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WHITE PAPER · CALIBRATION IN PRACTICE

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Serial or independent dilutions: how standard prep error gets inside the curve.

A calibration curve can only be as true as the standards it was built from. Some preparation errors show up as scatter. Others bend the curve, and one kind never shows up at all.

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SUMMARY

Calibration standards are usually made by diluting a stock, either step by step down a series or each directly from the stock. This paper works through how preparation error behaves under each scheme. A small transfer bias compounds down a serial series and quietly bends the low end of the curve. An error in the stock itself is invisible to every ICAL statistic and is caught only by a truly independent second source, and then only if it is large enough.

THE SHORT VERSION

01 In a serial series, a transfer bias repeats at every step, so the lowest standard carries all of it.

02 An error in the stock moves every standard by the same factor, so RSD, r^2 and every refit still pass.

03 Only a truly independent second source can see a stock error, and a wide ICV window will still let a modest one through.

A clean ICAL from a dirty stock.

The ICAL is the best the instrument has produced all year. RSD under 3%, every standard inside ±5% on refit. The ICV comes back at 93%, well inside the window, and the run goes ahead.

The stock was made 8% strong. Nothing in the calibration could have shown it. Every standard was 8% strong in exactly the same way, so the curve was perfectly linear and perfectly wrong.

Two ways to make a calibration series.

There are two common schemes. In a serial series, each standard is made from the one above it. In an independent series, each standard is made directly from the stock, or from a small number of intermediate stocks.

EPA Method 8000D notes that standards prepared by serial dilution of a stock typically form a geometric series. It suggests a partly arithmetic series at the top, because geometric spacing can mask a detector reaching saturation [1].

Serial series are popular because they use less solvent and less stock. In a 2022 analysis, Snow showed that each added dilution step adds to the uncertainty of the result. His serial example raised it from 0.6% to 0.9% over a single dilution [2].

How a small bias compounds down a series.

Random error grows slowly down a series. Bias does not. If a pipette or a technique delivers 1% low, an independent series puts every standard 1% low. A serial series puts the first standard 1% low and the eighth 7.7% low.

LABELLED (MG/L)	SERIAL STEPS	ACTUAL VS LABEL, SERIAL	ACTUAL VS LABEL, INDEPENDENT	SAMPLE BIAS ON SERIAL CURVE	RANDOM UNCERTAINTY, SERIAL
100	1	−1.0%	−1.0%	+1.0%	0.30%
50	2	−2.0%	−1.0%	+2.0%	0.42%
20	3	−3.0%	−1.0%	+3.1%	0.52%
10	4	−3.9%	−1.0%	+4.1%	0.60%
5	5	−4.9%	−1.0%	+5.2%	0.67%
2	6	−5.9%	−1.0%	+6.2%	0.73%
1	7	−6.8%	−1.0%	+7.3%	0.79%
0.5	8	−7.7%	−1.0%	+8.4%	0.85%

Illustrative calculation for an eight-level series with a 1% low delivery at every transfer. Random uncertainty assumes 0.3% relative standard uncertainty per dilution, combined in quadrature. Sample bias is for a sample at that true concentration read on the serial curve.

The serial curve is still linear and still passes. RF RSD is 2.5%, and the worst refit error on a $1/x^2$ line is +2.1%. But the low standards hold less analyte than their labels say. A real sample at 0.5 µg/L reads +8.4% on that curve.

−7.7%
LOWEST SERIAL STANDARD VS ITS LABEL

2.5%
RF RSD, STILL PASSING

+8.4%
SAMPLE BIAS AT THE LOWEST LEVEL

The stock error no statistic can see.

Now make the stock 8% strong and prepare everything else perfectly. Every standard is 8% strong. Every response is 8% higher than its label predicts, by the same factor.

The response factors are all equal, so the RSD does not change. The line passes through every point, so r^2 and refit errors do not change. Every sample reads −7.4%.

A stock error moves every standard by the same factor. A curve cannot see a factor.

This is why serial and independent schemes share one weakness. Both start from the same stock. The scheme decides how preparation error is distributed along the curve. It does nothing about an error that came in at the top.

What a second source can and cannot catch.

The only witness to a stock error is a standard made from a different stock. ICH M10 says calibration standards and QCs should be prepared from separate stock solutions [3]. EPA Method 8260D requires a second-source ICV after each ICAL, with a suggested range of 70% to 130% [4].

A 70% to 130% window will not flag an 8% stock error. An ICV at 93% looks fine. A QC limit of ±15% will not flag it either. The second source is there to catch gross errors: the wrong ampoule, a missed dilution, a decimal in the wrong place.

To see smaller errors, trend the ICV recovery across many calibrations. A stock that is consistently a few percent off shows up as an offset that does not average away.

Preparation habits that keep the curve honest.

Limit serial steps. Two or three steps from an intermediate stock keeps compounding small. Use Class A glassware, and verify pipettes and syringes gravimetrically at the volumes you actually use.

Prepare by mass where you can. A balance reading is easier to trace than a meniscus. Record every step with the device, lot and volume, so a bad standard can be traced to the step that made it.

A standard preparation checklist.

01 **Limit serial steps.**
Use intermediate stocks rather than long chains.

02 **Verify delivery devices.**
Gravimetrically, at the volumes the SOP uses.

03 **Use a truly independent second source.**
Different lot, ideally a different supplier.

04 **Trend ICV recovery.**
A consistent offset is a stock problem until shown otherwise.

05 **Check the low end on the curve.**
Compounded bias shows first in the lowest standards.

06 **Record the preparation chain.**
Device, lot, volume and analyst for every step.

Where WLTR fits.

A calibration curve reports how standards behaved, not whether they were made right. Seeing each standard's back-calculated error under every model, run after run, is what shows a low end that has drifted from its labels.

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

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SOURCES

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