The Systematic Study of Response Factor Variation for Extractables and Leachables

Maximizing Quantitative Accuracy using Liquid Chromatography Mass Spectroscopy (LCMS), Ultraviolet (UV) and Charged Aerosol (CAD) Detection

Jordi Labs

Material Solutions. Uncompromising Integrity.

Introduction

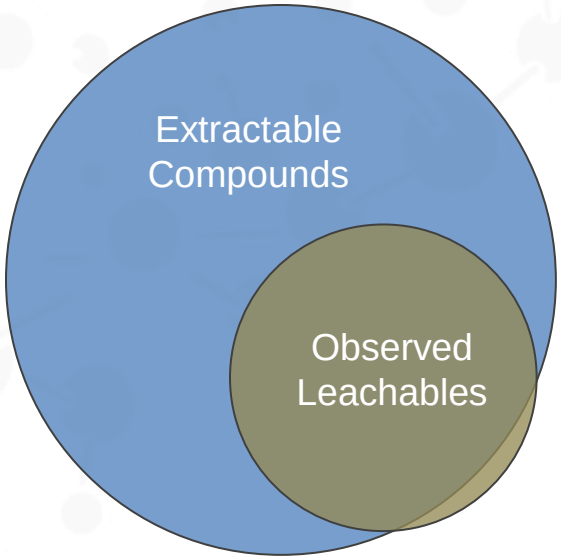
Overview

- Background
  - E&L Standards
    - Quantitation Methods
      - Internal Std
      - Relative
      - Formal
    - The relative quant problem
  - Response Factor Variation
    - Triple Detection (UV, CAD, MS)
    - Irganox Response Factors
      - Response Factor Variation
      - Detector Linearity
      - LOD/LOQ Comparison
    - Variation with Instrument Platform

Extractables and Leachables

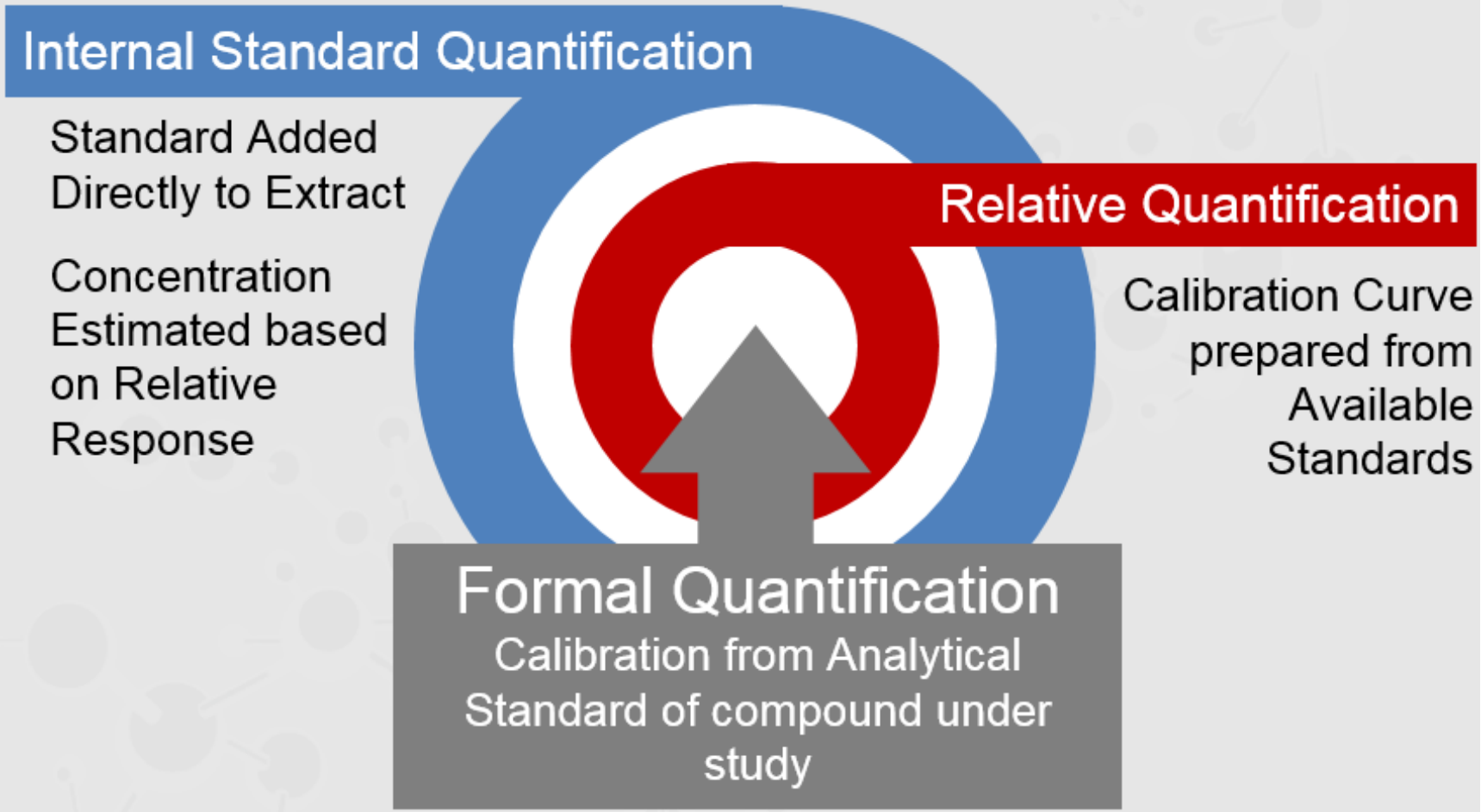
Examples of E&Ls

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



- Standards are not commercially available for many common E&L's

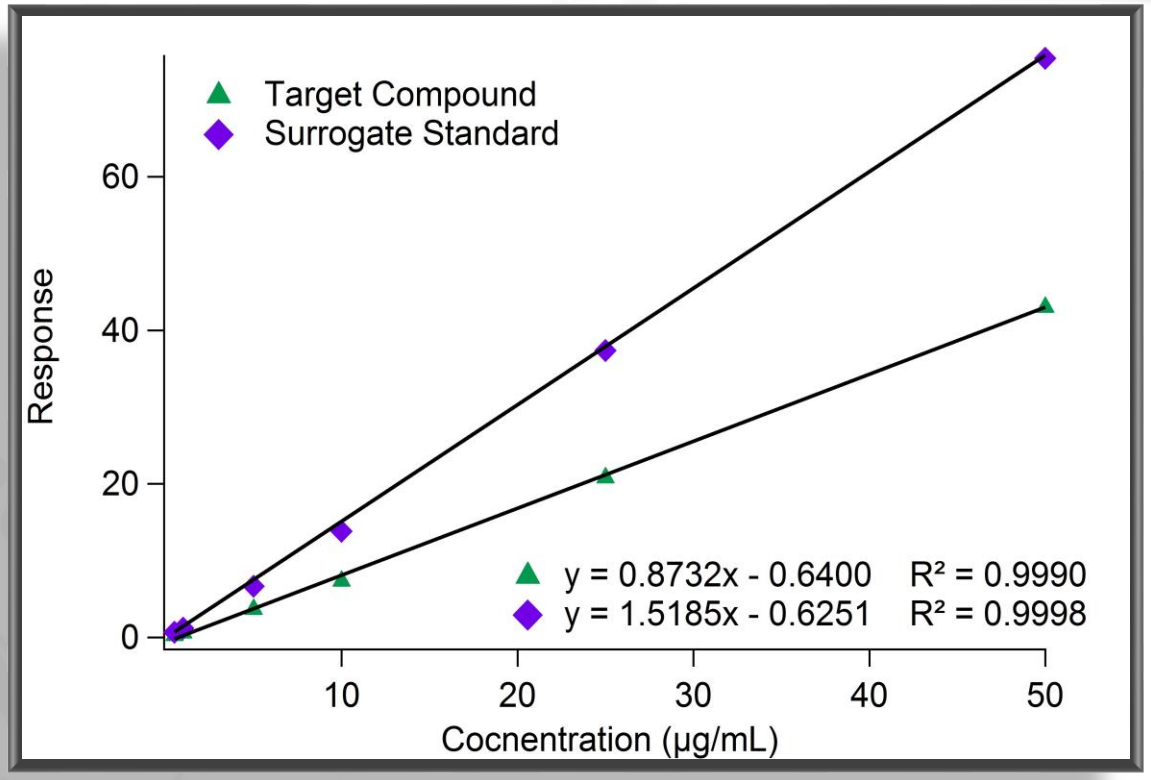
Methods of Quantitation



Response Factor

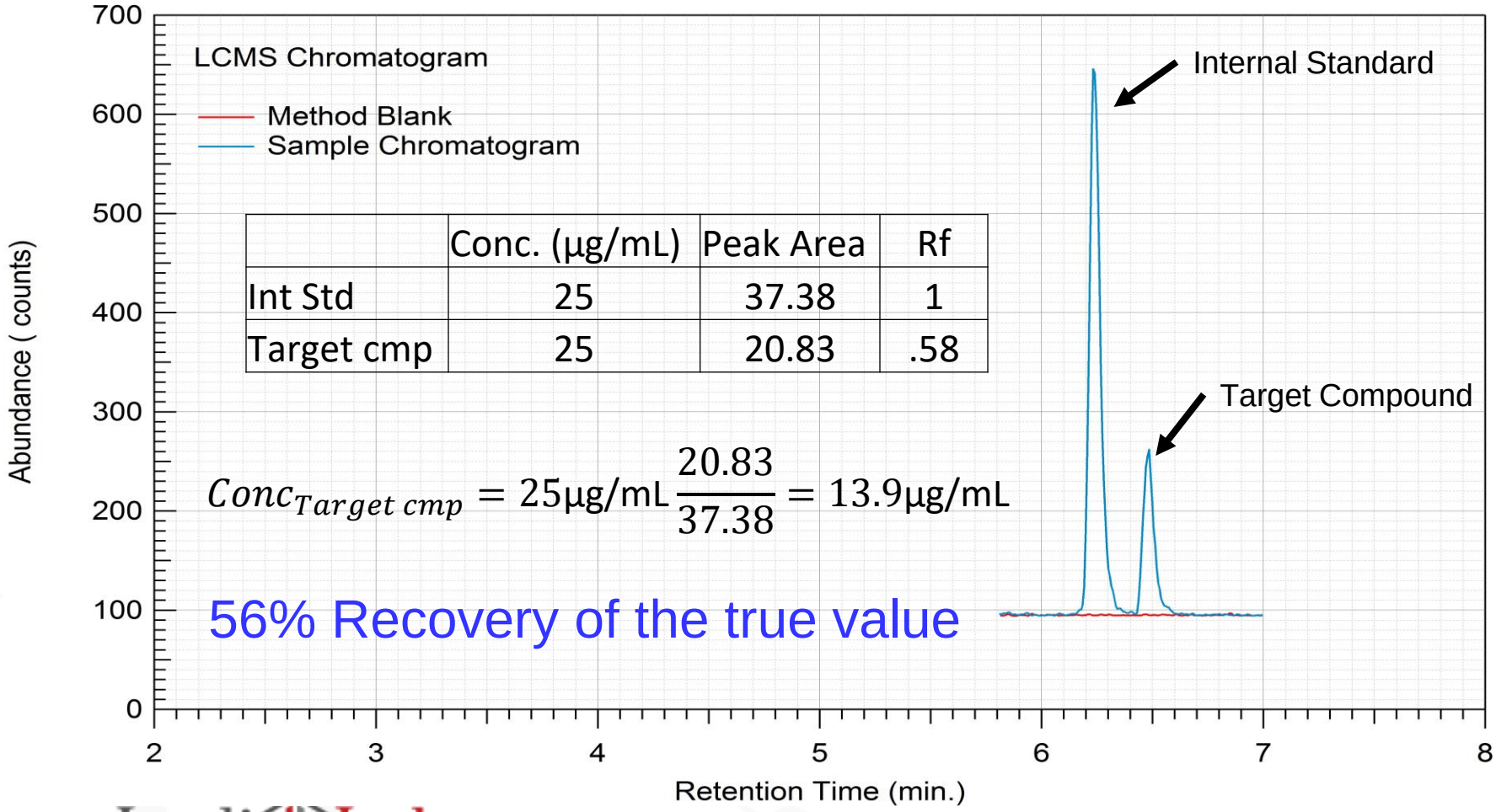
UV Response Factors

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



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

Internal Standard Quantitation



	Conc. (µg/mL)	Peak Area	Rf
Int Std	25	37.38	1
Target cmp	25	20.83	.58

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

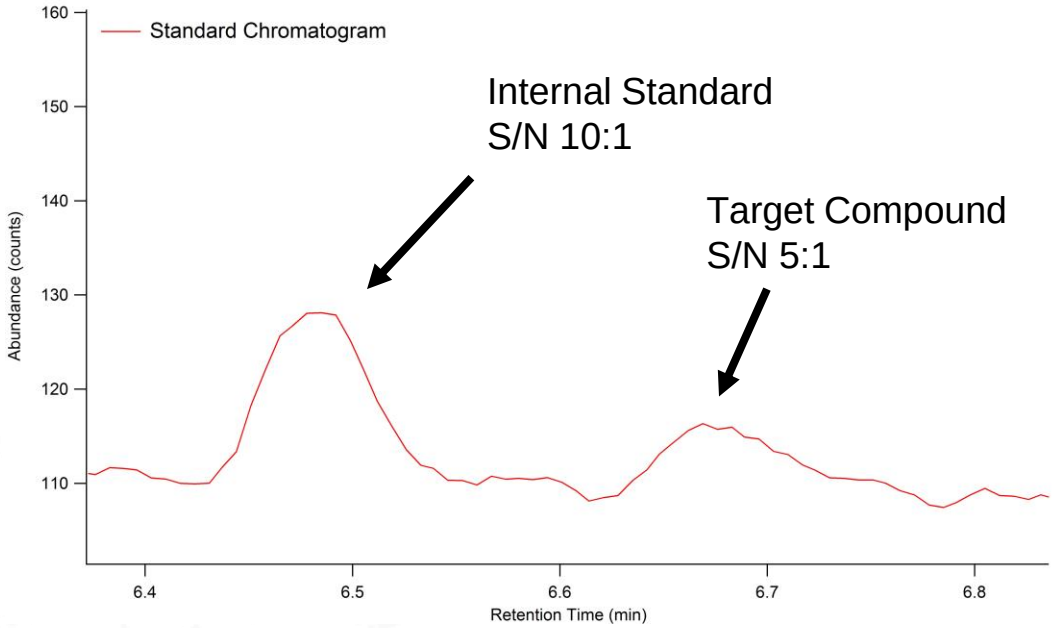
56% Recovery of the true value

Internal Standard Quantitation

$$\text{Conc} = 25\mu g/mL \frac{Peak\ Area}{37.38}$$

LOD is determined using the signal to noise ratio.

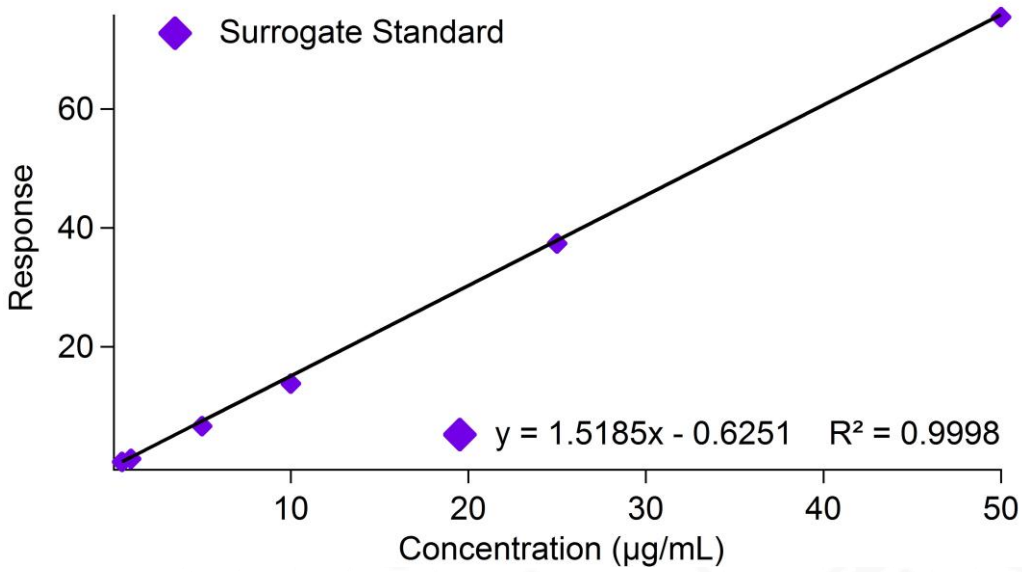
Any positive peak area can be used in the above equation to calculate a value no matter how small.



A 10 ppb LOQ would require a peak area of .015 which is below the noise level.

This method of calculation effectively assumes an infinite LOD.

Relative Quantitation



	Actual Conc. (µg/mL)	Peak Area
Target cmp	25	20.83

$$Conc = \left(\frac{Peak\ area - intercept}{slope}\right)$$

$$Conc_{Target\ cmp} = \left(\frac{20.83 + .6251}{1.5185}\right) = 14.1\ \mu g/mL$$

56% Recovery of the true value

Internal Standard vs Relative Quantitation

$$\text{Conc} = 25\mu g/mL \frac{Peak\ Area}{37.38}$$

$$= \frac{Peak\ Area}{.669}$$

$$Conc = \left(\frac{Peak\ area + .6251}{1.5185}\right)$$

Internal Standard Quantitation

Peak Area	Conc.	Calc. conc	Abs. Error	% Recov.
0.3	0.5	0.2	0.3	40
0.66	1.0	0.4	0.6	44
3.655	5.0	2.4	2.6	49
7.25	10.0	4.8	5.2	48
20.83	25.0	13.9	11.1	56

Relative Quantitation

Peak Area	Conc.	Calc. conc	Abs. Error	% Recov.
0.3	0.5	0.6	0.7	122
0.66	1.0	0.8	1.0	85
3.655	5.0	2.8	3.0	56
7.25	10.0	5.2	5.6	52
20.83	25.0	14.1	11.7	57

The intercept changes the error magnitude significantly when the peak area approaches the magnitude of the intercept.

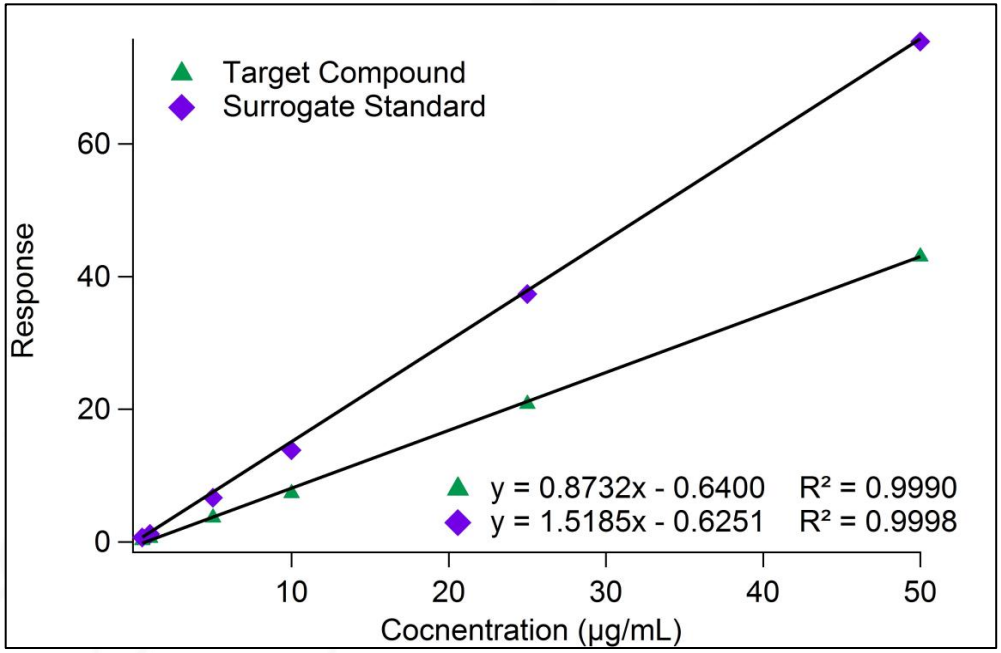
Conclusions for Relative Quantitation

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

Not a worst case estimate
Overestimates LOD

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

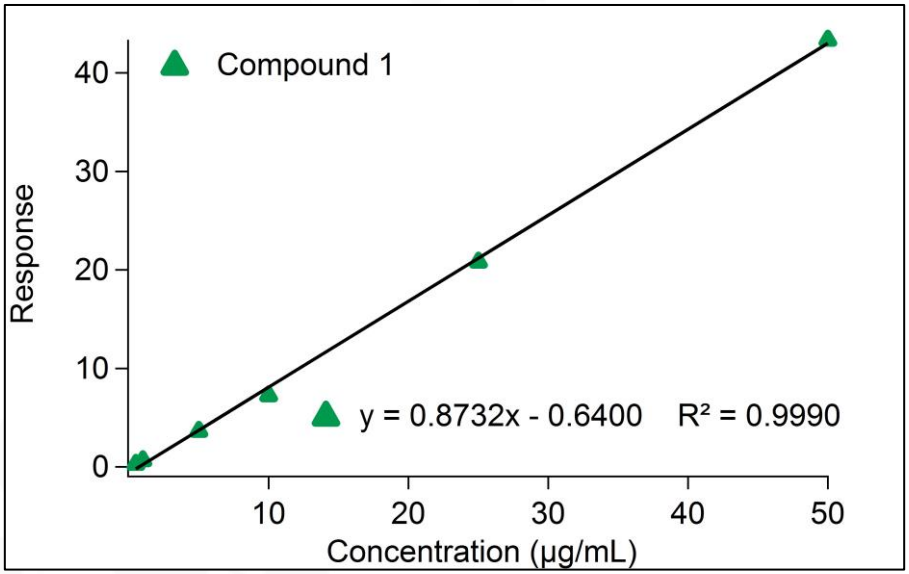
Is a worst case estimate
Underestimates LOD



Quantitation with an internal standard (no curves) provides no proof that AET can be reached for the standard compound.

Formal Quantitation

$$Conc = \left(\frac{Peak\ area - intercept}{slope}\right)$$



Replicate	Actual Conc. (µg/mL)	Calculated Value	Ave. Value	% Recovery
1	5	4.94	4.95%	99%
2		4.90		
3		5.03		

Formal Quant Eliminates Error due to Response Factor Variation

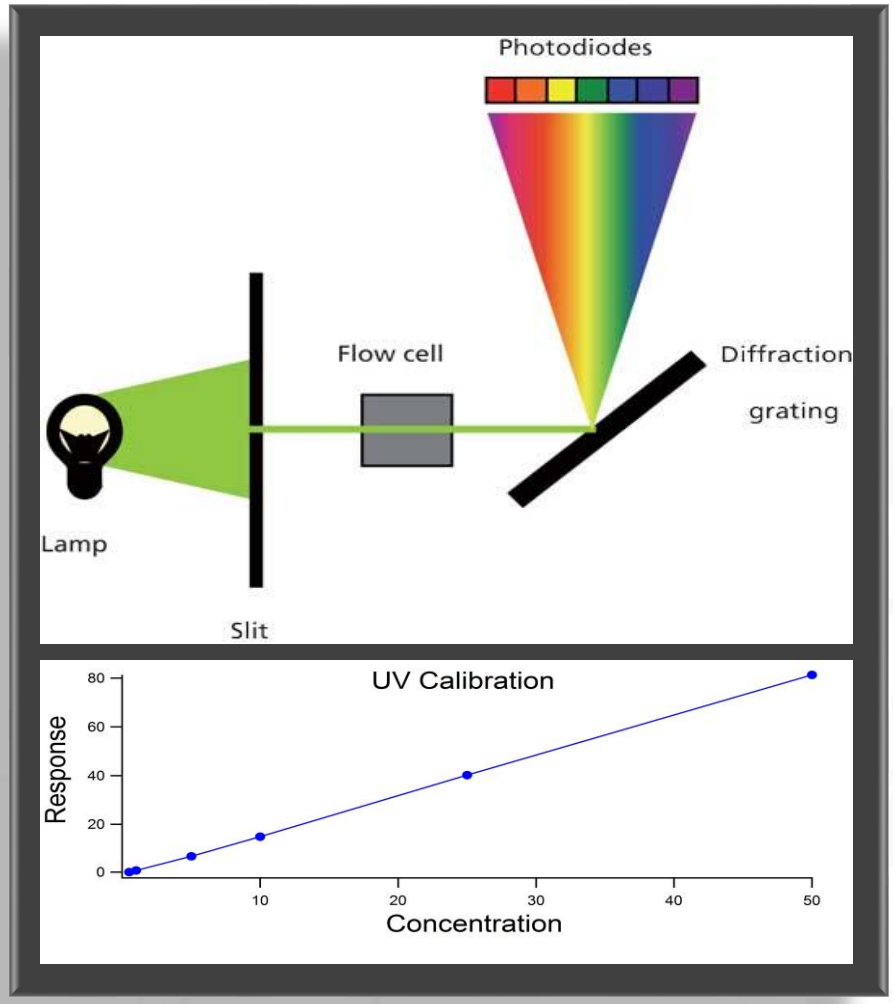
Response Factors for Triple Detection

UV Detection

Charged Aerosol Detection

QTOF LCMS Detection

UV Detection



Detector Attributes

Principle 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

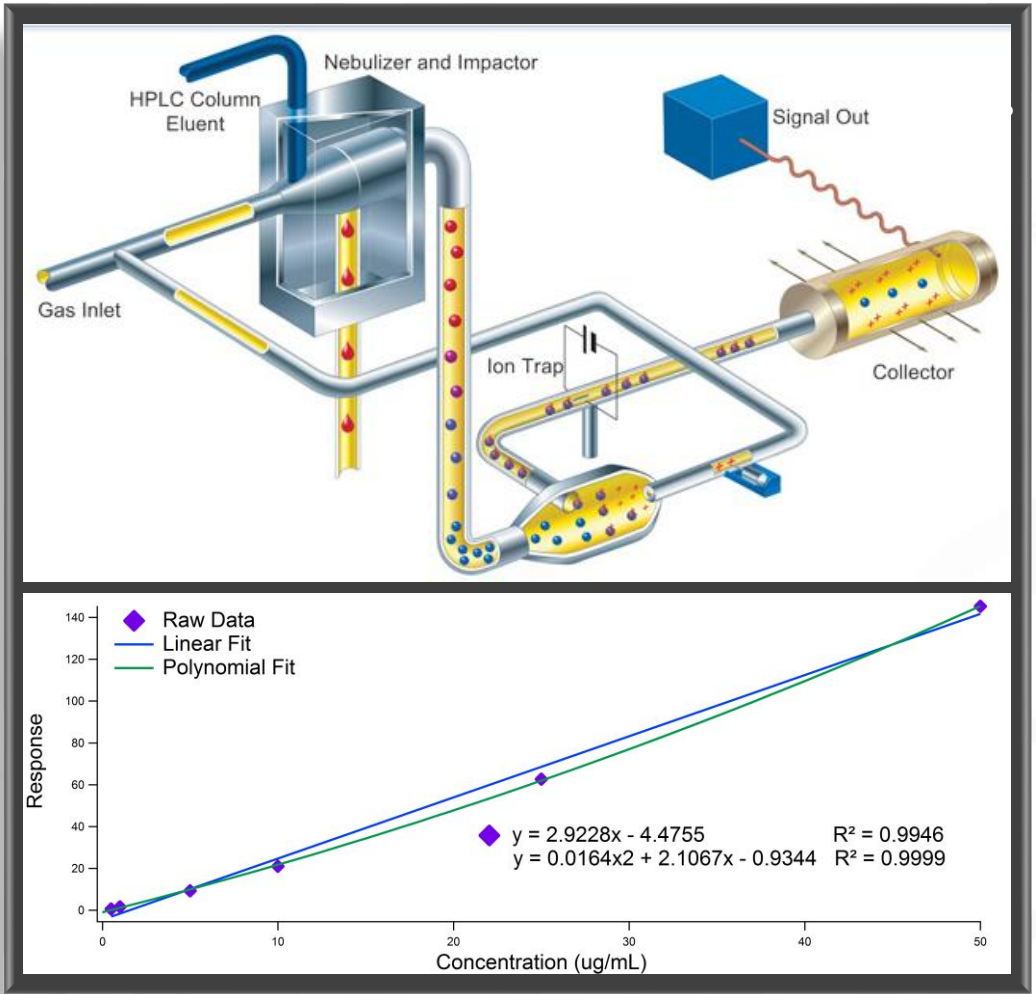
  L = path length

  c = analyte concentration

Attributes

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

CAD Detection



Detector Attributes

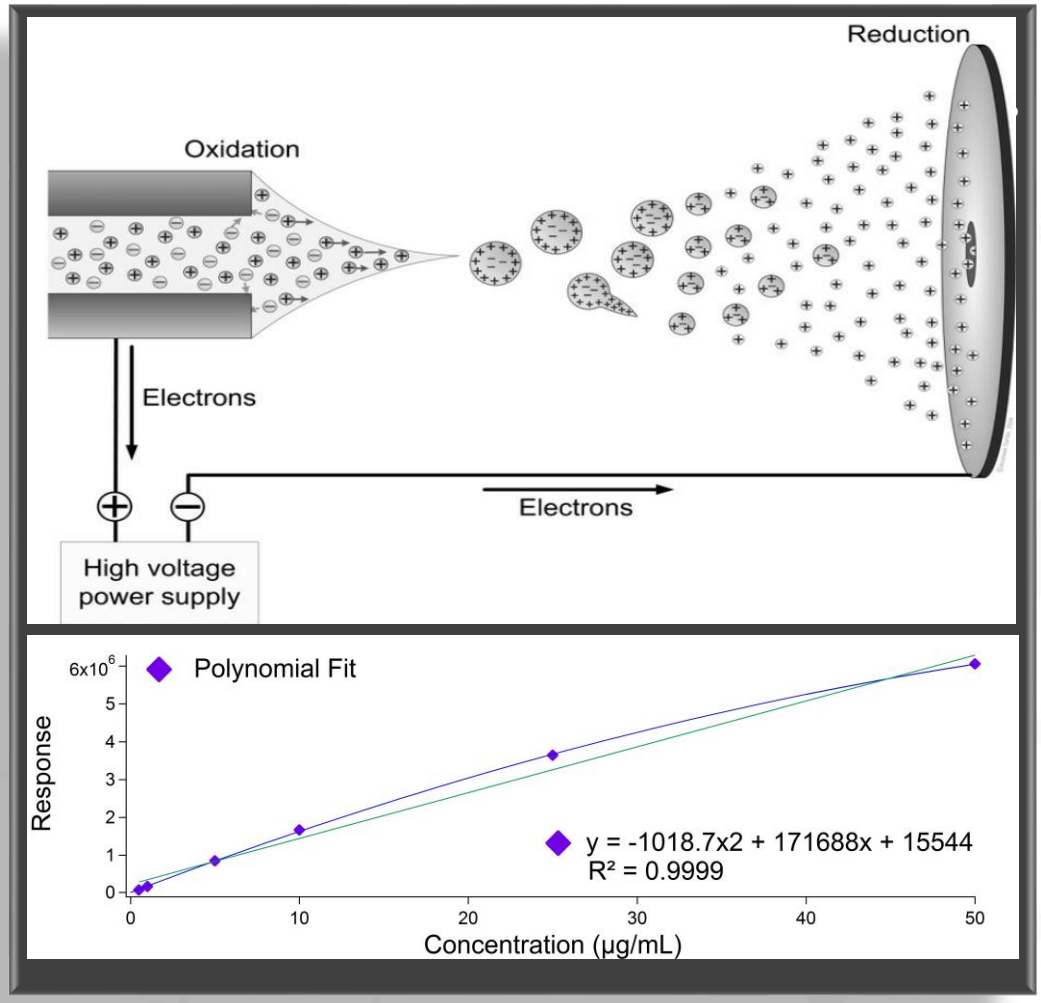
Principle of Detection

- 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 (<5%)
- Not subject to matrix effects
- Widely applicable
- Nanogram sensitivity
- Affected by Mobile Phase Composition

ESI-MS-TOF Detection



Detector Attributes

Principle of Detection

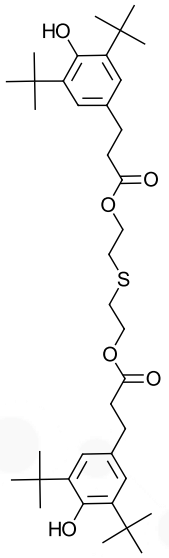
- Measures charged molecules
- Proportional to mass of analyte reaching the detector but behaves as a concentration detector due to loss during nebulization
- Measures only species which can associate with the charge carrier

Attributes

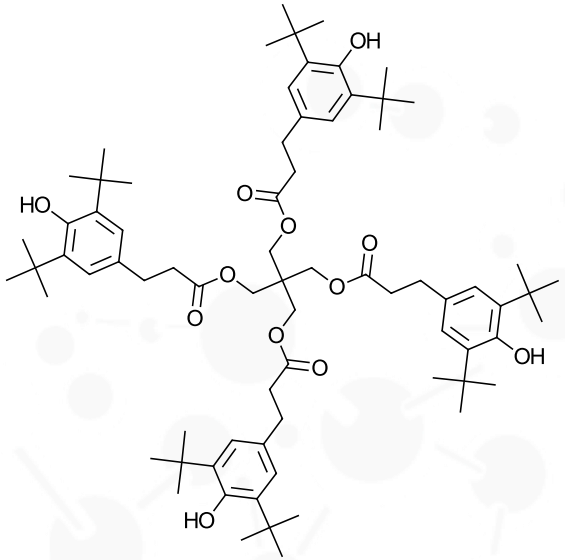
- Polynomial curve
- Moderate precision (<20%)
- Subject to matrix effects
- Applicable only to heteroatom containing species
- Picogram sensitivity

Response Factors for Related Polymer Antioxidants

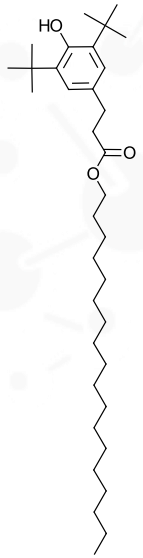
Irganox 1035



Irganox 1010

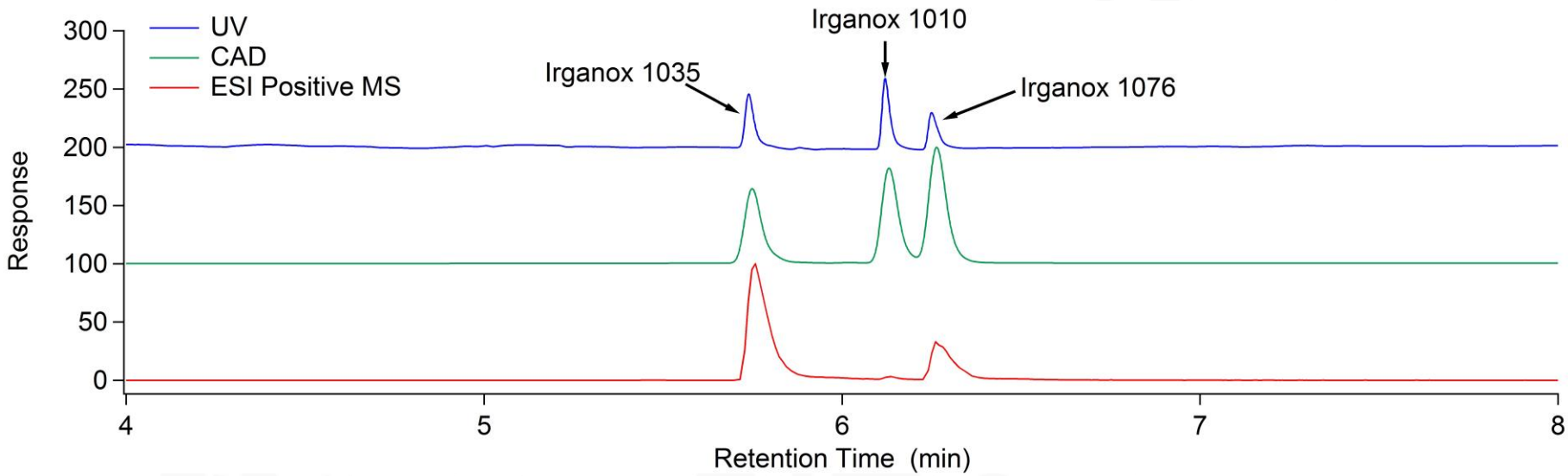


Irganox 1076



Standard	Rt	MW	Boiling point (°C)	Log P
Irganox 1035	5.678	642	665	8.7
Irganox 1010	6.063	1177	1005	14.4
Irganox 1076	6.193	531	568	13.9

Triple Detection Chromatogram



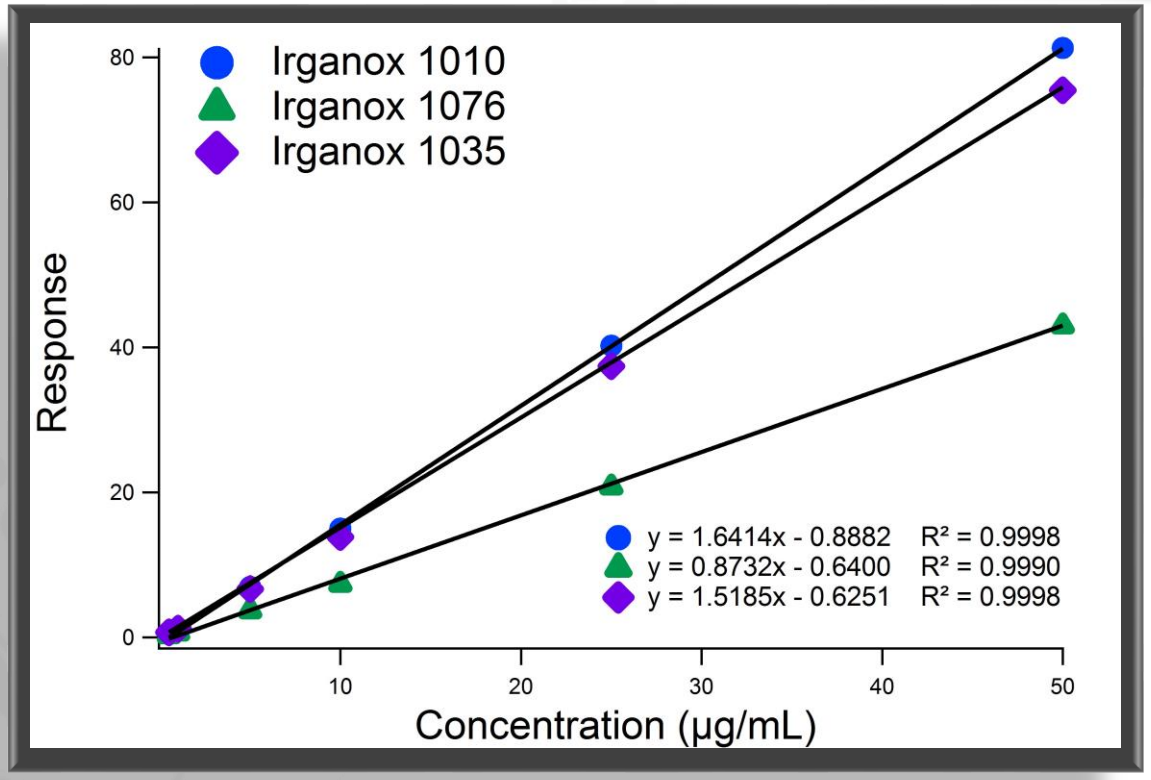
- MS response shows much greater variability than CAD or UV

UV Detection

UV Response Factors

Standard	Rf
Irganox 1035	1.00
Irganox 1010	1.08
Irganox 1076	0.58

- Linear response results in a consistent Rf vs conc.
- Molar Absorptivity determines Rf
- Rf scales with the # of chromophores per unit mass for equivalent chromophores

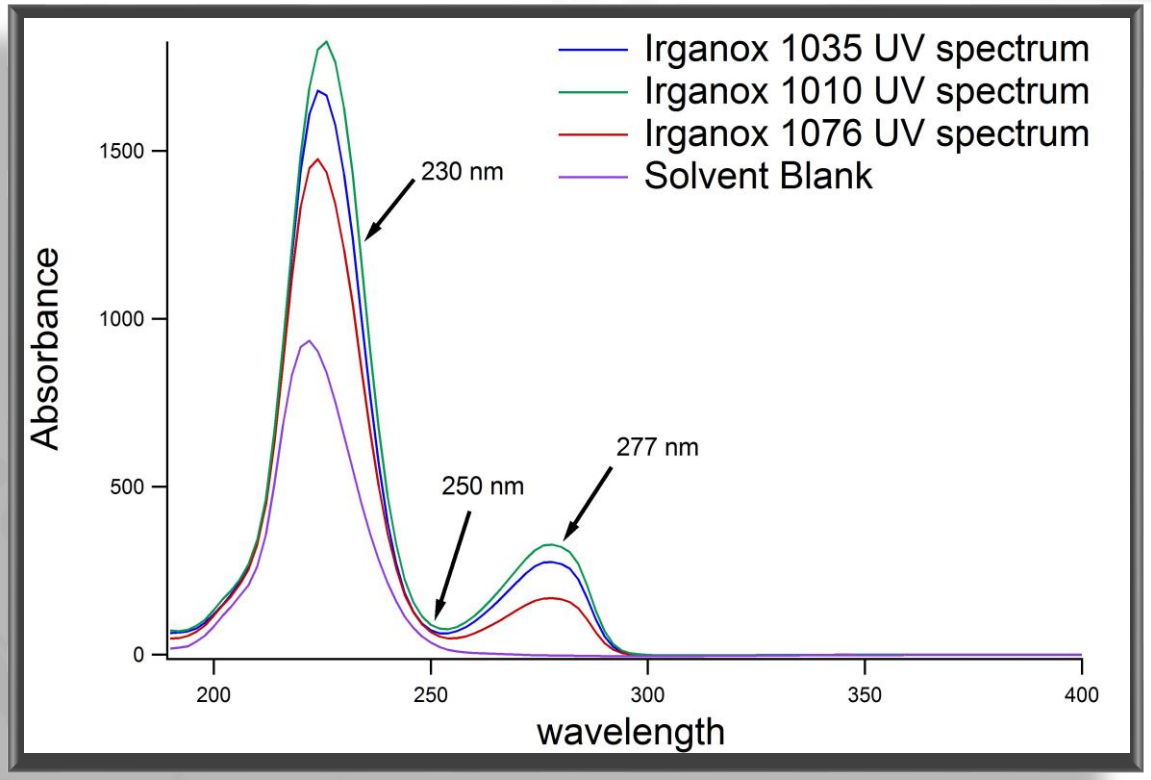


Standard	Mw	Chromophores	Mw/Chrom.	Ratio Mw /Chrom.
Irganox 1035	642	2	321	1.00
Irganox 1010	1177	4	294	1.09
Irganox 1076	531	1	521	0.62

UV Detection – 230 nm

UV Response Factors

- For equivalent chromophores, the response factors remain relatively constant with wavelength
- For different Chromophores, the response factor will vary with wavelength

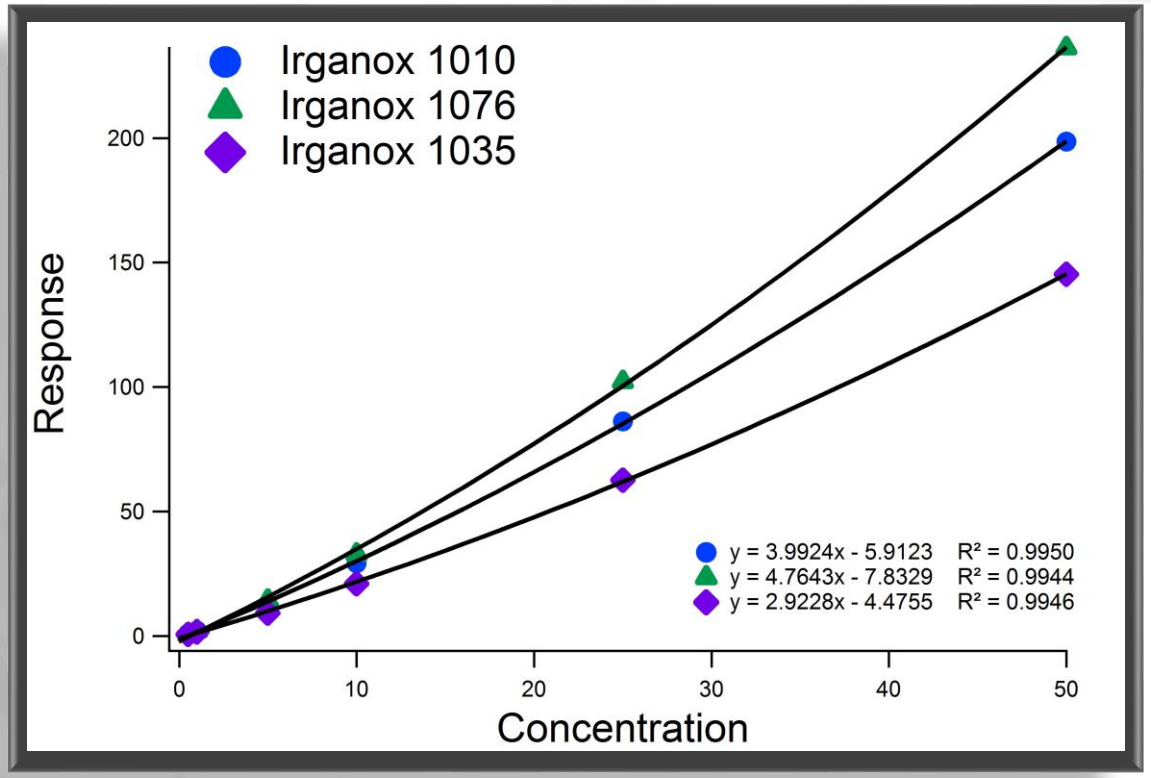


Standard	Rf 230 nm	Rf 250 nm	Rf 277 nm
Irganox 1035	1.00	1.00	1.00
Irganox 1010	1.05	1.08	1.06
Irganox 1076	.61	0.58	0.61

CAD Detection

CAD Response Factors

Conc (ppm)	Irganox		
	1035	1010	1076
.5	1.00	1.51	1.60
1	1.00	1.36	1.35
5	1.00	1.37	1.55
10	1.00	1.39	1.56
25	1.00	1.37	1.63
50	1.00	1.37	1.63
Ave	1.00	1.39	1.55
Std. Dev.	N.A.	0.06	0.11



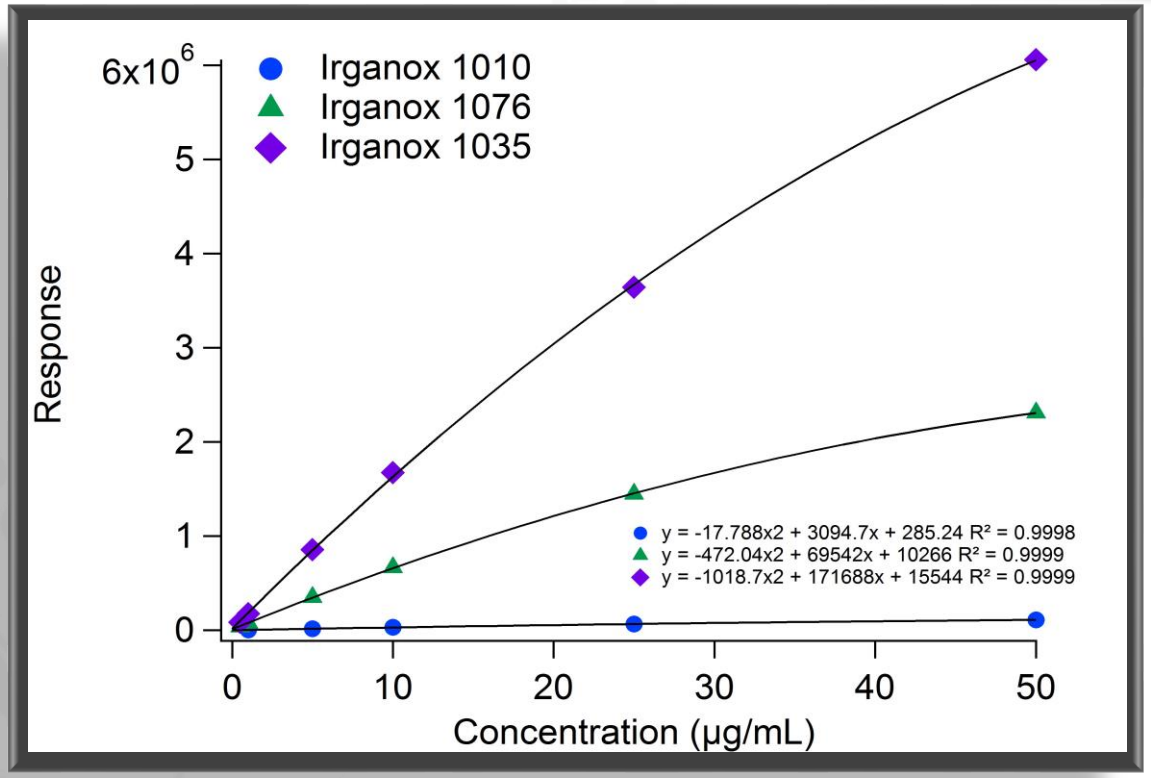
- Rf are stable over the concentration range.

Standard	Boiling Point
Irganox 1035	665
Irganox 1010	1005
Irganox 1076	568

MS ESI Positive Detection

MS Response Factors

Conc (ppm)	Irganox		
	1035	1010	1076
.5	1	<LOD	0.45
1	1	0.017	0.44
5	1	0.018	0.41
10	1	0.018	0.40
25	1	0.018	0.40
50	1	0.018	0.38
Ave	1	0.018	0.41
Std. Dev.	N.A.	0.001	0.03



- Rf varies strongly (56X) even for similar structures
- Non-linear curve results in additional error for internal std quantitation
- Possibility of Matrix effects

Limit of Detection

ID	Comparison of Detector LOD (µg/mL)			
	Quantitation Method	UV 250 nm	MS	CAD
	Irganox 1035	.17	.01	.45
	Irganox 1076	.28	.06	.22
	Irganox 1010	.14	1	.23

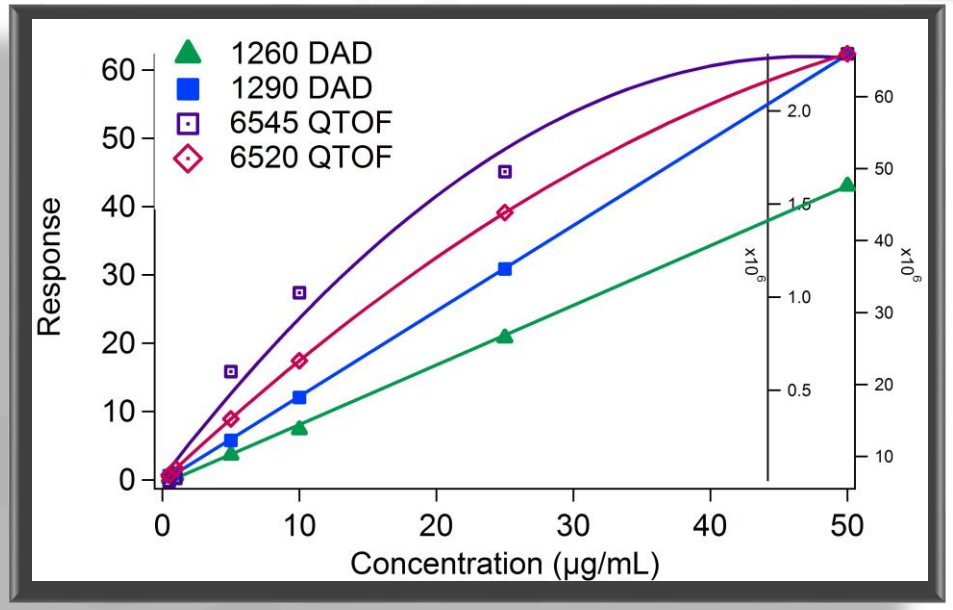
Quantification Results

ID	Comparison of Quant Methods Irganox 1010 (5 µg/mL)				
	Quantitation Method	Int. Standard Irganox 1035	Int. Standard Irganox 1076	Relative 1 Irganox 1035	Relative 2 Irganox 1076
	UV	5.1	9.3	4.9	8.5
	CAD	6.9	4.4	6.2	4.2
	MS	0.1	0.2	< LOQ	0.07

Response Factors (Second Instrument)

Response Factors

- UV Rf changed < 2%
- CAD Rf changed < 11%
- MS Rf showed larger variability and a 60X change in sensitivity



Standard	UV Rf 250 nm		CAD		MS	
Instrument	1260 DAD	1290 DAD	1	2	6520	6545
Irganox 1010	1.08	1.07	1.39	1.24	.018	.554
Irganox 1076	0.58	0.59	1.55	1.54	.41	.47

Sources of Error

Sources of Error

Error Sources	MS	UV	CAD
Response Factor Var.	5000x	200x[1]	50x[2]
Matrix Effects	yes	no	no
Technique Precision	<20%	<5%	<10%
Signal Drift	High	Low	Low
Ion Selection	Yes	No	No
Wavelength Selection	No	Yes	No

[1] Excludes compounds without a chromophore
[2] Excludes highly volatile compounds

Response Factors Database

Preliminary Results

- 94 Extractables
- LCMS-ESI-UV-CAD
- All 94 were detectable by at least one detector
- 1 compound missed without UV
- 4 compounds missed without CAD

Detector	Number Detected	Number Not Detected	Total	Percent Detected	Percent Detected Only by this Detector
MS Pos	80	14	94	85%	1%
MS Neg	40	54		43%	13%
UV 250 nm	44	50		47%	1%
CAD	53	41		56%	4%

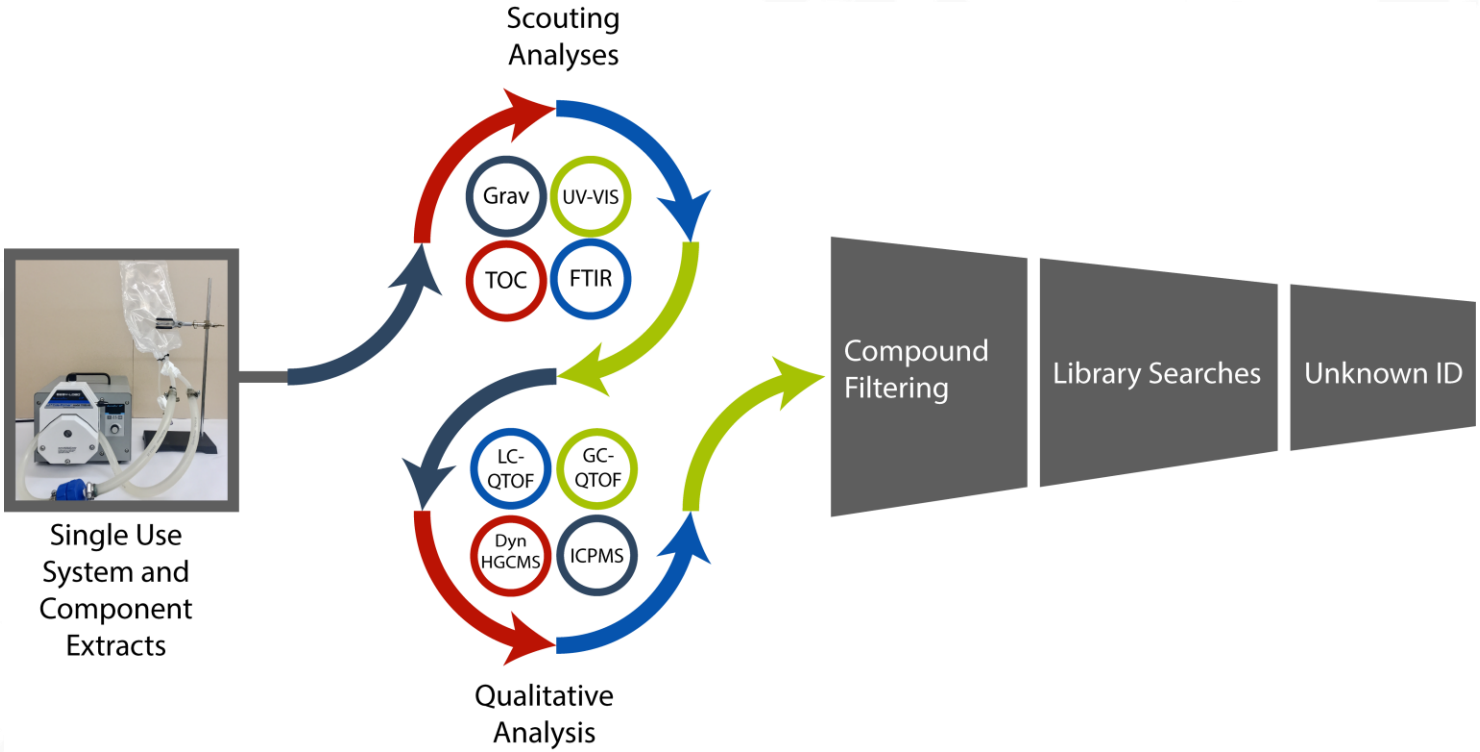
Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

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



Thank You!

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