Evaluation of the Effect of Regression Model Selection on Quantitative LC-MS/MS Data

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Overview

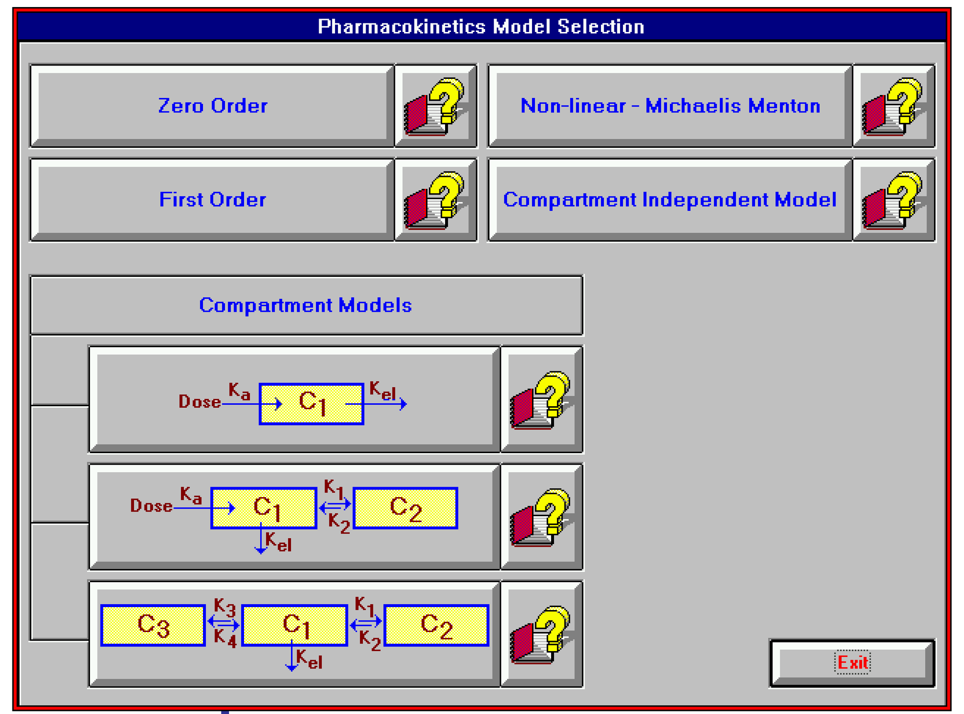
Purpose

- To evaluate the effects of regression model selection on statistical measures traditionally used to assess quantitative data set acceptability including bias of standards, r^2 and QC percent recovery.
- Explore the variability introduced into pharmacokinetic end-point parameters from regression model selection.
- Evaluate the use of an interactive calibration optimization software as a tool for CRO's performing method development research.

Procedures

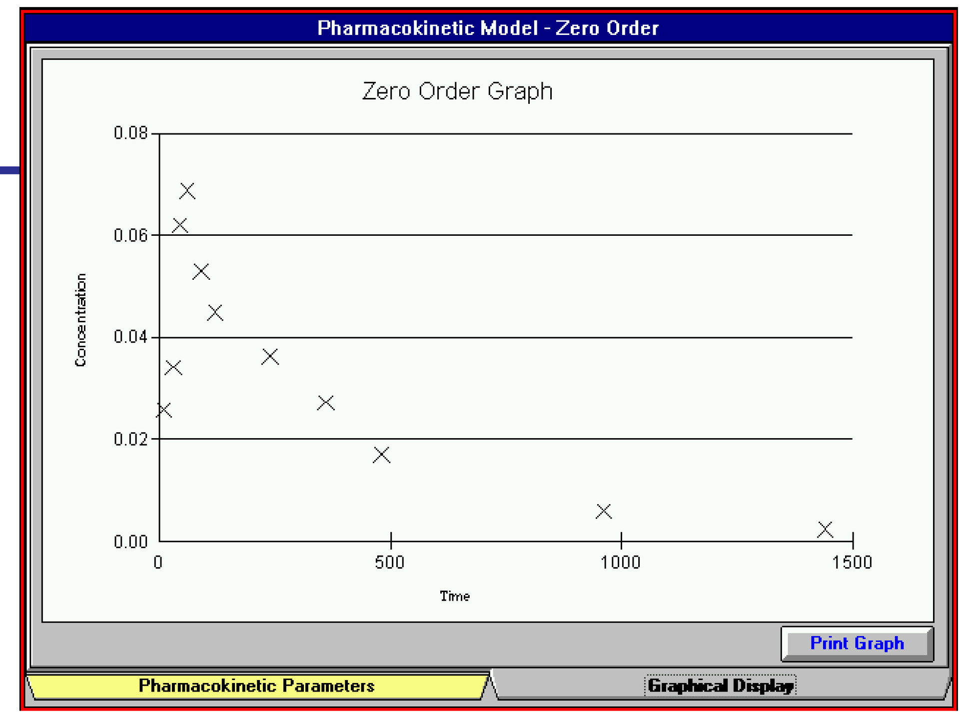
LCMS/MS data from a single dose safety and pharmacokinetic study was evaluated using the LCMS Report Writer, ALIS-DMPRA 4.0. Designs details of ALIS including a description of functionalities has been presented elsewhere[1]. The data which consisted of five analytical runs and 16 subjects were generated on a PE-Sciex API III^+ operating in turboionspray tandem mass spectrometry mode. Standard concentrations ranged over three orders of magnitude (0.005 - 5.0 ng/mL). Calibration sets were processed using weighted, non-weighted, linear, quadratic regression and log-log quadratic regression. All regression calculations were performed using the *ALIS Calibration Optimizer* module and verified by Jandel's TableCurve 4.0 for Windows (Figure 1).

The coefficient of determination (r^2) and the fit standard error were used to assess goodness of fit for each calibration curve-regression model combination. In addition, the bias of the lowest calibration standards and QC samples were determined to examine the effect of curve fit on data acceptability. Subject data were processed within the *ALIS Pharmacokinetic/SGI Data Analysis* module to generate $t_{1/2}$ for all subjects and three PK models: zero-order, first-order and compartment independent. To compare the results across regression types, the relative percent difference was calculated using the $t_{1/2}$ from the weighted linear regression $1/x^2$ as a reference point. PK algorithms had been previously validated using data sets from two independent sources[2,3].



Pharmacokinetic/SGI Data Analysis

Half-Life
Rate Constant
C_{max}, C_{min}
T_{max}, T_{min}
Terminal Half-Life

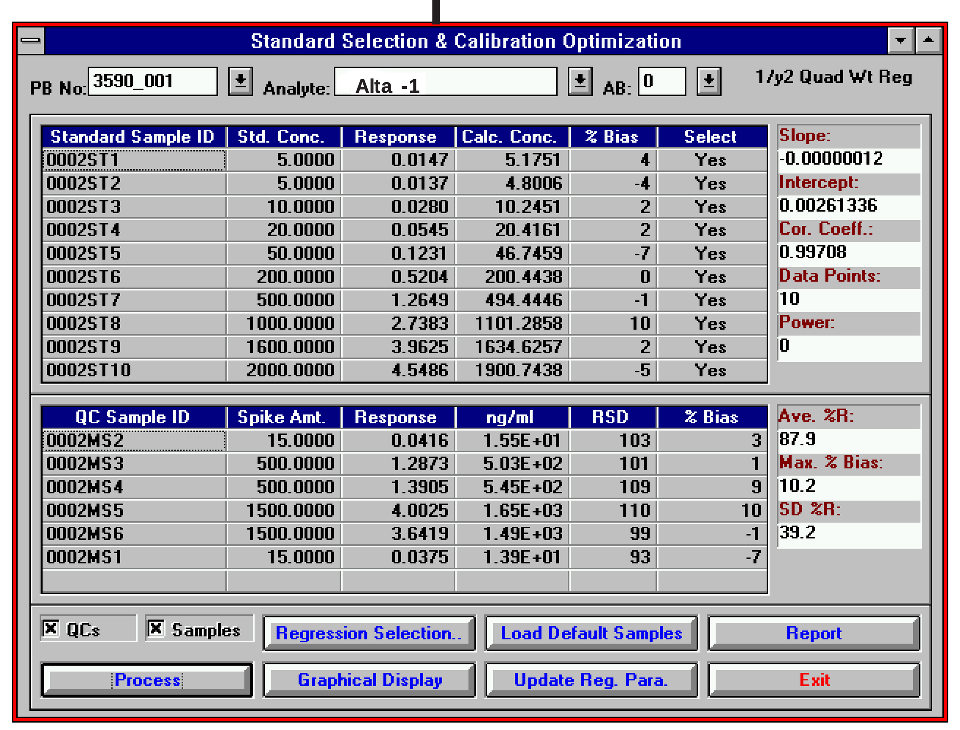


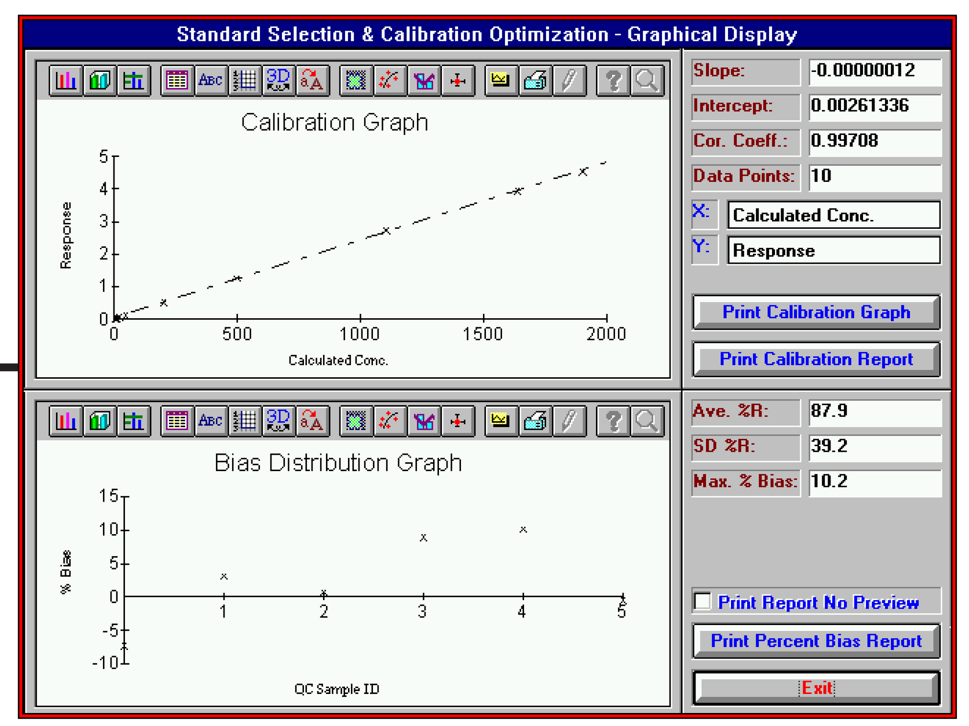
ALIS 4.0

Sample Log-In
Sample Preparation
Instrument Upload
Pre-Processing
Data Review
Reporting

Calibration Optimizer

Linear Regression
Quadratic Regression
Weighted Regression:
$1/x$, $1/x^2$, $1/y$, $1/y^2$
Ln-Ln Quad. Regression
Power Series





Effect of Regression Model on Bias

Set	Reg-Type	r^2	Fit Std Err	% Bias Standard 0.005 ng/ml	% Bias QC 0.015 ng/ml
Set 2	Lin. Reg.	0.999782	0.104184623	222, 211	62.0, 64.9
	1/x Wt. Lin. Reg.	0.999328	0.014561876	16.6, 5.04	-6.06, -3.14
	1/(x*x) Wt. Lin. Reg.	0.993894	0.002328733	8.90, -3.07	-6.71, -3.69
	1/y Wt. Lin. Reg.	0.999264	0.01572952	18.6, 6.99	-5.37, -2.45
	1/(y*y) Wt. Lin. Reg.	0.993894	0.002587961	10.6, -1.40	-5.89, -2.84
	Quad. Reg.	0.999881	0.088313992	88.9, 77.0	20.0, 23.0
	1/x Wt. Quad. Reg.	0.999665	0.011109061	10.4, -1.83	-5.02, -1.93
	1/(x*x) Wt. Quad. Reg.	0.995123	0.00224802	8.60, -3.82	-4.72, -1.58
	1/y Wt. Quad. Reg.	0.999642	0.011847325	11.3, -0.921	-4.54, -1.45
	1/(y*y) Wt. Quad. Reg.	0.995260	0.002462818	10.2, -2.32	-3.90, -0.742
	Log-Log Quad. Reg.	0.999509	0.07109393	9.83, -1.75	-6.68, -3.62
Set 3	Lin. Reg.	0.998701	0.342008475	-87.3, -109	-42.1, -30.6
	1/x Wt. Lin. Reg.	0.999051	0.023597511	17.4, -3.98	-7.41, 4.02
	1/(x*x) Wt. Lin. Reg.	0.991062	0.003922124	14.2, -7.55	-7.71, 3.89
	1/y Wt. Lin. Reg.	0.999062	0.024546591	19.4, -2.03	-6.71, 4.73
	1/(y*y) Wt. Lin. Reg.	0.992356	0.004055417	17.3, -4.47	-6.55, 5.08
	Quad. Reg.	0.998975	0.328084897	167, 147	39.0, 49.7
	1/x Wt. Quad. Reg.	0.999138	0.024283635	20.3, -0.507	-7.87, 3.26
	1/(x*x) Wt. Quad. Reg.	0.991283	0.004183766	14.1, -7.93	-6.90, 4.88
	1/y Wt. Quad. Reg.	0.999149	0.025254652	22.6, 1.76	-7.06, 4.08
	1/(y*y) Wt. Quad. Reg.	0.992588	0.004313413	17.2, -4.91	-5.74, 6.07
	Log-Log Quad. Reg.	0.999177	0.091892957	16.5, -4.20	-8.22, 3.27
Set 4	Lin. Reg.	0.998984	0.267582192	-81.2	-24.3, -20.4
	1/x Wt. Lin. Reg.	0.999277	0.022348336	-17.6	-3.31, 0.576
	1/(x*x) Wt. Lin. Reg.	0.995950	0.003150059	-6.25	-1.50, 2.27
	1/y Wt. Lin. Reg.	0.999269	0.022931679	-16.6	-2.94, 0.948
	1/(y*y) Wt. Lin. Reg.	0.996435	0.002942606	-4.93	-0.836, 2.95
	Quad. Reg.	0.999015	0.288507549	-217	-67.8, -63.8
	1/x Wt. Quad. Reg.	0.999303	0.024045181	-14.4	-3.24, 0.582
	1/(x*x) Wt. Quad. Reg.	0.996779	0.003077167	-5.19	-3.07, 0.600
	1/y Wt. Quad. Reg.	0.999294	0.024696317	-13.5	-2.89, 0.937
	1/(y*y) Wt. Quad. Reg.	0.997212	0.002850598	-4.06	-2.48, 1.20
	Log-Log Quad. Reg.	0.999678	0.056412019	-6.42	-3.85, -0.199
Set 5	Lin. Reg.	0.99885	0.255539663	-79.2, -94.1	-34.8, -29.8
	1/x Wt. Lin. Reg.	0.998736	0.021623212	19.5, 4.56	-2.13, 2.86
	1/(x*x) Wt. Lin. Reg.	0.991959	0.00288404	11.0, -4.46	-2.69, 2.48
	1/y Wt. Lin. Reg.	0.998756	0.021607572	21.4, 6.45	-1.42, 3.57
	1/(y*y) Wt. Lin. Reg.	0.992368	0.002980247	13.0, -2.56	-1.65, 3.56
	Quad. Reg.	0.998921	0.267390727	48.1, 33.7	5.64, 10.5
	1/x Wt. Quad. Reg.	0.998810	0.022657058	22.1, 7.56	-2.67, 2.19
	1/(x*x) Wt. Quad. Reg.	0.993471	0.002807121	10.8, -5.35	-0.311, 5.08
	1/y Wt. Quad. Reg.	0.998811	0.022815638	23.8, 9.25	-1.84, 3.05
	1/(y*y) Wt. Quad. Reg.	0.994010	0.002851817	12.6, -3.58	0.655, 6.07
	Log-Log Quad. Reg.	0.999430	0.077501643	12.8, -2.76	-0.464, 4.92

Variation (RPD) in $t_{1/2}$ vs Regression Models[1]

PK Model	Subject	Linear Regression 1/x	Linear Regression $1/x^2$	Linear Regression 1/y	Linear Regression $1/y^2$	Quadratic Regression LnLn	Quadratic Regression 1/x	Quadratic Regression $1/x^2$	Quadratic Regression 1/y	Quadratic Regression $1/y^2$
Zero	A	-43.9	-	-46.9	-9.6	-35.6	-32.2	47.6	-36.0	29.3
	B	-7.3	-	-6.8	0.5	1.6	-4.9	2.0	-4.4	2.5
	C	-15.6	-	-14.7	1.0	3.3	-10.4	3.9	-9.5	4.7
	D	-4.4	-	-4.4	0.5	1.1	-2.7	1.1	-2.7	1.6
First	A	-9.8	-	-9.1	0.7	2.1	-6.5	2.6	-5.9	3.1
	B	-6.7	-	-6.1	0.5	1.6	-4.4	2.1	-3.9	2.1
Compartment Independent	A	-9.8	-	-9.1	0.7	2.1	-6.5	2.6	-5.9	3.1
	B	-6.7	-	-6.1	0.5	1.6	-4.4	2.1	-3.9	2.1

[1] *Relative Percent Difference* (RPD) = $(\text{Half-Life}_{[RegModel n]} - \text{Half-Life}_{[LinReg 1/x2]})/((\text{Half-Life}_{[RegModel n]} + \text{Half-Life}_{[LinReg 1/x2]})/2)*100$

Results & Discussion

- For this compound, $1/x^2$ weighted linear regression appeared to provide the best fit across all calibration data sets based on the standard error of the fit and minimization of bias of the low standards.
- The coefficient of determination, r^2 appeared to be a relatively insensitive predictor of bias for both standards and QC samples. This suggests that r^2 should not be used as the sole criterion for regression model selection.
- QC sample bias was similiar for all weighted regression models tested (<15%). QC sample bias based on unweighted linear and quadratic regression was greater than 20% even though the corresponding r^2 values were 0.993 or higher.
- For the 16 subjects examined, variation in $t_{1/2}$ values were less than 10% across calculations based on weighted regression models for all but three subjects (Table 2). Variation in $t_{1/2}$ values across weighted regression models varied considerably for subjects A and C.
- These results suggest curve fit selection should be based on a goodness of fit measure such as Standard Error (Root MSE). In this study, regression model selection had a significant effect on the bias of standards and QC samples. Future work will examine procedures for outlier selection and rejection.
- The calibration optimizer and pharmacokinetic modules provide significant savings of time during method development and PK data analysis.

References

[1] ALIS-DMA-DMPRA: A New LC/MS Pharmaceutical and Agrochemical Data Report Writer, Poster Presentation, 44th ASMS Conference on Mass Spectrometry & Allied Topics, May 12-16 1996.

[2] Clinical Pharmacokinetic: Concepts and Applications. Roland, M. and Tozer, T. 3rd Edition, 1995. Williams & Wilkins.

[3] Handbook of Pharmacokinetic. Jean-Pierre Labaune. 1st Edition, 1989. Ellis Harwood.