (°C at 760mmHg)	pKa	logP (pH 7)	Refractive Index	Polar Surface Area	Molecular Complexity	Hydrogen Bond Donor	Hydrogen Bond Acceptor	Rotatable Bond Count
Min	-7	46.07	0	-24	-1.6	1.35555	0	2.8	0	0	0
Max	17	784.1	740.109	19.2	11.38	1.595	122	1170	4	17	20

Cihan Sorkun, M., Mullaj, D., Koelman, J., & Er, S. (2022). ChemPlot, a Python Library for Chemical Space Visualization. Chemistry–Methods, 2(7), e202200005].

Relative Response Factor and Variability

Relative Response Factor (RRF) and Uncertainty Factor (UF) Determination:

Calculated at the start of each analytical sequence per method as a function of the calibration for each Reference standard. Applicable only to that specific data set

Factors Affecting RRFs:

RRFs are a function of detection at that moment

Changes due to instrument differences, including model quirks and instrument health

Impacted by instrument health, unforeseen issues, and/or contaminants can dramatically alter RRFs

External Databases:

Not used as they do not account for normal variability and day-to-day changes

Determine UF each time data is collected for a curated set of Reference standards covering the expected chemical space (+)

RRF and UF Variability:

Studying RRFs over the years shows variability in internal standard corrected responses; Impacting study-UF

SVOC [GC-EI] UF: 2 to 4

NVOC [ESI+ and ESI- QTOF] UF: 2 to ∞

What is “Adequate” Coverage in the Context of E&L Studies?

Broad Detection:

Ensure that potential extractables are detected, especially those with toxicological significance

Use a range of Reference standards to cover the expected chemical space (+)

Proposed Quantitation Models:

Closest RT: Bracket non-targeted extractables (NTEs) with standards throughout the chromatography to ensure accurate retention time matching

Single Compound Quant: Select a compound that minimize the omission of relevant extractables from analytical evaluation thresholds (AET)

Similar Chemistry: Define criteria for selecting surrogate standards that are “similar” enough to the target compounds

Ionization Modeling: Modify ionization to apply effectively to the E&L space, ensuring that ionization efficiency is accounted for across different compounds

Toxicological Considerations:

Coverage should be sufficient to identify compounds with low tolerable intake (TI), which may require more stringent quantitation limits

Prior knowledge of extractables can help define the necessary coverage to meet safety and regulatory requirements

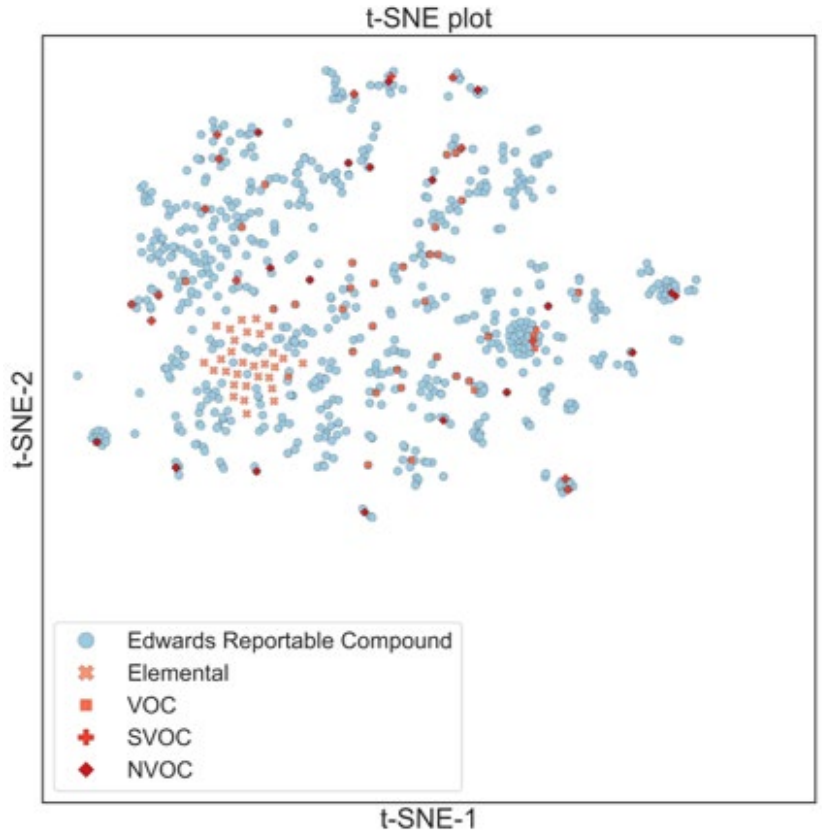
Instrument and Method Variability:

Account for variability in instrument performance and method sensitivity to ensure consistent and reliable quantitation across different runs and instruments

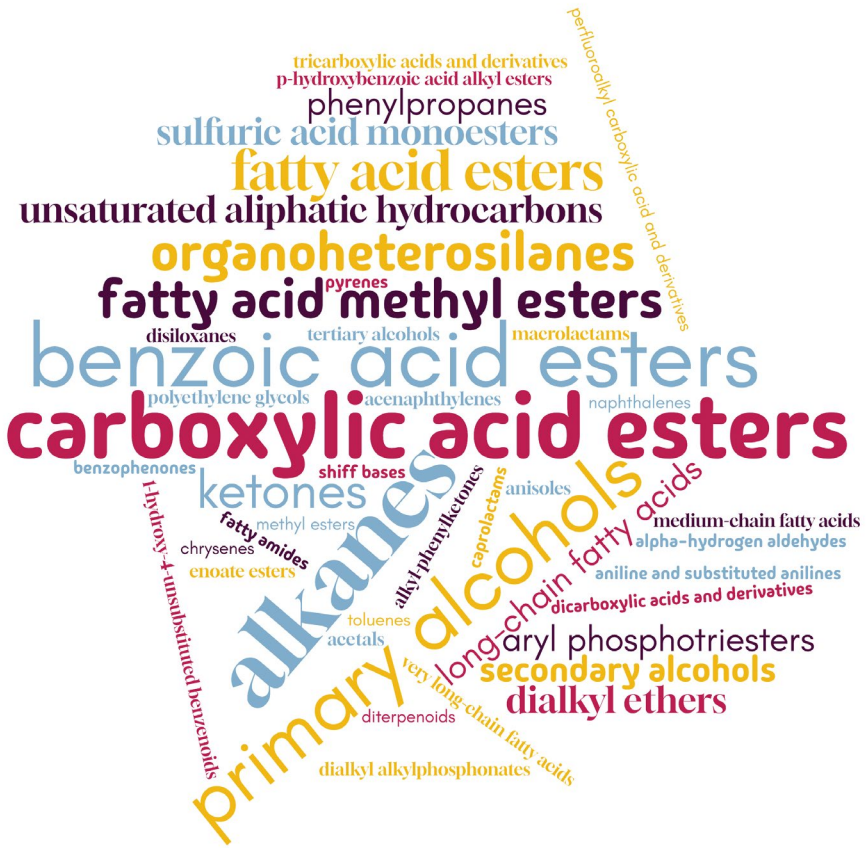
Kruve et al. Analytical Chemistry **2024** *96* (41), 16215-16226 DOI: 10.1021/acs.analchem.4c02902

Proposed Visual Representations of Chemical Coverage

t-SNE Plots from SMILES/InChI Codes



Ontological Taxonomy Using InChI Codes



Cihan Sorkun, M., Mullaj, D., Koelman, J., & Er, S. (2022). ChemPlot, a Python Library for Chemical Space Visualization. Chemistry–Methods, 2(7), e202200005].

Djoumbou Feunang, Y., Eisner, R., Knox, C. et al. ClassyFire: automated chemical classification with a comprehensive, computable taxonomy. J Cheminform 8, 61 (2016). https://doi.org/10.1186/s13321-016-0174-y

Acknowledgment:

- Michael Rush
- David Ricker
- Nuwan Kothalawala
- Cary Watterson
- Jiao Chen
- Neil Brasfield
- Vihara Wijekularatne

Edwards

Helping Patients is Our Life's Work, and

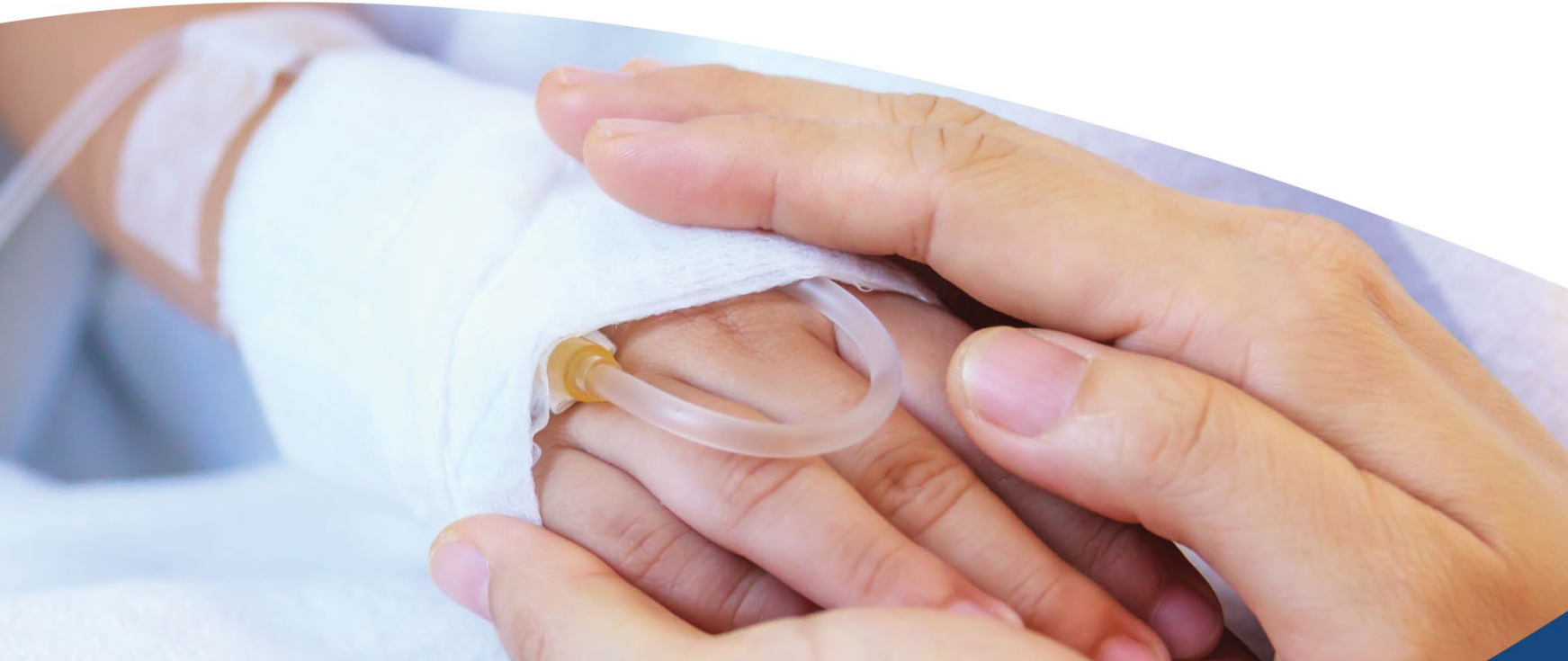
life is now

Nelson Labs®
A Sotera Health company

The use of the CLAP list: Coverage Maps, Databases and Beyond

DR PIET CHRISTIAENS, NELSON LABS

FDA Workshop (ASCA), November 06, 2024



Coverage

What is Generally Accepted as Minimum “Coverage” – Per Technique

Coverage per Technique

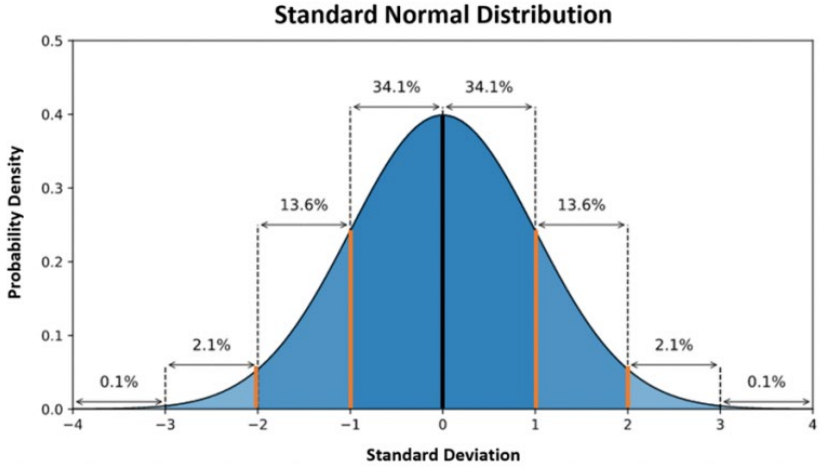
AET: The Practice in Non-Targeted Analysis

***STEP 1*: Use mean response of the population**

ISO 10993-18: "ideal situation is when mean response factor is 1"

$$AET = DBT \times \frac{A}{BC}$$

For **Targeted** Analysis with
Calibration Curves: No Uncertainty



AET (with mean RF=1, no UF) would be 50% Not Underestimated/ Protective

Nelson Labs
A Sotera Health company

AET: The Practice in Non-Targeted Analysis

***STEP 2*: Correct the mean response with UF to account for variation in Responses**

$$AET = \frac{DBT \times \frac{A}{BC}}{\boldsymbol{UF}}$$

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

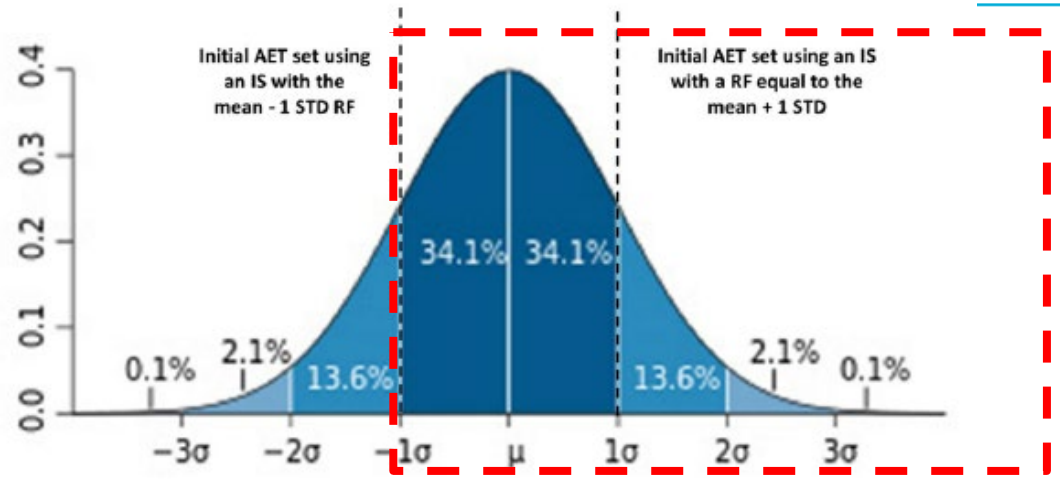
How protective is the UF corrected AET ?

$$\frac{\boldsymbol{mean}}{\boldsymbol{UF}} = \frac{\mathrm{mean}}{\frac{1}{1-\mathrm{RSD}}} = \frac{\mathrm{mean}}{\frac{1}{\left(1-\frac{\mathrm{SD}}{\mathrm{mean}}\right)}} = \mathrm{mean}\left(1-\frac{\mathrm{SD}}{\mathrm{mean}}\right) = \boldsymbol{mean - SD}$$

$$\frac{\mathrm{mean}}{\mathrm{UF}} = mean - SD$$

Nelson Labs
A Sotera Health company

Coverage per Technique: The 84% rule



84.1%

Assumes a Normal RRF distribution

- **Mean – 1xSD** offers 50% (mean) + 34.1% (1xSD) = 84.1% protectiveness,
- 84.1% of the population will have an RRF > mean-1xSD
- **currently accepted use of the uncertainty factor to correct the AET** (**mean/UF**) downwards offers **84.1% protectiveness – per Technique**

Hence, the *meaning that per technique 84.1% of the population will be detected above AET.*

However, currently there is no guidance for coverage of an overall orthogonal and complementary methodology

The 84% Rule in action: example for SVOC CLAP-Compounds

Parameter	CLAP *Nelson RRF results*	NELSON *RRF Database*
# of Compounds	84	2724
Mean RRF	0.64	0.57
Median RRF	0.64	0.54
Standard Deviation	0.32	0.32
% RSD	51	56
UF	2.0	2.27
Mean RRF/UF	**0.32**	**0.25**
Coverage using mean RRF/UF	**81%**	**84.1%**

- Only GC/MS (SVOC)
- Mean RRF for NL-Database is about 10% lower than CLAP-List RRF (Contribution of Low LogP Compounds?)
- UF for NL database is 10% Higher than UF derived from RRF of CLAP-Compounds
- Difference: Physico-Chemical Properties in distribution? See later

*Currently, there is **no consensus** on when to decide that a compound is not amenable to a certain analytical method and can be discarded from the population.*

This is impacting the statistics & coverage calculations substantially

Nelson Labs
A Sotera Health company

CLAP: Nelson Labs RRFs per technique

- Record RRF of CLAP compounds for GC/MS and LC/MS (APCI + ESI)
  - 90 of the 106 Compounds were in the Nelson Labs DB already, however, not always with full data
- Data for all 106 CLAP compounds (2 compounds are qualitatively detected)

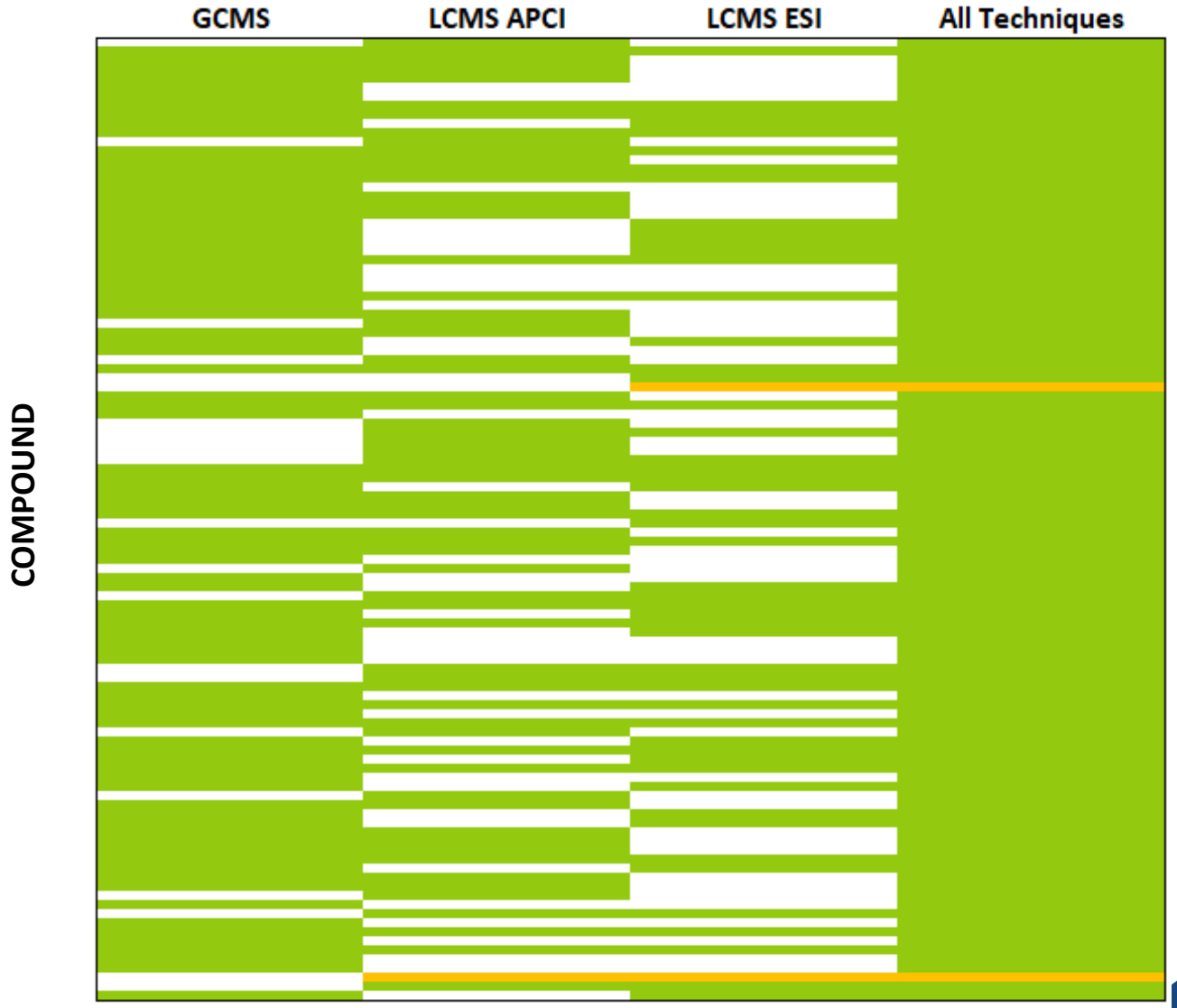
Parameter	SVOC	NVOC ESI±	NVOC APCI±
# of Compounds	84	50	63
Mean RRF	0.64	0.73	0.63
Median RRF	0.63	0.32	0.50
Standard Deviation	0.32	0.84	0.46
% RSD	50	115	74
UF	2.0	10	3.8
Mean RRF/UF	**0.32**	**0.073**	**0.16**
Incremental Coverage	**81%**	**90%**	**100%**

Nelson Labs
A Sotera Health company

Coverage map Nelson Labs database

- Nelson Labs data for CLAP Compounds
- Green: $RRF_{technique} > 0.1$
- **GCMS, LCMS-APCI± and LCMS-ESI±**
- Quantitative Coverage for 104/106 compounds
- 2 Compounds “Qualitatively” Detected *(no RRF, because of purity/solubility issues: technical grade)*

TOTAL COVERAGE = 100%

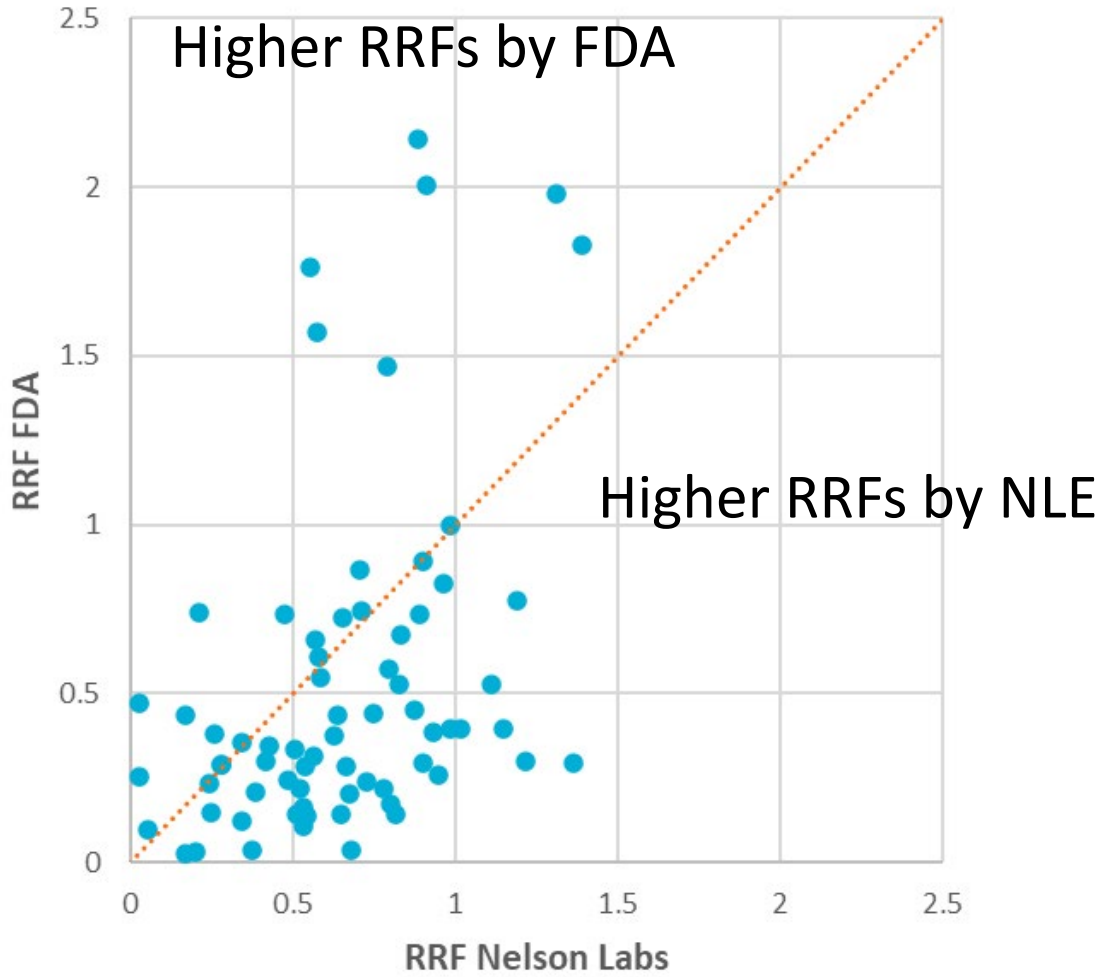


Nelson Labs
A Sotera Health company

Obervation: RRFs are Protocol Dependent: example GC/MS

Comparison GCMS RRFs FDA vs Nelson Labs

- Substantial differences
- Possible causes:
  - Injection technique
  - Inlet temperature
  - Injection volume
  - Type of liner used
  - Mass range
  - Model of MS detector



CONCLUSION: RRF VALUES (AND ASSOCIATED UF-VALUES) ARE LAB SPECIFIC!!

Nelson Labs
A Sotera Health company

Frequency of Occurrence (Reporting) of CLAP Compounds

NELSON LABS: EVALUATION OF FREQUENCY OF REPORTING - LAST 5 YEARS

370 Compounds represent 80% of all reported compounds (confirmed identity)

- **6 CLAP-Compounds in TOP 10 frequency of reporting**
- **17 CLAP-Compounds in TOP 25 frequency of reporting**
- **23 CLAP-Compounds in TOP 100 frequency of reporting**
- **61 CLAP-Compounds NOT in the top 370-list**

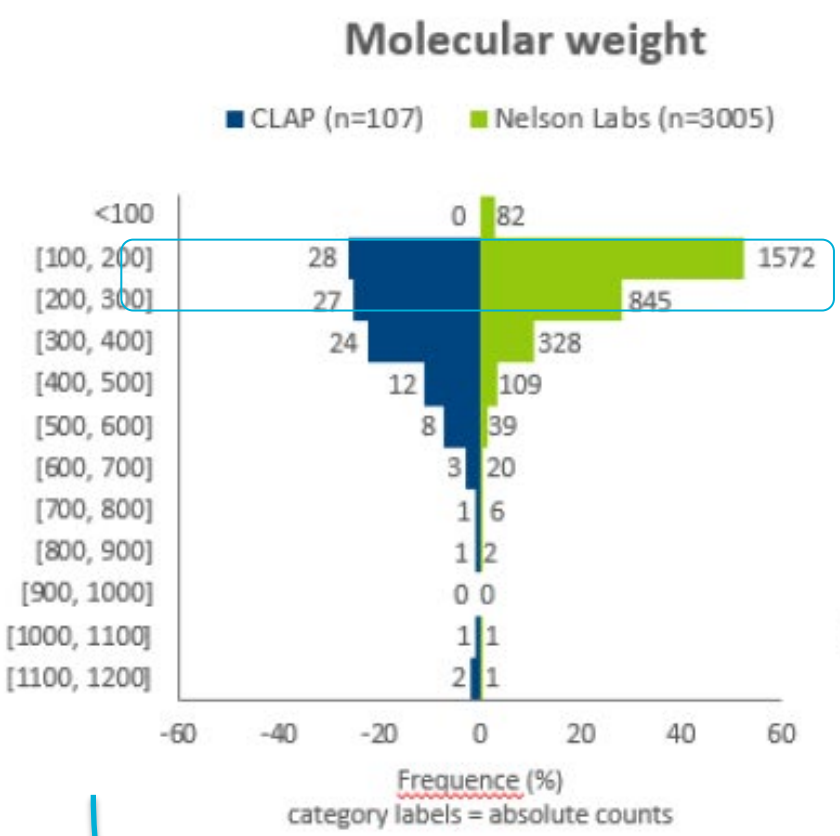
While the CLAP Compounds are relevant because (1) it are extractables, (2) their pchem properties are broad, they may not always represent the most frequently occurring compounds

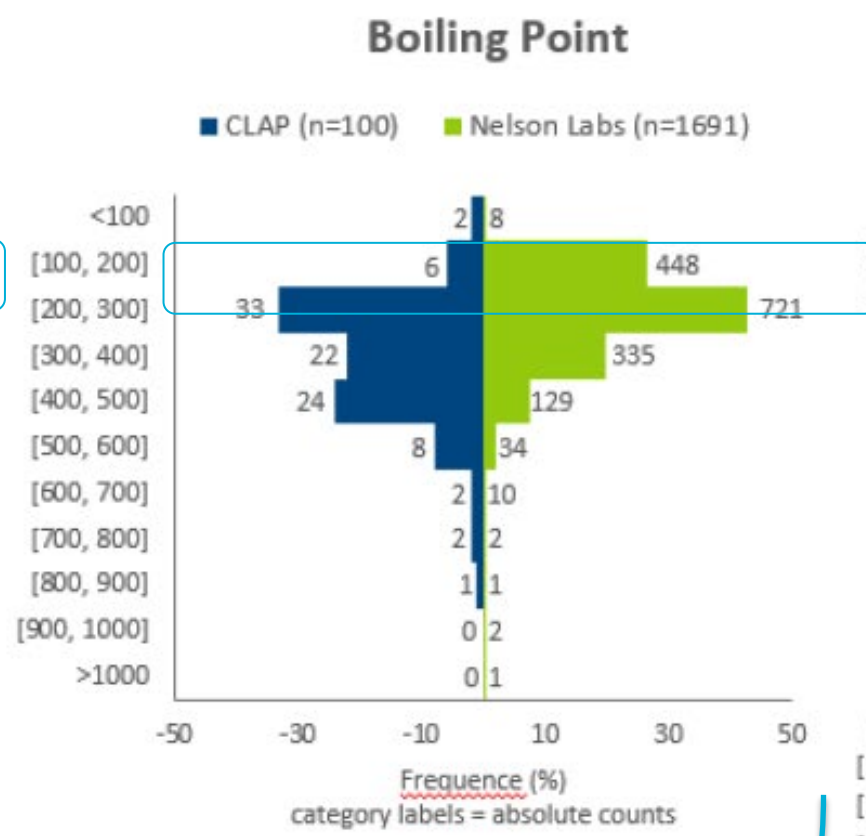
PART 1

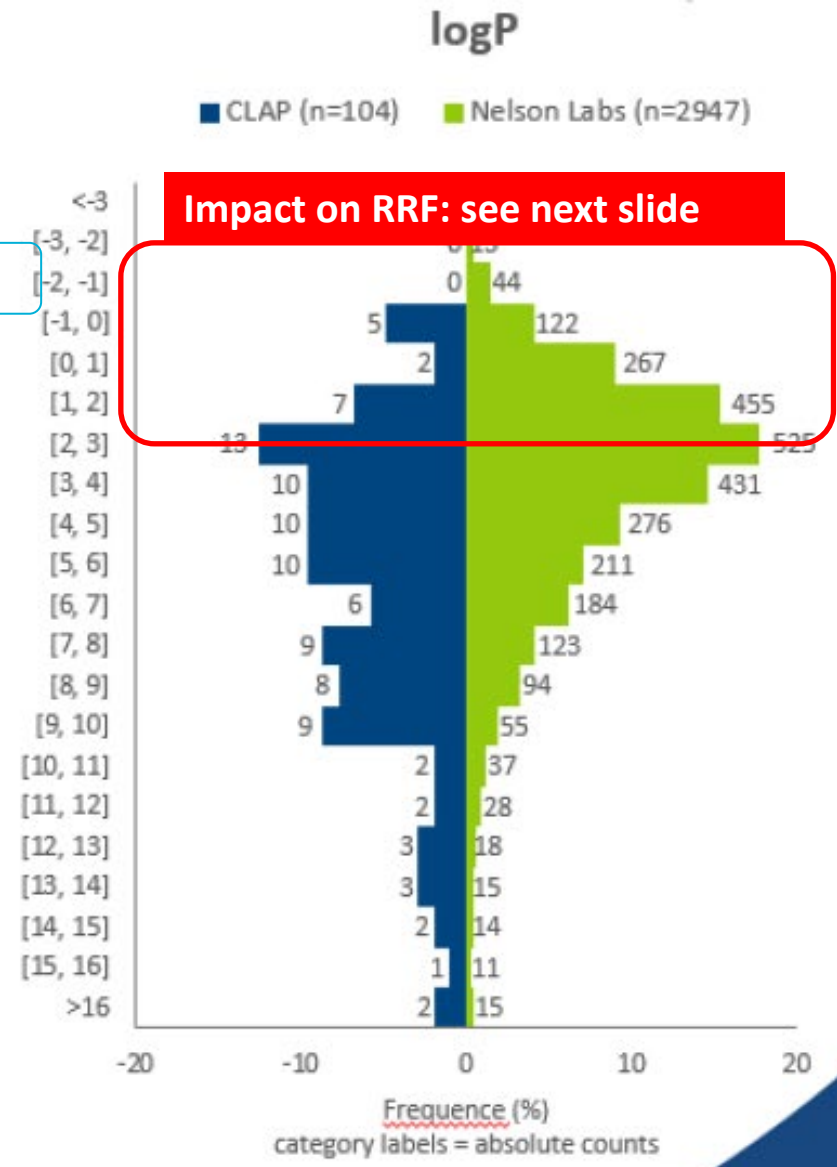
FDA's CLAP-List: Comparing Physico-Chemical Population Characteristics with Nelson Labs Data Base

Nelson Labs
A Sotera Health company

Broad range of physicochemical properties/ CLAP vs NL DATABASE





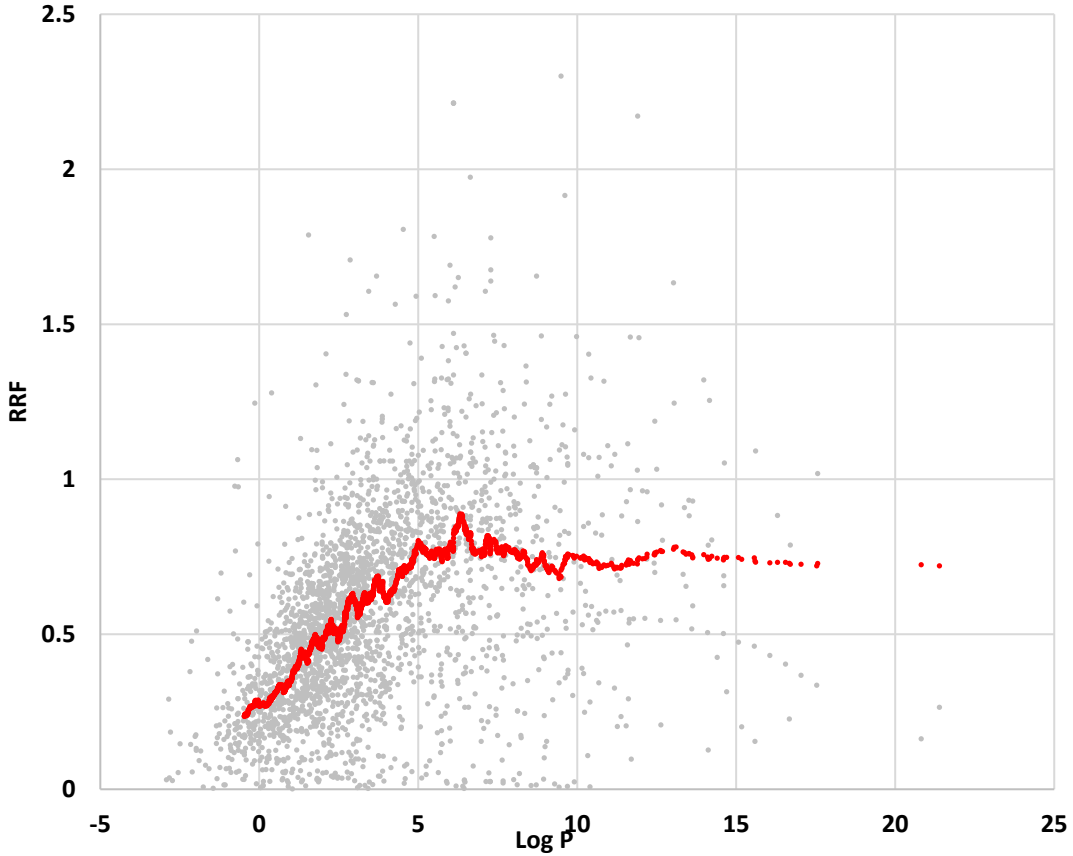


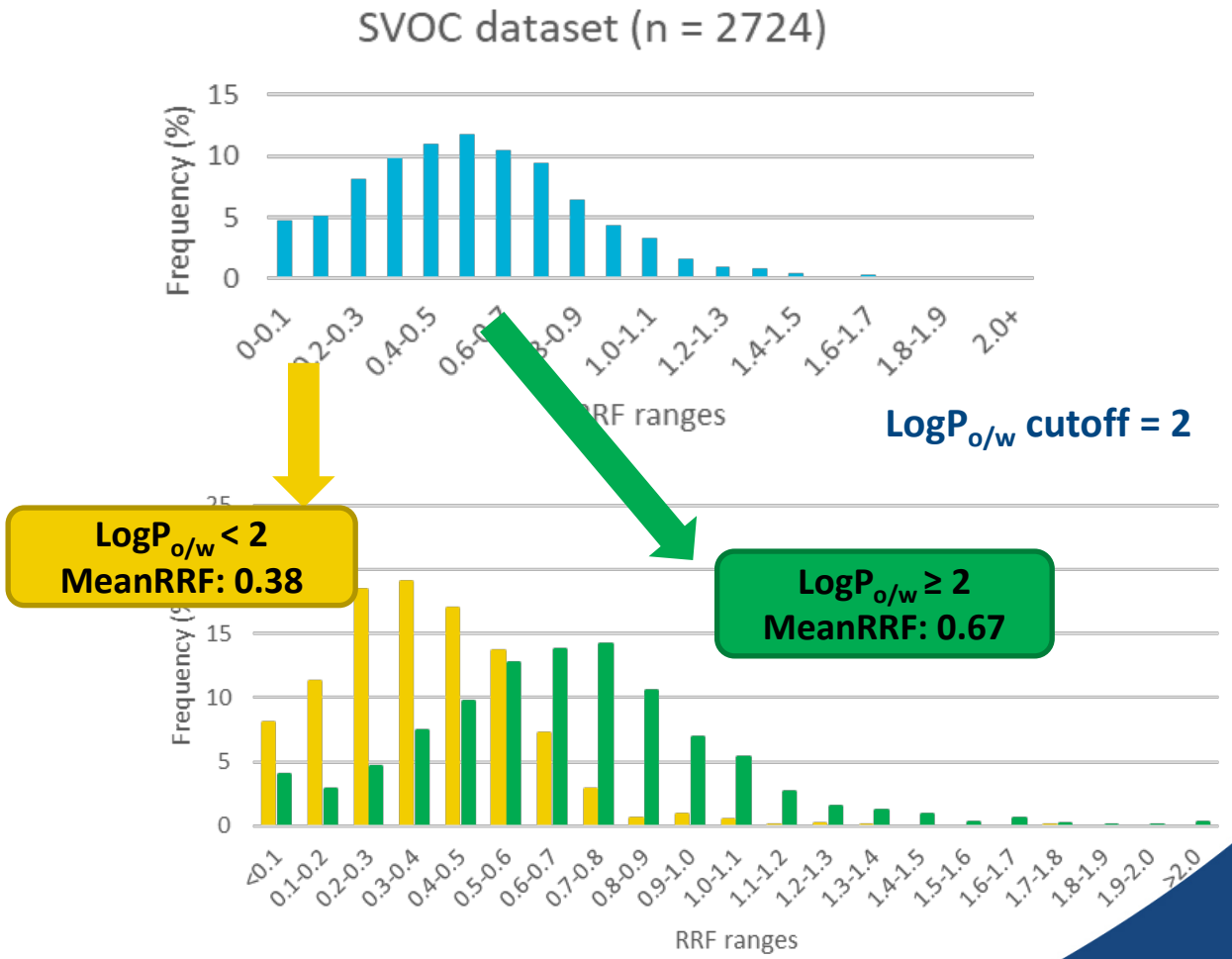
NELSON LABS DB is also for VOC-Compounds
(low MW, low BP)

Nelson Labs
A Sotera Health company

RRF versus $LogP_{o/w}$

- **Clear relationship** between **RRF and $LogP_{o/w}$**
- Molecules with a **high hydrophilicity** (low $LogP_{o/w}$ value): associated with **poor responders** in GCMS





Nelson Labs
A Sotera Health company

PART 2

The Use of the **CLAP List** to support **Identifications** in NTA-Procedures, based on Mass Spectral Matching (GC/MS)

Nelson Labs
A Sotera Health company

CLAP List and Identifications

Indirect benefit:

FDA Draft Guidance 2024

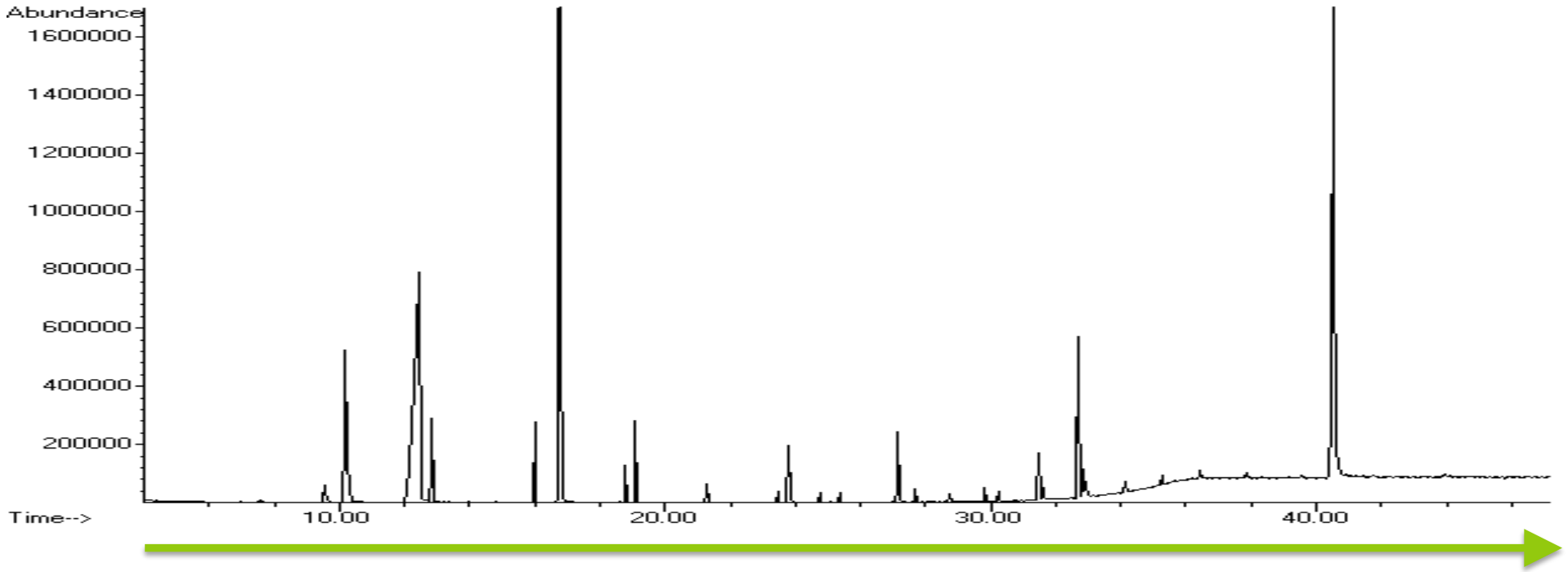
Supporting information[96, 97] for identification can include one or more of the following:

- molecular formula generation (based on accurate mass) and/or confirmation (with an authentic reference standard of the candidate structure or a close structural analog)
- RT or retention index matching
- isomer assignment based on interpretation of data
- spectral interpretation (e.g., for MS spectra)
- fragmentation spectra interpretation based on data (e.g., for EI-based MS spectra)
- MS^n elucidation of fragments

Could be the basis to **establish acceptance/rejection criteria** for **Retention Index Confirmation**.
However, more data would be needed than the current set of CLAP-standards

Retention Time as identification property

So Far, **Retention time** is merely a **'byproduct'** of Chemical Analysis



Retention Time

REASON: Retention Time depends on Scientific Protocol
(GC oven programme, stationary phase, carrier gas viscosity/velocity, column pressure...)

Nelson Labs
A Sotera Health company

Retention Index as (more) universal retention property for GCMS

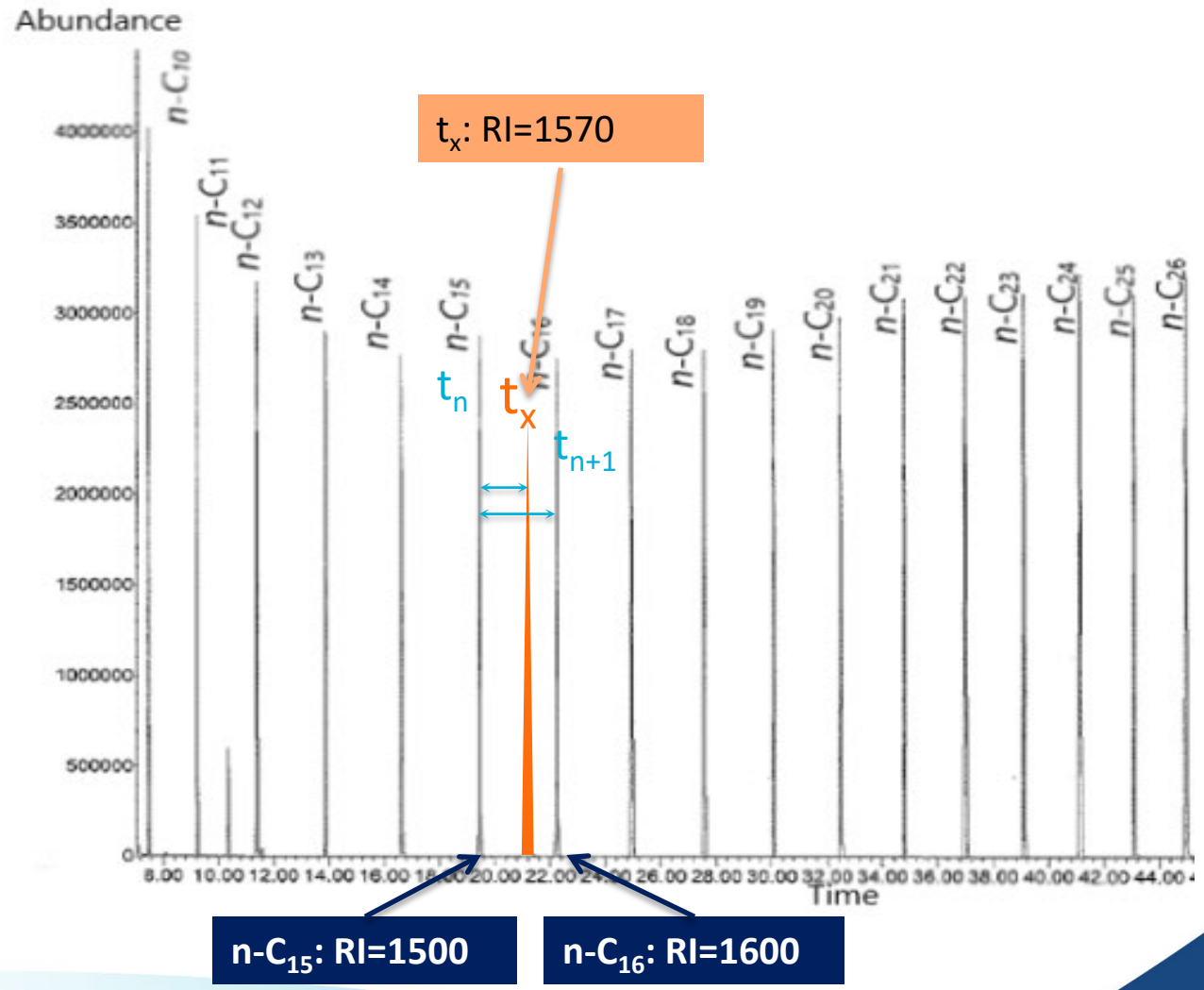
Kovàts Retention Index

= RT of compound relative to RT of linear alkanes

$$I_x = 100n + \frac{100(t_x - t_n)}{(t_{n+1} - t_n)}$$

Independent of GC oven programme

Still dependent on stationary phase



Source of Reference RI-Values: NIST23

1. **Experimental RI-values**:
   - most accurate
   - not for all compounds

2. Estimated RI-values: least accurate
   (not taken into consideration)

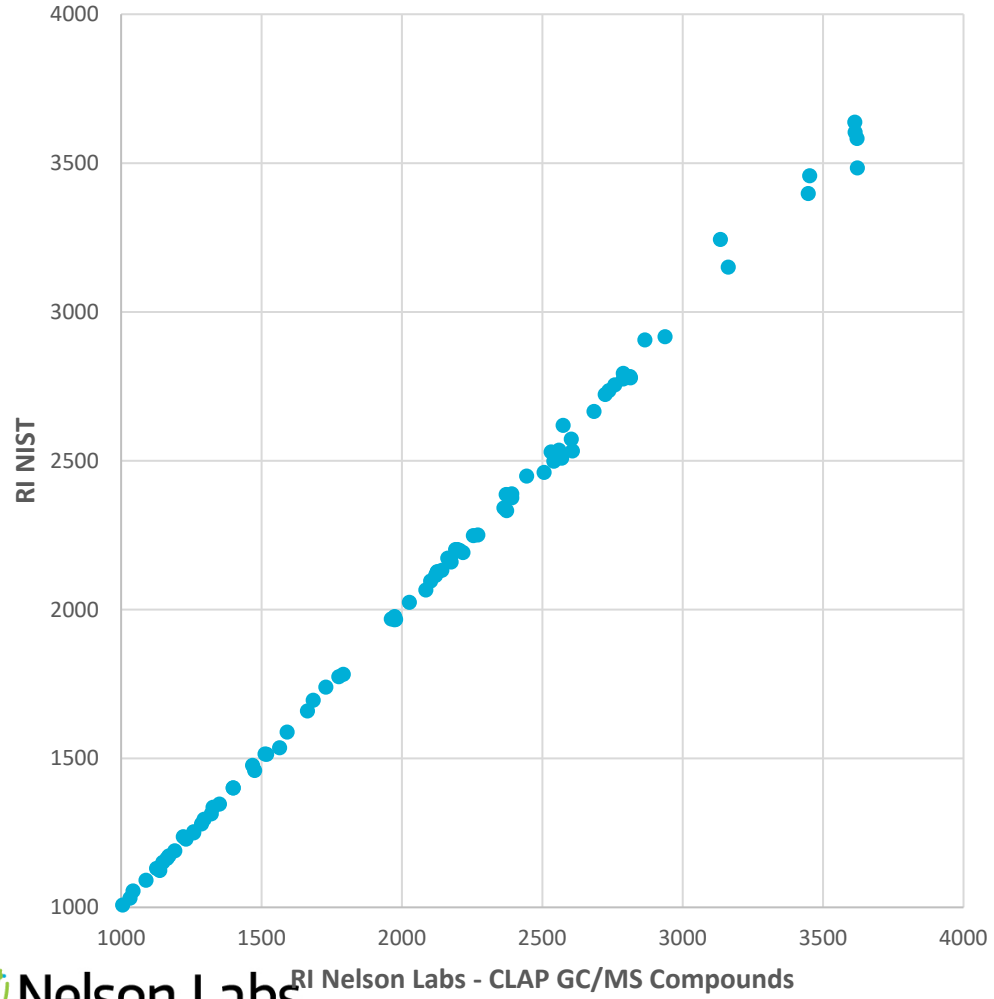
3. **AI-RI-values**:
   - Accurate
   - all compounds!

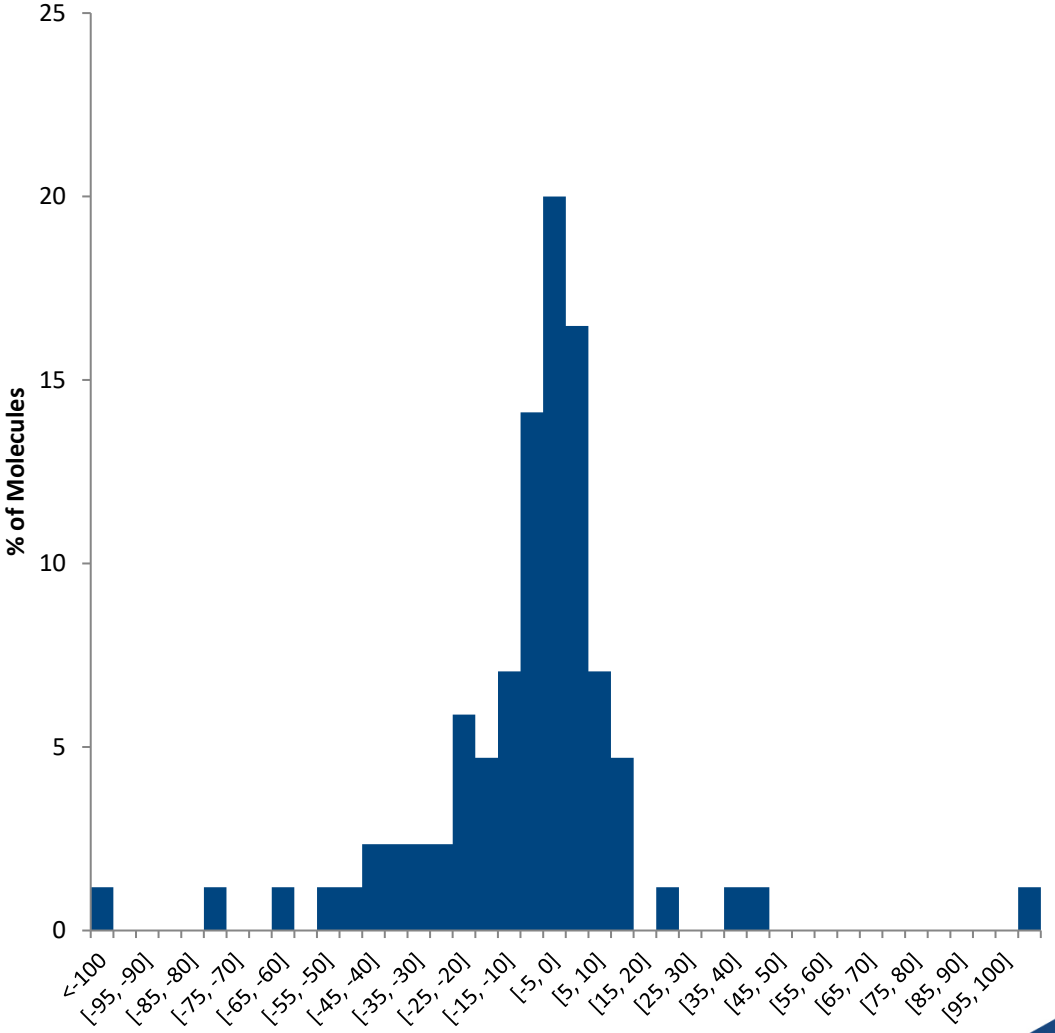
Stationary phase categories

Semi-standard non polar	Dimethyl silicone with 5% phenyl
Standard non polar	Dimethyl silicone
Polar	Polyethylene glycol

RI correlation Nelson Labs versus NIST – CLAP CGC/MS ompounds

- **Based on CLAP Compound list**
- NIST RI = **Preferably Experimental RI** (semi-standard non-polar), otherwise **AI RI**





Nelson Labs
A Sotera Health company

Distribution RI errors Nelson Labs versus NIST

GC/MS CLAP COMPOUNDS:

80% RI-Supported
94% RI-Supported / Accepted
6% False Negatives

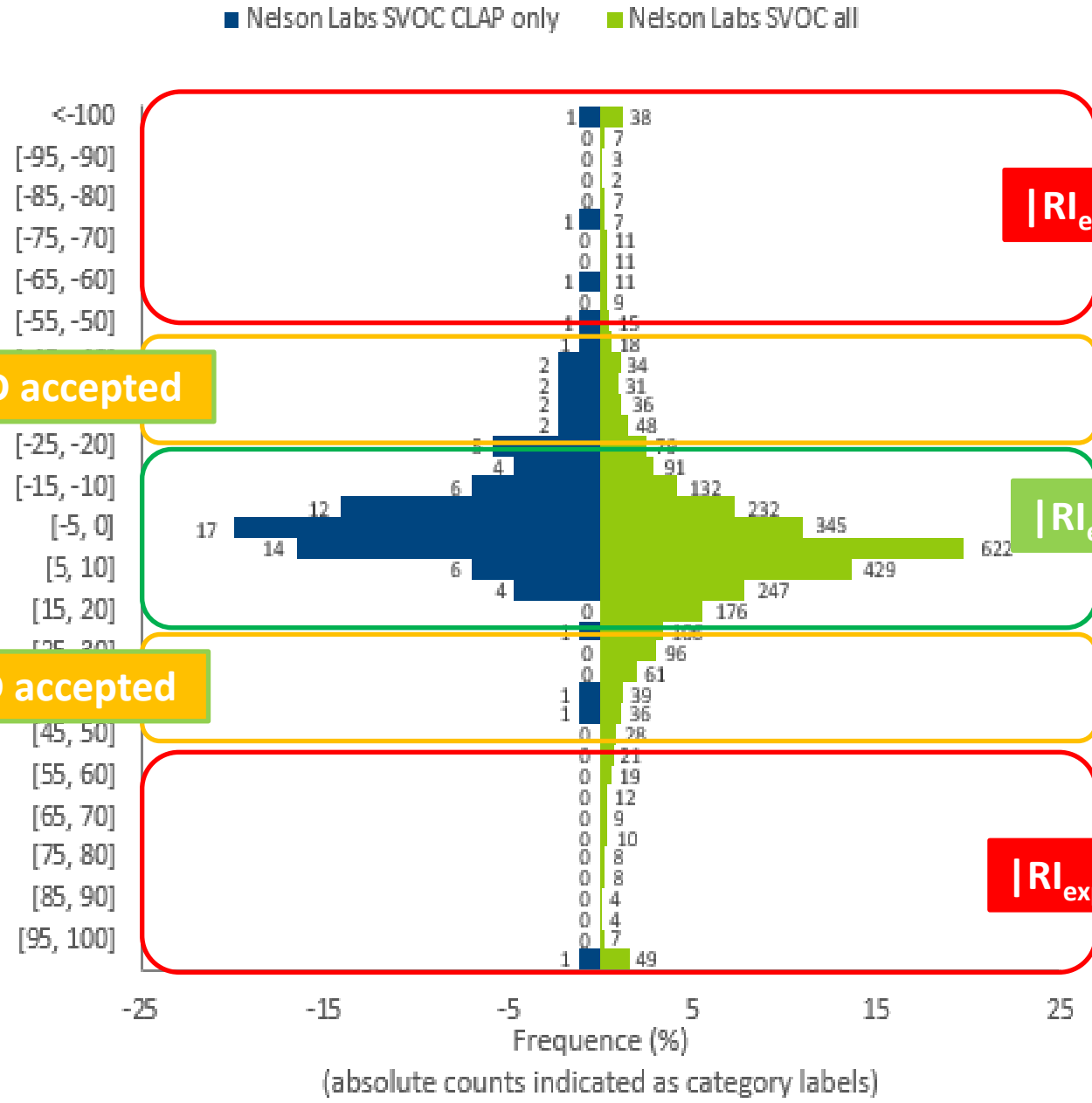
$|RI_{exp} - RI_{NIST}| > 50$ ID rejected

$20 \leq |RI_{exp} - RI_{NIST}| \leq 50$: ID accepted

$|RI_{exp} - RI_{NIST}| < 20$: ID SUPPORTED

$20 \leq |RI_{exp} - RI_{NIST}| \leq 50$: ID accepted

$|RI_{exp} - RI_{NIST}| > 50$ ID rejected



NELSON LABS DATABASE:

80% RI-Supported (ΔRI<20)
94% RI-Supported / Accepted (20≤ΔRI≤50)
6% False Negatives (RI>50)

Nelson Labs
A Sotera Health company

Questions?

Dr. Piet Christiaens, Scientific Director - Nelson Labs Europe

e-mail: pchristiaens@nelsonlabs.com

Nelson Labs
A Sotera Health company

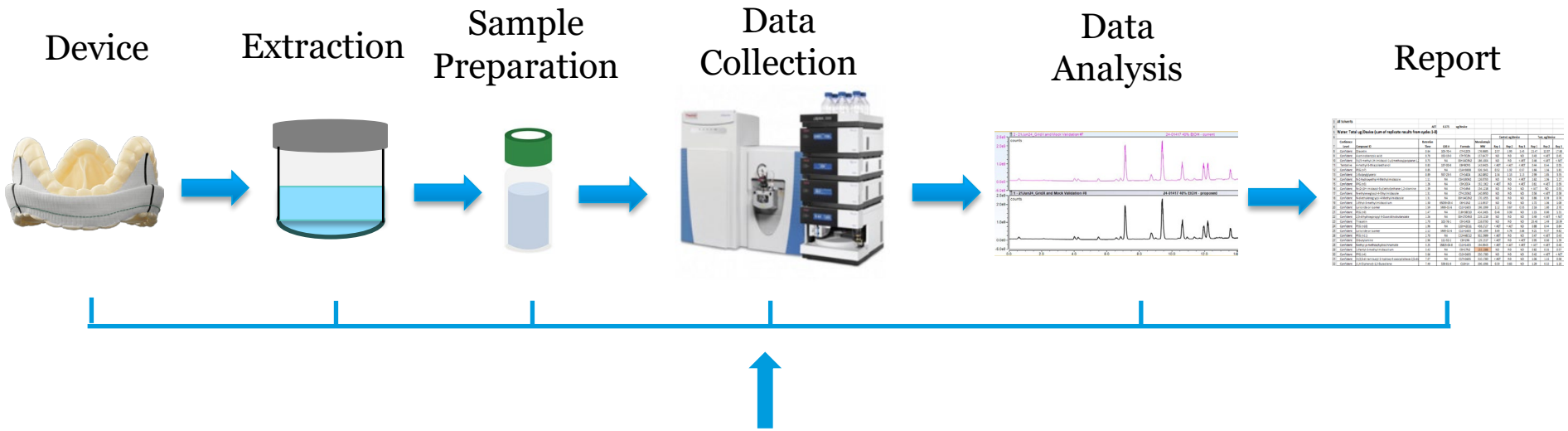
2024 ASCA

Developing a Coverage Map for the Analysis of Extractables for Abbott Devices and the Analytical Testing Strategy

Siyi Zhang PhD
Abbott Laboratories, Global Biocompatibility

BACKGROUND

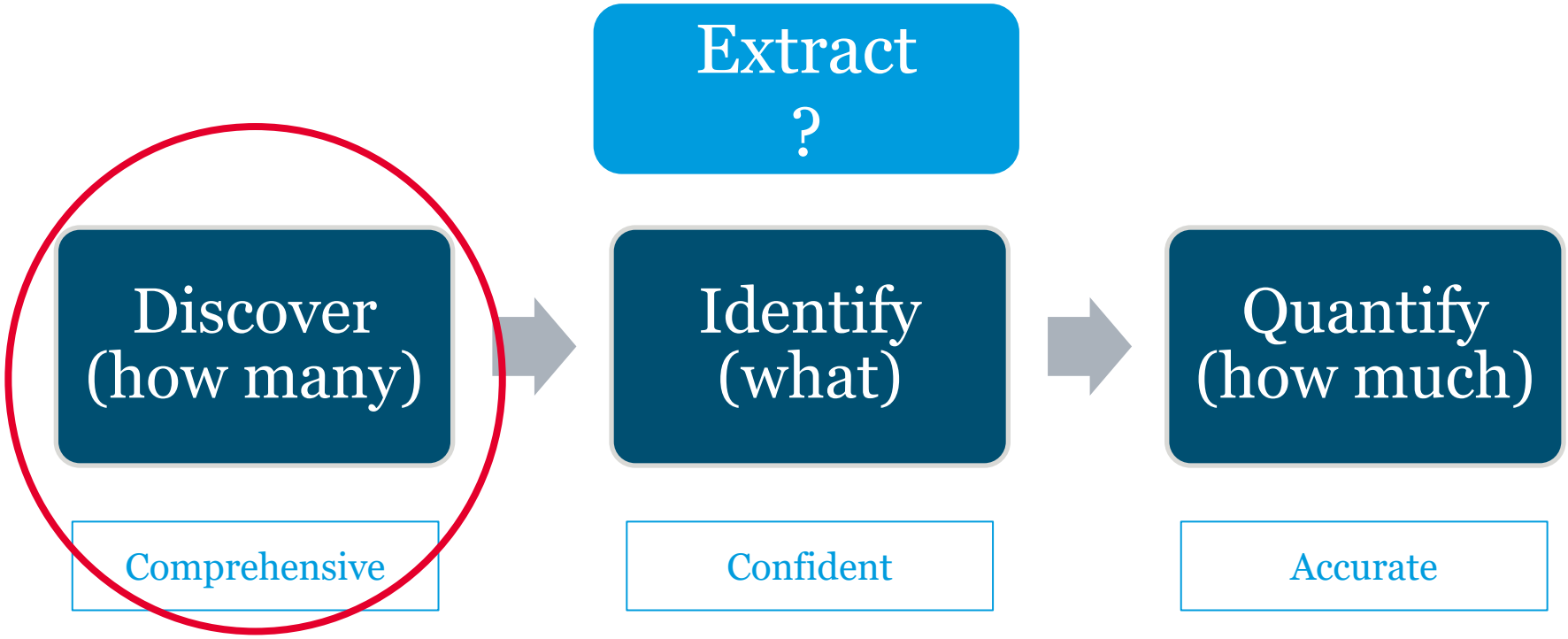
Extractables and Leachables Analysis



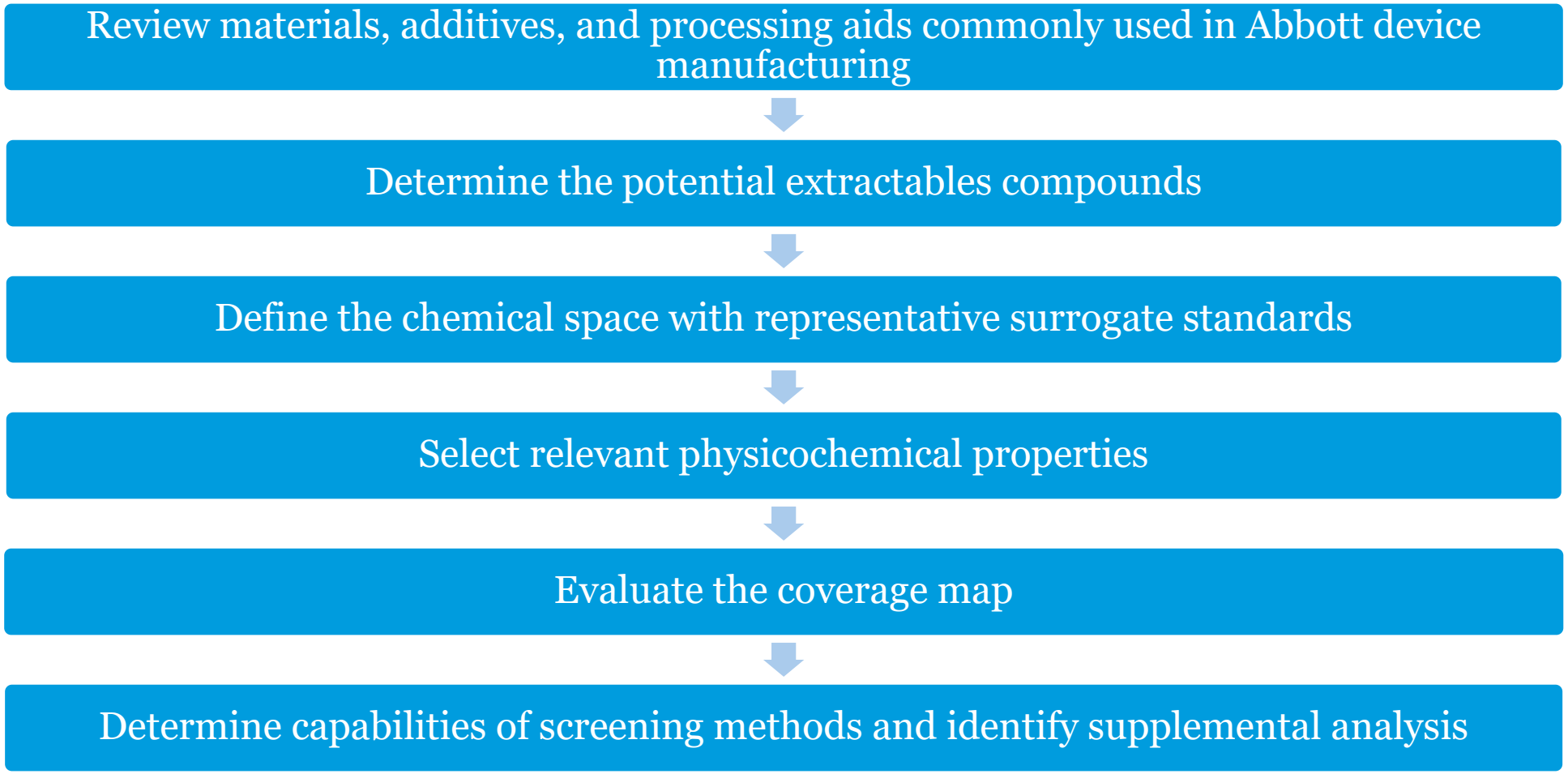
Information Gathering

Intended use and indicated population
Materials of composition
Manufacturing process and processing aids
Sterilization, packaging
Device use conditions (preconditioning/deployment)

Extractables and Leachables Analysis



Process to Develop a Extractables Coverage Map



Abbott Device Materials, Additives and Processing Aids

Polymeric Materials
Polyurethane
Silicone
Polyester
Polyamide/Pebax

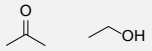
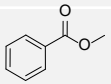
Metal/Alloy Materials
Nitinol
Titanium
Stainless Steel

Additives
Plasticizer
Antioxidant
UV Stabilizer
Colorant

Biological Materials
Tissue
Gelatin

Processing and Sterilization
Lubricant and surfactant
Cleaning agent
Passivation and electropolishing
EO and chemical Sterilization

Select Representative Surrogate Standards to Define the Chemical Space

Chemical Class	Structure	Representative Surrogate Standards	Potential Source
Hydrocarbon		Dodecane, Tetradecane, Octadecane	Residual processing aids such as petroleum oil Residual oligomers from polyethylene materials
		Cyclohexane, Heptane	Residual processing aids such as solvents
Aliphatic ester		Methyl stearate, Hexadecanoic acid, methyl ester	Residual processing aids
		Bis(2-ethylhexyl) adipate, Acetyl tributyl citrate	Plasticizer for various polymers
Aliphatic amide/amine		Oleamide, Erucamide	Residual processing aids such as slip agents
		Caprolactam, Laurolactam	Residual oligomers from polyamide materials
		1-(2-Hydroxyethyl)-2,2,6,6-tertramethylpiperidin-4-ol	Hindered amine light stabilizers
Fatty acids		Stearic acid, Palmitic acid	Residual processing aids such as surfactants Residuals from animal tissue materials
Ether		Polyethylene glycol, Polypropylene glycol	Residual processing aids such as surfactants
		Polytetrahydrofuran	Residual oligomers from polyurethane and Pebax materials
Alcohol and ketone		6-Undecanone, 1-Hexanol, 2-ethyl-	Residual processing aids such as solvents
Siloxane		Siloxane D4, D5, L5	Residual oligomers from silicone materials
Phenol		Butylated hydroxytoluene, Irganox 1010, Irganox 1076	Antioxidants for various polymers
		Bisphenol A, Bisphenol A diglycidyl ether	Plasticizer for various polymers Residual oligomers from epoxy materials
Aromatic esters		Bis(2-ethylhexyl) phthalate, Trioctyl trimellitate	Plasticizer for various polymers
		Dimethyl terephthalate, Bis(2-hydroxyethyl)terephthalate	Residual oligomers from PET materials
Polyaromatic hydrocarbon (PAH)		Naphthalene, Phenanthrene	Potential contaminants from processing aids such as petroleum oil
Aromatic amines or urethane		Methylene diphenyl diamine, Phenylurethane	Residual oligomers from polyurethane materials
		1,4-Bis(ethylamino)-9,10-anthraquinone	Colorants for various polymers
Aromatic heterocycles		1,2,3-benzotriazole, 2-Mercaptobenzothiazole	UV absorber
Aldehyde		Formaldehyde, Glutaraldehyde	Residual processing aids such as tissue fixation and sterilization
Sulfonic acid/sulfate		Sodium decyl sulfate, Sodium 4-dodecylbenzenesulfonate	Residual processing aids such as surfactants
Phosphite/Phosphate		Irgafos 168	Antioxidant for various polymers
		Dibutyl phosphate, Dibenzyl phophsate	Residual processing aids such as surfactants

PROCESS

Select Relevant Physicochemical Properties

Physical Properties

Melting Point
Boiling Point
Viscosity
Density
Polarity (Log P)
Vapor Pressure
Refractive Index

Chemical Properties

Acidity and Basicity (Pka)
Flammability
Reactivity

Molecular Attributes

Molecular Weight
Ring plus double bonds

Correlates to Analytical Method Separation and Detection

Most Relevant Properties

Molecular Weight
Boiling Point
Log P

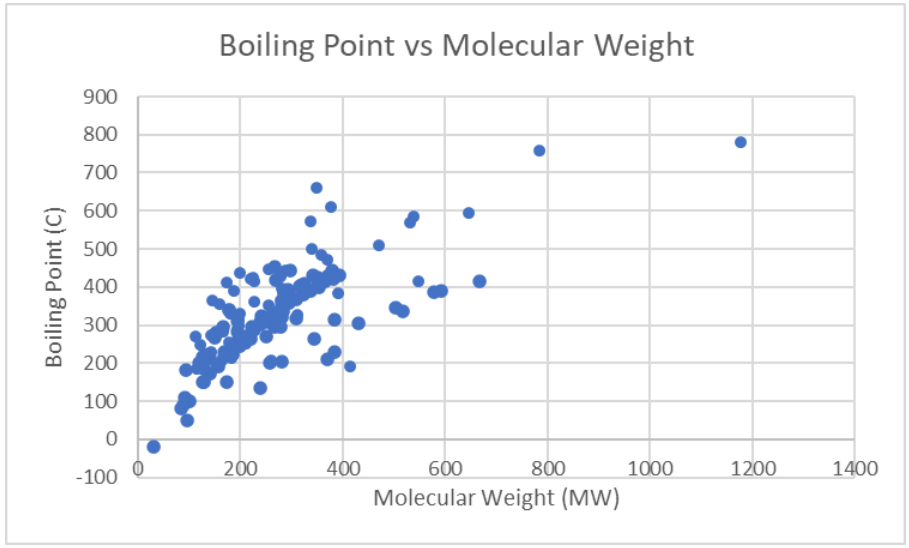
Other Relevant Properties

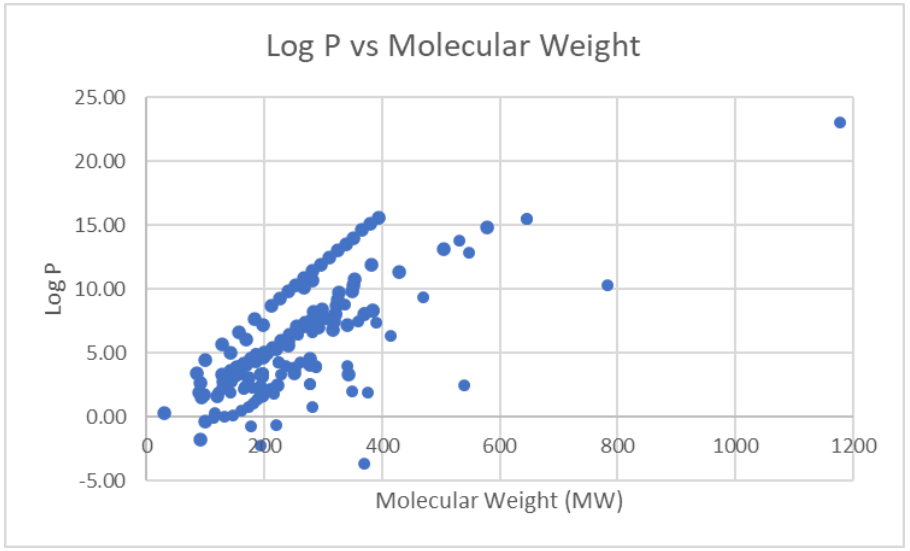
Vapor pressure
Pka

Developing Coverage Map

Surrogate Standard	CAS	Compound Class	Formula	MW	B.P. (°C)	LogP
Formaldehyde	50-00-0	Aldehyde	CH2O	30	-19	0.35
Glutaraldehyde	111-30-8	Aldehyde	C5H8O2	100	100	-0.34
Cyclohexane	110-82-7	Hydrocarbon	C6H12	84	81	3.39
Toluene	110-88-3	Hydrocarbon	C7H8	92	111	2.68
Hexanoic acid, methyl ester	106-70-7	Aliphatic Ester	C7H14O2	130	150	2.30
Octanoic acid, methyl ester	111-11-5	Aliphatic Ester	C9H18O2	158	193	3.40
Naphthalene	91-20-3	PAH	C10H8	128	218	3.30
Acenaphthylene	208-96-8	PAH	C12H8	152	280	3.90
Dodecane	112-40-3	Hydrocarbon	C12H26	170	216	6.10
Tetradecane	629-59-4	Hydrocarbon	C14H30	198	254	7.20
Diethyl phthalate	84-66-2	Aromatic Ester	C12H14O4	222	295	2.50
Cyclotetrasiloxane, 2,4,6,8-tetramethyl-	2370-88-9	Siloxane	C4H16O4Si4	240	135	5.54
Tetrasiloxane, 1,1,3,3,5,5,7,7-octamethyl-	1000-05-1	Siloxane	C8H26O3Si4	282	205	7.70
1-Hexanol, 2-ethyl-	104-76-7	Aliphatic Alcohol	C8H18O	130	184	2.80
Caprolactam	105-60-2	Aliphatic Amide	C6H11NO	113.2	270	-0.1
Laurolactam	947-04-6	Aliphatic Amide	C12H23NO	197.3	314.9	3.1
Tetraethylene glycol	112-60-7	Ether	C8H18O5	194.2	314	-2.23
Triethylene glycol monomethyl ether	112-35-6	Ether	C7H16O4	178.2	256	-0.70
Heptanoic acid	111-14-8	Aliphatic Acids	C7H14O2	130.2	222.0	2.4
Lauric acid	143-07-7	Aliphatic Acids	C12H24O2	200.3	330.0	4.6
Dibenzyl phosphate	1623-08-1	Phosphate	C14H15O4P	278.2	427.0	2.6
Bisphenol A bis(2,3-dihydroxypropyl) ether	5581-32-8	Phenol	C21H28O6	376.4	611.0	1.9

Developing Coverage Map





160 Compounds in the Coverage Map

Property	Range
MW	30 – 1200 amu
Boiling Point	-19 – 779 °C
Log P	-3.7 – 23

APPLICATION

Optimize the Detection of Compounds in the Coverage Map

Analytical Method Considerations

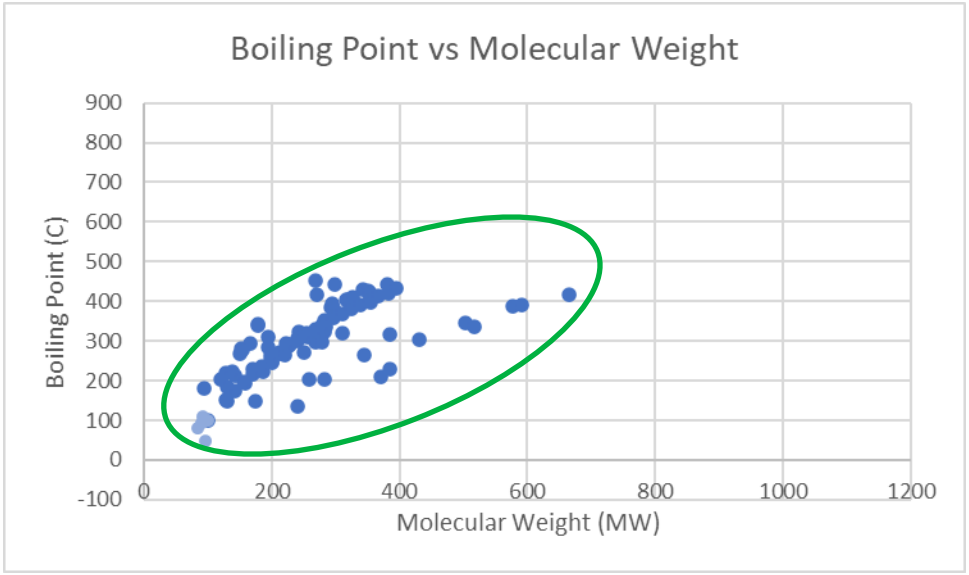
- Comprehensive
- Sensitive – Able to detect concentrations at AET
- Adequate accuracy for semi-quantification

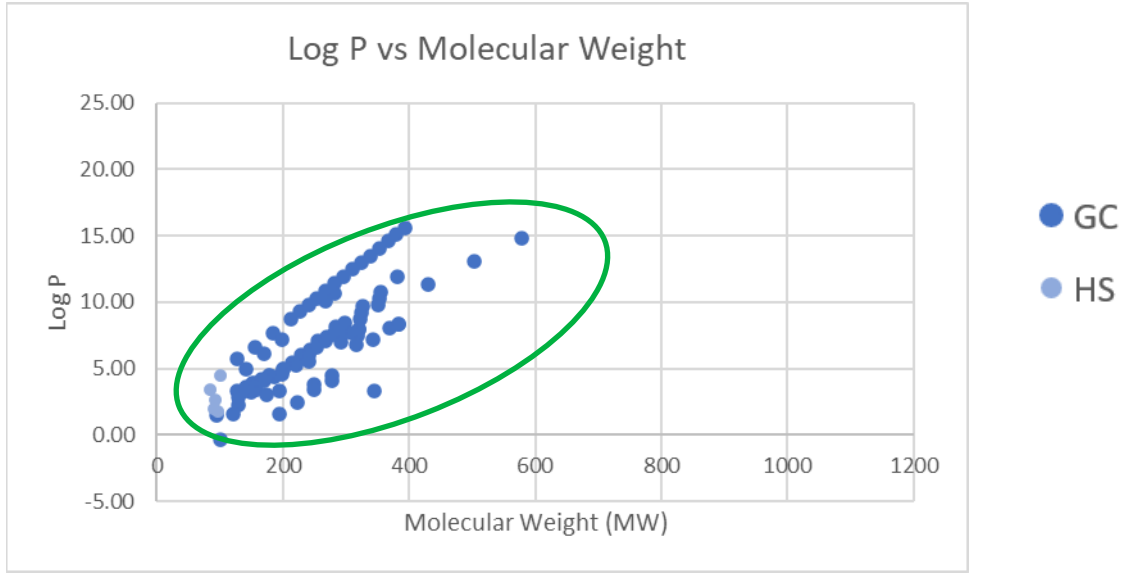
Method	Separation Mechanism	Physicochemical Property	Detection Mechanism	Suited Chemical Classes
GC Based Methods	Volatility	Boiling point	EI: Universal ionization	Siloxane, hydrocarbon, Alcohol, ether and ester, ketone, amide, phenolic antioxidant, phthalates, PAH
LC Based Methods	Polarity	Log P	ESI: functional group for protonation or deprotonation UV: chromophore	Amine and amide, ether and ester, phenolic antioxidant, phthalates, carboxylic acid, sulfate, phosphate, sulphone, thioether
ICP-MS	Targeted analysis for metal elements			

APPLICATION

Optimize the Detection of Compounds in the Coverage Map

Optimal Detection Range for GC Based Methods

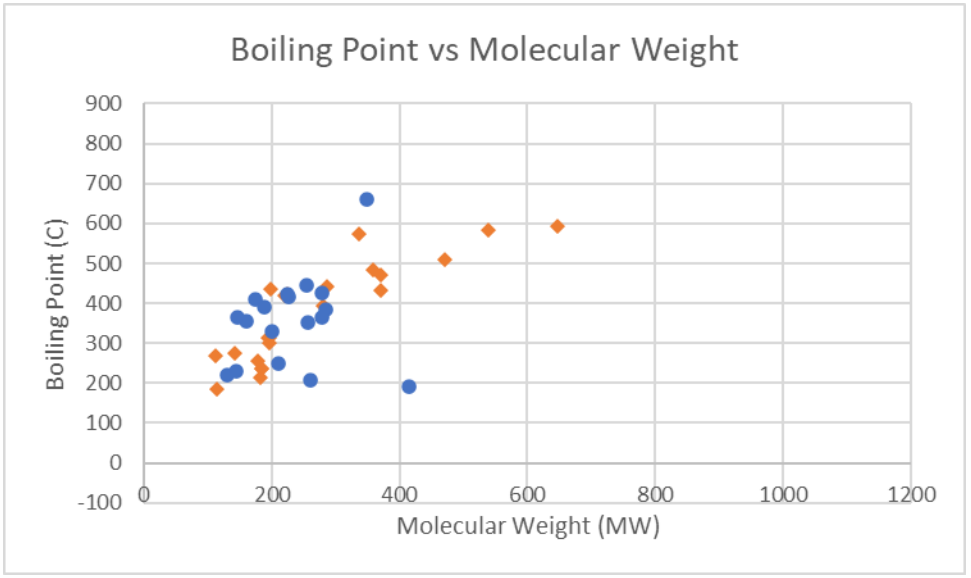


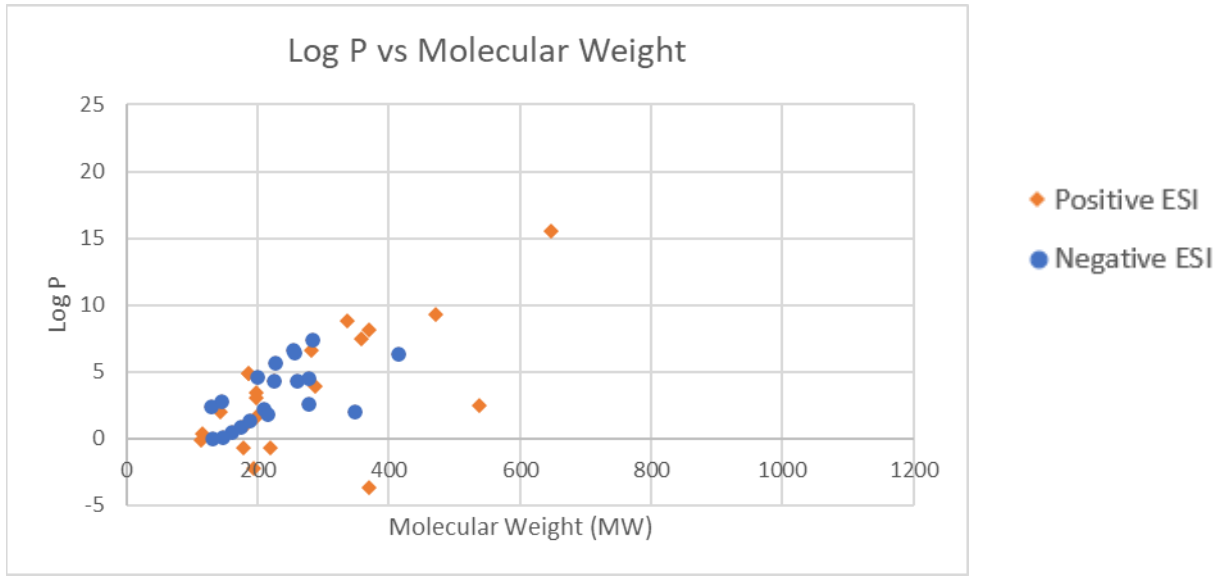


APPLICATION

Optimize the Detection of Compounds in the Coverage Map

Optimal Detection Range for LC Based Methods

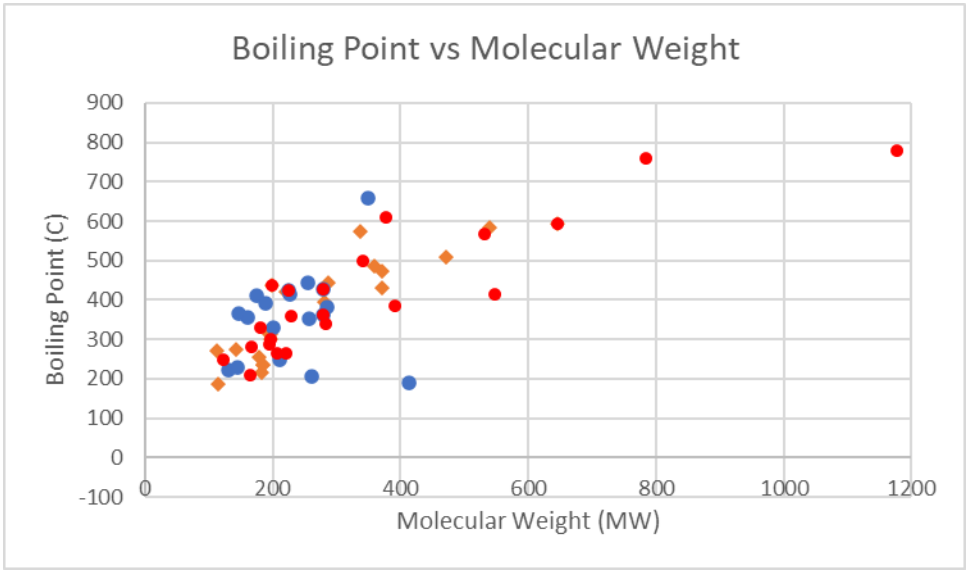


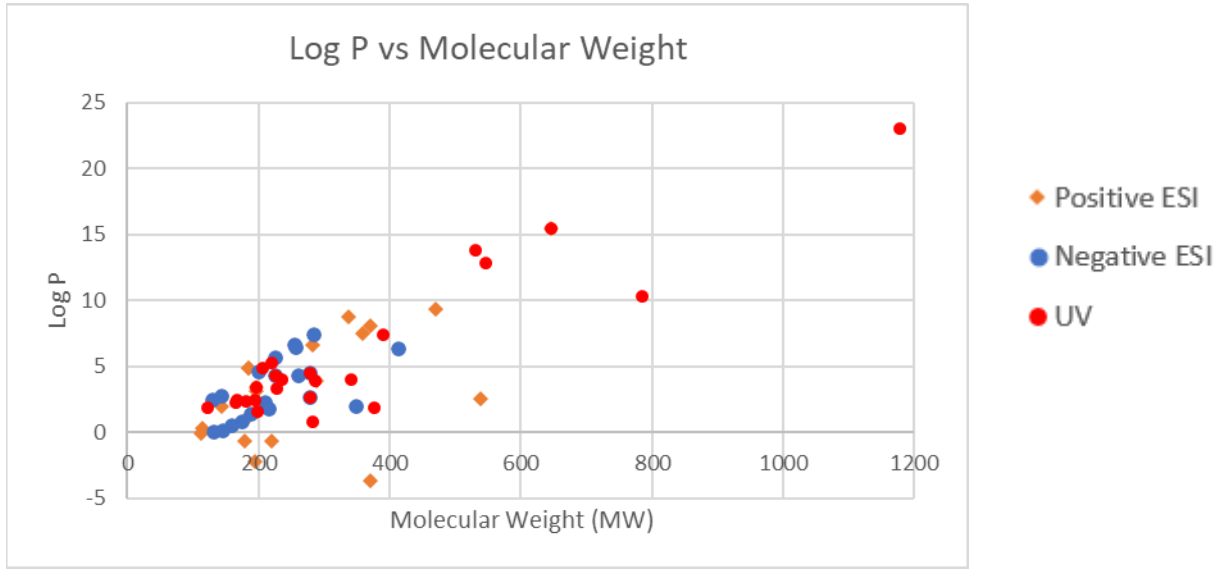


APPLICATION

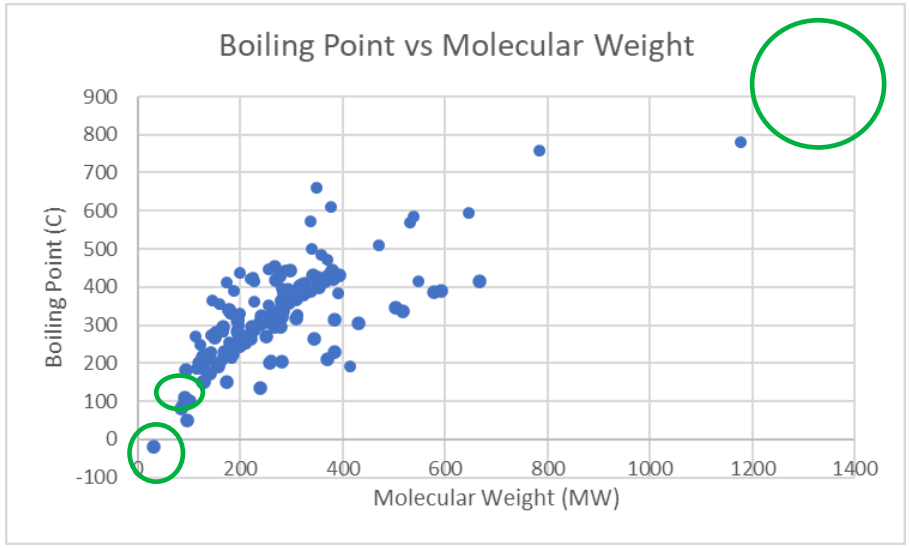
Optimize the Detection of Compounds in the Coverage Map

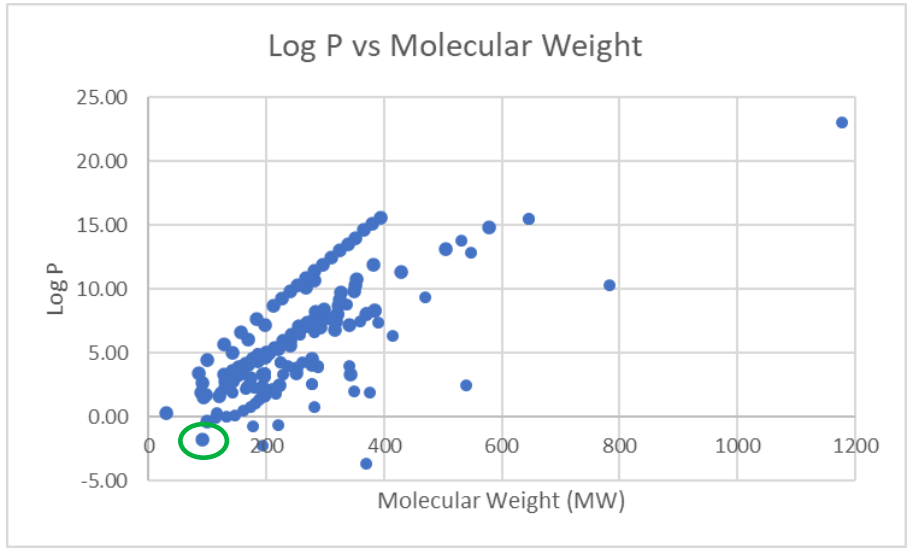
Optimal Detection Range for LC Based Methods





Supplemental Analysis for Selected Compounds in the Coverage Map





Compounds	Challenge to the Screening Methods	Supplemental Analysis
Formaldehyde	Very low MW and BP	HPLC-UV with DNPH derivatization
High MW polymer residues (MW>1500)	Non-volatile, do not elute/outside detection range by reversed phase LC based methods	GPC, Pyrolysis GC-MS
Glycerol	Very polar, poor peak shape and low sensitivity	HPLC-RI method

Future Work

- Increase the number of surrogate standards in the coverage map to better understand the response factor (RF) variation
- Fill the “gaps” in the coverage map
- Expand the material/processing aid database
- Expand the coverage map for novel materials/processing aids
- Evaluate additional unique physicochemical properties that could provide insights into compounds’ behavior
  - Vapor pressure
  - Pka

Acknowledgement

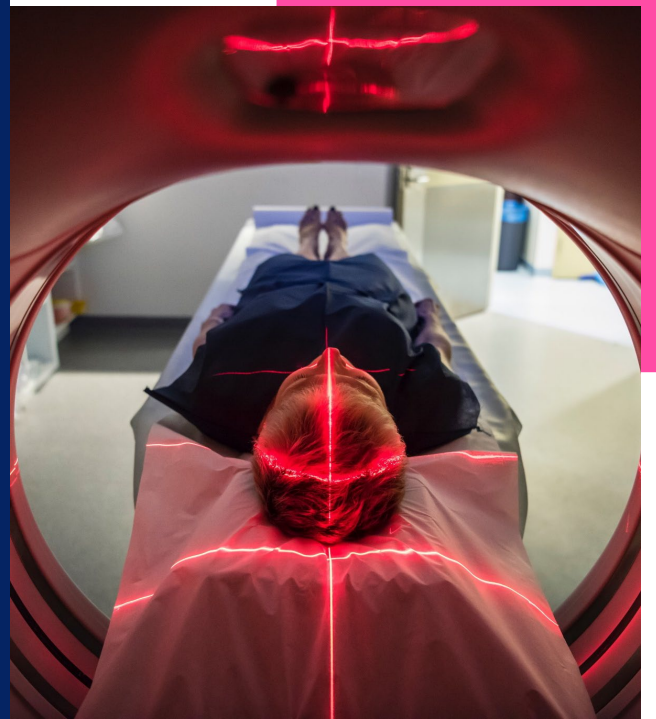
- Chemistry Team at Global Biocompatibility, Abbott Laboratories
  - Changhe Xiao, Makenzie Larch, Yan Jia,
- Sr. Management
  - Scott Citrowske
  - Robert Shulfer
  - Marika Kamberi

Jordi Labs
AN RQM+ COMPANY

Building a list of chemicals for assessment of extractables method coverage

Kevin Rowland
Executive VP & General Manager
6 November 2024





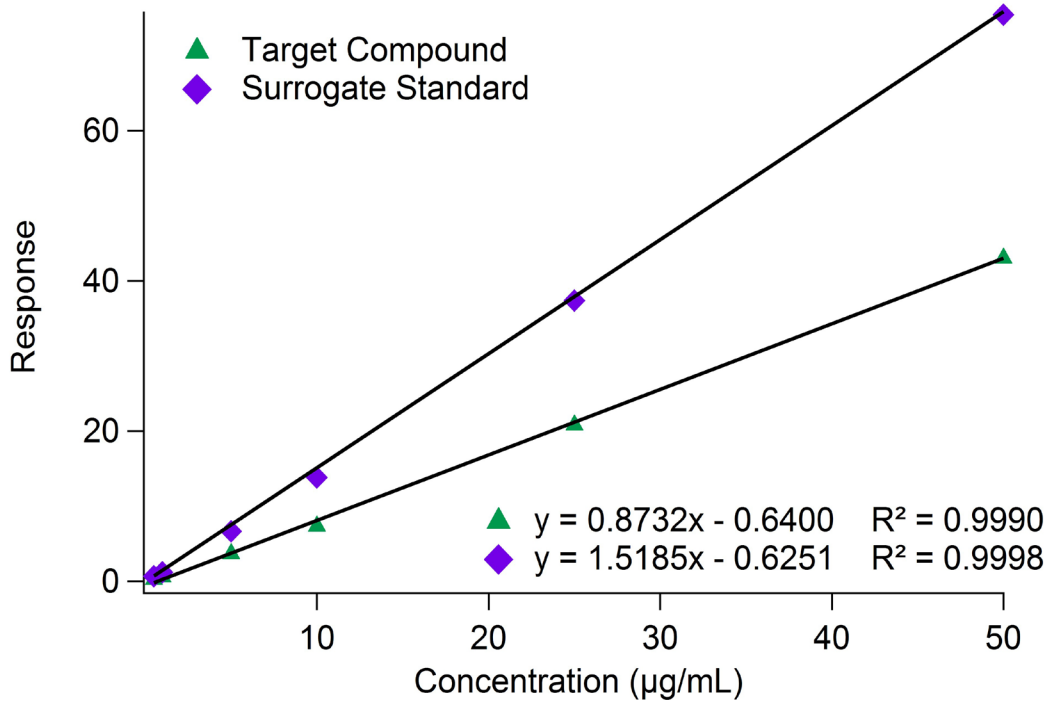

Agenda

- Background
- Relative Response Factor
- Determining Database Coverage
- Physicochemical Property Coverage
- Data Visualization
- Effects on omission of methods or properties on database coverage

Jordi Labs
AN RQM+ COMPANY

Relative Response Factor

$$RRF = \frac{Target\ Compound\ Slope}{Surrogate\ Compound\ Slope} = \frac{0.8732}{1.5185} = 0.575$$



Jordi Labs
AN RQM+ COMPANY

Use of RRF Database for Coverage Determination

Compound			Relative Response Factor				True Positive above AET by at least 1 Method
Name	Molecular Formula	CAS	LCMS Positive Mode RRF	LCMS Negative Mode RRF	LC-UV RRF	GCMS RRF	
2-Hydroxy-4′-(2-hydroxyethoxy)-2-methylpropiophenone	C12H16O4	106797-53-9	0.8484562	0	0.5468471	1.0911225	***Yes***
Irganox 1035	C38H58O6S	41484-35-9	5.3419812	1.2844742	0.4848434	0	***Yes***
Caprolactam	C6H11NO	105-60-2	1.2326767	0	0	0.8568995	***Yes***
5-Amino-1-pentanol	C5H13NO	2508-29-4	0.1495388	0	0	0.1235195	***No***
Tinuvin 571	C25H35N3O	125304-04-3	2.2147435	0.0838224	0.590812	0	***Yes***
Diphenyl sulfone	C12H10O2S	127-63-9	0.8643549	0	1.7626184	1.1529792	***Yes***
Decamethylcyclopentasiloxane	C10H30O5Si5	541-02-6	0	0	0	1.0030802	***Yes***
Glyceryl Monostearate	C21H42O4	31566-31-1	0.566631	0.0065647	0	0	***Yes***
3-Chloro-4-methoxybenzoic acid	C8H7ClO3	37908-96-6	0	1.3677914	0.644729	0.6879877	***Yes***

UFs applied:
GCMS: 3, LCMS: 4, LC-UV: 2

Jordi Labs
AN RQM+ COMPANY

Use of RRF Database for Coverage Determination

Compound			Relative Response Factor				True Positive above AET by at least 1 Method
Name	Molecular Formula	CAS	LCMS Positive Mode RRF	LCMS Negative Mode RRF	LC-UV RRF	GCMS RRF	
2-Hydroxy-4′-(2-hydroxyethoxy)-2-methylpropiophenone	C12H16O4	106797-53-9	0.8484562	0	0.5468471	1.0911225	***Yes***
Irganox 1035	C38H58O6S	41484-35-9	5.3419812	1.2844742	0.4848434	0	*Yes*
Caprolactam	C6H11NO	105-60-2	1.2326767	0	0	0.8568995	*Yes*
5-Amino-1-pentanol	C5H13NO	2508-29-4	0.1495388	0	0	0.1235195	*No*
Tinuvin 571	C25H35N3O	125304-04-3	2.2147435	0.0838224	0.590812	0	*Yes*
Diphenyl sulfone	C12H10O2S	127-63-9	0.8643549	0	1.7626184	1.1529792	*Yes*
Decamethylcyclopentasiloxane	C10H30O5Si5	541-02-6	0	0	0	1.0030802	*Yes*
Glyceryl Monostearate	C21H42O4	31566-31-1	0.566631	0.0065647	0	0	*Yes*
3-Chloro-4-methoxybenzoic acid	C8H7ClO3	37908-96-6	0	1.3677914	0.644729	0.6879877	*Yes*

UFs applied:
GCMS: 3, LCMS: 4, LC-UV: 2

Jordi Labs
AN RQM+ COMPANY

Use of RRF Database for Coverage Determination

Compound			Relative Response Factor				True Positive above AET by at least 1 Method
Name	Molecular Formula	CAS	LCMS Positive Mode RRF	LCMS Negative Mode RRF	LC-UV RRF	GCMS RRF	
2-Hydroxy-4'-(2-hydroxyethoxy)-2-methylpropiophenone	C12H16O4	106797-53-9	0.8484562	0	0.5468471	1.0911225	Yes
Irganox 1035	C38H58O6S	41484-35-9	5.3419812	1.2844742	0.4848434	0	Yes
Caprolactam	C6H11NO	105-60-2	1.2326767	0	0	0.8568995	Yes
5-Amino-1-pentanol	C5H13NO	2508-29-4	0.1495388	0	0	0.1235195	***No***
Tinuvin 571	C25H35N3O	125304-04-3	2.2147435	0.0838224	0.590812	0	Yes
Diphenyl sulfone	C12H10O2S	127-63-9	0.8643549	0	1.7626184	1.1529792	Yes
Decamethylcyclopentasiloxane	C10H30O5Si5	541-02-6	0	0	0	1.0030802	Yes
Glyceryl Monostearate	C21H42O4	31566-31-1	0.566631	0.0065647	0	0	Yes
3-Chloro-4-methoxybenzoic acid	C8H7ClO3	37908-96-6	0	1.3677914	0.644729	0.6879877	Yes

UFs applied:
GCMS: 3, LCMS: 4, LC-UV: 2

Jordi Labs
AN RQM+ COMPANY

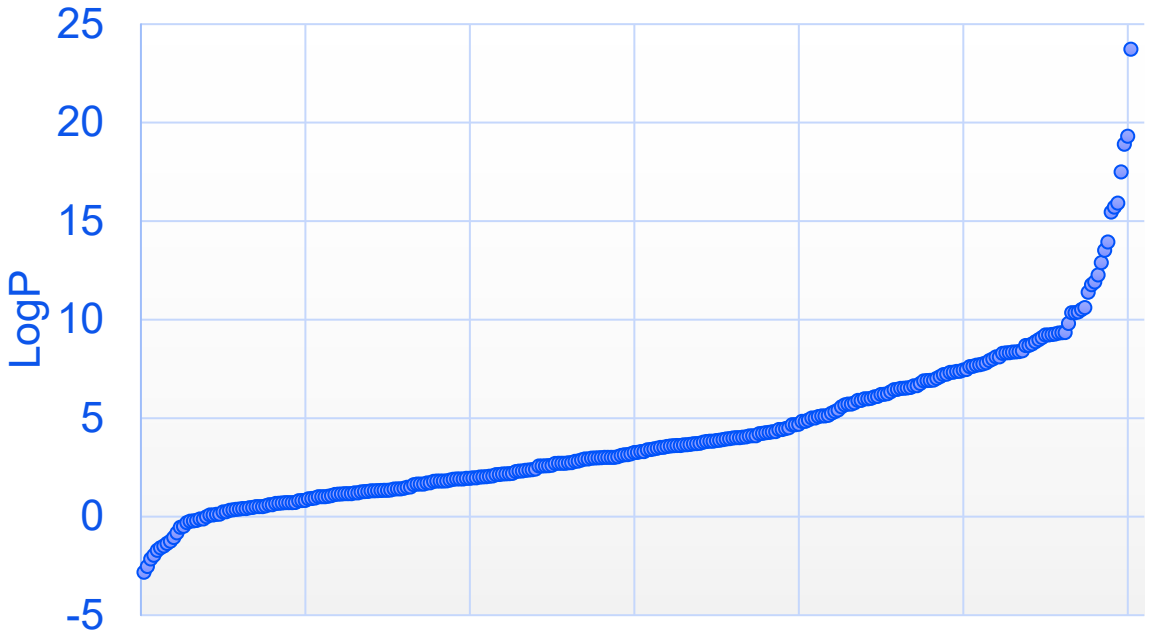
Example Database

- Constructed from authentic reference standards with high purity.
- RRF values determined based on calibration curve slope.
- Chemicals selected based on continuous coverage of properties:
  - Molecular Weight
  - Boiling Point
  - Log P
  - pKa
  - Vapor Pressure
  - Double Bond Equivalent (DBE)

Jordi Labs AN RQM+ COMPANY

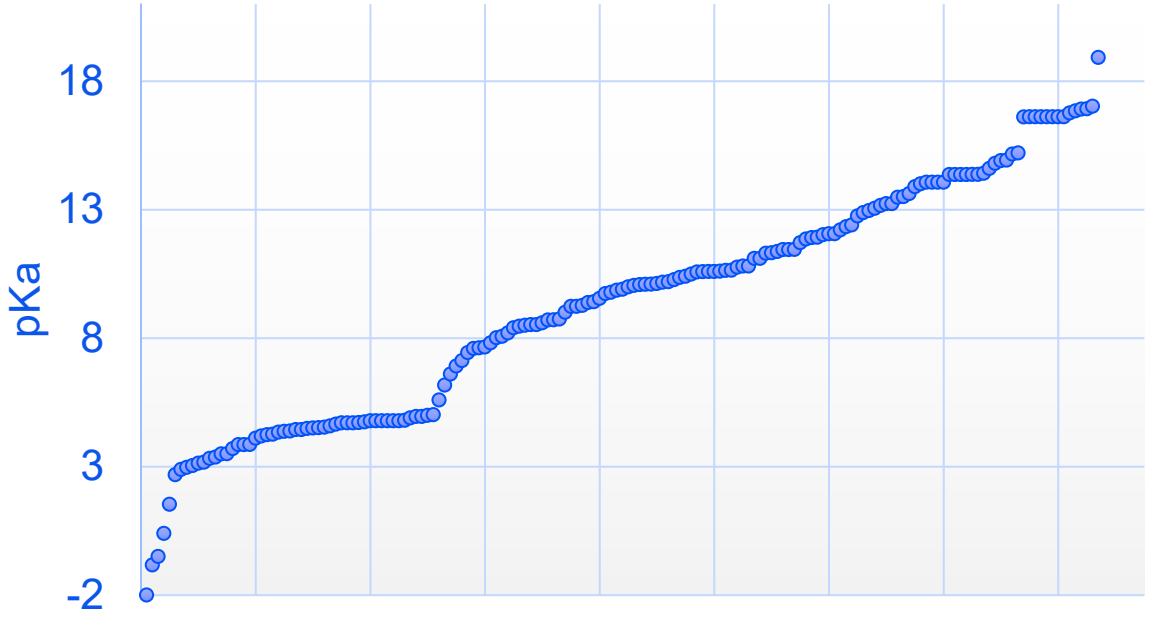
Physicochemical Properties

LogP Range



Polarity (LogP) range of compounds (-2.83 to 23.71)

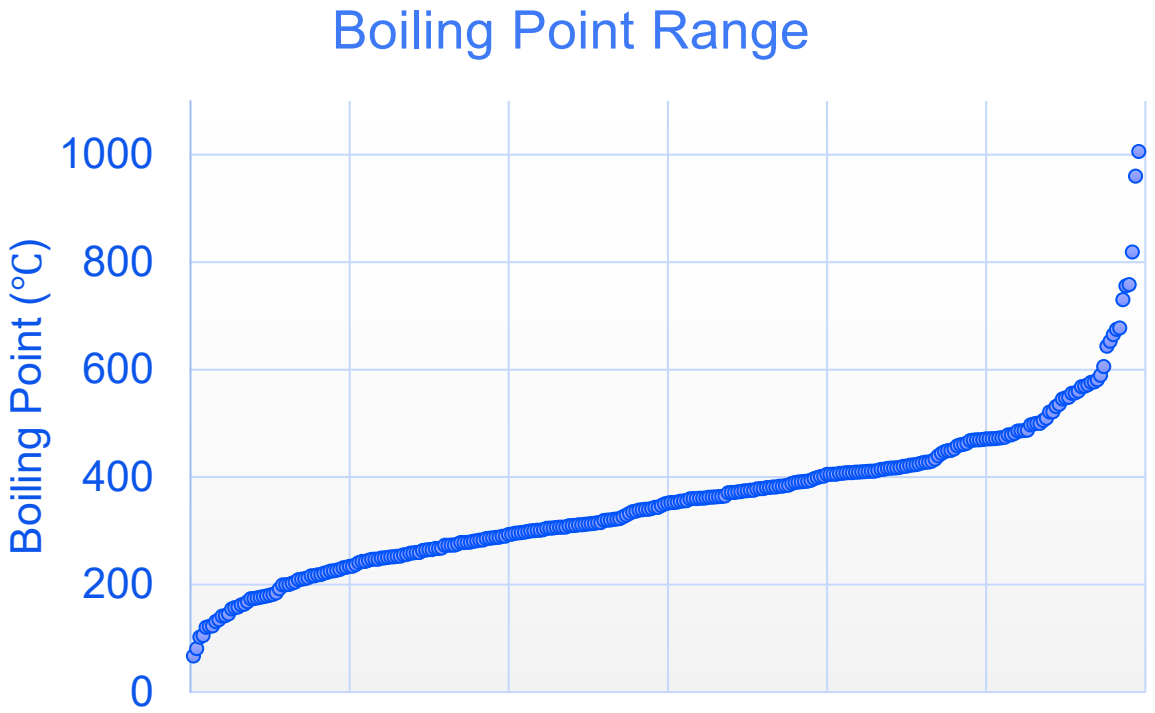
Acidity Range



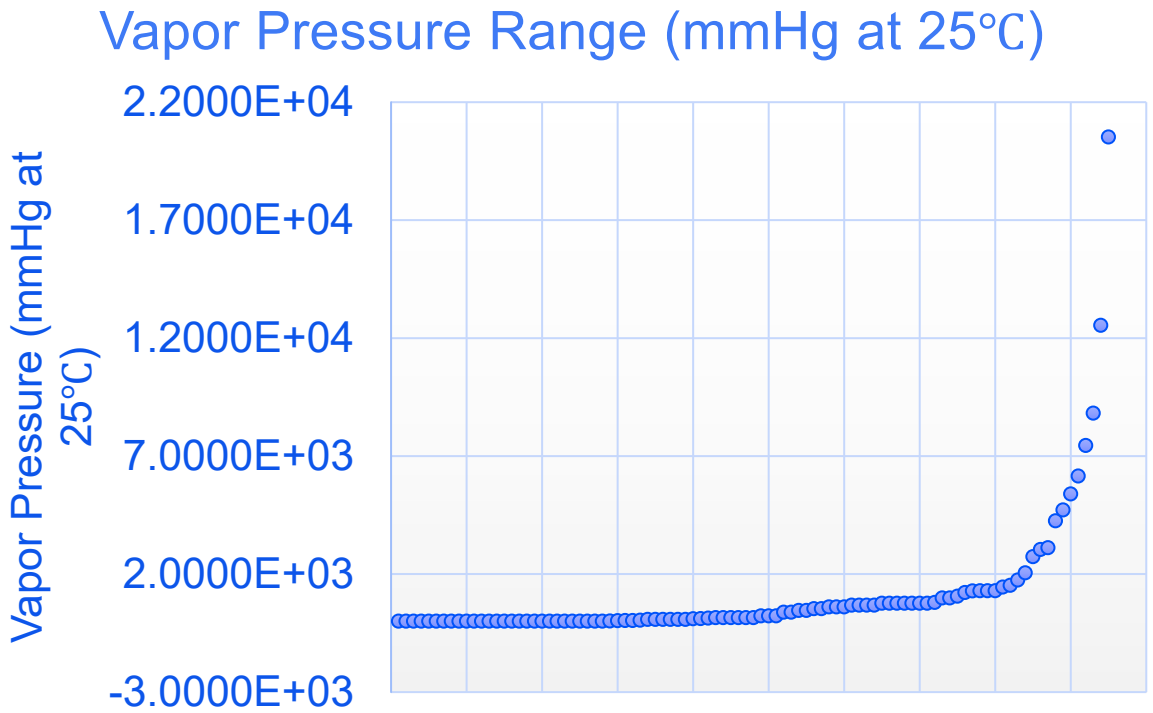
Ionizability range (shown as pKa) (pKa of -2 to 18.92)

Jordi Labs
AN RQM+ COMPANY

Physicochemical Properties

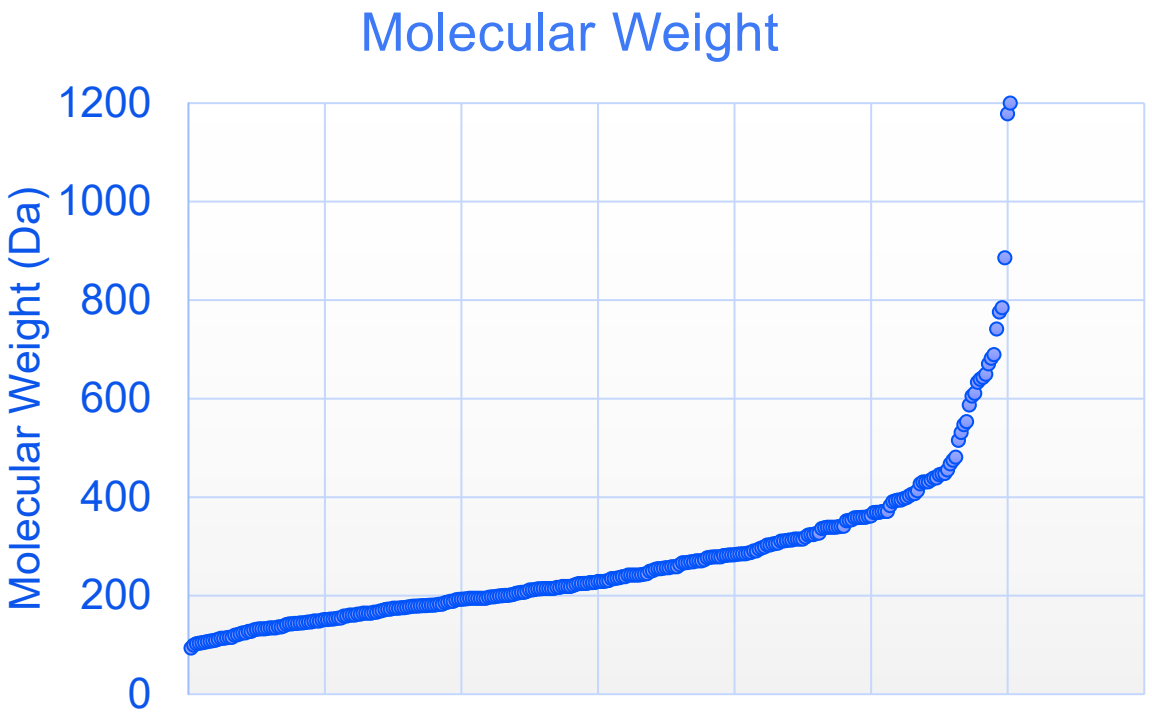


Volatility range (shown as boiling point) (67°C to 1005.8°C)

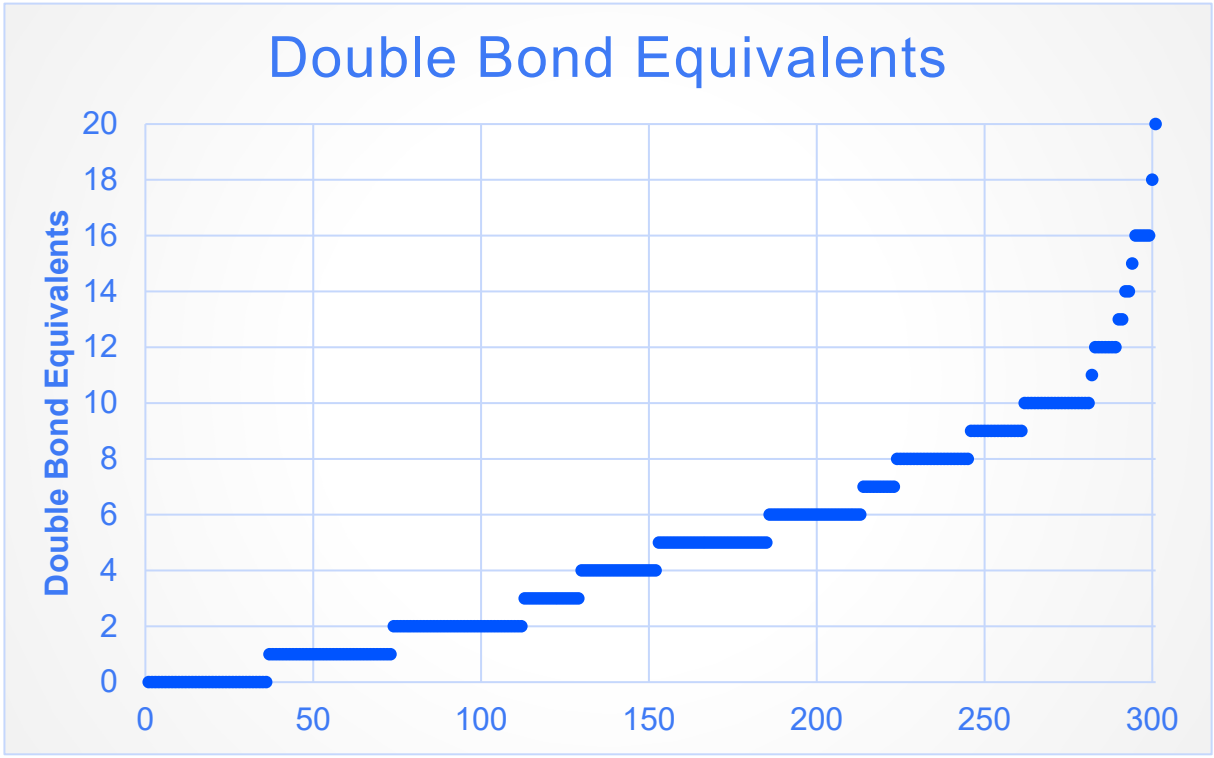


Volatility range (shown as Vapor Pressure in mmHG at 25°C)
(0.000000044764 mmHg to 20,520 mmHg)

Physicochemical Properties

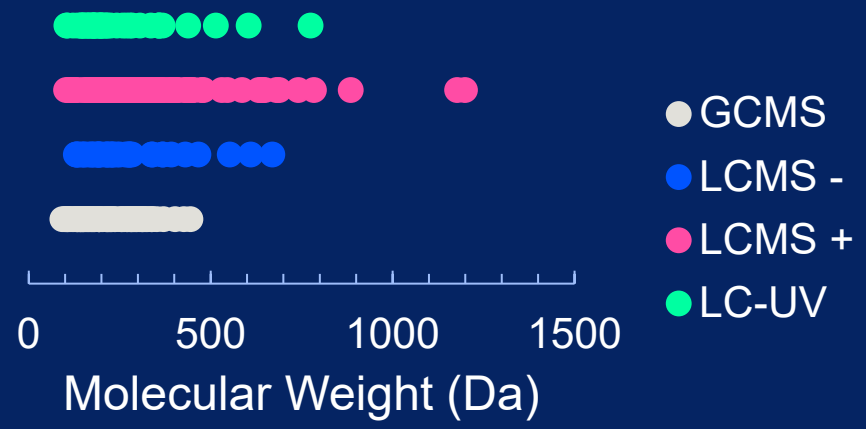


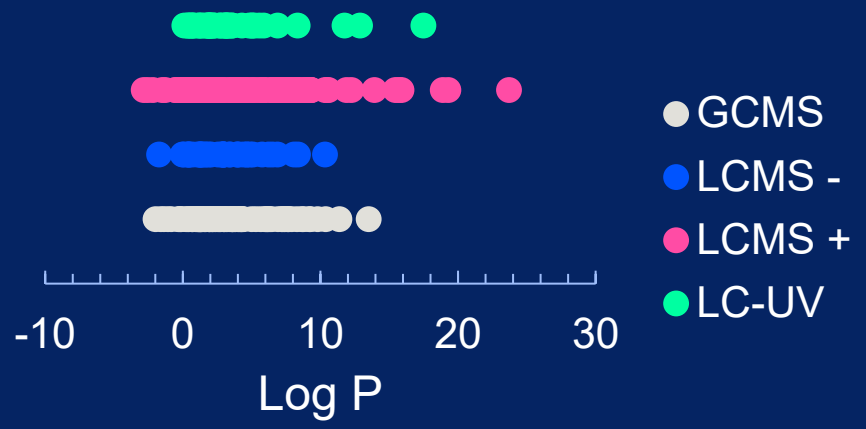
Molecular weight range (93.12832 Da to 1199.56052 Da)

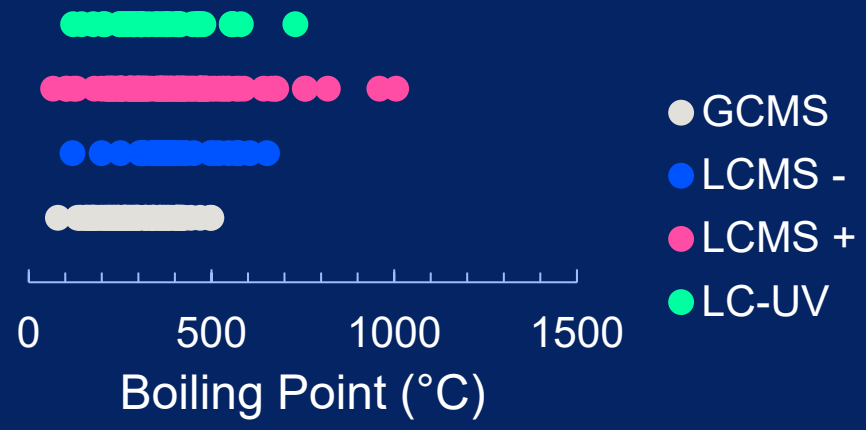


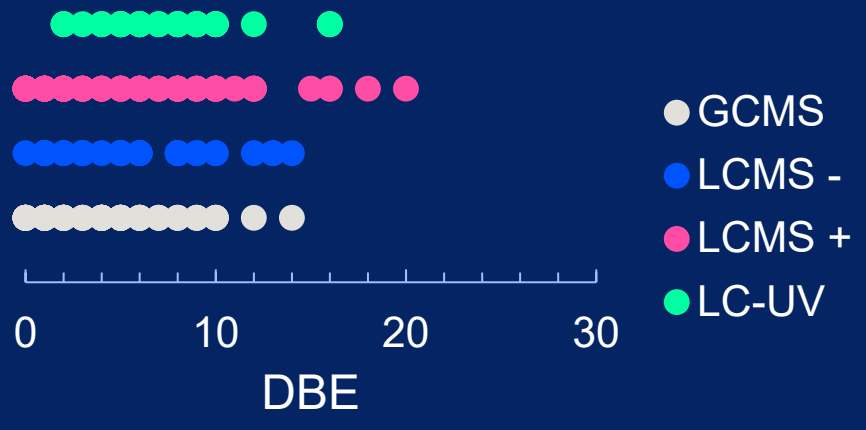
Aromaticity range (shown as double bond equivalents) (0 to 20)

Data Visualization









Jordi Labs AN RQM+ COMPANY

Effect of Methods

	UF GCMS	UF LCMS+	UF LCMS-	UF LC-UV	Apparent Coverage
All Modes Considered	3	4	4	2	95.0%
MS Only Approach	3	4	4	N/A	89.7%
MS Only Approach	4	10	10	N/A	94.9%

Jordi Labs AN RQM+ COMPANY

Effect of Limiting Properties

	UF GCMS	UF LCMS+	UF LCMS-	UF LC-UV	Apparent Coverage
Full 301 Compounds	3	4	4	2	95%
Only MW between 200-400 Da	3	4	4	2	97%
Only Log P between 2-8	3	4	4	2	97%
No Antioxidants or Plasticizers	3	4	4	2	94%

Jordi Labs AN RQM+ COMPANY

Observations

- Determination of uncertainty factors based on coverage of a well-constructed database appears to be a robust approach

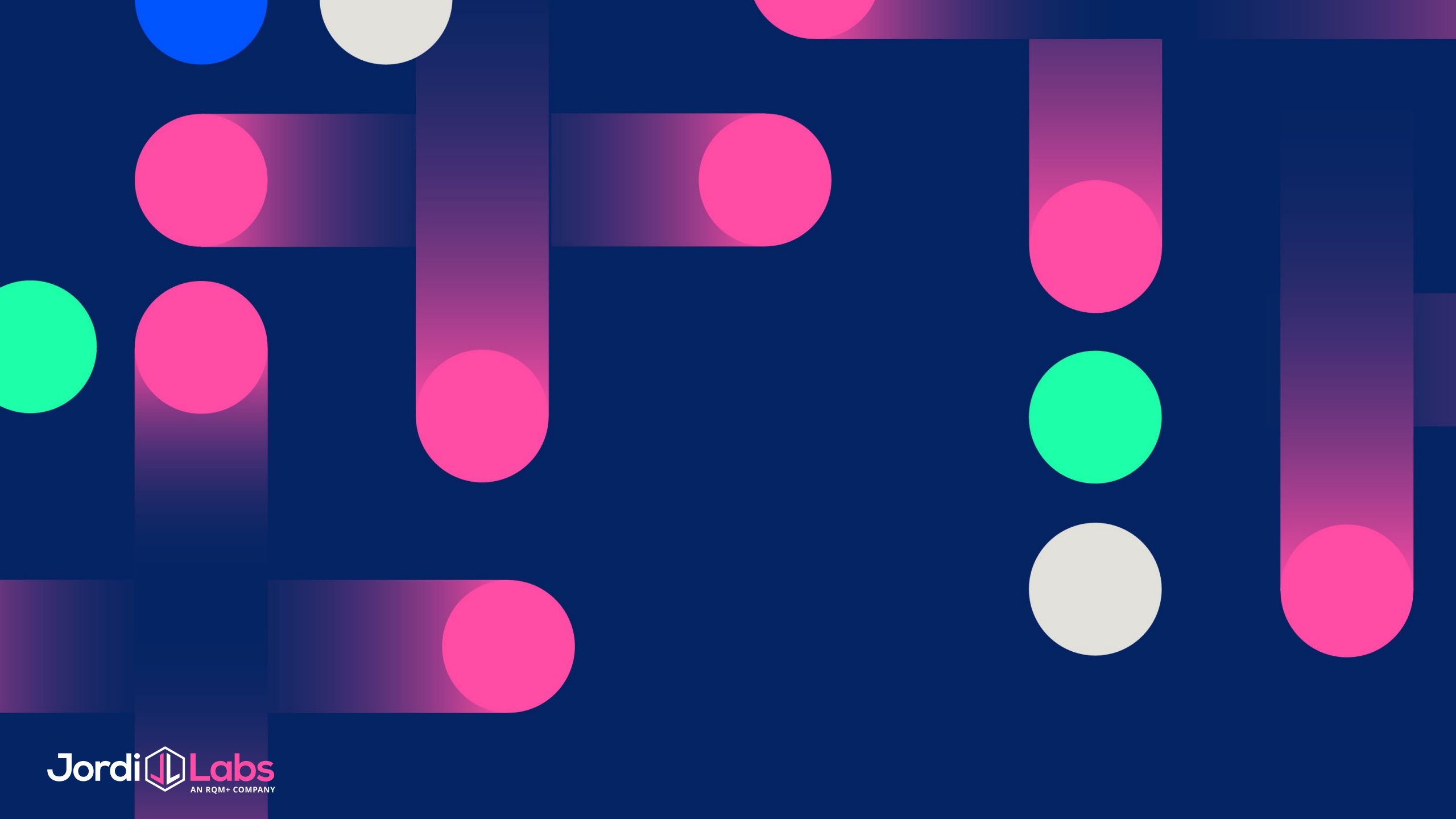
Coverage provides a simple metric to communicate the effectiveness of the method, but only if the database is well constructed.

- Range of physical properties included is a logical way to evaluate the database construction.

Properties included and the covered range of those properties are both important. Otherwise, apparent coverage is inflated.

- MS methods have RRF values that are difficult to correlate with a single property.

Lack of simple property dependence suggests a group of properties is required to ensure chemical space is accurately described


Jordi Labs
AN RQM+ COMPANY