

WEIGHTING • INSIGHTS 07

Calibration curve weighting: when to use $1/x$ and when to use $1/x^2$.

Unweighted regression assumes every standard has the same absolute scatter. Chromatographic detectors rarely behave that way. The weighting factor is how you tell the model the truth about your data.

SUMMARY

Least squares regression assumes constant variance across the calibration range. Chromatographic data usually show scatter that grows with concentration, and an unweighted fit then favors the top of the range at the expense of the reporting limit. This paper explains the rule for matching the weighting factor to the variance, works a ten-level example with replicate data, and sets out a documented procedure for selecting and defending the weight.

THE SHORT VERSION

- 01 Chromatographic scatter usually grows with concentration, so an unweighted fit favors the top standards and sacrifices the reporting limit.
- 02 Match the weight to the variance: when percent RSD is constant across the range, $1/x^2$ is the right factor.
- 03 Without replicates, pick the least weighting that minimizes the sum of absolute %RE, then fix it during validation.

The assumption inside every unweighted fit.

The ICAL looks clean at the top. The 500 ppb standard back-calculates at +0.1%. The 0.5 ppb standard back-calculates at -91.8%. Same data, same detector. The only problem is the regression.

Ordinary least squares places the line to minimize the sum of squared residuals. It treats every residual as equally trustworthy. That holds only when the scatter of the response is the same at every concentration, a condition statisticians call homoscedasticity.

Chromatographic data rarely meet it. The absolute scatter of a peak area at 500 ppb is far larger than at 0.5 ppb, even when relative precision is the same. An unweighted fit spends its effort on the high standards, where residuals are largest. The low standards absorb the error.

Method 8000D describes exactly this tendency and names weighting as the compensation ^[1]. If your lowest standard keeps failing, check the weighting as well as the instrument.

Match the weighting factor to the variance.

A weighting factor tells the regression how much to trust each standard. It is how you tell the regression the truth about your data. Gu and colleagues give the rule that connects the factor to the data ^[2]:

HOW THE SCATTER BEHAVES ACROSS THE RANGE	WEIGHTING FACTOR
Standard deviation is constant	1 (unweighted)
Variance grows in proportion to concentration	1/x
Standard deviation grows in proportion to concentration	1/x ²

The last row describes constant relative precision: a fixed percent RSD at every level. That is the common case for chromatographic detectors. It is why 1/x² is so often the right answer.

Burrows and Parr note that 1/x² weighting gives every calibration point the same weight in relative terms. They also show that an average response factor calibration is equivalent to a 1/x²-weighted regression through the origin [3].

So read the weight from the scatter you measure. Habit is not evidence.

Weighted regression on a ten-level curve.

The data below are a ten-level calibration from 0.5 to 500 ppb, with each level injected in triplicate. Four levels are shown with the scatter of their replicates.

LEVEL (PPB)	MEAN RESPONSE	SD OF TRIPLICATES	RELATIVE SD
0.5	771	17.5	2.3%
5	6,120	409.7	6.7%
50	59,880	1,869.1	3.1%
500	578,958	19,448.9	3.4%

The standard deviation grows by roughly three orders of magnitude across the range. The relative standard deviation stays in single digits. That is the third row of the rule: scatter proportional to concentration, which points to 1/x².

The weighting factor is how you tell the regression the truth about your data.

To test that prediction, the mean responses were fit three ways and each standard was back-calculated.

LEVEL (PPB)	UNWEIGHTED	1/X	1/X ²
0.5	-91.8%	-7.8%	+0.6%
1	-47.6%	-5.8%	-2.5%
2.5	-14.2%	+2.2%	+2.3%
5	-6.6%	+1.4%	+0.5%
10	-0.9%	+2.9%	+1.4%
25	+3.4%	+4.6%	+2.8%
50	+2.4%	+2.8%	+0.9%
100	+0.9%	+0.9%	-1.0%
250	-0.7%	-1.0%	-2.9%
500	+0.1%	-0.2%	-2.2%
SUM OF %RE	168.7	29.7	17.2
RSE	37.0%	4.2%	2.2%

Illustrative data set constructed for this article. Every derived value is computed from the mean responses shown.

-91.8%

ERROR AT 0.5 PPB, UNWEIGHTED

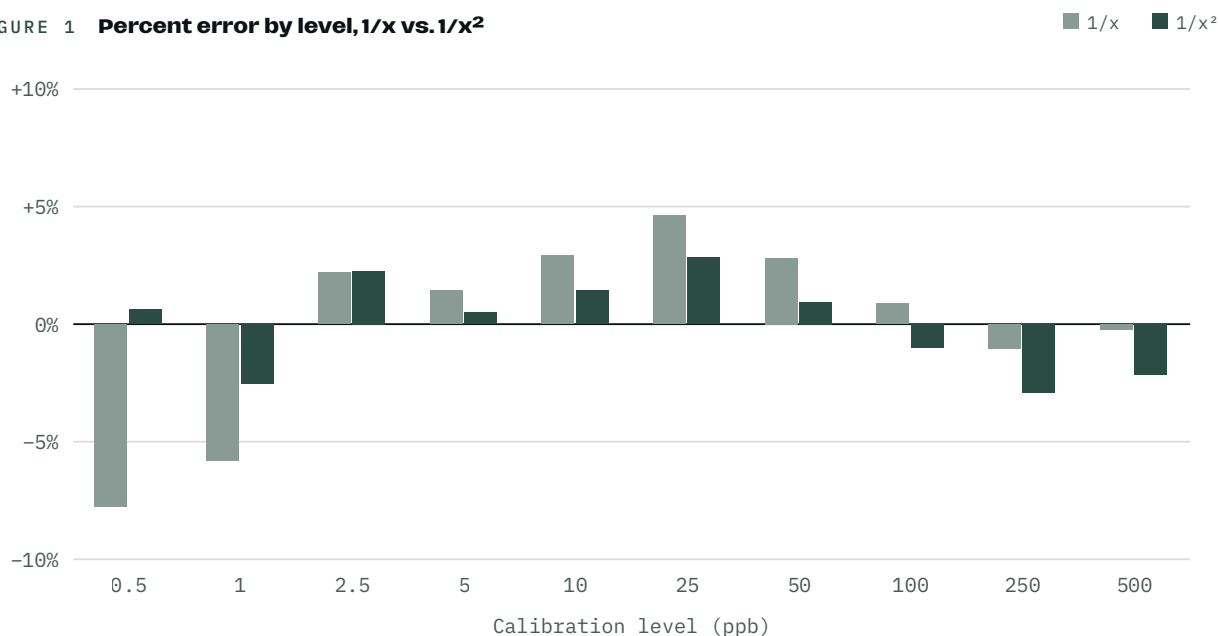
169 → 17

SUM OF |%RE|, UNWEIGHTED TO 1/X²

2.9%

LARGEST ERROR AT ANY LEVEL, 1/X²

FIGURE 1 Percent error by level, 1/x vs. 1/x²



The unweighted fit misses the lowest standard by 91.8%. That fails Method 8000D's 30% limit at the bottom of the range. $1/x$ brings every level inside 10%, though its largest errors still sit at the low end.

$1/x^2$ spreads the error evenly and holds every level within 2.9%. That is what the replicate data predicted. When the replicates and the fit agree, the weight is easy to defend.

Choosing the weight with the relative error test.

Replicates are the best evidence, but most routine ICAIs have a single injection per level. For those, the practical test comes from the bioanalytical literature.

Almeida, Castel-Branco and Falcão fit each candidate weight. They chose the one with the smallest sum of absolute percent relative error (%RE) across the range ^[4]. Dolan recommends the same comparison and adds a constraint: use the least amount of weighting that minimizes the error ^[5].

In this example the test agrees with the replicates. The sum of absolute errors falls from 169 unweighted to 30 with $1/x$ and 17 with $1/x^2$.

Gu and colleagues add a caution worth keeping in the record. In their LC-MS/MS assays, an incorrect weight changed reported concentrations only modestly, yet it significantly affected assay performance ^[2]. A wrong weight may move the numbers only a little. It still leaves a method that is harder to defend and less reliable near its limits.

What EPA, FDA and ICH say about weighting.

SOURCE	POSITION ON WEIGHTING
EPA SW-846 Method 8000D, Sec. 11.5.2	$1/x$ or $1/x^2$ weighting may be used to compensate for an unweighted fit's bias toward the upper levels.
FDA Bioanalytical Method Validation, 2018, Sec. III.B.2	Use the simplest model that adequately describes the concentration-response relationship, with an appropriate weighting scheme.
ICH M10, 2022, Sec. 3.2.4	The regression model, weighting scheme and any transformation are determined during method validation.

None of these documents mandates one factor for every method. All of them expect the choice to be made deliberately and recorded ^{[1][6][7]}. If an auditor asks why your method uses its weight, the answer should already be in the validation file.

A weighted regression checklist for the ICAI record.

01 Characterize the scatter.

Use replicate injections from method development to see whether SD or RSD is constant.

02 Match the factor to the variance.

Constant SD: unweighted. Variance proportional to x : $1/x$. Constant RSD: $1/x^2$.

03 Compare candidates on relative error.

Back-calculate every standard under each weight. Record the sum of absolute %RE.

04 Use the least weighting that minimizes error.

Do not pick a heavier weight than the data support.

05 Fix the weight in the method.

Set it during validation, never batch by batch to rescue a failing curve.

06 Check the low standard.

Weighting exists to protect accuracy near the reporting limit. Confirm it does.

Where WLTR fits.

Few labs compare three weightings for every analyte, in every ICAL, by hand. Most keep the weighting set when the method was first built, whether or not current data still support it.

WLTR is calibration data integrity infrastructure for regulated analytical laboratories. For each analyte, it derives nine calibration regression models: average response factor, plus linear and quadratic fits across four weightings each. It scores every model against fifteen ICAL parameters, including RSE. All of it is computed orthogonally to the CDS, under U.S. Patent 12,038,448 B2 ^[P].

CALIBRATION VERIFICATION

See every weighting for every analyte in your ICAL, with the error each one introduces at your reporting limit.

[GET COMPLIANCE GAP ASSESSMENT](#)

WLTR.TECH

SOURCES

1. U.S. EPA, SW-846 Test Method 8000D, *Determinative Chromatographic Separations*, Revision 5, March 2018, Sec. 11.5.2. www.epa.gov/hw-sw846/sw-846-test-method-8000d-determinative-chromatographic-separations
2. H. Gu, G. Liu, J. Wang, A.-F. Aubry, M. E. Arnold, "Selecting the Correct Weighting Factors for Linear and Quadratic Calibration Curves with Least-Squares Regression Algorithm in Bioanalytical LC-MS/MS Assays," *Analytical Chemistry* 86(18), 2014, 8959–8966. DOI 10.1021/ac5018265. pubs.acs.org/doi/10.1021/ac5018265
3. R. Burrows and J. Parr, "Evaluating the Goodness of Instrument Calibration for Chromatography Procedures," *LCGC Supplements* 38(11), November 2020. www.chromatographyonline.com/view/evaluating-the-goodness-of-instrument-calibration-for-chromatography-procedures
4. A. M. Almeida, M. M. Castel-Branco, A. C. Falcão, "Linear regression for calibration lines revisited: weighting schemes for bioanalytical methods," *Journal of Chromatography B* 774(2), 2002, 215–222. DOI 10.1016/S1570-0232(02)00244-1. [doi.org/10.1016/S1570-0232\(02\)00244-1](https://doi.org/10.1016/S1570-0232(02)00244-1)
5. J. W. Dolan, "Calibration Curves, Part V: Curve Weighting," *LCGC North America* 27(7), July 2009. www.chromatographyonline.com/view/calibration-curves-part-v-curve-weighting
6. U.S. FDA, *Bioanalytical Method Validation, Guidance for Industry*, May 2018. www.fda.gov/media/70858/download
7. ICH M10, *Bioanalytical Method Validation and Study Sample Analysis*, May 2022, Sec. 3.2.4. database.ich.org/sites/default/files/M10_Guideline_Step4_2022_0524.pdf
8. U.S. Patent 12,038,448 B2, inventor Walter "Chip" Hinchman Sr. patents.google.com/patent/US12038448B2

Walter Hinchman, MBA

WLTR, INC. • NEVADA C-CORP

WLTR