Mitigating Response Variation and the need for Uncertainty Factors (UFs) in Extractables and Leachables Analysis

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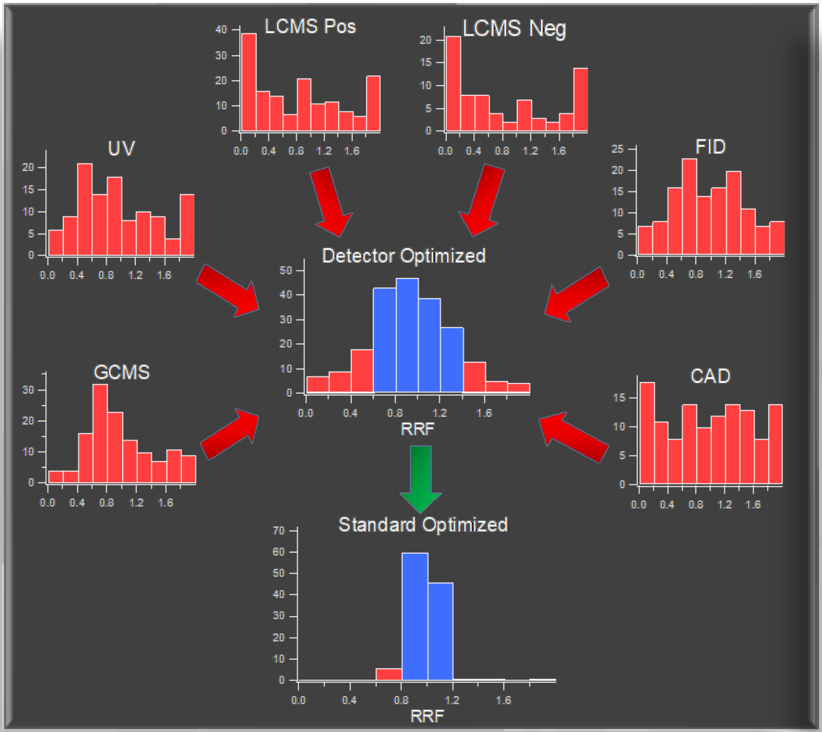
Introduction



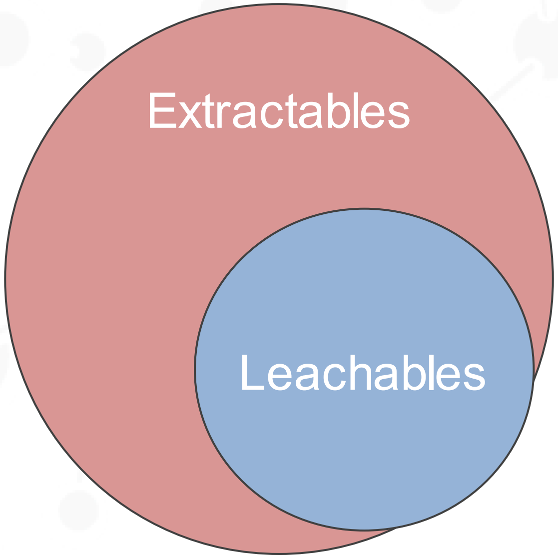
Overview

- Background
  - Response Factor (RF) Variation
  - The Two RF Problems
    - 1) RF and the AET
    - 2) RF and Quant Accuracy
  - Reducing RF Variation
    - Multidetector Approach
      - Triple Detection LC (UV, CAD, MS)
      - Dual Detection GC (-MS/FID)
    - Mitigating the RF AET problem
    - Improving Quantitative Accuracy

Extractables and Leachables

Examples of E&Ls

- Small molecules present in a polymer system include:
  - Antioxidants
  - Surfactants
  - Slip agents
  - Plasticizers
  - Cross linking agents
  - Residual monomers and *oligomers*
  - *Polymerization side products*
  - *Process Impurities*
  - *Degradation products*



- Standards are not commercially available for many common E&L's and thus can't be put into an RF database

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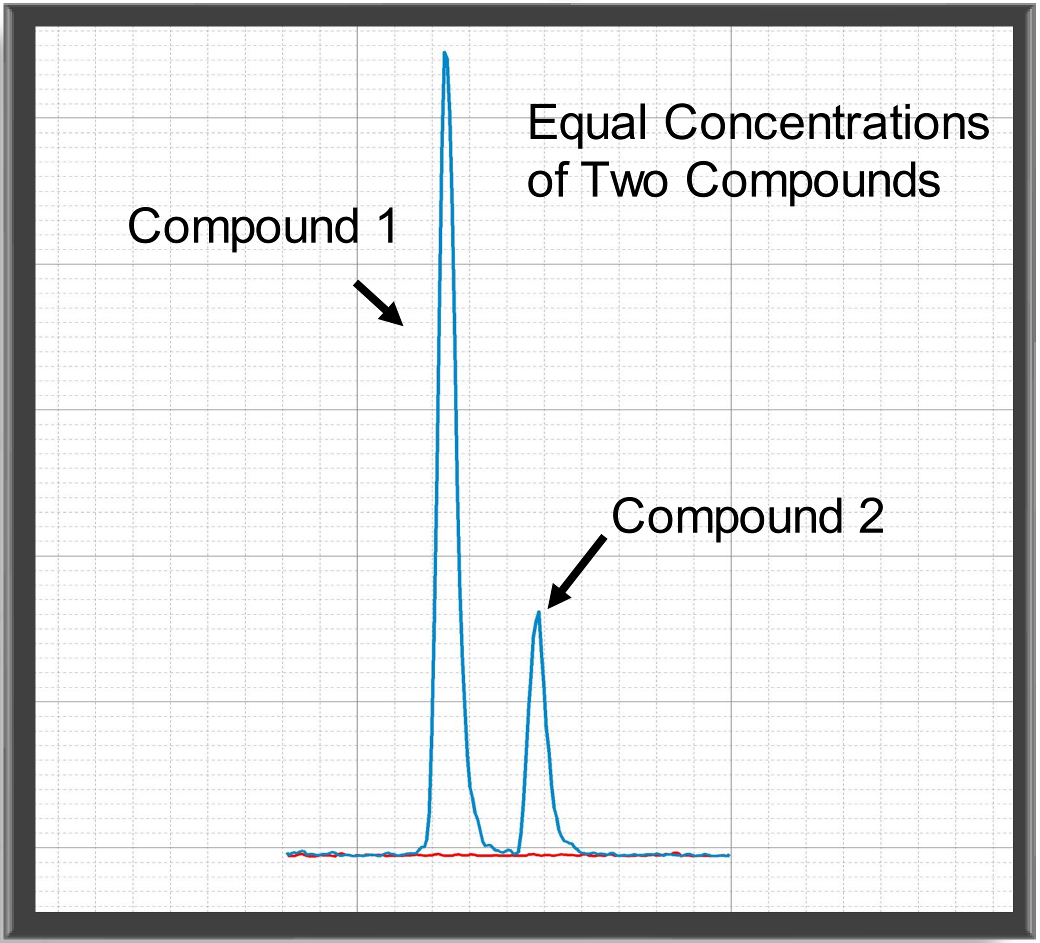
Response Factor Variation

Response Factors

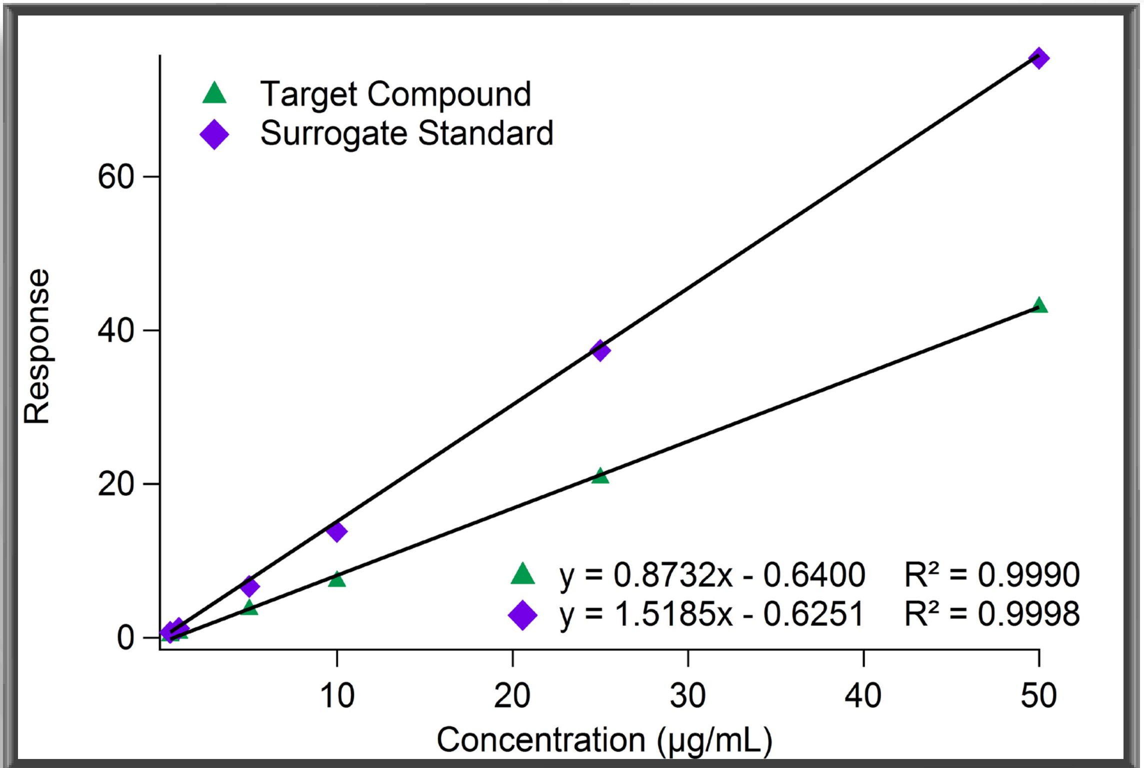
- The amount of signal per unit concentration
- Most detectors don't provide <u>equal response at equal concentrations</u> for <u>different compounds</u>



Relative Response Factor Definition

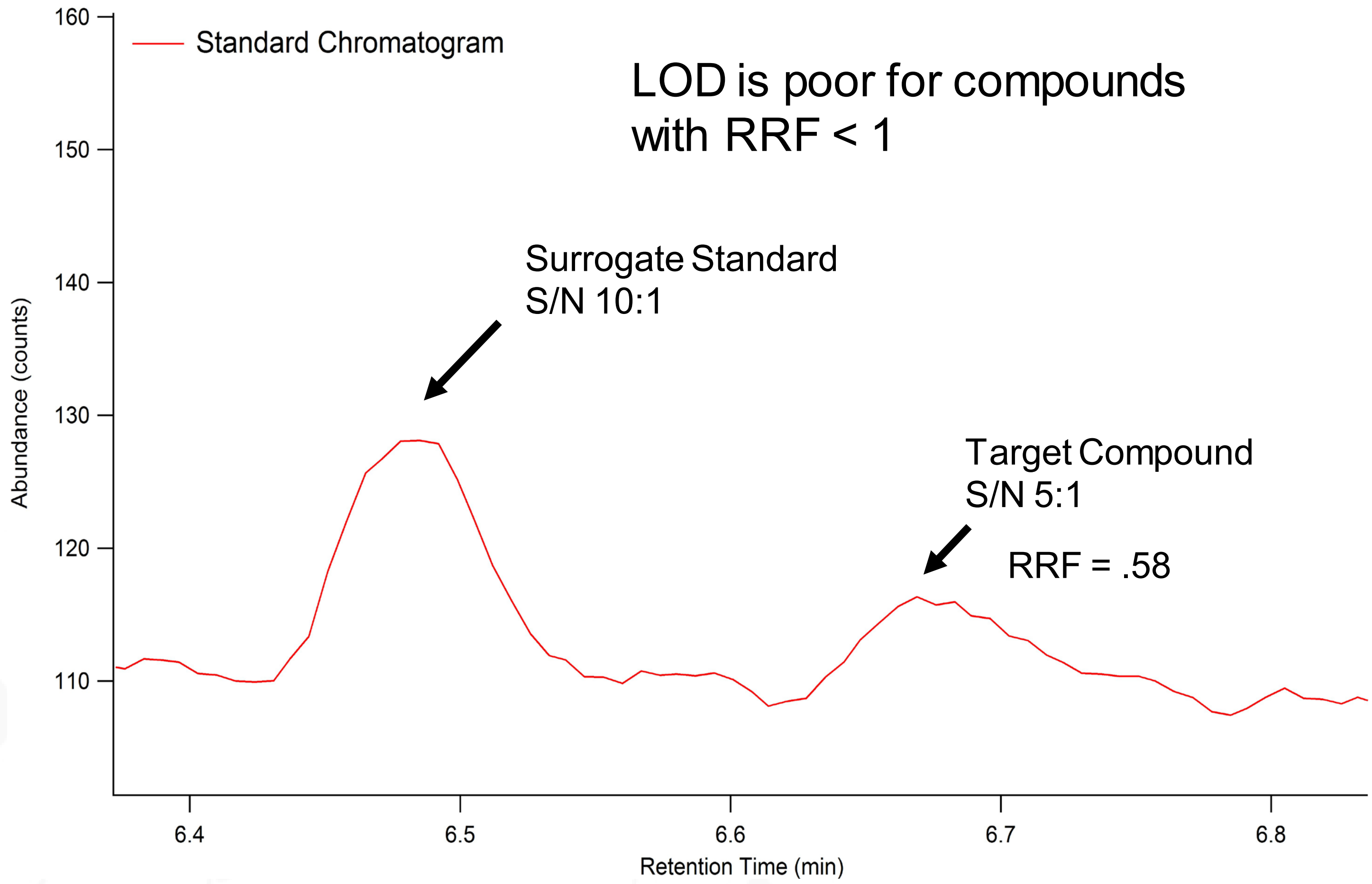
Response Factors

- Response factor directly correlates to quantitative accuracy
- RRF = .58 means that the calculated value will be 58% of the true value
- Only linear detectors provide consistent response factors



$$Relative\ Response\ Factor = RRF = \frac{Slope\ (Target\ Compound)}{Slope\ (Surrogate\ Standard)} = \frac{.8732}{1.5185} = .58$$

RF Variation and LOD Variation



Analytical Evaluation Threshold (AET)

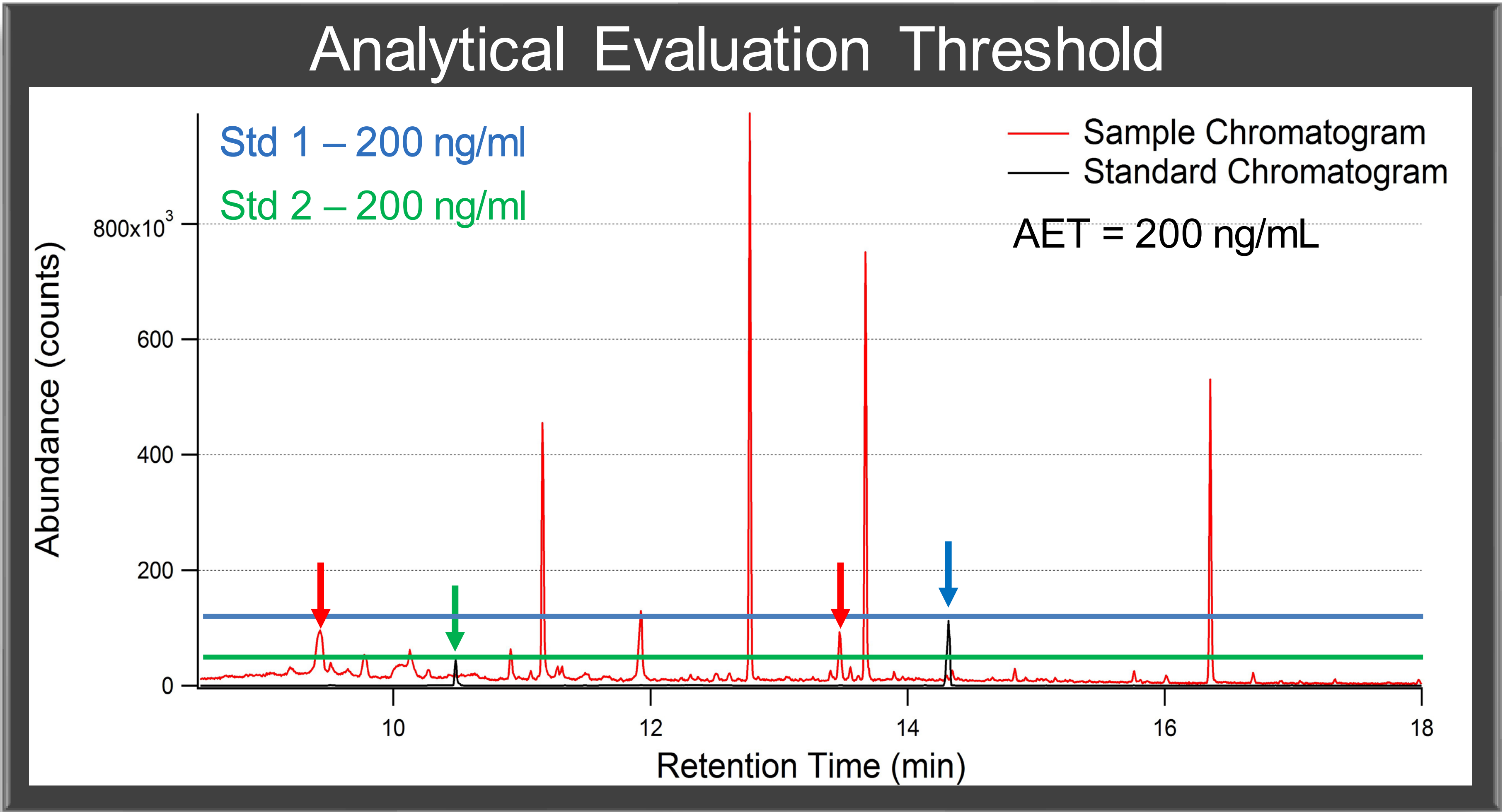
$$AET\left(\frac{\mu g}{mL}\right) = DBT\left(\frac{\mu g}{day}\right) \times \frac{A}{B \times C \times D} \times \frac{1}{UF}$$

DBT = Dose Based Threshold
A = Number of Devices Extracted
B = Extraction Volume
C = Number of Devices Used per Patient
D = Concentration/Dilution Factor
UF = Uncertainty Factor due to RF variation

PQRI Definition

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

RF Variation and the AET Value



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Qualitative assessment of extractables from single-use components and the impact of reference standard selection

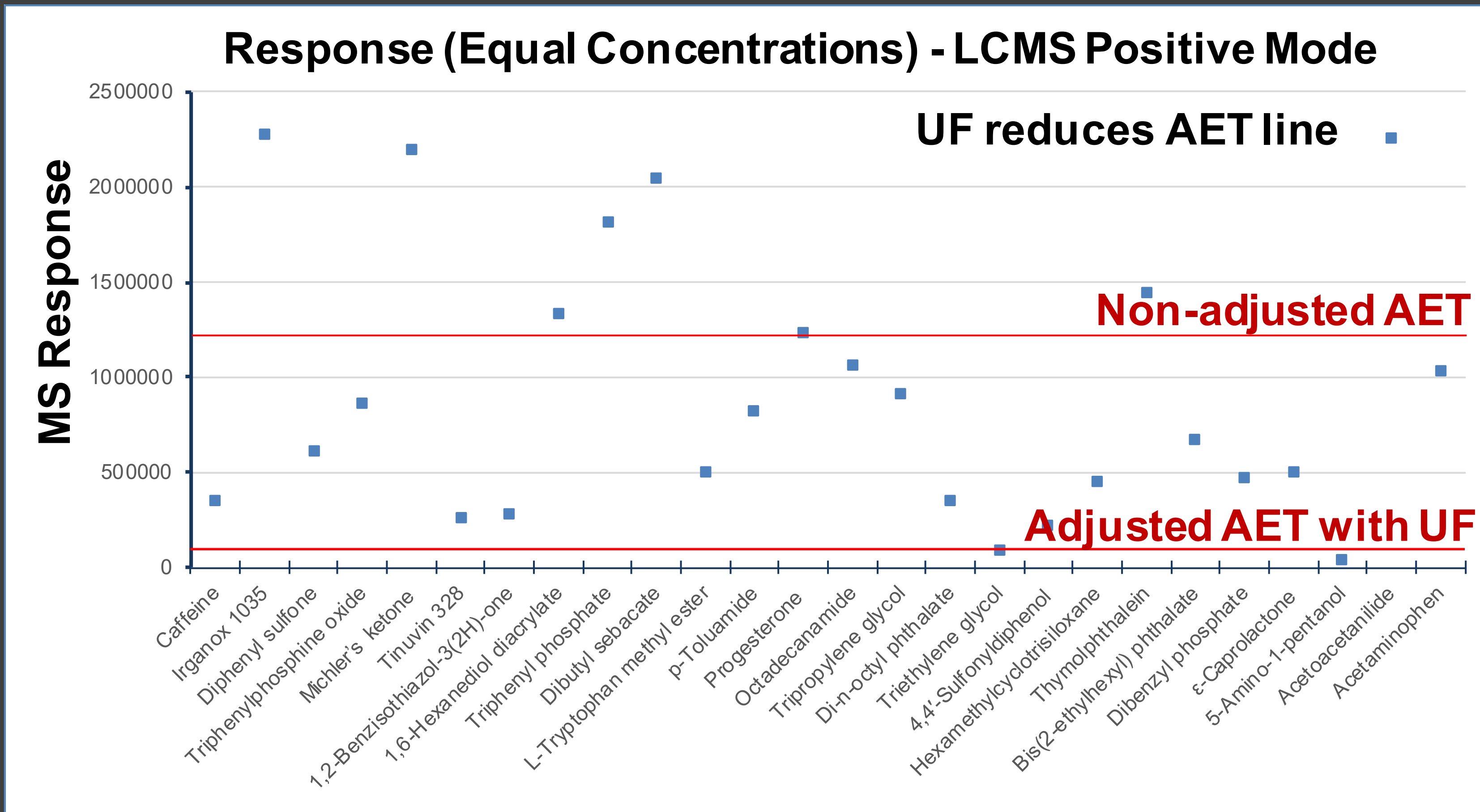
Journal of Pharmaceutical and Biomedical Analysis 150 (2018) 368–376

Sample	Extraction Conditions	Number of Extractables after different filters						
		No AET Applied	AET filter standard(s)					
			Erucamide	Irganox 1010	Irganox 1141	Stearic acid	Sodium Benzene sulfonate	Response Factor Database
Tubing	Water	35	0	0	34			1
		6				0	1	6
	Saline	41	1	2	41			3
		14				1	5	11
	50% Ethanol	145	0	1	135			2
		36				3	13	31
	100% Ethanol	488	5	9	486			94
		161				74	117	159

GCMS RF Study:

Jenke and Odufu, Journal of Chromatographic Science 2012;50:206–212

Adjusted AET and the UF factor



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Example of Revised AET

$$\text{AET}\left(\mu\frac{\text{g}}{\text{mL}}\right) = 1.5\mu\text{g/day} \times \frac{1}{30/3 \times 1 \times 1} \times \frac{1}{10} = .015\ \mu\text{g/mL}$$

DBT = TTC of 1.5 ug/day
A = Number of Devices Extracted = 1
B = Extraction Volume = 30 cm^2 / 3 cm^2/mL = 10 mL
C = Number of Devices Used per Patient = 1
D = Concentration/Dilution Factor = 1

FDA is frequently citing:
UF = 10 for LCMS
UF = 4 for GCMS

.015 µg/mL is beyond the sensitivity of most MS systems!

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Summary of RF Problem 1: AET Underreporting

We need a universal (non-targeted) method for a universe of extractables!

For cases in which an **insufficient UF** is applied:

- May not be sufficiently protective to include compounds **> AET**

For cases in which a **large UF** is applied (4 & 10):

- May extend beyond the sensitivity of the MS system
- Requires large concentration factors which could cause loss
  - May result in false positives for compounds below AET

RF Databases allow calculation of a method specific UF, but <u>do not reduce the UF required</u>.

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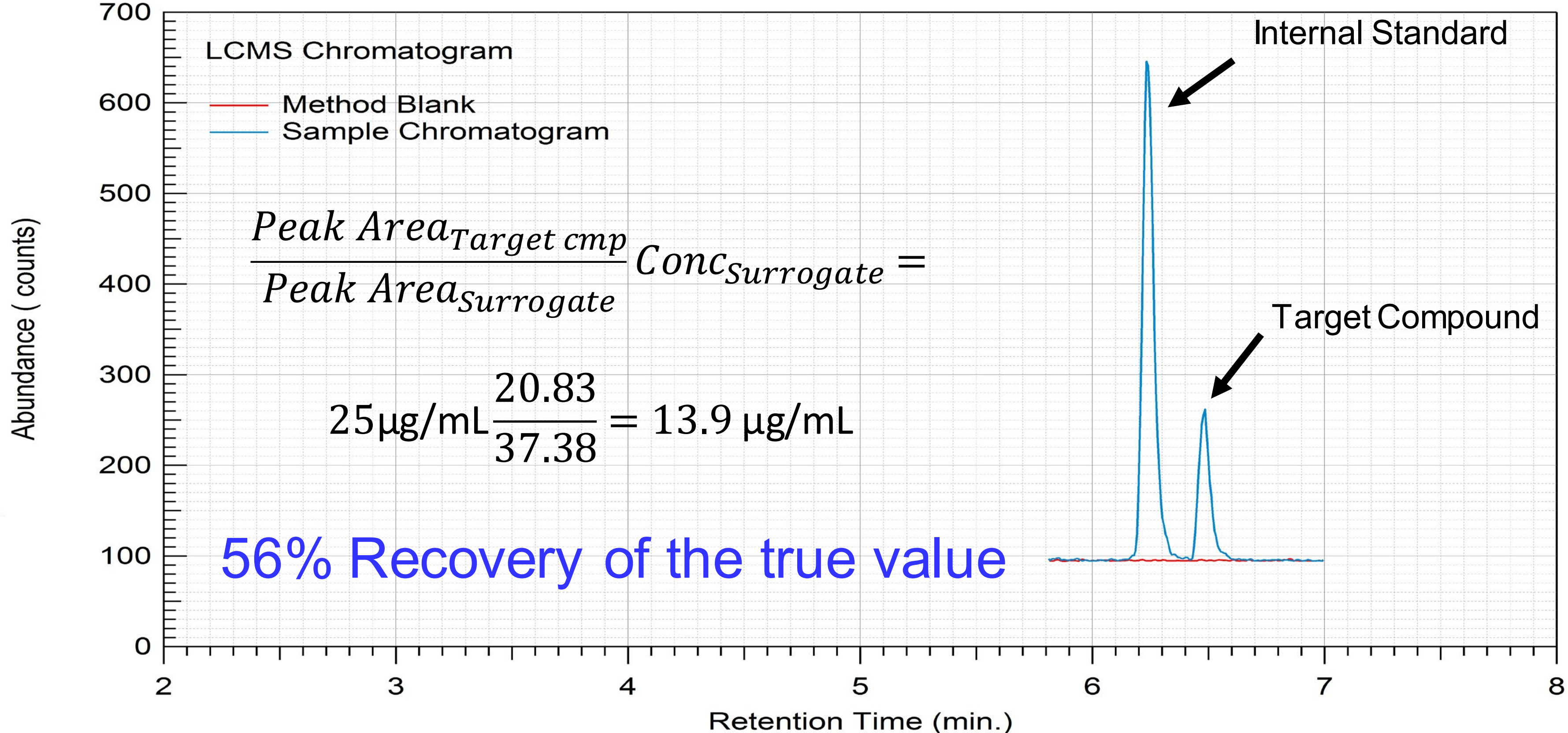
Summary of RF Problem 2: Relative Quantitation

	Conc. (µg/mL)	Peak Area	Rf
Int Std	25	37.38	1
Target cmp	25	20.83	.56



$$\frac{Peak\ Area_{Target\ cmp}}{Peak\ Area_{Surrogate}} Conc_{Surrogate} =$$

$$25\mu g/mL \frac{20.83}{37.38} = 13.9\ \mu g/mL$$

56% Recovery of the true value

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Summary of RF Problem 2: Relative Quantitation

For cases in which the $\mathbf{Rf_{cmp} < Rf_{std}}$

Not a worst case estimate
Overestimates LOQ

For cases in which the $\mathbf{Rf_{cmp} > Rf_{std}}$

Is a worst case estimate
Underestimates LOQ

Overestimates concentration.
Could result in MOS < 1.

- RF Databases only correct quantitative error <u>for compounds in the database</u>
  - UF Factors do nothing to correct quantitative inaccuracy.

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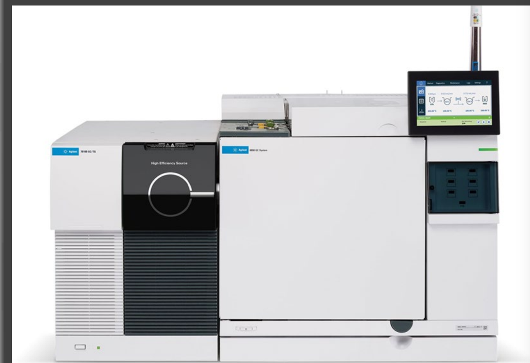
The Multidetector Approach

UV Detection

GCMS Detection



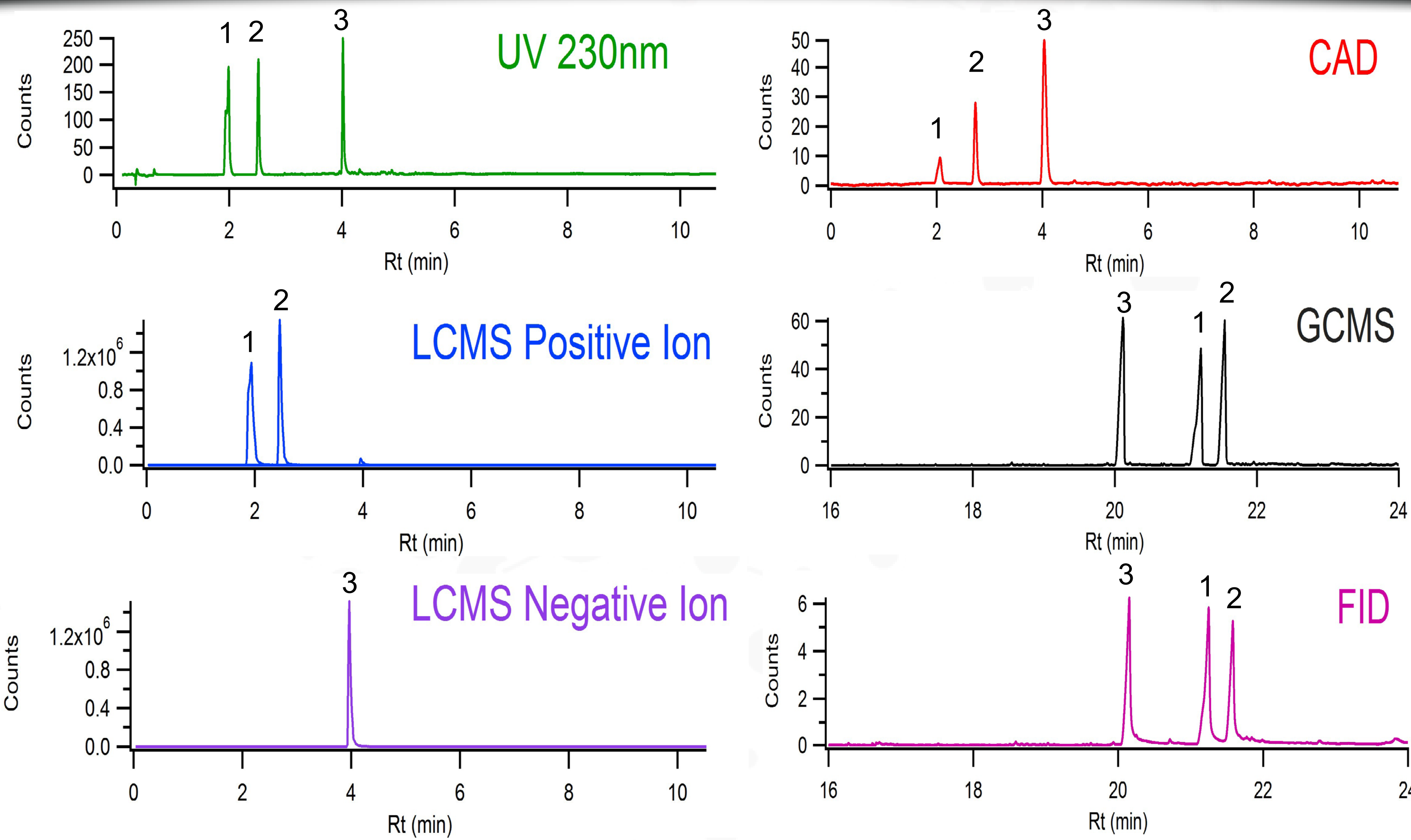
FID Detection

Charged Aerosol Detection

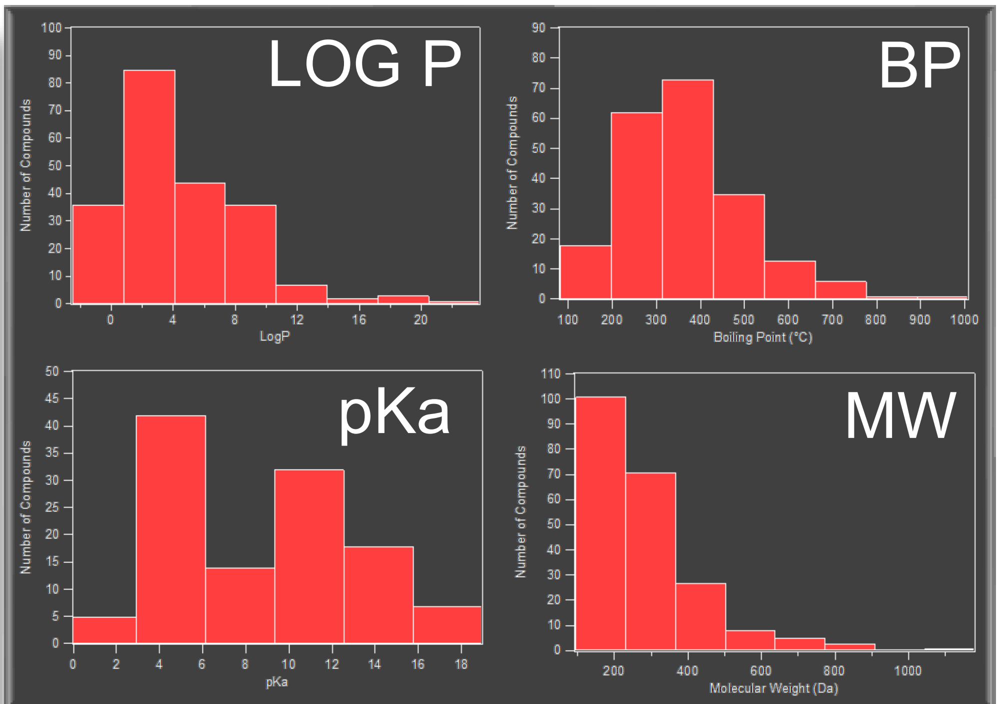
QTOF LCMS

QTOF LCMS

Multidetector Chromatograms



Estimating the RRF Distributions



Determination of RRF Factors:

- 217 Unique Compounds
- 6 Different Detectors

  - MW (93-1177 amu)
  - LogP (-2.38-25.2)
  - Boiling Point (102 - >600)
  - DBE (0-20)
  - pKa (-0.8-18.9)

Includes - monomers, slip agents, plasticizers, oligomers, surfactants, UV screens, initiators, etc.

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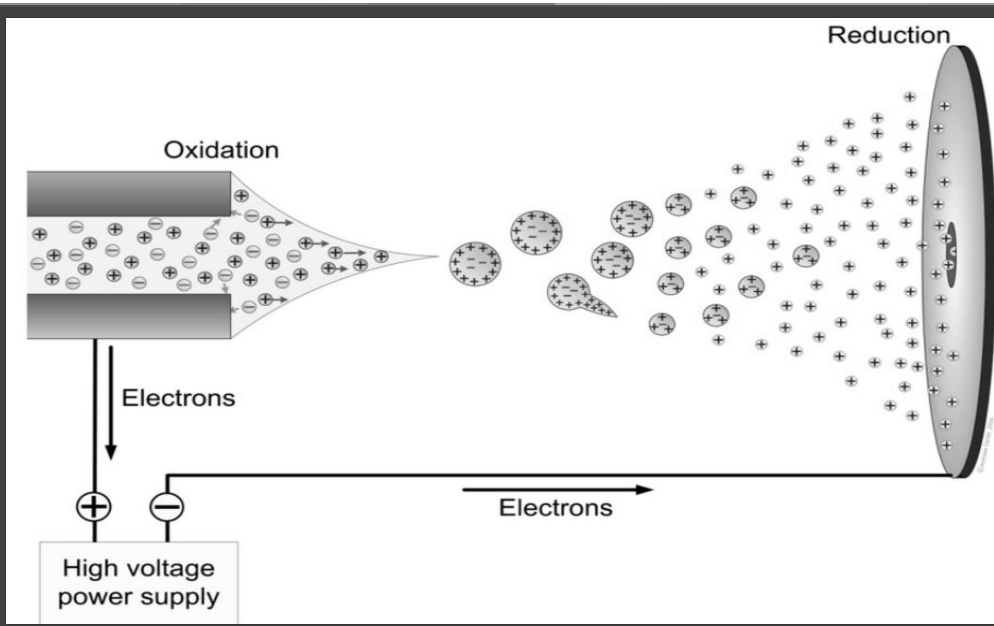
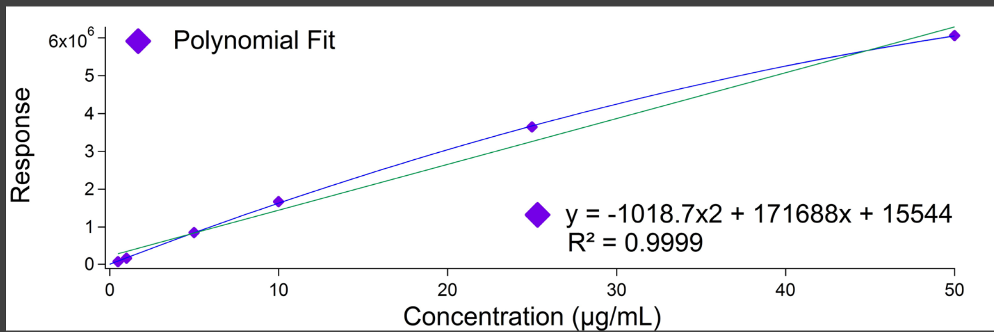
ESI-MS-TOF Detection





Principles of Detection

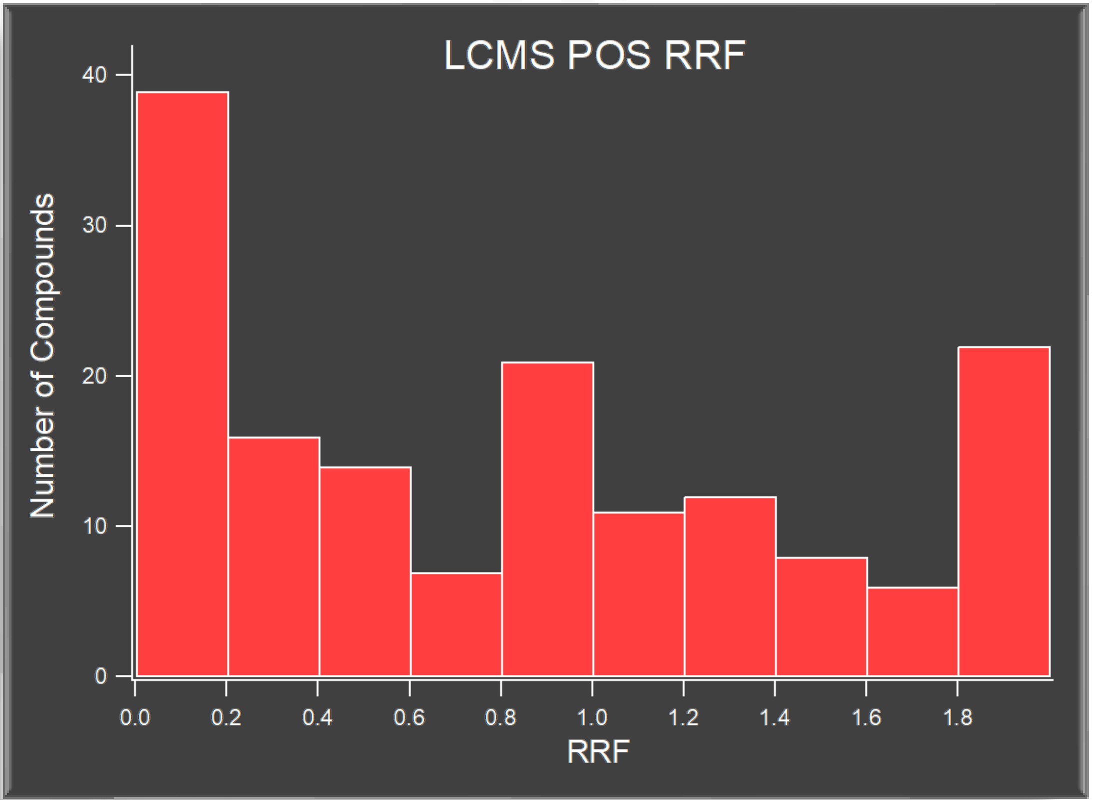
- Measures charged molecules
- Proportional to mass of analyte reaching the detector but behaves as a concentration detector due to loss during nebulization
- Measures only species which can associate with the charge carrier

Attributes

- Broadly Applicable
- Excellent Identification Capabilities
- Picogram sensitivity
- Polynomial Curve
- Subject to matrix effects

LCMS Positive Mode – Normalized RRF Factors

RRF Distribution Information	
Average	1.00
%RSD	108%
Min	0.0013
Max	6.11
Percent Detected	72%
UF	NA



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

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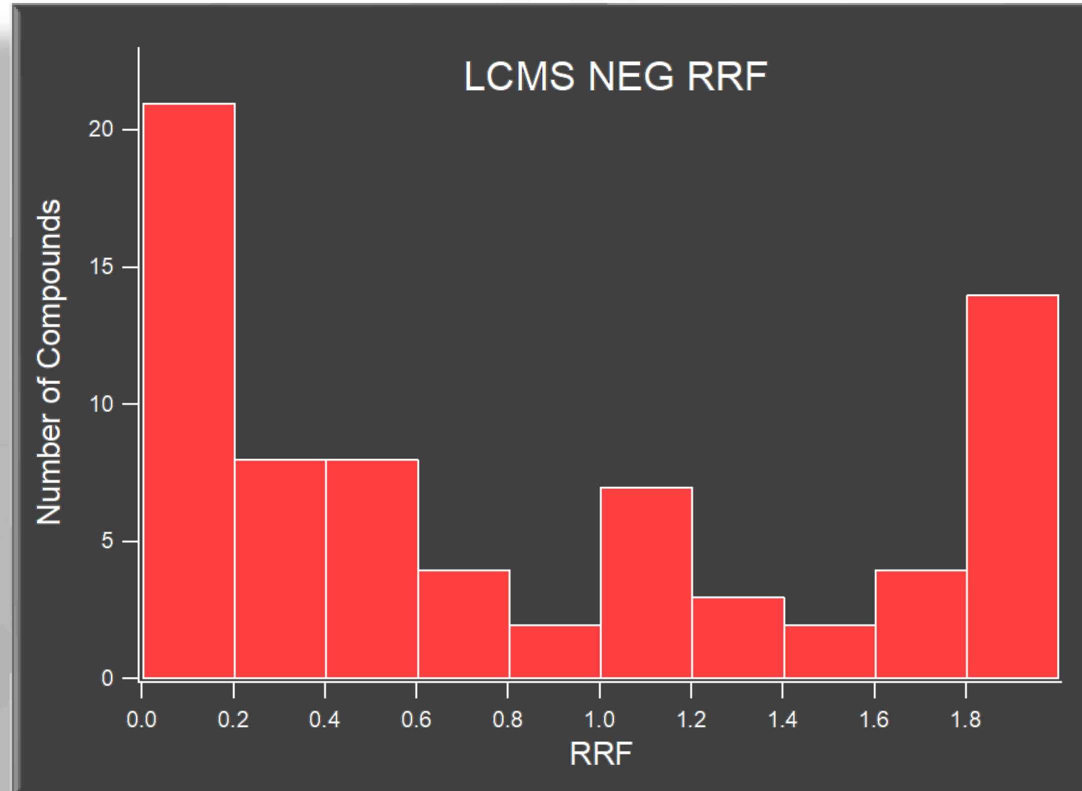
LCMS Negative Mode – Normalized RRF Factors

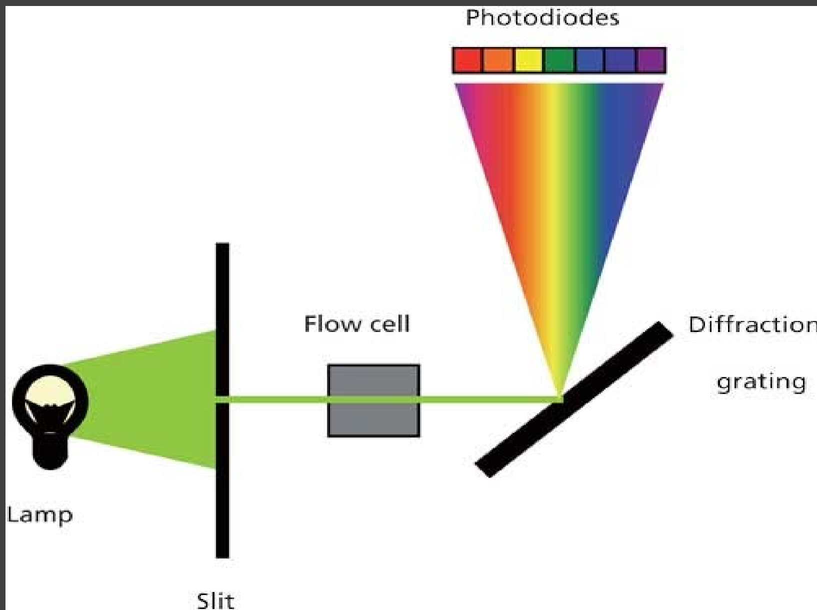
RRF Distribution Information	
Average	1.00
%RSD	119%
Min	0.0065
Max	7.98
Percent Detected	34%
UF	NA

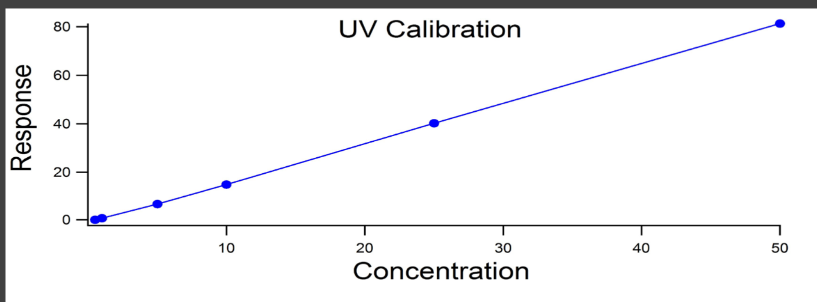


54% of compounds observed by LCMS are seen in GCMS!

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

UV Detection





Principles of Detection

- Absorption of UV light by a chromophore
- Response is proportional to concentration according to Beer's law:

  A = absorption = εLc

  ε = molar extinction coefficient

  L = path length

  c = analyte concentration

Attributes

- Highly linear
- Highly precise (<5%)
- Not subject to matrix effects
- Widely applicable
- Nanogram sensitivity with Max-Light

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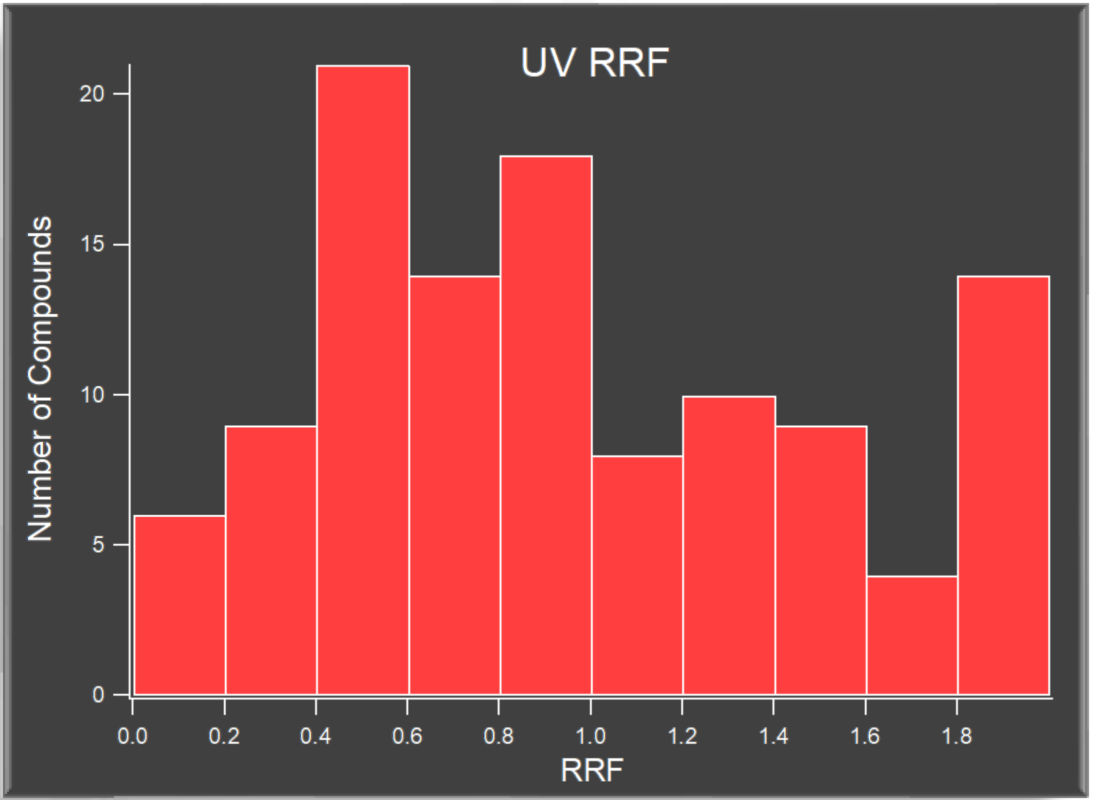
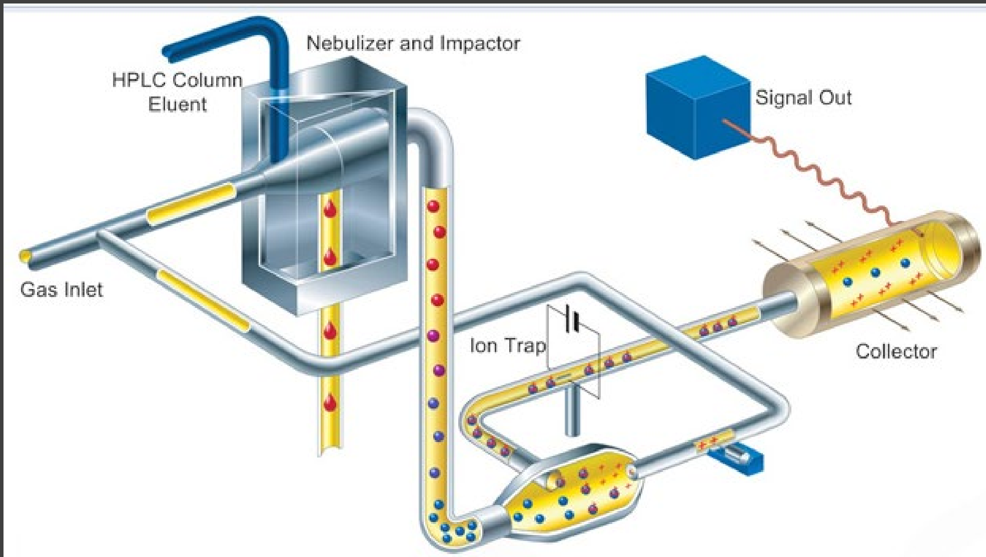
UV 230 nm – Normalized RRF Factors

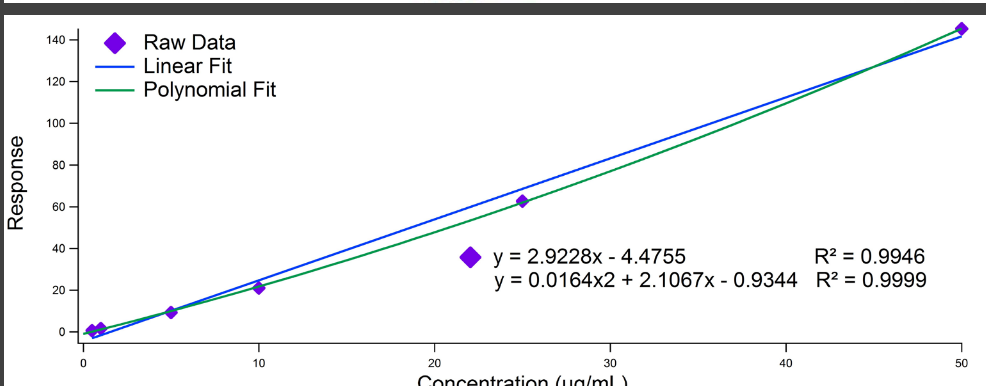
RRF Distribution Information	
Average	1.00
%RSD	60%
Min	0.036
Max	2.84
Percent Detected	52%
UF	2.5



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

CAD Detection





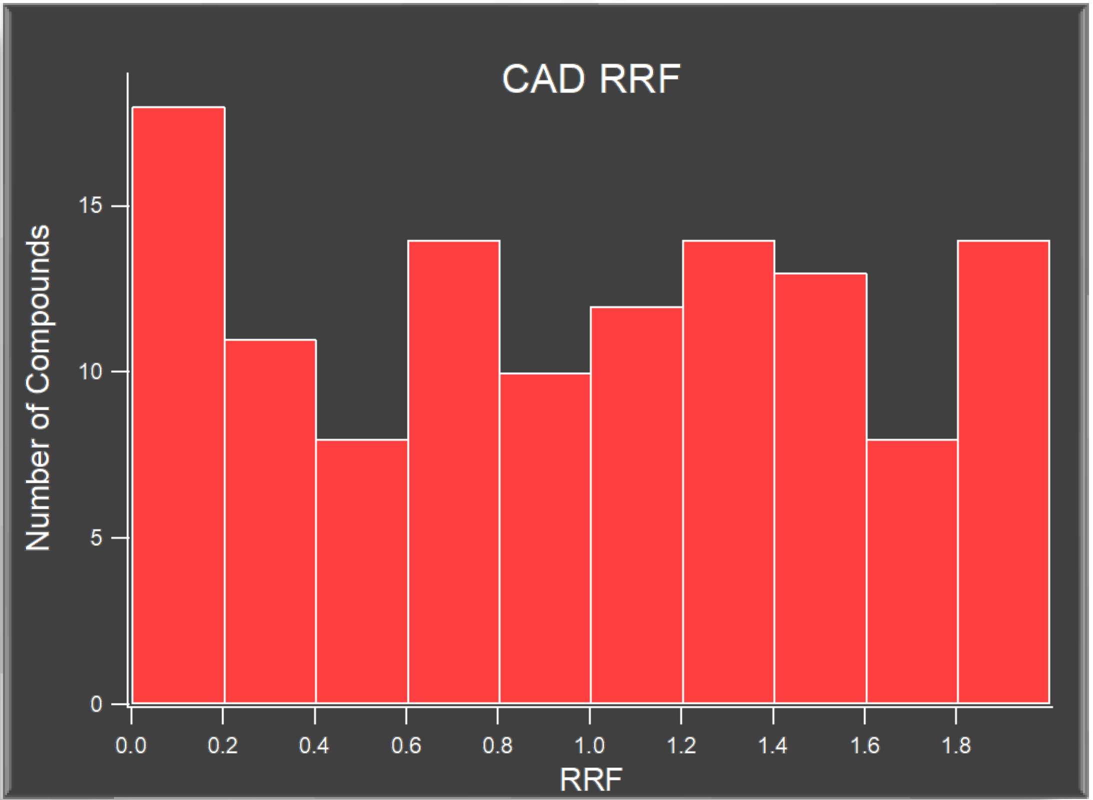
Principles of Detection

- Measures charge on vaporized analyte particles
- Response is proportional to mass of analyte reaching the detector
- Measures all non-volatile species

Attributes

- Curvilinear
- Highly precise (<10%)
- Widely applicable
- Nanogram sensitivity
- Affected by Mobile Phase Composition

CAD – Normalized RRF Factors



RRF Distribution Information	
Average	1.00
%RSD	65%
Min	0.034
Max	2.65
Percent Detected	56%
UF	2.9

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

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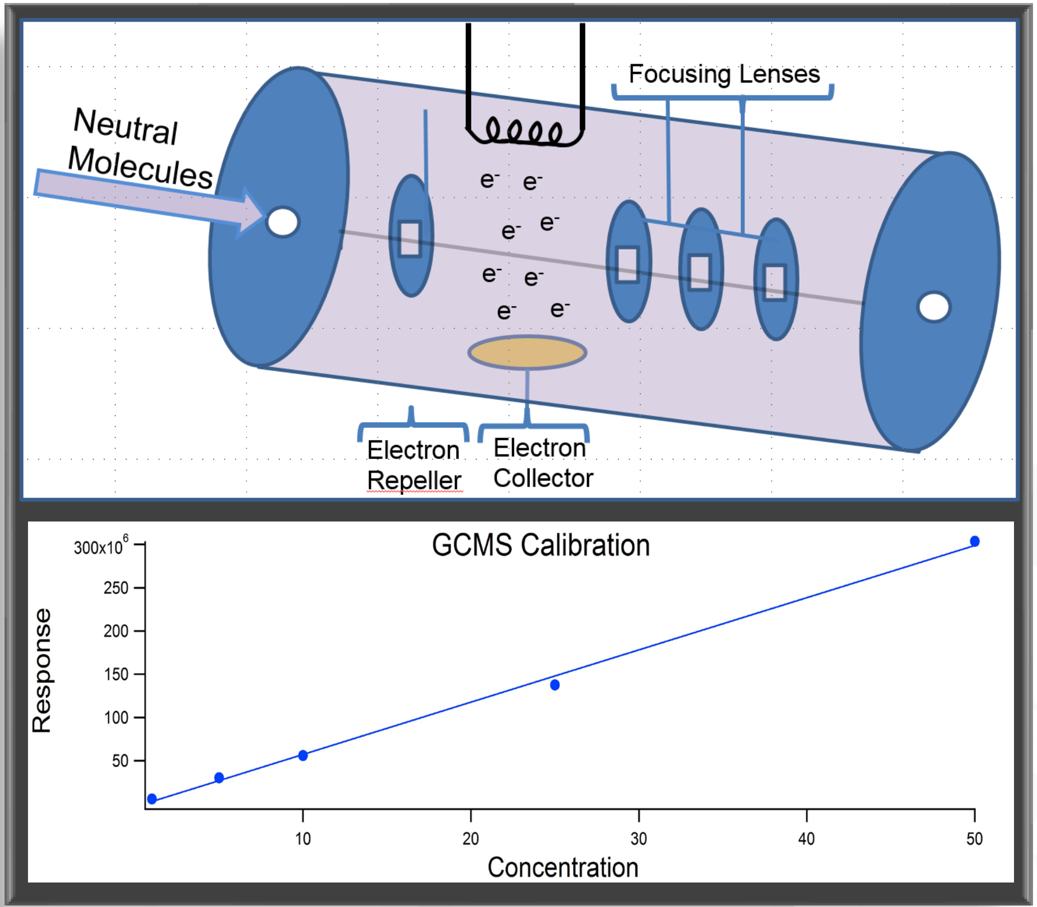
GCMS Detection



Principles of Detection

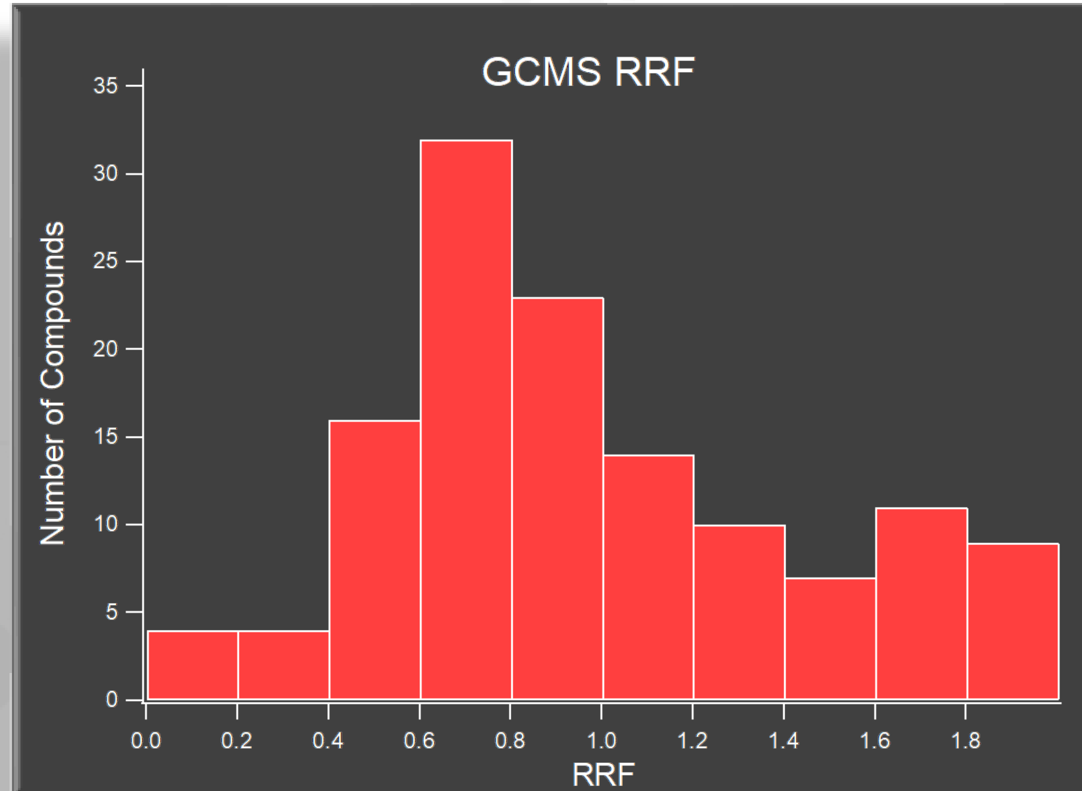
- Measures ionized molecules
- Response is proportional to mass of analyte reaching the detector
- Measures volatile and semi-volatile species

Attributes

- Wide Applicability
- Excellent Identification Capabilities
- Linear
- Moderate precision (<20%)
- Nanogram sensitivity

GCMS – Normalized RRF Factors

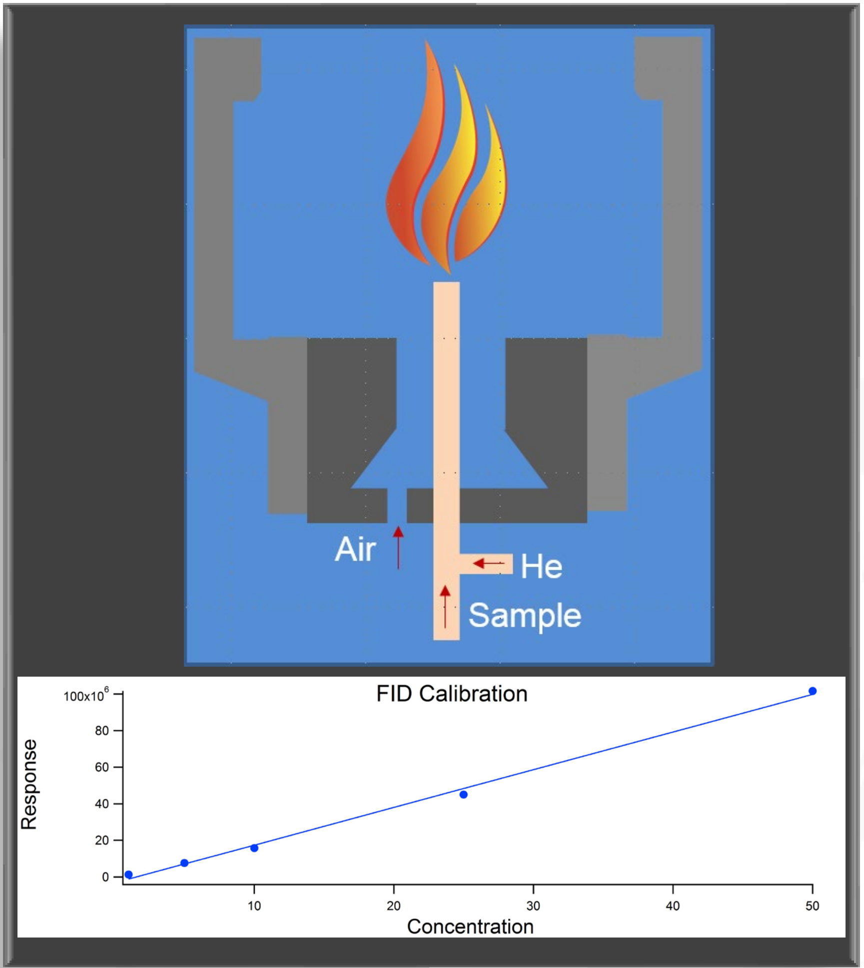
RRF Distribution Information	
Average	1.00
%RSD	52%
Min	0.032
Max	2.84
Percent Detected	59%
UF	2.1



71% of compounds observed by GCMS are seen in LCMS!

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

GC-FID Detection



Principles of Detection

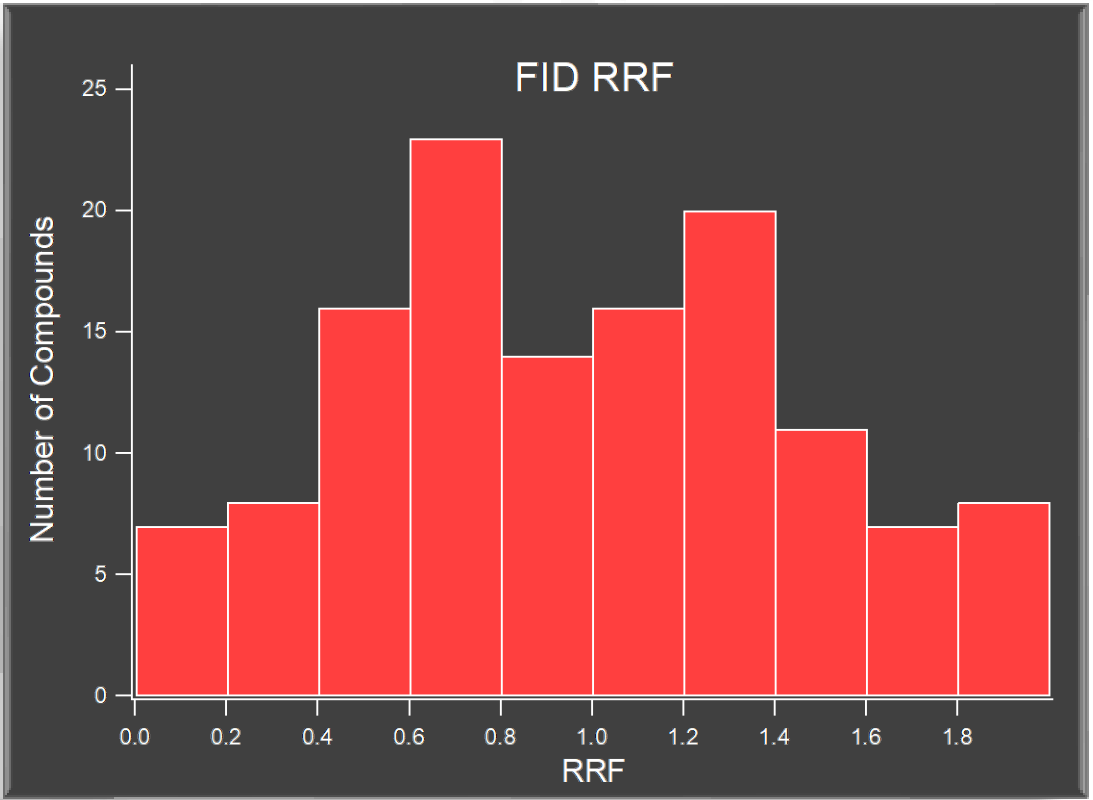
- Measures ion current produced in a flame
- Response is proportional to mass of carbon
- Measures total volatile carbon

Attributes

- Linear response
- Highly precise (<5%)
- Not subject to matrix effects
- Widely applicable
- Picogram C/sec
- Linear dynamic range of 10^7

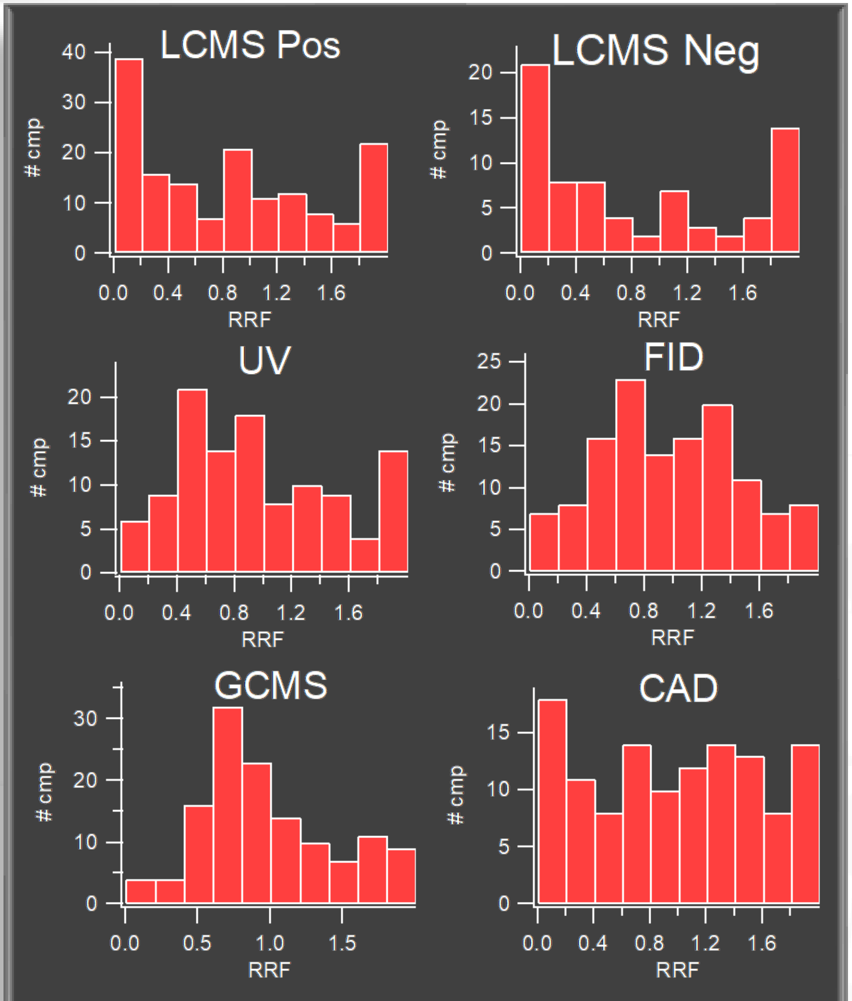
FID – Normalized RRF Factors

RRF Distribution Information	
Average	1.00
%RSD	54%
Min	0.010
Max	3.12
Percent Detected	59%
UF	2.2



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

The RRF Distributions



There is no universal detector!

- Synergies between detectors
  - Enhance Detection
  - Mitigate RF variation
  - Reduce Under Reporting

RRF %RSD	
LCMS POS	108%
LCMS Neg	119%
UV	60%
CAD	65%
GCMS	52%
FID	54%

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Multidetector Approach: Putting it All Together

Detector Synergy Mitigates AET Underreporting

Multidetector AET Approach							
Name	Min UF	RRF Values					
		LCMS Pos	LCMS Neg	GCMS	CAD	UV	FID
Bis(2-ethylhexyl) phthalate	1	1.921	ND	1.706	0.802	0.622	1.855
5-Amino-1-pentanol	8	0.126	ND	0.095	2.597	ND	0.663
1,3,5-Triphenylbenzene	∞	ND	ND	ND	0.813	1.940	ND

Min UF = The minimum UF required to detect the compound using only LCMS and GCMS
Colors denote positive detection above AET at UF= 1 using alternative detectors (green) or LCMS/GCMS (blue).

To be identified a compound must:

Have a normalized RRF value greater than 1/UF on any one detector

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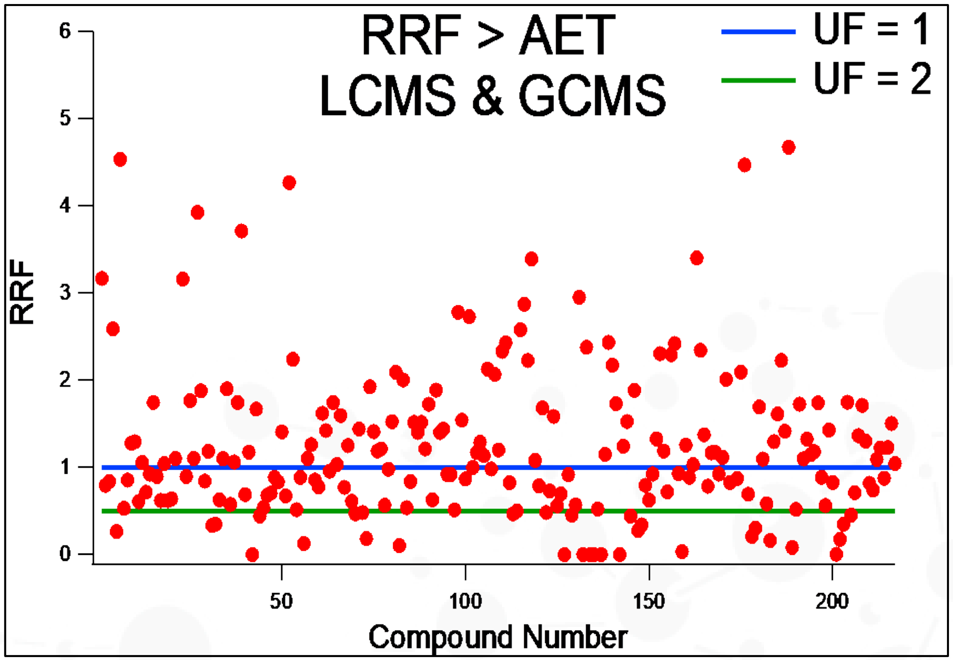
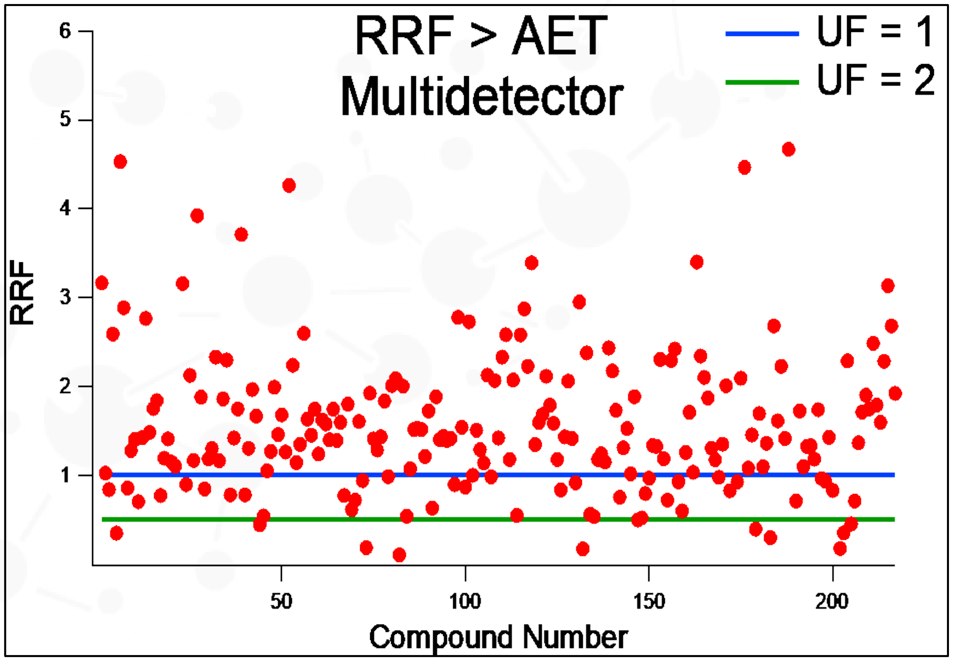
Multidetector Approach: Improved Coverage, Lower UF

94% of Compounds Detected at AET for UF = 2





UF	Percent RF > AET
1	54%
2	85%

UF	Percent RF > AET
1	76%
2	94%

Multidetector Strategy: Reducing Underreporting

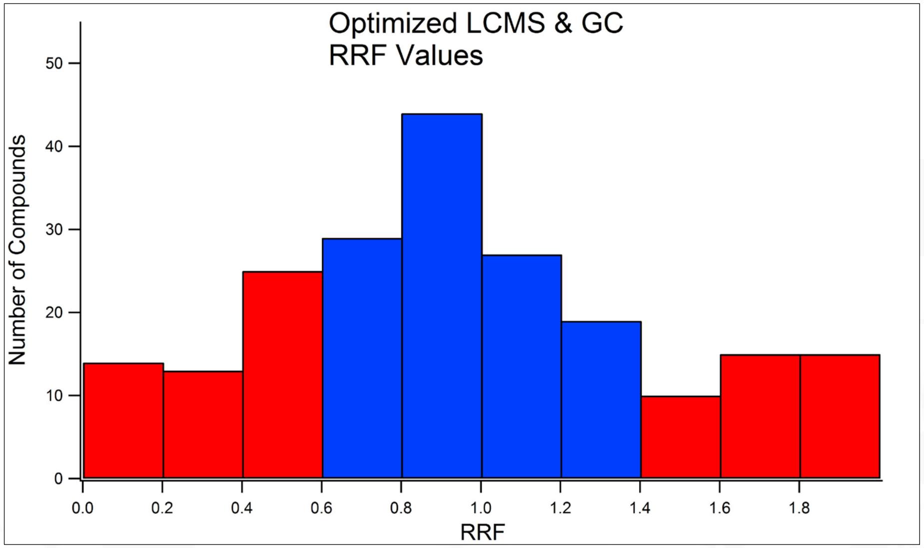
Percent Coverage vs Detector Configuration

Percentage of Compounds Above AET								
Detector	UF = 1		UF = 2		UF = 3		UF = 4	
	GCMS	GCMS-FID	GCMS	GCMS-FID	GCMS	GCMS-FID	GCMS	GCMS-FID
LCMS	54%	63%	85%	86%	91%	92%	93%	93%
LCMS-UV	63%	70%	90%	91%	95%	95%	95%	96%
LCMS-CAD	62%	71%	90%	91%	95%	95%	96%	96%
LCMS-UV-CAD	69%	75%	93%	94%	97%	97%	97%	97%

Optimized Detector Selection: Solution to Problem 2

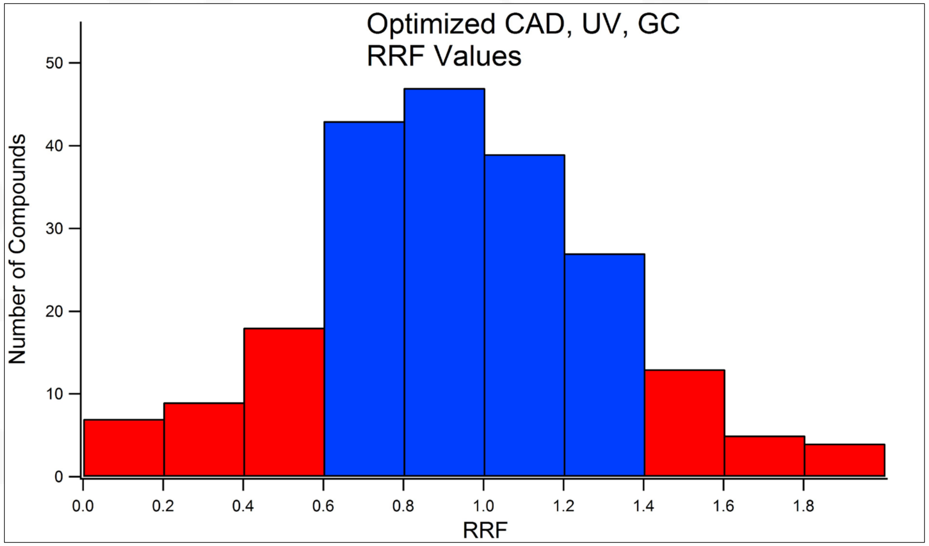
Improved Quantitative Accuracy

LCMS/GCMS Only



33% are within +/- 20% of true value
55% are within +/- 40%

UV/CAD + GCMS/FID



40% are within +/- 20% of true value
72% are within +/- 40%

Only 7% of compounds are significantly under reported.

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Optimized Standard Selection: Solution to Problem 2

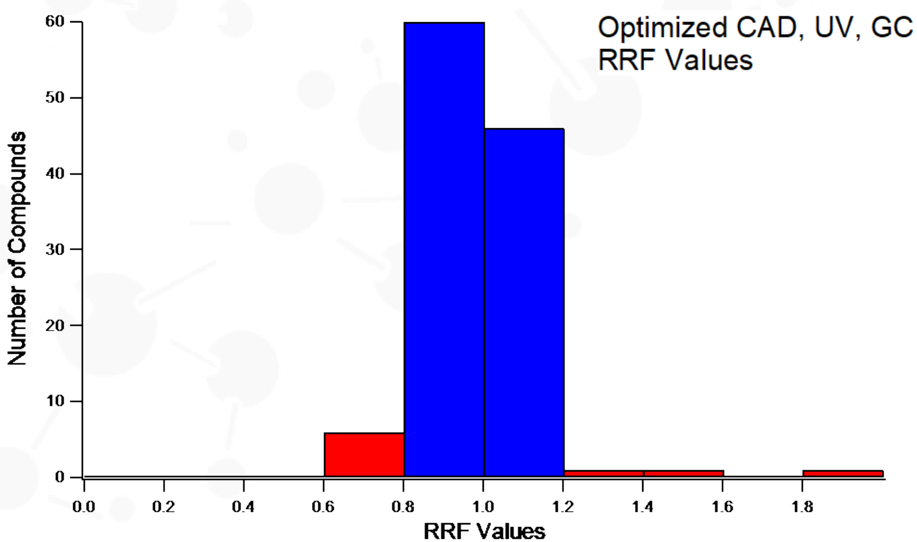
Improved Quantitative Accuracy

Standard	Designation	UV Peak Area
Bisphenol A	STD1	121.007
Irganox 245	STD2	42.079

Compound	UV Peak Area	RF1 STD1	RF2 STD2
Bisphenol A diglycidyl ether	100.827	0.833	2.396
Irganox 1010	38.569	0.319	.917

Blue equals optimum standard selection

UV/CAD + GCMS/FID



85% are within +/- 20% of true value
91% are within +/- 40%

No compounds < 60% of true value!

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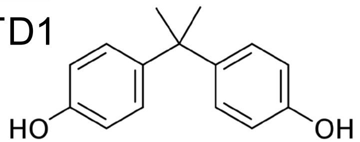
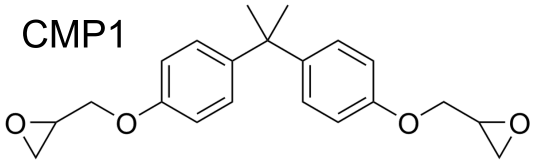
LCMS Relative Quant?

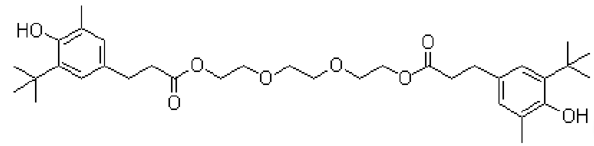
STD1



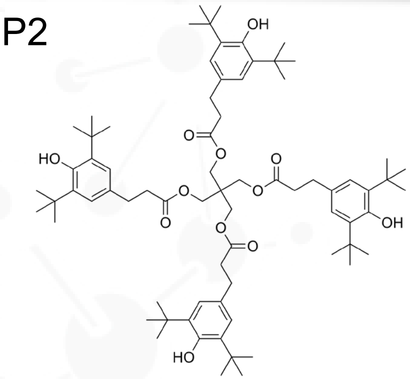
CMP1



STD2



CMP2



Compound	Designation	Slope LCMS POS	Slope LCMS NEG	RRF1 Pos	RRF2 Neg
Bisphenol A	STD1	ND	381.867	?	?
Bisphenol A diglycidyl ether	CMP1	544994.457	ND		

Compound	Designation	Slope LCMS POS	Slope LCMS NEG	RRF1 Pos	RRF2 Neg
Irganox 245	STD2	855316.4	230966.1	.147	0.024
Irganox 1010	CMP2	125698.8	5524.5		

<14.7% of the true value!

Conclusions

Multidetection: A universal method for the universe of extractables

- Problem 1: Under Reporting at AET
  - Weak detector response on one detector is overcome by stronger response of a different detector
  - UF of 2 gives 94% coverage for the combination of GCMS/FID, LCMS-UV-CAD
  - Equivalent to UF of 10 for LCMS/GCMS alone
  - Mitigates the negative effects of a large UF
  - RF Databases need to validate proper standard selection and method specific UF values
  - Verification of method sensitivity at AET is essential

Conclusions

- **Multidetector Solution to Problem 2: Quantitative Accuracy**
  - Use Detectors with less RF variation – UV/CAD
  - Use appropriate surrogate standards

- 85% of compounds were withing 20% of true value
- No compounds <60% of accurate value
- RF Databases are limited in that they only correct quantitative error for compounds in the database
- UF values do not correct quantitative inaccuracy
- E&L Labs should confirm the ability of their method to obtain reasonable accuracy for a range of compounds not in their database.

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Reducing Relative Response Factor Variation using a Multidetector Approach for Extractables and Leachables (E&L) Analysis to Mitigate the Need for Uncertainty Factors

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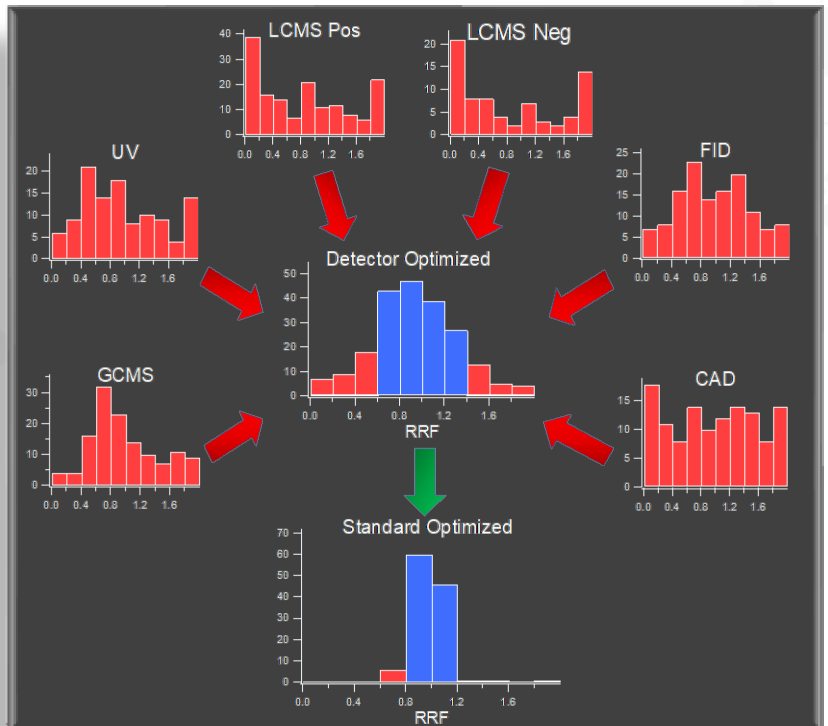

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