

Should a calibration curve go through zero? When forcing it is justified, and when it is a finding.

Zero concentration should give zero response. Real instruments do not always agree, and forcing the curve through the origin can move the error to the one place your lab can least afford it.

SUMMARY

Forcing a regression through the origin removes one parameter from the calibration model. It is acceptable when the true intercept is indistinguishable from zero and harmful when it is not. This paper sets out the regulatory position, explains the standard-error test for the intercept, and works two seven-level examples: one where forcing makes no difference and one where it introduces a 40% error at the lowest standard.

THE SHORT VERSION

- 01 Fit with a free intercept first, then compare the intercept with its own standard error.
- 02 If the intercept sits within about one standard error of zero, forcing the curve through the origin is reasonable.
- 03 Never add a zero point, and never use a forced curve to report below the lowest standard.

Why forcing a curve through zero is tempting.

The ICAL is done, and the low end of the plot sags a little below the line. There is a checkbox in the CDS that would pin the curve to the origin and tidy it up.

The logic feels sound. A line has two parameters, a slope and an intercept. A sample with no analyte should give no signal, so the intercept should be zero. Forcing it there removes a parameter and makes the curve look the way theory says it should.

The intercept, though, is a measurement. A positive intercept can come from background or a co-eluting interference. A negative one can come from adsorptive losses that consume a fixed amount of analyte before any reaches the detector.

Forcing zero removes neither effect. It hides them, and the error moves to the low standards.

What the regulations say.

EPA SW-846 Method 8000D allows a linear regression to be forced through the origin, provided the calibration criteria are met. It attaches two firm conditions ^[1]:

01 No zero calibration point.

Whatever model you choose, do not include an X value of zero as a calibration point.

02 No reporting below the range.

Forcing through the origin is not a reason to report results below the lowest calibration standard.

Bioanalytical guidance takes the same line on blanks. ICH M10 states that blank and zero samples should not be included in the regression ^[2]. A blank is evidence of contamination or carryover. It is judged on its own criteria and is not a calibration standard.

One link is useful to keep in mind. An average response factor calibration is, mathematically, a $1/x^2$ -weighted regression forced through the origin ^[3]. If your lab uses average RF, you already use a zero-intercept model, and the RSD criterion is what tests whether that model holds.

Test the intercept against its own error.

The intercept is never exactly zero. What matters is whether it can be told apart from zero, given the scatter in the data.

Dolan gives the practical test. Compare the intercept with its own standard error, and not with the overall standard error of the regression ^[4].

STANDARD ERROR OF THE INTERCEPT, UNWEIGHTED LEAST SQUARES

$$SE(b) = s_{y/x} \times \sqrt{1/n + \bar{x}^2 / \sum(x_i - \bar{x})^2}$$

$s_{y/x}$ is the residual standard deviation of the regression, n the number of standards and $\bar{x}$ their mean concentration. If the intercept lies within about one $SE(b)$ of zero, forcing through the origin is reasonable. If it lies further out, keep the intercept.

Using the wrong standard error leads to the wrong call. In Dolan's worked example, the regression's overall standard error would have suggested forcing a curve whose intercept was real ^[4]. Check the intercept against $SE(b)$ before you touch the checkbox.

Two calibrations, two answers.

Two seven-level calibrations from 1 to 100 ppb were each fit twice: once with a free intercept and once forced through zero. Case A is a compound that loses a fixed amount to active sites in the system. Case B has no such loss.

LEVEL (PPB)	CASE A, FREE INTERCEPT	CASE A, FORCED ZERO	CASE B, FREE INTERCEPT	CASE B, FORCED ZERO
1	-7.5%	-40.0%	-0.1%	+1.2%
2	-13.3%	-29.3%	+1.9%	+2.6%
5	-0.9%	-7.0%	-2.3%	-2.0%
10	-0.6%	-3.4%	+0.8%	+1.0%
20	+0.5%	-0.6%	+1.1%	+1.2%
50	+1.4%	+1.2%	-0.7%	-0.7%
100	-0.3%	-0.2%	+0.1%	+0.1%
INTERCEPT ± SE		-273 ± 148		11 ± 89
INTERCEPT / SE		1.8		0.1

Illustrative data sets constructed for this article. Unweighted linear regression. Responses, Case A: 498, 1,173, 3,858, 8,018, 16,492, 41,983, 82,813. Case B: 863, 1,750, 4,178, 8,612, 17,263, 42,336, 85,417.

1.8 SE

CASE A INTERCEPT DISTANCE FROM ZERO

-40.0%

CASE A ERROR AT 1 PPB WHEN FORCED THROUGH ZERO

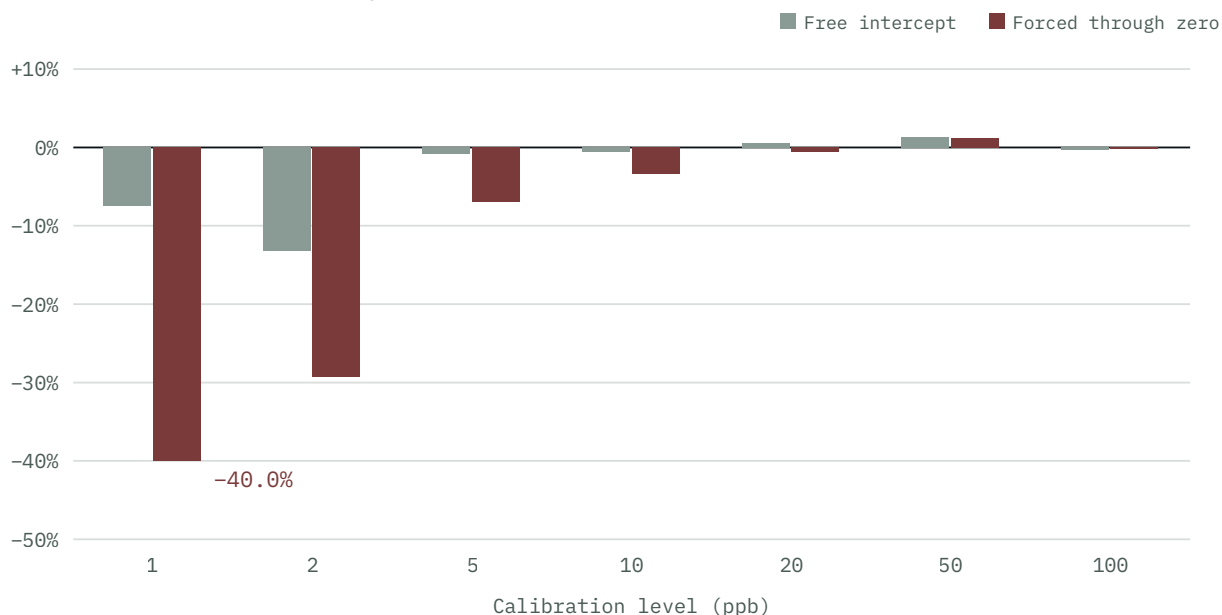
0.1 SE

CASE B INTERCEPT DISTANCE FROM ZERO

In Case B the intercept is 0.1 standard errors from zero. Forcing it moves no standard by more than 1.3 percentage points, and either model is defensible.

Case A is different. Its intercept is 1.8 standard errors from zero. Forcing it pushes the 1 ppb standard to -40.0%, outside Method 8000D's 30% limit. The high standards barely move.

FIGURE 1 Case A: percent error by level, free intercept vs. forced zero



The whole cost of the forced model lands at the reporting limit.

The reporting limit is the one place your lab can least afford that error. Check the low standard before you accept a forced fit.

What a non-zero intercept is telling you.

A significant intercept is information about the method. A negative one points to adsorption, degradation or incomplete transfer, which matter most at low concentration. A positive one points to background, contamination or an interference.

Each has a corrective action in the method or the instrument. Record it and fix it, rather than model it away.

The intercept also sets what a zero response would report. A curve with a negative intercept reports a positive concentration for a blank. A curve with a positive intercept reports a negative one.

Neither is a reason to report below the lowest standard. Method 8000D does not allow that under any model ^[1].

A checklist for forcing a curve through zero.

01 **Fit with a free intercept first.**

Record the intercept and its standard error.

02 **Compare the intercept with its own SE.**

Within about one SE of zero, forcing is reasonable. Beyond it, keep the intercept.

03 **Back-calculate both fits.**

Compare the percent error at the low and mid standards before you choose.

04 **Never add a zero point.**

Judge blanks on their own criteria. Keep them out of the regression.

05 **Investigate a significant intercept.**

Adsorption, background and interference each have a fix.

06 **Report only within the calibrated range.**

Forcing zero never extends the range below the lowest standard.

Where WLTR fits.

Most data systems offer forced zero as a checkbox. They report neither the intercept's standard error nor the error it adds at the low standard, so the choice is usually made by habit.

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 the intercept, its standard error and the effect of forcing zero for every analyte in your ICAL.

[GET COMPLIANCE GAP ASSESSMENT](#)

WLTR.TECH

SOURCES

1. U.S. EPA, SW-846 Test Method 8000D, *Determinative Chromatographic Separations*, Revision 5, March 2018, Secs. 11.5 and 11.5.2.1. www.epa.gov/hw-sw846/sw-846-test-method-8000d-determinative-chromatographic-separations
2. 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
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. J. W. Dolan, "Calibration Curves, Part I: To b or Not to b?," *LCGC North America* 27(3), March 2009. www.chromatographyonline.com/view/calibration-curves-part-i-b-or-not-b
5. 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

