

WHITE PAPER · CALIBRATION IN PRACTICE

OVER-CURVE SAMPLES · INSIGHTS 33

Over-curve samples: dilution, extrapolation and the internal standard problem.

A result above the top standard is not a measurement. It is an estimate from a part of the curve that was never calibrated, and with an internal standard, dilution may not fix it.

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SUMMARY

A sample that reads above the highest calibration standard has to be brought back inside the range. This paper shows, with an illustrative detector that begins to saturate, how far extrapolated results can fall below the truth. It then covers the trap specific to internal standard methods: diluting a sample after the internal standard is added leaves the ratio, and the result, unchanged. It closes with what EPA Methods 8000D and 8260D and ICH M10 require.

THE SHORT VERSION

01 Above the top standard the curve is a guess, and detectors usually bend there, so extrapolated results read low.

02 With an internal standard, diluting the extract after the IS is added does not change the ratio or the result.

03 Dilute the sample before the IS is added, or add more IS, and use a dilution scheme the lab has shown to work.

The result above the top standard.

The sample reads 236 µg/mL. The top standard is 100. The analyst dilutes the vial five-fold, re-injects, multiplies back, and gets 236 again. The repeat agrees with the first result, so it gets reported.

Both numbers are wrong for the same reason. The true value is 300, and neither injection ever measured it.

Why extrapolation reads low.

EPA Method 8000D warns that extrapolating the calibration beyond its range may lead to significant quantitative errors. Instrument response must fall within the range set by the initial calibration [1].

The reason is the detector. Many detectors start to flatten near the top of their range. Inside the calibration, a slight bend barely shows. Every standard here back-calculates within −4.8%. Beyond the top standard, the line keeps going straight while the response does not.

TRUE (MG/ML)	REPORTED (MG/ML)	ERROR		POSITION
100	95	−4.8%	PASS	Inside range
125	116	−6.9%	PASS	1.2x the top standard
150	137	−8.9%	PASS	1.5x the top standard
200	174	−13.1%	PASS	2x the top standard
300	236	−21.3%	FAIL	3x the top standard
400	282	−29.6%	FAIL	4x the top standard

Illustrative detector with a slight onset of saturation, calibrated from 1 to 100 µg/mL with a 1/x² linear fit. Not instrument data.

At the top standard the error is −4.8%. At 400 µg/mL, four times the top of the curve, the sample reads −29.6%. The result is low, and nothing about it looks unusual.

A result above the top standard is not a measurement. It is an estimate from a part of the curve no one calibrated.

The internal standard dilution trap.

With external standards, the fix is simple: dilute the sample until it falls inside the range. With an internal standard, it depends on when the IS went in.

The IS method reports the ratio of analyte to IS. If the extract already contains the IS, a five-fold dilution lowers both peaks five-fold. The ratio does not move, so the result does not move. The diluted injection reads 236 µg/mL, the same as before.

LCGC's LC Troubleshooting column made this point in 2015. Compensating for dilution is why the IS was added, and over-curve samples are where that same property defeats the analyst [2]. Dilute the sample with blank matrix before the IS is added. The five-fold dilution then reads 295 µg/mL, measured inside the range where the curve is real.

What the methods require.

01 **8000D.**
Some samples need dilution to bring target analytes within the calibration range [1].

02 **8260D.**
Prepare and analyse a dilution, aimed so the analytes that saturated fall near the middle of the range (Sec. 11.5.4) [3].

03 **M10 dilution integrity.**
Dilute with the same matrix used for the QCs. Test at least five replicates per dilution factor; mean accuracy within ±15% and precision within 15% [4].

04 **M10 study samples.**
Samples above the ULOQ are diluted using the validated dilution method. If a large number of study samples fall above the ULOQ, M10 says the analysis should be stopped [4].

Carryover after a high sample.

A sample high enough to be over-curve is high enough to leave some of itself behind. 8260D asks for at least one method or instrument blank after a sample that may cause carryover. If a blank cannot be run, the next sample is reviewed, and the absence of the high analytes shows carryover did not occur [3].

Check the sample after every over-curve result before reporting it. A low hit after a high one is a carryover question first.

An over-curve sample checklist.

01 **Never report above the top standard.**
Dilute and re-analyse, or qualify as estimated.

02 **Dilute before the IS goes in.**
Or add IS in proportion, as the validated method sets out.

03 **Aim for the middle of the range.**
Not just under the top standard.

04 **Prove the dilution scheme.**
Dilution integrity data for each factor in use.

05 **Check the next sample.**
Or run a blank, for carryover.

06 **Watch for clustering.**
Many over-curve samples mean the range is wrong.

Where WLTR fits.

The top of a curve is where detectors bend and models disagree. Seeing every standard back-calculated under every model, quadratic options included, shows how much of the range can be trusted.

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.

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SOURCES

1. U.S. EPA, SW-846 Test Method 8000D, Determinative Chromatographic Separations, Revision 5, March 2018, Secs. 9.5.2.6 and 11.4. www.epa.gov/sites/production/files/2015-12/documents/8000d.pdf
2. Internal Standard Calibration Problems, LC Troubleshooting, LCGC North America 33(6), June 2015. www.chromatographyonline.com/view/internal-standard-calibration-problems-0
3. U.S. EPA, SW-846 Test Method 8260D, Volatile Organic Compounds by Gas Chromatography/Mass Spectrometry, Revision 4, June 2018, Secs. 4.3, 11.5.4 and 11.5.5. www.epa.gov/sites/default/files/2018-06/documents/method_8260d_update_vi_final_06-11-2018.pdf
4. ICH, M10: Bioanalytical Method Validation and Study Sample Analysis, final version adopted 24 May 2022, Secs. 3.2.7 and 3.3.3. www.pmda.go.jp/files/000246792.pdf
5. U.S. Patent 12,038,448 B2, inventor Walter “Chip” Hinchman Sr. patents.google.com/patent/US12038448B2

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