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Regression and weighting under ICH M10: building the justification.



ICH M10 asks for a simple regression model, selected under written procedures and fixed with its weighting during method validation. It does not name a weighting factor. This paper sets out the evidence that makes the choice defensible to a sponsor or an auditor.

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THE SHORT VERSION

- 01 ICH M10 Section 3.2.4 asks for a simple regression model that adequately describes the data, selected under written procedures, with the model, weighting and any transformation fixed during method validation.
- 02 Bioanalytical LC-MS/MS data spanning three or more orders of magnitude usually show a roughly constant %CV, the variance pattern that $1/x^2$ weighting matches.
- 03 A quadratic term or a different weighting needs replicate, residual and lack-of-fit evidence in the validation report, not just a lower sum of |%RE|.

01 • WHAT ICH M10 SAYS ABOUT THE MODEL

What ICH M10 says about the model.

Section 3.2.4 of ICH M10 sets the requirement in three sentences: “A simple regression model that adequately describes the concentration-response relationship should be used.” “The selection of the regression model should be directed by written procedures.” “The regression model, weighting scheme and transformation should be determined during the method validation.”¹ FDA adopted the same text as final guidance in November 2022².

M10 names no weighting factor, never uses the word “simplest,” and does not ask in Section 3.2.4 for the weighting to be “justified.” Where M10 asks for a model choice to be justified, it is in the LBA section, for models other than the 4- or 5-parameter logistic (Section 4.2.3)¹. The M10 Questions and Answers add nothing on regression or weighting³. The justification lives where M10 puts the decision: in the written procedure and the validation record.

Earlier guidance was similar: FDA in 2018 asked for “the simplest model that adequately describes the concentration-response relationship”⁴, and EMA in 2011 for a relationship that can “simply and adequately describe the response,” without mentioning weighting⁵. M10 is now the reference. EMA lists its 2011 guideline as superseded⁶, M10 took legal effect in the EU on January 21, 2023⁷, and FDA’s biomarker guidance states that the May 2018 guidance “will be withdrawn”⁸.

02 • WHY LC-MS/MS CALIBRATION DATA NEED WEIGHTING

Why LC-MS/MS calibration data need weighting.

Bioanalytical calibration ranges are usually broad, “presenting three or more orders of magnitude”⁹. Measurement error over such a range has an additive part that dominates near zero and a multiplicative part whose standard deviation grows in proportion to concentration¹⁰. Where the proportional part dominates, the %CV is roughly constant, so over 1 to 1,000 ng/mL the standard deviation at the ULOQ is about 1,000 times that at the LLOQ.

Unweighted least squares treats every standard as equally precise, so the top standards control the fit and the error concentrates at the low end. Weighting counteracts “the greater influence of the greater concentrations” and, in Almeida’s data, improved accuracy at the lower end⁹. Gu and colleagues give the rule: weight by 1, $1/x$ or $1/x^2$ when the standard deviation is constant, the variance is proportional to concentration, or the standard deviation is

proportional to concentration. They concluded that $1/x^2$ “should always be used” for bioanalytical LC-MS/MS assays, and the correct weighting gave the most stable curves¹¹. Under M10, the method’s own data still have to show it; WLTR’s primer covers the arithmetic¹².

03 • WHY QUADRATIC FITS APPEAR IN LC-MS/MS

Why quadratic fits appear in LC-MS/MS.

Three mechanisms bend LC-MS/MS calibrations, mostly at the top of the range.

- 01 **Ionization.** In electrospray, the factors that limit the calibration curve at high concentrations include droplet surface area and excess charge concentration¹³.
- 02 **Detection.** The linear dynamic range of mass analyzers typically spans three orders of magnitude; monitoring multiple isotopologue transitions has been proposed to extend LC-MS/MS ranges “particularly when the detector is saturated”¹⁴.
- 03 **Isotope contribution.** Natural isotopes of the analyte can add to the signal of a stable-isotope-labeled IS and create “nonlinear calibration behavior that may bias quantitative results”; the effect is more pronounced at high analyte to IS ratios and for higher molecular weight compounds or those containing sulfur, chlorine or bromine¹⁵. M10 also asks that the IS be checked for unlabeled analyte (Section 3.1)¹.

A quadratic term absorbs any of these without removing the cause, and it assumes study samples curve as the calibrators do. Liu and colleagues described an assay in which matrix ion enhancement differed between standards and QCs; when a quadratic model had to be used, the assay failed on “unacceptable positive bias” in the middle and high QCs¹⁶. WLTR’s linear versus quadratic paper covers the general case¹⁷.

04 • BUILD THE JUSTIFICATION DURING VALIDATION

Build the justification during validation.

Build the case from the method’s own validation data: the replicate standards (M10 puts every acceptable replicate into the regression) and the acceptable curves from at least three independent runs that it requires to be

reported¹. Score every candidate against the same criteria.

EVIDENCE	WHAT IT SHOWS	BASIS
Replicate %CV by level	How scatter scales; a constant %CV points to $1/x^2$	Gu et al. ¹¹
Slope of log SD on log concentration	Near 0: none; near 0.5: $1/x$; near 1: $1/x^2$	Gu et al. ¹¹
Variance F test, ULOQ against LLOQ	Whether the data are heteroscedastic	Almeida et al. ⁹
%RE at every level	Where each candidate is biased, above all at the LLOQ	M10 Sec. 3.2.4 ¹
Sum of %RE	Which weighting gives the best overall accuracy	Almeida et al. ⁹
Residual pattern	A systematic shape that suggests the wrong weighting or model	Almeida et al. ⁹
Lack-of-fit F test	Whether level means stray beyond replicate scatter	Analytical Methods Committee ¹⁸
Quadratic term test (Mandel's test)	Whether the extra parameter is supported	Van Loco et al. ¹⁹
Curves across validation runs	Whether the curves are stable run to run	Gu et al. ¹¹
Analyte and IS cross-contribution	Whether curvature has a physical cause	Rule et al. ¹⁵

Write the decision rule into the SOP before validation data exist.

01 Weighting from variance. Choose the weighting the replicate data support and confirm it with %RE at every level^{9,11}.

02 Linear first. Fit the linear model with that weighting and test it for lack of fit¹⁸.

03 Quadratic on evidence only. Add the term only when the linear model shows lack of fit, the term is significant, the curvature recurs across runs and its cause has been examined^{15,19}.

05 • AN ILLUSTRATIVE EIGHT-LEVEL EXAMPLE

An illustrative eight-level example.

The data set below is illustrative: an LC-MS/MS assay in human plasma with eight non-zero levels from 1 to 1,000 ng/mL, three standards per level, and the analyte to IS peak area ratio as the response. A script computed every

value.

LEVEL (NG/ML)	REPLICATE %CV	LINEAR, UNWEIGHTED	LINEAR, 1/X	LINEAR, 1/X ²	QUADRATIC, 1/X ²
1 (LLOQ)	7.1	-84.8	-4.9	-0.6	-0.1
2	2.3	-41.0	-1.3	+0.3	-0.1
5	2.3	-13.7	+2.0	+1.9	+0.9
20	4.8	-2.4	+1.3	+0.3	-0.9
100	3.2	+2.8	+3.3	+2.1	+1.1
400	2.9	-0.1	-0.2	-1.4	-1.4
800	4.3	+0.3	+0.2	-1.0	+0.2
1,000 (ULOQ)	5.9	-0.2	-0.4	-1.6	+0.3
Sum of %RE		145.4	13.5	9.2	5.0
Standards within limits		17 of 24	24 of 24	24 of 24	24 of 24

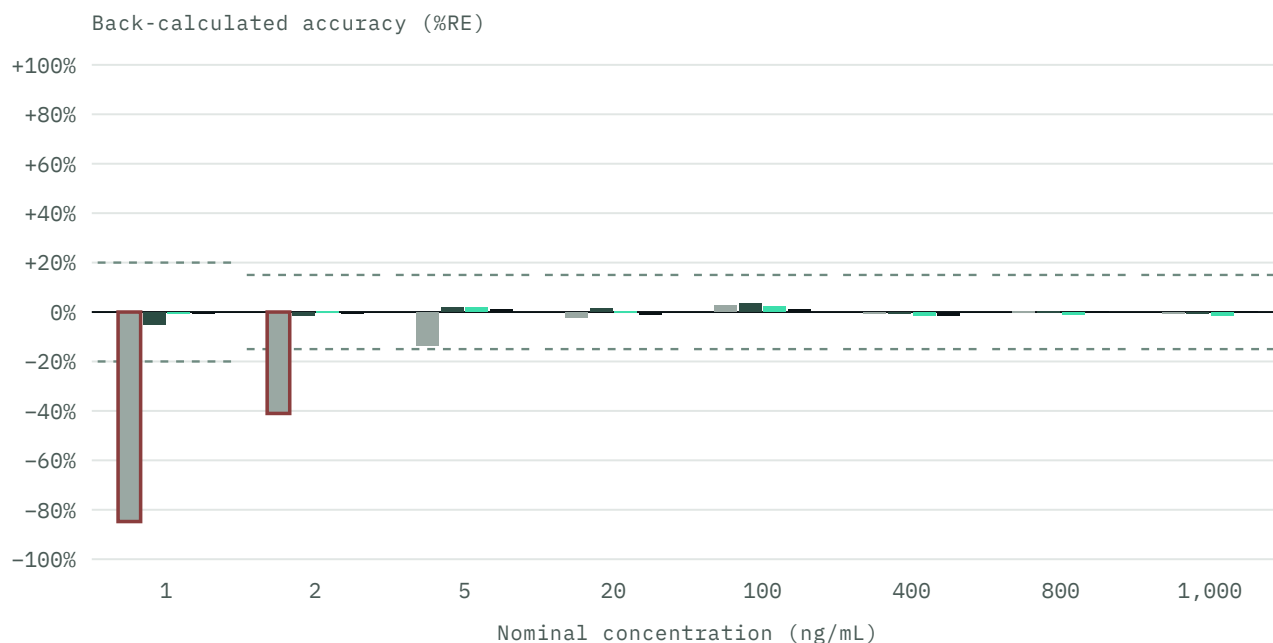
Illustrative data. Mean %RE of three standards per level; sums add the eight absolute level means before rounding. M10 limits: ±15%, ±20% at the LLOQ.

The variance evidence comes first. Across the 1,000-fold range the replicate %CV stays between 2.3% and 7.1% while the standard deviation grows 762-fold, the slope of log standard deviation on log concentration is 1.01, and the ULOQ to LLOQ variance ratio (580,316) far exceeds the 99% critical value of 99.0. The data call for $1/x^2$.

Accuracy agrees. The unweighted fit misses the LLOQ by -84.8%, and only 17 of 24 standards (70.8%) meet the M10 limits, short of the 75% required. The $1/x$ fit passes with a sum of 13.5 and a -4.9% error at the LLOQ; $1/x^2$ keeps every level within 2.1%, with a sum of 9.2.

FIGURE 1 Back-calculated accuracy by level for four candidate models

■ Linear, unweighted ■ Linear, $1/x$ ■ Linear, $1/x^2$ ■ Quadratic, $1/x^2$ ■ Outside the M10 limit



Illustrative data. Only the unweighted fit leaves the M10 band, at the low end.

The quadratic lowers the sum to 5.0, 46% below linear $1/x^2$, but that is not a justification. The linear $1/x^2$ residuals form a shallow arch, yet a lack-of-fit test using pure error from the triplicates finds none ($F = 0.34$; 6 and 16 df; $p = 0.91$), and the quadratic term is not significant ($F = 1.67$; 1 and 21 df; $p = 0.21$). The extra parameter fits replicate scatter. With all three weighted models passing, the variance evidence selects linear $1/x^2$ over the equally simple linear $1/x$.

06 • RECORD THE DECISION SO AN AUDITOR CAN FOLLOW IT

Record the decision so an auditor can follow it.

M10 sets the documentary floor. Its Table 1 asks the validation and bioanalytical reports for tables of all runs, including failed runs, and of calibration standard response function results, and it lists run summary tables “including calibration curve, regression, weighting function” among the documentation kept at the analytical site²⁰. Section 8 expects the basis for changing or reprocessing data to be documented “with sufficient detail” and the original record maintained²⁰.

To let a sponsor or auditor follow the decision, the report should also hold the evidence above for every candidate, not only the one chosen, plus the SOP decision rule, its result, and who made and approved the choice. The choice should be reproducible from the record alone.

The model also stays fixed from run to run. M10 expects study samples, QCs and calibration standards to be processed “in accordance with the validated analytical method,” under run acceptance criteria defined in the protocol, study plan or an SOP (Sections 3.3 and 3.3.2)²¹. A model picked after a run’s results are known is chosen on the data it is meant to judge, and FDA’s 2018 guidance was explicit: “Exclusion should not change the model used”⁴.

07 • WHEN ROUTINE RUNS START FAILING

When routine runs start failing.

Inside a run, M10’s steps for calibration failures leave the model alone. Where more than six levels are run, a failing standard is rejected and a new regression performed; if the LLOQ or ULOQ is rejected, the next acceptable standard becomes the limit, the revised range must cover at least three QC levels, and study samples outside it are reanalyzed (Section 3.3.2)²¹.

Repeated failures call for more. In validation, if every LLOQ or ULOQ replicate in an accuracy and precision run is rejected, the run is rejected and “the possible source of the failure should be determined” (Section 3.2.4)¹. In study sample analysis, a QC overall mean accuracy or precision outside the 15% criterion calls for an investigation (Section 3.3.2)²¹.

If the investigation changes the method, partial validation follows. M10’s examples, “not limited to” these, include changes in detection system or platform, sample processing and calibration range (Section 6.1)²²; a range change requires partial validation “to verify the response function” (Section 3.3.3)²¹. M10 does not list a new regression model or weighting, but it fixes both in validation, so changing either needs a documented method change with validation data, not a single run.

08 • WHERE WLTR FITS

Where WLTR fits.

WLTR is a verification layer that evaluates the calibration data your CDS already produces. It reads the raw instrument export directly and records a SHA-256 hash of the original file; nothing is retyped. It evaluates every candidate regression and weighting choice against the same acceptance criteria at once, 15 per analyte with each acceptance value set to your SOPs, and shows them side by side, with back-calculated accuracy at every level²³.

WLTR recommends the simplest model that meets every criterion; the analyst makes the final decision, and your validated method and SOPs still decide which model is reported. The full evaluation is kept, method configurations are frozen once results exist, and each exclusion is recorded with the user and the reason. WLTR verifies. Your lab validates.

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