Assessing Calibration-Related Measurement Bias Near the Limit of Quantitation

Troy Strock[1], Wayne Whipple[1], Steve Reimer[2], Diane Gregg[3]

[1]US Environmental Protection Agency, Region V Laboratory
536 S. Clark St. 10th floor (ML-10C), Chicago IL 60605

[2] US Environmental Protection Agency, Region X Manchester Laboratory
7411 Beach Drive East (LAB), Port Orchard, WA 98366

[3] US Environmental Protection Agency, Region VI Laboratory, Houston Branch
10625 Fallstone Road (6MD), Houston, TX 77099

Disclaimer

e opinions expressed herein are the author's and (due to limited
ne for review and busy schedules) do not necessarily reflect the
nion of the co-authors, let alone the US EPA.

How low can you go?

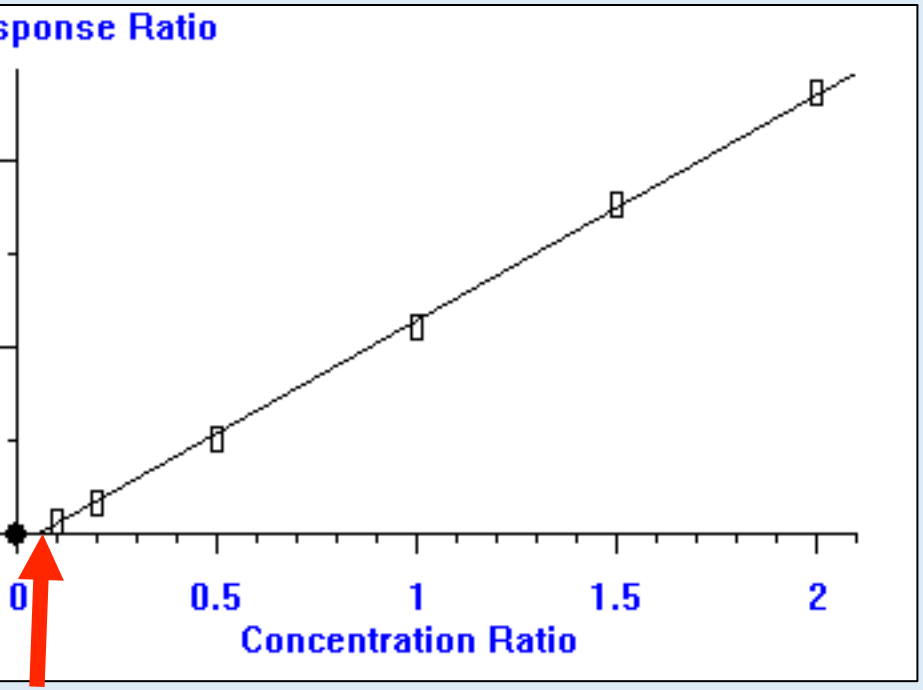
- Measurement sensitivity is an important consideration for many analytical chemistry applications
- Environmental laboratories and instrumentation with lower sensitivity may have a competitive advantage
- However, instrument sensitivity and background in reagents and standards can vary over time, depending on the analyte
- Allowing intercept of calibration function to float (and weighting) enables regression line to fit lowest data points better; However, this can lead to measurement bias in applying the calibration curve, especially when extrapolated outside the calibration range

Definitions

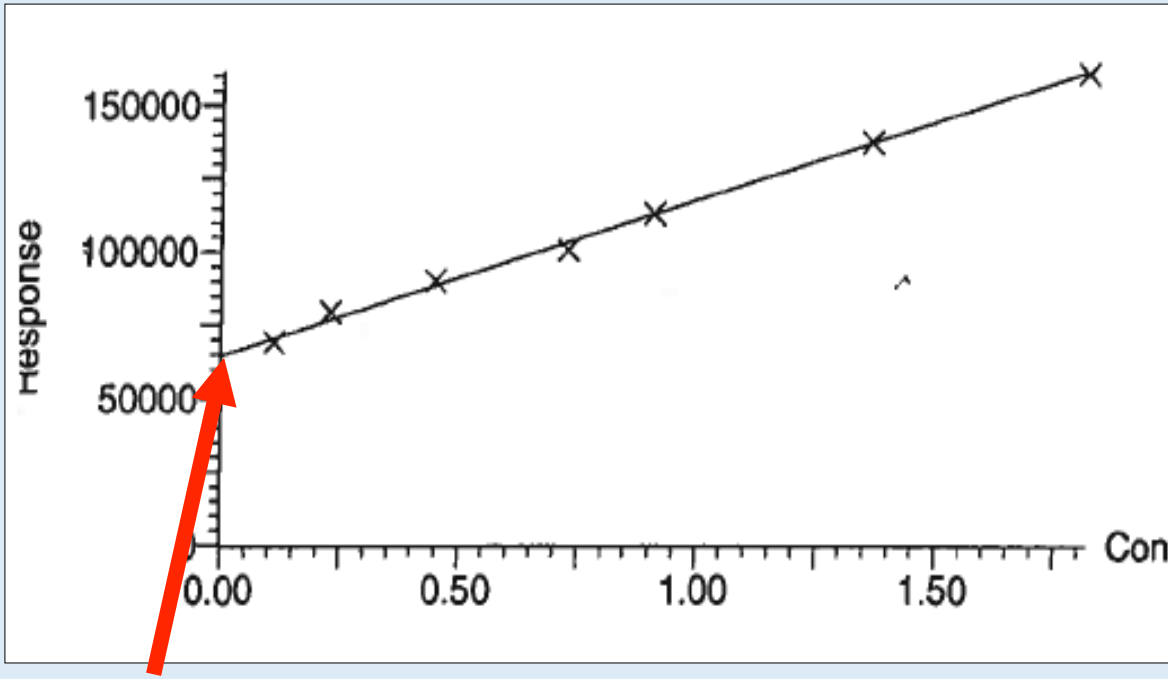
- Calibration—The act of evaluating and adjusting the precision and accuracy of measurement equipment. Instrument calibration is intended to eliminate or reduce bias in an instrument's readings over a range of continuous values. http://www.chemwiki.ucdavis.edu/Analytical_chemistry/Data_analysis/Instrument_Calibration_Over_A_Regime/
- Bias—a systematic error that contributes to the difference between the mean of a large number of test results and an accepted reference value. http://www.astm.org/ILS/precisionbias.html
- Limit of quantitation— The lowest concentration at which the analyte can not only be reliably detected but at which some predefined goals for bias and imprecision are met. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2556583/

Calibration related measurement bias -- Loss of proportionality between measured response and calculated concentration due to application of the calibration model

o types of calibration related measurement bi



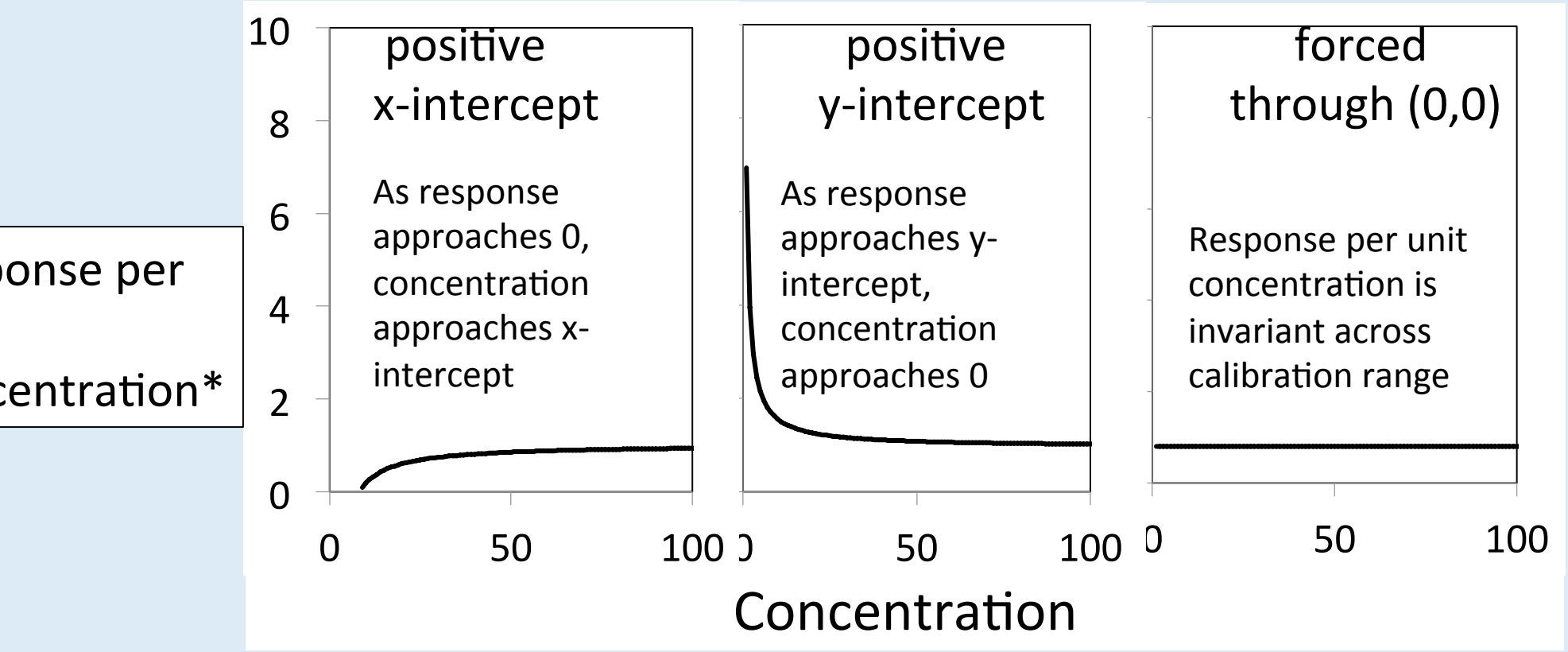
sitive x-intercept: Curve
duces a positive calculated
centration at response = 0



Positive y-intercept: Curve produces a calculated concentration of = 0 at a response > 0

o types of calibration related measurement bi

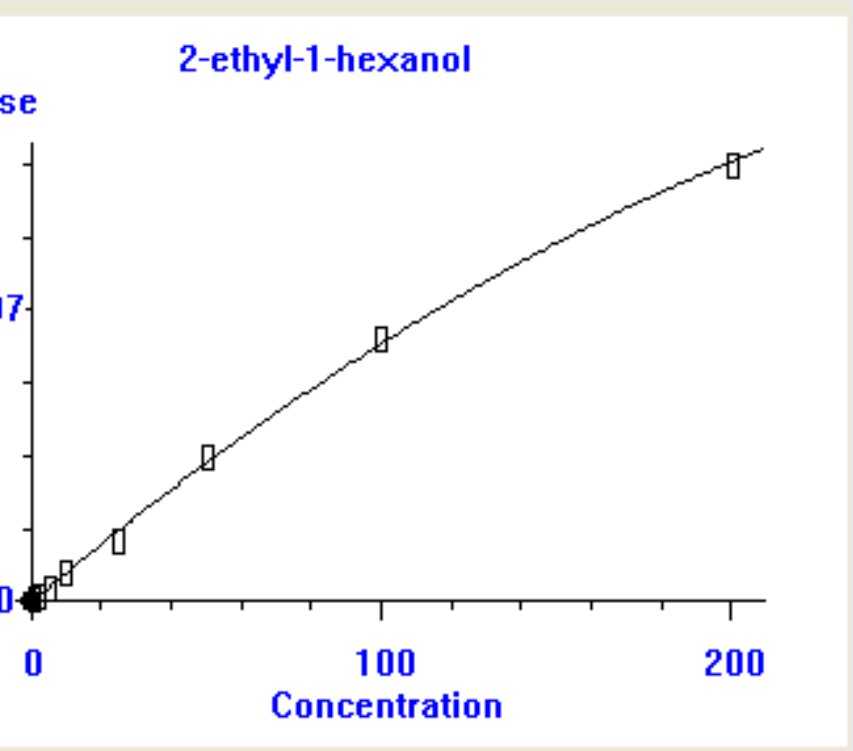
For calibration models that do not pass through (0,0), modeled response per unit concentration is not linear

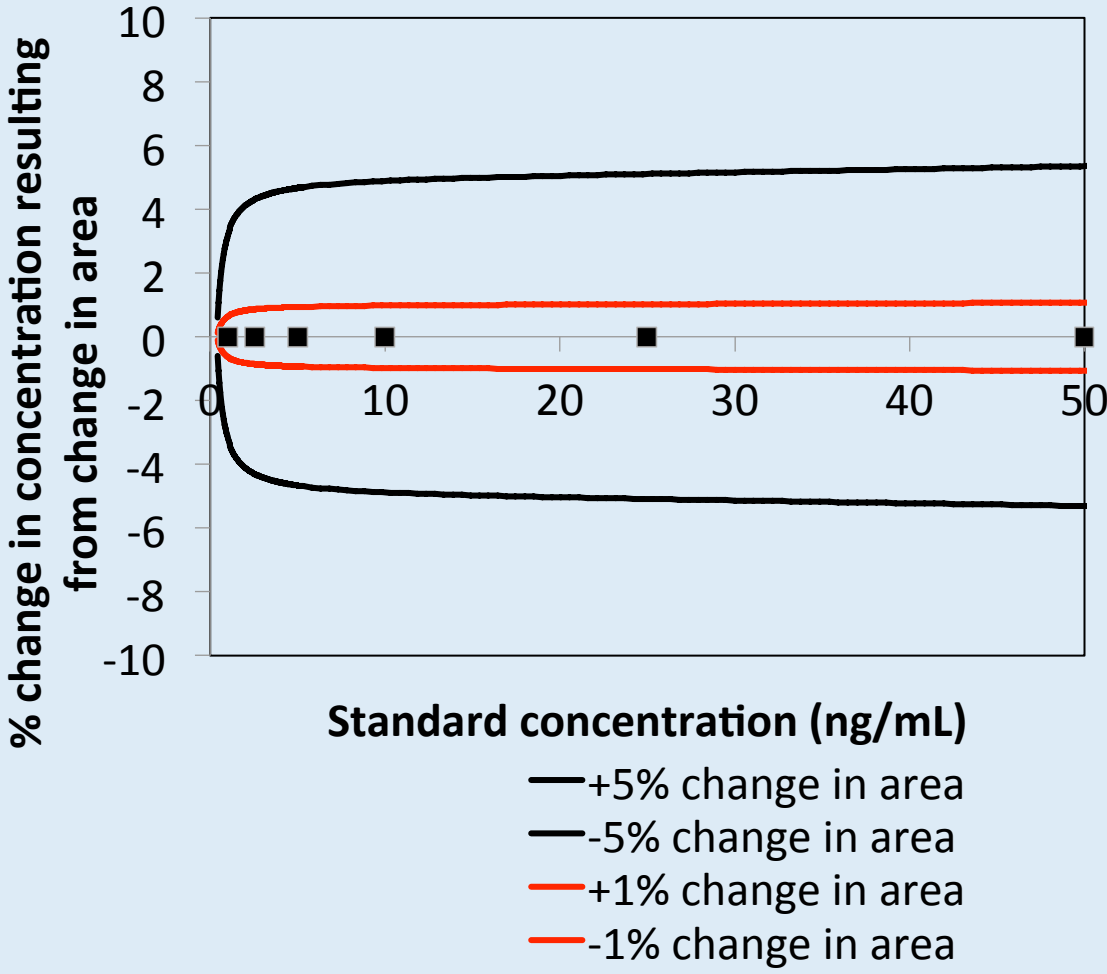


*Recalculated expected response across calibration range based on calibration function, then divide expected response by concentration

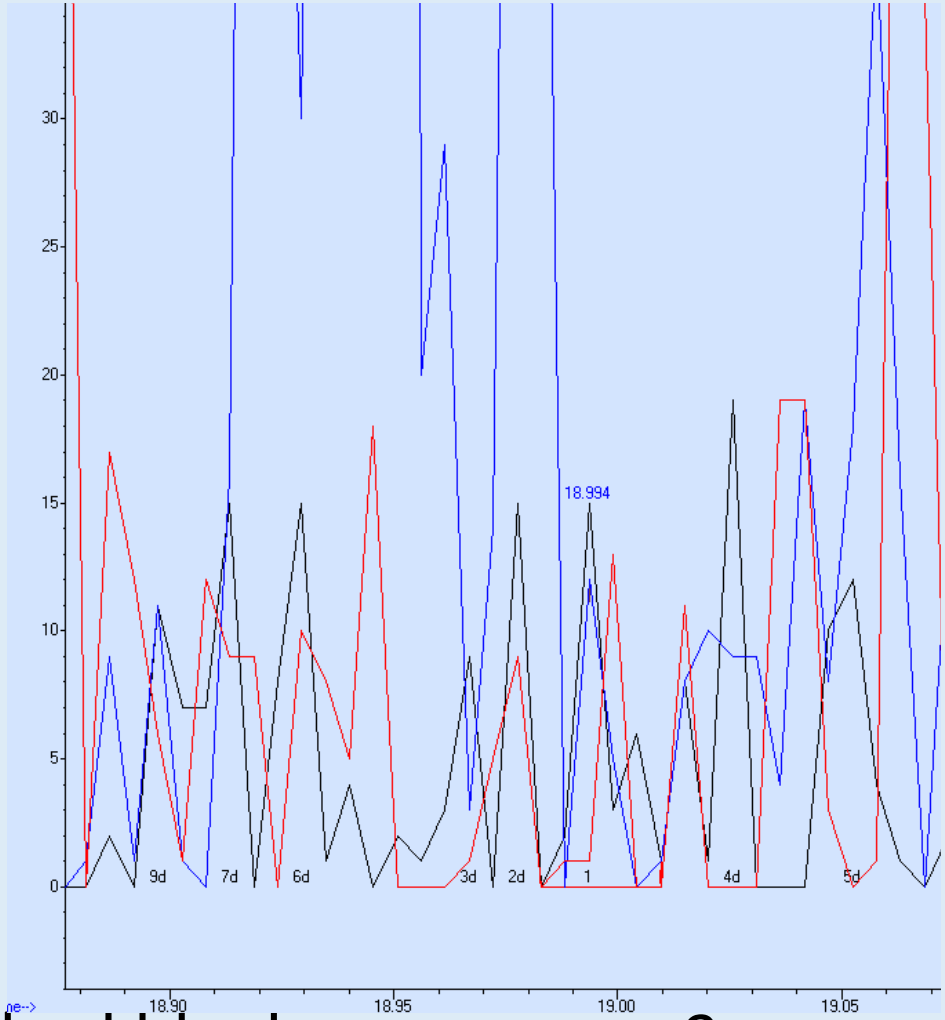
urves with Positive X-Intercepts

2-ethyl-1-hexanol by 5030/8260, 80 deg C
(x-intercept is ~60% of 1 ppb standard concentration)



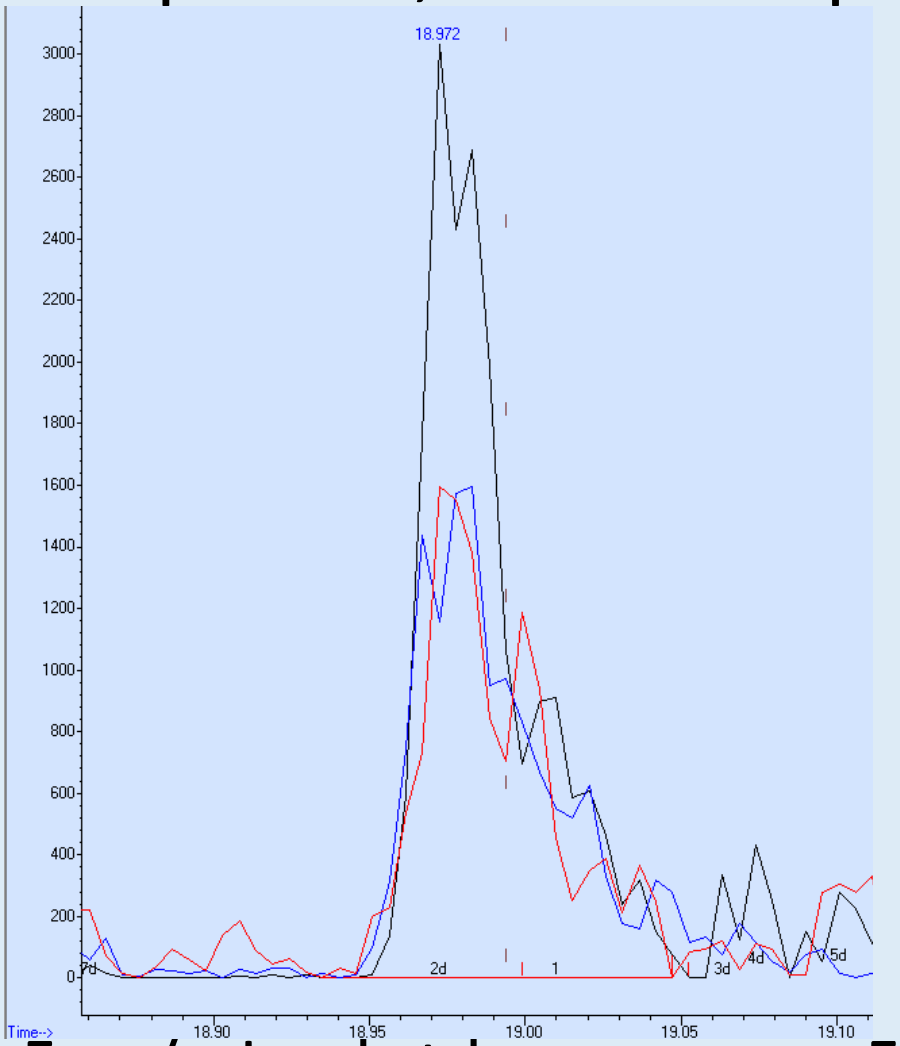


rves with Positive X-Intercepts: 2,4-dintrophe



ethod blank, response = 9

lc concentration = 2.05 ug/mL



2.5 ug/mL cal std, response = 5962

Calc concentration = 3.05 ug/mL

rves with Positive x-Intercepts: Pentachlorophen

Concentration (ug/mL)	Calc RRF
0.5	0.031
1	0.031
2.5	0.056
5	0.076
10	0.093
25	0.119
50	0.128
75	0.137
100	0.134

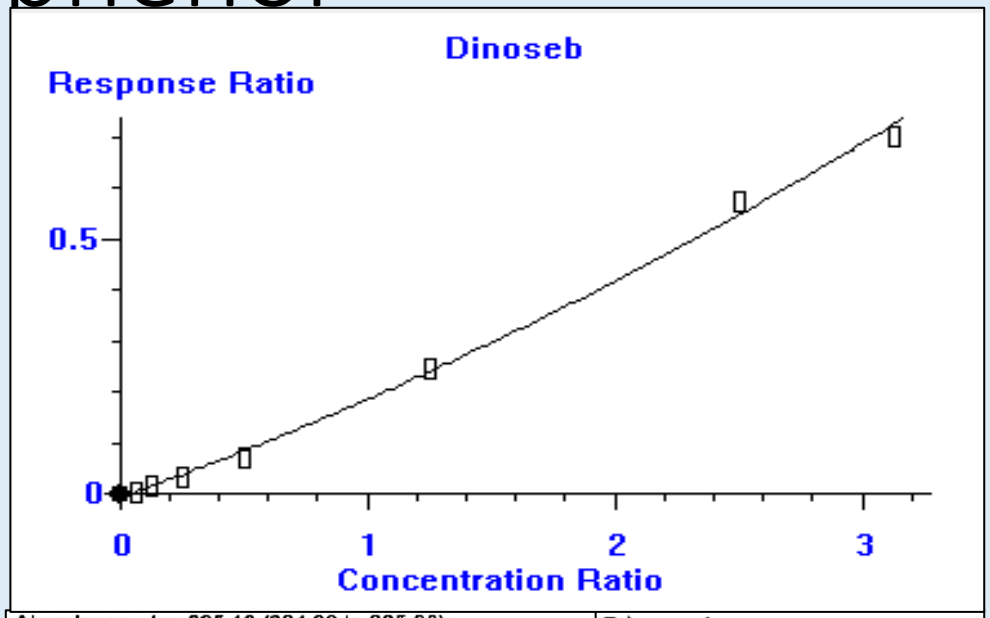
Calibration range (ug/mL)	LLOQ standard* conc (ug/mL)	Blank concentration (ug/mL)	Blank ar low stan area
0.5-100	1	0.80	24%
1-100	2.5	1.28	14%
2.5-100	2.5	2.03	2.1%
5-100	5	2.77	0.7%

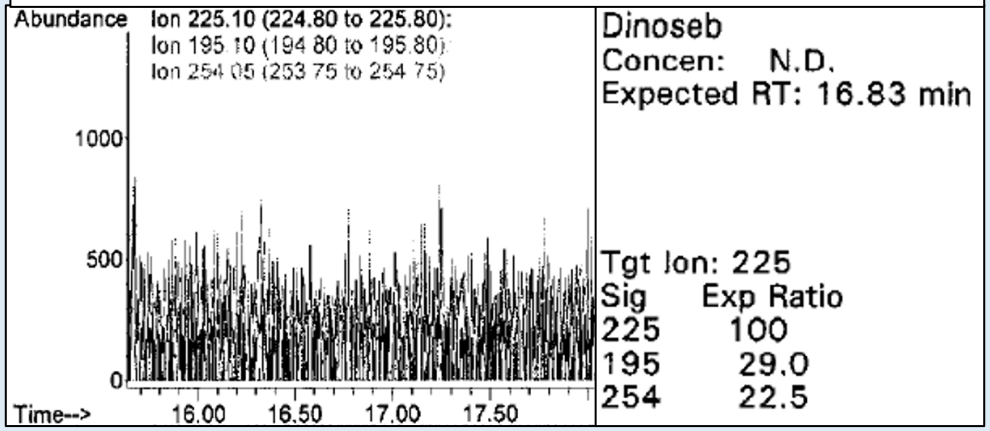
*LLOQ: Lower Limit of Quantitation, set as lowest standard whose calculated concentration was within ±50% of expected. From SW-846 method 8000D.

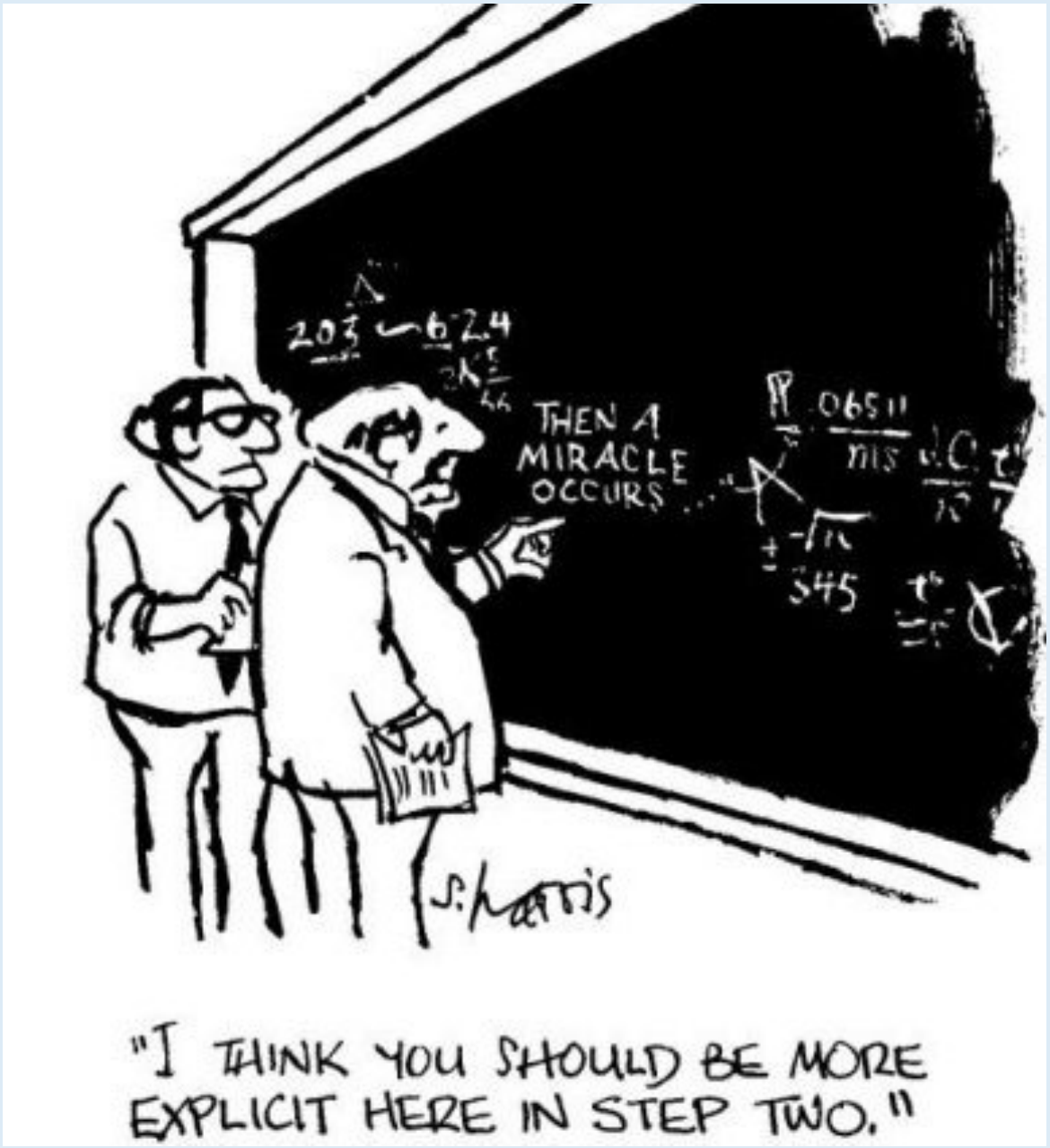
ves with Positive x-intercepts: Methylated -dinitro-2-sec-butyl phenol

ad 1/x weighted
-125 ug/L
= 0.998
=9.1%
nt) (IS conc) = 1.7 ug/L

thod Detection Limit Study 2.5 ug/L spike level, n=7)	
d Dev (ug/L)	0.086
DL ($s*t_{\alpha=0.01, df=6}$)	0.27







"I THINK YOU SHOULD BE MORE EXPLICIT HERE IN STEP TWO."

urves with Positive x-Intercepts – Measurement uality Considerations

concentration below the x-intercept can be calculated with the curve,
ess the data system recognizes no signal and software returns
ndetect’.

sponses close to the x-intercept can change by orders of magnitude
hout much change in concentration

nen concentrations of interest are near or below the LOQ, use care in
ablishing integration parameters and signal thresholds, otherwise low
ponses that produce (biased) blank concentrations potentially near th
samples will be ignored

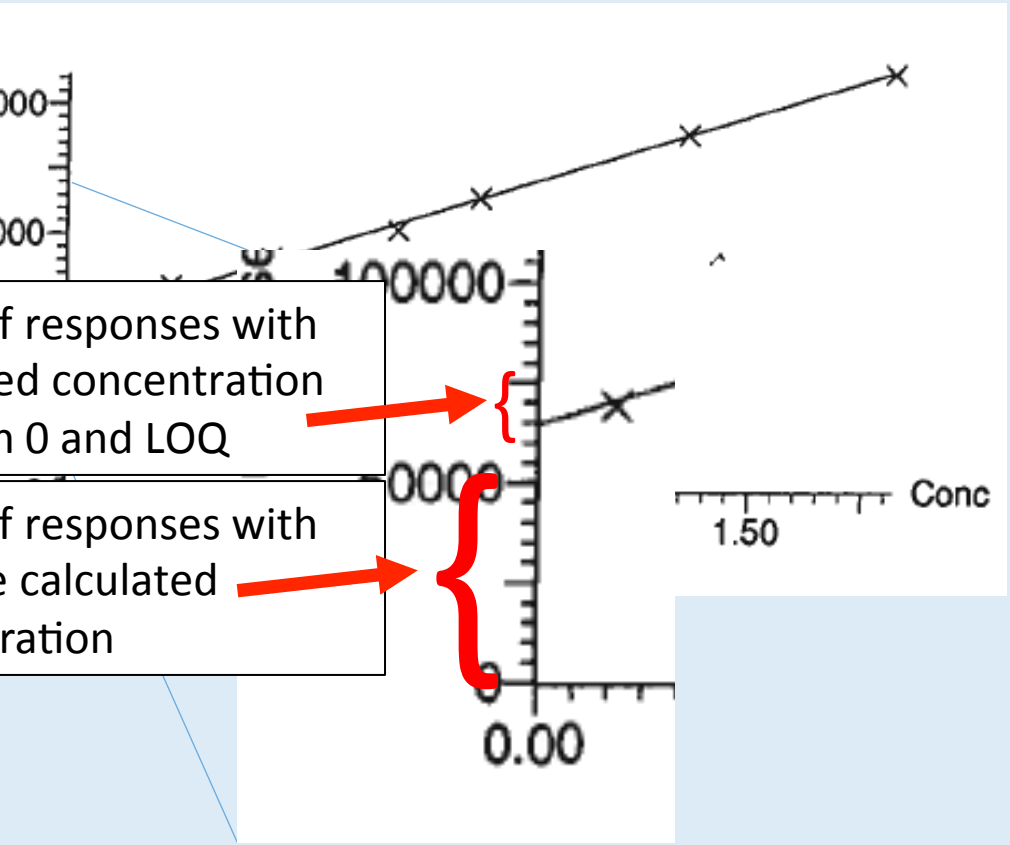
urves with Positive x-Intercepts – Measurement uality Considerations (cont.)

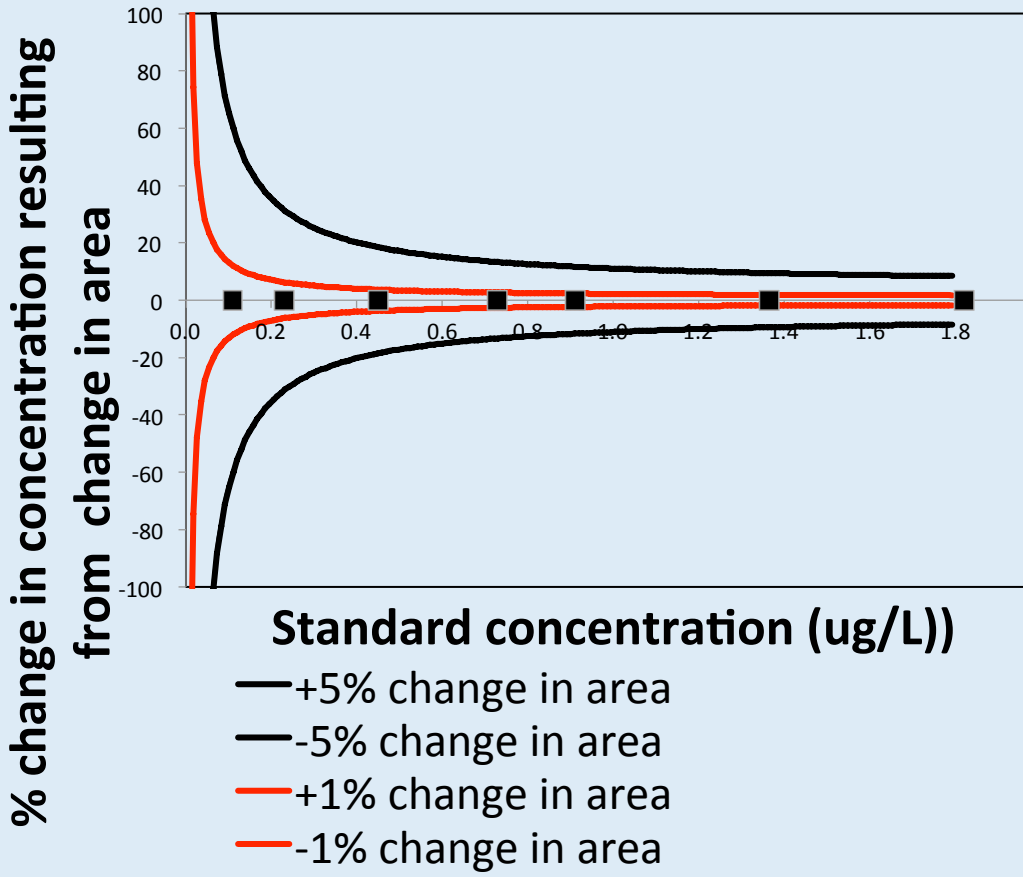
Calibrating to a lower concentration and weighting regression tend to push the x-intercept or the regression line closer to the origin, in turn decreasing positive bias in calculated concentration for extrapolated responses (i.e., method blanks)

Response is useful for data evaluation (e.g., comparing blanks are to samples

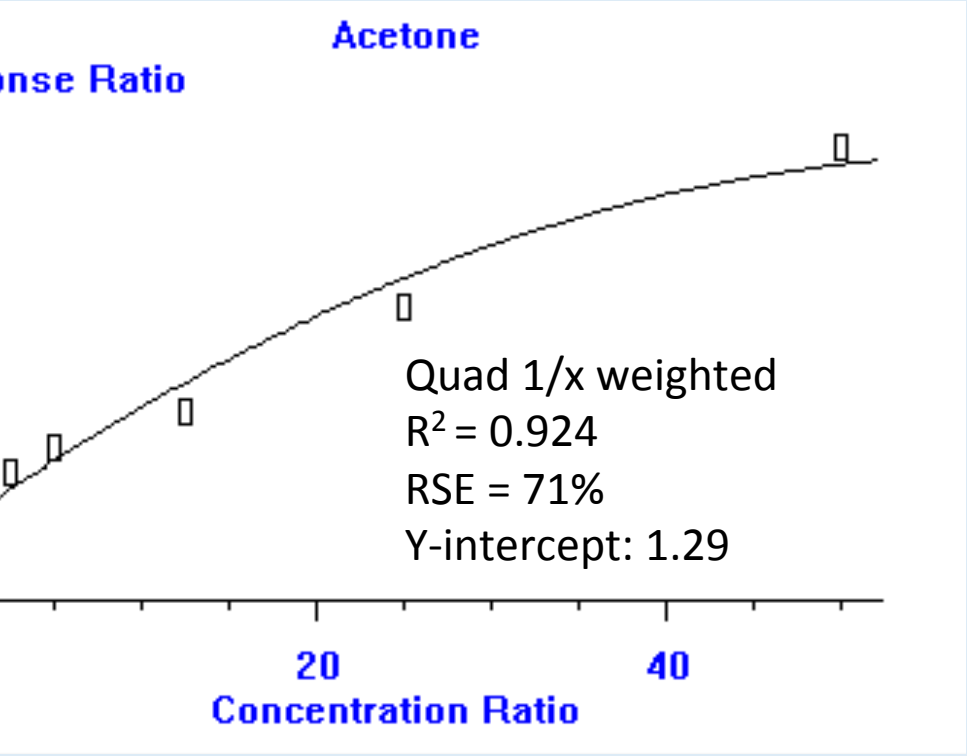
urves with Positive y-intercepts

Octylphenol 12-ethoxylate by LC/MS/MS
(y-intercept is 93% of low standard response)

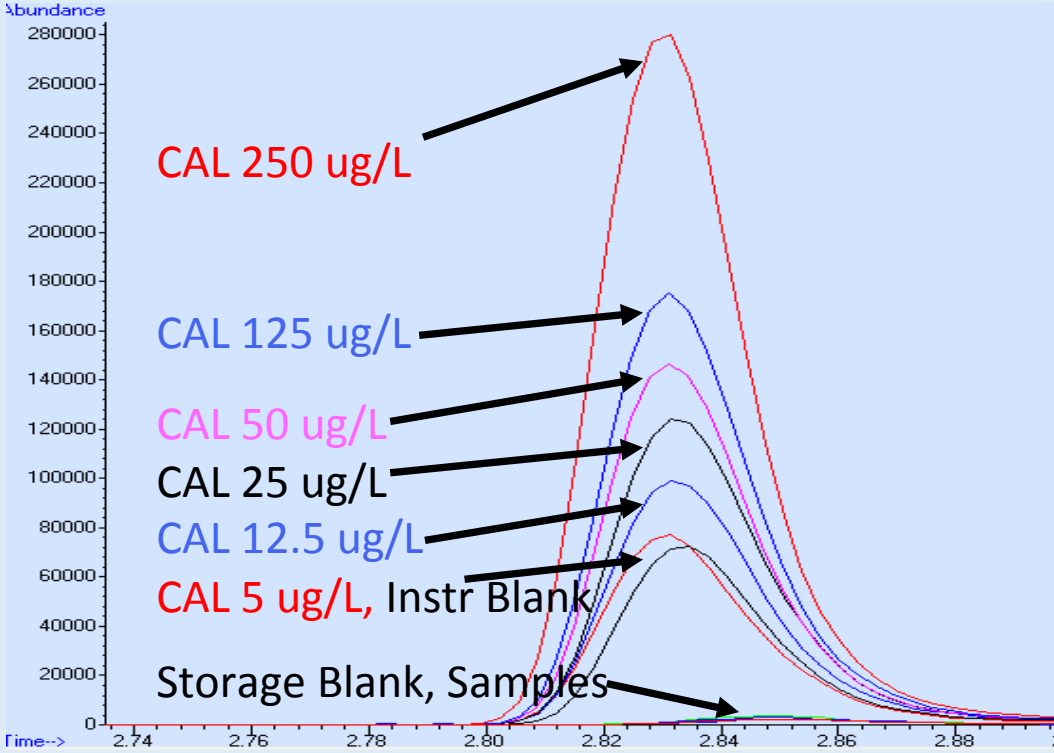




rves with Positive Y-intercepts: Acetone in ater by 5030/8260



Extracted ion (m/z 43) chromatogram



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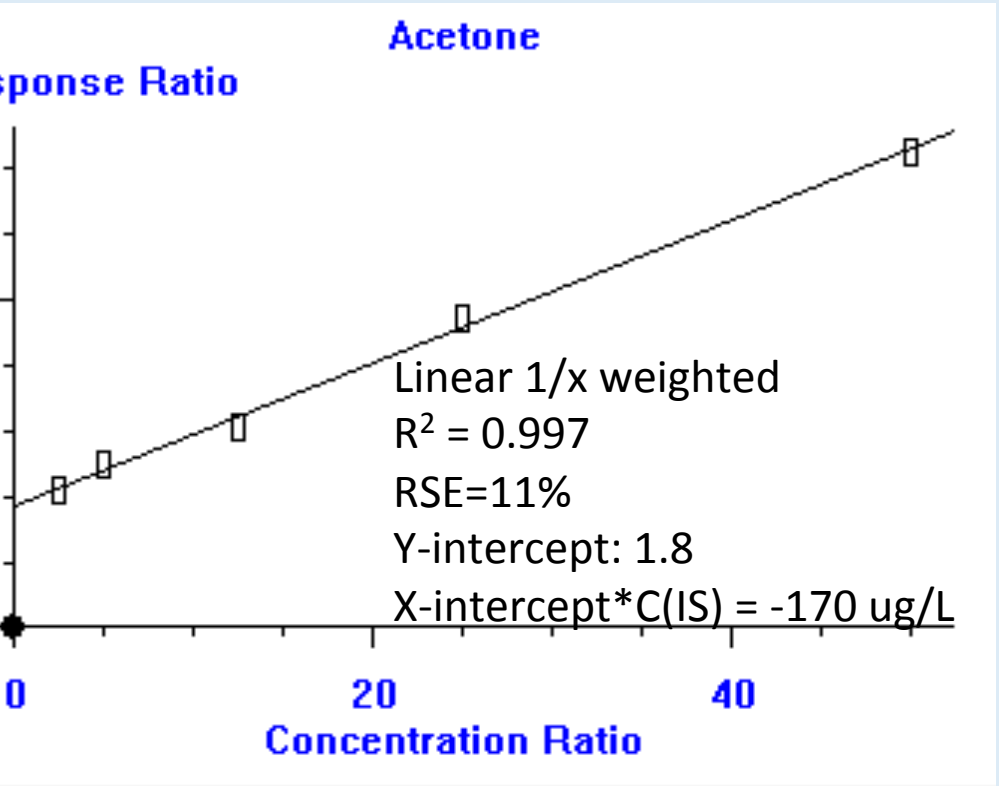
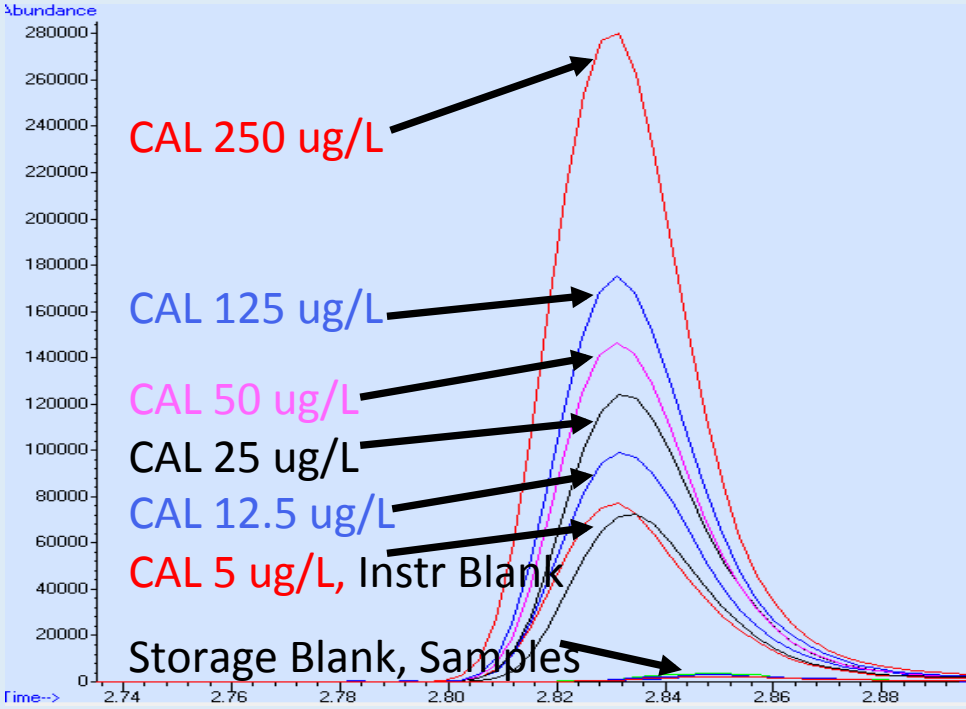
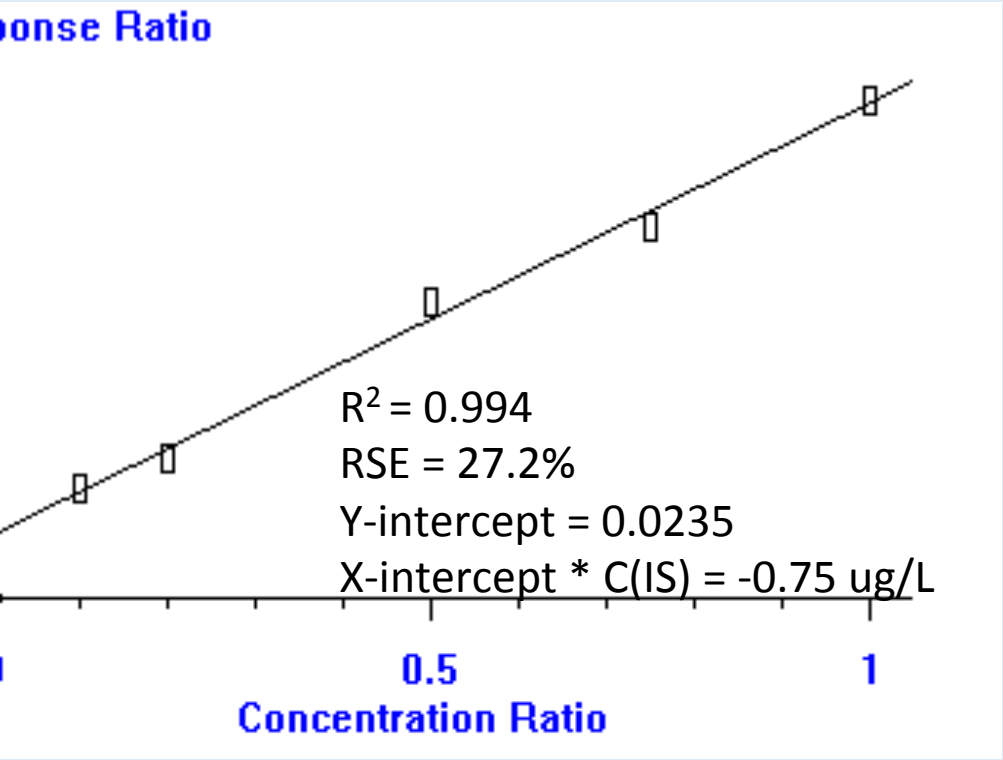


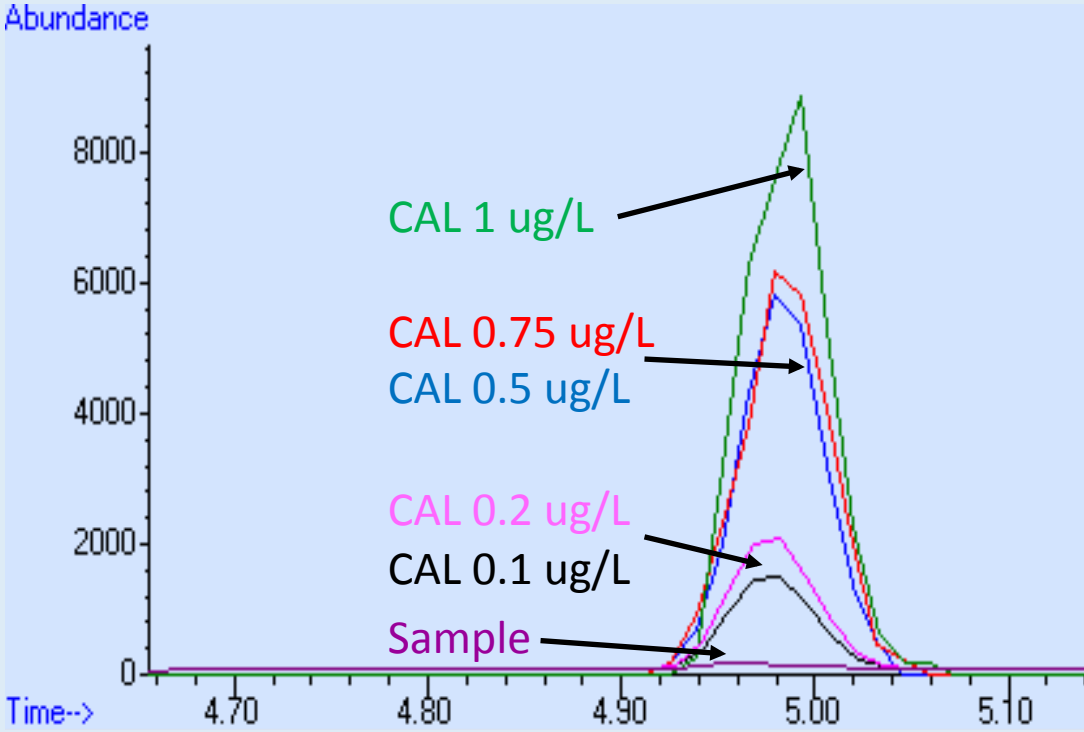
Extracted ion (m/z 43) chromatogram



urves with positive Y-intercepts: Vinyl chloride water by 5030/8260 (Single Quad SIM)



Extracted ion m/z 62 response



positive Y-Intercepts – Measurement Quality Considerations

ckground present in calibration standards can be calibrated out, which may eate a measurement bias problem. Matrix spikes can reveal this bias, but pends on matrix spike level relative to the LOQ

hen necessary for data application, limit bias by raising effective LOQ to a ibration standard level clearly distinguishable from the y-intercept.

- For example, raising LOQ to lowest calibration standard with response > twice the y-intercept of calibration function limits bias at the LOQ to factor 2 (true concentration of 10 = measured concentration of 5)

refully evaluate sources of background, and minimize any sources associate th standards that are not also in samples, if possible

e response instead of or in addition to concentration for data evaluation (e. comparing blanks to samples).

other Approach: FDA Method for Preparation and /MS/MS Analysis of Honey for Fluoroquinolone sidues (enrofloxacin, cyprofloxacin)

xternal standard calibration in blank honey matrix
near calibration model, not forced through zero.
needed, a 1/x weighting may be used to more accurately quantitate low oncentrations. Acceptable curves have correlation coefficients of 0.99 or greater.

- Calibration range: 2.5-50 ng/g
- Concentration limit: 5 ng/g
- < 2.5 ng/g reported as non-detect
- 2.5 ng/g - 5.0 ng/g reported as positively identified, but below quantification limi
- ≥ 5.0 ng/g reported as a numerical concentration value

http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm071495.htm

ecommendation to instrument software evelopers

Proximity of calibration intercept (x or y) to low calibration standard concentration or response is not always apparent

Need: a software option that lets analysts calculate and display the proximity of the LOQ standard response or concentration to the intercept of calibration function

With this data, we could track proximity of LOQ standard to X-intercept or Y-intercept of calibration function over time, which would provide an indication of trends in measurement bias near the LOQ that might otherwise be hard to identify

onclusions

r calibration models not forced through the origin, the intercept can
an important indicator of the potential for measurement bias near
e LOQ.

ckground in reagents and standards used for instrument calibration
d instrument sensitivity can vary by over time, and the cause is not
ways apparent.

aluating the calibration intercept and comparing responses of blanks,
mples and standards during data evaluation will result in more
fensible decisions about how to address non-linear behavior near the
Q

Conclusions (cont.)

hen calibration fit is acceptable, calibration models forced through the
gin avoid sticky problems associated with the intercepts

ncentration estimates extrapolated outside the calibration range
ould be used with caution due to potential for measurement bias.

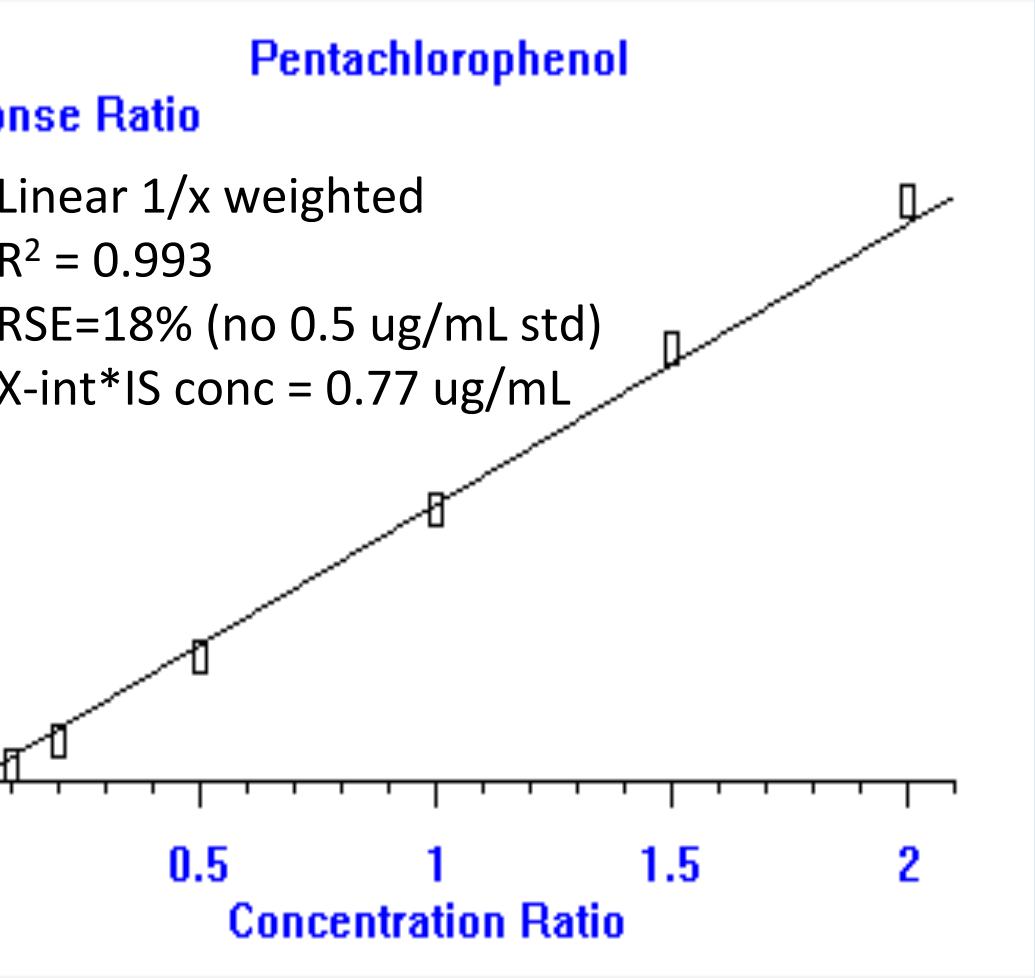
owever, even for curves with non-zero intercepts, as long as target
alytes responses are not close to the intercept (i.e., where the
ationship between response and concentration is proportional),
ncentration estimates below the LOQ may still be useful.

ost important: Define data quality needs first. Then consider how
easurement bias or uncertainty near the LOQ may impact them.

Thanks for listening!

sitive x-intercept: Pentachlorophenol by 8270

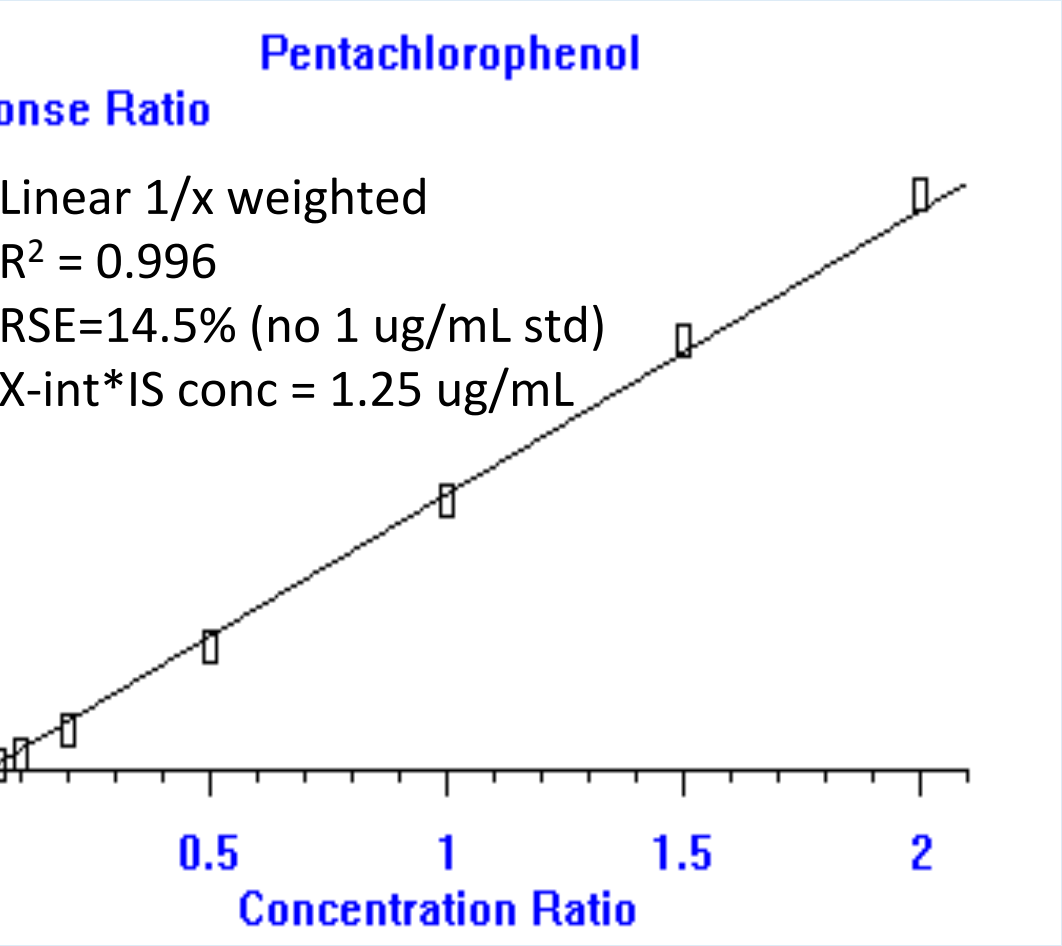
Calibration Range: 0.5-100 ug/mL



Conc (ug/mL)	Calc RRF	Calc conc (ug/mL)	% e
0.5	0.031	0.9	8
1	0.031	1.0	4
2.5	0.056	2.0	-2
5	0.076	4.1	-1
10	0.093	8.9	-1
25	0.119	26.7	6
50	0.128	56.8	1
75	0.137	90.7	2
100	0.134	118	1

sitive X-intercept: Pentachlorophenol by 8270

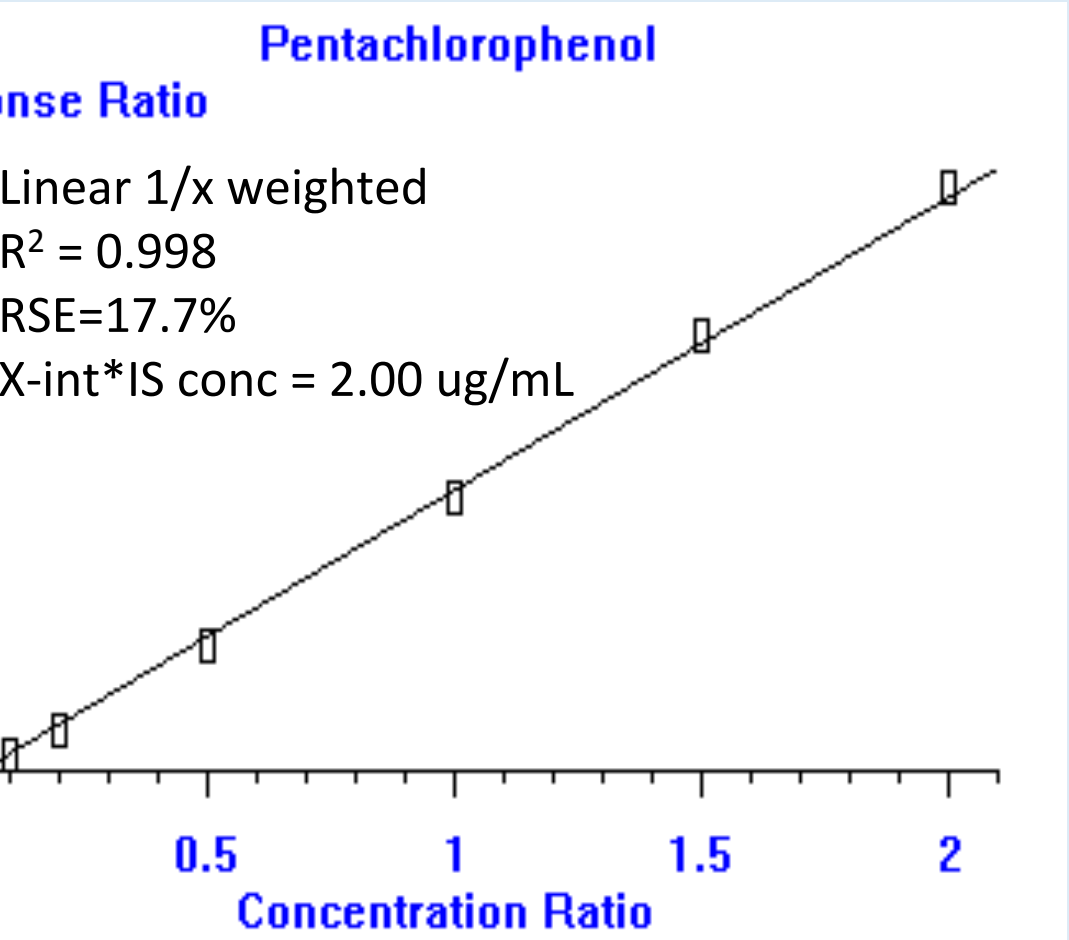
Calibration Range : 1-100 ug/mL



Conc (ug/mL)	Calc RRF	Calc conc (ug/mL)	% e
0.5	0.031	1.38	17
1	0.031	1.52	5
2.5	0.056	2.45	-2
5	0.076	4.51	-9
10	0.093	9.30	-7
25	0.119	26.8	7
50	0.128	56.5	1
75	0.137	90.0	2
100	0.134	117	1

sitive X-intercept: Pentachlorophenol by 8270

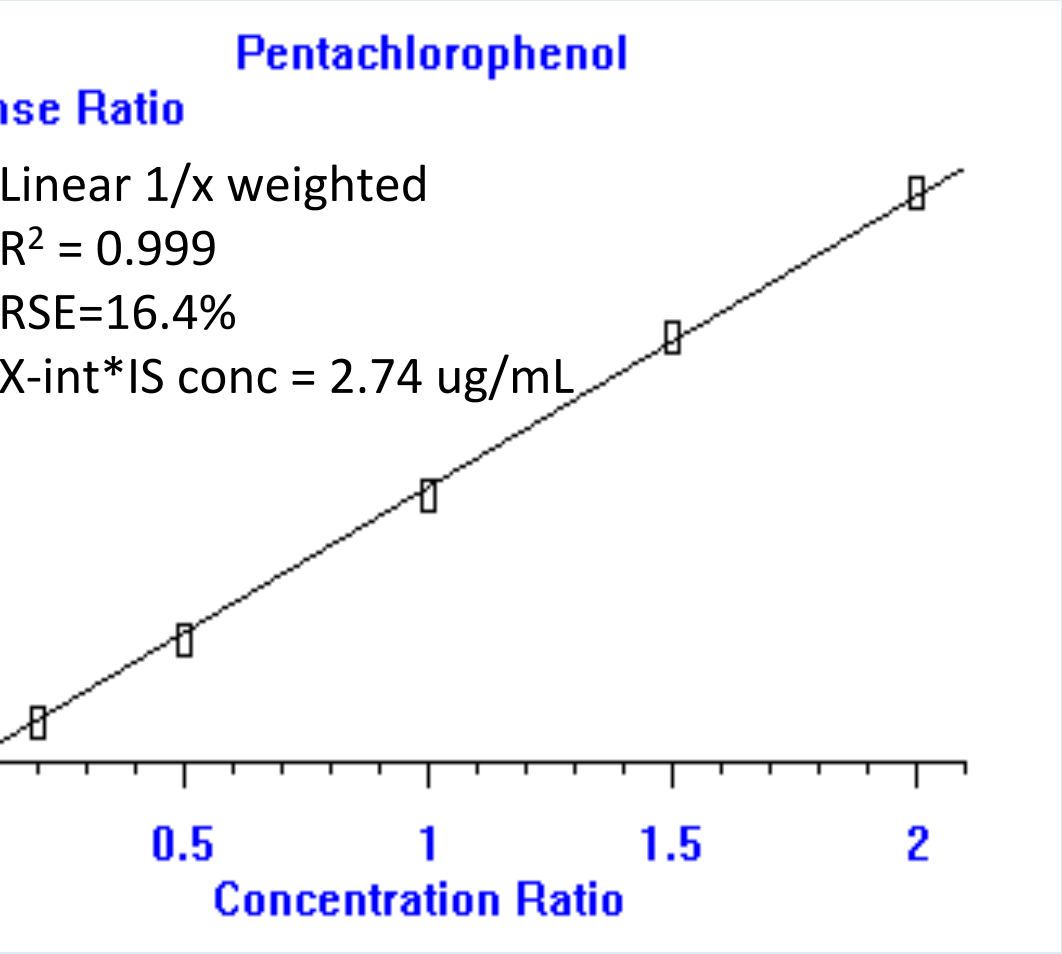
Calibration Range : 2.5-100 ug/mL



Conc (ug/mL)	Calc RRF	Calc conc (ug/mL)	% e
0.5	0.031	2.1	32
1	0.031	2.3	12
2.5	0.056	3.2	2
5	0.076	5.2	3
10	0.093	9.9	-
25	0.119	27.1	8
50	0.128	56.1	1
75	0.137	89.0	1
100	0.134	115	1

sitive X-intercept: Pentachlorophenol by 8270

Calibration Range : 5-100 ug/mL

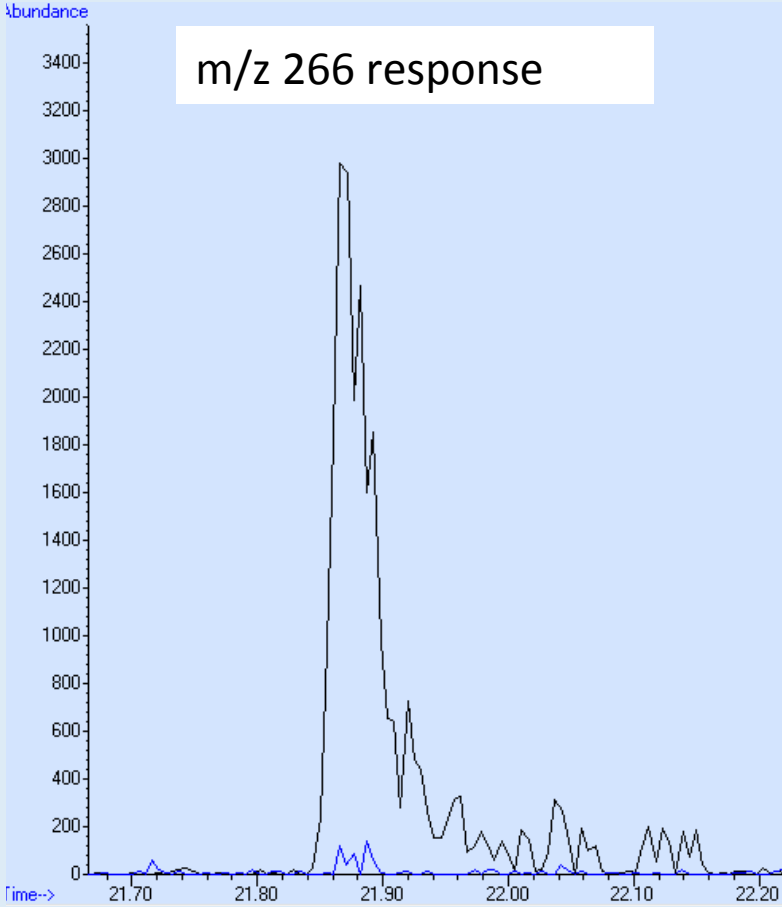


Conc (ug/mL)	Calc RRF	Calc conc (ug/mL)	% e
0.5	0.031	2.9	47
1	0.031	3.0	20
2.5	0.056	3.9	5
5	0.076	5.9	1
10	0.093	10.5	5
25	0.119	27.4	9
50	0.128	56.0	1
75	0.137	88.3	1
100	0.134	113.9	1

itive x-intercept: Pentachlorophenol by 8270D

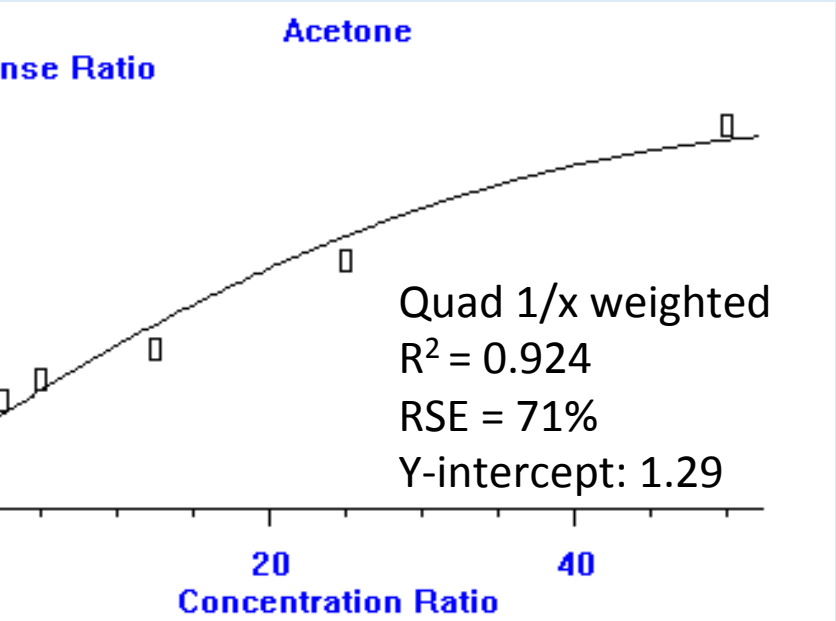
tion (ug/)	LLOQ standard* conc (ug/mL)	Blank conc (ug/mL, at area of 149)	Blank area / low standard area
00	1	0.80	24%
0	2.5	1.28	14%
00	2.5	2.03	2.1%
0	5	2.77	0.7%

Q: Lower Limit of Quantitation, set as lowest
ard whose calculated concentration was within
of expected. From SW-846 method 8000D.

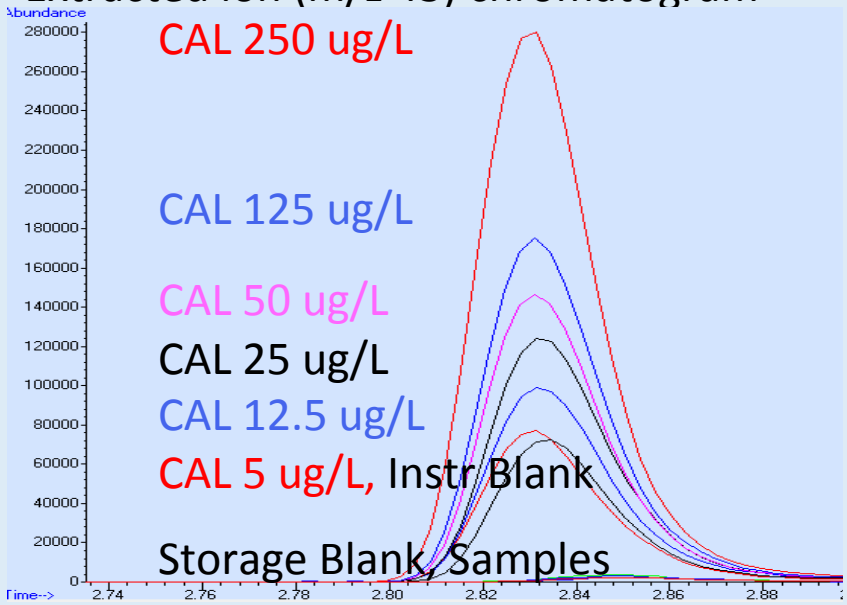


Black: 2.5 ug/mL cal stand
Blue: method blank

positive Y-intercept: Acetone in water by 5030/8260

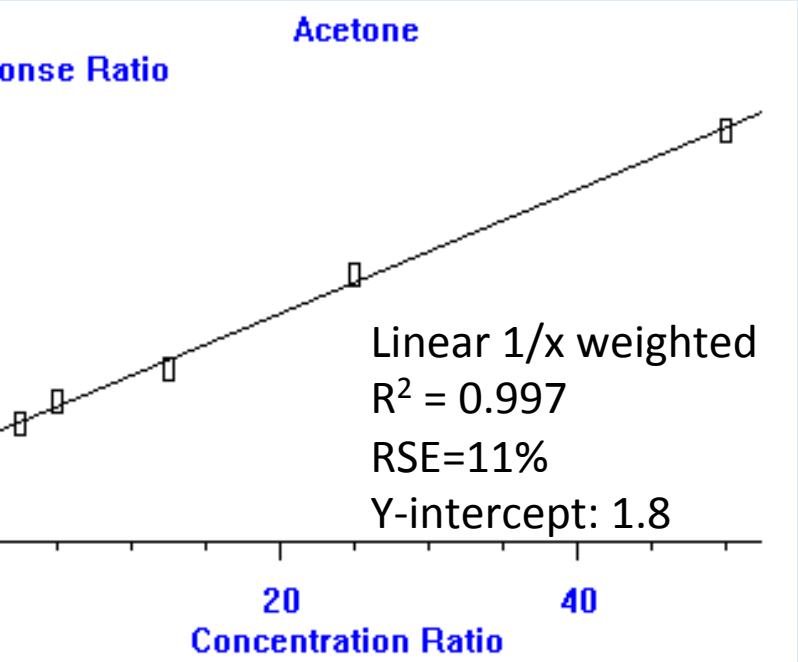


Extracted ion (m/z 43) chromatogram

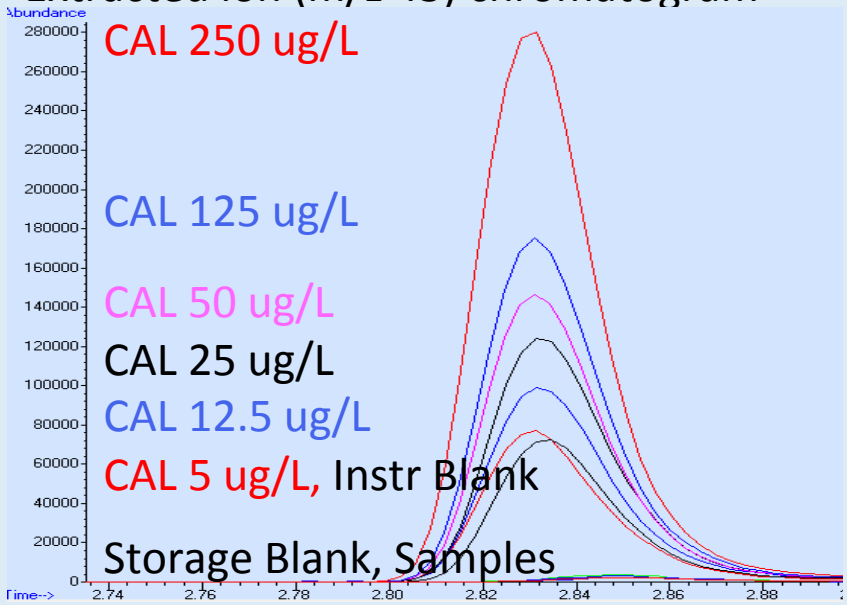


	Acetone (m/z 43) Area	Fluorobenzene (m/z 96) area	Response ratio	Calc (µg/L)	% of expecte
L 5 ug/L	1435462	1134699	1.27	-1.4	
12.5 ug/L	1886403	1122258	1.68	20.4	163.1
25 ug/L	2343751	1126067	2.08	42.2	168.7
50 ug/L	2711673	1099304	2.47	64.0	127.9
125 ug/L	3221382	1065216	3.02	97.2	77.7
250 ug/L	5139976	1095717	4.69	213.1	85.2
500 ug/L	7685080	1063448	7.23	undefined	

positive Y-intercept: Acetone in water by 5030/8260

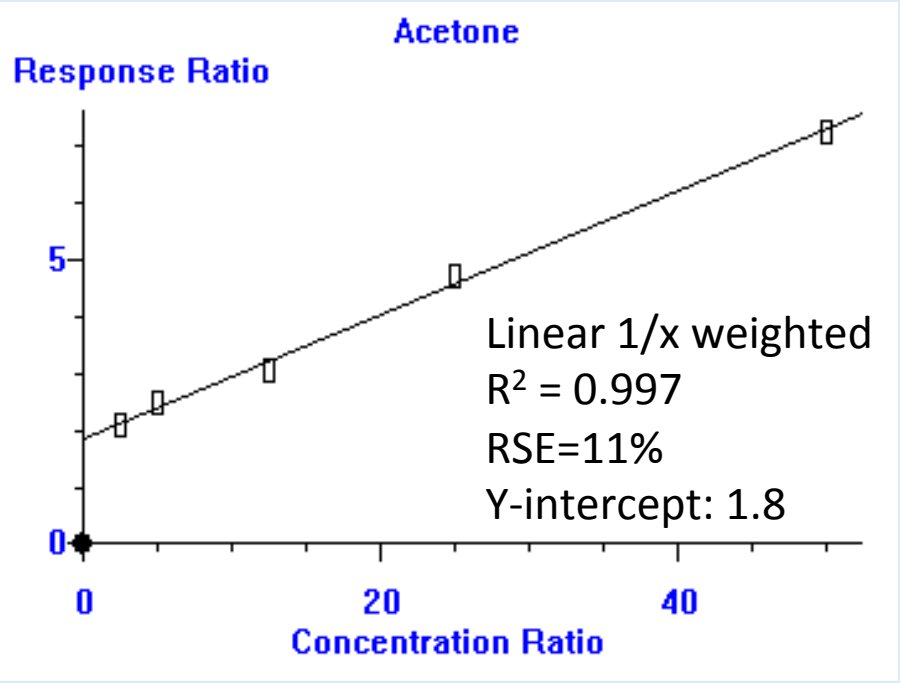


Extracted ion (m/z 43) chromatogram

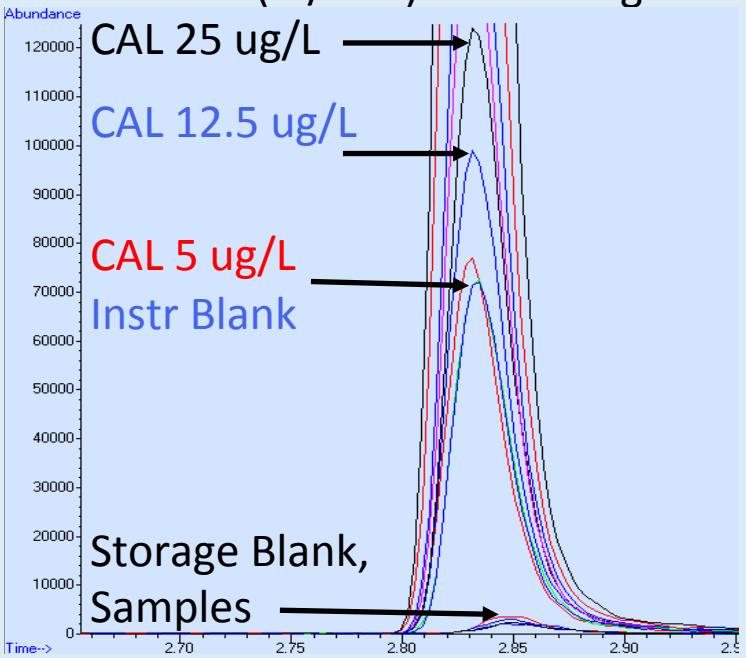


	Acetone (m/z 43) Area	Fluorobenzene (m/z 96) area	Response ratio	Calc (µg/L)	% of expecte
L 5 ug/L	1435462	1134699	1.27	-52.8	
12.5 ug/L	1886403	1122258	1.68	-14.5	
25 ug/L	2343751	1126067	2.08	22.4	89.7
50 ug/L	2711673	1099304	2.47	57.9	115.9
125 ug/L	3221382	1065216	3.02	109.3	87.5
250 ug/L	5139976	1095717	4.69	262.9	105.2
500 ug/L	7685080	1063448	7.23	496.6	99.3

ositive Y-Intercept: Acetone in water by 5030/8260



Extracted ion (m/z 43) chromatogram



	Acetone (m/z 43) Area	Fluorobenzene (m/z 96) area	Response ratio	Calc (µg/L) Quad 1/x	Calc (µg/L) Linear 1/x
. 25 ug/L	2343751	1126067	2.08	42.2	22.4
ment Blank	1373321	1090711	1.26	-1.7	-53.4
age Blank	80798	1006630	0.08	-59.5	-162
mple 1	67660	936560	0.07	-59.9	-163
mple 2	48775	941101	0.05	-60.8	-165
mple 3	46067	889486	0.05	-60.8	-165

ositive Y-Intercept: Acetone in water by 5030/8260

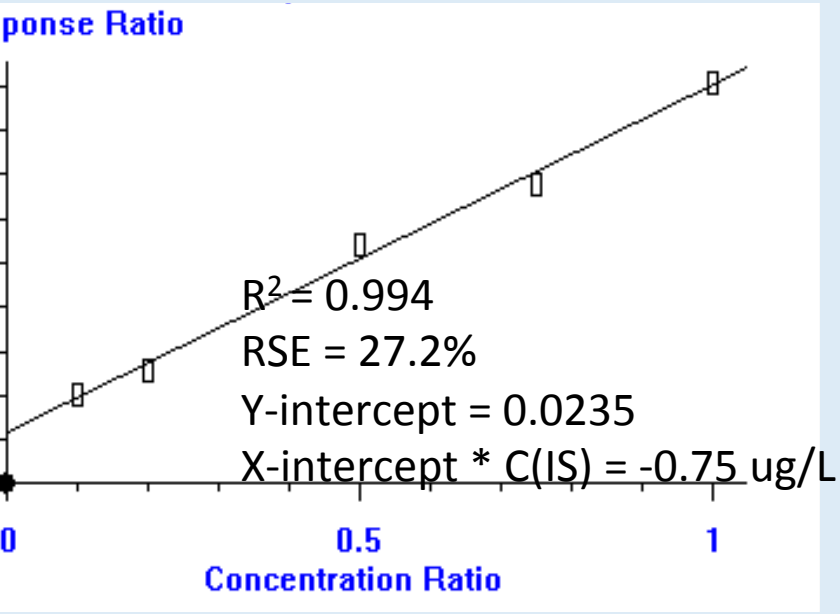
ed acetone ple (ug/L)	Actual (including background) (ug/L)	10% variability (Meas ±10% True)	Best case for true value		Worse case for true	
			% of Measured (no variability)	% of Measured (10% variability)	% of Measured (no variability)	% of M (10% va
0	170	-17 - 17		-	-	
25	195	5.5 - 44.5	100%	45-178%	13%	3-2
125	295	95.5 - 128	100%	76-124%	42%	32-
500	670	433 - 567	100%	86-113%	75%	65-

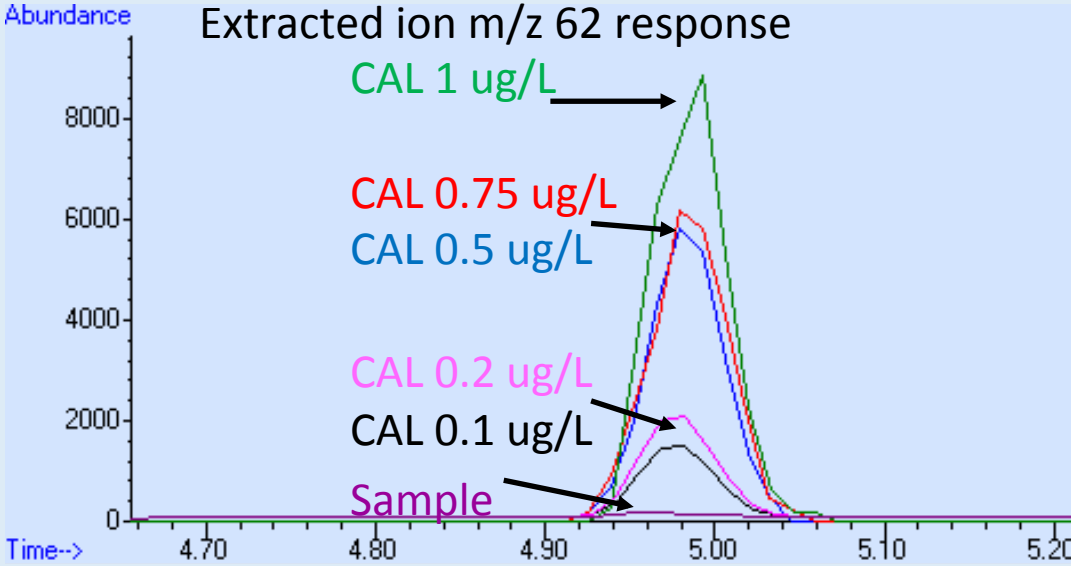
Using method of standard additions, -(x intercept) = Calculated aceton
background in the calibration standards = ~170 ug/L

Best case: Background in the calibration standards is also present in samples.

Worst case: Background in calibration standards is <u>absent</u> from sample

sitive Y-intercept: Vinyl chloride in water by 30/8260 (Single Quad SIM)





	Vinyl Chloride m/z 62 Area	Chloroethane-d5 m/z 69 area	Relative response	Calc (µg/L)	% of expected
CAL 0.1 ug/L	4512	117771	0.038	0.09	94%
CAL 0.2 ug/L	5526	126996	0.044	0.09	43%
CAL 0.5 ug/L	12844	120084	0.107	0.53	107%
CAL 0.75 ug/L	20846	149072	0.140	0.74	99%
CAL 1.0 ug/L	24426	132886	0.184	1.03	103%
Sample 1	311	116725	0.003	-0.13	

tive Y-Intercept: Vinyl Chloride in water by 5030/8

ured loride le (ug/)	Actual (including std addition backgrnd) (ug/L)	Measured, with 10% Variability in Response (Meas ±10% Actual)	Best case		Worst case	
			% of Measured (no variability) (=meas/meas)	% measured (with 10% variability) =(meas)±10%(actual)	% of Measured (no variability) =(meas/actual)	% meas (with 10% v =meas/act (actu
	0.75	-0.075-0.075		-	-	-
1	0.85	0.02-0.17	100%	15-185%	12%	1.8-2
2	0.95	0.11-0.29	100%	53-148%	21%	11-3
5	1.25	0.38-0.66	100%	75-125%	40%	30-5
	1.75	0.83-1.31	100%	83-118%	57%	47-7

Calculated equivalent background in calibration standards (based on method of standard additions) = 0.75 ug/L

Best case: Background in the calibration standards is also present in samples.

Worst case: Background in calibration standards is absent from samples.