

WHITE PAPER · CALIBRATION IN PRACTICE

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Internal standard response drift: when the correction stops correcting.

An internal standard can lose almost half its area and still pass the method window. If the analytes do not drift with it, every result in the run moves, and nothing in the sequence turns red.

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SUMMARY

Internal standard calibration assumes the analyte and its internal standard gain or lose signal together. This paper sets out what EPA Method 8260D, ICH M10 and FDA guidance say about internal standard response, and works an illustrative sequence in which the internal standard drifts and the analyte does not. The internal standard stays inside the method window for the whole run while reported results climb well past any acceptance limit a lab would accept for a CCV.

THE SHORT VERSION

01 An internal standard corrects only the signal changes it shares with the analyte.

02 8260D allows the IS area to fall to half its midpoint value, which is far wider than any result tolerance.

03 Trend IS area by injection, compare it with the calibration standards, and set an SOP limit tighter than the method's.

The CCV at hour nine.

The CCV comes off at hour nine. The internal standard area is 75% of the ICAL midpoint. The method window is 50% to 200%, so the check passes and the batch goes on.

Three of the late-eluting analytes read high. Not by much at first. By the last injection, the worst of them reads +54.5%. Nothing in the sequence turned red, because nothing in the sequence was looking at the right ratio.

What the methods say about internal standard response.

EPA Method 8260D sets two checks on each internal standard in the calibration verification [1]:

01 **Retention time.**
If it moves by more than 30 seconds from the midpoint standard of the most recent ICAL, inspect the system (Sec. 11.4.4).

02 **Response.**
If the area changes by more than a factor of 2, that is outside 50% to 200% of the midpoint ICAL standard, inspect the system (Sec. 11.4.5). The lab may compare against the average of the ICAL standards instead, if its SOP says so.

ICH M10 asks bioanalytical labs to monitor IS responses in study samples for systemic variability. An IS response that differs significantly from the calibration standards and QCs, as defined in an SOP, is a listed reason for reanalysis [2].

FDA's 2019 question-and-answer guidance on IS response sets no numeric IS limit. It asks whether sample IS responses fall in the same range as the calibration standards and QCs in the same run. Drift is a concern when it moves outside that range, or when no QCs sit in the affected part of the run [3].

The one assumption every IS calibration makes.

The internal standard method reports a ratio. Analyte area over IS area, read against the same ratio in the standards. If injection volume falls by 10%, both areas fall by 10%, the ratio holds, and the result is right.

That is the whole mechanism. The IS corrects the part of the signal change it shares with the analyte. It cannot correct the part it does not share. When the IS loses signal faster than the analyte, the ratio rises and the result reads high. When it loses signal more slowly, the result reads low.

A deuterated analogue that co-elutes with its analyte shares almost everything. A single IS assigned to a group of compounds across a wide retention window shares much less. Most multi-analyte methods use the second kind.

A drift that passes every window.

Here is an illustrative run of 20 injections after the ICAL. The IS area falls steadily to 55% of its midpoint value. One analyte assigned to it falls only to 85%. A second analyte has its own labelled analogue, which drifts exactly with it.

INJECTION	IS AREA VS ICAL MIDPOINT	ANALYTE AREA VS ICAL	REPORTED BIAS, UNMATCHED IS	REPORTED BIAS, MATCHED IS
1	100% PASS	100%	+0.0% PASS	+0.0%
5	94% PASS	98%	+4.2% PASS	+0.0%
9	85% PASS	95%	+11.4% PASS	+0.0%
13	75% PASS	92%	+21.9% FAIL	+0.0%
17	64% PASS	88%	+37.5% FAIL	+0.0%
20	55% PASS	85%	+54.5% FAIL	+0.0%

Illustrative sequence constructed for this article, not instrument data. Reported bias is the ratio of analyte to IS signal change; the matched case assumes the analyte and its labelled analogue lose signal identically.

55%	+54.5%	Injection 13
LOWEST IS AREA, STILL INSIDE 50 TO 200%	REPORTED BIAS AT THE LAST INJECTION	FIRST RESULT MORE THAN 20% HIGH

The IS never leaves the 8260D window. The unmatched analyte crosses 20% high at injection 13 and reaches +54.5% by the end. The matched analyte reads +0.0% the whole way.

The CCV would catch this if it sat in the affected stretch, and if that analyte were one of the compounds that failed. In a long run with one CCV every twelve hours, much of the drift falls between checks.

The IS window is a factor of two. No result in your report is allowed a factor of two.

Drift inside the initial calibration itself.

Drift does not wait for the samples. If the IS area changes from level to level inside the ICAL itself, the response factors carry that change. A trap or source that responds differently at high load can make the IS area rise or fall with standard concentration.

The result looks like a calibration problem, not an IS problem. Response factors trend with level, the RSD widens, and the analyst reaches for a quadratic. The curve fits. The cause is still there, and it will behave differently in samples that do not follow the standards' pattern.

So read the IS areas across the ICAL before you read the RSD. A flat IS across all levels is the starting condition the rest of the review depends on.

Trend it, and set a limit that means something.

A pass against 50% to 200% tells you the instrument has not failed. It does not tell you the results are right. Three habits close that gap.

First, plot IS area against injection number for every run, with the calibration standards and QCs marked. FDA's test is whether the samples sit in the same range as the standards and QCs [3]. The plot answers that at a glance.

Second, write a tighter internal limit into the SOP for the compounds that matter, and say why. ICH M10 expects the threshold for a significant difference to be set in advance [2]. Third, assign internal standards by retention time and chemistry, not by habit, and record which analytes each one serves.

An internal standard review checklist.

01 **Check IS area across the ICAL.**
Before the RSD, before the model choice.

02 **Plot IS area by injection.**
Every run, with standards and QCs marked.

03 **Compare samples with standards.**
Same range, or a documented reason why not.

04 **Set an SOP limit tighter than the method.**
And write down the basis for it.

05 **Map analytes to their IS.**
By retention time and chemistry, and review the map.

06 **Bracket long runs.**
Enough CCVs that drift cannot hide between them.

07 **Reanalyze on evidence.**
Follow the pre-set repeat criteria and record the outcome.

Where WLTR fits.

An internal standard hides drift by design. That is its job when the drift is shared, and its weakness when it is not. Seeing each analyte's back-calculated error under every model, run after run, is what shows when the correction has stopped working.

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 analyte in your ICAL 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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