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ICH M10 calibration curve acceptance criteria, explained.



ICH M10 states its calibration curve acceptance criteria in four places: for chromatography and ligand binding assays, in validation and in study runs. This paper sets out each rule with its section number and works one illustrative LC-MS/MS curve through a failed mid-level standard and a failed LLOQ.

ICH M10

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THE SHORT VERSION

- 01 M10 sets calibration rules for validation and for study runs, and they differ at the ends of the curve: losing every LLOQ or ULOQ standard rejects a validation accuracy and precision run, while in a study run it narrows the range (Sections 3.2.4 and 3.3.2).
- 02 For chromatography, at least 75% of standards across at least six levels must back-calculate within $\pm 15\%$ of nominal, or $\pm 20\%$ at the LLOQ; ligand binding assays allow $\pm 20\%$, or $\pm 25\%$ at the LLOQ and ULOQ, and leave anchor points out of the count (Sections 3.3.2 and 4.3.2).
- 03 M10 sets no r or r^2 criterion: a curve passes or fails on the back-calculated accuracy of its standards, level by level.

01 · WHERE THE CALIBRATION RULES SIT IN ICH M10

Where the calibration rules sit in ICH M10.

ICH M10, adopted at Step 4 on May 24, 2022, covers methods that measure chemical and biological drugs and their metabolites in biological samples for regulatory submissions¹. Its calibration curve acceptance criteria sit in four sections: 3.2.4 and 3.3.2 for chromatographic validation and study runs, and 4.2.3 and 4.3.2 for the ligand binding assay (LBA) equivalents^{2,3,4,5}. Sections 3.3.1 and 4.3.1 define the analytical run^{6,7}, Section 3.3.4 lists reasons for reanalysis⁸, and Section 8.2 points to the documentation in Table 1⁹.

In the EU, M10 came into effect on January 21, 2023, superseding EMA's 2011 Guideline on bioanalytical method validation (EMA/CHMP/EWP/192217/2009 Rev. 1 Corr. 2), in effect since February 1, 2012^{10,11}.

FDA issued M10 as final guidance in November 2022¹². Its guidance page and Federal Register notice say it replaces the June 27, 2019 draft; neither mentions FDA's May 2018 Bioanalytical Method Validation guidance^{13,14}. FDA's biomarker guidance, first issued in January 2025, addresses it in Section II: M10 "was expected to supersede" the 2018 guidance, M10 excludes biomarkers, and the 2018 guidance "will be withdrawn" on the biomarker guidance's publication¹⁵. As of October 26, 2026, FDA's page for the 2018 guidance still lists it as final¹⁶. The M10 Questions and Answers (FDA, June 2024) leave the calibration criteria unchanged¹⁷.

02 · THE CHROMATOGRAPHIC RULES, ITEM BY ITEM

The chromatographic rules, item by item.

Section 3.2.4 sets the rules for validation; Section 3.3.2 applies the same limits to each study run^{2,3}.

- 01 **Blank and zero samples.** Each curve includes a blank and a zero sample (a blank spiked with IS); neither enters the regression².
- 02 **Six levels minimum.** Each curve has at least six concentration levels, including the LLOQ (the lowest standard) and the ULOQ (the highest), prepared in the same matrix as the study samples; each analyte has its own curve in each run².

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- 03 Back-calculated limits.** Each standard must back-calculate within $\pm 15\%$ of nominal, or $\pm 20\%$ at the LLOQ^{2,3}.
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- 04 Share that must pass.** At least 75% of the standards, across at least six levels, must meet those limits^{2,3}. With single standards, an eight-level curve can therefore lose two standards and a six-level curve none.
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- 05 Replicates.** All acceptable replicates go into the regression; in validation, at least 50% of the standards at each level must pass².
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- 06 Rejection and re-regression.** A failing standard is rejected and the curve re-evaluated without it, including the regression^{2,3}.
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- 07 Model and weighting.** M10 asks for “a simple regression model that adequately describes the concentration-response relationship” (Section 3.2.4). Written procedures direct the choice, and the regression model, weighting scheme and transformation are determined during validation². Insights 07 covers how to choose the weighting factor¹⁸.
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- 08 Fresh standards.** At least one validation assessment uses freshly spiked standards².
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03 · LIGAND BINDING ASSAYS USE WIDER LIMITS AND ANCHOR POINTS

Ligand binding assays use wider limits and anchor points.

Section 4 keeps the structure and changes the numbers. An LBA curve needs at least six levels, including the LLOQ and ULOQ, plus a blank; no zero sample is called for. Anchor points below the LLOQ and above the ULOQ may be added to improve curve fitting, and they carry no acceptance criteria because they lie outside the quantifiable range. A four- or five-parameter logistic model is most common when data points lie near the asymptotes; other models need justification⁴.

The limits widen to $\pm 20\%$, or $\pm 25\%$ at the LLOQ and ULOQ. At least 75% of the standards, excluding anchor points, must meet them across at least six levels, which in validation must include the LLOQ and ULOQ^{4,5}.

CRITERION	CHROMATOGRAPHY	LIGAND BINDING ASSAY
Blank and zero samples	Blank and zero (blank plus IS), neither in the regression	Blank only, not in the curve parameters
Minimum levels	6, including the LLOQ and ULOQ	6, including the LLOQ and ULOQ; anchor points optional
Back-calculated limits	$\pm 15\%$; $\pm 20\%$ at the LLOQ	$\pm 20\%$; $\pm 25\%$ at the LLOQ and ULOQ
Share that must pass	75%, minimum 6 levels	75% excluding anchor points, minimum 6 levels (including the LLOQ and ULOQ in validation)
Replicates	All acceptable replicates used; 50% per level pass (validation)	Wells per standard and QC match each study sample (study runs)
Regression model	A simple model that adequately describes the response	Most often 4- or 5-parameter logistic
Weighting	Determined during validation	Not specified in Section 4.2.3
Validation runs	Curves from at least 3 runs reported	At least 6 runs evaluated
LLOQ or ULOQ rejected (study run)	Next acceptable standard becomes the limit, keeps $\pm 15\%$; range covers at least 3 QC levels	Next acceptable standard becomes the limit, keeps 20%; range covers all QCs
QC rule (study run)	2/3 of all QCs and 50% per level within $\pm 15\%$	2/3 of QCs and 50% per level within 20%

Summarized from Sections 3.2.4, 3.3.1, 3.3.2, 4.2.3, 4.3.1 and 4.3.2^{2,3,4,5,6,7}.

04 · AN ANALYTICAL RUN PASSES TWO SEPARATE TESTS

An analytical run passes two separate tests.

A chromatographic study run contains a blank, a zero sample, standards at six or more levels, and low, medium and high QCs in duplicate or at least 5% of the study samples, whichever is higher⁶. Its acceptance criteria are defined in the protocol, the study plan or an SOP³.

The first test is the curve, against the limits above. If the LLOQ is rejected, the next lowest acceptable standard becomes the run's lower limit and, per Section 3.3.2, "will retain its original acceptance criteria (i.e., $\pm 15\%$)."³ A rejected ULOQ moves the upper limit down the same way. The revised range should still cover at least three QC levels, and study samples outside it are reanalyzed³; Section 3.3.4 lists a result below a revised LLOQ as a reason for reanalysis⁸. With replicate standards, losing only one LLOQ or ULOQ replicate leaves the range unchanged³.

Validation is stricter: in an accuracy and precision run, rejecting all replicates of the LLOQ or ULOQ standard rejects the run².

The second test is the QCs: at least two thirds of all QCs, and at least 50% at each level, must be within $\pm 15\%$ of nominal, or the run is rejected³. A passing curve cannot rescue failing QCs, and standards in a failed batch cannot support other batches in the run³.

05 · M10 SETS NO CORRELATION COEFFICIENT CRITERION

M10 sets no correlation coefficient criterion.

None of the four calibration sections mentions r or r^2 . In Sections 1 to 8, the word “correlation” appears once, in Section 6.2, where the concordance correlation coefficient is one option for assessing agreement between two methods in cross validation¹⁹. Elsewhere, “coefficient” means the coefficient of variation. The calibration test is the back-calculated accuracy of every standard, plus the 75% and six-level minimums^{2,3}.

An SOP may still set an r^2 limit, but r^2 says little about the ends of the curve. In Case B below, both LLOQ standards fail, yet the unweighted r^2 is 0.999765, unchanged to six decimal places from the base run; the $1/x^2$ -weighted r^2 falls from 0.9995 to 0.985. Insights 01 explains why r^2 is not enough²⁰.

06 · ONE ILLUSTRATIVE CURVE, TWO KINDS OF FAILURE

One illustrative curve, two kinds of failure.

Illustrative data. An LC-MS/MS assay in human plasma is calibrated at 1, 2, 5, 10, 50, 100, 500 and 1,000 ng/mL in duplicate (one set before the study samples, one after), with a linear model and $1/x^2$ weighting set during validation. QCs are at 3, 400 and 800 ng/mL. In the base run, all 16 standards back-calculate between -3.1% and $+2.9\%$. Case A changes one response; Case B changes both LLOQ responses.

The duplicates are deliberate. With single standards, $1/x^2$ weighting holds the fit so close to the LLOQ that, on this design, the LLOQ cannot read beyond $\pm 12.0\%$ while every other standard is within $\pm 15\%$. A single LLOQ standard therefore cannot fail alone.

NOMINAL (NG/ML)	SET 1 AREA RATIO	SET 2 AREA RATIO
1	0.01296	0.01236
2	0.02400	0.02496
5	0.06180	0.05880
10	0.11940	0.12360
50	0.60960	0.59760
100	1.17660	1.21260
500	6.06060	5.91060
1,000	11.94060	12.24060

Illustrative data. Peak area ratio (analyte/IS), base run. Case A sets 50 ng/mL set 2 to 0.73260. Case B sets the 1 ng/mL values to 0.01620 and 0.00948.

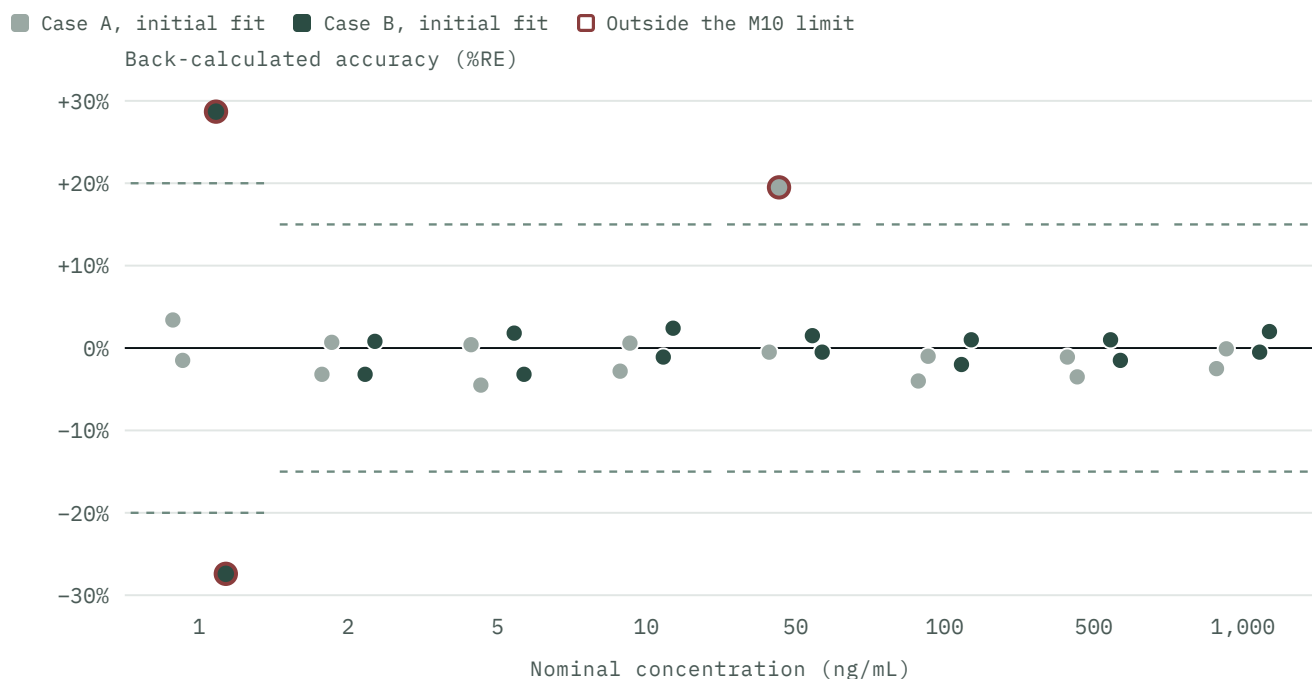
Case A. The 50 ng/mL set 2 standard reads +19.5%, outside $\pm 15\%$; the other 15 fall between -4.5% and +3.4%. After it is rejected and the curve re-regressed, every remaining standard lies between -3.1% and +2.9%. With 15 of 16 standards (93.8%) passing across all eight levels, the run meets both minimums and keeps its 1 to 1,000 ng/mL range³.

Case B. The two 1 ng/mL standards scatter in opposite directions, to +28.7% and -27.4%, both outside $\pm 20\%$. Both are rejected and the curve re-regressed. The 2 ng/mL standards become the lower limit and must now meet $\pm 15\%$; they read -2.0% and +2.0%. With 14 of 16 standards (87.5%) passing across seven levels, the run's range becomes 2 to 1,000 ng/mL and still covers all three QC levels. Study samples that read below 2 ng/mL in this run are reanalyzed^{3,8}.

NOMINAL (NG/ML)	A, INITIAL	A, RE-FIT	B, INITIAL	B, RE-FIT
1	+3.4 / -1.5	+2.9 / -2.1	+28.7 / -27.4	Both rejected
2	-3.2 / +0.7	-2.6 / +1.4	-3.2 / +0.8	-2.0 / +2.0
5	+0.4 / -4.5	+1.9 / -3.1	+1.8 / -3.2	+2.1 / -2.9
10	-2.8 / +0.6	-1.1 / +2.4	-1.1 / +2.4	-1.1 / +2.4
50	-0.5 / +19.5	+1.4 / rejected	+1.5 / -0.5	+1.3 / -0.7
100	-4.0 / -1.0	-2.1 / +0.9	-2.0 / +1.0	-2.2 / +0.8
500	-1.1 / -3.5	+0.9 / -1.6	+1.0 / -1.5	+0.8 / -1.7
1,000	-2.5 / -0.1	-0.6 / +1.9	-0.5 / +2.0	-0.7 / +1.8

Illustrative data. Back-calculated %RE, set 1 / set 2. Limits: $\pm 20\%$ at 1 ng/mL, $\pm 15\%$ elsewhere. Intercept and slope: A 0.000287 and 0.012252 (re-fit 0.000599 and 0.012016); B 0.000766 and 0.011996 (re-fit 0.000422 and 0.012026).

FIGURE 1 Back-calculated accuracy in the initial fits



Illustrative data. Each point is one standard; points outside the band ($\pm 15\%$, $\pm 20\%$ at the LLQ) fail and are rejected before the re-fit.

Three variations show where the rules bind. Had only one 1 ng/mL standard failed, the range would have stayed 1 to 1,000 ng/mL³. Had the 2 ng/mL standards also failed, the new lower limit of 5 ng/mL would sit above the 3 ng/mL low QC, and the range would no longer cover three QC levels³. In a validation accuracy and precision run, losing both 1 ng/mL standards would reject the run².

07 · WHAT THE REPORTS MUST DOCUMENT ABOUT CALIBRATION

What the reports must document about calibration.

Section 8.2 points to Table 1, which splits documentation among the analytical site, the validation report and the bioanalytical report⁹.

- 01 Validation report.** Curve parameters (for example, slope and intercept), back-calculated concentrations with mean accuracy and precision, and all acceptable curves from at least three runs²; a table of all runs, including failed runs, with analysis dates; a table of calibration standard concentrations and response function results (curve parameters) for all accepted runs, with accuracy and precision; and values outside the acceptance criteria clearly marked in the accuracy and precision run tables⁹.

- 02 Bioanalytical report.** A table of all runs with status (accepted or failed), reason for failure and analysis dates, and the same calibration table for accepted runs⁹; back-calculated concentrations of the standards and QCs of accepted runs³; and a repeat analysis table with sample IDs, reason, original and repeat values, reason for the reported value and run IDs⁹.
- 03 Comparative BA/BE studies.** Both reports add the run summary table for 100% of accepted and failed runs⁹.
- 04 At the analytical site.** Procedures that set acceptance criteria for runs, calibration curves and QCs, and run summary tables for 100% of accepted and failed runs, including the calibration curve, regression and weighting function⁹.

In Case B, a study sample reanalyzed because it read below 2 ng/mL appears in the repeat analysis table with its reason⁹.

08 · WHERE WLTR FITS

Where WLTR fits.

WLTR is a verification layer for the calibration data your CDS already produces. It reads the raw instrument export directly, records a SHA-256 hash of the original file and checks back-calculated accuracy at every calibration level, so bias at the LLOQ and ULOQ surfaces before it reaches a study report. It evaluates every candidate calibration model against the same acceptance criteria at once, 15 per analyte, each with an acceptance value the lab sets to its SOPs²¹, and recommends the simplest model that meets every criterion. The analyst makes the final decision.

Each exclusion, like the rejected standards in Cases A and B, is recorded with the user who made it and the reason given, and it travels with the record into QA approval. Method configurations are frozen once results exist, so historical results never shift. WLTR does not replace your CDS, your LIMS or your method validation. WLTR verifies. Your lab validates.

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