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WHITE PAPER · CALIBRATION FUNDAMENTALS

LOW END · INSIGHTS 31

Negative or missing results at the low end: when the intercept outweighs the response.

A blank reads below zero, a low standard comes back empty, and the data system flags it as indeterminate. The cause is usually the intercept, and the intercept is usually the weighting.

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SUMMARY

A calibration line with an intercept larger than the response of a low sample returns a negative concentration, and many data systems blank or flag the result. This paper explains where that intercept comes from, works an illustrative eight-level curve under three weightings, and sets out what EPA Methods 8000D and 8260D and ICH M10 allow near the bottom of the range. Forcing the line through zero hides the symptom; weighting usually removes the cause.

THE SHORT VERSION

01 An unweighted fit lets the top of the curve set the intercept, and a few percent of error there swamps the bottom.

02 When a low response sits below the intercept, the result is negative, and many data systems leave it blank.

03 Weight the fit, judge the low standard by its back-calculated error, and do not force through zero to make the symptom go away.

The blank that came back below zero.

The method blank reads −0.32 µg/L. A low spike, made at half the reporting limit, comes back negative. The data system leaves the field empty and adds a flag. The ICAL passed with r^2 of 0.9996.

Nothing is wrong with the blank, and nothing is wrong with the spike. The line is wrong at the bottom, in a way that r^2 is not built to notice.

Where the intercept comes from.

An unweighted least-squares fit minimises absolute residuals. A 2% error at 100 µg/L is 2 µg/L of response. A 2% error at 0.5 µg/L is 0.01. The top standards outvote the bottom ones by a factor of hundreds, and they decide where the line crosses the axis.

So a few percent of scatter at the top can push the intercept up or down by more than the entire signal of the lowest standard. Results near the bottom inherit that offset. If it is positive and large enough, a low response falls below it and back-calculates to a negative number.

Some data systems report the negative value. Others leave the concentration blank, or mark the result as indeterminate. The flag is honest. It is telling you the line does not describe the low end.

One data set, three weightings.

Here is an illustrative eight-level curve, 0.5 to 100 µg/L, with no more than 3.5% scatter at any level. It was fitted three ways.

LEVEL (MG/L)	UNWEIGHTED	1/X	1/X²
0.5	−61.8% FAIL	+0.8% PASS	+1.7% PASS
1	−34.5% FAIL	−3.8% PASS	−3.4% PASS
2	−14.2% PASS	+0.4% PASS	+0.4% PASS
5	−7.5% PASS	−2.4% PASS	−2.6% PASS
10	−0.3% PASS	+1.6% PASS	+1.4% PASS
25	+3.2% PASS	+3.1% PASS	+2.8% PASS
50	+2.8% PASS	+2.1% PASS	+1.8% PASS
100	−0.9% PASS	−1.9% PASS	−2.1% PASS
r²	0.9996	0.9994	0.9993
Intercept (µg/L)	+0.324	+0.004	−0.002

Illustrative data set constructed for this article, not instrument data. Limits shown are the 8000D refit criteria: ±30% at every level, with ±50% allowed at the lowest point for some data uses.

0.9996
R², UNWEIGHTED

−61.8%
LOWEST STANDARD, UNWEIGHTED

−0.07 µg/L
SAMPLE AT HALF THE LLOQ, UNWEIGHTED

The unweighted fit has the highest r^2 of the three. It also reads the lowest standard at −61.8%, and a sample at half the LLOQ at −0.07 µg/L. Every point above 5 µg/L is fine.

With $1/x^2$ weighting, the same data put the lowest standard at +1.7% and the half-LLOQ sample at 0.25 µg/L. Nothing about the data changed. Only the question the fit was asked to answer.

The flag is honest. It is telling you the line does not describe the low end.

Why forcing through zero is the wrong fix.

The quick fix is to force the line through the origin. The negative results disappear. So does the evidence that the model was wrong at the bottom.

EPA Method 8000D is direct on this. Forcing the regression through the origin may not be used as a rationale for reporting results below the calibration range [1]. Our paper on forcing a calibration curve through zero covers when a zero intercept is justified.

Forcing through zero is a model choice with its own conditions. It belongs in the SOP with evidence that the true intercept is zero, not in the review as a way to clear a flag.

What the methods allow near the LLOQ.

The methods agree on the shape of the low end, even where the numbers differ:

01 **The lowest standard sets the LLOQ.**
8000D ties the LLOQ to the lowest standard in the ICAL. Results below it should not be reported unless qualified as estimated [1].

02 **Refit error at the low point.**
8000D asks for 30% or less at every standard, with up to 50% acceptable at the lowest point for some data uses. 8260D holds the lowest point to ±50% [2].

03 **Bioanalysis is tighter.**
ICH M10 holds the LLOQ standard to ±20% of nominal, and every other level to ±15% [3].

All three judge the low end by back-calculated error at the low standard. None of them judges it by r^2. That is the test to run first.

A low-end review checklist.

01 **Back-calculate the lowest standard.**
Under the model you report, every time.

02 **Compare the intercept with the LLOQ.**
In concentration units, side by side.

03 **Weight wide ranges.**
Choose $1/x$ or $1/x^2$ from the residuals, and record why.

04 **Do not force through zero to clear a flag.**
Treat it as a model choice with evidence.

05 **Never report below the LLOQ unqualified.**
Estimated, flagged, or not at all.

06 **Read blank flags as model evidence.**
A negative blank is a fit problem first.

Where WLTR fits.

A curve can be right at the top and wrong at the bottom, and r^2 will rate it highly. Seeing the low standard back-calculated under every model and weighting, side by side, is what shows which fit describes the end of the range you report from.

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 calibration standard back-calculated under every regression model and weighting, computed orthogonally to your CDS.

GET COMPLIANCE GAP ASSESSMENT WLTR.TECH

SOURCES

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2. U.S. EPA, SW-846 Test Method 8260D, Volatile Organic Compounds by Gas Chromatography/Mass Spectrometry, Revision 4, June 2018, Sec. 11.3.5.4. www.epa.gov/sites/default/files/2018-06/documents/method_8260d_update_vi_final_06-11-2018.pdf
3. ICH, M10: Bioanalytical Method Validation and Study Sample Analysis, final version adopted 24 May 2022, Sec. 3.2.4. www.pmda.go.jp/files/000246792.pdf
4. U.S. Patent 12,038,448 B2, inventor Walter “Chip” Hinchman Sr. patents.google.com/patent/US12038448B2

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