Impact of Calibrator Concentrations and Their Distribution on Accuracy of Quadratic Regression for LC-MS Bioanalysis

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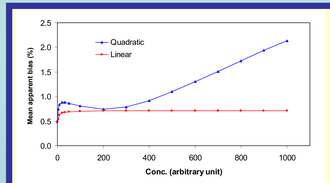
Introduction

Despite the common use of quadratic regression in LC-MS bioanalysis, how calibrator concentrations should be determined is still vague. Both the number and concentrations of calibrators are usually selected arbitrarily to each bioanalytical scientist's preference, without necessary regards to their possible impact on the accuracy of quadratic regression. The purpose of this research is to evaluate the impact of calibrator concentrations on accuracy of quadratic regression and to find new approaches with improved accuracy and robustness for LC-MS bioanalysis.

Are all calibrators equally important?



Mean Apparent Bias (MAB):

$$MAB=\sqrt{\frac{1}{n}\left(\sum_{i=1}^{n} b_i^2\right)}$$

b_i: *bias for individual sample*

Quadratic: The two limits plus geometric mean are **3** critical concentrations.

Linear: The two limits are **2** critical concentrations. Contrary to conventional thinking, extrapolation to linearity-known region at high concentration end is usually acceptable because the impact of high concentrations (e.g. 200 to 1000) is almost identical, which confirms our earlier finding (J. Chromatogr. B, 911, 2012, p.192).

Calibration Schemes and CS/QC Concentrations

Use 1-1000 range as an example (other ranges are similar).

Calibration scheme	CS Conc.	QC Conc.
10×2	1, 2, 5, 10, 20, 50, 100, 400, 800, 1000 (**2** replicates each)	1, 3, 31.62, 350, 750, 1000 (**6** replicates each)
$7+6_{gm}+7$	1 (**7***), 31.62 (**6**), 1000 (**7**)	
3×2 ($2+2_{gm}+2$)	1 (**2**), 31.62 (**2**), 1000 (**2**)	
$7+6_{am}+7$	1 (**7**), 500 (**6**), 1000 (**7**)	

*: *no. of replicates; CS=calibration standard; QC=quality control*

Comparison of Different Calibration Schemes (Simulation)

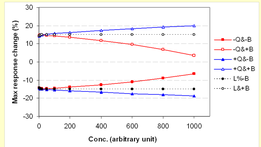
Degree of quadratic	Precision	Parameter	10×2	$7+6_{gm}+7$	$7+6_{am}+7$	3×2
-40%	Good	Failed batches	9 (3*)	5 (0)	5 (0)	12 (2)
	Very Good	No. of NRV	22	8	23	17
		Failed batches	1 (0)	1 (0)	2 (0)	4 (0)
-25%	Poor	Failed batches	18 (11)	8 (2)	16 (7)	18 (10)
	Good	MAB (%)	6.0	5.8	6.1	6.5 (0.5**)
	Very Good	MAB (%)	3.3	3.2	3.3	3.6 (0.3)
-10%	Poor	Failed batches	8 (3)	0	8 (8)	6 (4)
	Good	MAB (%)	5.4	5.3	5.4	5.8 (0.4)
	Very Good	MAB (%)	2.9	2.8	3.2	3.1 (0.2)
+10%	Poor	MAB (%)	10.6	9.7	10.3	11.3 (0.7)
		Failed batches	4 (3)	1 (1)	2 (2)	9 (7)
	Good	MAB (%)	5.2	5.1	5.4	5.6 (0.4)
	Very Good	MAB (%)	2.9	2.7	2.8	3.2 (0.3)
+25%	Poor	MAB (%)	10.7	9.8	11.3	11.1 (0.4)
		Failed batches	4 (4)	2 (2)	8 (8)	5 (5)
	Good	MAB (%)	5.4	5.2	5.4	5.7 (0.3)
	Very Good	MAB (%)	2.7	2.6	2.8	2.9 (0.2)
+40%	Poor	MAB (%)	10.0	9.6	10.9	11.2 (1.2)
		Failed batches	3 (3)	3 (3)	7 (7)	6 (4)
	Good	MAB (%)	5.0	5.0	5.5	5.4 (0.4)
	Very Good	MAB (%)	2.8	2.7	3.0	3.0 (0.2)

*: *no. of failed batches when ULQC failure not considered.* **: *difference from the MAB of 10×2*

- $7+6_{gm}+7$ is consistently better than 10×2 and $7+6_{am}+7$.
- The majority of failed runs for downward quadratic were due to failure of ULQC.
- It is necessary to differentiate down- (-) and up- (+) quadratic.
- In many cases, the accuracy of 3×2 is not significantly different from that of 10×2.

Concentration range	Calibration scheme	10×2	$7+6_{gm}+7$	$7+6_{am}+7$	3×2
1-2000	MAB of LLQC (%)	8.4	7.8	7.8	9.0
	MAB of QC1 (%)	4.9	4.8	5.2	5.2
	MAB of QC_{int} (%)	4.0	4.4	5.1	5.1
	MAB of QC (%)	4.9	4.9	5.4	5.6
	MAB of QC3 (%)	6.3	5.4	5.4	6.3
	MAB of ULQC (%)	8.6	6.8	6.8	7.9
	Overall MAB (%)	6.4	**5.8**	6.0	6.7 (0.3)
	No. of failed batches	1 (0)	0	0	0
1-1000	MAB of LLQC (%)	8.0	8.1	8.1	8.3
	MAB of QC1 (%)	4.3	4.3	4.9	4.6
	MAB of QC_{int} (%)	4.2	4.1	4.9	4.7
	MAB of QC (%)	4.9	4.9	5.4	5.5
	MAB of QC3 (%)	6.0	5.7	5.7	6.2
	MAB of ULQC (%)	7.7	6.9	7.1	8.5
	Overall MAB (%)	6.0	**5.8**	6.1	6.5 (0.5)
1-500	MAB of LLQC (%)	9.7	8.7	8.5	10.2
	MAB of QC1 (%)	4.4	4.7	4.8	5.0
	MAB of QC_{int} (%)	4.4	4.7	5.0	4.6
	MAB of QC (%)	4.6	4.8	5.2	5.0
	MAB of QC3 (%)	5.3	5.6	5.6	5.9
	MAB of ULQC (%)	6.9	6.7	7.0	7.5
	Overall MAB (%)	6.2	**6.0**	6.2	6.6 (0.4)
1-250	MAB of LLQC (%)	9.3	8.6	8.5	9.9
	MAB of QC1 (%)	4.3	4.4	4.9	5.3
	MAB of QC_{int} (%)	4.3	4.3	5.0	4.6
	MAB of QC (%)	4.5	4.5	5.4	5.2
	MAB of QC3 (%)	5.6	5.4	5.5	6.1
	MAB of ULQC (%)	8.1	6.8	6.9	7.8
	Overall MAB (%)	6.3	**5.9**	6.2	6.7 (0.4)
	No. of failed batches	1 (0)	0	0	0

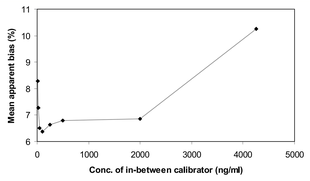
Acceptable Response Window *vs.* Concentration



-Q: -40% quadratic;
+Q: +40% quadratic;
L: linear;
-B: -15% bias in conc.;
+B: +15% bias in conc.

- Efforts should be made to reduce or eliminate downward quadratic while reproducible upward quadratic is not bad.

Retrospective Data Analysis: Impact of Calibrators



Original raw data from Cunliffe et al., J. Pharm. Biomed. Anal., 50 (2009) 46

For 3×2, best overall accuracy is achieved with 100 ng/ml as the in-between calibrator because it is the closest existing conc. to the geometric mean.

Retrospective Data Analysis: 3×2 vs. 10×2

	LLQC	QCL	QCM-L	QCM	QCH
Norm. Conc. (ng/ml)	**5**	**15**	**150**	**400**	**4000**
Run 1	5.23[a] (5.27[b])	14.5 (14.7)	151 (153)	418 (423)	4290 (4230)
	5.05 (5.09)	15.3 (15.5)	157 (159)	412 (417)	4310 (4250)
	5.04 (5.08)	15.4 (15.6)	155 (157)	413 (418)	4080 (4030)
	4.85 (4.89)	15.0 (15.2)	154 (156)	417 (422)	4310 (4250)
	4.89 (4.93)	15.4 (15.6)	144 (146)	402 (407)	4170 (4120)
	5.25 (5.29)	14.6 (14.8)	150 (152)	401 (406)	4100 (4050)
Run 2	5.16 (5.20)	13.9 (13.7)	154 (151)	408 (399)	4390 (4330)
	5.55 (5.58)	14.3 (14.1)	153 (150)	410 (401)	4300 (4240)
	4.50 (4.55)	14.5 (14.3)	148 (145)	414 (405)	4160 (4100)
	4.40 (4.45)	15.9 (15.7)	152 (149)	421 (412)	4210 (4150)
	4.77 (4.82)	15.5 (15.3)	155 (152)	414 (405)	4260 (4200)
	4.53 (4.58)	14.5 (14.3)	160 (157)	428 (419)	4280 (4220)
Run 3	4.38 (4.53)	13.9 (14.1)	146 (147)	400 (403)	4420 (4310)
	4.33 (4.48)	13.8 (14.0)	143 (144)	400 (403)	4160 (4070)
	4.06 (4.21)	14.2 (14.4)	147 (148)	391 (394)	4010 (3930)
	4.13 (4.28)	13.5 (13.7)	150 (151)	391 (394)	4170 (4080)
	3.95 (4.10)	14.7 (14.9)	146 (147)	395 (398)	3810 (3740)
	4.44 (4.60)	13.7 (13.9)	149 (150)	396 (399)	3820 (3750)
Mean	**4.69 (4.77)**	**14.6 (14.7)**	**151 (151)**	**407 (407)**	**4180 (4110)**
% CV	**9.8 (8.7)**	**4.9 (4.7)**	**3.2 (2.9)**	**2.6 (2.3)**	**4.0 (4.1)**
% Norm.	**93.9 (95.4)**	**97.1 (98.0)**	**100.5 (100.7)**	**101.8 (101.8)**	**104.5 (102.8)**

a: Results from 3×2 regression (three standards in duplicate, 5, 1000, and 5000 ng/ml)
b: Original ones based on 10×2 calibration (Cunliffe et al., J. Pharm. Biomed. Anal., 50 (2009) 46)

Conclusions

- The two limits and their geometric mean are 3 critical conc. for quadratic regression.
- When quadratic model is established, the best accuracy and lowest batch failure rate are achieved using the 3 critical conc. with multiple replicate/conc. (e.g. $7+6_{gm}+7$), instead of multiple conc. in duplicate like 10×2.
- ULQC should be included in each batch for downward quadratic regression.
- Using the critical conc. can potentially save a lot of time & cost.

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