One Curve Fit to Rule them All

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Historical Calibration Guidance

Method 8000A

"Prepare a calibration curve"

- If the % RSD is less than 20% over the working range, the average calibration factor (response factor) may be used in place of a calibration curve

In practice, Average RF was preferred, then unweighted linear

Method 8000B

"SW-846 chromatographic methods allow the use of both linear and non-linear models."

Options

- Linear using average RF or CF, < 20% RSD
- Linear using least squares regression
  - Weighting may be employed if replicate multi-point calibrations have been performed
  - $1/(SD)^2$
  - Evaluated with the correlation coefficient, $r \geq 0.990$
- Non-Linear
  - Up to third order
  - Evaluated with the Coefficient of Determination (COD, $r2 \geq 0.990$)
  - No mention of weighting for non-linear

In practice, Average was preferred, then unweighted linear, then unweighted quadratic

Method 8000C

"SW-846 chromatographic methods allow the use of both linear and non-linear models."

Options

- Linear using average RF or CF, < 20% RSD
- Linear using least squares regression
  - Weighting the sum of the squares may significantly improve the ability of the least quuares regression to fir the linear model to the data. The mathematics of the least squares regression has a tendency to favor numbers of larger value over numbers of smaller value. Thus the regression curves that are generated will tend to fit points that are at the upper calibration levels better than points at the lower calibration levels. To compensate a weighting factor can be used
    $1/X$ or $1/X^2$
  - In practice, this meant some weighting
  - Evaluated with the correlation coefficient, r $\geq$ 0.990 or COD $\geq$ 0.990
- Non-Linear
  - Up to third order
  - Evaluated with the Coefficient of Determination (COD, r2 $\geq$ 0.990)
  - Weighting in a calibration model may significantly improve the ability of the least squares regression to fit the data

Weighting begins to be used

Method 8000D

“SW-846 chromatographic methods allow the use of both linear and non-linear models.”

Options

- Linear using average RF or CF, < 20% RSD
- Linear using least squares regression
  - Weighting the sum of the squares may significantly improve the ability of the least quuares regression to fit the linear model to the data. Mathematics used in least squares regression favors numbers of larger value over numbers of smaller value. Thus, unweighted regression curves will tend to fit points that are at upper calibration levels better than those points at lower calibration levels. If concentrations of concern are at lower calibration levels, an unweighted regression curve tends to give less accurate results. A weighting factor which reduces this tendency can be used as compensation.
  - Evaluated with the correlation coefficient, $r \geq 0.995$ or COD ≥ 0.990
- Non-Linear
  - Up to third order
  - Evaluated with the Coefficient of Determination (COD, $r2 \geq 0.990$)
  - the curve may either be weighted or forced through the origin as long as calibration criteria are met
- RSE – introduced as an alternative evaluation criterion for linear and non-linear curves – Use same criterion as RSD.

What are we actually getting with various weighting options?

$$S_{min} = \sum_{i=1}^{n} r_i^2$$

Unweighted - Minimize the sum of the squares of the absolute residuals

$1/(\mathrm{Conc})^2$ weighting – Minimize the sum of the squares of the relative residuals

1/Conc weighting – Intermediate, but in relative terms, higher concentrations are weighted more

1/(Concentration)^2

$$S_{min} = \sum_{i=1}^{n} r_i^2$$

Calibration point	5	100
Error	10%	10%
Error	0.5	10
Error squared	0.25	100
Weighting factor 1/(X)2	0.04	0.0001
Weight factor x Error squared	0.01	0.01

If we want to minimize % error across the calibration range, then we should use $1/(\text{Concentration})^2$ weighting

One constant through SW-846 revisions – Average RF

$$s_C^2 = \frac{\sum_{i=1}^{n}(C_i - \bar{C})^2}{n-1}$$ Equation 4

If $\hat{y}_i$ is defined as the expected response for calibration level i from the relationship $\hat{y}_i = x_i\bar{C}$ by substitution the variance can also be expressed as:

$$s_C^2 = \frac{\sum\left[\frac{y_i - \hat{y}}{x_i}\right]^2}{n-1}$$ Equation 5

Using the method of least squares we can determine a mathematical relationship between the dependent and independent variables which minimizes the residual variance. By definition the residual variance represents the variability due to experimental error[5] and does not include that contribution to variance which is attributable to differences in the independent variable. The residual variance of *y* on *x* is defined as:

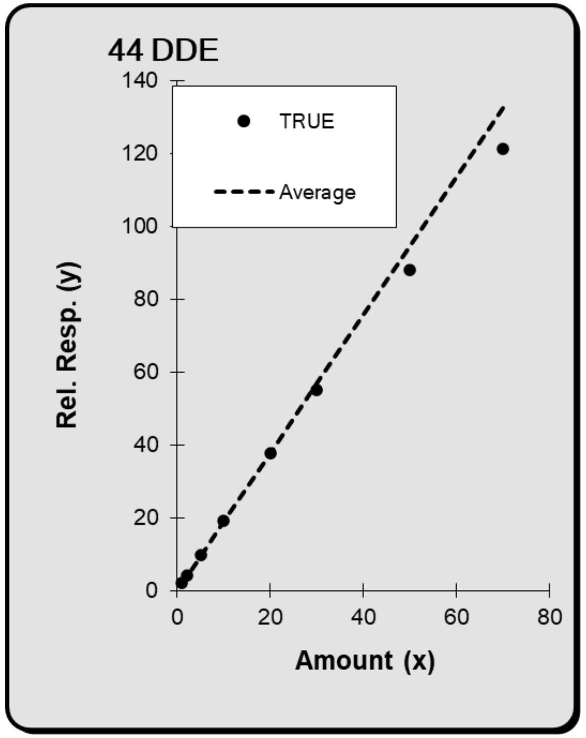
$$s_{yx}^2 = \frac{\sum_{i=1}^{n}(y_i - \hat{y})^2}{n-1}$$ Equation 6

A comparison of equations 5 and 6 demonstrates that the average value is the same as a coefficient derived from least squares regression if a weighting of $1/x^2$ is applied. The average calibration factor gives a result which is identical to that produced by the method of least squares using (1/concentration^2) weighting and intercept forced through zero.

More on Average RF

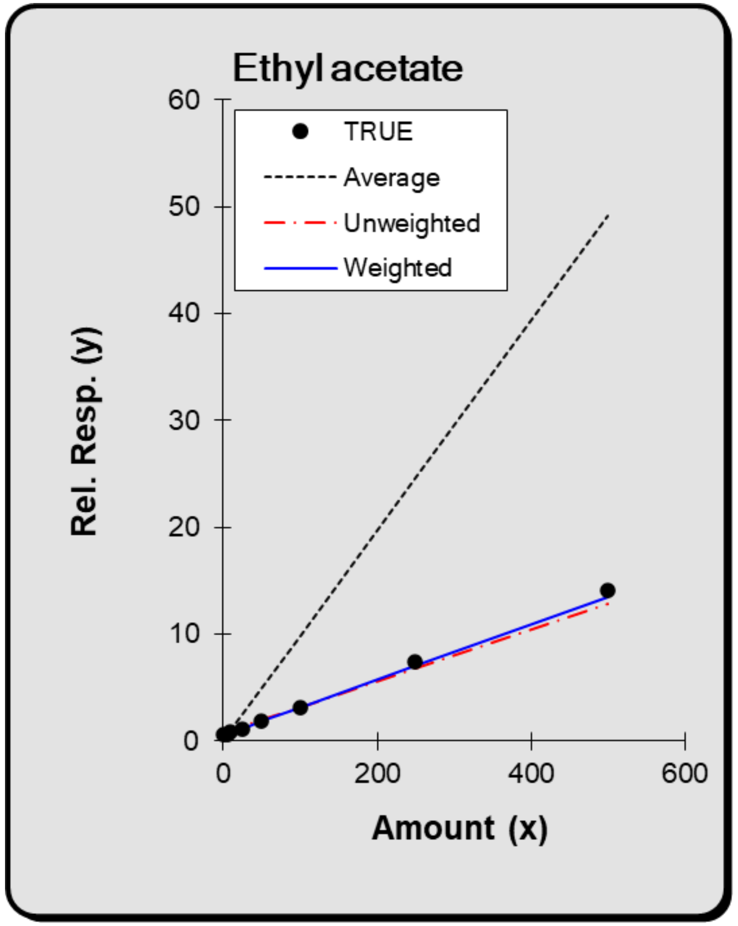
Commonly Average is a very good curve fit, but…

- Forced through zero
- Linear



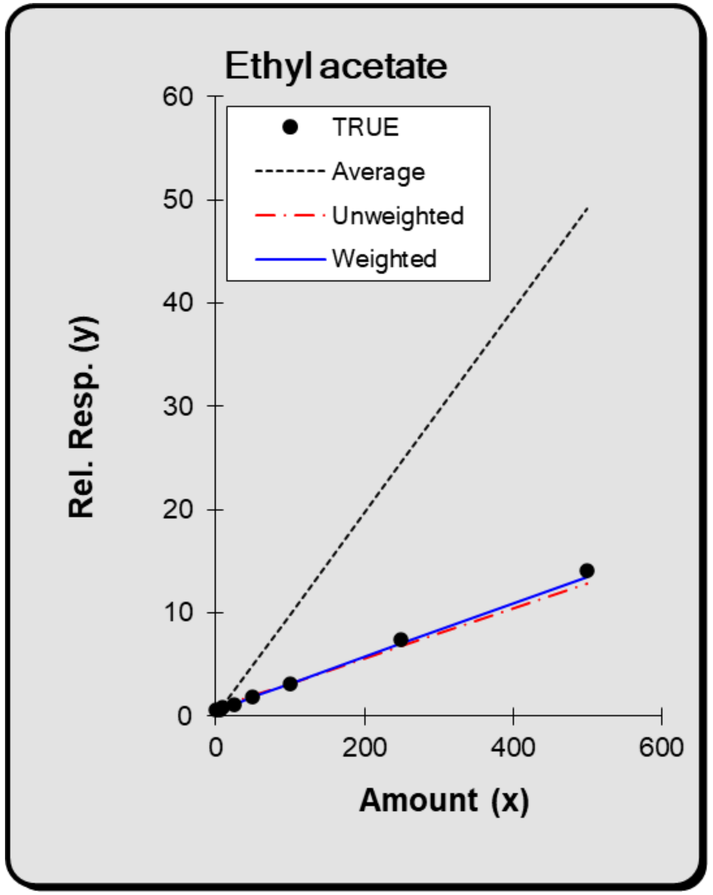
Ethyl acetate

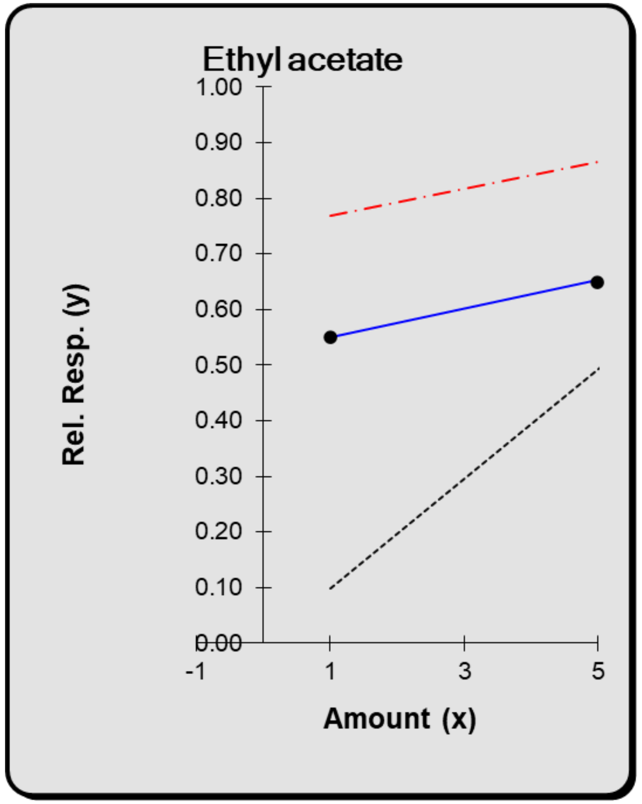
Amount	Response	Rel. Resp.
1.00	30954	0.5508
4.99	36122	0.6482
9.97	44514	0.7955
24.93	65450	1.1631
49.85	105507	1.8612
99.70	179154	3.1417
249.25	418289	7.3527
498.50	775959	14.1399
747.75	1058840	18.1501
997.00	1385597	24.6614



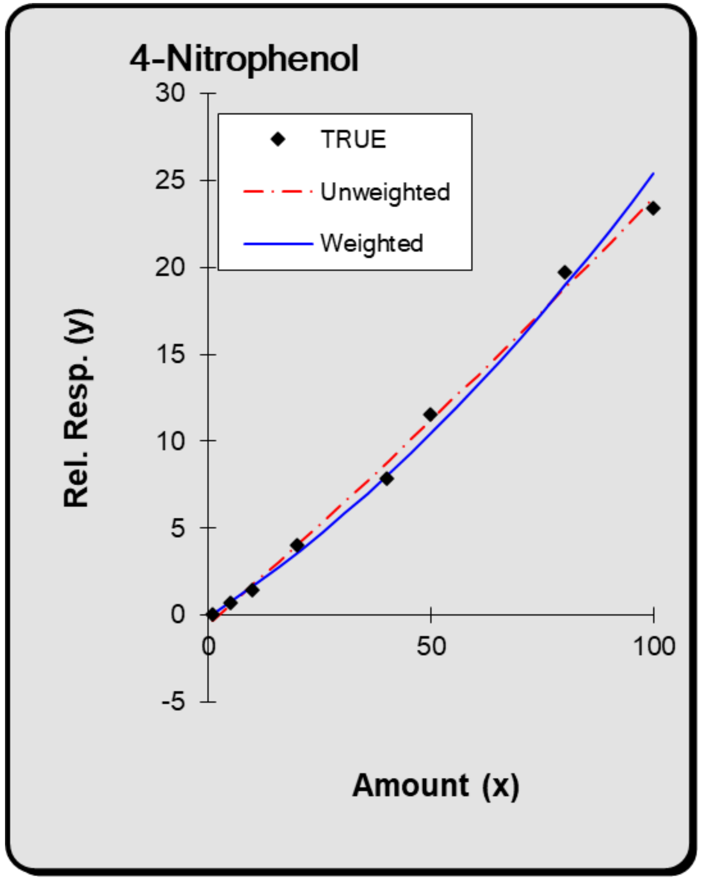
Ethyl acetate

	Average	**Linear 1/X2**	Linear
1	461.08%	**0.37%**	-898.59%
5	32.05%	**-4.51%**	-179.12%
10	-18.97%	**4.79%**	-78.60%
25	-52.61%	**-1.15%**	-30.60%
50	-62.08%	**3.49%**	-7.52%
100	-68.00%	**1.33%**	-0.77%
250	-70.04%	**5.76%**	9.40%
500	-71.19%	**5.44%**	10.87%
750	-75.35%	**-9.00%**	-3.96%
1000	-74.88%	**-6.53%**	-1.02%
RSE	166%	**5.5%**	325%
r2		**0.997**	0.996





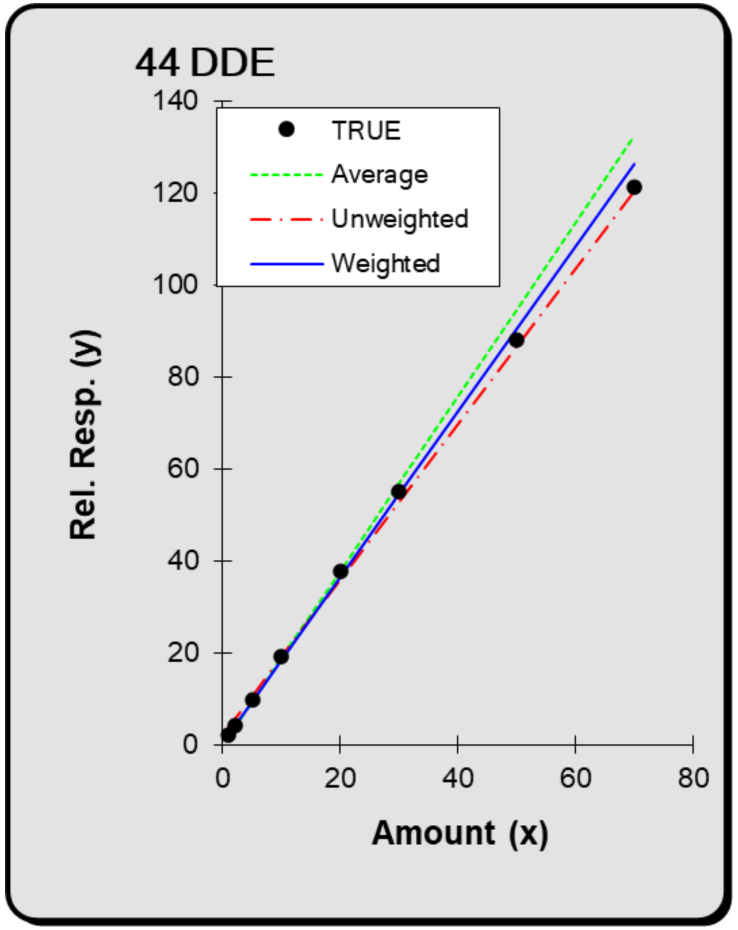
4-Nitrophenol



Amount	Response	Rel. Resp.	Average	Linear 1/X2	Quadratic	Quadratic 1/X2
1.00	784	0.0677	-62.94%	**5.91%**	165.42%	**2.10%**
5.00	9623	0.6806	-25.44%	**-20.43%**	7.30%	**-8.52%**
10.00	19629	1.4677	-19.60%	**-22.73%**	-11.74%	**-10.40%**
20.00	59142	4.0266	10.29%	**-0.43%**	-0.35%	**10.49%**
40.00	87892	7.8553	7.58%	**-4.63%**	-9.64%	**-1.51%**
50.00	149575	11.5396	26.43%	**11.39%**	2.65%	**8.34%**
80.00	268521	19.7625	35.32%	**18.58%**	4.84%	**3.49%**
100.00	344399	23.4307	28.35%	**12.33%**	-2.15%	**-5.83%**
	RSE		34%	**16%**	74%	**9.1%**
	r^2			0.974		0.993

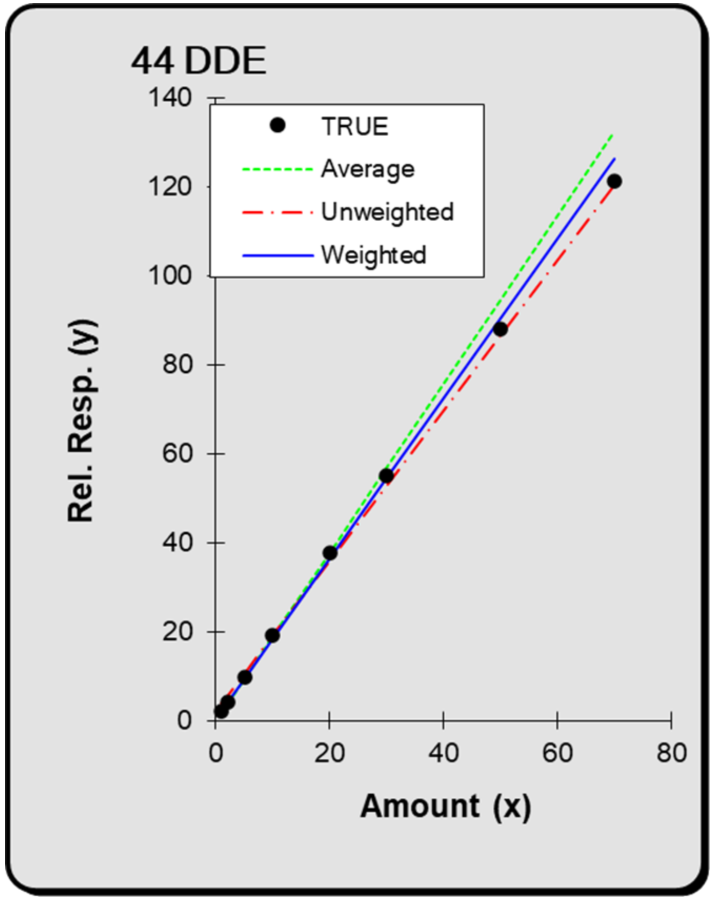
DDE

Amount	Rel. Resp.
1.00	2.1986
2.00	4.0603
5.00	9.8468
10.00	19.2339
20.00	37.7398
30.00	55.0850
50.00	88.0265
70.00	121.1937
100.00	168.3699



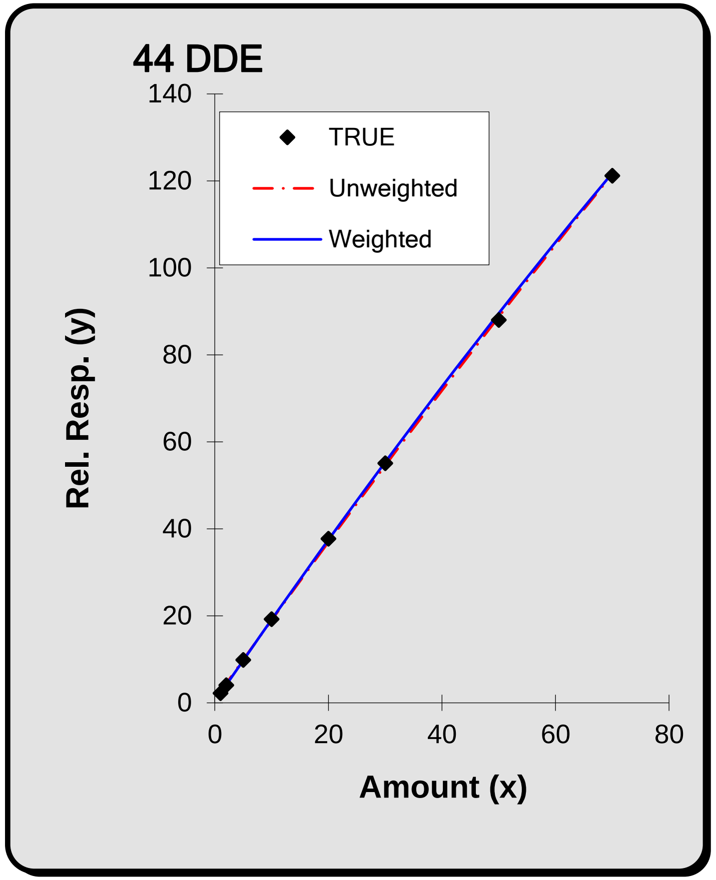
DDE

Amount	Rel. Resp.	Average	Linear 1/X2	Linear unweighted
1.00	2.1986	16.26%	-1.86%	-105.05%
2.00	4.0603	7.35%	0.83%	-47.27%
5.00	9.8468	4.14%	4.69%	-10.22%
10.00	19.2339	1.71%	4.55%	0.61%
20.00	37.7398	-0.22%	3.73%	5.23%
30.00	55.0850	-2.91%	1.31%	4.47%
50.00	88.0265	-6.91%	-2.58%	1.79%
70.00	121.1937	-8.45%	-4.06%	0.83%
100.00	168.3699	-10.97%	-6.61%	-1.42%
RSE		8.6%	4.3%	44%
r^2		0.989	0.998	0.999



DDE

Amount	Rel. Resp.	Quad 1/X2	Quad unweighted
1.00	2.1986	**0.24%**	-19.69%
2.00	4.0603	**-0.91%**	-9.31%
5.00	9.8468	**0.83%**	-0.65%
10.00	19.2339	**0.59%**	1.24%
20.00	37.7398	**0.75%**	2.21%
30.00	55.0850	**-0.44%**	1.01%
50.00	88.0265	**-1.82%**	-0.84%
70.00	121.1937	**-0.54%**	-0.29%
100.00	168.3699	**1.15%**	0.17%
RSE		**1.2%**	9.0%
r^2		**0.999**	0.999



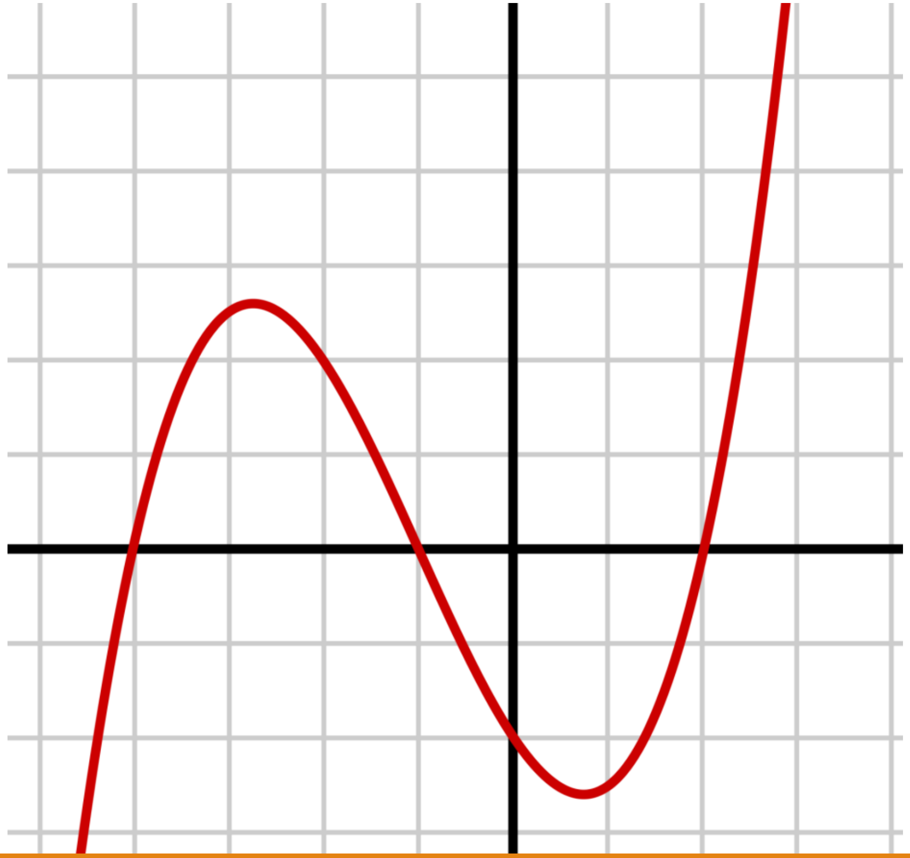
$Y = -0.0024661x^2 + 1.9083x + 0.34989$

QC for a quadratic curve

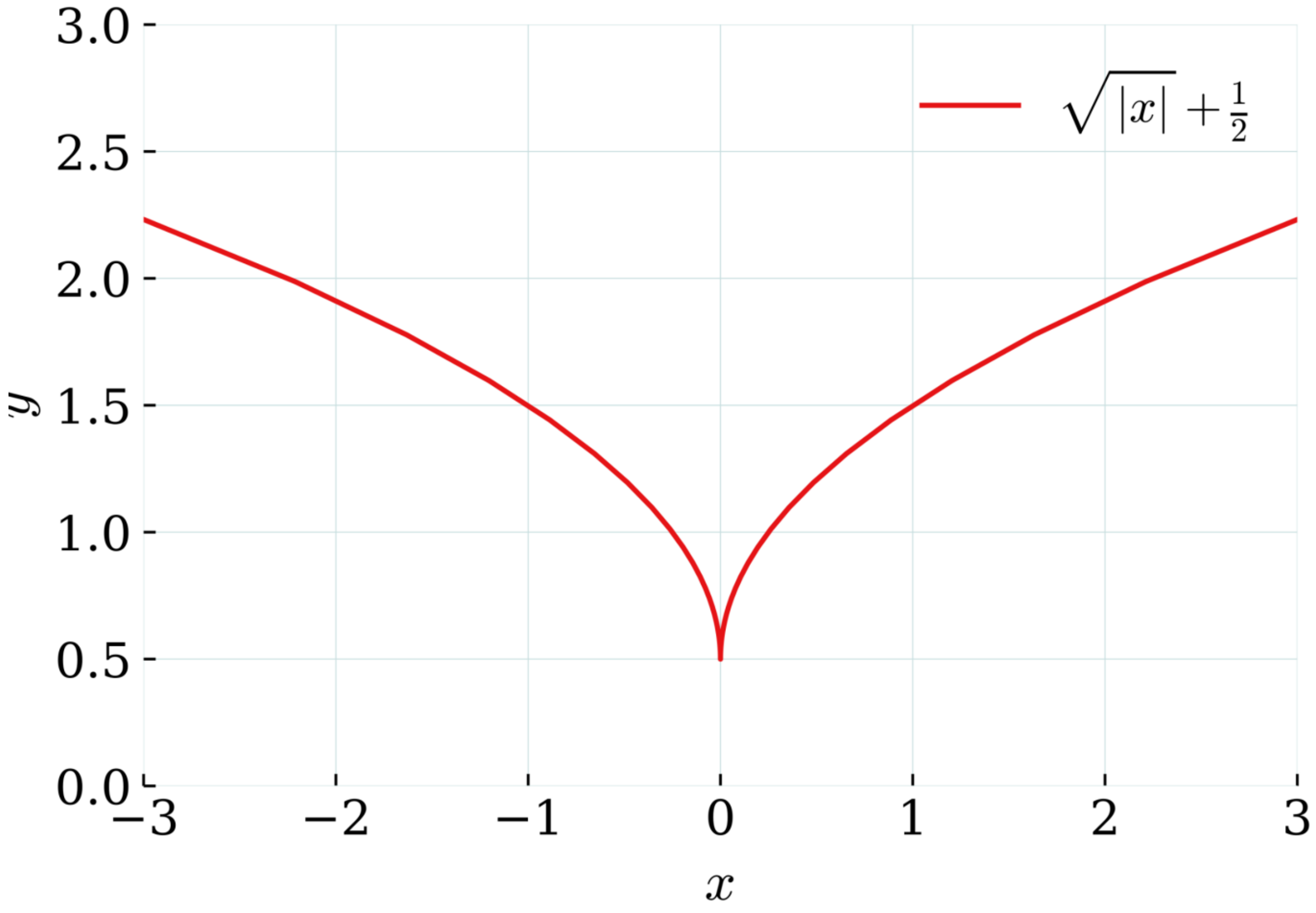
8000D

- When a calibration model for quantitation is used, the curve must be continuous: continuously differentiable and monotonic over the calibration range.

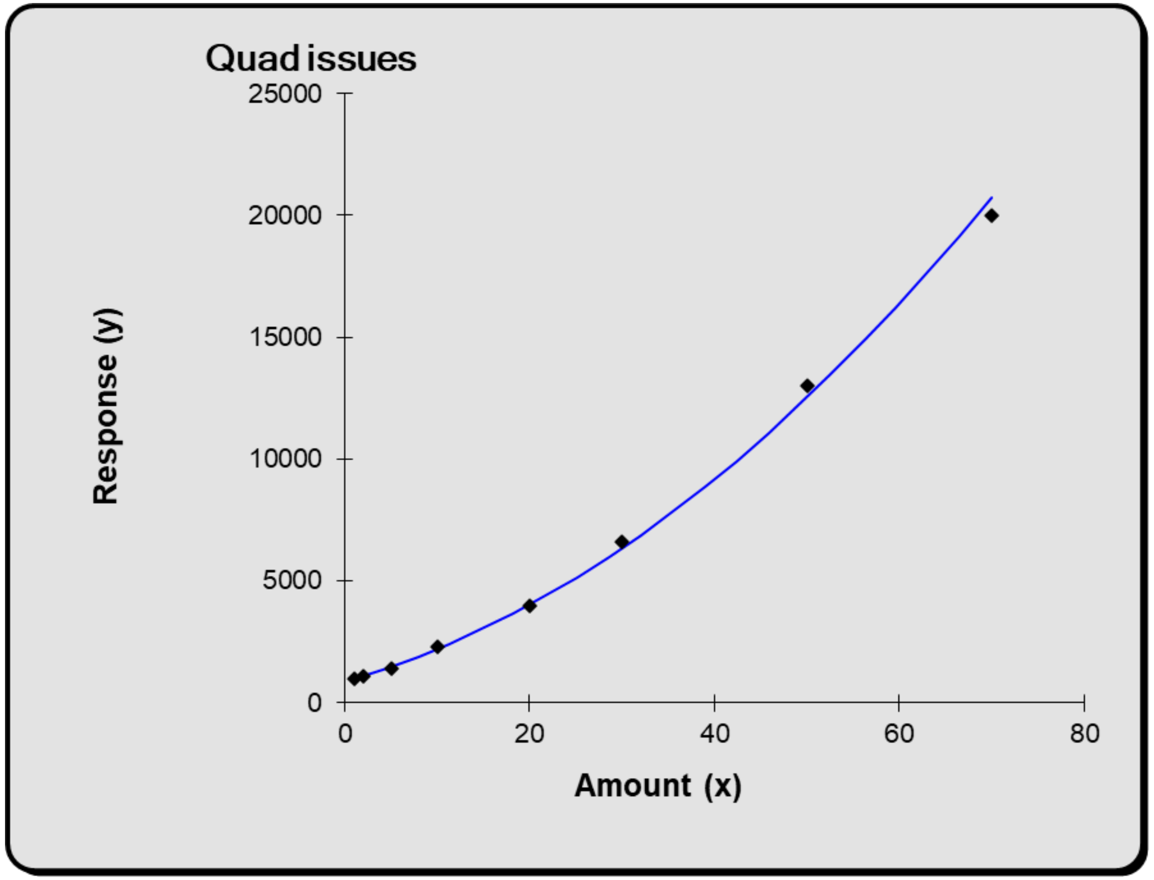
Not Monotonic

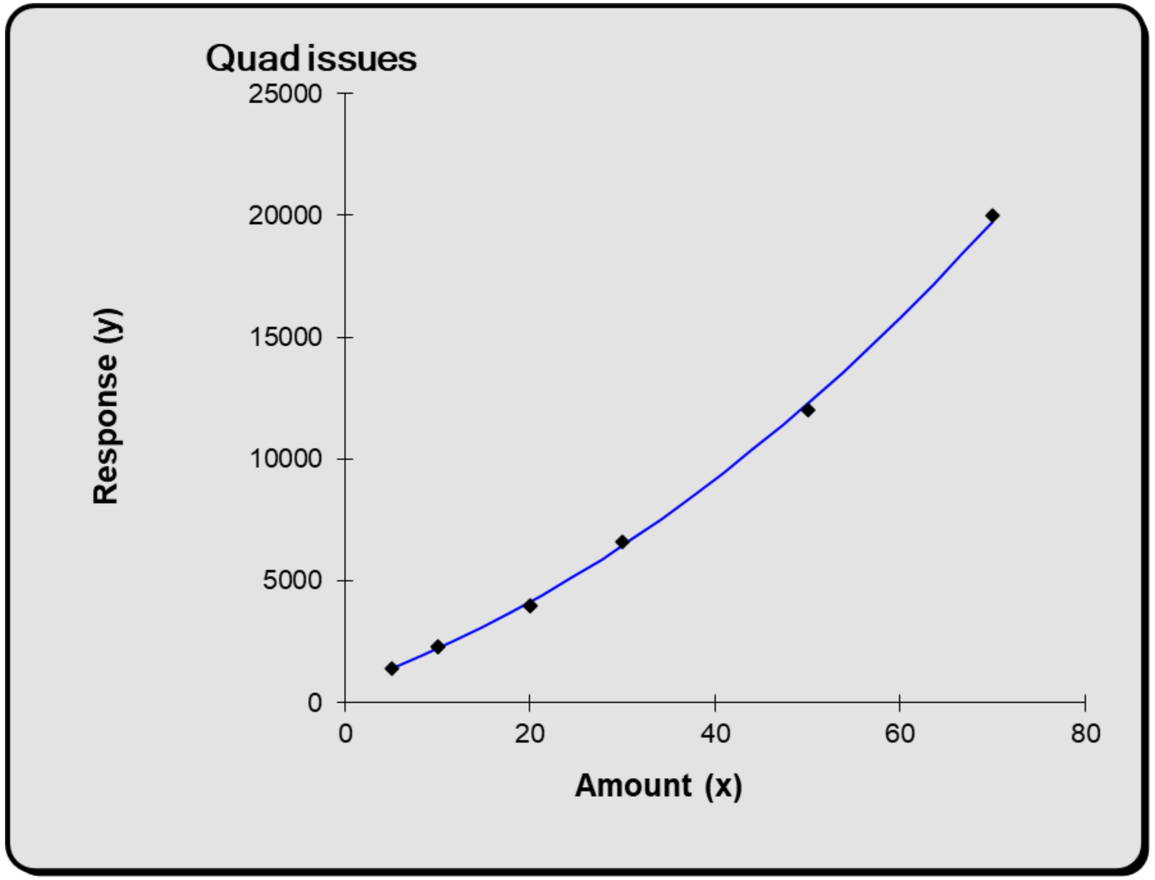


Not Continuously Differentiable (or monotonic)

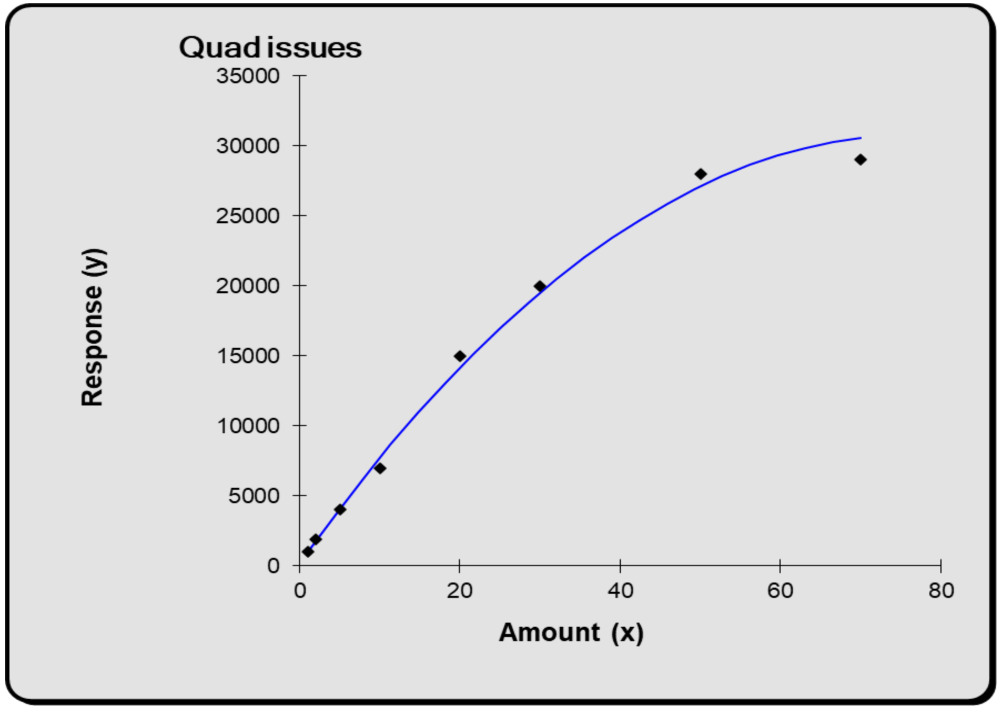


Is that enough?





Quadratic issues



$Y = 202 + 808x - 5.3x2$

RSE = 10.5%

Response factors	
1	991.0
2	937.5
5	810.2
10	701.2
20	756.6
30	677.4
50	564.3
70	418.6

Response factor for any two points on the curve should not differ by more than 2X
or
The response factors for any two adjacent points should not differ be more than 20%

In summary:

Regressions should be weighted by 1/(Concentration)^2

- In order to have the same relative weight for each calibration point

Forcing through zero may work but commonly reduces the quality of the calibration

- Therefore Average RF should be used with caution if at all

Linear regressions introduce unnecessary error with even quite slight amounts of curvature

There is no penalty to using a quadratic even if the function is completely linear

The best curve fit to use in almost all cases is 1/X^2 weighted Quadratic