A Quantum Leap in the Development of Quadratic External Calibration Models by EZSTATSG2, an Innovative Tool for ANSI/ASB Standard 036 Method Validation Using Microsoft Excel

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Special Issue

Abstract

The first generation of this Microsoft (MS) Excel (Redmond, WA, USA) tool for method validation, EZSTATSG1, was designed for methods utilizing only linear calibration curves requiring seven calibration levels, and quadratic calibration models were not supported. This significantly improved version, EZSTATSG2, includes all of the features of the original template, such as: weighted linear calibration models; bias and precision data for the lower limit of quantitation; quality controls; dilution integrity and ion suppression, while new features in this version include: flexible five-, six- or seven-point calibration curves; six weighted quadratic calibration models; normality testing of residuals by use of frequency plots overlaid with the normal distribution function along with five-number summary data; a processed sample stability function; and the implementation of Visual Basic for Applications (VBA) in Excel UserForms for easier data entry, to prevent accidental alteration of existing formulas and the VBA codes also ensure that pertinent cells are relocked every time a file is reopened. The quadratic models are fully-characterized by providing the equations for the axis of symmetry, directrix, and coordinates for vertex and focus. Example data of α-hydroxymidazolam demonstrates that the quadratic calibration models generate a more accurate representation of the results than the linear models for this method. This second-generation tool summarizes all of the validation parameters of a method for both linear and quadratic calibration models, and the better model representation is color-coded green. Like the EZSTATSG1, the redesigned EZSTATSG2.xltm MS Excel self-actuating validation tool and a completed PDF example are available to the scientific community for download as Supplementary data.

Introduction

A new era in evaluating method validation data is hindering many forensic laboratories from completing comprehensive and full method validations. According to Shug, complete method validation is still a glaring deficiency in many forensic laboratories (1). While Shug mentions that obtaining the required validation parameters, according to the Academy Standards Board (ASB) 036 Standard's guidelines, is not hard, the difficulty in processing and assessing the validation data may be one reason for the lack of complete method validations. Like the predecessor EZSTATSG1, the intent of this second-generation autonomous tool is to aid the scientific community with these challenging data evaluation steps, which are an integral part of the method validation process, whether a calibration model is linear or quadratic.

The Mayflower edition of this Microsoft (MS) Excel validation template was developed for linear calibration models only and was published by Sofalvi and Schueler in order to make the template available to the scientific community so that method validations could be completed quickly, easily, efficiently and economically (2). The first published use of EZSTATSG1 in a newly developed method was recently cited by Waters *et al.* (3). According to the American National Standards Institute (ANSI)/ASB Standard 036, the simplest calibration model that best fits the concentration-response relationship should be used. Until lately, this has been most frequently a simple linear calibration model; however, quadratic calibration models are also acceptable (4) and are becoming more prevalent. As instrument manufacturers begin to introduce various quadratic curve fitting options into their data processing software, many laboratories are discovering that the quadratic calibration models are often more rigorous or stable than the linear counterpart. All quantitative analytes in an article published by Garcia *et al.* for the analysis of novel and nonroutine benzodiazepines utilized quadratic calibration curves (5). Jakobsson *et al.* published a method for the analysis of heroin-related compounds and determined, based on the residual plots, that quadratic regression with $1/x$ or $1/x^2$ were the best fits for their method (6). Sullinger *et al*. also

used a quadratic calibration curve for the quantitation of suvorexant in urine (7). MS Excel includes many non-weighted trendline curve fitting options (i.e., linear, exponential, logarithmic, polynomial, power and moving average) but does not contain a weighted quadratic curve fitting selection. EZSTATSG2 not only provides verification of both weighted linear and quadratic calibration curves resulting from a laboratory's instrument software; but also, this tool automatically performs all of the statistical calculations required for each validation parameter of a complete method validation.

The debate over using linear versus quadratic or non-weighted versus weighted calibration models will continue; however, the selection should not be based on which model is "desired" but instead the best model which represents the trending data. Several authors have provided numerous examples and justifications for the use of non-weighted and weighted quadratic curves (8,9,10). Desharnais *et al*. noted that both physical and chemical phenomenon such as detector saturation or competition in the LC−MS-MS ionization process can produce data which fits quadratic models better than linear models (11). The choice of calibration model is especially significant when the accuracy of the low end of the calibration curve is critically important, as in cases involving very low blood drug concentrations such as: interpreting pharmaceutical compliance; evaluating extremely potent novel psychoactive substances (NPS); and in drug-facilitated sexual assaults (DFSA) or drug-facilitated crimes (DFC), where the difference between an unreportable negative and a reportable positive result should be based on objective science. For example, any case sample with an area ratio less than the y-intercept of a linear calibration curve produces a negative result which cannot be reported even qualitatively (Figure 1). According to LeBeau and Montgomery, the acknowledgment of the many challenges of DFSA investigations may serve as an avenue to improve the chances of successfully investigating these types of cases (12).

The key new features of this tool compared to the predecessor, EZSTATSG1, are support for weighted quadratic calibration models, the implementation of Visual Basic for Applications

(VBA) codes which lock the cells for security purposes every time the file is reopened, data entry UserForms and the ability to validate methods with variable (five, six or seven) calibration points. The template has the flexibility to drop a calibration point (the same concentration level in all five trials) during validation processing by only deleting a target concentration in Trial 1 without deleting the peak areas, which is analogous to toggling a point using instrument software. If the change is not desirable, the previous calibration curves can be easily restored by only reentering the deleted target concentration. An additional tab for evaluating processed sample stability has also been incorporated into EZSTATSG2.

Development of the Template

Quadratic Calibration Model

When a newly developed method exhibits non-linear behavior, a quadratic model may fit the data better than a linear model. The relevant form of the quadratic equation is yhat = $ax^2 + bx + c$, where yhat is the predicted value and x is the concentration. The coefficients of the quadratic calibration equation (a, b and c) with equal weighting were found by solving a system of three equations (13) represented by matrices A and B shown in Table I. The system of equations has the form AX = B where A is the coefficient matrix, B is the constant matrix and X is the variable matrix which in this case is the unknown column vector holding the values for a, b and c shown in the equation below, where n is the number of calibration points:

$$\begin{bmatrix} \sum_{i=1}^{n} x_i^4 & \sum_{i=1}^{n} x_i^3 & \sum_{i=1}^{n} x_i^2 \\ \sum_{i=1}^{n} x_i^3 & \sum_{i=1}^{n} x_i^2 & \sum_{i=1}^{n} x_i \\ \sum_{i=1}^{n} x_i^2 & \sum_{i=1}^{n} x_i & n \end{bmatrix} X = \begin{bmatrix} \sum_{i=1}^{n} x_i^2 y_i \\ \sum_{i=1}^{n} x_i y_i \\ \sum_{i=1}^{n} y_i \end{bmatrix}$$

Multiplying both sides of this equation by the inverse (A^{-1}) of matrix A provides the solutions for a, b and c since X = (A^{-1}) B. This is accomplished in Excel on each Quadratic Curves worksheet by first finding A^{-1} by clicking cell B28 and entering = MINVERSE(B24:D26) which returns the inverse value of matrix A. Next, X was found by first selecting cell H24 and entering = MMULT(B28:D30,F24:F26) which calculates the matrix product of the A^{-1} and B arrays. The same procedure was employed to find the coefficients of the quadratic calibration equation with 1/*x* weighting (w), but it required different A and B matrices shown in equation below and in B32:D34 and F32:F34, respectively:

$$\begin{bmatrix} \sum_{i=1}^{n} w_i x_i^4 & \sum_{i=1}^{n} w_i x_i^3 & \sum_{i=1}^{n} w_i x_i^2 \\ \sum_{i=1}^{n} w_i x_i^3 & \sum_{i=1}^{n} w_i x_i^2 & \sum_{i=1}^{n} w_i x_i \\ \sum_{i=1}^{n} w_i x_i^2 & \sum_{i=1}^{n} w_i x_i & \sum_{i=1}^{n} w_i \end{bmatrix} X = \begin{bmatrix} \sum_{i=1}^{n} w_i x_i^2 y_i \\ \sum_{i=1}^{n} w_i x_i y_i \\ \sum_{i=1}^{n} w_i y_i \end{bmatrix}$$

A^{-1} for the weighted calibration was determined by clicking cell B36 and entering = MINVERSE(B32:D34). X for the weighted calibration was found by clicking cell H32 and entering = MMULT(B36:D38,F32:F34). Each calculated calibrator concentration was obtained using the following equation:

$$x_{calculated} = \frac{-b + \sqrt{b^2 - 4a(c - y_i)}}{2a}$$

The predicted values for each of the seven calibrator target concentrations were calculated using the quadratic equation, yhat = $ax^2 + bx + c$. The residuals (y - yhat) were obtained from the difference between the area ratio (y) and the predicted value. The coefficient of determination (R^2) for equal weighting was calculated in the next equation:

$$R^2_{equal} = 1 - \frac{\sum_{i=1}^{n}(y_i - \hat{y}_i)^2}{\sum_{i=1}^{n}(y_i - \bar{y})^2}$$

R^2 for $1/x$ weighting was calculated using equation:

$$R^2_{1/x} = 1 - \frac{\sum_{i=1}^{n} w_i (y_i - \hat{y}_i)^2}{\sum_{i=1}^{n} w_i (y_i - \bar{y}_w)^2}; \qquad where \qquad \bar{y}_w = \frac{\sum_{i=1}^{n} w_i y_i}{\sum_{i=1}^{n} w_i}$$

Visual Basic for Applications (VBA) in Excel UserForms

EZSTATSG2 employs VBA UserForms to facilitate data entry and reduce manipulation of equations within worksheets which could result in inadvertent alterations of cells. In the EZSTATSG2 template, the results of a validation can only be entered by clicking the "Data Entry" button located at the top of the Instructions tab which displays a UserForm for data entry; however, if any data need to be changed, the entries can be edited by clicking the appropriate "EDIT" button on either the Instructions tab or at the top of the corresponding worksheet where the data are located. In case of outliers in the limit of detection (LOD), lower limit of quantitation (LLOQ) or dilution integrity results, the data fields should be left blank, and the mean and bias are shown in real time. The quality control (QC) outliers must be entered as zeros. After all data have been entered, the location where the file is to be saved is selected, and the Excel file is saved as the analyte name and the template version number in that location. The validation data can be captured as electronic records in PDF form immediately after all data entry is completed by clicking "Yes" when prompted, or at any time by clicking on the "Save Workbook as PDF" button located on the Instructions worksheet. The PDF will be saved as the analyte and assay names in the same location as the workbook. The Validation Summary Report includes the results of both quadratic and linear calibration models. The better model, which has the lower average sum of relative errors and higher R^2 values, is color coded green.

The UserForms are simply a data transfer mechanism and do not manipulate the data in any way except to transfer what is entered onto the corresponding worksheet. If data on the spreadsheet

must be changed, the cell in question may be unlocked by right-clicking the cell, selecting "Format Cells" and deselecting the "Locked" radio button on the Protection tab.

The drop-down menus in the Areas of Trials 1-5 UserForm can be edited directly, however, the drop-down options can also be customized. To customize the Instrument and Toxicologist options, the EZSTATSG2 Excel Macro-Enabled Template (*.xltm) must be opened. To do this, right click on the EZSTATSG2 file and select "Open". If the file is simply double clicked, it will open an Excel Macro-Enabled Workbook (*.xlsm) and changes to the template will not be saved. Once the template is open, the Developer tab must be selected. If the Developer tab is not visible in the toolbar, it can be enabled in Excel by clicking File > Options > Customize Ribbon and under Customize the Ribbon, selecting the Developer checkbox. Under the Developer tab, select the Visual Basic icon, and a new window will open. In the left-hand Project Explorer window, select the CalibrationModel form, right click, and select "View Code". The code associated with this UserForm will appear in the window. At the top of this window, the Private Sub UserForm_Initialize should appear. Where indicated, the existing presets can be changed and/or toxicologist and instrument names can be added. When finished, close the VBA window and save the file, ensuring that it is saved as an Excel Macro-Enabled Template (*.xltm). To use the EZSTATSG2 template, simply double click the file from any location. This will open an Excel Macro-Enabled Workbook (*.xlsm) that contains any changes made to the original template.

Discussion

The data for the example PDF using α-hydroxymidazolam were obtained from validation trials published by Sofalvi *et al.* (14). Figure 1 shows the quadratic calibration model with 1/x weighting factor, the quadratic equations, R^2 values of the five trials and also indicates the low, medium and high QC target concentrations for visual inspection of the QC distribution throughout the dynamic range of the calibration curve. The standard residual plot of the linear calibration curves exhibits an inverted U-shaped distribution when the quadratic coefficient is negative which indicates a non-

linear correlation, as shown in Figure 2. However, the random distribution around the zero-line using quadratic calibration models suggest that the quadratic models are more appropriate than the linear models.

Robust methods can generate calibration curves which may overlap, as shown in Figure 1. The existence of the five different curves can be more clearly visualized by expanding the graph which also illustrates the parabolic shape of each of the five quadratic calibration curves, as shown in Figure 3. The parabolic curves were generated in the MathCAD 2001 Professional software package using the POLYROOTS function. Nonlinear calibration data obtained from bioanalytical method validation procedures for forensic toxicology applications which fit quadratic functions correspond to parabolas featuring a vertical axis of symmetry that can curve either downward or upward. Since these models are second-order functions, each area ratio less than the vertex corresponds to two calculated concentrations except for where the vertex curves downward, but only one of these concentrations is of interest and falls within the dynamic range of the calibration curve. The presented template has integrated equations which produce the calculated concentrations of interest only. In the case of parabolas which curve downward (Figure 3), there is one important detail to keep in mind. Whenever the area ratio is greater than the vertex of the parabola, which may happen in overdose cases, the quadratic model cannot predict an estimate of the concentration for dilution purposes because the response value is not on the curve. However, the maximum area ratio can be calculated up to where the model is still valid. At the vertex, the tangent line to the parabola has a zero slope; therefore, the solution of the first derivative of the quadratic equation, -b/2a, coincides with the vertical axis of symmetry ($x = h$) and is equal to the concentration at which the parabola has the maximum value. Solving the quadratic model at the axis of symmetry quantifies the maximum usable area ratio. EZSTATSG2 also generates similar figures showing the parabolic model closest to the origin and crossing the horizontal axis twice for each of the six weighting factors regardless of whether the models curve upward or downward.

This is accomplished in Excel using variables for the variable concentrations since two sets of concentration ranges are necessary. On the one hand, when the quadratic functions curve downward, the concentration range is calculated from the lowest calibrator up to the width of the parabola with the smallest axis of symmetry when the curve crosses the x-axis the second time. On the other hand, when the models curve upward, the range is obtained from the width of the parabola with the smallest axis of symmetry crossing the horizontal axis on the negative side up to the highest calibrator concentration. This design generates a full view of both types of parabolic models having the smallest axis of symmetry whether they curve upward or downward. The vertical lines indicate the axis of symmetry, and the intersection of the axis of symmetry with the model represents the vertex. The general form of a parabolic equation with vertical axis of symmetry is $4p\,(y - k) = (x - h)^2$. After rearranging the quadratic equation to the parabolic version, the three parameters (h, k and p) can be obtained as a function of the three coefficients (a, b and c) as $h = -b/(2a)$, $k = c - b^2/(4a)$ and $p = 1/(4a)$. From this information, each quadratic model can be fully-characterized by providing the equations for the vertical axis of symmetry ($x = h$) and directrix ($y = k - p$) and the coordinates for the vertex (h, k) and focus (h, $k + p$), where p is defined as the distance between the directrix and the vertex which is also equal to the distance between the focus and vertex. Since the vertex and focus are located on the axis of symmetry, only the vertical coordinate is shown; whereas, the horizontal coordinate corresponds to the symmetry value (h). As an example, the location of the directrix and focus was also generated in MathCAD 2001 Professional and is shown in Figure 4.

The normality of residuals graph offers an analyst-independent view of the residuals as suggested by Desharnais *et al.* (15). The frequency plot is created using Excel's FREQUENCY function which is overlaid with the normal probability distribution using the NORM.DIST function. The five-number summary representing the minimum, Q1, median, Q3 and maximum data is created using the QUARTILE function (Figure 5).

The weighted average response equation was obtained from the Handbook of Chemometrics and Qualimetrics (16). The simplest model is not always necessarily the best one. The better model is the one which has lower calibrator and QC bias and consequently generates more accurate results for case samples.

Conclusions

To the authors' knowledge, this comprehensive validation tool represents the first publication which can create weighted quadratic calibration models utilizing five-, six-, or seven-point calibration curves in MS Excel for bioanalytical method validation purposes in forensic toxicology. The EZSTATSG2 template provides an independent verification of both linear and quadratic weighted calibration models regardless of instrument manufacturers' software platforms. According to the ANSI/ASB Standard 036, when there is a notable difference between variances at the lowest and highest concentrations, a weighted least squares model or another appropriate non-linear model should be applied. Kiser and Dolan (17) state that bioanalytical calibration data are very unlikely to be homoscedastic; therefore, these weighted linear and quadratic models should become the norm. With this method validation tool being readily accessible to the scientific community; as well as, other commercially available validation software programs, the authors recommend the ANSI/ASB Standard 036 be updated from "the simplest calibration model that best fits the concentration-response relationship should be used" to "the best curve fit model representing the trending data should be used". If the better model is weighted quadratic which curves downward, this can resolve any unreportable negative concentration values often obtained from linear calibration curves by generating reportable positive low-concentration results. This could have a significant impact on the outcome of many pharmaceutical compliance, NPS, DFSA or DFC cases.

Supplementary data

Supplementary data includes EZSTATSG2 template and PDF example which are available at *Journal of Analytical Toxicology* online.

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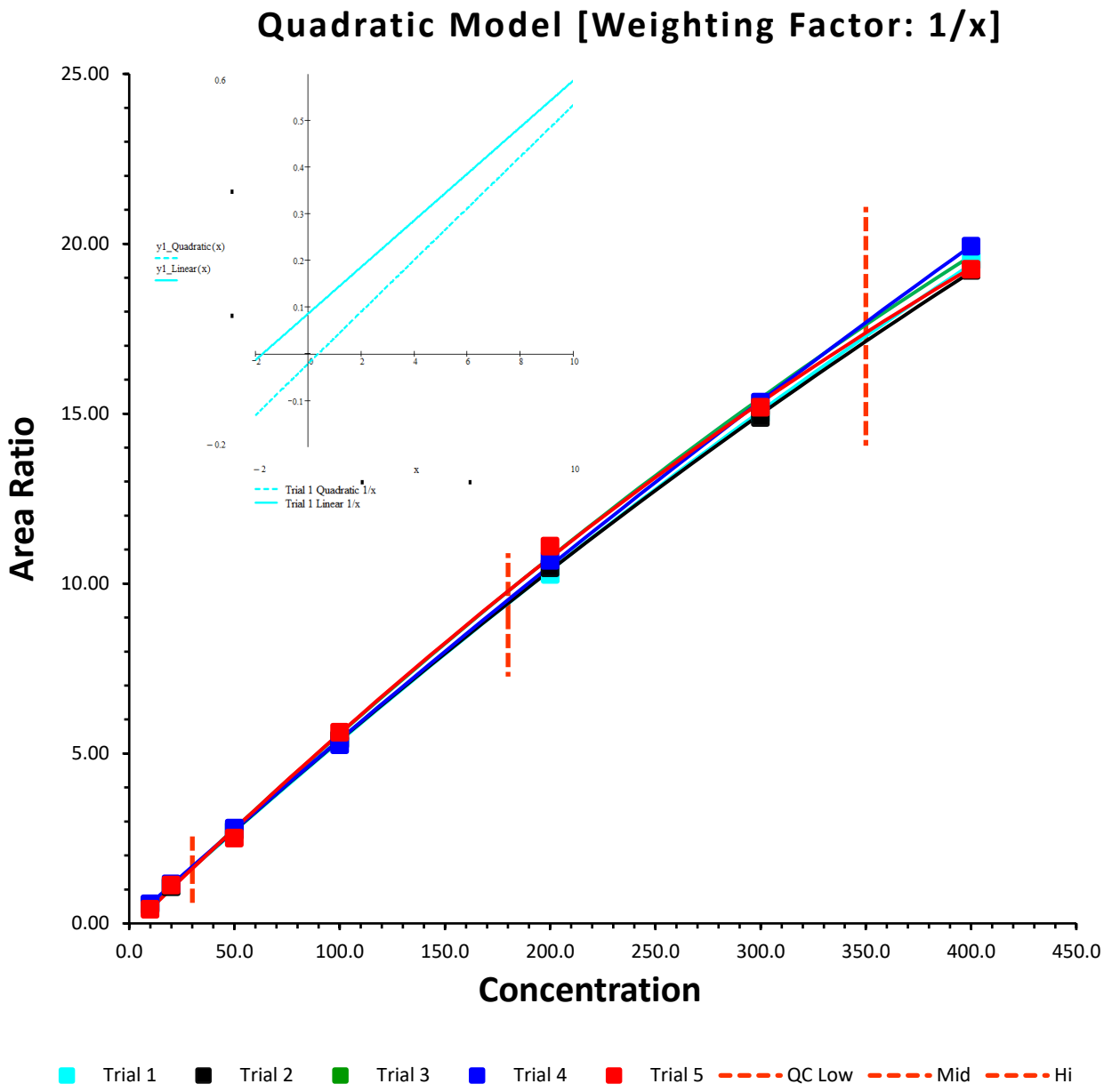


	a		b			c	R^2
Trial 1	-1.761E-05	X^2 +	5.559E-02	X	+	-2.011E-02	0.9999
Trial 2	-2.041E-05	X^2 +	5.614E-02	X	+	-2.023E-02	1.0000
Trial 3	-2.532E-05	X^2 +	5.956E-02	X	+	-1.284E-01	1.0000
Trial 4	-1.342E-05	X^2 +	5.514E-02	X	+	3.190E-02	0.9998
Trial 5	-3.030E-05	X^2 +	6.079E-02	X	+	-1.818E-01	0.9987

Figure 1. Alpha-hydroxymidazolam calibration model with 1/x weighting factor for a dynamic range of 10─400 ng/mL and QC targets where x and y represent the concentration and area ratio, respectively. The embedded figure zooms in to the zero and LLOQ concentration range and illustrates the difference between quadratic and linear calibration curves.

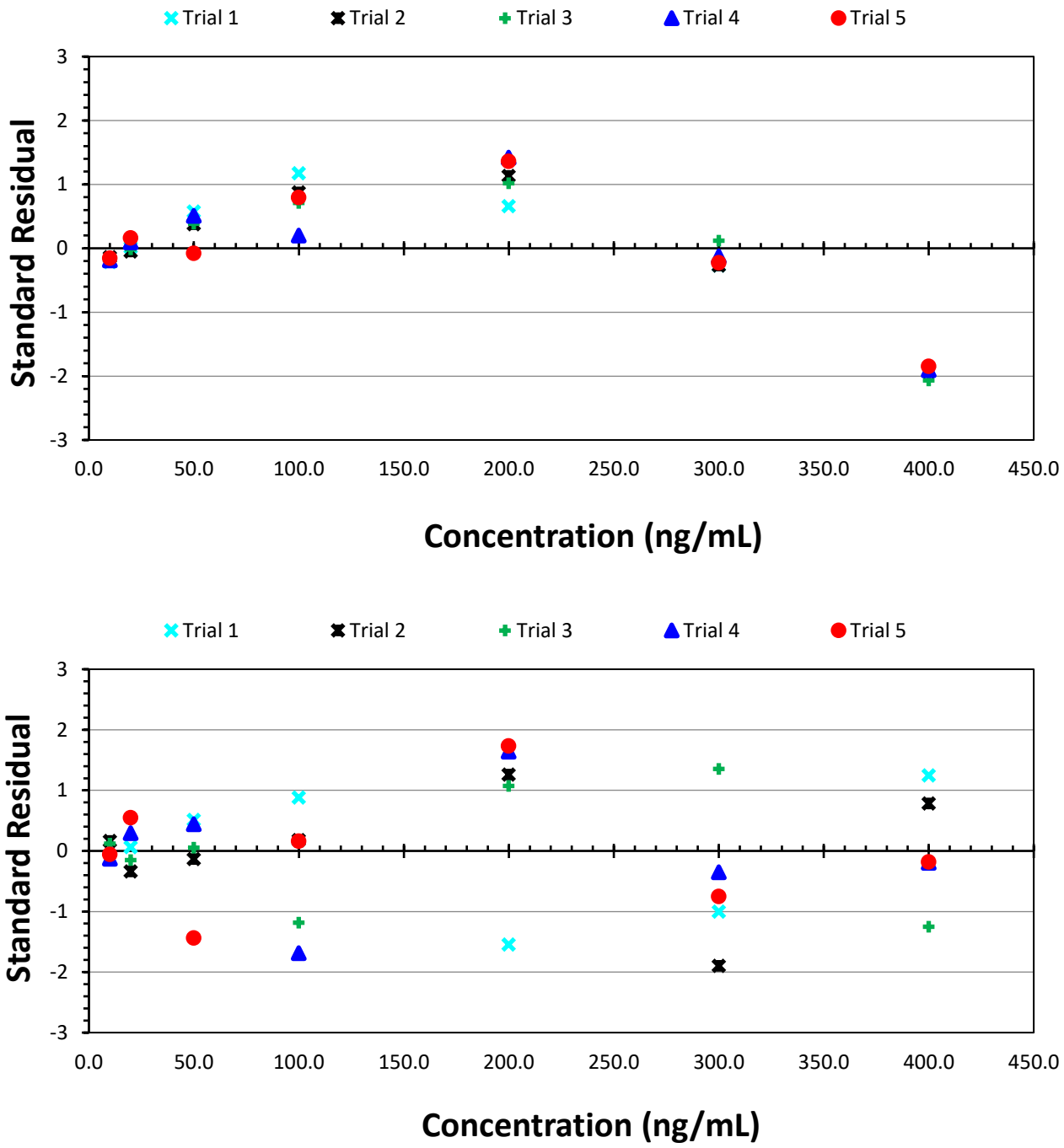


Figure 2. Standard residuals for alpha-hydroxymidazolam with 1/x weighting factor from the linear (top) and the quadratic (bottom) calibration models.

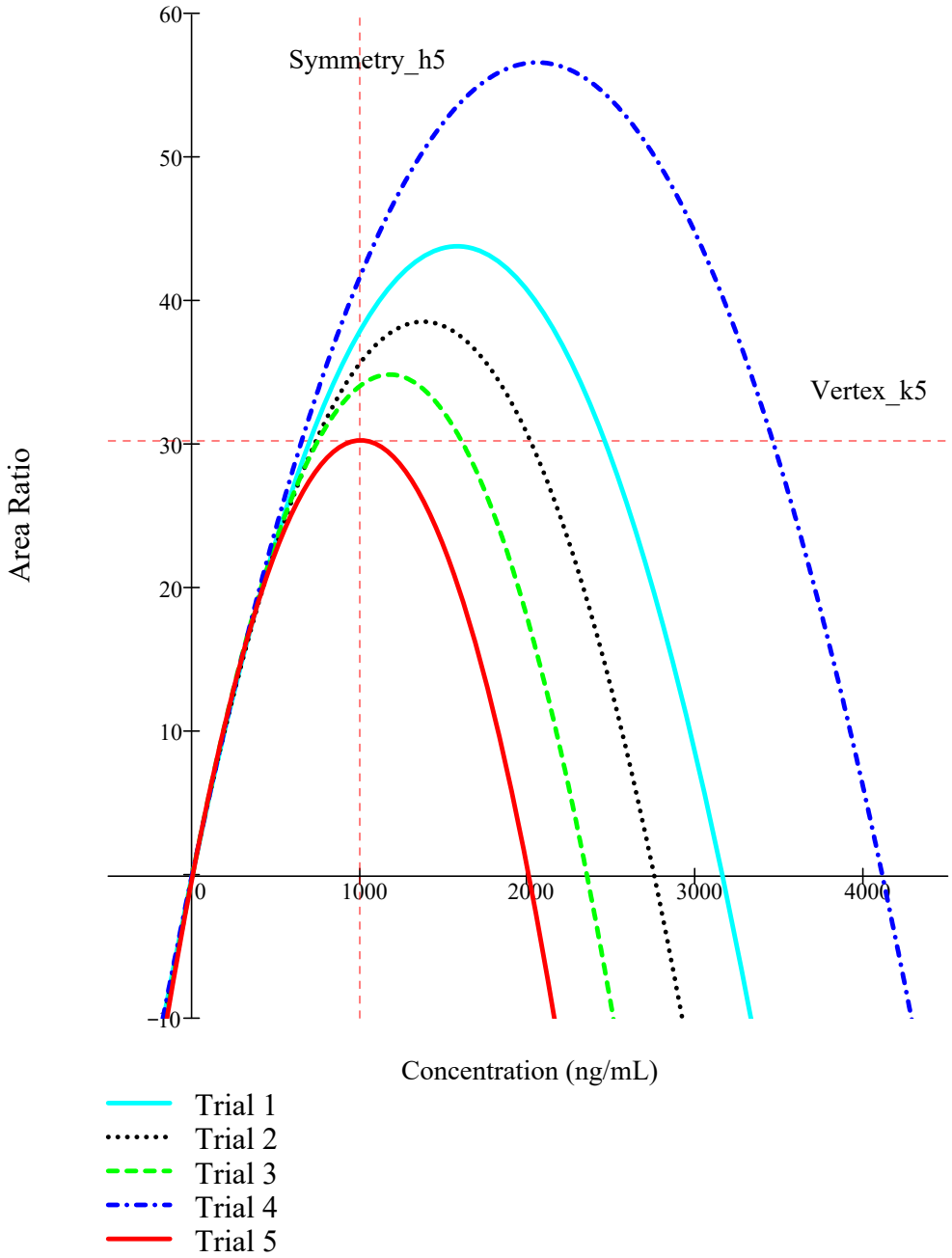


Figure 3. Quadratic calibration model with 1/x weighting factor beyond the dynamic range for alpha-hydroxymidazolam. The vertex and vertical axis of symmetry is shown for Trial 5.

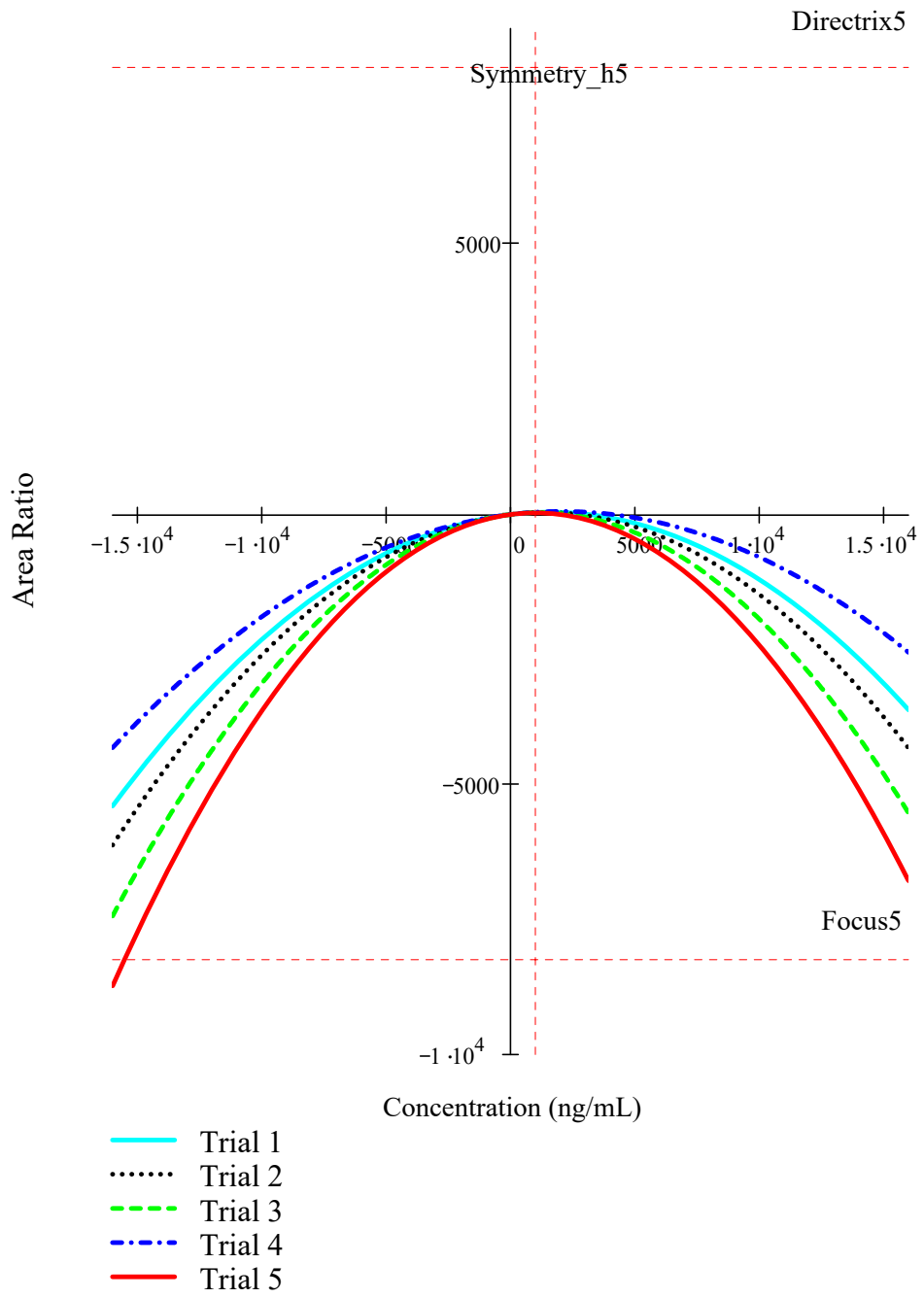


Figure 4. Quadratic calibration model with 1/x weighting factor illustrating the focus and directrix for Trial 5.

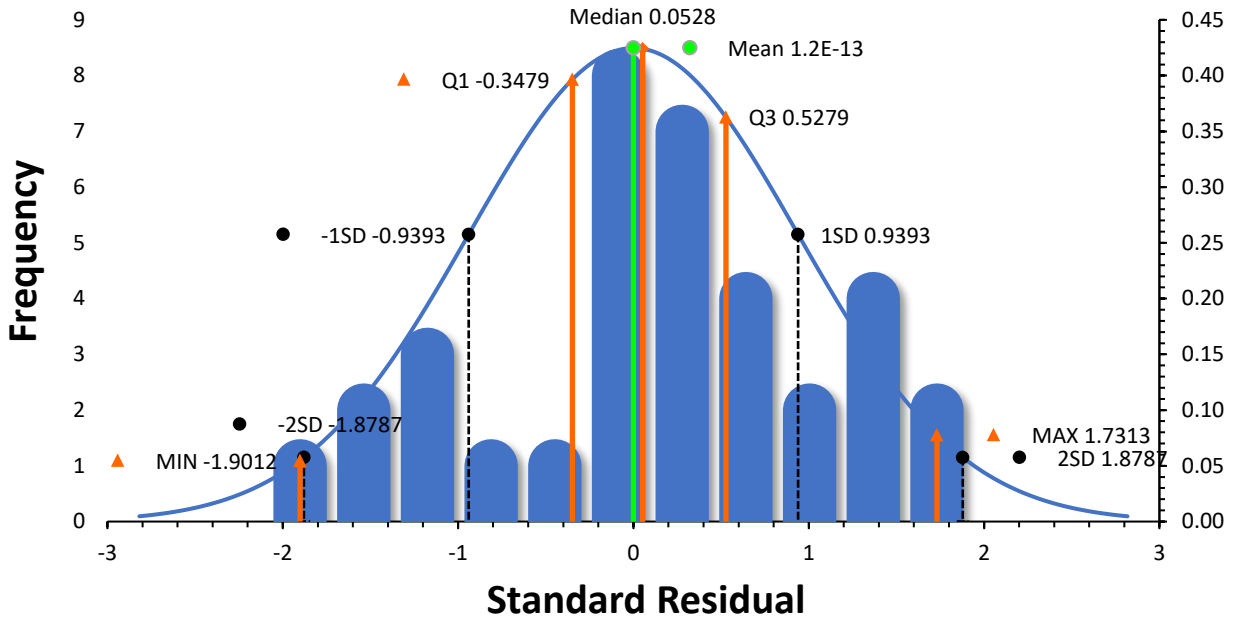


Figure 5. Normality of residuals plot for alpha-hydroxymidazolam with quadratic 1/x weighting.

	A	B	C	D	E	F	G	H	I	J
1	Drug:	α-Hydroxymidazolam								
2	Unit:	ng/mL								
3										
4	Trial 1	x	y	w (1/x)	x^2	x^3	x^4	xy	x^2y	$(y\text{-}ybar)^2$
5	L1	10.000	5.23E-01	1.00E-01	1.00E+02	1.00E+03	1.00E+04	5.23E+00	5.23E+01	5.29E+01
6	L2	20.000	1.09E+00	5.00E-02	4.00E+02	8.00E+03	1.60E+05	2.18E+01	4.36E+02	4.50E+01
7	L3	50.000	2.76E+00	2.00E-02	2.50E+03	1.25E+05	6.25E+06	1.38E+02	6.89E+03	2.54E+01
8	L4	100.000	5.43E+00	1.00E-02	1.00E+04	1.00E+06	1.00E+08	5.43E+02	5.43E+04	5.59E+00
9	L5	200.000	1.03E+01	5.00E-03	4.00E+04	8.00E+06	1.60E+09	2.05E+03	4.11E+05	6.15E+00
10	L6	300.000	1.50E+01	3.33E-03	9.00E+04	2.70E+07	8.10E+09	4.50E+03	1.35E+06	5.18E+01
11	L7	400.000	1.95E+01	2.50E-03	1.60E+05	6.40E+07	2.56E+10	7.80E+03	3.12E+06	1.37E+02
12	7	1.08E+03	5.46E+01	1.91E-01	3.03E+05	1.00E+08	3.54E+10	1.51E+04	4.94E+06	3.24E+02
13										
14		wx	wy		wx^2	wx^3	wx^4	wxy	wx^2y	$(y\text{-}ybarw)^2$
15		1.00E+00	5.23E-02		1.00E+01	1.00E+02	1.00E+03	5.23E-01	5.23E+00	1.95E+00
16		1.00E+00	5.45E-02		2.00E+01	4.00E+02	8.00E+03	1.09E+00	2.18E+01	6.89E-01
17		1.00E+00	5.51E-02		5.00E+01	2.50E+03	1.25E+05	2.76E+00	1.38E+02	6.98E-01
18		1.00E+00	5.43E-02		1.00E+02	1.00E+04	1.00E+06	5.43E+00	5.43E+02	1.23E+01
19		1.00E+00	5.14E-02		2.00E+02	4.00E+04	8.00E+06	1.03E+01	2.05E+03	6.98E+01
20		1.00E+00	5.00E-02		3.00E+02	9.00E+04	2.70E+07	1.50E+01	4.50E+03	1.71E+02
21		1.00E+00	4.87E-02		4.00E+02	1.60E+05	6.40E+07	1.95E+01	7.80E+03	3.09E+02
22		7.00E+00	3.66E-01		1.08E+03	3.03E+05	1.00E+08	5.46E+01	1.51E+04	
23										
24		3.54E+10	1.00E+08	3.03E+05		4.94E+06		-1.443E-05	a	
25	A=	1.00E+08	3.03E+05	1.08E+03	B =	1.51E+04	X =	5.435E-02	b	
26		3.03E+05	1.08E+03	7.00E+00		5.46E+01		3.426E-02	c	Equal
27										
28		7.18E-10	-2.81E-07	1.23E-05						
29	A^{-1} =	-2.81E-07	1.17E-04	-5.94E-03				0.9999	R^2	
30		1.23E-05	-5.94E-03	5.28E-01						
31										
32		1.00E+08	3.03E+05	1.08E+03		1.51E+04		-1.761E-05	a	
33	A =	3.03E+05	1.08E+03	7.00E+00	B =	5.46E+01	X =	5.559E-02	b	
34		1.08E+03	7.00E+00	1.91E-01		3.66E-01		-2.011E-02	c	Weighted
35										
36		1.03E-07	-3.28E-05	6.23E-04						
37	A^{-1} =	-3.28E-05	1.17E-02	-2.44E-01				0.9999	R^2	
38		6.23E-04	-2.44E-01	1.07E+01						
39										
40	Standard			1/x				Equal		
41	Residual	y-yhat	Target	Calculated	Response	Bias	y-yhat	Calculated	Response	Bias
42	-0.1402	-0.0109	10.000	9.803	5.23E-01	**-1.97%**	-0.0532	9.017	5.23E-01	**-9.83%**
43	0.0611	0.0048	20.000	20.087	1.09E+00	**0.43%**	-0.0260	19.516	1.09E+00	**-2.42%**
44	0.5081	0.0395	50.000	50.734	2.76E+00	**1.47%**	0.0394	50.745	2.76E+00	**1.49%**
45	0.8777	0.0683	100.000	101.312	5.43E+00	**1.31%**	0.1065	102.071	5.43E+00	**2.07%**
46	-1.5454	-0.1202	200.000	197.526	1.03E+01	**-1.24%**	-0.0529	198.911	1.03E+01	**-0.54%**
47	-1.0056	-0.0782	300.000	298.264	1.50E+01	**-0.58%**	-0.0454	299.006	1.50E+01	**-0.33%**
48	1.2444	0.0968	400.000	402.335	1.95E+01	**0.58%**	0.0316	400.739	1.95E+01	**0.18%**
49						7.59%				16.87%

BZDG1 in Blood

α-OH-Midazolam

Parameter:		Target:	Weighting	QUADRATIC Average ΣRE (%)	QUADRATIC R^2	LINEAR LOD Estimated	LINEAR Average ΣRE (%)	LINEAR R^2
Calibration Model	(1)	L1 to ULOQ	Equal	14%	0.9998	N/A	95%	0.9975
	(2)	L1 to ULLOQ	1/x	11%	0.9997	3.232	38%	0.9974
	(3)	L1 to ULLOQ	$1/x^2$	10%	0.9988	4.087	30%	0.9959
	(4)	L1 to ULLOQ	$1/x^{1/2}$	11%	0.9998	1.980	53%	0.9977
	(5)	L1 to ULLOQ	1/y	11%	0.9997	3.685	37%	0.9975
	(6)	L1 to ULLOQ	$1/y^2$	10%	0.9989	4.409	30%	0.9959
	(7)	L1 to ULLOQ	$1/y^{1/2}$	11%	0.9998	2.352	53%	0.9977

LOD Target	5.000 ng/mL	LOD Mean	5.367	
Bias	10.000 ng/mL	LLOQ		2.2%
	30.000 ng/mL	Low QC		1.0%
	180.000 ng/mL	Medium QC		0.9%
	350.000 ng/mL	High QC		0.8%
	400.000 ng/mL	ULOQ		
Precision	LLOQ	Within & Between run ≤ ± 20%	LLOQ	Within 8.0%
				Between 9.9%
	Low	Within & Between run ≤ ± 20%	Low QC	Within 1.4%
				Between 3.1%
	Medium	Within & Between run ≤ ± 20%	Medium QC	Within 2.2%
				Between 4.0%
	High	Within & Between run ≤ ± 20%	High QC	Within 2.8%
				Between 3.8%

BZDG1 in Blood

α-OH-Midazolam

Parameter:	Desired Limit:		Result:		
Interference Studies	No interfering signals from the matrix, isotopically-labelled internal standards, common drugs of abuse, OTC drugs and prescription medications				
Carryover	No significant carryover detected in a blank control analyzed after a L7x10 calibrator fortified sample.	L7x1	N/A	ng/mL	
		L7x2	N/A	ng/mL	
		L7x10	N/A	ng/mL	
Recovery		Low QC			90.5%
		High QC			72.6%
Dilution Integrity	Accuracy and Precision criteria of ≤ ± 20% met at x2, x5, and x10 dilution levels	2		Bias	1.1%
		Precision		Within	3.3%
				Between	4.3%
		5		Bias	-0.6%
		Precision		Within	8.3%
				Between	9.1%
		10		Bias	-4.0%
		Precision		Within	6.3%
				Between	8.8%
Ion Suppression/ Enhancement	% Ionization Low QC:				0.1%
	% Ionization ISTD Low QC:				2.0%
	% Ionization High QC:				-13.3%
	% Ionization ISTD Hi QC:				-16.2%

Instructions

Data entry fields are colored beige and will be automatically filled through the VBA Data Entry forms

Acceptable values are colored green and will automatically turn green when values are within defined limits

Cells calculated by the template (editable)

1. Click the "Data Entry" button below to begin entering your data.
2. When you are finished entering all your data, the workbook will be saved in the location that you specify as both an Excel workbook and as a PDF.
3. If you need to edit any of your data, select the appropriate button below or on the page that you wish to edit.
4. If you edit any data, you can save your changes in a PDF by clicking the button below. Clicking this button will overwrite any existing PDF in the specified location.

If you've been dreaming about processing method validation data the easy way, you have come to the right place! Dreams do come true, every once in a while, outside of Magic Kingdom!

BZDG1 in Blood

Drug: α-OH-Midazolam

Calibration Model:

Toxicologist(s): JD1 JD2 **Instrument:** LC-MS/MS

Trial 1

Calibrator Level & Concentration		Drug Response	Internal Standard Response	Area Ratio
	ng/mL		α-OH-Midazolam-D4	
L1	10.000	664,643	1,270,477	5.23E-01
L2	20.000	3,146,960	2,888,586	1.09E+00
L3	50.000	4,057,919	1,472,920	2.76E+00
L4	100.000	14,840,592	2,732,414	5.43E+00
L5	200.000	38,386,825	3,736,413	1.03E+01
L6	300.000	60,819,316	4,056,140	1.50E+01
L7	400.000	83,302,623	4,272,834	1.95E+01
n=	7			

Trial 2

Calibrator Level & Concentration		Drug Response	Internal Standard Response	Area Ratio
	ng/mL		α-OH-Midazolam-D4	
L1	10.000	1,497,304	2,734,108	5.48E-01
L2	20.000	1,864,359	1,731,890	1.08E+00
L3	50.000	3,778,555	1,384,672	2.73E+00
L4	100.000	18,682,499	3,460,214	5.40E+00
L5	200.000	33,811,104	3,232,865	1.05E+01
L6	300.000	37,694,288	2,532,239	1.49E+01
L7	400.000	71,216,868	3,706,644	1.92E+01

BZDG1 in Blood

Drug: α-OH-Midazolam

Calibration Model:

Toxicologist(s): JD3 JD4 **Instrument:** LC-MS/MS

Trial 3

Calibrator Level & Concentration		Drug Response	Internal Standard Response	Area Ratio
	ng/mL		α-OH-Midazolam-D4	
L1	10.000	1,528,218	3,252,267	4.70E-01
L2	20.000	3,061,601	2,927,407	1.05E+00
L3	50.000	8,521,585	3,055,977	2.79E+00
L4	100.000	16,568,812	3,000,927	5.52E+00
L5	200.000	37,170,400	3,436,073	1.08E+01
L6	300.000	51,069,553	3,290,626	1.55E+01
L7	400.000	73,406,342	3,747,788	1.96E+01

Trial 4

Calibrator Level & Concentration		Drug Response	Internal Standard Response	Area Ratio
	ng/mL		α-OH-Midazolam-D4	
L1	10.000	1,824,020	3,201,916	5.70E-01
L2	20.000	3,840,469	3,319,421	1.16E+00
L3	50.000	8,338,371	2,981,261	2.80E+00
L4	100.000	20,855,628	3,970,161	5.25E+00
L5	200.000	36,229,504	3,393,047	1.07E+01
L6	300.000	54,950,906	3,583,801	1.53E+01
L7	400.000	68,271,268	3,426,831	1.99E+01

BZDG1 in Blood

Drug: α-OH-Midazolam

Calibration Model:

Toxicologist(s): JD5 **Instrument:** LC-MS/MS

Trial 5

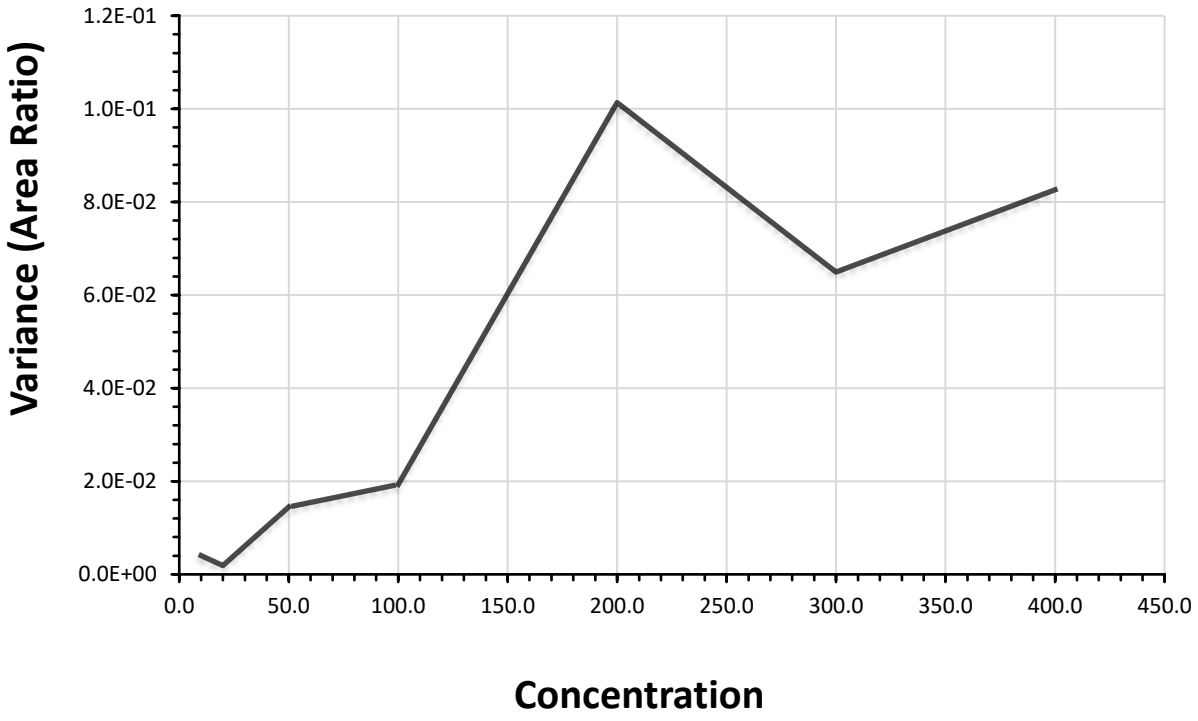
Calibrator Level & Concentration		Drug Response	Internal Standard Response	Area Ratio
	ng/mL		α-OH-Midazolam-D4	
L1	10.000	152,292	369,869	4.12E-01
L2	20.000	81,147	72,003	1.13E+00
L3	50.000	804,147	320,972	2.51E+00
L4	100.000	5,025,214	893,454	5.62E+00
L5	200.000	14,222,870	1,281,743	1.11E+01
L6	300.000	28,984,152	1,908,954	1.52E+01
L7	400.000	38,185,288	1,983,618	1.93E+01

BZDG1 in Blood

Drug: **α-OH-Midazolam**

Unit: **ng/mL**

Variance in Instrument Response

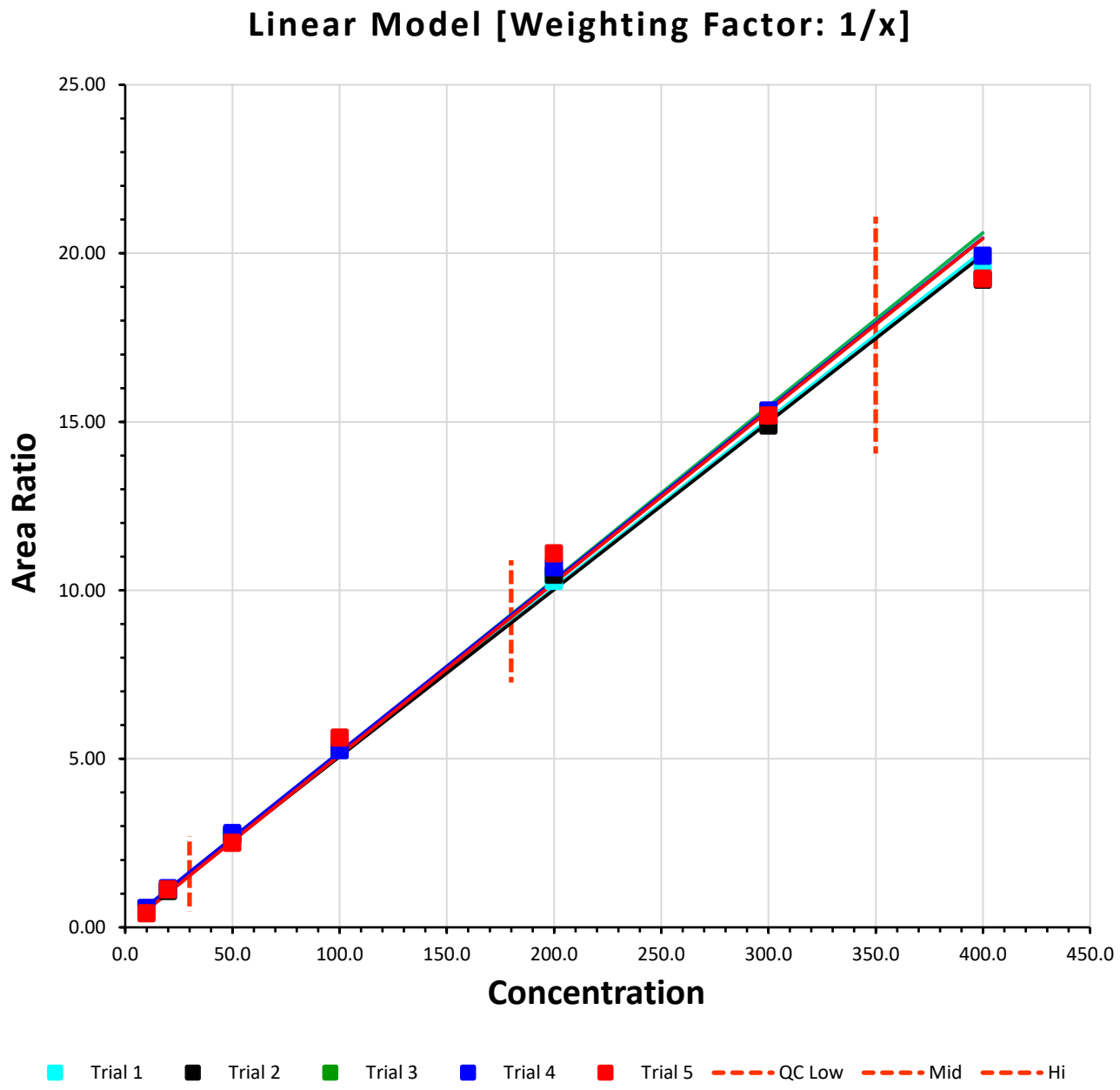


	Concentration	Variance
L1	10.000	4.06E-03
L2	20.000	1.89E-03
L3	50.000	1.45E-02
L4	100.000	1.93E-02
L5	200.000	1.01E-01
L6	300.000	6.50E-02
L7	400.000	8.26E-02

BZDG1 in Blood

Drug: α-OH-Midazolam

Unit: ng/mL



		slope			y-intercept	R^2
Trial 1	Y=	4.996E-02	X	+	8.690E-02	0.9984
Trial 2	Y=	4.961E-02	X	+	1.038E-01	0.9980
Trial 3	Y=	5.145E-02	X	+	2.552E-02	0.9971
Trial 4	Y=	5.085E-02	X	+	1.135E-01	0.9990
Trial 5	Y=	5.110E-02	X	+	2.243E-03	0.9946

Drug: α-OH-Midazolam

Unit: ng/mL

Trial 1	x	y	w (1/x)	xy	wxy	wy	wx^2	wy^2	x^2
L1	10.000	5.23E-01	1.00E-01	5.23E+00	5.23E-01	5.23E-02	1.00E+01	2.74E-02	1.00E+02
L2	20.000	1.09E+00	5.00E-02	2.18E+01	1.09E+00	5.45E-02	2.00E+01	5.93E-02	4.00E+02
L3	50.000	2.76E+00	2.00E-02	1.38E+02	2.76E+00	5.51E-02	5.00E+01	1.52E-01	2.50E+03
L4	100.000	5.43E+00	1.00E-02	5.43E+02	5.43E+00	5.43E-02	1.00E+02	2.95E-01	1.00E+04
L5	200.000	1.03E+01	5.00E-03	2.05E+03	1.03E+01	5.14E-02	2.00E+02	5.28E-01	4.00E+04
L6	300.000	1.50E+01	3.33E-03	4.50E+03	1.50E+01	5.00E-02	3.00E+02	7.49E-01	9.00E+04
L7	400.000	1.95E+01	2.50E-03	7.80E+03	1.95E+01	4.87E-02	4.00E+02	9.50E-01	1.60E+05
7	1.08E+03	5.46E+01	1.91E-01	1.51E+04	5.46E+01	3.66E-01	1.08E+03	2.76E+00	3.03E+05

8.690E-02	intercept1	2.813E-01	intercept1
4.996E-02	slope1	4.870E-02	slope1
0.9984	R^2	0.9990	R^2

Standard Residual	y-yhat	w (1/x) Target	Calculated	yhat	Bias	Equal Target	Calculated	Response	Bias
-0.2134	-0.0633	10.000	8.732	5.86E-01	-12.68%	10.000	4.967	5.23E-01	-50.33%
0.0114	0.0034	20.000	20.068	1.09E+00	0.34%	20.000	16.596	1.09E+00	-17.02%
0.5734	0.1702	50.000	53.407	2.58E+00	6.81%	50.000	50.798	2.76E+00	1.60%
1.1744	0.3486	100.000	106.978	5.08E+00	6.98%	100.000	105.754	5.43E+00	5.75%
0.6576	0.1952	200.000	203.908	1.01E+01	1.95%	200.000	205.191	1.03E+01	2.60%
-0.2691	-0.0799	300.000	298.401	1.51E+01	-0.53%	300.000	302.129	1.50E+01	0.71%
-1.9343	-0.5742	400.000	388.506	2.01E+01	-2.87%	400.000	394.565	1.95E+01	-1.36%
					32.17%				79.37%

Drug: α-OH-Midazolam

Unit: ng/mL

Trial 2	x	y	w (1/x)	xy	wxy	wy	wx^2	wy^2	x^2
L1	10.000	5.48E-01		5.48E+00	5.48E-01	5.48E-02		3.00E-02	
L2	20.000	1.08E+00		2.15E+01	1.08E+00	5.38E-02		5.79E-02	
L3	50.000	2.73E+00		1.36E+02	2.73E+00	5.46E-02		1.49E-01	
L4	100.000	5.40E+00		5.40E+02	5.40E+00	5.40E-02		2.92E-01	
L5	200.000	1.05E+01		2.09E+03	1.05E+01	5.23E-02		5.47E-01	
L6	300.000	1.49E+01		4.47E+03	1.49E+01	4.96E-02		7.39E-01	
L7	400.000	1.92E+01		7.69E+03	1.92E+01	4.80E-02		9.23E-01	
		5.43E+01		1.49E+04	5.43E+01	3.67E-01		2.74E+00	

1.038E-01	intercept2	3.290E-01	intercept2
4.961E-02	slope2	4.815E-02	slope2
0.9980	R^2	0.9982	R^2

Standard Residual	y-yhat	Target	w (1/x) Calculated	yhat	Bias	Equal Target	Calculated	Response	Bias
-0.1369	-0.0523	10.000	8.946	6.00E-01	-10.54%	10.000	**4.541**	5.48E-01	**-54.59%**
-0.0513	-0.0196	20.000	19.606	1.10E+00	-1.97%	20.000	**15.523**	1.08E+00	**-22.39%**
0.3781	0.1444	50.000	52.910	2.58E+00	5.82%	50.000	**49.837**	2.73E+00	-0.33%
0.8749	0.3340	100.000	106.733	5.07E+00	6.73%	100.000	**105.291**	5.40E+00	5.29%
1.1314	0.4319	200.000	208.706	1.00E+01	4.35%	200.000	**210.355**	1.05E+01	5.18%
-0.2680	-0.1023	300.000	297.938	1.50E+01	-0.69%	300.000	**302.293**	1.49E+01	0.76%
-1.9283	-0.7362	400.000	385.162	1.99E+01	-3.71%	400.000	**392.161**	1.92E+01	-1.96%
					33.81%				90.50%

Drug: α-OH-Midazolam

Unit: ng/mL

Trial 3	x	y	w (1/x)	xy	wxy	wy	wx^2	wy^2	x^2
L1	10.000	4.70E-01		4.70E+00	4.70E-01	4.70E-02		2.21E-02	
L2	20.000	1.05E+00		2.09E+01	1.05E+00	5.23E-02		5.47E-02	
L3	50.000	2.79E+00		1.39E+02	2.79E+00	5.58E-02		1.56E-01	
L4	100.000	5.52E+00		5.52E+02	5.52E+00	5.52E-02		3.05E-01	
L5	200.000	1.08E+01		2.16E+03	1.08E+01	5.41E-02		5.85E-01	
L6	300.000	1.55E+01		4.66E+03	1.55E+01	5.17E-02		8.03E-01	
L7	400.000	1.96E+01		7.83E+03	1.96E+01	4.90E-02		9.59E-01	
		5.57E+01		1.54E+04	5.57E+01	3.65E-01		2.88E+00	

2.552E-02	intercept3	3.050E-01	intercept3
5.145E-02	slope3	4.964E-02	slope3
0.9971	R^2	0.9970	R^2

Standard Residual	y-yhat	w (1/x) Target	Calculated	yhat	Bias	Equal Target	Calculated	Response	Bias
-0.1422	-0.0702	10.000	8.636	5.40E-01	-13.64%	10.000	3.322	4.70E-01	-66.78%
-0.0178	-0.0088	20.000	19.830	1.05E+00	-0.85%	20.000	14.924	1.05E+00	-25.38%
0.3856	0.1903	50.000	53.698	2.60E+00	7.40%	50.000	50.027	2.79E+00	0.05%
0.7100	0.3503	100.000	106.807	5.17E+00	6.81%	100.000	105.075	5.52E+00	5.07%
1.0161	0.5013	200.000	209.742	1.03E+01	4.87%	200.000	211.766	1.08E+01	5.88%
0.1173	0.0579	300.000	301.124	1.55E+01	0.37%	300.000	306.482	1.55E+01	2.16%
-2.0689	-1.0207	400.000	380.163	2.06E+01	-4.96%	400.000	388.404	1.96E+01	-2.90%
					38.90%				108.23%

Drug: α-OH-Midazolam

Unit: ng/mL

Trial 4	x	y	w (1/x)	xy	wxy	wy	wx^2	wy^2	x^2
L1	10.000	5.70E-01		5.70E+00	5.70E-01	5.70E-02		3.25E-02	
L2	20.000	1.16E+00		2.31E+01	1.16E+00	5.78E-02		6.69E-02	
L3	50.000	2.80E+00		1.40E+02	2.80E+00	5.59E-02		1.56E-01	
L4	100.000	5.25E+00		5.25E+02	5.25E+00	5.25E-02		2.76E-01	
L5	200.000	1.07E+01		2.14E+03	1.07E+01	5.34E-02		5.70E-01	
L6	300.000	1.53E+01		4.60E+03	1.53E+01	5.11E-02		7.84E-01	
L7	400.000	1.99E+01		7.97E+03	1.99E+01	4.98E-02		9.92E-01	
		5.57E+01		1.54E+04	5.57E+01	3.78E-01		2.88E+00	

1.135E-01	intercept4	2.616E-01	intercept4
5.085E-02	slope4	4.989E-02	slope4
0.9990	R^2	0.9990	R^2

Standard Residual	y-yhat	w (1/x) Target	Calculated	yhat	Bias	Equal Target	Calculated	Response	Bias
-0.1880	-0.0523	10.000	8.972	6.22E-01	-10.28%	10.000	**6.174**	5.70E-01	**-38.26%**
0.0954	0.0265	20.000	20.522	1.13E+00	2.61%	20.000	**17.947**	1.16E+00	-10.26%
0.5072	0.1411	50.000	52.774	2.66E+00	5.55%	50.000	**50.820**	2.80E+00	1.64%
0.1972	0.0548	100.000	101.079	5.20E+00	1.08%	100.000	**100.054**	5.25E+00	0.05%
1.4186	0.3945	200.000	207.759	1.03E+01	3.88%	200.000	**208.789**	1.07E+01	4.39%
-0.1247	-0.0347	300.000	299.318	1.54E+01	-0.23%	300.000	**302.110**	1.53E+01	0.70%
-1.9057	-0.5300	400.000	389.576	2.05E+01	-2.61%	400.000	**394.105**	1.99E+01	-1.47%
					26.23%				56.79%

Drug: α-OH-Midazolam

Unit: ng/mL

Trial 5	x	y	w (1/x)	xy	wxy	wy	wx^2	wy^2	x^2
L1	10.000	4.12E-01		4.12E+00	4.12E-01	4.12E-02		1.70E-02	
L2	20.000	1.13E+00		2.25E+01	1.13E+00	5.63E-02		6.35E-02	
L3	50.000	2.51E+00		1.25E+02	2.51E+00	5.01E-02		1.26E-01	
L4	100.000	5.62E+00		5.62E+02	5.62E+00	5.62E-02		3.16E-01	
L5	200.000	1.11E+01		2.22E+03	1.11E+01	5.55E-02		6.16E-01	
L6	300.000	1.52E+01		4.55E+03	1.52E+01	5.06E-02		7.68E-01	
L7	400.000	1.93E+01		7.70E+03	1.93E+01	4.81E-02		9.26E-01	
		5.52E+01		1.52E+04	5.52E+01	3.58E-01		2.83E+00	

2.243E-03	intercept5	3.366E-01	intercept5
5.110E-02	slope5	4.893E-02	slope5
0.9946	R^2	0.9944	R^2

Standard Residual	y-yhat	w (1/x) Target	Calculated	yhat	Bias	Equal Target	Calculated	Response	Bias
-0.1575	-0.1015	10.000	8.014	5.13E-01	-19.86%	10.000	1.536	4.12E-01	-84.64%
0.1596	0.1028	20.000	22.013	1.02E+00	10.06%	20.000	16.154	1.13E+00	-19.23%
-0.0802	-0.0517	50.000	48.989	2.56E+00	-2.02%	50.000	44.325	2.51E+00	-11.35%
0.7958	0.5127	100.000	110.034	5.11E+00	10.03%	100.000	108.074	5.62E+00	8.07%
1.3585	0.8752	200.000	217.129	1.02E+01	8.56%	200.000	219.912	1.11E+01	9.96%
-0.2291	-0.1476	300.000	297.112	1.53E+01	-0.96%	300.000	303.438	1.52E+01	1.15%
-1.8472	-1.1901	400.000	376.709	2.04E+01	-5.82%	400.000	386.560	1.93E+01	-3.36%
					57.33%				137.75%

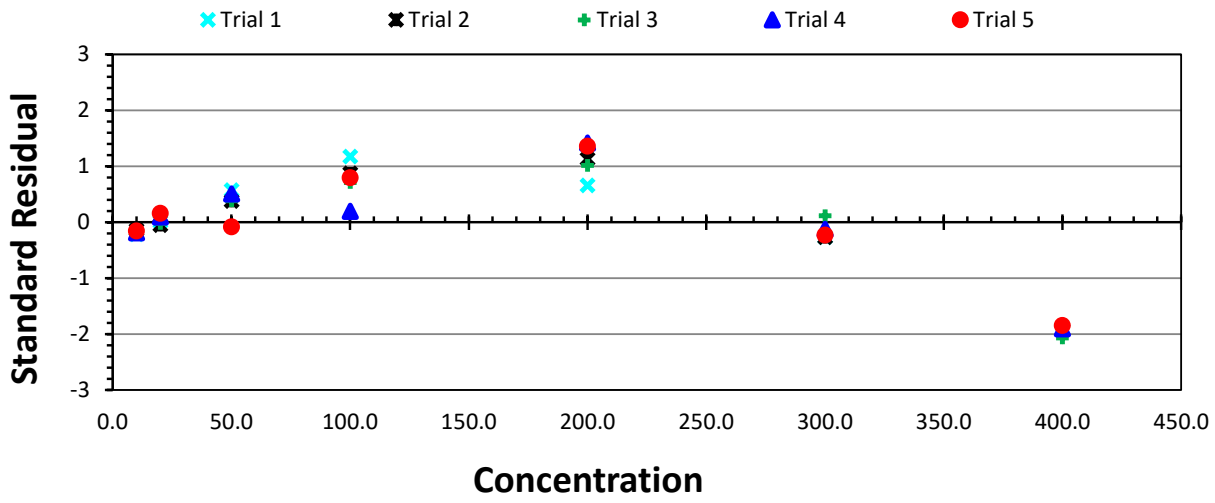
BZDG1 in Blood

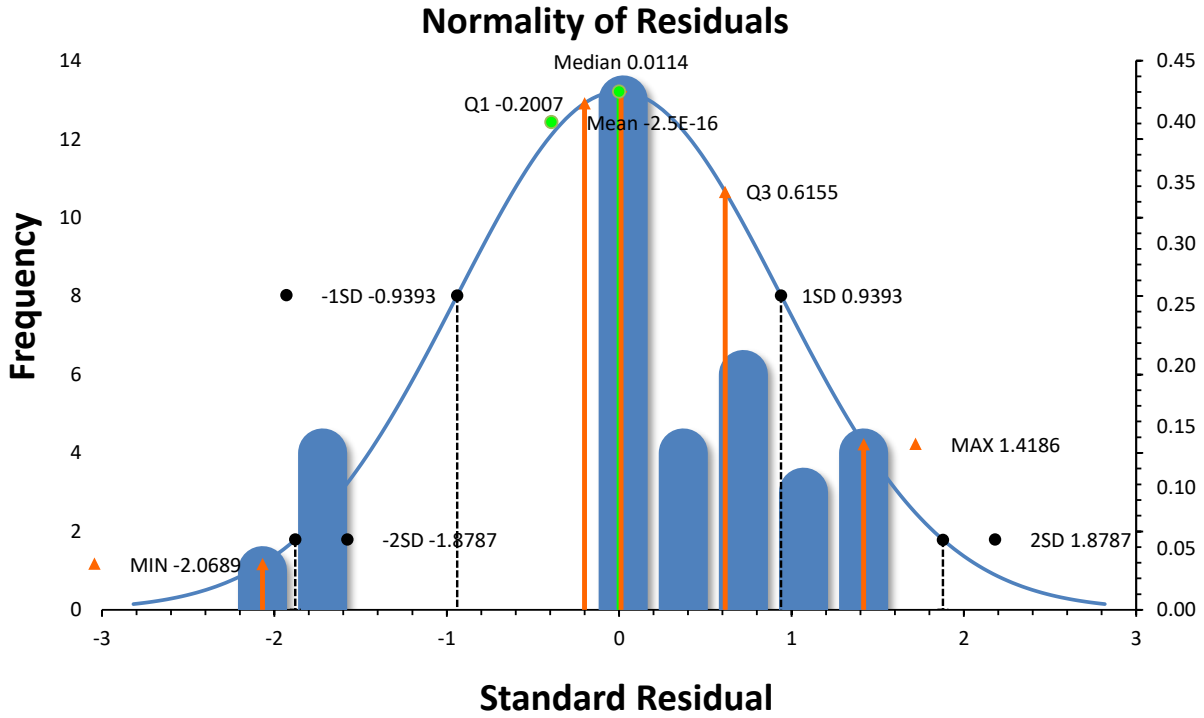
Drug: α-OH-Midazolam

Unit: ng/mL

Calibration Model: Linear (1/x)

Toxicologist(s): JD1 JD2 JD3 JD4 JD5 **Instrument:** LC-MS/MS

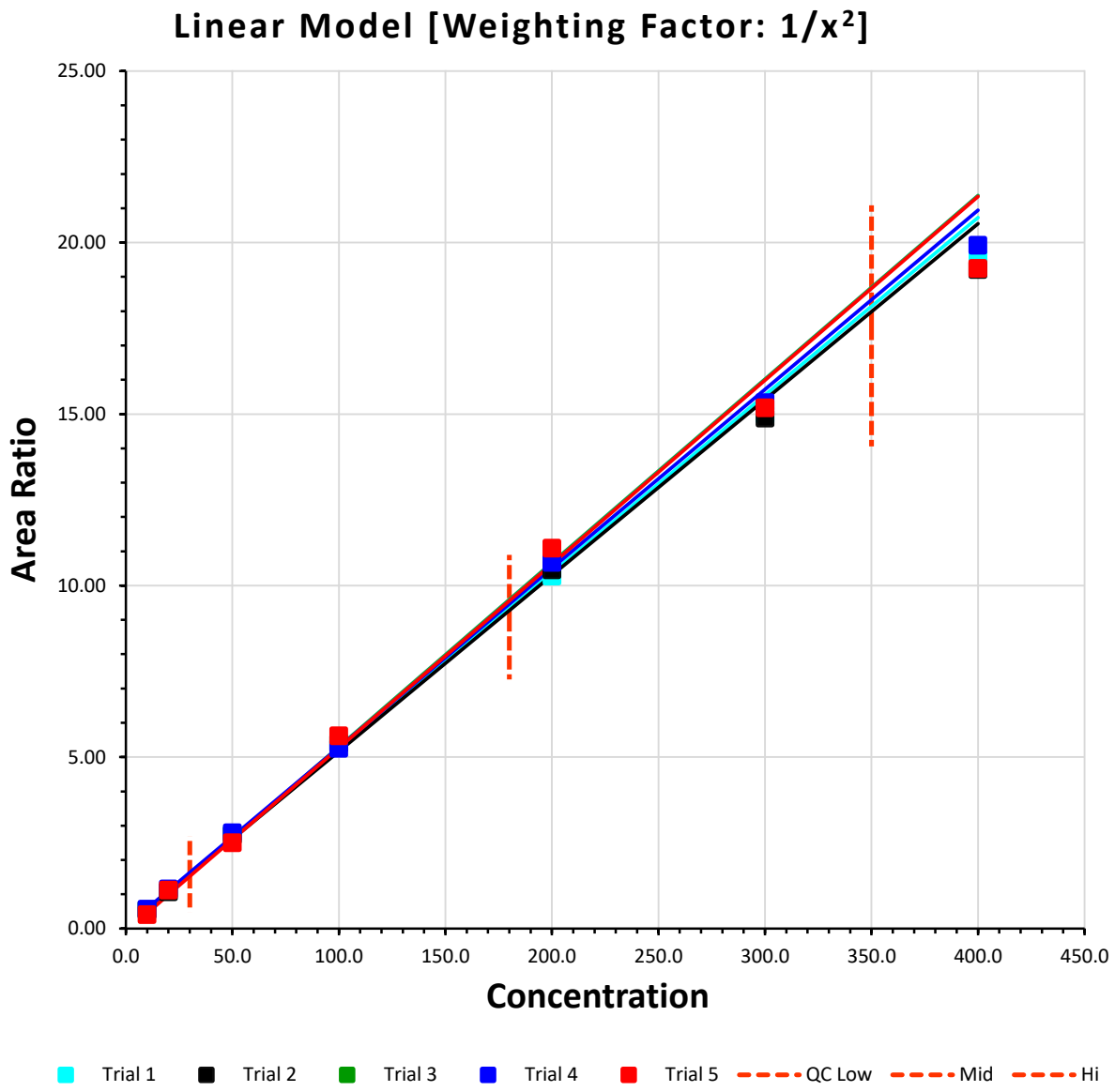




BZDG1 in Blood

Drug: α-OH-Midazolam

Unit: ng/mL



		slope			y-intercept	R^2
Trial 1	Y=	5.177E-02	X	+	2.038E-02	0.9971
Trial 2	Y=	5.127E-02	X	+	4.313E-02	0.9976
Trial 3	Y=	5.356E-02	X	+	-5.174E-02	0.9965
Trial 4	Y=	5.220E-02	X	+	6.393E-02	0.9979
Trial 5	Y=	5.358E-02	X	+	-8.883E-02	0.9902

Drug: α-OH-Midazolam

Unit: ng/mL

Trial 1	x	y	w (1/x^2)	xy	wxy	wy	wx^2	wy^2	x^2
L1	10.000	5.23E-01	1.00E-02	5.23E+00	5.23E-02	5.23E-03	1.00E+00	2.74E-03	1.00E+02
L2	20.000	1.09E+00	2.50E-03	2.18E+01	5.45E-02	2.72E-03	1.00E+00	2.97E-03	4.00E+02
L3	50.000	2.76E+00	4.00E-04	1.38E+02	5.51E-02	1.10E-03	1.00E+00	3.04E-03	2.50E+03
L4	100.000	5.43E+00	1.00E-04	5.43E+02	5.43E-02	5.43E-04	1.00E+00	2.95E-03	1.00E+04
L5	200.000	1.03E+01	2.50E-05	2.05E+03	5.14E-02	2.57E-04	1.00E+00	2.64E-03	4.00E+04
L6	300.000	1.50E+01	1.11E-05	4.50E+03	5.00E-02	1.67E-04	1.00E+00	2.50E-03	9.00E+04
L7	400.000	1.95E+01	6.25E-06	7.80E+03	4.87E-02	1.22E-04	1.00E+00	2.38E-03	1.60E+05
7	1.08E+03	5.46E+01	1.30E-02	1.51E+04	3.66E-01	1.01E-02	7.00E+00	1.92E-02	3.03E+05

2.038E-02	intercept1
5.177E-02	slope1
0.9971	R^2

2.813E-01	intercept1
4.870E-02	slope1
0.9990	R^2

Standard Residual	y-yhat	w (1/x^2) Target	Calculated	yhat	Bias	Equal Target	Calculated	Response	Bias
-0.0289	-0.0149	10.000	9.711	5.38E-01	-2.89%	10.000	4.967	5.23E-01	-50.33%
0.0651	0.0336	20.000	20.650	1.06E+00	3.25%	20.000	16.596	1.09E+00	-17.02%
0.2828	0.1461	50.000	52.821	2.61E+00	5.64%	50.000	50.798	2.76E+00	1.60%
0.4526	0.2338	100.000	104.515	5.20E+00	4.52%	100.000	105.754	5.43E+00	5.75%
-0.1955	-0.1010	200.000	198.049	1.04E+01	-0.98%	200.000	205.191	1.03E+01	2.60%
-1.0794	-0.5575	300.000	289.232	1.56E+01	-3.59%	300.000	302.129	1.50E+01	0.71%
-2.3876	-1.2332	400.000	376.181	2.07E+01	-5.95%	400.000	394.565	1.95E+01	-1.36%
					26.81%				79.37%

Drug: α-OH-Midazolam

Unit: ng/mL

Trial 2	x	y	w (1/x²)	xy	wxy	wy	wx²	wy²	x²
L1	10.000	5.48E-01		5.48E+00	5.48E-02	5.48E-03		3.00E-03	
L2	20.000	1.08E+00		2.15E+01	5.38E-02	2.69E-03		2.90E-03	
L3	50.000	2.73E+00		1.36E+02	5.46E-02	1.09E-03		2.98E-03	
L4	100.000	5.40E+00		5.40E+02	5.40E-02	5.40E-04		2.92E-03	
L5	200.000	1.05E+01		2.09E+03	5.23E-02	2.61E-04		2.73E-03	
L6	300.000	1.49E+01		4.47E+03	4.96E-02	1.65E-04		2.46E-03	
L7	400.000	1.92E+01		7.69E+03	4.80E-02	1.20E-04		2.31E-03	
		5.43E+01		1.49E+04	3.67E-01	1.03E-02		1.93E-02	

4.313E-02	intercept2	3.290E-01	intercept2
5.127E-02	slope2	4.815E-02	slope2
0.9976	R^2	0.9982	R^2

Standard Residual	y-yhat	w (1/x²) Target	Calculated	yhat	Bias	Equal Target	Calculated	Response	Bias
-0.0145	-0.0082	10.000	9.841	5.56E-01	-1.59%	10.000	4.541	5.48E-01	-54.59%
0.0142	0.0080	20.000	20.156	1.07E+00	0.78%	20.000	15.523	1.08E+00	-22.39%
0.2171	0.1223	50.000	52.386	2.61E+00	4.77%	50.000	49.837	2.73E+00	-0.33%
0.4070	0.2293	100.000	104.474	5.17E+00	4.47%	100.000	105.291	5.40E+00	5.29%
0.2873	0.1619	200.000	203.158	1.03E+01	1.58%	200.000	210.355	1.05E+01	5.18%
-0.9540	-0.5376	300.000	289.513	1.54E+01	-3.50%	300.000	302.293	1.49E+01	0.76%
-2.3722	-1.3369	400.000	373.924	2.06E+01	-6.52%	400.000	392.161	1.92E+01	-1.96%
					23.21%				90.50%

Drug: α-OH-Midazolam

Unit: ng/mL

Trial 3	x	y	w (1/x²)	xy	wxy	wy	wx²	wy²	x²
L1	10.000	4.70E-01		4.70E+00	4.70E-02	4.70E-03		2.21E-03	
L2	20.000	1.05E+00		2.09E+01	5.23E-02	2.61E-03		2.73E-03	
L3	50.000	2.79E+00		1.39E+02	5.58E-02	1.12E-03		3.11E-03	
L4	100.000	5.52E+00		5.52E+02	5.52E-02	5.52E-04		3.05E-03	
L5	200.000	1.08E+01		2.16E+03	5.41E-02	2.70E-04		2.93E-03	
L6	300.000	1.55E+01		4.66E+03	5.17E-02	1.72E-04		2.68E-03	
L7	400.000	1.96E+01		7.83E+03	4.90E-02	1.22E-04		2.40E-03	
		5.57E+01		1.54E+04	3.65E-01	9.55E-03		1.91E-02	

-5.174E-02	intercept3	3.050E-01	intercept3
5.356E-02	slope3	4.964E-02	slope3
0.9965	R^2	0.9970	R^2

Standard Residual	y-yhat	w (1/x²) Target	Calculated	yhat	Bias	Equal Target	Calculated	Response	Bias
-0.0194	-0.0140	10.000	9.739	4.84E-01	-2.61%	10.000	3.322	4.70E-01	-66.78%
0.0366	0.0264	20.000	20.492	1.02E+00	2.46%	20.000	14.924	1.05E+00	-25.38%
0.2254	0.1622	50.000	53.028	2.63E+00	6.06%	50.000	50.027	2.79E+00	0.05%
0.3015	0.2169	100.000	104.050	5.30E+00	4.05%	100.000	105.075	5.52E+00	5.07%
0.2186	0.1573	200.000	202.937	1.07E+01	1.47%	200.000	211.766	1.08E+01	5.88%
-0.6904	-0.4967	300.000	290.726	1.60E+01	-3.09%	300.000	306.482	1.55E+01	2.16%
-2.4823	-1.7859	400.000	366.656	2.14E+01	-8.34%	400.000	388.404	1.96E+01	-2.90%
					28.07%				108.23%

Drug: α-OH-Midazolam

Unit: ng/mL

Trial 4	x	y	w (1/x²)	xy	wxy	wy	wx²	wy²	x²
L1	10.000	5.70E-01		5.70E+00	5.70E-02	5.70E-03		3.25E-03	
L2	20.000	1.16E+00		2.31E+01	5.78E-02	2.89E-03		3.35E-03	
L3	50.000	2.80E+00		1.40E+02	5.59E-02	1.12E-03		3.13E-03	
L4	100.000	5.25E+00		5.25E+02	5.25E-02	5.25E-04		2.76E-03	
L5	200.000	1.07E+01		2.14E+03	5.34E-02	2.67E-04		2.85E-03	
L6	300.000	1.53E+01		4.60E+03	5.11E-02	1.70E-04		2.61E-03	
L7	400.000	1.99E+01		7.97E+03	4.98E-02	1.25E-04		2.48E-03	
		5.57E+01		1.54E+04	3.78E-01	1.08E-02		2.04E-02	

6.393E-02	intercept4	2.616E-01	intercept4
5.220E-02	slope4	4.989E-02	slope4
0.9979	R^2	0.9990	R^2

Standard Residual	y-yhat	w (1/x²) Target	Calculated	yhat	Bias	Equal Target	Calculated	Response	Bias
-0.0385	-0.0162	10.000	9.689	5.86E-01	-3.11%	10.000	6.174	5.70E-01	-38.26%
0.1164	0.0491	20.000	20.940	1.11E+00	4.70%	20.000	17.947	1.16E+00	-10.26%
0.2919	0.1231	50.000	52.358	2.67E+00	4.72%	50.000	50.820	2.80E+00	1.64%
-0.0728	-0.0307	100.000	99.412	5.28E+00	-0.59%	100.000	100.054	5.25E+00	0.05%
0.4125	0.1739	200.000	203.332	1.05E+01	1.67%	200.000	208.789	1.07E+01	4.39%
-0.9258	-0.3904	300.000	292.522	1.57E+01	-2.49%	300.000	302.110	1.53E+01	0.70%
-2.4209	-1.0208	400.000	380.444	2.09E+01	-4.89%	400.000	394.105	1.99E+01	-1.47%
					22.16%				56.79%

Drug: α-OH-Midazolam

Unit: ng/mL

Trial 5	x	y	w (1/x²)	xy	wxy	wy	wx²	wy²	x²
L1	10.000	4.12E-01		4.12E+00	4.12E-02	4.12E-03		1.70E-03	
L2	20.000	1.13E+00		2.25E+01	5.63E-02	2.82E-03		3.18E-03	
L3	50.000	2.51E+00		1.25E+02	5.01E-02	1.00E-03		2.51E-03	
L4	100.000	5.62E+00		5.62E+02	5.62E-02	5.62E-04		3.16E-03	
L5	200.000	1.11E+01		2.22E+03	5.55E-02	2.77E-04		3.08E-03	
L6	300.000	1.52E+01		4.55E+03	5.06E-02	1.69E-04		2.56E-03	
L7	400.000	1.93E+01		7.70E+03	4.81E-02	1.20E-04		2.32E-03	
		5.52E+01		1.52E+04	3.58E-01	9.07E-03		1.85E-02	

-8.883E-02	intercept5	3.366E-01	intercept5
5.358E-02	slope5	4.893E-02	slope5
0.9902	R^2	0.9944	R^2

Standard Residual	y-yhat	w (1/x²) Target	Calculated	yhat	Bias	Equal Target	Calculated	Response	Bias
-0.0394	-0.0352	10.000	9.343	4.47E-01	-6.57%	10.000	1.536	4.12E-01	-84.64%
0.1613	0.1443	20.000	22.693	9.83E-01	13.46%	20.000	16.154	1.13E+00	-19.23%
-0.0947	-0.0847	50.000	48.419	2.59E+00	-3.16%	50.000	44.325	2.51E+00	-11.35%
0.3975	0.3555	100.000	106.635	5.27E+00	6.64%	100.000	108.074	5.62E+00	8.07%
0.5252	0.4697	200.000	208.767	1.06E+01	4.38%	200.000	219.912	1.11E+01	9.96%
-0.8961	-0.8013	300.000	285.044	1.60E+01	-4.99%	300.000	303.438	1.52E+01	1.15%
-2.3394	-2.0921	400.000	360.953	2.13E+01	-9.76%	400.000	386.560	1.93E+01	-3.36%
					48.96%				137.75%

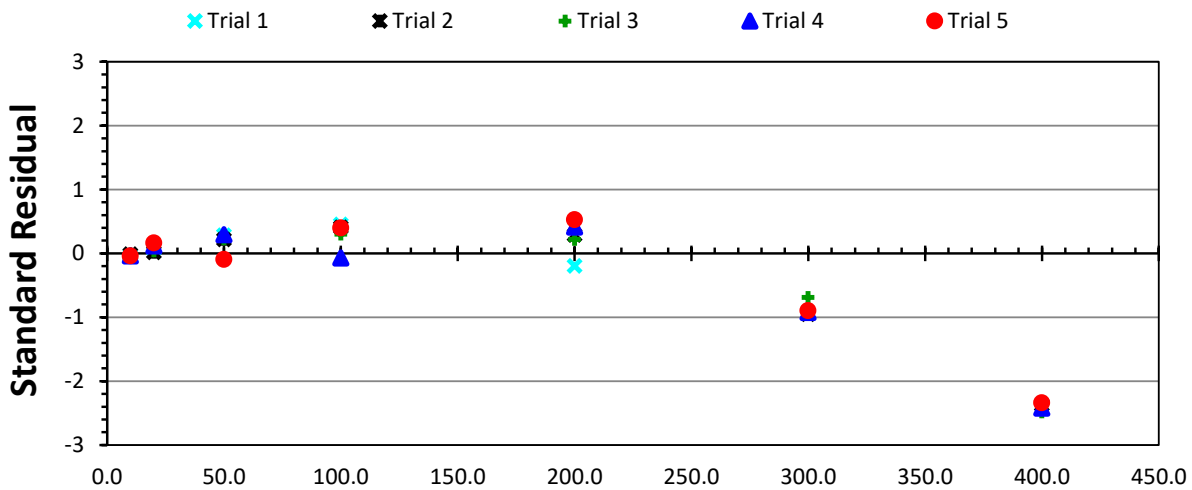
BZDG1 in Blood

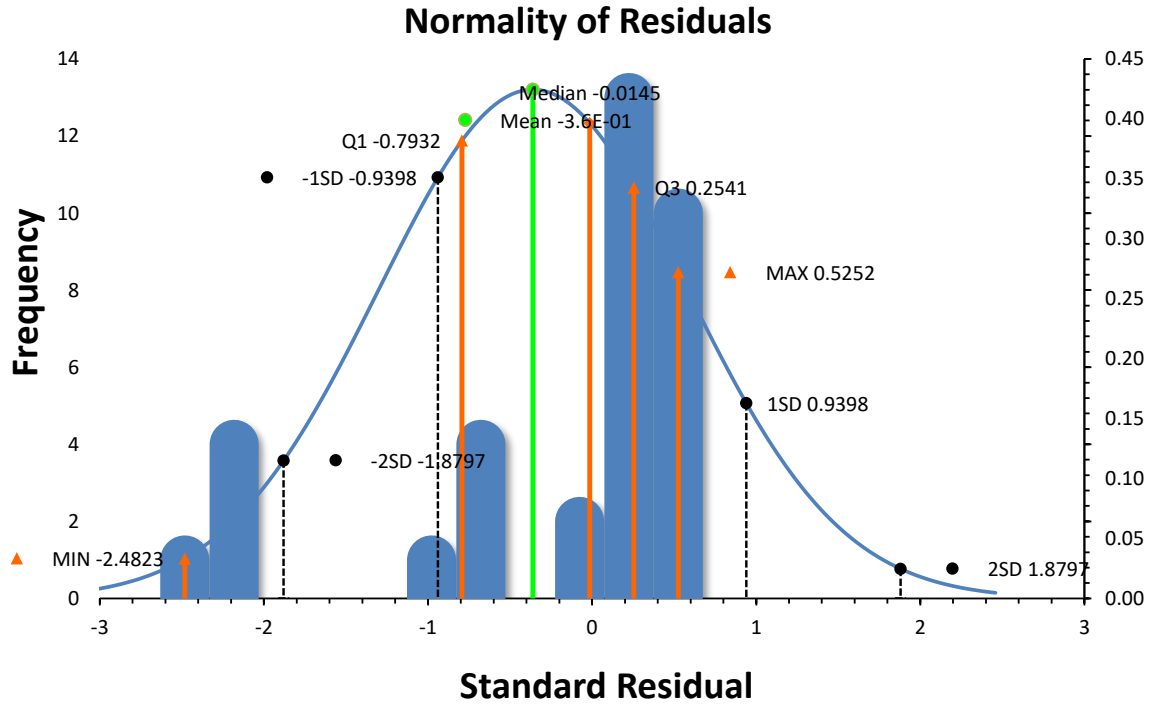
Drug: α-OH-Midazolam

Unit: ng/mL

Calibration Model: Linear ($1/x^2$)

Toxicologist(s): JD1 JD2 JD3 JD4 JD5 **Instrument:** LC-MS/MS

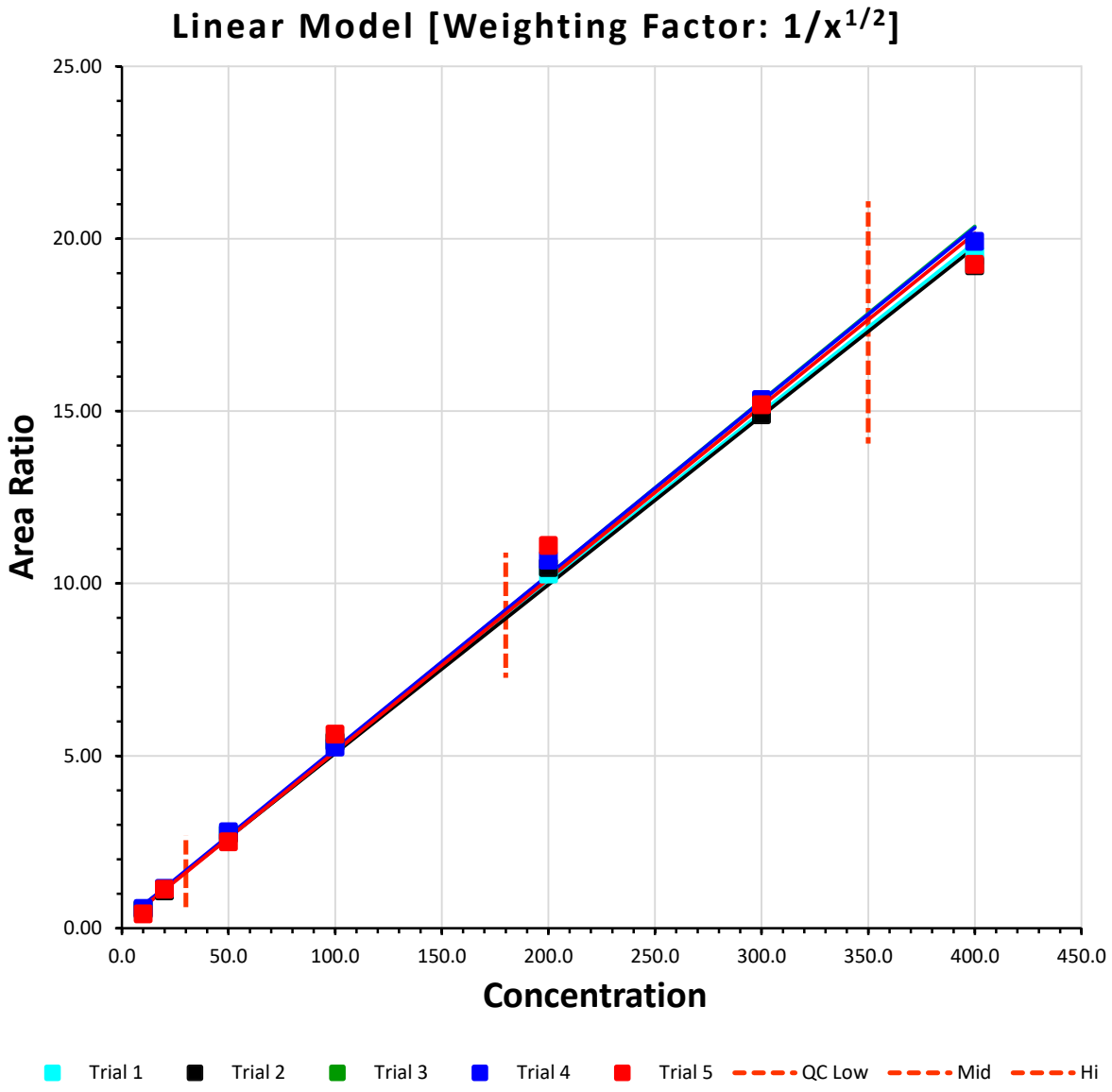




BZDG1 in Blood

Drug: **α-OH-Midazolam**

Unit: **ng/mL**



		slope			y-intercept	R^2
Trial 1	Y=	4.933E-02	X	+	1.578E-01	0.9988
Trial 2	Y=	4.892E-02	X	+	1.803E-01	0.9982
Trial 3	Y=	5.060E-02	X	+	1.198E-01	0.9973
Trial 4	Y=	5.039E-02	X	+	1.647E-01	0.9991
Trial 5	Y=	5.010E-02	X	+	1.109E-01	0.9949

Drug: α-OH-Midazolam

Unit: ng/mL

Trial 1	x	y	w ($1/x^{1/2}$)	xy	wxy	wy	wx^2	wy^2	x^2
L1	10.000	5.23E-01	3.16E-01	5.23E+00	1.65E+00	1.65E-01	3.16E+01	8.65E-02	1.00E+02
L2	20.000	1.09E+00	2.24E-01	2.18E+01	4.87E+00	2.44E-01	8.94E+01	2.65E-01	4.00E+02
L3	50.000	2.76E+00	1.41E-01	1.38E+02	1.95E+01	3.90E-01	3.54E+02	1.07E+00	2.50E+03
L4	100.000	5.43E+00	1.00E-01	5.43E+02	5.43E+01	5.43E-01	1.00E+03	2.95E+00	1.00E+04
L5	200.000	1.03E+01	7.07E-02	2.05E+03	1.45E+02	7.26E-01	2.83E+03	7.46E+00	4.00E+04
L6	300.000	1.50E+01	5.77E-02	4.50E+03	2.60E+02	8.66E-01	5.20E+03	1.30E+01	9.00E+04
L7	400.000	1.95E+01	5.00E-02	7.80E+03	3.90E+02	9.75E-01	8.00E+03	1.90E+01	1.60E+05
7	1.08E+03	5.46E+01	9.60E-01	1.51E+04	8.75E+02	3.91E+00	1.75E+04	4.38E+01	3.03E+05

1.578E-01	intercept1	2.813E-01	intercept1
4.933E-02	slope1	4.870E-02	slope1
0.9988	R^2	0.9990	R^2

Standard Residual	y-yhat	w ($1/x^{1/2}$) Target	Calculated	yhat	Bias	Equal Target	Calculated	Response	Bias
-0.5176	-0.1279	10.000	7.406	6.51E-01	-25.94%	10.000	4.967	5.23E-01	-50.33%
-0.2222	-0.0549	20.000	18.887	1.14E+00	-5.57%	20.000	16.596	1.09E+00	-17.02%
0.5290	0.1308	50.000	52.651	2.62E+00	5.30%	50.000	50.798	2.76E+00	1.60%
1.3779	0.3406	100.000	106.905	5.09E+00	6.90%	100.000	105.754	5.43E+00	5.75%
1.0117	0.2501	200.000	205.070	1.00E+01	2.53%	200.000	205.191	1.03E+01	2.60%
0.1530	0.0378	300.000	300.767	1.50E+01	0.26%	300.000	302.129	1.50E+01	0.71%
-1.5923	-0.3936	400.000	392.021	1.99E+01	-1.99%	400.000	394.565	1.95E+01	-1.36%
					48.49%				79.37%

Drug: α-OH-Midazolam

Unit: ng/mL

Trial 2	x	y	w ($1/x^{1/2}$)	xy	wxy	wy	wx^2	wy^2	x^2
L1	10.000	5.48E-01		5.48E+00	1.73E+00	1.73E-01		9.48E-02	
L2	20.000	1.08E+00		2.15E+01	4.81E+00	2.41E-01		2.59E-01	
L3	50.000	2.73E+00		1.36E+02	1.93E+01	3.86E-01		1.05E+00	
L4	100.000	5.40E+00		5.40E+02	5.40E+01	5.40E-01		2.92E+00	
L5	200.000	1.05E+01		2.09E+03	1.48E+02	7.40E-01		7.73E+00	
L6	300.000	1.49E+01		4.47E+03	2.58E+02	8.59E-01		1.28E+01	
L7	400.000	1.92E+01		7.69E+03	3.84E+02	9.61E-01		1.85E+01	
		5.43E+01		1.49E+04	8.70E+02	3.90E+00		4.33E+01	

1.803E-01	intercept2	3.290E-01	intercept2
4.892E-02	slope2	4.815E-02	slope2
0.9982	R^2	0.9982	R^2

Standard Residual	y-yhat	w ($1/x^{1/2}$) Target	Calculated	yhat	Bias	Equal Target	Calculated	Response	Bias
-0.3663	-0.1219	10.000	7.509	6.70E-01	-24.91%	10.000	4.541	5.48E-01	-54.59%
-0.2472	-0.0823	20.000	18.319	1.16E+00	-8.41%	20.000	15.523	1.08E+00	-22.39%
0.3078	0.1024	50.000	52.094	2.63E+00	4.19%	50.000	49.837	2.73E+00	-0.33%
0.9818	0.3267	100.000	106.678	5.07E+00	6.68%	100.000	105.291	5.40E+00	5.29%
1.4839	0.4938	200.000	210.093	9.96E+00	5.05%	200.000	210.355	1.05E+01	5.18%
0.0864	0.0287	300.000	300.587	1.49E+01	0.20%	300.000	302.293	1.49E+01	0.76%
-1.6107	-0.5360	400.000	389.045	1.97E+01	-2.74%	400.000	392.161	1.92E+01	-1.96%
					52.17%				90.50%

Drug: α-OH-Midazolam

Unit: ng/mL

Trial 3	x	y	w ($1/x^{1/2}$)	xy	wxy	wy	wx^2	wy^2	x^2
L1	10.000	4.70E-01		4.70E+00	1.49E+00	1.49E-01		6.98E-02	
L2	20.000	1.05E+00		2.09E+01	4.68E+00	2.34E-01		2.45E-01	
L3	50.000	2.79E+00		1.39E+02	1.97E+01	3.94E-01		1.10E+00	
L4	100.000	5.52E+00		5.52E+02	5.52E+01	5.52E-01		3.05E+00	
L5	200.000	1.08E+01		2.16E+03	1.53E+02	7.65E-01		8.27E+00	
L6	300.000	1.55E+01		4.66E+03	2.69E+02	8.96E-01		1.39E+01	
L7	400.000	1.96E+01		7.83E+03	3.92E+02	9.79E-01		1.92E+01	
		5.57E+01		1.54E+04	8.95E+02	3.97E+00		4.58E+01	

1.198E-01	intercept3	3.050E-01	intercept3
5.060E-02	slope3	4.964E-02	slope3
0.9973	R^2	0.9970	R^2

Standard Residual	y-yhat	Target	w ($1/x^{1/2}$) Calculated	yhat	Bias	Target	Equal Calculated	Response	Bias
-0.3579	-0.1559	10.000	6.919	6.26E-01	-30.81%	10.000	3.322	4.70E-01	-66.78%
-0.1974	-0.0860	20.000	18.301	1.13E+00	-8.50%	20.000	14.924	1.05E+00	-25.38%
0.3182	0.1386	50.000	52.739	2.65E+00	5.48%	50.000	50.027	2.79E+00	0.05%
0.7834	0.3412	100.000	106.744	5.18E+00	6.74%	100.000	105.075	5.52E+00	5.07%
1.3258	0.5775	200.000	211.413	1.02E+01	5.71%	200.000	211.766	1.08E+01	5.88%
0.5035	0.2193	300.000	304.334	1.53E+01	1.44%	300.000	306.482	1.55E+01	2.16%
-1.7770	-0.7740	400.000	384.703	2.04E+01	-3.82%	400.000	388.404	1.96E+01	-2.90%
					62.50%				108.23%

Drug: α-OH-Midazolam

Unit: ng/mL

Trial 4	x	y	w (1/x^1/2)	xy	wxy	wy	wx^2	wy^2	x^2
L1	10.000	5.70E-01		5.70E+00	1.80E+00	1.80E-01		1.03E-01	
L2	20.000	1.16E+00		2.31E+01	5.17E+00	2.59E-01		2.99E-01	
L3	50.000	2.80E+00		1.40E+02	1.98E+01	3.96E-01		1.11E+00	
L4	100.000	5.25E+00		5.25E+02	5.25E+01	5.25E-01		2.76E+00	
L5	200.000	1.07E+01		2.14E+03	1.51E+02	7.55E-01		8.06E+00	
L6	300.000	1.53E+01		4.60E+03	2.66E+02	8.85E-01		1.36E+01	
L7	400.000	1.99E+01		7.97E+03	3.98E+02	9.96E-01		1.98E+01	
		5.57E+01		1.54E+04	8.94E+02	4.00E+00		4.57E+01	

1.647E-01	intercept4	2.616E-01	intercept4
5.039E-02	slope4	4.989E-02	slope4
0.9991	R^2	0.9990	R^2

Standard Residual	y-yhat	w (1/x^1/2) Target	Calculated	yhat	Bias	Equal Target	Calculated	Response	Bias
-0.3969	-0.0989	10.000	8.037	6.69E-01	-19.63%	10.000	6.174	5.70E-01	-38.26%
-0.0622	-0.0155	20.000	19.692	1.17E+00	-1.54%	20.000	17.947	1.16E+00	-10.26%
0.4525	0.1128	50.000	52.238	2.68E+00	4.48%	50.000	50.820	2.80E+00	1.64%
0.1985	0.0495	100.000	100.982	5.20E+00	0.98%	100.000	100.054	5.25E+00	0.05%
1.7454	0.4350	200.000	208.633	1.02E+01	4.32%	200.000	208.789	1.07E+01	4.39%
0.2073	0.0517	300.000	301.025	1.53E+01	0.34%	300.000	302.110	1.53E+01	0.70%
-1.5962	-0.3978	400.000	392.105	2.03E+01	-1.97%	400.000	394.105	1.99E+01	-1.47%
					33.26%				56.79%

Drug: α-OH-Midazolam

Unit: ng/mL

Trial 5	x	y	w ($1/x^{1/2}$)	xy	wxy	wy	wx^2	wy^2	x^2
L1	10.000	4.12E-01		4.12E+00	1.30E+00	1.30E-01		5.36E-02	
L2	20.000	1.13E+00		2.25E+01	5.04E+00	2.52E-01		2.84E-01	
L3	50.000	2.51E+00		1.25E+02	1.77E+01	3.54E-01		8.88E-01	
L4	100.000	5.62E+00		5.62E+02	5.62E+01	5.62E-01		3.16E+00	
L5	200.000	1.11E+01		2.22E+03	1.57E+02	7.85E-01		8.71E+00	
L6	300.000	1.52E+01		4.55E+03	2.63E+02	8.77E-01		1.33E+01	
L7	400.000	1.93E+01		7.70E+03	3.85E+02	9.63E-01		1.85E+01	
		5.52E+01		1.52E+04	8.85E+02	3.92E+00		4.49E+01	

1.109E-01	intercept5	3.366E-01	intercept5
5.010E-02	slope5	4.893E-02	slope5
0.9949	R^2	0.9944	R^2

Standard Residual	y-yhat	w ($1/x^{1/2}$) Target	Calculated	yhat	Bias	Equal Target	Calculated	Response	Bias
-0.3435	-0.2002	10.000	6.004	6.12E-01	-39.96%	10.000	1.536	4.12E-01	-84.64%
0.0240	0.0140	20.000	20.280	1.11E+00	1.40%	20.000	16.154	1.13E+00	-19.23%
-0.1900	-0.1107	50.000	47.790	2.62E+00	-4.42%	50.000	44.325	2.51E+00	-11.35%
0.8634	0.5032	100.000	110.044	5.12E+00	10.04%	100.000	108.074	5.62E+00	8.07%
1.6555	0.9649	200.000	219.258	1.01E+01	9.63%	200.000	219.912	1.11E+01	9.96%
0.0709	0.0413	300.000	300.824	1.51E+01	0.27%	300.000	303.438	1.52E+01	1.15%
-1.5475	-0.9020	400.000	381.997	2.02E+01	-4.50%	400.000	386.560	1.93E+01	-3.36%
					70.22%				137.75%

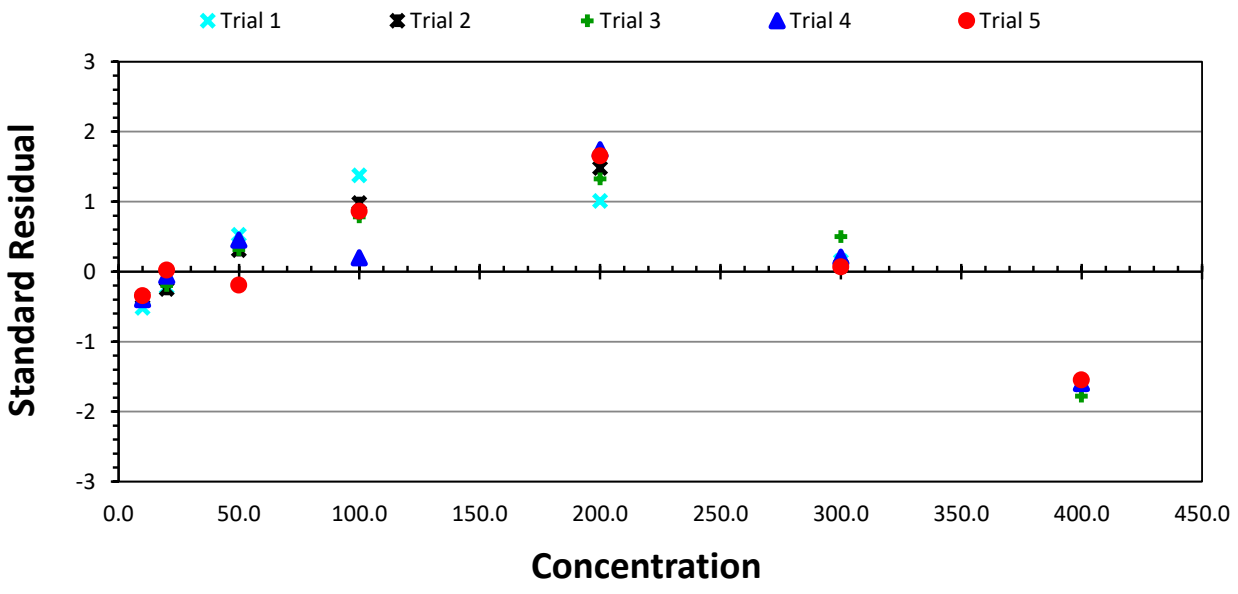
BZDG1 in Blood

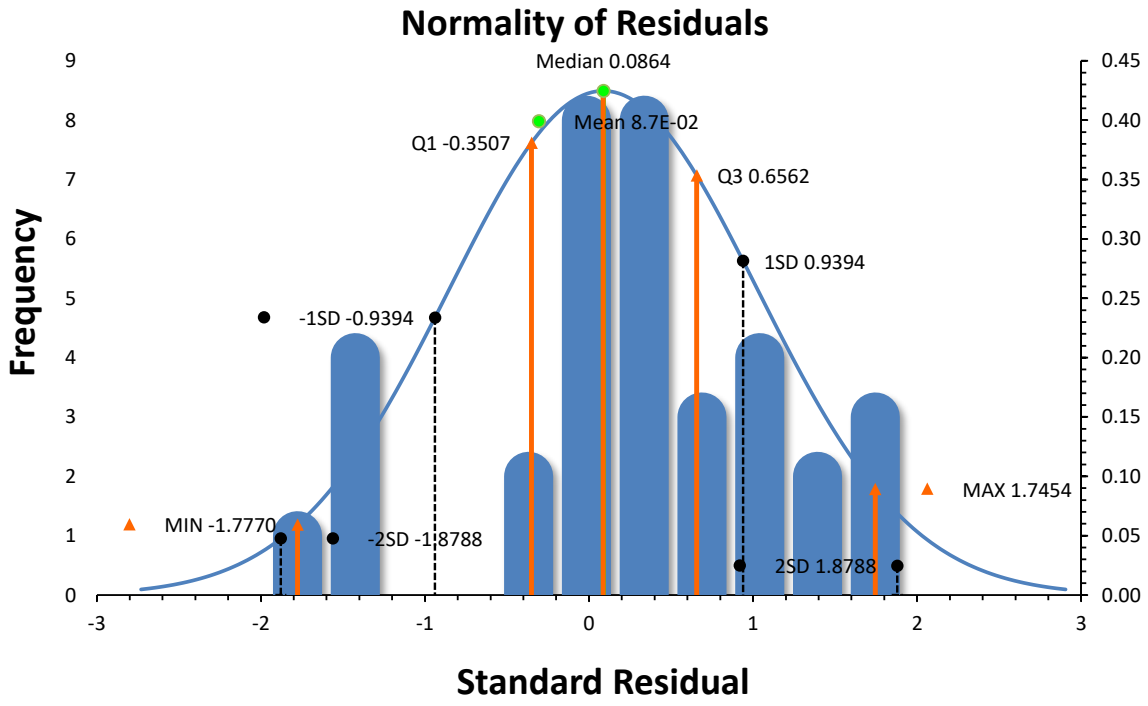
Drug: α-OH-Midazolam

Unit: ng/mL

Calibration Model: Linear ($1/x^{1/2}$)

Toxicologist(s): JD1 JD2 JD3 JD4 JD5 **Instrument:** LC-MS/MS

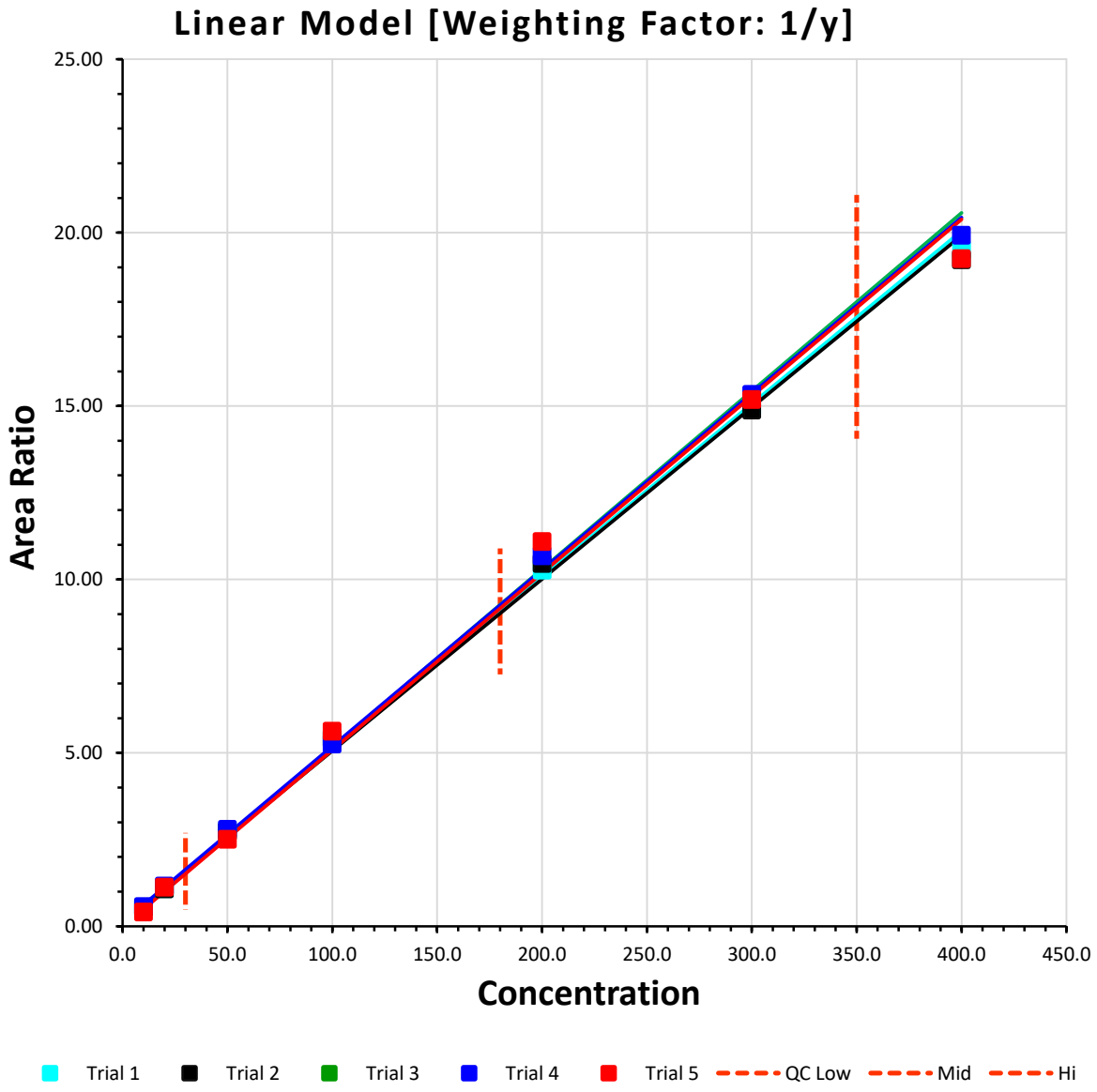




BZDG1 in Blood

Drug: **α-OH-Midazolam**

Unit: **ng/mL**



		slope			y-intercept	R^2
Trial 1	Y=	4.990E-02	X	+	8.685E-02	0.9985
Trial 2	Y=	4.952E-02	X	+	1.065E-01	0.9980
Trial 3	Y=	5.137E-02	X	+	2.117E-02	0.9972
Trial 4	Y=	5.080E-02	X	+	1.155E-01	0.9990
Trial 5	Y=	5.099E-02	X	+	-1.289E-02	0.9948

Drug: α-OH-Midazolam

Unit: ng/mL

Trial 1	x	y	w (1/y)	xy	wxy	wy	wx^2	wy^2	x^2
L1	10.000	5.23E-01	1.91E+00	5.23E+00	1.00E+01	1.00E+00	1.91E+02	5.23E-01	1.00E+02
L2	20.000	1.09E+00	9.18E-01	2.18E+01	2.00E+01	1.00E+00	3.67E+02	1.09E+00	4.00E+02
L3	50.000	2.76E+00	3.63E-01	1.38E+02	5.00E+01	1.00E+00	9.07E+02	2.76E+00	2.50E+03
L4	100.000	5.43E+00	1.84E-01	5.43E+02	1.00E+02	1.00E+00	1.84E+03	5.43E+00	1.00E+04
L5	200.000	1.03E+01	9.73E-02	2.05E+03	2.00E+02	1.00E+00	3.89E+03	1.03E+01	4.00E+04
L6	300.000	1.50E+01	6.67E-02	4.50E+03	3.00E+02	1.00E+00	6.00E+03	1.50E+01	9.00E+04
L7	400.000	1.95E+01	5.13E-02	7.80E+03	4.00E+02	1.00E+00	8.21E+03	1.95E+01	1.60E+05
7	1.08E+03	5.46E+01	3.59E+00	1.51E+04	1.08E+03	7.00E+00	2.14E+04	5.46E+01	3.03E+05

8.685E-02	intercept1	2.813E-01	intercept1
4.990E-02	slope1	4.870E-02	slope1
0.9985	R^2	0.9990	R^2

Standard Residual	y-yhat	Target	w (1/y) Calculated	yhat	Bias	Equal Target	Calculated	Response	Bias
-0.2152	-0.0627	10.000	8.743	5.86E-01	-12.57%	10.000	4.967	5.23E-01	-50.33%
0.0157	0.0046	20.000	20.092	1.08E+00	0.46%	20.000	16.596	1.09E+00	-17.02%
0.5939	0.1731	50.000	53.469	2.58E+00	6.94%	50.000	50.798	2.76E+00	1.60%
1.2157	0.3543	100.000	107.101	5.08E+00	7.10%	100.000	105.754	5.43E+00	5.75%
0.7089	0.2066	200.000	204.140	1.01E+01	2.07%	200.000	205.191	1.03E+01	2.60%
-0.2156	-0.0628	300.000	298.741	1.51E+01	-0.42%	300.000	302.129	1.50E+01	0.71%
-1.8921	-0.5515	400.000	388.948	2.00E+01	-2.76%	400.000	394.565	1.95E+01	-1.36%
					32.32%				79.37%

Drug: α-OH-Midazolam

Unit: ng/mL

Trial 2	x	y	w (1/y)	xy	wxy	wy	wx^2	wy^2	x^2
L1	10.000	5.48E-01	1.83E+00	5.48E+00	1.00E+01	1.00E+00	1.83E+02	5.48E-01	1.00E+02
L2	20.000	1.08E+00	9.29E-01	2.15E+01	2.00E+01	1.00E+00	3.72E+02	1.08E+00	4.00E+02
L3	50.000	2.73E+00	3.66E-01	1.36E+02	5.00E+01	1.00E+00	9.16E+02	2.73E+00	2.50E+03
L4	100.000	5.40E+00	1.85E-01	5.40E+02	1.00E+02	1.00E+00	1.85E+03	5.40E+00	1.00E+04
L5	200.000	1.05E+01	9.56E-02	2.09E+03	2.00E+02	1.00E+00	3.82E+03	1.05E+01	4.00E+04
L6	300.000	1.49E+01	6.72E-02	4.47E+03	3.00E+02	1.00E+00	6.05E+03	1.49E+01	9.00E+04
L7	400.000	1.92E+01	5.20E-02	7.69E+03	4.00E+02	1.00E+00	8.33E+03	1.92E+01	1.60E+05
		5.43E+01	3.52E+00	1.49E+04	1.08E+03	7.00E+00	2.15E+04	5.43E+01	3.03E+05

1.065E-01	intercept2	3.290E-01	intercept2
4.952E-02	slope2	4.815E-02	slope2
0.9980	R^2	0.9982	R^2

Standard Residual	y-yhat	Target	w (1/y) Calculated	yhat	Bias	Equal Target	Calculated	Response	Bias
-0.1446	-0.0541	10.000	8.908	6.02E-01	-10.92%	10.000	4.541	5.48E-01	-54.59%
-0.0547	-0.0204	20.000	19.587	1.10E+00	-2.06%	20.000	15.523	1.08E+00	-22.39%
0.3911	0.1462	50.000	52.953	2.58E+00	5.91%	50.000	49.837	2.73E+00	-0.33%
0.9105	0.3405	100.000	106.876	5.06E+00	6.88%	100.000	105.291	5.40E+00	5.29%
1.1969	0.4476	200.000	209.038	1.00E+01	4.52%	200.000	210.355	1.05E+01	5.18%
-0.2071	-0.0774	300.000	298.436	1.50E+01	-0.52%	300.000	302.293	1.49E+01	0.76%
-1.8776	-0.7021	400.000	385.822	1.99E+01	-3.54%	400.000	392.161	1.92E+01	-1.96%
					34.35%				90.50%

Drug: α-OH-Midazolam

Unit: ng/mL

Trial 3	x	y	w (1/y)	xy	wxy	wy	wx^2	wy^2	x^2
L1	10.000	4.70E-01	2.13E+00	4.70E+00	1.00E+01	1.00E+00	2.13E+02	4.70E-01	1.00E+02
L2	20.000	1.05E+00	9.56E-01	2.09E+01	2.00E+01	1.00E+00	3.82E+02	1.05E+00	4.00E+02
L3	50.000	2.79E+00	3.59E-01	1.39E+02	5.00E+01	1.00E+00	8.97E+02	2.79E+00	2.50E+03
L4	100.000	5.52E+00	1.81E-01	5.52E+02	1.00E+02	1.00E+00	1.81E+03	5.52E+00	1.00E+04
L5	200.000	1.08E+01	9.24E-02	2.16E+03	2.00E+02	1.00E+00	3.70E+03	1.08E+01	4.00E+04
L6	300.000	1.55E+01	6.44E-02	4.66E+03	3.00E+02	1.00E+00	5.80E+03	1.55E+01	9.00E+04
L7	400.000	1.96E+01	5.11E-02	7.83E+03	4.00E+02	1.00E+00	8.17E+03	1.96E+01	1.60E+05
		5.57E+01	3.83E+00	1.54E+04	1.08E+03	7.00E+00	2.10E+04	5.57E+01	3.03E+05

2.117E-02	intercept3	3.050E-01	intercept3
5.137E-02	slope3	4.964E-02	slope3
0.9972	R^2	0.9970	R^2

Standard Residual	y-yhat	Target	w (1/y) Calculated	yhat	Bias	Equal Target	Calculated	Response	Bias
-0.1336	-0.0650	10.000	8.735	5.35E-01	-12.65%	10.000	3.322	4.70E-01	-66.78%
-0.0056	-0.0027	20.000	19.947	1.05E+00	-0.26%	20.000	14.924	1.05E+00	-25.38%
0.4088	0.1988	50.000	53.871	2.59E+00	7.74%	50.000	50.027	2.79E+00	0.05%
0.7465	0.3631	100.000	107.068	5.16E+00	7.07%	100.000	105.075	5.52E+00	5.07%
1.0744	0.5226	200.000	210.173	1.03E+01	5.09%	200.000	211.766	1.08E+01	5.88%
0.1802	0.0877	300.000	301.706	1.54E+01	0.57%	300.000	306.482	1.55E+01	2.16%
-2.0198	-0.9824	400.000	380.875	2.06E+01	-4.78%	400.000	388.404	1.96E+01	-2.90%
					38.16%				108.23%

Drug: α-OH-Midazolam

Unit: ng/mL

Trial 4	x	y	w (1/y)	xy	wxy	wy	wx^2	wy^2	x^2
L1	10.000	5.70E-01	1.76E+00	5.70E+00	1.00E+01	1.00E+00	1.76E+02	5.70E-01	1.00E+02
L2	20.000	1.16E+00	8.64E-01	2.31E+01	2.00E+01	1.00E+00	3.46E+02	1.16E+00	4.00E+02
L3	50.000	2.80E+00	3.58E-01	1.40E+02	5.00E+01	1.00E+00	8.94E+02	2.80E+00	2.50E+03
L4	100.000	5.25E+00	1.90E-01	5.25E+02	1.00E+02	1.00E+00	1.90E+03	5.25E+00	1.00E+04
L5	200.000	1.07E+01	9.37E-02	2.14E+03	2.00E+02	1.00E+00	3.75E+03	1.07E+01	4.00E+04
L6	300.000	1.53E+01	6.52E-02	4.60E+03	3.00E+02	1.00E+00	5.87E+03	1.53E+01	9.00E+04
L7	400.000	1.99E+01	5.02E-02	7.97E+03	4.00E+02	1.00E+00	8.03E+03	1.99E+01	1.60E+05
		5.57E+01	3.38E+00	1.54E+04	1.08E+03	7.00E+00	2.10E+04	5.57E+01	3.03E+05

1.155E-01	intercept4	2.616E-01	intercept4
5.080E-02	slope4	4.989E-02	slope4
0.9990	R^2	0.9990	R^2

Standard Residual	y-yhat	w (1/y) Target	w (1/y) Calculated	yhat	Bias	Equal Target	Equal Calculated	Equal Response	Equal Bias
-0.1963	-0.0538	10.000	8.941	6.23E-01	-10.59%	10.000	6.174	5.70E-01	-38.26%
0.0932	0.0255	20.000	20.503	1.13E+00	2.51%	20.000	17.947	1.16E+00	-10.26%
0.5166	0.1416	50.000	52.788	2.66E+00	5.58%	50.000	50.820	2.80E+00	1.64%
0.2116	0.0580	100.000	101.142	5.20E+00	1.14%	100.000	100.054	5.25E+00	0.05%
1.4696	0.4029	200.000	207.931	1.03E+01	3.97%	200.000	208.789	1.07E+01	4.39%
-0.0771	-0.0211	300.000	299.584	1.54E+01	-0.14%	300.000	302.110	1.53E+01	0.70%
-1.8651	-0.5113	400.000	389.934	2.04E+01	-2.52%	400.000	394.105	1.99E+01	-1.47%
					26.44%				56.79%

Drug: α-OH-Midazolam

Unit: ng/mL

Trial 5	x	y	w (1/y)	xy	wxy	wy	wx^2	wy^2	x^2
L1	10.000	4.12E-01	2.43E+00	4.12E+00	1.00E+01	1.00E+00	2.43E+02	4.12E-01	1.00E+02
L2	20.000	1.13E+00	8.87E-01	2.25E+01	2.00E+01	1.00E+00	3.55E+02	1.13E+00	4.00E+02
L3	50.000	2.51E+00	3.99E-01	1.25E+02	5.00E+01	1.00E+00	9.98E+02	2.51E+00	2.50E+03
L4	100.000	5.62E+00	1.78E-01	5.62E+02	1.00E+02	1.00E+00	1.78E+03	5.62E+00	1.00E+04
L5	200.000	1.11E+01	9.01E-02	2.22E+03	2.00E+02	1.00E+00	3.60E+03	1.11E+01	4.00E+04
L6	300.000	1.52E+01	6.59E-02	4.55E+03	3.00E+02	1.00E+00	5.93E+03	1.52E+01	9.00E+04
L7	400.000	1.93E+01	5.19E-02	7.70E+03	4.00E+02	1.00E+00	8.31E+03	1.93E+01	1.60E+05
		5.52E+01	4.10E+00	1.52E+04	1.08E+03	7.00E+00	2.12E+04	5.52E+01	3.03E+05

-1.289E-02	intercept5	3.366E-01	intercept5
5.099E-02	slope5	4.893E-02	slope5
0.9948	R^2	0.9944	R^2

Standard Residual	y-yhat	Target	w (1/y) Calculated	yhat	Bias	Equal Target	Calculated	Response	Bias
-0.1340	-0.0852	10.000	8.329	4.97E-01	-16.71%	10.000	1.536	4.12E-01	-84.64%
0.1890	0.1202	20.000	22.357	1.01E+00	11.79%	20.000	16.154	1.13E+00	-19.23%
-0.0488	-0.0310	50.000	49.391	2.54E+00	-1.22%	50.000	44.325	2.51E+00	-11.35%
0.8472	0.5388	100.000	110.568	5.09E+00	10.57%	100.000	108.074	5.62E+00	8.07%
1.4344	0.9123	200.000	217.893	1.02E+01	8.95%	200.000	219.912	1.11E+01	9.96%
-0.1564	-0.0995	300.000	298.048	1.53E+01	-0.65%	300.000	303.438	1.52E+01	1.15%
-1.7782	-1.1310	400.000	377.817	2.04E+01	-5.55%	400.000	386.560	1.93E+01	-3.36%
					55.43%				137.75%

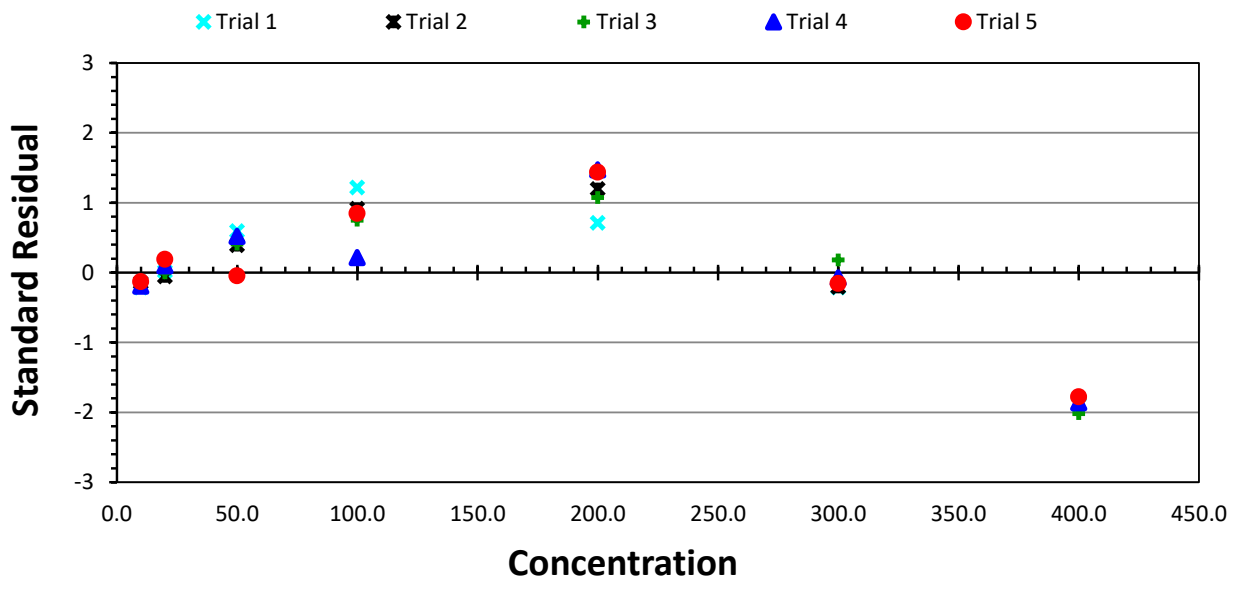
BZDG1 in Blood

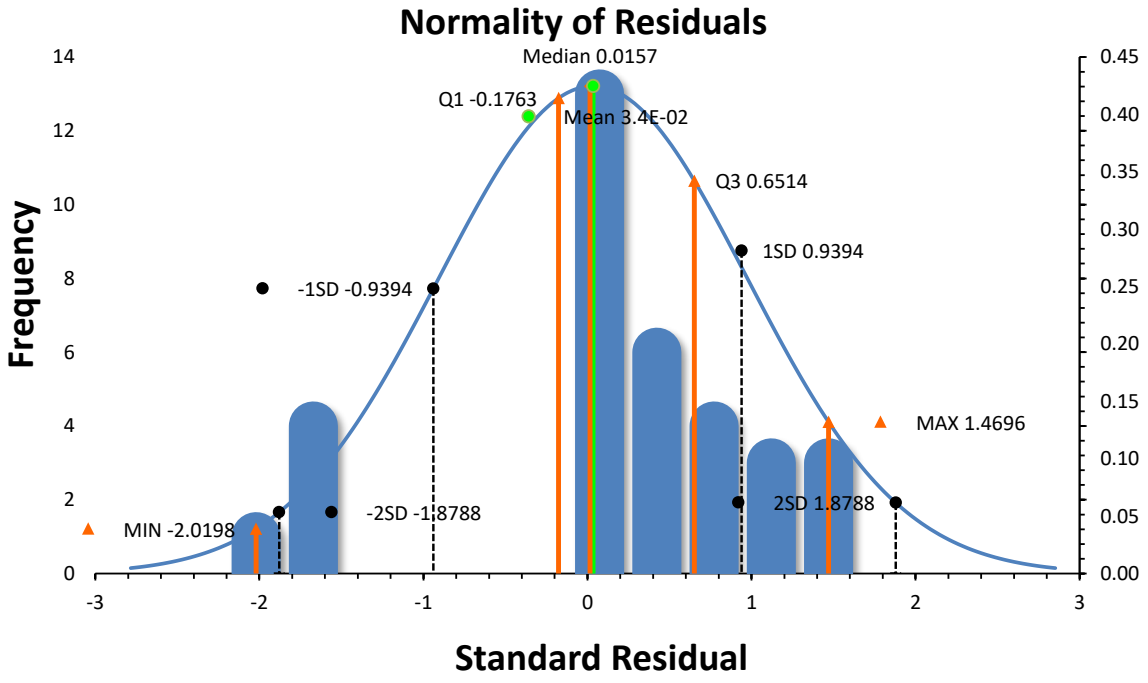
Drug: α-OH-Midazolam

Unit: ng/mL

Calibration Model: Linear (1/y)

Toxicologist(s): JD1 JD2 JD3 JD4 JD5 **Instrument:** LC-MS/MS

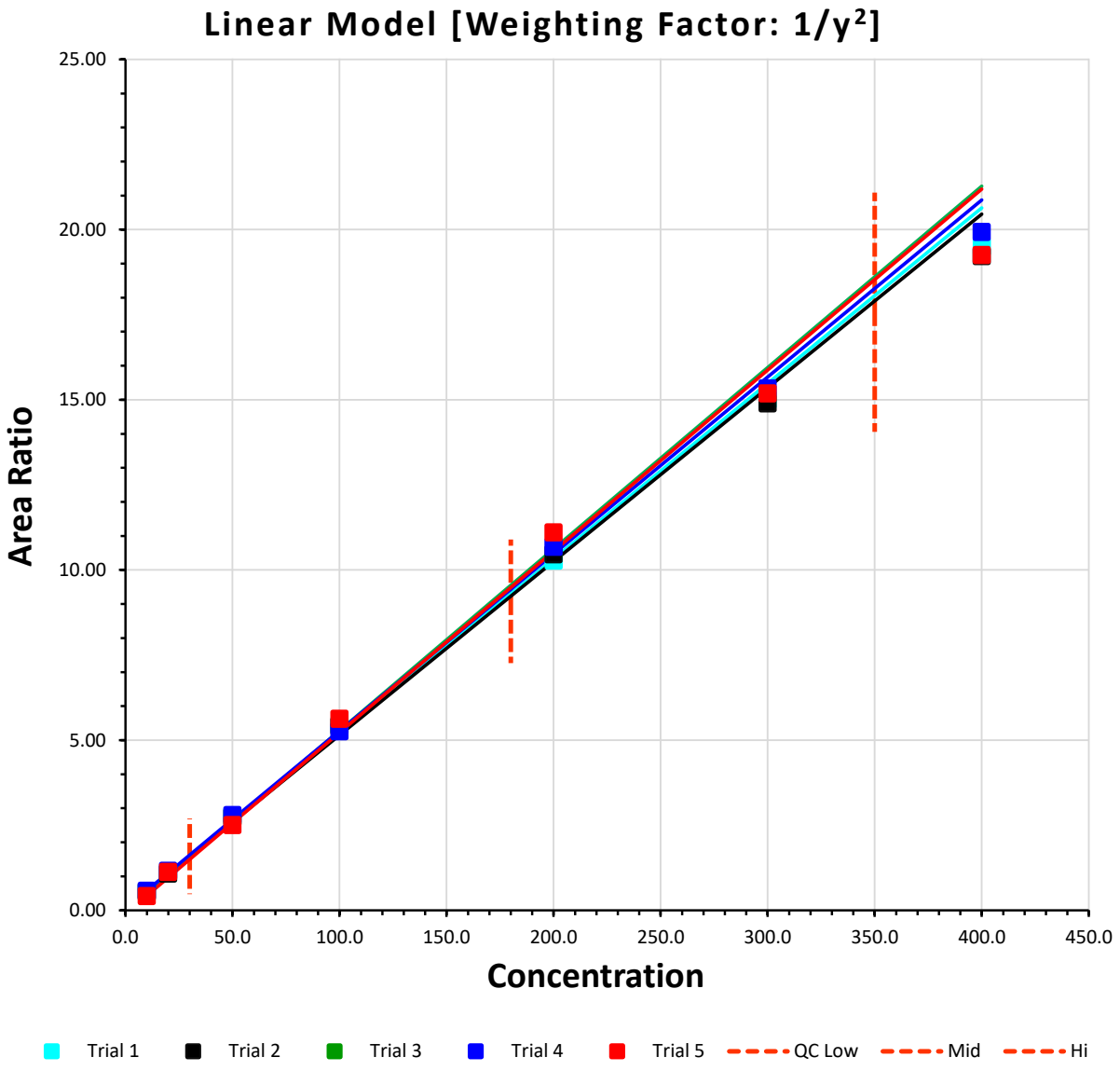




BZDG1 in Blood

Drug: α-OH-Midazolam

Unit: ng/mL



		slope			y-intercept	R^2
Trial 1	Y=	5.154E-02	X	+	2.253E-02	0.9972
Trial 2	Y=	5.102E-02	X	+	4.669E-02	0.9975
Trial 3	Y=	5.331E-02	X	+	-5.129E-02	0.9964
Trial 4	Y=	5.201E-02	X	+	6.639E-02	0.9981
Trial 5	Y=	5.322E-02	X	+	-9.893E-02	0.9905

Drug: α-OH-Midazolam

Unit: ng/mL

Trial 1	x	y	w (1/y²)	xy	wxy	wy	wx²	wy²	x²
L1	10.000	5.23E-01	3.65E+00	5.23E+00	1.91E+01	1.91E+00	3.65E+02	1.00E+00	1.00E+02
L2	20.000	1.09E+00	8.43E-01	2.18E+01	1.84E+01	9.18E-01	3.37E+02	1.00E+00	4.00E+02
L3	50.000	2.76E+00	1.32E-01	1.38E+02	1.81E+01	3.63E-01	3.29E+02	1.00E+00	2.50E+03
L4	100.000	5.43E+00	3.39E-02	5.43E+02	1.84E+01	1.84E-01	3.39E+02	1.00E+00	1.00E+04
L5	200.000	1.03E+01	9.47E-03	2.05E+03	1.95E+01	9.73E-02	3.79E+02	1.00E+00	4.00E+04
L6	300.000	1.50E+01	4.45E-03	4.50E+03	2.00E+01	6.67E-02	4.00E+02	1.00E+00	9.00E+04
L7	400.000	1.95E+01	2.63E-03	7.80E+03	2.05E+01	5.13E-02	4.21E+02	1.00E+00	1.60E+05
7	1.08E+03	5.46E+01	4.68E+00	1.51E+04	1.34E+02	3.59E+00	2.57E+03	7.00E+00	3.03E+05

2.253E-02	intercept1	2.813E-01	intercept1
5.154E-02	slope1	4.870E-02	slope1
0.9972	R^2	0.9990	R^2

Standard Residual	y-yhat	w (1/y²) Target	Calculated	yhat	Bias	Equal Target	Calculated	Response	Bias
-0.0304	-0.0148	10.000	9.714	5.38E-01	-2.86%	10.000	4.967	5.23E-01	-50.33%
0.0746	0.0362	20.000	20.702	1.05E+00	3.51%	20.000	16.596	1.09E+00	-17.02%
0.3209	0.1556	50.000	53.020	2.60E+00	6.04%	50.000	50.798	2.76E+00	1.60%
0.5260	0.2551	100.000	104.950	5.18E+00	4.95%	100.000	105.754	5.43E+00	5.75%
-0.1159	-0.0562	200.000	198.910	1.03E+01	-0.55%	200.000	205.191	1.03E+01	2.60%
-1.0087	-0.4892	300.000	290.508	1.55E+01	-3.16%	300.000	302.129	1.50E+01	0.71%
-2.3535	-1.1414	400.000	377.853	2.06E+01	-5.54%	400.000	394.565	1.95E+01	-1.36%
					26.61%				79.37%

Drug: α-OH-Midazolam

Unit: ng/mL

Trial 2	x	y	w (1/y^2)	xy	wxy	wy	wx^2	wy^2	x^2
L1	10.000	5.48E-01	3.33E+00	5.48E+00	1.83E+01	1.83E+00	3.33E+02	1.00E+00	1.00E+02
L2	20.000	1.08E+00	8.63E-01	2.15E+01	1.86E+01	9.29E-01	3.45E+02	1.00E+00	4.00E+02
L3	50.000	2.73E+00	1.34E-01	1.36E+02	1.83E+01	3.66E-01	3.36E+02	1.00E+00	2.50E+03
L4	100.000	5.40E+00	3.43E-02	5.40E+02	1.85E+01	1.85E-01	3.43E+02	1.00E+00	1.00E+04
L5	200.000	1.05E+01	9.14E-03	2.09E+03	1.91E+01	9.56E-02	3.66E+02	1.00E+00	4.00E+04
L6	300.000	1.49E+01	4.51E-03	4.47E+03	2.02E+01	6.72E-02	4.06E+02	1.00E+00	9.00E+04
L7	400.000	1.92E+01	2.71E-03	7.69E+03	2.08E+01	5.20E-02	4.33E+02	1.00E+00	1.60E+05
		5.43E+01	4.38E+00	1.49E+04	1.34E+02	3.52E+00	2.56E+03	7.00E+00	3.03E+05

4.669E-02	intercept2	3.290E-01	intercept2
5.102E-02	slope2	4.815E-02	slope2
0.9975	R^2	0.9982	R^2

Standard Residual	y-yhat	w (1/y^2) Target	Calculated	yhat	Bias	Equal Target	Calculated	Response	Bias
-0.0173	-0.0092	10.000	9.819	5.57E-01	-1.81%	10.000	4.541	5.48E-01	-54.59%
0.0177	0.0094	20.000	20.185	1.07E+00	0.92%	20.000	15.523	1.08E+00	-22.39%
0.2463	0.1312	50.000	52.572	2.60E+00	5.14%	50.000	49.837	2.73E+00	-0.33%
0.4705	0.2507	100.000	104.913	5.15E+00	4.91%	100.000	105.291	5.40E+00	5.29%
0.3907	0.2081	200.000	204.079	1.03E+01	2.04%	200.000	210.355	1.05E+01	5.18%
-0.8758	-0.4666	300.000	290.855	1.54E+01	-3.05%	300.000	302.293	1.49E+01	0.76%
-2.3294	-1.2409	400.000	375.678	2.05E+01	-6.08%	400.000	392.161	1.92E+01	-1.96%
					23.96%				90.50%

Drug: α-OH-Midazolam

Unit: ng/mL

Trial 3	x	y	w (1/y^2)	xy	wxy	wy	wx^2	wy^2	x^2
L1	10.000	4.70E-01	4.53E+00	4.70E+00	2.13E+01	2.13E+00	4.53E+02	1.00E+00	1.00E+02
L2	20.000	1.05E+00	9.14E-01	2.09E+01	1.91E+01	9.56E-01	3.66E+02	1.00E+00	4.00E+02
L3	50.000	2.79E+00	1.29E-01	1.39E+02	1.79E+01	3.59E-01	3.22E+02	1.00E+00	2.50E+03
L4	100.000	5.52E+00	3.28E-02	5.52E+02	1.81E+01	1.81E-01	3.28E+02	1.00E+00	1.00E+04
L5	200.000	1.08E+01	8.55E-03	2.16E+03	1.85E+01	9.24E-02	3.42E+02	1.00E+00	4.00E+04
L6	300.000	1.55E+01	4.15E-03	4.66E+03	1.93E+01	6.44E-02	3.74E+02	1.00E+00	9.00E+04
L7	400.000	1.96E+01	2.61E-03	7.83E+03	2.04E+01	5.11E-02	4.17E+02	1.00E+00	1.60E+05
		5.57E+01	5.62E+00	1.54E+04	1.35E+02	3.83E+00	2.60E+03	7.00E+00	3.03E+05

-5.129E-02	intercept3	3.050E-01	intercept3
5.331E-02	slope3	4.964E-02	slope3
0.9964	R^2	0.9970	R^2

Standard Residual	y-yhat	w (1/y^2) Target	Calculated	yhat	Bias	Equal Target	Calculated	Response	Bias
-0.0173	-0.0120	10.000	9.776	4.82E-01	-2.24%	10.000	3.322	4.70E-01	-66.78%
0.0448	0.0309	20.000	20.579	1.01E+00	2.89%	20.000	14.924	1.05E+00	-25.38%
0.2526	0.1741	50.000	53.266	2.61E+00	6.53%	50.000	50.027	2.79E+00	0.05%
0.3499	0.2412	100.000	104.524	5.28E+00	4.52%	100.000	105.075	5.52E+00	5.07%
0.2993	0.2063	200.000	203.870	1.06E+01	1.93%	200.000	211.766	1.08E+01	5.88%
-0.6138	-0.4230	300.000	292.065	1.59E+01	-2.64%	300.000	306.482	1.55E+01	2.16%
-2.4485	-1.6875	400.000	368.347	2.13E+01	-7.91%	400.000	388.404	1.96E+01	-2.90%
					28.68%				108.23%

Drug: α-OH-Midazolam

Unit: ng/mL

Trial 4	x	y	w (1/y^2)	xy	wxy	wy	wx^2	wy^2	x^2
L1	10.000	5.70E-01	3.08E+00	5.70E+00	1.76E+01	1.76E+00	3.08E+02	1.00E+00	1.00E+02
L2	20.000	1.16E+00	7.47E-01	2.31E+01	1.73E+01	8.64E-01	2.99E+02	1.00E+00	4.00E+02
L3	50.000	2.80E+00	1.28E-01	1.40E+02	1.79E+01	3.58E-01	3.20E+02	1.00E+00	2.50E+03
L4	100.000	5.25E+00	3.62E-02	5.25E+02	1.90E+01	1.90E-01	3.62E+02	1.00E+00	1.00E+04
L5	200.000	1.07E+01	8.77E-03	2.14E+03	1.87E+01	9.37E-02	3.51E+02	1.00E+00	4.00E+04
L6	300.000	1.53E+01	4.25E-03	4.60E+03	1.96E+01	6.52E-02	3.83E+02	1.00E+00	9.00E+04
L7	400.000	1.99E+01	2.52E-03	7.97E+03	2.01E+01	5.02E-02	4.03E+02	1.00E+00	1.60E+05
		5.57E+01	4.01E+00	1.54E+04	1.30E+02	3.38E+00	2.43E+03	7.00E+00	3.03E+05

6.639E-02	intercept4	2.616E-01	intercept4
5.201E-02	slope4	4.989E-02	slope4
0.9981	R^2	0.9990	R^2

Standard Residual	y-yhat	w (1/y^2) Target	Calculated	yhat	Bias	Equal Target	Calculated	Response	Bias
-0.0422	-0.0168	10.000	9.677	5.86E-01	-3.23%	10.000	6.174	5.70E-01	-38.26%
0.1266	0.0504	20.000	20.969	1.11E+00	4.85%	20.000	17.947	1.16E+00	-10.26%
0.3267	0.1301	50.000	52.502	2.67E+00	5.00%	50.000	50.820	2.80E+00	1.64%
-0.0355	-0.0141	100.000	99.728	5.27E+00	-0.27%	100.000	100.054	5.25E+00	0.05%
0.5260	0.2095	200.000	204.028	1.05E+01	2.01%	200.000	208.789	1.07E+01	4.39%
-0.8431	-0.3358	300.000	293.544	1.57E+01	-2.15%	300.000	302.110	1.53E+01	0.70%
-2.3781	-0.9472	400.000	381.788	2.09E+01	-4.55%	400.000	394.105	1.99E+01	-1.47%
					22.07%				56.79%

Drug: α-OH-Midazolam

Unit: ng/mL

Trial 5	x	y	w (1/y²)	xy	wxy	wy	wx²	wy²	x²
L1	10.000	4.12E-01	5.90E+00	4.12E+00	2.43E+01	2.43E+00	5.90E+02	1.00E+00	1.00E+02
L2	20.000	1.13E+00	7.87E-01	2.25E+01	1.77E+01	8.87E-01	3.15E+02	1.00E+00	4.00E+02
L3	50.000	2.51E+00	1.59E-01	1.25E+02	2.00E+01	3.99E-01	3.98E+02	1.00E+00	2.50E+03
L4	100.000	5.62E+00	3.16E-02	5.62E+02	1.78E+01	1.78E-01	3.16E+02	1.00E+00	1.00E+04
L5	200.000	1.11E+01	8.12E-03	2.22E+03	1.80E+01	9.01E-02	3.25E+02	1.00E+00	4.00E+04
L6	300.000	1.52E+01	4.34E-03	4.55E+03	1.98E+01	6.59E-02	3.90E+02	1.00E+00	9.00E+04
L7	400.000	1.93E+01	2.70E-03	7.70E+03	2.08E+01	5.19E-02	4.32E+02	1.00E+00	1.60E+05
		5.52E+01	6.89E+00	1.52E+04	1.38E+02	4.10E+00	2.77E+03	7.00E+00	3.03E+05

-9.893E-02	intercept5	3.366E-01	intercept5
5.322E-02	slope5	4.893E-02	slope5
0.9905	R^2	0.9944	R^2

Standard Residual	y-yhat	w (1/y²) Target	Calculated	yhat	Bias	Equal Target	Calculated	Response	Bias
-0.0253	-0.0215	10.000	9.595	4.33E-01	-4.05%	10.000	1.536	4.12E-01	-84.64%
0.1894	0.1615	20.000	23.034	9.66E-01	15.17%	20.000	16.154	1.13E+00	-19.23%
-0.0666	-0.0568	50.000	48.933	2.56E+00	-2.13%	50.000	44.325	2.51E+00	-11.35%
0.4705	0.4013	100.000	107.540	5.22E+00	7.54%	100.000	108.074	5.62E+00	8.07%
0.6463	0.5512	200.000	210.356	1.05E+01	5.18%	200.000	219.912	1.11E+01	9.96%
-0.8023	-0.6842	300.000	287.144	1.59E+01	-4.29%	300.000	303.438	1.52E+01	1.15%
-2.2741	-1.9393	400.000	363.562	2.12E+01	-9.11%	400.000	386.560	1.93E+01	-3.36%
					47.47%				137.75%

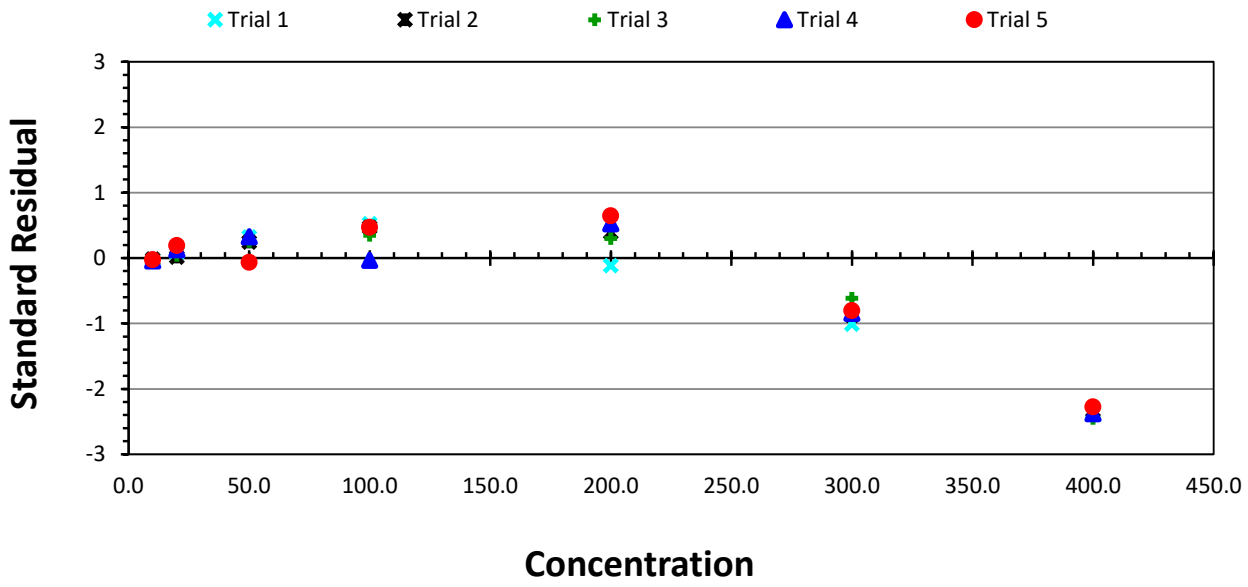
BZDG1 in Blood

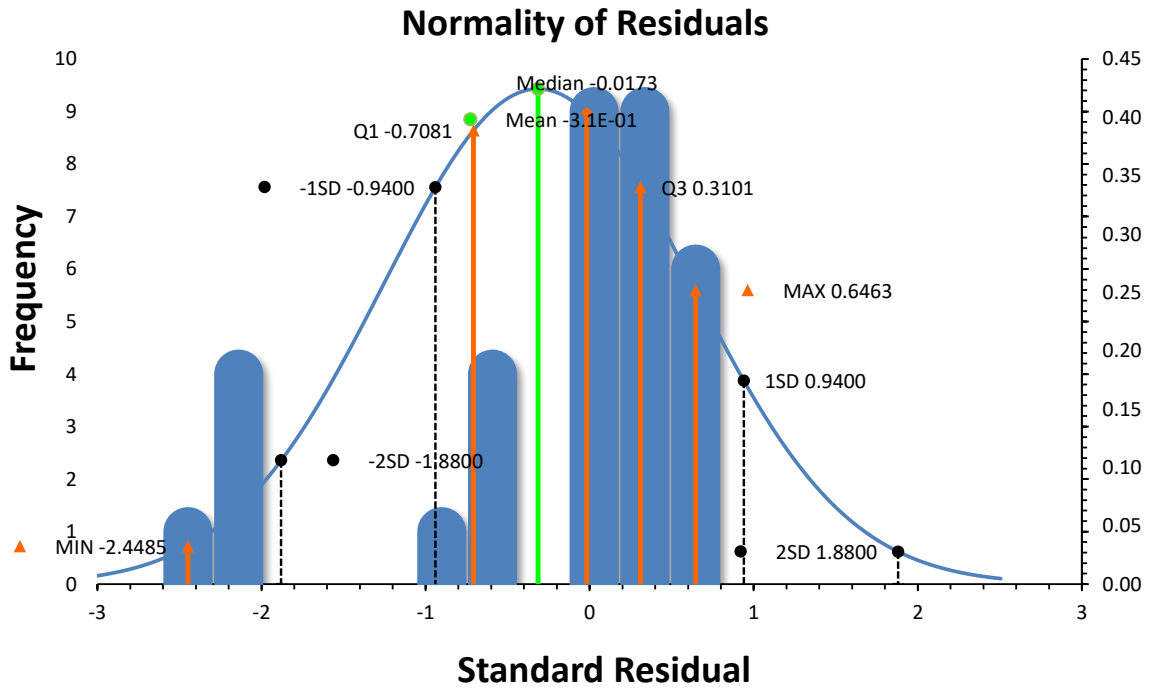
Drug: α-OH-Midazolam

Unit: ng/mL

Calibration Model: Linear ($1/y^2$)

Toxicologist(s): JD1 JD2 JD3 JD4 JD5 **Instrument:** LC-MS/MS

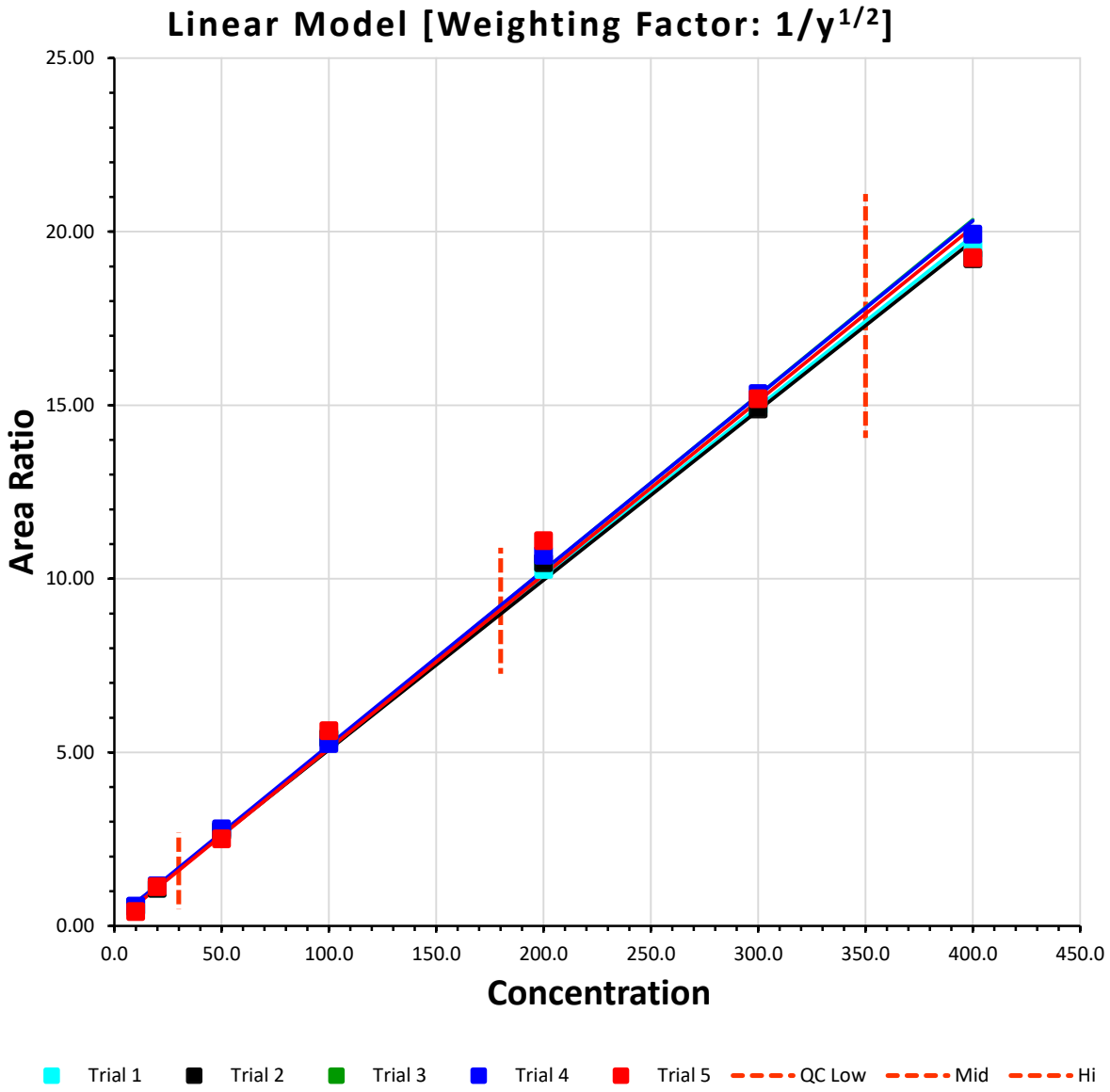




BZDG1 in Blood

Drug: α-OH-Midazolam

Unit: ng/mL



		slope			y-intercept	R^2
Trial 1	Y=	4.931E-02	X	+	1.578E-01	0.9989
Trial 2	Y=	4.888E-02	X	+	1.825E-01	0.9982
Trial 3	Y=	5.058E-02	X	+	1.154E-01	0.9973
Trial 4	Y=	5.037E-02	X	+	1.668E-01	0.9991
Trial 5	Y=	5.008E-02	X	+	9.883E-02	0.9951

Drug: α-OH-Midazolam

Unit: ng/mL

Trial 1	x	y	w ($1/y^{1/2}$)	xy	wxy	wy	wx^2	wy^2	x^2
L1	10.000	5.23E-01	1.38E+00	5.23E+00	7.23E+00	7.23E-01	1.38E+02	3.78E-01	1.00E+02
L2	20.000	1.09E+00	9.58E-01	2.18E+01	2.09E+01	1.04E+00	3.83E+02	1.14E+00	4.00E+02
L3	50.000	2.76E+00	6.02E-01	1.38E+02	8.30E+01	1.66E+00	1.51E+03	4.57E+00	2.50E+03
L4	100.000	5.43E+00	4.29E-01	5.43E+02	2.33E+02	2.33E+00	4.29E+03	1.27E+01	1.00E+04
L5	200.000	1.03E+01	3.12E-01	2.05E+03	6.41E+02	3.21E+00	1.25E+04	3.29E+01	4.00E+04
L6	300.000	1.50E+01	2.58E-01	4.50E+03	1.16E+03	3.87E+00	2.32E+04	5.81E+01	9.00E+04
L7	400.000	1.95E+01	2.26E-01	7.80E+03	1.77E+03	4.42E+00	3.62E+04	8.61E+01	1.60E+05
7	1.08E+03	5.46E+01	4.17E+00	1.51E+04	3.91E+03	1.73E+01	7.83E+04	1.96E+02	3.03E+05

1.578E-01	intercept1	2.813E-01	intercept1
4.931E-02	slope1	4.870E-02	slope1
0.9989	R^2	0.9990	R^2

Standard Residual	y-yhat	Target	w ($1/y^{1/2}$) Calculated	yhat	Bias	Equal Target	Calculated	Response	Bias
-0.5191	-0.1278	10.000	7.409	6.51E-01	-25.91%	10.000	4.967	5.23E-01	-50.33%
-0.2217	-0.0546	20.000	18.893	1.14E+00	-5.53%	20.000	16.596	1.09E+00	-17.02%
0.5348	0.1316	50.000	52.670	2.62E+00	5.34%	50.000	50.798	2.76E+00	1.60%
1.3908	0.3424	100.000	106.943	5.09E+00	6.94%	100.000	105.754	5.43E+00	5.75%
1.0303	0.2536	200.000	205.143	1.00E+01	2.57%	200.000	205.191	1.03E+01	2.60%
0.1753	0.0432	300.000	300.875	1.50E+01	0.29%	300.000	302.129	1.50E+01	0.71%
-1.5700	-0.3865	400.000	392.162	1.99E+01	-1.96%	400.000	394.565	1.95E+01	-1.36%
					48.55%				79.37%

Drug: α-OH-Midazolam

Unit: ng/mL

Trial 2	x	y	w ($1/y^{1/2}$)	xy	wxy	wy	wx^2	wy^2	x^2
L1	10.000	5.48E-01	1.35E+00	5.48E+00	7.40E+00	7.40E-01	1.35E+02	4.05E-01	1.00E+02
L2	20.000	1.08E+00	9.64E-01	2.15E+01	2.08E+01	1.04E+00	3.86E+02	1.12E+00	4.00E+02
L3	50.000	2.73E+00	6.05E-01	1.36E+02	8.26E+01	1.65E+00	1.51E+03	4.51E+00	2.50E+03
L4	100.000	5.40E+00	4.30E-01	5.40E+02	2.32E+02	2.32E+00	4.30E+03	1.25E+01	1.00E+04
L5	200.000	1.05E+01	3.09E-01	2.09E+03	6.47E+02	3.23E+00	1.24E+04	3.38E+01	4.00E+04
L6	300.000	1.49E+01	2.59E-01	4.47E+03	1.16E+03	3.86E+00	2.33E+04	5.74E+01	9.00E+04
L7	400.000	1.92E+01	2.28E-01	7.69E+03	1.75E+03	4.38E+00	3.65E+04	8.42E+01	1.60E+05
		5.43E+01	4.15E+00	1.49E+04	3.90E+03	1.72E+01	7.85E+04	1.94E+02	3.03E+05

1.825E-01	intercept2	3.290E-01	intercept2
4.888E-02	slope2	4.815E-02	slope2
0.9982	R^2	0.9982	R^2

Standard Residual	y-yhat	Target	w ($1/y^{1/2}$) Calculated	yhat	Bias	Equal Target	Calculated	Response	Bias
-0.3740	-0.1237	10.000	7.469	6.71E-01	-25.31%	10.000	4.541	5.48E-01	-54.59%
-0.2530	-0.0837	20.000	18.288	1.16E+00	-8.56%	20.000	15.523	1.08E+00	-22.39%
0.3086	0.1021	50.000	52.089	2.63E+00	4.18%	50.000	49.837	2.73E+00	-0.33%
0.9922	0.3282	100.000	106.715	5.07E+00	6.71%	100.000	105.291	5.40E+00	5.29%
1.5087	0.4991	200.000	210.210	9.96E+00	5.10%	200.000	210.355	1.05E+01	5.18%
0.1143	0.0378	300.000	300.774	1.48E+01	0.26%	300.000	302.293	1.49E+01	0.76%
-1.5813	-0.5231	400.000	389.299	1.97E+01	-2.68%	400.000	392.161	1.92E+01	-1.96%
					52.80%				90.50%

Drug: α-OH-Midazolam

Unit: ng/mL

Trial 3	x	y	w ($1/y^{1/2}$)	xy	wxy	wy	wx^2	wy^2	x^2
L1	10.000	4.70E-01	1.46E+00	4.70E+00	6.85E+00	6.85E-01	1.46E+02	3.22E-01	1.00E+02
L2	20.000	1.05E+00	9.78E-01	2.09E+01	2.05E+01	1.02E+00	3.91E+02	1.07E+00	4.00E+02
L3	50.000	2.79E+00	5.99E-01	1.39E+02	8.35E+01	1.67E+00	1.50E+03	4.66E+00	2.50E+03
L4	100.000	5.52E+00	4.26E-01	5.52E+02	2.35E+02	2.35E+00	4.26E+03	1.30E+01	1.00E+04
L5	200.000	1.08E+01	3.04E-01	2.16E+03	6.58E+02	3.29E+00	1.22E+04	3.56E+01	4.00E+04
L6	300.000	1.55E+01	2.54E-01	4.66E+03	1.18E+03	3.94E+00	2.28E+04	6.11E+01	9.00E+04
L7	400.000	1.96E+01	2.26E-01	7.83E+03	1.77E+03	4.43E+00	3.62E+04	8.67E+01	1.60E+05
		5.57E+01	4.24E+00	1.54E+04	3.96E+03	1.74E+01	7.74E+04	2.02E+02	3.03E+05

1.154E-01	intercept3	3.050E-01	intercept3
5.058E-02	slope3	4.964E-02	slope3
0.9973	R^2	0.9970	R^2

Standard Residual	y-yhat	Target	w ($1/y^{1/2}$) Calculated	yhat	Bias	Equal Target	Calculated	Response	Bias
-0.3484	-0.1513	10.000	7.008	6.21E-01	-29.92%	10.000	3.322	4.70E-01	-66.78%
-0.1868	-0.0811	20.000	18.396	1.13E+00	-8.02%	20.000	14.924	1.05E+00	-25.38%
0.3321	0.1442	50.000	52.852	2.64E+00	5.70%	50.000	50.027	2.79E+00	0.05%
0.8016	0.3481	100.000	106.883	5.17E+00	6.88%	100.000	105.075	5.52E+00	5.07%
1.3514	0.5869	200.000	211.605	1.02E+01	5.80%	200.000	211.766	1.08E+01	5.88%
0.5325	0.2313	300.000	304.573	1.53E+01	1.52%	300.000	306.482	1.55E+01	2.16%
-1.7487	-0.7595	400.000	384.983	2.03E+01	-3.75%	400.000	388.404	1.96E+01	-2.90%
					61.61%				108.23%

Drug: α-OH-Midazolam

Unit: ng/mL

Trial 4	x	y	w ($1/y^{1/2}$)	xy	wxy	wy	wx^2	wy^2	x^2
L1	10.000	5.70E-01	1.32E+00	5.70E+00	7.55E+00	7.55E-01	1.32E+02	4.30E-01	1.00E+02
L2	20.000	1.16E+00	9.30E-01	2.31E+01	2.15E+01	1.08E+00	3.72E+02	1.24E+00	4.00E+02
L3	50.000	2.80E+00	5.98E-01	1.40E+02	8.36E+01	1.67E+00	1.49E+03	4.68E+00	2.50E+03
L4	100.000	5.25E+00	4.36E-01	5.25E+02	2.29E+02	2.29E+00	4.36E+03	1.20E+01	1.00E+04
L5	200.000	1.07E+01	3.06E-01	2.14E+03	6.54E+02	3.27E+00	1.22E+04	3.49E+01	4.00E+04
L6	300.000	1.53E+01	2.55E-01	4.60E+03	1.17E+03	3.92E+00	2.30E+04	6.00E+01	9.00E+04
L7	400.000	1.99E+01	2.24E-01	7.97E+03	1.79E+03	4.46E+00	3.58E+04	8.89E+01	1.60E+05
		5.57E+01	4.07E+00	1.54E+04	3.96E+03	1.74E+01	7.74E+04	2.02E+02	3.03E+05

1.668E-01	intercept4	2.616E-01	intercept4
5.037E-02	slope4	4.989E-02	slope4
0.9991	R^2	0.9990	R^2

Standard Residual	y-yhat	w ($1/y^{1/2}$) Target	Calculated	yhat	Bias	Equal Target	Calculated	Response	Bias
-0.4062	-0.1008	10.000	7.998	6.70E-01	-20.02%	10.000	6.174	5.70E-01	-38.26%
-0.0691	-0.0172	20.000	19.659	1.17E+00	-1.70%	20.000	17.947	1.16E+00	-10.26%
0.4507	0.1118	50.000	52.221	2.69E+00	4.44%	50.000	50.820	2.80E+00	1.64%
0.2004	0.0497	100.000	100.987	5.20E+00	0.99%	100.000	100.054	5.25E+00	0.05%
1.7636	0.4377	200.000	208.690	1.02E+01	4.34%	200.000	208.789	1.07E+01	4.39%
0.2284	0.0567	300.000	301.125	1.53E+01	0.38%	300.000	302.110	1.53E+01	0.70%
-1.5732	-0.3904	400.000	392.248	2.03E+01	-1.94%	400.000	394.105	1.99E+01	-1.47%
					33.80%				56.79%

Drug: α-OH-Midazolam

Unit: ng/mL

Trial 5	x	y	w ($1/y^{1/2}$)	xy	wxy	wy	wx^2	wy^2	x^2
L1	10.000	4.12E-01	1.56E+00	4.12E+00	6.42E+00	6.42E-01	1.56E+02	2.64E-01	1.00E+02
L2	20.000	1.13E+00	9.42E-01	2.25E+01	2.12E+01	1.06E+00	3.77E+02	1.20E+00	4.00E+02
L3	50.000	2.51E+00	6.32E-01	1.25E+02	7.91E+01	1.58E+00	1.58E+03	3.97E+00	2.50E+03
L4	100.000	5.62E+00	4.22E-01	5.62E+02	2.37E+02	2.37E+00	4.22E+03	1.33E+01	1.00E+04
L5	200.000	1.11E+01	3.00E-01	2.22E+03	6.66E+02	3.33E+00	1.20E+04	3.70E+01	4.00E+04
L6	300.000	1.52E+01	2.57E-01	4.55E+03	1.17E+03	3.90E+00	2.31E+04	5.92E+01	9.00E+04
L7	400.000	1.93E+01	2.28E-01	7.70E+03	1.76E+03	4.39E+00	3.65E+04	8.45E+01	1.60E+05
		5.52E+01	4.34E+00	1.52E+04	3.93E+03	1.73E+01	7.79E+04	1.99E+02	3.03E+05

9.883E-02	intercept5	3.366E-01	intercept5
5.008E-02	slope5	4.893E-02	slope5
0.9951	R^2	0.9944	R^2

Standard Residual	y-yhat	Target	w ($1/y^{1/2}$) Calculated	yhat	Bias	Equal Target	Calculated	Response	Bias
-0.3230	-0.1878	10.000	6.249	6.00E-01	-37.51%	10.000	1.536	4.12E-01	-84.64%
0.0458	0.0267	20.000	20.532	1.10E+00	2.66%	20.000	16.154	1.13E+00	-19.23%
-0.1672	-0.0972	50.000	48.058	2.60E+00	-3.88%	50.000	44.325	2.51E+00	-11.35%
0.8909	0.5181	100.000	110.347	5.11E+00	10.35%	100.000	108.074	5.62E+00	8.07%
1.6895	0.9826	200.000	219.623	1.01E+01	9.81%	200.000	219.912	1.11E+01	9.96%
0.1063	0.0618	300.000	301.235	1.51E+01	0.41%	300.000	303.438	1.52E+01	1.15%
-1.5108	-0.8787	400.000	382.453	2.01E+01	-4.39%	400.000	386.560	1.93E+01	-3.36%
					69.01%				137.75%

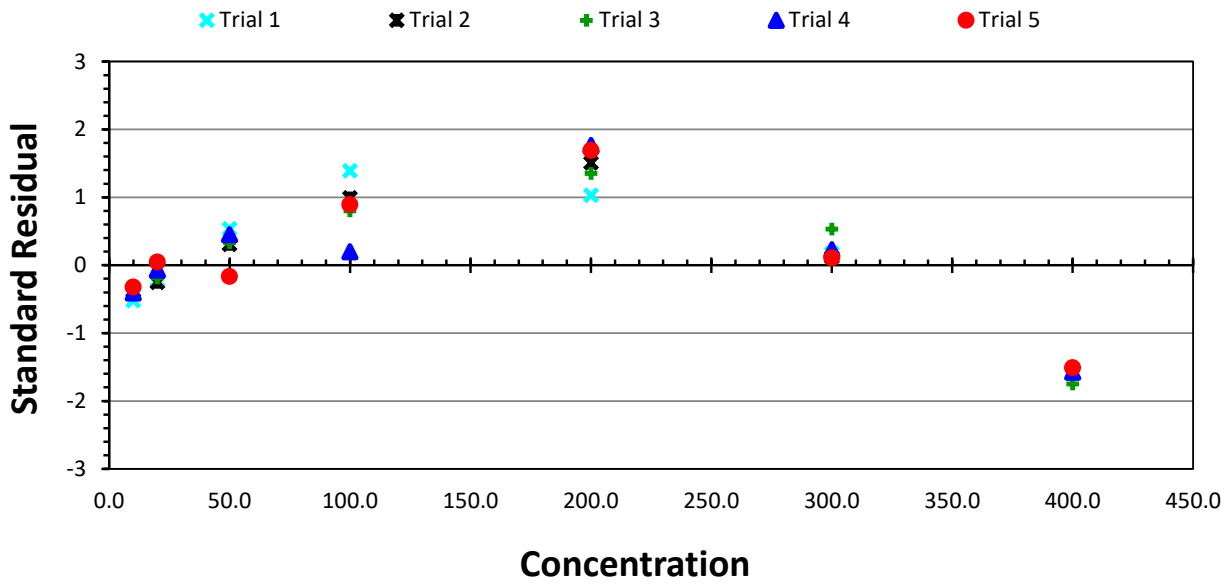
BZDG1 in Blood

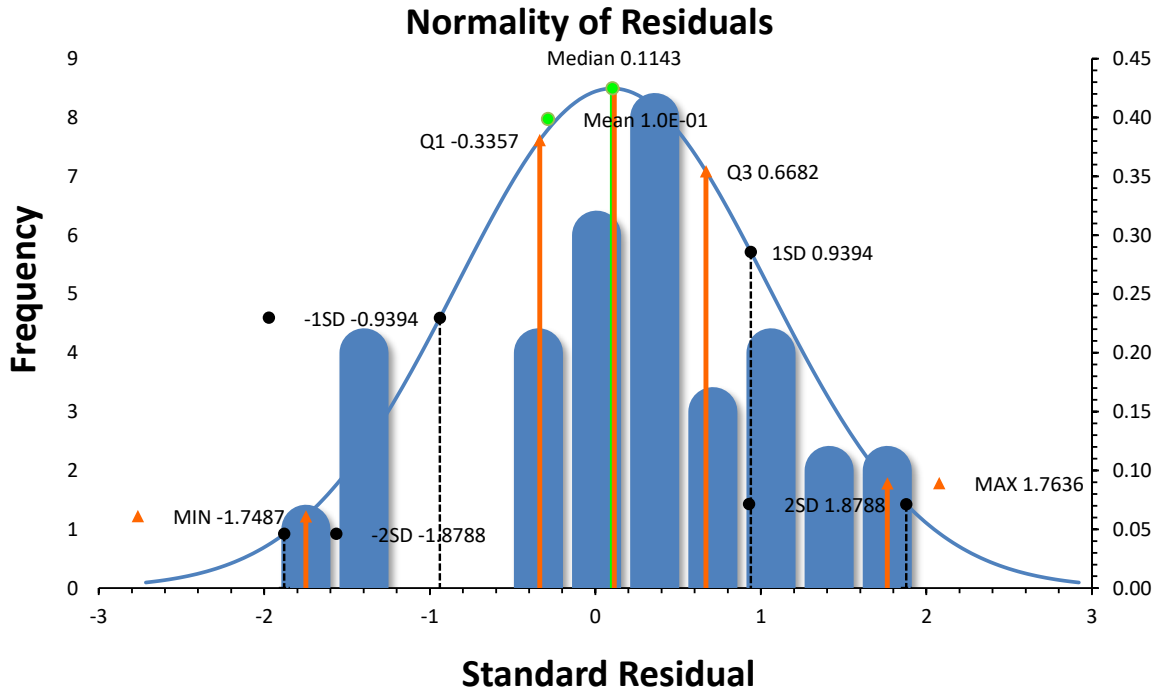
Drug: α-OH-Midazolam

Unit: ng/mL

Calibration Model: Linear ($1/y^{1/2}$)

Toxicologist(s): JD1 JD2 JD3 JD4 JD5 **Instrument:** LC-MS/MS

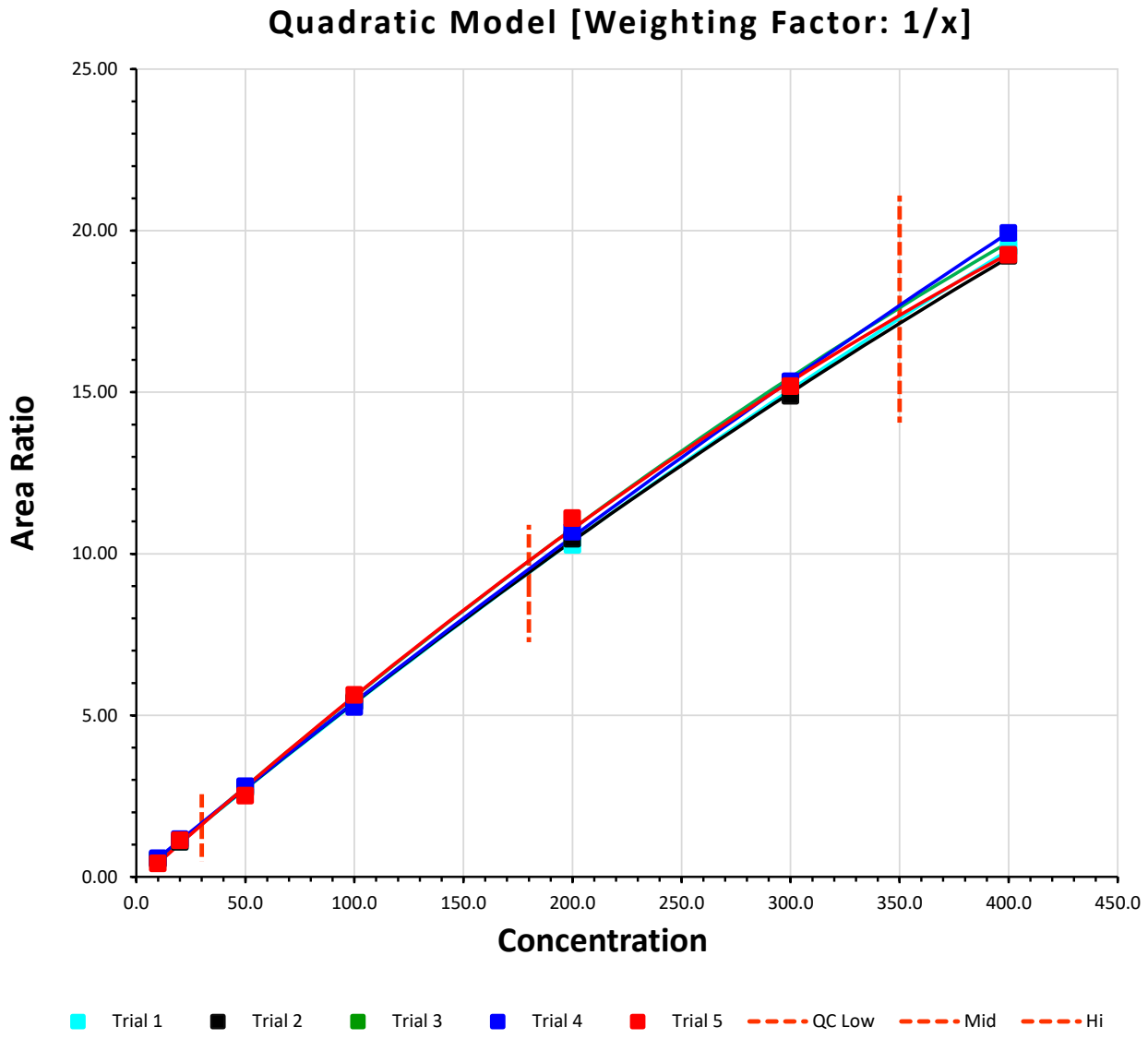




BZDG1 in Blood

Drug: α-OH-Midazolam

Unit: ng/mL

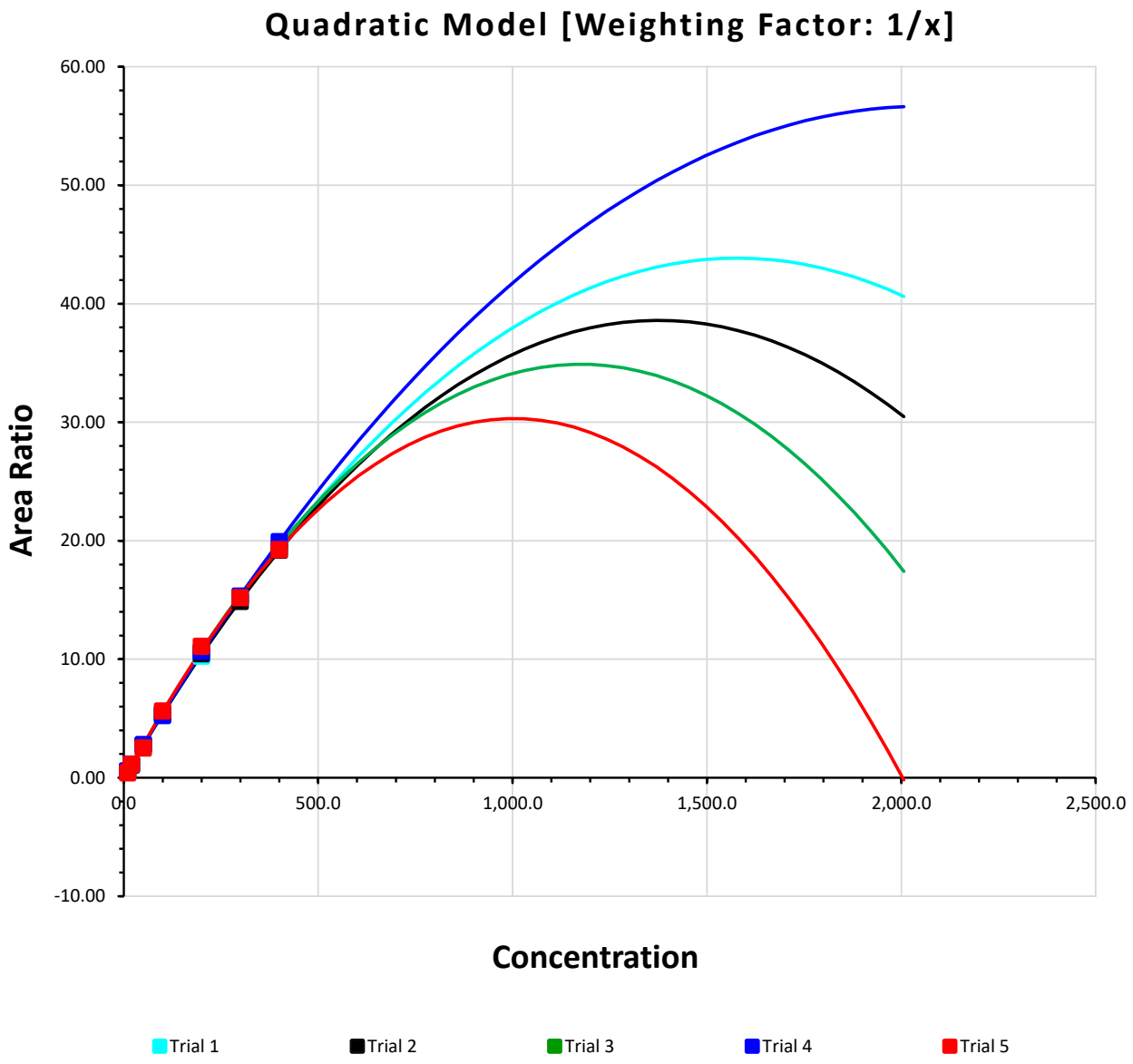


	a		b			c	R^2
Trial 1	-1.761E-05	X^2 +	5.559E-02	X	+	-2.011E-02	0.9999
Trial 2	-2.041E-05	X^2 +	5.614E-02	X	+	-2.023E-02	1.0000
Trial 3	-2.532E-05	X^2 +	5.956E-02	X	+	-1.284E-01	1.0000
Trial 4	-1.342E-05	X^2 +	5.514E-02	X	+	3.190E-02	0.9998
Trial 5	-3.030E-05	X^2 +	6.079E-02	X	+	-1.818E-01	0.9987

BZDG1 in Blood

Drug: α-OH-Midazolam

Unit: ng/mL



		Symmetry, h [Concentration]	Vertex, k [Area Ratio]	Focus [Area Ratio]		Directrix [Area Ratio]
Trial 1	x=	1.58E+03	4.39E+01	-1.42E+04	y=	1.42E+04
Trial 2	x=	1.38E+03	3.86E+01	-1.22E+04	y=	1.23E+04
Trial 3	x=	1.18E+03	3.49E+01	-9.84E+03	y=	9.91E+03
Trial 4	x=	2.05E+03	5.67E+01	-1.86E+04	y=	1.87E+04
Trial 5	x=	1.00E+03	3.03E+01	-8.22E+03	y=	8.28E+03

Drug: α-OH-Midazolam
Unit: ng/mL

Trial 1	x	y	w (1/x)	x^2	x^3	x^4	xy	x^2y	$(y\text{-}ybar)^2$
L1	10.000	5.23E-01	1.00E-01	1.00E+02	1.00E+03	1.00E+04	5.23E+00	5.23E+01	5.29E+01
L2	20.000	1.09E+00	5.00E-02	4.00E+02	8.00E+03	1.60E+05	2.18E+01	4.36E+02	4.50E+01
L3	50.000	2.76E+00	2.00E-02	2.50E+03	1.25E+05	6.25E+06	1.38E+02	6.89E+03	2.54E+01
L4	100.000	5.43E+00	1.00E-02	1.00E+04	1.00E+06	1.00E+08	5.43E+02	5.43E+04	5.59E+00
L5	200.000	1.03E+01	5.00E-03	4.00E+04	8.00E+06	1.60E+09	2.05E+03	4.11E+05	6.15E+00
L6	300.000	1.50E+01	3.33E-03	9.00E+04	2.70E+07	8.10E+09	4.50E+03	1.35E+06	5.18E+01
L7	400.000	1.95E+01	2.50E-03	1.60E+05	6.40E+07	2.56E+10	7.80E+03	3.12E+06	1.37E+02
7	1.08E+03	5.46E+01	1.91E-01	3.03E+05	1.00E+08	3.54E+10	1.51E+04	4.94E+06	3.24E+02

wx	wy	wx^2	wx^3	wx^4	wxy	wx^2y	$(y\text{-}ybarw)^2$
1.00E+00	5.23E-02	1.00E+01	1.00E+02	1.00E+03	5.23E-01	5.23E+00	1.95E+00
1.00E+00	5.45E-02	2.00E+01	4.00E+02	8.00E+03	1.09E+00	2.18E+01	6.89E-01
1.00E+00	5.51E-02	5.00E+01	2.50E+03	1.25E+05	2.76E+00	1.38E+02	6.98E-01
1.00E+00	5.43E-02	1.00E+02	1.00E+04	1.00E+06	5.43E+00	5.43E+02	1.23E+01
1.00E+00	5.14E-02	2.00E+02	4.00E+04	8.00E+06	1.03E+01	2.05E+03	6.98E+01
1.00E+00	5.00E-02	3.00E+02	9.00E+04	2.70E+07	1.50E+01	4.50E+03	1.71E+02
1.00E+00	4.87E-02	4.00E+02	1.60E+05	6.40E+07	1.95E+01	7.80E+03	3.09E+02
7.00E+00	3.66E-01	1.08E+03	3.03E+05	1.00E+08	5.46E+01	1.51E+04	

Equal

A=	3.54E+10	1.00E+08	3.03E+05	B =	4.94E+06	X =	**-1.443E-05**	a
	1.00E+08	3.03E+05	1.08E+03		1.51E+04		**5.435E-02**	b
	3.03E+05	1.08E+03	7.00E+00		5.46E+01		**3.426E-02**	c
A^{-1} =	7.18E-10	-2.81E-07	1.23E-05					
	-2.81E-07	1.17E-04	-5.94E-03				**0.9999**	R^2
	1.23E-05	-5.94E-03	5.28E-01					

Weighted

A =	1.00E+08	3.03E+05	1.08E+03	B =	1.51E+04	X =	**-1.761E-05**	a
	3.03E+05	1.08E+03	7.00E+00		5.46E+01		**5.559E-02**	b
	1.08E+03	7.00E+00	1.91E-01		3.66E-01		**-2.011E-02**	c
A^{-1} =	1.03E-07	-3.28E-05	6.23E-04					
	-3.28E-05	1.17E-02	-2.44E-01				**0.9999**	R^2
	6.23E-04	-2.44E-01	1.07E+01					

Standard			w (1/x)			Equal			
Residual	y-yhat	Target	Calculated	yhat	Bias	y-yhat	Calculated	Response	Bias
-0.1402	-0.0109	10.000	**9.803**	5.34E-01	**-1.97%**	-0.0532	**9.017**	5.23E-01	**-9.83%**
0.0611	0.0048	20.000	**20.087**	1.08E+00	**0.43%**	-0.0260	**19.516**	1.09E+00	**-2.42%**
0.5081	0.0395	50.000	**50.734**	2.72E+00	**1.47%**	0.0394	**50.745**	2.76E+00	**1.49%**
0.8777	0.0683	100.000	**101.312**	5.36E+00	**1.31%**	0.1065	**102.071**	5.43E+00	**2.07%**
-1.5454	-0.1202	200.000	**197.526**	1.04E+01	**-1.24%**	-0.0529	**198.911**	1.03E+01	**-0.54%**
-1.0056	-0.0782	300.000	**298.264**	1.51E+01	**-0.58%**	-0.0454	**299.006**	1.50E+01	**-0.33%**
1.2444	0.0968	400.000	**402.335**	1.94E+01	**0.58%**	0.0316	**400.739**	1.95E+01	**0.18%**
					7.59%				16.87%

Drug: α-OH-Midazolam
Unit: ng/mL

Trial 2	x	y	w (1/x)	x^2	x^3	x^4	xy	x^2y	$(y-ybar)^2$
L1	10.000	5.48E-01		1.00E+02	1.00E+03	1.00E+04	5.48E+00	5.48E+01	5.20E+01
L2	20.000	1.08E+00		4.00E+02	8.00E+03	1.60E+05	2.15E+01	4.31E+02	4.46E+01
L3	50.000	2.73E+00		2.50E+03	1.25E+05	6.25E+06	1.36E+02	6.82E+03	2.53E+01
L4	100.000	5.40E+00		1.00E+04	1.00E+06	1.00E+08	5.40E+02	5.40E+04	5.57E+00
L5	200.000	1.05E+01		4.00E+04	8.00E+06	1.60E+09	2.09E+03	4.18E+05	7.29E+00
L6	300.000	1.49E+01		9.00E+04	2.70E+07	8.10E+09	4.47E+03	1.34E+06	5.08E+01
L7	400.000	1.92E+01		1.60E+05	6.40E+07	2.56E+10	7.69E+03	3.07E+06	1.31E+02
7	1.08E+03	5.43E+01		3.03E+05	1.00E+08	3.54E+10	1.49E+04	4.89E+06	3.17E+02

wx	wy	wx^2	wx^3	wx^4	wxy	wx^2y	$(y-ybarw)^2$
1.00E+00	5.48E-02	1.00E+01	1.00E+02	1.00E+03	5.48E-01	5.48E+00	1.89E+00
1.00E+00	5.38E-02	2.00E+01	4.00E+02	8.00E+03	1.08E+00	2.15E+01	7.18E-01
1.00E+00	5.46E-02	5.00E+01	2.50E+03	1.25E+05	2.73E+00	1.36E+02	6.48E-01
1.00E+00	5.40E-02	1.00E+02	1.00E+04	1.00E+06	5.40E+00	5.40E+02	1.21E+01
1.00E+00	5.23E-02	2.00E+02	4.00E+04	8.00E+06	1.05E+01	2.09E+03	7.28E+01
1.00E+00	4.96E-02	3.00E+02	9.00E+04	2.70E+07	1.49E+01	4.47E+03	1.68E+02
1.00E+00	4.80E-02	4.00E+02	1.60E+05	6.40E+07	1.92E+01	7.69E+03	2.99E+02
7.00E+00	3.67E-01	1.08E+03	3.03E+05	1.00E+08	5.43E+01	1.49E+04	

Equal

A=	3.54E+10	1.00E+08	3.03E+05	B =	4.89E+06	X =	**-2.019E-05**	a
	1.00E+08	3.03E+05	1.08E+03		1.49E+04		**5.606E-02**	b
	3.03E+05	1.08E+03	7.00E+00		5.43E+01		**-1.647E-02**	c
A^{-1} =	7.18E-10	-2.81E-07	1.23E-05					
	-2.81E-07	1.17E-04	-5.94E-03				**0.9999**	R^2
	1.23E-05	-5.94E-03	5.28E-01					

Weighted

A =	1.00E+08	3.03E+05	1.08E+03	B =	1.49E+04	X =	**-2.041E-05**	a
	3.03E+05	1.08E+03	7.00E+00		5.43E+01		**5.614E-02**	b
	1.08E+03	7.00E+00	1.91E-01		3.67E-01		**-2.023E-02**	c
A^{-1} =	1.03E-07	-3.28E-05	6.23E-04					
	-3.28E-05	1.17E-02	-2.44E-01				**1.0000**	R^2
	6.23E-04	-2.44E-01	1.07E+01					

Standard Residual	y-yhat	Target	w (1/x) Calculated	yhat	Bias	Equal y-yhat	Calculated	Response	Bias
0.1606	0.0084787	10.000	**10.152**	5.39E-01	**1.52%**	0.0056	**10.100**	5.48E-01	**1.00%**
-0.3406	-0.017984	20.000	**19.675**	1.09E+00	**-1.63%**	-0.0201	**19.636**	1.08E+00	**-1.82%**
-0.1340	-0.007074	50.000	**49.869**	2.74E+00	**-0.26%**	-0.0071	**49.869**	2.73E+00	**-0.26%**
0.1742	0.0091984	100.000	**100.177**	5.39E+00	**0.18%**	0.0118	**100.228**	5.40E+00	**0.23%**
1.2578	0.0664037	200.000	**201.385**	1.04E+01	**0.69%**	0.0711	**201.482**	1.05E+01	**0.74%**
-1.9012	-0.100372	300.000	**297.716**	1.50E+01	**-0.76%**	-0.0981	**297.770**	1.49E+01	**-0.74%**
0.7832	0.041349	400.000	**401.039**	1.92E+01	**0.26%**	0.0368	**400.923**	1.92E+01	**0.23%**
					5.30%				5.02%

Drug: α-OH-Midazolam
Unit: ng/mL

Trial 3	x	y	w (1/x)	x^2	x^3	x^4	xy	x^2y	$(y-ybar)^2$
L1	10.000	4.70E-01		1.00E+02	1.00E+03	1.00E+04	4.70E+00	4.70E+01	5.62E+01
L2	20.000	1.05E+00		4.00E+02	8.00E+03	1.60E+05	2.09E+01	4.18E+02	4.79E+01
L3	50.000	2.79E+00		2.50E+03	1.25E+05	6.25E+06	1.39E+02	6.97E+03	2.68E+01
L4	100.000	5.52E+00		1.00E+04	1.00E+06	1.00E+08	5.52E+02	5.52E+04	5.97E+00
L5	200.000	1.08E+01		4.00E+04	8.00E+06	1.60E+09	2.16E+03	4.33E+05	8.14E+00
L6	300.000	1.55E+01		9.00E+04	2.70E+07	8.10E+09	4.66E+03	1.40E+06	5.71E+01
L7	400.000	1.96E+01		1.60E+05	6.40E+07	2.56E+10	7.83E+03	3.13E+06	1.35E+02
7	1.08E+03	5.57E+01		3.03E+05	1.00E+08	3.54E+10	1.54E+04	5.03E+06	3.37E+02

	wx	wy		wx^2	wx^3	wx^4	wxy	wx^2y	$(y-ybarw)^2$
	1.00E+00	4.70E-02		1.00E+01	1.00E+02	1.00E+03	4.70E-01	4.70E+00	2.08E+00
	1.00E+00	5.23E-02		2.00E+01	4.00E+02	8.00E+03	1.05E+00	2.09E+01	7.52E-01
	1.00E+00	5.58E-02		5.00E+01	2.50E+03	1.25E+05	2.79E+00	1.39E+02	7.67E-01
	1.00E+00	5.52E-02		1.00E+02	1.00E+04	1.00E+06	5.52E+00	5.52E+02	1.30E+01
	1.00E+00	5.41E-02		2.00E+02	4.00E+04	8.00E+06	1.08E+01	2.16E+03	7.93E+01
	1.00E+00	5.17E-02		3.00E+02	9.00E+04	2.70E+07	1.55E+01	4.66E+03	1.85E+02
	1.00E+00	4.90E-02		4.00E+02	1.60E+05	6.40E+07	1.96E+01	7.83E+03	3.12E+02
	7.00E+00	3.65E-01		1.08E+03	3.03E+05	1.00E+08	5.57E+01	1.54E+04	

Equal

A=	3.54E+10	1.00E+08	3.03E+05	B =	5.03E+06	X =	**-2.686E-05**	**a**	
	1.00E+08	3.03E+05	1.08E+03		1.54E+04		**6.016E-02**	**b**	
	3.03E+05	1.08E+03	7.00E+00		5.57E+01		**-1.547E-01**	**c**	
A^{-1} =	7.18E-10	-2.81E-07	1.23E-05						
	-2.81E-07	1.17E-04	-5.94E-03				**1.0000**	**R^2**	
	1.23E-05	-5.94E-03	5.28E-01						

Weighted

A =	1.00E+08	3.03E+05	1.08E+03	B =	1.54E+04	X =	**-2.532E-05**	**a**	
	3.03E+05	1.08E+03	7.00E+00		5.57E+01		**5.956E-02**	**b**	
	1.08E+03	7.00E+00	1.91E-01		3.65E-01		**-1.284E-01**	**c**	
A^{-1} =	1.03E-07	-3.28E-05	6.23E-04						
	-3.28E-05	1.17E-02	-2.44E-01				**1.0000**	**R^2**	
	6.23E-04	-2.44E-01	1.07E+01						

Standard Residual	y-yhat	Target	w (1/x) Calculated	yhat	Bias	Equal y-yhat	Calculated	Response	Bias
0.1171	0.0052	10.000	**10.088**	4.65E-01	**0.88%**	0.0257	**10.431**	4.70E-01	**4.31%**
-0.1527	-0.0068	20.000	**19.884**	1.05E+00	**-0.58%**	0.0081	**20.137**	1.05E+00	**0.69%**
0.0528	0.0023	50.000	**50.041**	2.79E+00	**0.08%**	0.0024	**50.042**	2.79E+00	**0.08%**
-1.1856	-0.0528	100.000	**99.031**	5.57E+00	**-0.97%**	-0.0713	**98.699**	5.52E+00	**-1.30%**
1.0715	0.0477	200.000	**200.966**	1.08E+01	**0.48%**	0.0151	**200.306**	1.08E+01	**0.15%**
1.3526	0.0602	300.000	**301.359**	1.55E+01	**0.45%**	0.0444	**301.008**	1.55E+01	**0.34%**
-1.2557	-0.0559	400.000	**398.578**	1.96E+01	**-0.36%**	-0.0243	**399.371**	1.96E+01	**-0.16%**
					3.81%				7.02%

Drug: α-OH-Midazolam
Unit: ng/mL

Trial 4	x	y	w (1/x)	x^2	x^3	x^4	xy	x^2y	$(y-ybar)^2$
L1	10.000	5.70E-01		1.00E+02	1.00E+03	1.00E+04	5.70E+00	5.70E+01	5.46E+01
L2	20.000	1.16E+00		4.00E+02	8.00E+03	1.60E+05	2.31E+01	4.63E+02	4.63E+01
L3	50.000	2.80E+00		2.50E+03	1.25E+05	6.25E+06	1.40E+02	6.99E+03	2.66E+01
L4	100.000	5.25E+00		1.00E+04	1.00E+06	1.00E+08	5.25E+02	5.25E+04	7.32E+00
L5	200.000	1.07E+01		4.00E+04	8.00E+06	1.60E+09	2.14E+03	4.27E+05	7.39E+00
L6	300.000	1.53E+01		9.00E+04	2.70E+07	8.10E+09	4.60E+03	1.38E+06	5.44E+01
L7	400.000	1.99E+01		1.60E+05	6.40E+07	2.56E+10	7.97E+03	3.19E+06	1.43E+02
7	1.08E+03	5.57E+01		3.03E+05	1.00E+08	3.54E+10	1.54E+04	5.05E+06	3.40E+02

	wx	wy		wx^2	wx^3	wx^4	wxy	wx^2y	$(y-ybarw)^2$
	1.00E+00	5.70E-02		1.00E+01	1.00E+02	1.00E+03	5.70E-01	5.70E+00	1.99E+00
	1.00E+00	5.78E-02		2.00E+01	4.00E+02	8.00E+03	1.16E+00	2.31E+01	6.75E-01
	1.00E+00	5.59E-02		5.00E+01	2.50E+03	1.25E+05	2.80E+00	1.40E+02	6.70E-01
	1.00E+00	5.25E-02		1.00E+02	1.00E+04	1.00E+06	5.25E+00	5.25E+02	1.07E+01
	1.00E+00	5.34E-02		2.00E+02	4.00E+04	8.00E+06	1.07E+01	2.14E+03	7.57E+01
	1.00E+00	5.11E-02		3.00E+02	9.00E+04	2.70E+07	1.53E+01	4.60E+03	1.78E+02
	1.00E+00	4.98E-02		4.00E+02	1.60E+05	6.40E+07	1.99E+01	7.97E+03	3.22E+02
	7.00E+00	3.78E-01		1.08E+03	3.03E+05	1.00E+08	5.57E+01	1.54E+04	

Equal

A=	3.54E+10	1.00E+08	3.03E+05	B =	5.05E+06	X =	**-1.435E-05**	**a**	
	1.00E+08	3.03E+05	1.08E+03		1.54E+04		**5.550E-02**	**b**	
	3.03E+05	1.08E+03	7.00E+00		5.57E+01		**1.614E-02**	**c**	
A^{-1} =	7.18E-10	-2.81E-07	1.23E-05						
	-2.81E-07	1.17E-04	-5.94E-03				**0.9998**	R^2	
	1.23E-05	-5.94E-03	5.28E-01						

Weighted

A =	1.00E+08	3.03E+05	1.08E+03	B =	1.54E+04	X =	**-1.342E-05**	**a**	
	3.03E+05	1.08E+03	7.00E+00		5.57E+01		**5.514E-02**	**b**	
	1.08E+03	7.00E+00	1.91E-01		3.78E-01		**3.190E-02**	**c**	
A^{-1} =	1.03E-07	-3.28E-05	6.23E-04						
	-3.28E-05	1.17E-02	-2.44E-01				**0.9998**	R^2	
	6.23E-04	-2.44E-01	1.07E+01						

Standard Residual	y-yhat	Target	w (1/x) Calculated	yhat	Bias	Equal y-yhat	Calculated	Response	Bias
-0.1310	-0.0123	10.000	**9.775**	5.82E-01	**-2.25%**	-0.0001	**9.999**	5.70E-01	**-0.01%**
0.2933	0.0276	20.000	**20.505**	1.13E+00	**2.53%**	0.0365	**20.665**	1.16E+00	**3.32%**
0.4407	0.0414	50.000	**50.770**	2.76E+00	**1.54%**	0.0415	**50.767**	2.80E+00	**1.53%**
-1.6892	-0.1589	100.000	**96.974**	5.41E+00	**-3.03%**	-0.1699	**96.774**	5.25E+00	**-3.23%**
1.6384	0.1541	200.000	**203.098**	1.05E+01	**1.55%**	0.1346	**202.706**	1.07E+01	**1.35%**
-0.3552	-0.0334	300.000	**299.291**	1.54E+01	**-0.24%**	-0.0429	**299.085**	1.53E+01	**-0.30%**
-0.1970	-0.0185	400.000	**399.583**	1.99E+01	**-0.10%**	0.0004	**400.009**	1.99E+01	**0.00%**
					11.23%				9.76%

Drug: α-OH-Midazolam
Unit: ng/mL

Trial 5	x	y	w (1/x)	x^2	x^3	x^4	xy	x^2y	$(y-ybar)^2$
L1	10.000	4.12E-01		1.00E+02	1.00E+03	1.00E+04	4.12E+00	4.12E+01	5.59E+01
L2	20.000	1.13E+00		4.00E+02	8.00E+03	1.60E+05	2.25E+01	4.51E+02	4.57E+01
L3	50.000	2.51E+00		2.50E+03	1.25E+05	6.25E+06	1.25E+02	6.26E+03	2.89E+01
L4	100.000	5.62E+00		1.00E+04	1.00E+06	1.00E+08	5.62E+02	5.62E+04	5.11E+00
L5	200.000	1.11E+01		4.00E+04	8.00E+06	1.60E+09	2.22E+03	4.44E+05	1.03E+01
L6	300.000	1.52E+01		9.00E+04	2.70E+07	8.10E+09	4.55E+03	1.37E+06	5.33E+01
L7	400.000	1.93E+01		1.60E+05	6.40E+07	2.56E+10	7.70E+03	3.08E+06	1.29E+02
7	1.08E+03	5.52E+01		3.03E+05	1.00E+08	3.54E+10	1.52E+04	4.95E+06	3.28E+02

	wx	wy		wx^2	wx^3	wx^4	wxy	wx^2y	$(y-ybarw)^2$
	1.00E+00	4.12E-02		1.00E+01	1.00E+02	1.00E+03	4.12E-01	4.12E+00	2.15E+00
	1.00E+00	5.63E-02		2.00E+01	4.00E+02	8.00E+03	1.13E+00	2.25E+01	5.62E-01
	1.00E+00	5.01E-02		5.00E+01	2.50E+03	1.25E+05	2.51E+00	1.25E+02	3.95E-01
	1.00E+00	5.62E-02		1.00E+02	1.00E+04	1.00E+06	5.62E+00	5.62E+02	1.40E+01
	1.00E+00	5.55E-02		2.00E+02	4.00E+04	8.00E+06	1.11E+01	2.22E+03	8.50E+01
	1.00E+00	5.06E-02		3.00E+02	9.00E+04	2.70E+07	1.52E+01	4.55E+03	1.77E+02
	1.00E+00	4.81E-02		4.00E+02	1.60E+05	6.40E+07	1.93E+01	7.70E+03	3.02E+02
	7.00E+00	3.58E-01		1.08E+03	3.03E+05	1.00E+08	5.52E+01	1.52E+04	

Equal

A=	3.54E+10	1.00E+08	3.03E+05	B =	4.95E+06	X =	**-3.446E-05**	**a**	
	1.00E+08	3.03E+05	1.08E+03		1.52E+04		**6.242E-02**	**b**	
	3.03E+05	1.08E+03	7.00E+00		5.52E+01		**-2.531E-01**	**c**	
A^{-1} =	7.18E-10	-2.81E-07	1.23E-05						
	-2.81E-07	1.17E-04	-5.94E-03				**0.9994**	R^2	
	1.23E-05	-5.94E-03	5.28E-01						

Weighted

A =	1.00E+08	3.03E+05	1.08E+03	B =	1.52E+04	X =	**-3.030E-05**	**a**	
	3.03E+05	1.08E+03	7.00E+00		5.52E+01		**6.079E-02**	**b**	
	1.08E+03	7.00E+00	1.91E-01		3.58E-01		**-1.818E-01**	**c**	
A^{-1} =	1.03E-07	-3.28E-05	6.23E-04						
	-3.28E-05	1.17E-02	-2.44E-01				**0.9987**	R^2	
	6.23E-04	-2.44E-01	1.07E+01						

Standard Residual	y-yhat	Target	w (1/x) Calculated	yhat	Bias	Equal y-yhat	Calculated	Response	Bias
-0.0586	-0.0113	10.000	**9.813**	4.23E-01	**-1.87%**	0.0441	**10.714**	4.12E-01	**7.14%**
0.5477	0.1052	20.000	**21.767**	1.02E+00	**8.84%**	0.1455	**22.387**	1.13E+00	**11.93%**
-1.4393	-0.2765	50.000	**45.225**	2.78E+00	**-9.55%**	-0.2763	**45.327**	2.51E+00	**-9.35%**
0.1586	0.0305	100.000	**100.557**	5.59E+00	**0.56%**	-0.0196	**99.647**	5.62E+00	**-0.35%**
1.7313	0.3326	200.000	**206.862**	1.08E+01	**3.43%**	0.2444	**205.044**	1.11E+01	**2.52%**
-0.7534	-0.1447	300.000	**296.612**	1.53E+01	**-1.13%**	-0.1877	**295.520**	1.52E+01	**-1.49%**
-0.1863	-0.0358	400.000	**399.022**	1.93E+01	**-0.24%**	0.0496	**401.426**	1.93E+01	**0.36%**
					25.62%				33.15%

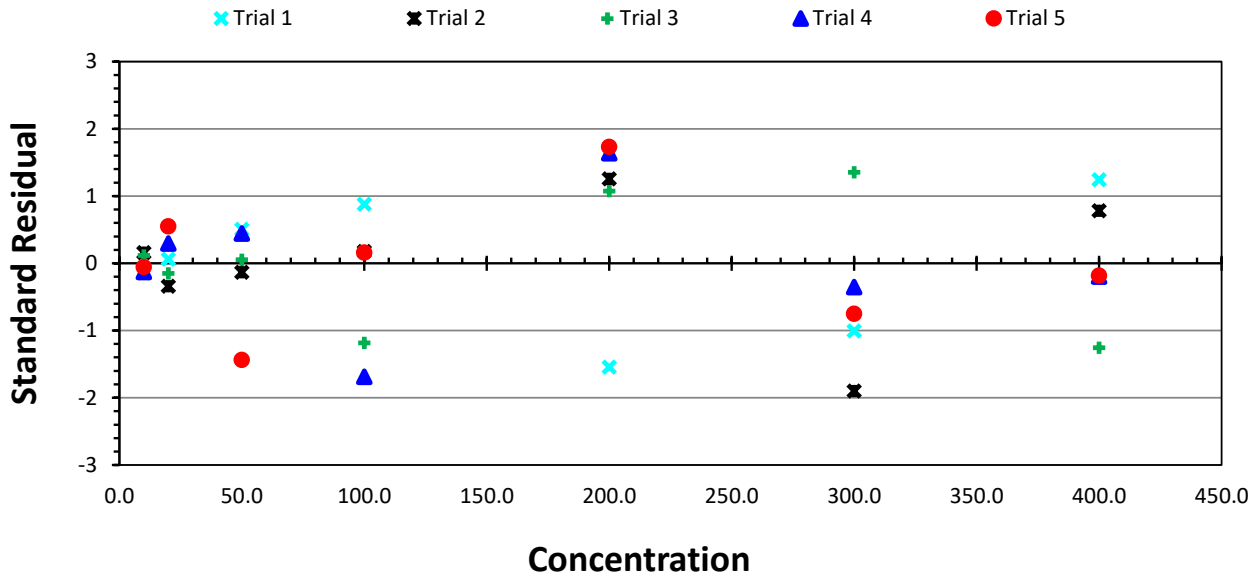
BZDG1 in Blood

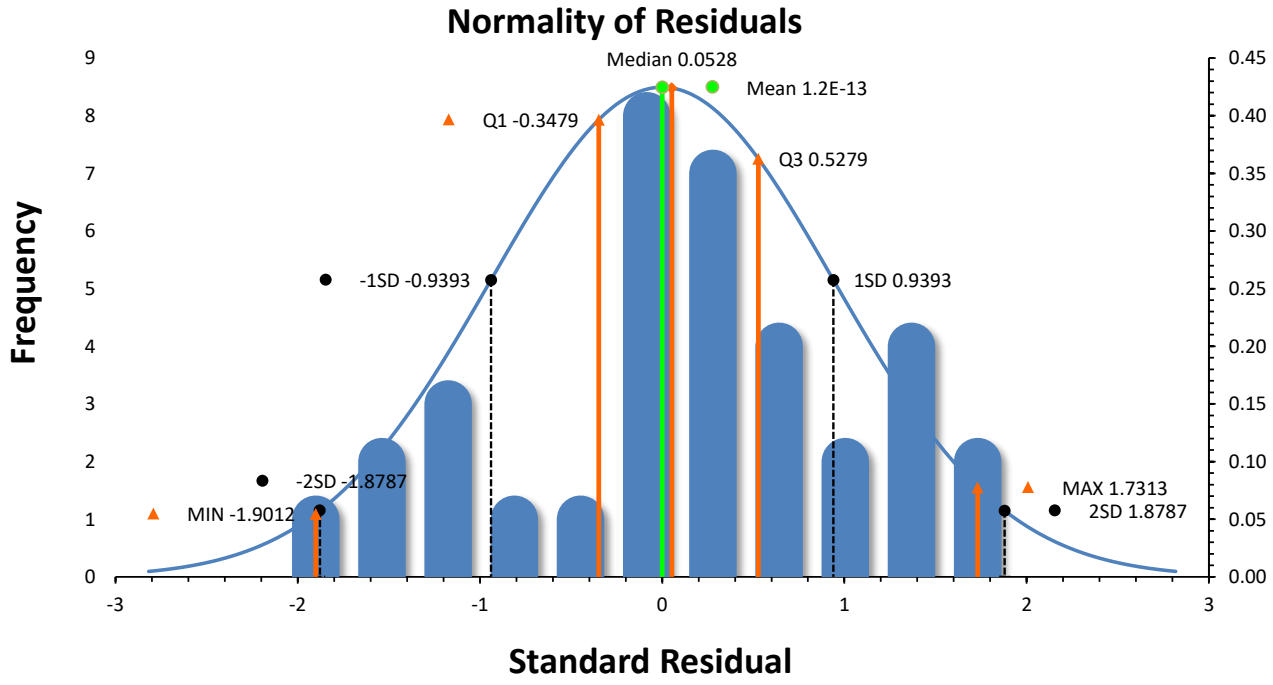
Drug: α-OH-Midazolam

Unit: ng/mL

Calibration Model: Quadratic (1/x)

Toxicologist(s): JD1 JD2 JD3 JD4 JD5 **Instrument:** LC-MS/MS

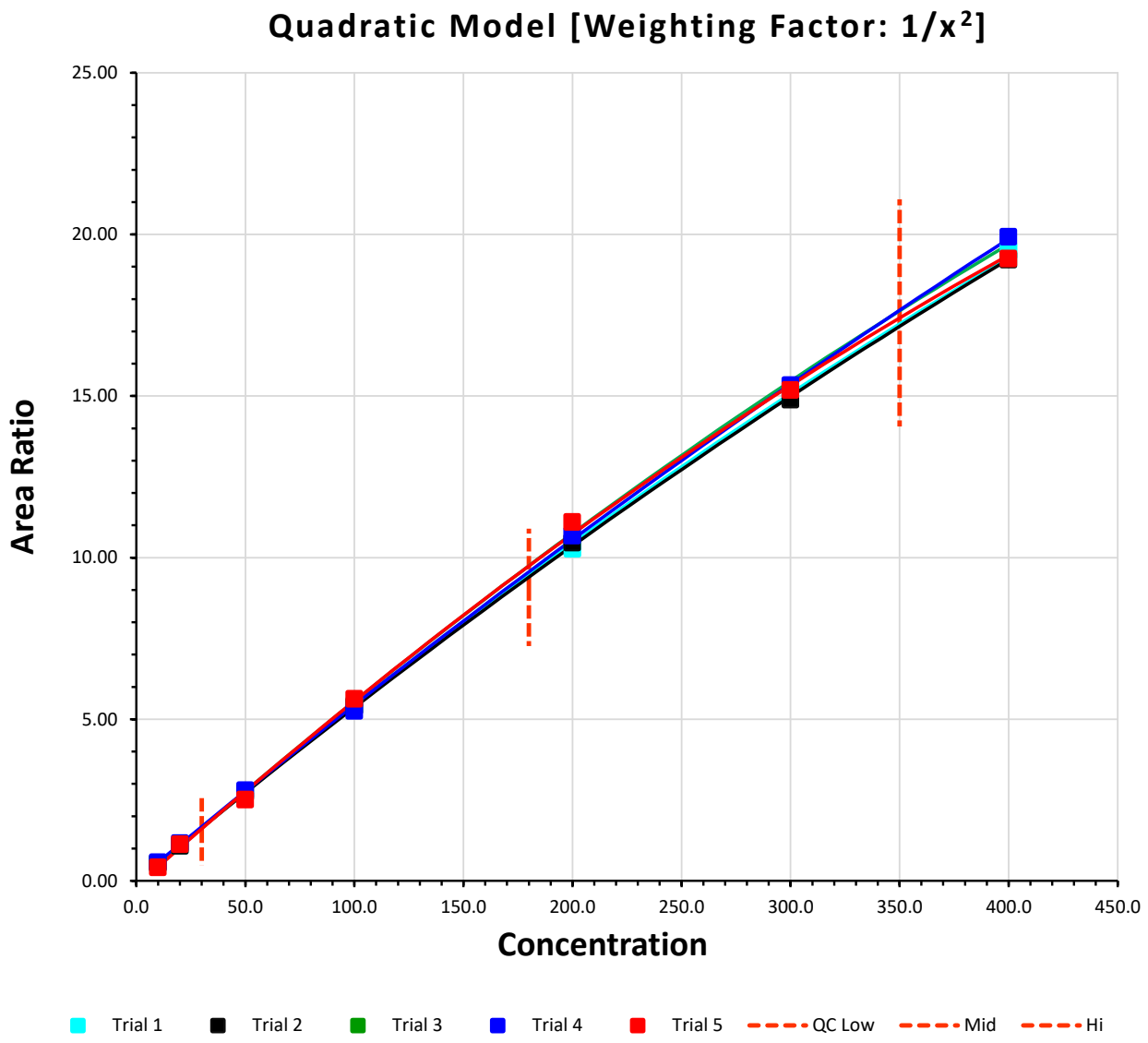




BZDG1 in Blood

Drug: α-OH-Midazolam

Unit: ng/mL

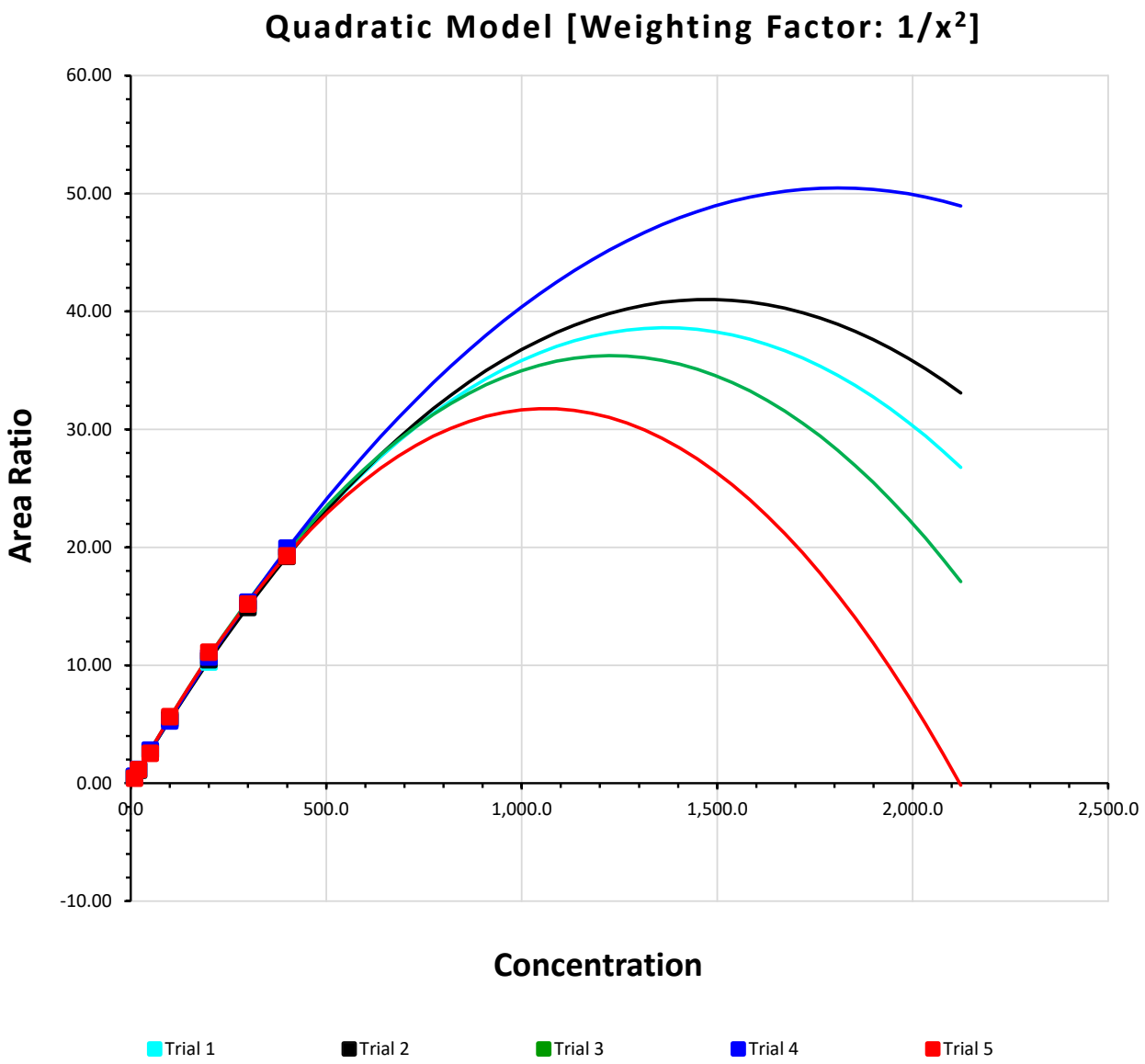


	a		b			c	R^2
Trial 1	-2.070E-05	X^2 +	5.658E-02	X	+	-3.889E-02	0.9999
Trial 2	-1.887E-05	X^2 +	5.565E-02	X	+	-1.089E-02	0.9999
Trial 3	-2.404E-05	X^2 +	5.915E-02	X	+	-1.206E-01	1.0000
Trial 4	-1.542E-05	X^2 +	5.578E-02	X	+	1.979E-02	0.9994
Trial 5	-2.834E-05	X^2 +	6.016E-02	X	+	-1.699E-01	0.9949

BZDG1 in Blood

Drug: **α-OH-Midazolam**

Unit: **ng/mL**



		Symmetry, h [Concentration]	Vertex, k [Area Ratio]	Focus [Area Ratio]		Directrix [Area Ratio]
Trial 1	x=	1.37E+03	3.86E+01	-1.20E+04	y=	1.21E+04
Trial 2	x=	1.47E+03	4.10E+01	-1.32E+04	y=	1.33E+04
Trial 3	x=	1.23E+03	3.63E+01	-1.04E+04	y=	1.04E+04
Trial 4	x=	1.81E+03	5.05E+01	-1.62E+04	y=	1.63E+04
Trial 5	x=	1.06E+03	3.18E+01	-8.79E+03	y=	8.85E+03

Drug: α-OH-Midazolam
Unit: ng/mL

Trial 1	x	y	w (1/x²)	x²	x³	x⁴	xy	x²y	(y-ybar)²
L1	10.000	5.23E-01	1.00E-02	1.00E+02	1.00E+03	1.00E+04	5.23E+00	5.23E+01	5.29E+01
L2	20.000	1.09E+00	2.50E-03	4.00E+02	8.00E+03	1.60E+05	2.18E+01	4.36E+02	4.50E+01
L3	50.000	2.76E+00	4.00E-04	2.50E+03	1.25E+05	6.25E+06	1.38E+02	6.89E+03	2.54E+01
L4	100.000	5.43E+00	1.00E-04	1.00E+04	1.00E+06	1.00E+08	5.43E+02	5.43E+04	5.59E+00
L5	200.000	1.03E+01	2.50E-05	4.00E+04	8.00E+06	1.60E+09	2.05E+03	4.11E+05	6.15E+00
L6	300.000	1.50E+01	1.11E-05	9.00E+04	2.70E+07	8.10E+09	4.50E+03	1.35E+06	5.18E+01
L7	400.000	1.95E+01	6.25E-06	1.60E+05	6.40E+07	2.56E+10	7.80E+03	3.12E+06	1.37E+02
7	1.08E+03	5.46E+01	1.30E-02	3.03E+05	1.00E+08	3.54E+10	1.51E+04	4.94E+06	3.24E+02

wx	wy	wx²	wx³	wx⁴	wxy	wx²y	(y-ybarw)²
1.00E-01	5.23E-03	1.00E+00	1.00E+01	1.00E+02	5.23E-02	5.23E-01	6.49E-02
5.00E-02	2.72E-03	1.00E+00	2.00E+01	4.00E+02	5.45E-02	1.09E+00	9.71E-02
2.00E-02	1.10E-03	1.00E+00	5.00E+01	2.50E+03	5.51E-02	2.76E+00	3.91E+00
1.00E-02	5.43E-04	1.00E+00	1.00E+02	1.00E+04	5.43E-02	5.43E+00	2.17E+01
5.00E-03	2.57E-04	1.00E+00	2.00E+02	4.00E+04	5.14E-02	1.03E+01	9.02E+01
3.33E-03	1.67E-04	1.00E+00	3.00E+02	9.00E+04	5.00E-02	1.50E+01	2.02E+02
2.50E-03	1.22E-04	1.00E+00	4.00E+02	1.60E+05	4.87E-02	1.95E+01	3.50E+02
1.91E-01	1.01E-02	7.00E+00	1.08E+03	3.03E+05	3.66E-01	5.46E+01	

Equal

A=	3.54E+10	1.00E+08	3.03E+05	B =	4.94E+06	X =	**-1.443E-05**	**a**
	1.00E+08	3.03E+05	1.08E+03		1.51E+04		**5.435E-02**	**b**
	3.03E+05	1.08E+03	7.00E+00		5.46E+01		**3.426E-02**	**c**
A^{-1} =	7.18E-10	-2.81E-07	1.23E-05					
	-2.81E-07	1.17E-04	-5.94E-03				**0.9999**	**R^2**
	1.23E-05	-5.94E-03	5.28E-01					

Weighted

A =	3.03E+05	1.08E+03	7.00E+00	B =	5.46E+01	X =	**-2.070E-05**	**a**
	1.08E+03	7.00E+00	1.91E-01		3.66E-01		**5.658E-02**	**b**
	7.00E+00	1.91E-01	1.30E-02		1.01E-02		**-3.889E-02**	**c**
A^{-1} =	1.39E-05	-3.22E-03	3.97E-02					
	-3.22E-03	9.86E-01	-1.27E+01				**0.9999**	**R^2**
	3.97E-02	-1.27E+01	2.41E+02					

Standard Residual	w (1/x²) y-yhat	Target	Calculated	yhat	Bias	Equal y-yhat	Calculated	Response	Bias
-0.0145	-0.0017	10.000	**9.970**	5.25E-01	**-0.30%**	-0.0532	**9.017**	5.23E-01	**-9.83%**
0.0423	0.0050	20.000	**20.090**	1.08E+00	**0.45%**	-0.0260	**19.516**	1.09E+00	**-2.42%**
0.1406	0.0166	50.000	**50.305**	2.74E+00	**0.61%**	0.0394	**50.745**	2.76E+00	**1.49%**
0.1618	0.0191	100.000	**100.364**	5.41E+00	**0.36%**	0.1065	**102.071**	5.43E+00	**2.07%**
-1.4874	-0.1756	200.000	**196.371**	1.04E+01	**-1.81%**	-0.0529	**198.911**	1.03E+01	**-0.54%**
-0.6603	-0.0779	300.000	**298.237**	1.51E+01	**-0.59%**	-0.0454	**299.006**	1.50E+01	**-0.33%**
1.8176	0.2145	400.000	**405.376**	1.93E+01	**1.34%**	0.0316	**400.739**	1.95E+01	**0.18%**
					5.47%				16.87%

Drug: α-OH-Midazolam
Unit: ng/mL

Trial 2	x	y	w (1/x²)	x²	x³	x⁴	xy	x²y	(y-ybar)²
L1	10.000	5.48E-01		1.00E+02	1.00E+03	1.00E+04	5.48E+00	5.48E+01	5.20E+01
L2	20.000	1.08E+00		4.00E+02	8.00E+03	1.60E+05	2.15E+01	4.31E+02	4.46E+01
L3	50.000	2.73E+00		2.50E+03	1.25E+05	6.25E+06	1.36E+02	6.82E+03	2.53E+01
L4	100.000	5.40E+00		1.00E+04	1.00E+06	1.00E+08	5.40E+02	5.40E+04	5.57E+00
L5	200.000	1.05E+01		4.00E+04	8.00E+06	1.60E+09	2.09E+03	4.18E+05	7.29E+00
L6	300.000	1.49E+01		9.00E+04	2.70E+07	8.10E+09	4.47E+03	1.34E+06	5.08E+01
L7	400.000	1.92E+01		1.60E+05	6.40E+07	2.56E+10	7.69E+03	3.07E+06	1.31E+02
7	1.08E+03	5.43E+01		3.03E+05	1.00E+08	3.54E+10	1.49E+04	4.89E+06	3.17E+02

wx	wy	wx²	wx³	wx⁴	wxy	wx²y	(y-ybarw)²
1.00E-01	5.48E-03	1.00E+00	1.00E+01	1.00E+02	5.48E-02	5.48E-01	6.03E-02
5.00E-02	2.69E-03	1.00E+00	2.00E+01	4.00E+02	5.38E-02	1.08E+00	8.02E-02
2.00E-02	1.09E-03	1.00E+00	5.00E+01	2.50E+03	5.46E-02	2.73E+00	3.75E+00
1.00E-02	5.40E-04	1.00E+00	1.00E+02	1.00E+04	5.40E-02	5.40E+00	2.12E+01
5.00E-03	2.61E-04	1.00E+00	2.00E+02	4.00E+04	5.23E-02	1.05E+01	9.34E+01
3.33E-03	1.65E-04	1.00E+00	3.00E+02	9.00E+04	4.96E-02	1.49E+01	1.99E+02
2.50E-03	1.20E-04	1.00E+00	4.00E+02	1.60E+05	4.80E-02	1.92E+01	3.39E+02
1.91E-01	1.03E-02	7.00E+00	1.08E+03	3.03E+05	3.67E-01	5.43E+01	

Equal

A=	3.54E+10	1.00E+08	3.03E+05	B =	4.89E+06	X =	**-2.019E-05**	a
	1.00E+08	3.03E+05	1.08E+03		1.49E+04		**5.606E-02**	b
	3.03E+05	1.08E+03	7.00E+00		5.43E+01		**-1.647E-02**	c
A^{-1} =	7.18E-10	-2.81E-07	1.23E-05					
	-2.81E-07	1.17E-04	-5.94E-03				**0.9999**	R^2
	1.23E-05	-5.94E-03	5.28E-01					

Weighted

A =	3.03E+05	1.08E+03	7.00E+00	B =	5.43E+01	X =	**-1.887E-05**	a
	1.08E+03	7.00E+00	1.91E-01		3.67E-01		**5.565E-02**	b
	7.00E+00	1.91E-01	1.30E-02		1.03E-02		**-1.089E-02**	c
A^{-1} =	1.39E-05	-3.22E-03	3.97E-02					
	-3.22E-03	9.86E-01	-1.27E+01				**0.9999**	R^2
	3.97E-02	-1.27E+01	2.41E+02					

Standard Residual	y-yhat	Target	w (1/x²) Calculated	yhat	Bias	Equal y-yhat	Calculated	Response	Bias
0.0664	0.0039	10.000	**10.071**	5.44E-01	**0.71%**	0.0056	**10.100**	5.48E-01	**1.00%**
-0.3081	-0.0181	20.000	**19.670**	1.09E+00	**-1.65%**	-0.0201	**19.636**	1.08E+00	**-1.82%**
0.0737	0.0043	50.000	**50.081**	2.72E+00	**0.16%**	-0.0071	**49.869**	2.73E+00	**-0.26%**
0.5728	0.0337	100.000	**100.649**	5.37E+00	**0.65%**	0.0118	**100.228**	5.40E+00	**0.23%**
1.5985	0.0939	200.000	**201.954**	1.04E+01	**0.98%**	0.0711	**201.482**	1.05E+01	**0.74%**
-1.7106	-0.1005	300.000	**297.735**	1.50E+01	**-0.76%**	-0.0981	**297.770**	1.49E+01	**-0.74%**
-0.2928	-0.0172	400.000	**399.576**	1.92E+01	**-0.11%**	0.0368	**400.923**	1.92E+01	**0.23%**
					5.00%				5.02%

Drug: α-OH-Midazolam
Unit: ng/mL

Trial 3	x	y	w (1/x²)	x²	x³	x⁴	xy	x²y	(y-ybar)²
L1	10.000	4.70E-01		1.00E+02	1.00E+03	1.00E+04	4.70E+00	4.70E+01	5.62E+01
L2	20.000	1.05E+00		4.00E+02	8.00E+03	1.60E+05	2.09E+01	4.18E+02	4.79E+01
L3	50.000	2.79E+00		2.50E+03	1.25E+05	6.25E+06	1.39E+02	6.97E+03	2.68E+01
L4	100.000	5.52E+00		1.00E+04	1.00E+06	1.00E+08	5.52E+02	5.52E+04	5.97E+00
L5	200.000	1.08E+01		4.00E+04	8.00E+06	1.60E+09	2.16E+03	4.33E+05	8.14E+00
L6	300.000	1.55E+01		9.00E+04	2.70E+07	8.10E+09	4.66E+03	1.40E+06	5.71E+01
L7	400.000	1.96E+01		1.60E+05	6.40E+07	2.56E+10	7.83E+03	3.13E+06	1.35E+02
7	1.08E+03	5.57E+01		3.03E+05	1.00E+08	3.54E+10	1.54E+04	5.03E+06	3.37E+02

	wx	wy		wx²	wx³	wx⁴	wxy	wx²y	(y-ybarw)²
	1.00E-01	4.70E-03		1.00E+00	1.00E+01	1.00E+02	4.70E-02	4.70E-01	6.87E-02
	5.00E-02	2.61E-03		1.00E+00	2.00E+01	4.00E+02	5.23E-02	1.05E+00	9.85E-02
	2.00E-02	1.12E-03		1.00E+00	5.00E+01	2.50E+03	5.58E-02	2.79E+00	4.23E+00
	1.00E-02	5.52E-04		1.00E+00	1.00E+02	1.00E+04	5.52E-02	5.52E+00	2.29E+01
	5.00E-03	2.70E-04		1.00E+00	2.00E+02	4.00E+04	5.41E-02	1.08E+01	1.02E+02
	3.33E-03	1.72E-04		1.00E+00	3.00E+02	9.00E+04	5.17E-02	1.55E+01	2.19E+02
	2.50E-03	1.22E-04		1.00E+00	4.00E+02	1.60E+05	4.90E-02	1.96E+01	3.55E+02
	1.91E-01	9.55E-03		7.00E+00	1.08E+03	3.03E+05	3.65E-01	5.57E+01	

Equal

A=	3.54E+10	1.00E+08	3.03E+05	B =	5.03E+06	X =	**-2.686E-05**	**a**	
	1.00E+08	3.03E+05	1.08E+03		1.54E+04		**6.016E-02**	**b**	
	3.03E+05	1.08E+03	7.00E+00		5.57E+01		**-1.547E-01**	**c**	
A^{-1} =	7.18E-10	-2.81E-07	1.23E-05						
	-2.81E-07	1.17E-04	-5.94E-03				**1.0000**	**R^2**	
	1.23E-05	-5.94E-03	5.28E-01						

Weighted

A =	3.03E+05	1.08E+03	7.00E+00	B =	5.57E+01	X =	**-2.404E-05**	**a**	
	1.08E+03	7.00E+00	1.91E-01		3.65E-01		**5.915E-02**	**b**	
	7.00E+00	1.91E-01	1.30E-02		9.55E-03		**-1.206E-01**	**c**	
A^{-1} =	1.39E-05	-3.22E-03	3.97E-02						
	-3.22E-03	9.86E-01	-1.27E+01				**1.0000**	**R^2**	
	3.97E-02	-1.27E+01	2.41E+02						

Standard Residual	y-yhat	Target	w (1/x²) Calculated	yhat	Bias	Equal y-yhat	Calculated	Response	Bias
0.0237	0.0014	10.000	**10.024**	4.68E-01	**0.24%**	0.0257	**10.431**	4.70E-01	**4.31%**
-0.1171	-0.0069	20.000	**19.881**	1.05E+00	**-0.59%**	0.0081	**20.137**	1.05E+00	**0.69%**
0.2014	0.0119	50.000	**50.209**	2.78E+00	**0.42%**	0.0024	**50.042**	2.79E+00	**0.08%**
-0.5496	-0.0324	100.000	**99.404**	5.55E+00	**-0.60%**	-0.0713	**98.699**	5.52E+00	**-1.30%**
1.1998	0.0707	200.000	**201.428**	1.07E+01	**0.71%**	0.0151	**200.306**	1.08E+01	**0.15%**
1.0202	0.0601	300.000	**301.345**	1.55E+01	**0.45%**	0.0444	**301.008**	1.55E+01	**0.34%**
-1.7784	-0.1048	400.000	**397.378**	1.97E+01	**-0.66%**	-0.0243	**399.371**	1.96E+01	**-0.16%**
					3.66%				7.02%

Drug: α-OH-Midazolam
Unit: ng/mL

Trial 4	x	y	w (1/x²)	x²	x³	x⁴	xy	x²y	(y-ybar)²
L1	10.000	5.70E-01		1.00E+02	1.00E+03	1.00E+04	5.70E+00	5.70E+01	5.46E+01
L2	20.000	1.16E+00		4.00E+02	8.00E+03	1.60E+05	2.31E+01	4.63E+02	4.63E+01
L3	50.000	2.80E+00		2.50E+03	1.25E+05	6.25E+06	1.40E+02	6.99E+03	2.66E+01
L4	100.000	5.25E+00		1.00E+04	1.00E+06	1.00E+08	5.25E+02	5.25E+04	7.32E+00
L5	200.000	1.07E+01		4.00E+04	8.00E+06	1.60E+09	2.14E+03	4.27E+05	7.39E+00
L6	300.000	1.53E+01		9.00E+04	2.70E+07	8.10E+09	4.60E+03	1.38E+06	5.44E+01
L7	400.000	1.99E+01		1.60E+05	6.40E+07	2.56E+10	7.97E+03	3.19E+06	1.43E+02
7	1.08E+03	5.57E+01		3.03E+05	1.00E+08	3.54E+10	1.54E+04	5.05E+06	3.40E+02

wx	wy	wx²	wx³	wx⁴	wxy	wx²y	(y-ybarw)²
1.00E-01	5.70E-03	1.00E+00	1.00E+01	1.00E+02	5.70E-02	5.70E-01	6.66E-02
5.00E-02	2.89E-03	1.00E+00	2.00E+01	4.00E+02	5.78E-02	1.16E+00	1.08E-01
2.00E-02	1.12E-03	1.00E+00	5.00E+01	2.50E+03	5.59E-02	2.80E+00	3.88E+00
1.00E-02	5.25E-04	1.00E+00	1.00E+02	1.00E+04	5.25E-02	5.25E+00	1.96E+01
5.00E-03	2.67E-04	1.00E+00	2.00E+02	4.00E+04	5.34E-02	1.07E+01	9.70E+01
3.33E-03	1.70E-04	1.00E+00	3.00E+02	9.00E+04	5.11E-02	1.53E+01	2.10E+02
2.50E-03	1.25E-04	1.00E+00	4.00E+02	1.60E+05	4.98E-02	1.99E+01	3.65E+02
1.91E-01	1.08E-02	7.00E+00	1.08E+03	3.03E+05	3.78E-01	5.57E+01	

Equal

A=	3.54E+10	1.00E+08	3.03E+05	B =	5.05E+06	X =	**-1.435E-05**	a
	1.00E+08	3.03E+05	1.08E+03		1.54E+04		**5.550E-02**	b
	3.03E+05	1.08E+03	7.00E+00		5.57E+01		**1.614E-02**	c
A^{-1} =	7.18E-10	-2.81E-07	1.23E-05					
	-2.81E-07	1.17E-04	-5.94E-03				**0.9998**	R^2
	1.23E-05	-5.94E-03	5.28E-01					

Weighted

A =	3.03E+05	1.08E+03	7.00E+00	B =	5.57E+01	X =	**-1.542E-05**	a
	1.08E+03	7.00E+00	1.91E-01		3.78E-01		**5.578E-02**	b
	7.00E+00	1.91E-01	1.30E-02		1.08E-02		**1.979E-02**	c
A^{-1} =	1.39E-05	-3.22E-03	3.97E-02					
	-3.22E-03	9.86E-01	-1.27E+01				**0.9994**	R^2
	3.97E-02	-1.27E+01	2.41E+02					

Standard Residual	y-yhat	Target	w (1/x²) Calculated	yhat	Bias	Equal y-yhat	Calculated	Response	Bias
-0.0660	-0.0064	10.000	**9.885**	5.76E-01	**-1.15%**	-0.0001	**9.999**	5.70E-01	**-0.01%**
0.2865	0.0277	20.000	**20.503**	1.13E+00	**2.51%**	0.0365	**20.665**	1.16E+00	**3.32%**
0.2753	0.0267	50.000	**50.492**	2.77E+00	**0.98%**	0.0415	**50.767**	2.80E+00	**1.53%**
-1.9682	-0.1906	100.000	**96.388**	5.44E+00	**-3.61%**	-0.1699	**96.774**	5.25E+00	**-3.23%**
1.2227	0.1184	200.000	**202.388**	1.06E+01	**1.19%**	0.1346	**202.706**	1.07E+01	**1.35%**
-0.3431	-0.0332	300.000	**299.286**	1.54E+01	**-0.24%**	-0.0429	**299.085**	1.53E+01	**-0.30%**
0.5927	0.0574	400.000	**401.322**	1.99E+01	**0.33%**	0.0004	**400.009**	1.99E+01	**0.00%**
					10.02%				9.76%

Drug: α-OH-Midazolam
Unit: ng/mL

Trial 5	x	y	w (1/x²)	x²	x³	x⁴	xy	x²y	(y-ybar)²
L1	10.000	4.12E-01		1.00E+02	1.00E+03	1.00E+04	4.12E+00	4.12E+01	5.59E+01
L2	20.000	1.13E+00		4.00E+02	8.00E+03	1.60E+05	2.25E+01	4.51E+02	4.57E+01
L3	50.000	2.51E+00		2.50E+03	1.25E+05	6.25E+06	1.25E+02	6.26E+03	2.89E+01
L4	100.000	5.62E+00		1.00E+04	1.00E+06	1.00E+08	5.62E+02	5.62E+04	5.11E+00
L5	200.000	1.11E+01		4.00E+04	8.00E+06	1.60E+09	2.22E+03	4.44E+05	1.03E+01
L6	300.000	1.52E+01		9.00E+04	2.70E+07	8.10E+09	4.55E+03	1.37E+06	5.33E+01
L7	400.000	1.93E+01		1.60E+05	6.40E+07	2.56E+10	7.70E+03	3.08E+06	1.29E+02
7	1.08E+03	5.52E+01		3.03E+05	1.00E+08	3.54E+10	1.52E+04	4.95E+06	3.28E+02

	wx	wy		wx²	wx³	wx⁴	wxy	wx²y	(y-ybarw)²
	1.00E-01	4.12E-03		1.00E+00	1.00E+01	1.00E+02	4.12E-02	4.12E-01	8.03E-02
	5.00E-02	2.82E-03		1.00E+00	2.00E+01	4.00E+02	5.63E-02	1.13E+00	1.87E-01
	2.00E-02	1.00E-03		1.00E+00	5.00E+01	2.50E+03	5.01E-02	2.51E+00	3.28E+00
	1.00E-02	5.62E-04		1.00E+00	1.00E+02	1.00E+04	5.62E-02	5.62E+00	2.43E+01
	5.00E-03	2.77E-04		1.00E+00	2.00E+02	4.00E+04	5.55E-02	1.11E+01	1.08E+02
	3.33E-03	1.69E-04		1.00E+00	3.00E+02	9.00E+04	5.06E-02	1.52E+01	2.10E+02
	2.50E-03	1.20E-04		1.00E+00	4.00E+02	1.60E+05	4.81E-02	1.93E+01	3.44E+02
	1.91E-01	9.07E-03		7.00E+00	1.08E+03	3.03E+05	3.58E-01	5.52E+01	

Equal

A=	3.54E+10	1.00E+08	3.03E+05	B =	4.95E+06	X =	**-3.446E-05**	a	
	1.00E+08	3.03E+05	1.08E+03		1.52E+04		**6.242E-02**	b	
	3.03E+05	1.08E+03	7.00E+00		5.52E+01		**-2.531E-01**	c	
A^{-1} =	7.18E-10	-2.81E-07	1.23E-05						
	-2.81E-07	1.17E-04	-5.94E-03				**0.9994**	R^2	
	1.23E-05	-5.94E-03	5.28E-01						

Weighted

A =	3.03E+05	1.08E+03	7.00E+00	B =	5.52E+01	X =	**-2.834E-05**	a	
	1.08E+03	7.00E+00	1.91E-01		3.58E-01		**6.016E-02**	b	
	7.00E+00	1.91E-01	1.30E-02		9.07E-03		**-1.699E-01**	c	
A^{-1} =	1.39E-05	-3.22E-03	3.97E-02						
	-3.22E-03	9.86E-01	-1.27E+01				**0.9949**	R^2	
	3.97E-02	-1.27E+01	2.41E+02						

Standard Residual	y-yhat	Target	w (1/x²) Calculated	yhat	Bias	Equal y-yhat	Calculated	Response	Bias
-0.0834	-0.0171	10.000	**9.713**	4.29E-01	**-2.87%**	0.0441	**10.714**	4.12E-01	**7.14%**
0.5125	0.1050	20.000	**21.781**	1.02E+00	**8.91%**	0.1455	**22.387**	1.13E+00	**11.93%**
-1.2780	-0.2619	50.000	**45.441**	2.77E+00	**-9.12%**	-0.2763	**45.327**	2.51E+00	**-9.35%**
0.3007	0.0616	100.000	**101.132**	5.56E+00	**1.13%**	-0.0196	**99.647**	5.62E+00	**-0.35%**
1.7936	0.3676	200.000	**207.562**	1.07E+01	**3.78%**	0.2444	**205.044**	1.11E+01	**2.52%**
-0.7070	-0.1449	300.000	**296.650**	1.53E+01	**-1.12%**	-0.1877	**295.520**	1.52E+01	**-1.49%**
-0.5385	-0.1104	400.000	**397.063**	1.94E+01	**-0.73%**	0.0496	**401.426**	1.93E+01	**0.36%**
					27.65%				33.15%

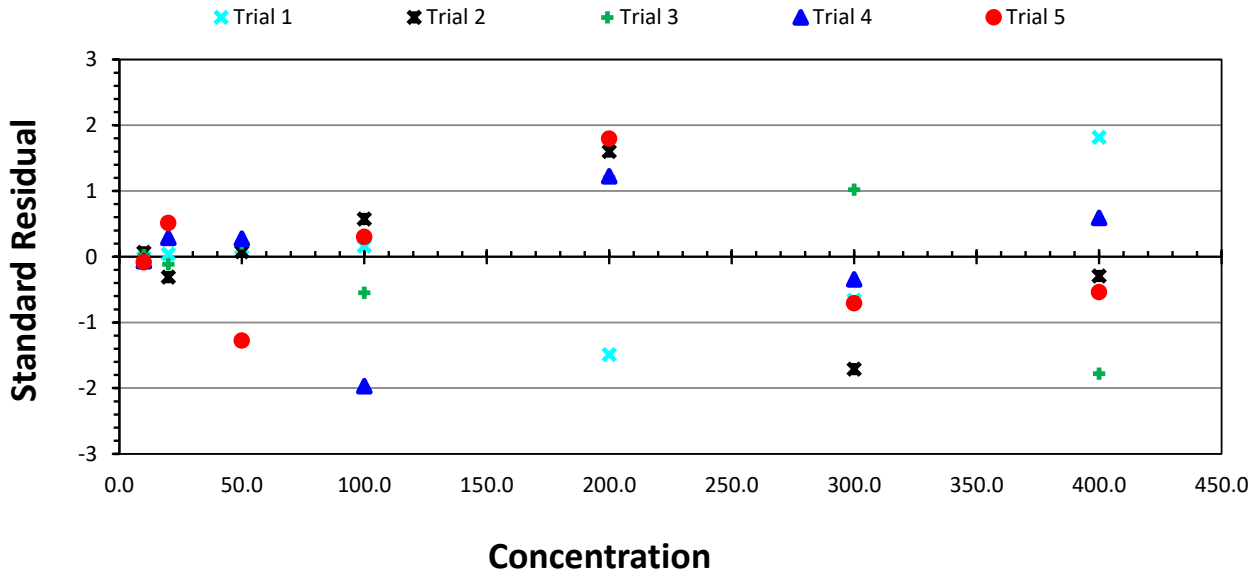
BZDG1 in Blood

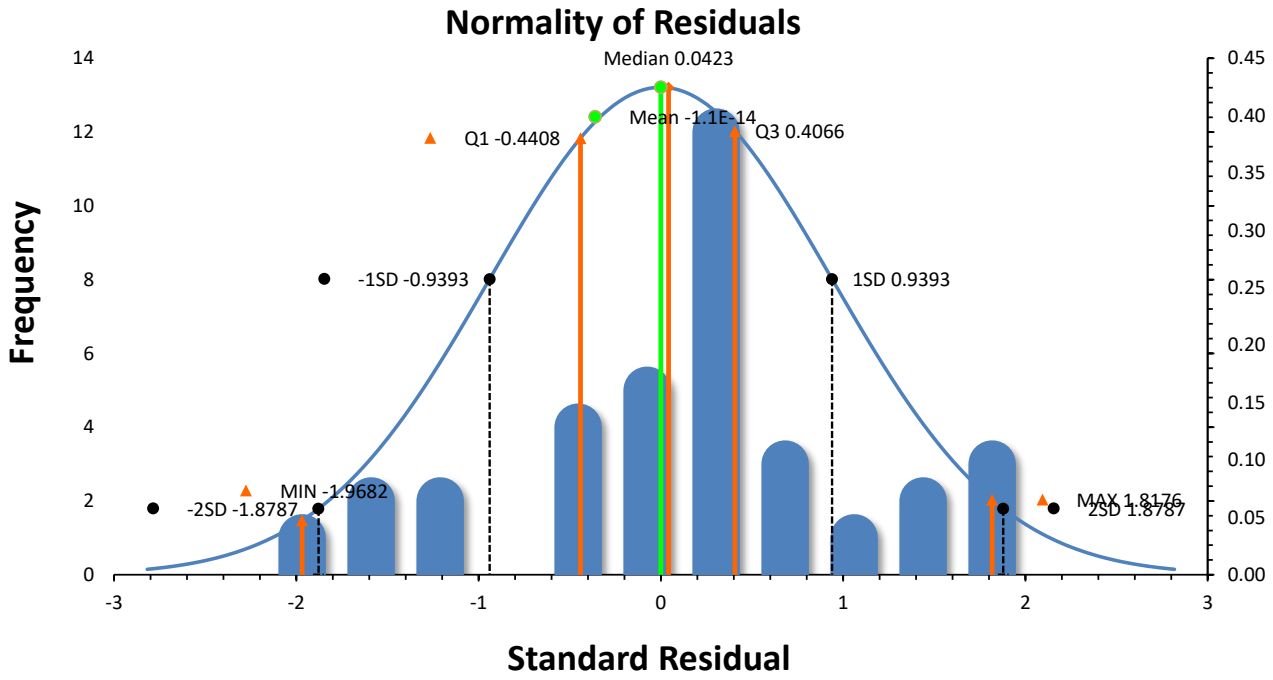
Drug: α-OH-Midazolam

Unit: ng/mL

Calibration Model: Quadratic $(1/x^2)$

Toxicologist(s): JD1 JD2 JD3 JD4 JD5 **Instrument:** LC-MS/MS

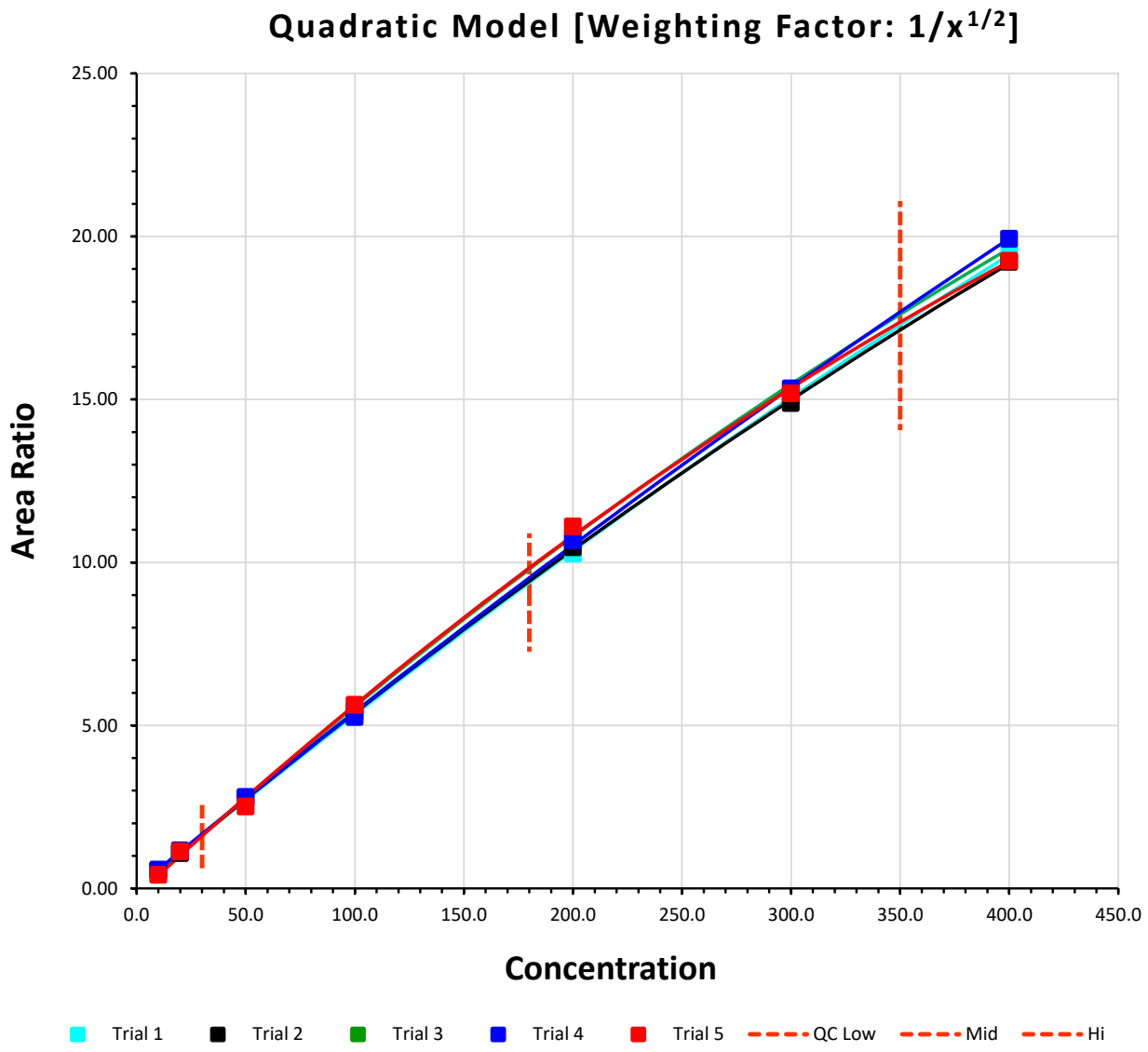




BZDG1 in Blood

Drug: α-OH-Midazolam

Unit: ng/mL

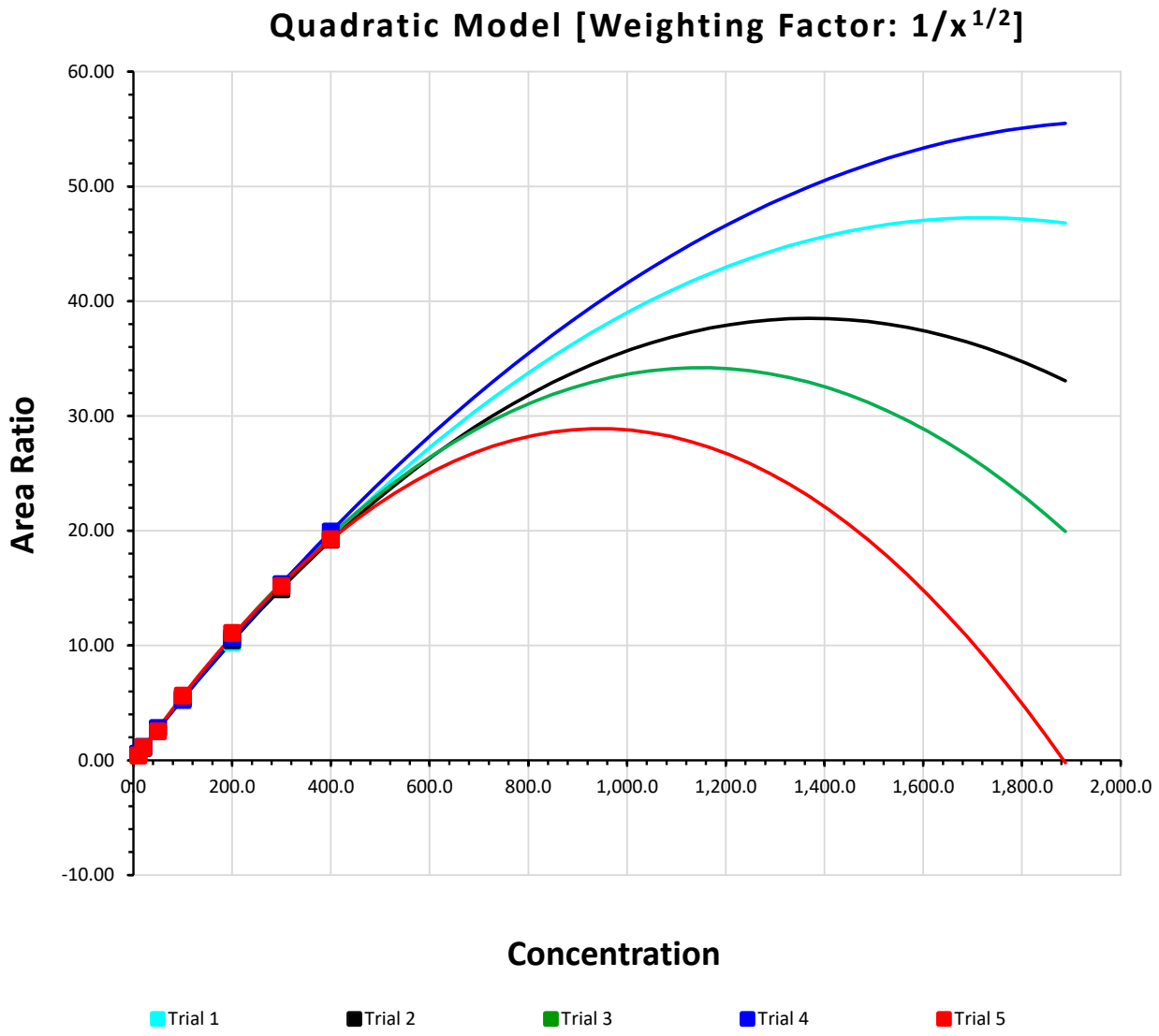


	a		b			c	R^2
Trial 1	-1.599E-05	X^2 +	5.499E-02	X	+	-2.923E-05	0.9999
Trial 2	-2.047E-05	X^2 +	5.617E-02	X	+	-2.171E-02	1.0000
Trial 3	-2.606E-05	X^2 +	5.983E-02	X	+	-1.373E-01	1.0000
Trial 4	-1.370E-05	X^2 +	5.524E-02	X	+	2.953E-02	0.9998
Trial 5	-3.267E-05	X^2 +	6.167E-02	X	+	-2.114E-01	0.9992

BZDG1 in Blood

Drug: α-OH-Midazolam

Unit: ng/mL



		Symmetry, h [Concentration]	Vertex, k [Area Ratio]	Focus [Area Ratio]		Directrix [Area Ratio]
Trial 1	x=	1.72E+03	4.73E+01	-1.56E+04	y=	1.57E+04
Trial 2	x=	1.37E+03	3.85E+01	-1.22E+04	y=	1.22E+04
Trial 3	x=	1.15E+03	3.42E+01	-9.56E+03	y=	9.63E+03
Trial 4	x=	2.02E+03	5.57E+01	-1.82E+04	y=	1.83E+04
Trial 5	x=	9.44E+02	2.89E+01	-7.62E+03	y=	7.68E+03

Drug: α-OH-Midazolam
Unit: ng/mL

Trial 1	x	y	w ($1/x^{1/2}$)	x^2	x^3	x^4	xy	x^2y	$(y\text{-}ybar)^2$
L1	10.000	5.23E-01	3.16E-01	1.00E+02	1.00E+03	1.00E+04	5.23E+00	5.23E+01	5.29E+01
L2	20.000	1.09E+00	2.24E-01	4.00E+02	8.00E+03	1.60E+05	2.18E+01	4.36E+02	4.50E+01
L3	50.000	2.76E+00	1.41E-01	2.50E+03	1.25E+05	6.25E+06	1.38E+02	6.89E+03	2.54E+01
L4	100.000	5.43E+00	1.00E-01	1.00E+04	1.00E+06	1.00E+08	5.43E+02	5.43E+04	5.59E+00
L5	200.000	1.03E+01	7.07E-02	4.00E+04	8.00E+06	1.60E+09	2.05E+03	4.11E+05	6.15E+00
L6	300.000	1.50E+01	5.77E-02	9.00E+04	2.70E+07	8.10E+09	4.50E+03	1.35E+06	5.18E+01
L7	400.000	1.95E+01	5.00E-02	1.60E+05	6.40E+07	2.56E+10	7.80E+03	3.12E+06	1.37E+02
7	1.08E+03	5.46E+01	9.60E-01	3.03E+05	1.00E+08	3.54E+10	1.51E+04	4.94E+06	3.24E+02

wx	wy	wx^2	wx^3	wx^4	wxy	wx^2y	$(y\text{-}ybarw)^2$
3.16E+00	1.65E-01	3.16E+01	3.16E+02	3.16E+03	1.65E+00	1.65E+01	1.26E+01
4.47E+00	2.44E-01	8.94E+01	1.79E+03	3.58E+04	4.87E+00	9.74E+01	8.90E+00
7.07E+00	3.90E-01	3.54E+02	1.77E+04	8.84E+05	1.95E+01	9.74E+02	1.74E+00
1.00E+01	5.43E-01	1.00E+03	1.00E+05	1.00E+07	5.43E+01	5.43E+03	1.85E+00
1.41E+01	7.26E-01	2.83E+03	5.66E+05	1.13E+08	1.45E+02	2.91E+04	3.85E+01
1.73E+01	8.66E-01	5.20E+03	1.56E+06	4.68E+08	2.60E+02	7.79E+04	1.19E+02
2.00E+01	9.75E-01	8.00E+03	3.20E+06	1.28E+09	3.90E+02	1.56E+05	2.38E+02
7.62E+01	3.91E+00	1.75E+04	5.44E+06	1.87E+09	8.75E+02	2.69E+05	

Equal

A=	3.54E+10	1.00E+08	3.03E+05	B =	4.94E+06	X =	**-1.443E-05**	**a**
	1.00E+08	3.03E+05	1.08E+03		1.51E+04		**5.435E-02**	**b**
	3.03E+05	1.08E+03	7.00E+00		5.46E+01		**3.426E-02**	**c**
A^{-1} =	7.18E-10	-2.81E-07	1.23E-05					
	-2.81E-07	1.17E-04	-5.94E-03				**0.9999**	R^2
	1.23E-05	-5.94E-03	5.28E-01					

Weighted

A =	1.87E+09	5.44E+06	1.75E+04	B =	2.69E+05	X =	**-1.599E-05**	**a**
	5.44E+06	1.75E+04	7.62E+01		8.75E+02		**5.499E-02**	**b**
	1.75E+04	7.62E+01	9.60E-01		3.91E+00		**-2.923E-05**	**c**
A^{-1} =	8.57E-09	-3.03E-06	8.45E-05					
	-3.03E-06	1.16E-03	-3.69E-02				**0.9999**	R^2
	8.45E-05	-3.69E-02	2.43E+00					

Standard Residual	y-yhat	Target	w ($1/x^{1/2}$) Calculated	yhat	Bias	Equal y-yhat	Calculated	Response	Bias
-0.3836	-0.0252	10.000	**9.540**	5.48E-01	**-4.60%**	-0.0532	**9.017**	5.23E-01	**-9.83%**
-0.0606	-0.0040	20.000	**19.927**	1.09E+00	**-0.37%**	-0.0260	**19.516**	1.09E+00	**-2.42%**
0.6925	0.0454	50.000	**50.851**	2.71E+00	**1.70%**	0.0394	**50.745**	2.76E+00	**1.49%**
1.4037	0.0920	100.000	**101.778**	5.34E+00	**1.78%**	0.1065	**102.071**	5.43E+00	**2.07%**
-1.2955	-0.0850	200.000	**198.253**	1.04E+01	**-0.87%**	-0.0529	**198.911**	1.03E+01	**-0.54%**
-0.9727	-0.0638	300.000	**298.596**	1.51E+01	**-0.47%**	-0.0454	**299.006**	1.50E+01	**-0.33%**
0.8860	0.0581	400.000	**401.377**	1.94E+01	**0.34%**	0.0316	**400.739**	1.95E+01	**0.18%**
					10.13%				16.87%

Drug: α-OH-Midazolam
Unit: ng/mL

Trial 2	x	y	w (1/x^1/2)	x^2	x^3	x^4	xy	x^2y	(y-ybar)^2
L1	10.000	5.48E-01		1.00E+02	1.00E+03	1.00E+04	5.48E+00	5.48E+01	5.20E+01
L2	20.000	1.08E+00		4.00E+02	8.00E+03	1.60E+05	2.15E+01	4.31E+02	4.46E+01
L3	50.000	2.73E+00		2.50E+03	1.25E+05	6.25E+06	1.36E+02	6.82E+03	2.53E+01
L4	100.000	5.40E+00		1.00E+04	1.00E+06	1.00E+08	5.40E+02	5.40E+04	5.57E+00
L5	200.000	1.05E+01		4.00E+04	8.00E+06	1.60E+09	2.09E+03	4.18E+05	7.29E+00
L6	300.000	1.49E+01		9.00E+04	2.70E+07	8.10E+09	4.47E+03	1.34E+06	5.08E+01
L7	400.000	1.92E+01		1.60E+05	6.40E+07	2.56E+10	7.69E+03	3.07E+06	1.31E+02
7	1.08E+03	5.43E+01		3.03E+05	1.00E+08	3.54E+10	1.49E+04	4.89E+06	3.17E+02

wx	wy	wx^2	wx^3	wx^4	wxy	wx^2y	(y-ybarw)^2
3.16E+00	1.73E-01	3.16E+01	3.16E+02	3.16E+03	1.73E+00	1.73E+01	1.24E+01
4.47E+00	2.41E-01	8.94E+01	1.79E+03	3.58E+04	4.81E+00	9.63E+01	8.92E+00
7.07E+00	3.86E-01	3.54E+02	1.77E+04	8.84E+05	1.93E+01	9.65E+02	1.78E+00
1.00E+01	5.40E-01	1.00E+03	1.00E+05	1.00E+07	5.40E+01	5.40E+03	1.79E+00
1.41E+01	7.40E-01	2.83E+03	5.66E+05	1.13E+08	1.48E+02	2.96E+04	4.09E+01
1.73E+01	8.59E-01	5.20E+03	1.56E+06	4.68E+08	2.58E+02	7.73E+04	1.17E+02
2.00E+01	9.61E-01	8.00E+03	3.20E+06	1.28E+09	3.84E+02	1.54E+05	2.30E+02
7.62E+01	3.90E+00	1.75E+04	5.44E+06	1.87E+09	8.70E+02	2.67E+05	

Equal

A=	3.54E+10	1.00E+08	3.03E+05	B =	4.89E+06	X =	**-2.019E-05**	a
	1.00E+08	3.03E+05	1.08E+03		1.49E+04		**5.606E-02**	b
	3.03E+05	1.08E+03	7.00E+00		5.43E+01		**-1.647E-02**	c
A^{-1} =	7.18E-10	-2.81E-07	1.23E-05					
	-2.81E-07	1.17E-04	-5.94E-03				**0.9999**	R^2
	1.23E-05	-5.94E-03	5.28E-01					

Weighted

A =	1.87E+09	5.44E+06	1.75E+04	B =	2.67E+05	X =	**-2.047E-05**	a
	5.44E+06	1.75E+04	7.62E+01		8.70E+02		**5.617E-02**	b
	1.75E+04	7.62E+01	9.60E-01		3.90E+00		**-2.171E-02**	c
A^{-1} =	8.57E-09	-3.03E-06	8.45E-05					
	-3.03E-06	1.16E-03	-3.69E-02				**1.0000**	R^2
	8.45E-05	-3.69E-02	2.43E+00					

Standard Residual	y-yhat	Target	w (1/x^1/2) Calculated	yhat	Bias	Equal y-yhat	Calculated	Response	Bias
0.1832	0.0097	10.000	**10.174**	5.38E-01	**1.74%**	0.0056	**10.100**	5.48E-01	**1.00%**
-0.3222	-0.0170	20.000	**19.692**	1.09E+00	**-1.54%**	-0.0201	**19.636**	1.08E+00	**-1.82%**
-0.1290	-0.0068	50.000	**49.874**	2.74E+00	**-0.25%**	-0.0071	**49.869**	2.73E+00	**-0.26%**
0.1618	0.0086	100.000	**100.164**	5.39E+00	**0.16%**	0.0118	**100.228**	5.40E+00	**0.23%**
1.2285	0.0649	200.000	**201.354**	1.04E+01	**0.68%**	0.0711	**201.482**	1.05E+01	**0.74%**
-1.9164	-0.1013	300.000	**297.694**	1.50E+01	**-0.77%**	-0.0981	**297.770**	1.49E+01	**-0.74%**
0.7987	0.0422	400.000	**401.062**	1.92E+01	**0.27%**	0.0368	**400.923**	1.92E+01	**0.23%**
					5.40%				5.02%

Drug: α-OH-Midazolam
Unit: ng/mL

Trial 3	x	y	w (1/x^1/2)	x^2	x^3	x^4	xy	x^2y	(y-ybar)^2
L1	10.000	4.70E-01		1.00E+02	1.00E+03	1.00E+04	4.70E+00	4.70E+01	5.62E+01
L2	20.000	1.05E+00		4.00E+02	8.00E+03	1.60E+05	2.09E+01	4.18E+02	4.79E+01
L3	50.000	2.79E+00		2.50E+03	1.25E+05	6.25E+06	1.39E+02	6.97E+03	2.68E+01
L4	100.000	5.52E+00		1.00E+04	1.00E+06	1.00E+08	5.52E+02	5.52E+04	5.97E+00
L5	200.000	1.08E+01		4.00E+04	8.00E+06	1.60E+09	2.16E+03	4.33E+05	8.14E+00
L6	300.000	1.55E+01		9.00E+04	2.70E+07	8.10E+09	4.66E+03	1.40E+06	5.71E+01
L7	400.000	1.96E+01		1.60E+05	6.40E+07	2.56E+10	7.83E+03	3.13E+06	1.35E+02
7	1.08E+03	5.57E+01		3.03E+05	1.00E+08	3.54E+10	1.54E+04	5.03E+06	3.37E+02

wx	wy	wx^2	wx^3	wx^4	wxy	wx^2y	(y-ybarw)^2
3.16E+00	1.49E-01	3.16E+01	3.16E+02	3.16E+03	1.49E+00	1.49E+01	1.34E+01
4.47E+00	2.34E-01	8.94E+01	1.79E+03	3.58E+04	4.68E+00	9.35E+01	9.55E+00
7.07E+00	3.94E-01	3.54E+02	1.77E+04	8.84E+05	1.97E+01	9.86E+02	1.82E+00
1.00E+01	5.52E-01	1.00E+03	1.00E+05	1.00E+07	5.52E+01	5.52E+03	1.92E+00
1.41E+01	7.65E-01	2.83E+03	5.66E+05	1.13E+08	1.53E+02	3.06E+04	4.46E+01
1.73E+01	8.96E-01	5.20E+03	1.56E+06	4.68E+08	2.69E+02	8.06E+04	1.30E+02
2.00E+01	9.79E-01	8.00E+03	3.20E+06	1.28E+09	3.92E+02	1.57E+05	2.39E+02
7.62E+01	3.97E+00	1.75E+04	5.44E+06	1.87E+09	8.95E+02	2.75E+05	

Equal

A=	3.54E+10	1.00E+08	3.03E+05	B =	5.03E+06	X =	**-2.686E-05**	**a**
	1.00E+08	3.03E+05	1.08E+03		1.54E+04		**6.016E-02**	**b**
	3.03E+05	1.08E+03	7.00E+00		5.57E+01		**-1.547E-01**	**c**
A^{-1} =	7.18E-10	-2.81E-07	1.23E-05					
	-2.81E-07	1.17E-04	-5.94E-03				**1.0000**	**R^2**
	1.23E-05	-5.94E-03	5.28E-01					

Weighted

A =	1.87E+09	5.44E+06	1.75E+04	B =	2.75E+05	X =	**-2.606E-05**	**a**
	5.44E+06	1.75E+04	7.62E+01		8.95E+02		**5.983E-02**	**b**
	1.75E+04	7.62E+01	9.60E-01		3.97E+00		**-1.373E-01**	**c**
A^{-1} =	8.57E-09	-3.03E-06	8.45E-05					
	-3.03E-06	1.16E-03	-3.69E-02				**1.0000**	**R^2**
	8.45E-05	-3.69E-02	2.43E+00					

Standard Residual	y-yhat	Target	w (1/x^1/2) Calculated	yhat	Bias	Equal y-yhat	Calculated	Response	Bias
0.2896	0.0115	10.000	**10.195**	4.58E-01	**1.95%**	0.0257	**10.431**	4.70E-01	**4.31%**
-0.0744	-0.0030	20.000	**19.950**	1.05E+00	**-0.25%**	0.0081	**20.137**	1.05E+00	**0.69%**
-0.0107	-0.0004	50.000	**49.993**	2.79E+00	**-0.01%**	0.0024	**50.042**	2.79E+00	**0.08%**
-1.5965	-0.0637	100.000	**98.835**	5.58E+00	**-1.16%**	-0.0713	**98.699**	5.52E+00	**-1.30%**
0.7954	0.0317	200.000	**200.642**	1.08E+01	**0.32%**	0.0151	**200.306**	1.08E+01	**0.15%**
1.3485	0.0538	300.000	**301.218**	1.55E+01	**0.41%**	0.0444	**301.008**	1.55E+01	**0.34%**
-0.9574	-0.0382	400.000	**399.021**	1.96E+01	**-0.24%**	-0.0243	**399.371**	1.96E+01	**-0.16%**
					4.35%				7.02%

Drug: α-OH-Midazolam
Unit: ng/mL

Trial 4	x	y	w ($1/x^{1/2}$)	x^2	x^3	x^4	xy	x^2y	$(y\text{-}ybar)^2$
L1	10.000	5.70E-01		1.00E+02	1.00E+03	1.00E+04	5.70E+00	5.70E+01	5.46E+01
L2	20.000	1.16E+00		4.00E+02	8.00E+03	1.60E+05	2.31E+01	4.63E+02	4.63E+01
L3	50.000	2.80E+00		2.50E+03	1.25E+05	6.25E+06	1.40E+02	6.99E+03	2.66E+01
L4	100.000	5.25E+00		1.00E+04	1.00E+06	1.00E+08	5.25E+02	5.25E+04	7.32E+00
L5	200.000	1.07E+01		4.00E+04	8.00E+06	1.60E+09	2.14E+03	4.27E+05	7.39E+00
L6	300.000	1.53E+01		9.00E+04	2.70E+07	8.10E+09	4.60E+03	1.38E+06	5.44E+01
L7	400.000	1.99E+01		1.60E+05	6.40E+07	2.56E+10	7.97E+03	3.19E+06	1.43E+02
7	1.08E+03	5.57E+01		3.03E+05	1.00E+08	3.54E+10	1.54E+04	5.05E+06	3.40E+02

	wx	wy		wx^2	wx^3	wx^4	wxy	wx^2y	$(y\text{-}ybarw)^2$
	3.16E+00	1.80E-01		3.16E+01	3.16E+02	3.16E+03	1.80E+00	1.80E+01	1.29E+01
	4.47E+00	2.59E-01		8.94E+01	1.79E+03	3.58E+04	5.17E+00	1.03E+02	9.04E+00
	7.07E+00	3.96E-01		3.54E+02	1.77E+04	8.84E+05	1.98E+01	9.89E+02	1.87E+00
	1.00E+01	5.25E-01		1.00E+03	1.00E+05	1.00E+07	5.25E+01	5.25E+03	1.19E+00
	1.41E+01	7.55E-01		2.83E+03	5.66E+05	1.13E+08	1.51E+02	3.02E+04	4.24E+01
	1.73E+01	8.85E-01		5.20E+03	1.56E+06	4.68E+08	2.66E+02	7.97E+04	1.25E+02
	2.00E+01	9.96E-01		8.00E+03	3.20E+06	1.28E+09	3.98E+02	1.59E+05	2.48E+02
	7.62E+01	4.00E+00		1.75E+04	5.44E+06	1.87E+09	8.94E+02	2.76E+05	

Equal

A=	3.54E+10	1.00E+08	3.03E+05	B =	5.05E+06	X =	**-1.435E-05**	a	
	1.00E+08	3.03E+05	1.08E+03		1.54E+04		**5.550E-02**	b	
	3.03E+05	1.08E+03	7.00E+00		5.57E+01		**1.614E-02**	c	
A^{-1} =	7.18E-10	-2.81E-07	1.23E-05						
	-2.81E-07	1.17E-04	-5.94E-03				**0.9998**	R^2	
	1.23E-05	-5.94E-03	5.28E-01						

Weighted

A =	1.87E+09	5.44E+06	1.75E+04	B =	2.76E+05	X =	**-1.370E-05**	a	
	5.44E+06	1.75E+04	7.62E+01		8.94E+02		**5.524E-02**	b	
	1.75E+04	7.62E+01	9.60E-01		4.00E+00		**2.953E-02**	c	
A^{-1} =	8.57E-09	-3.03E-06	8.45E-05						
	-3.03E-06	1.16E-03	-3.69E-02				**0.9998**	R^2	
	8.45E-05	-3.69E-02	2.43E+00						

Standard Residual	y-yhat	Target	w ($1/x^{1/2}$) Calculated	yhat	Bias	**Equal** y-yhat	Calculated	Response	Bias
-0.1164	-0.0109	10.000	**9.802**	5.81E-01	**-1.98%**	-0.0001	**9.999**	5.70E-01	**-0.01%**
0.3008	0.0281	20.000	**20.514**	1.13E+00	**2.57%**	0.0365	**20.665**	1.16E+00	**3.32%**
0.4242	0.0397	50.000	**50.737**	2.76E+00	**1.47%**	0.0415	**50.767**	2.80E+00	**1.53%**
-1.7471	-0.1634	100.000	**96.890**	5.42E+00	**-3.11%**	-0.1699	**96.774**	5.25E+00	**-3.23%**
1.5835	0.1481	200.000	**202.979**	1.05E+01	**1.49%**	0.1346	**202.706**	1.07E+01	**1.35%**
-0.3782	-0.0354	300.000	**299.248**	1.54E+01	**-0.25%**	-0.0429	**299.085**	1.53E+01	**-0.30%**
-0.1175	-0.0110	400.000	**399.752**	1.99E+01	**-0.06%**	0.0004	**400.009**	1.99E+01	**0.00%**
					10.94%				9.76%

Drug: α-OH-Midazolam
Unit: ng/mL

Trial 5	x	y	w (1/x^1/2)	x^2	x^3	x^4	xy	x^2y	(y-ybar)^2
L1	10.000	4.12E-01		1.00E+02	1.00E+03	1.00E+04	4.12E+00	4.12E+01	5.59E+01
L2	20.000	1.13E+00		4.00E+02	8.00E+03	1.60E+05	2.25E+01	4.51E+02	4.57E+01
L3	50.000	2.51E+00		2.50E+03	1.25E+05	6.25E+06	1.25E+02	6.26E+03	2.89E+01
L4	100.000	5.62E+00		1.00E+04	1.00E+06	1.00E+08	5.62E+02	5.62E+04	5.11E+00
L5	200.000	1.11E+01		4.00E+04	8.00E+06	1.60E+09	2.22E+03	4.44E+05	1.03E+01
L6	300.000	1.52E+01		9.00E+04	2.70E+07	8.10E+09	4.55E+03	1.37E+06	5.33E+01
L7	400.000	1.93E+01		1.60E+05	6.40E+07	2.56E+10	7.70E+03	3.08E+06	1.29E+02
7	1.08E+03	5.52E+01		3.03E+05	1.00E+08	3.54E+10	1.52E+04	4.95E+06	3.28E+02

wx	wy	wx^2	wx^3	wx^4	wxy	wx^2y	(y-ybarw)^2
3.16E+00	1.30E-01	3.16E+01	3.16E+02	3.16E+03	1.30E+00	1.30E+01	1.35E+01
4.47E+00	2.52E-01	8.94E+01	1.79E+03	3.58E+04	5.04E+00	1.01E+02	8.76E+00
7.07E+00	3.54E-01	3.54E+02	1.77E+04	8.84E+05	1.77E+01	8.86E+02	2.50E+00
1.00E+01	5.62E-01	1.00E+03	1.00E+05	1.00E+07	5.62E+01	5.62E+03	2.36E+00
1.41E+01	7.85E-01	2.83E+03	5.66E+05	1.13E+08	1.57E+02	3.14E+04	4.91E+01
1.73E+01	8.77E-01	5.20E+03	1.56E+06	4.68E+08	2.63E+02	7.89E+04	1.23E+02
2.00E+01	9.63E-01	8.00E+03	3.20E+06	1.28E+09	3.85E+02	1.54E+05	2.30E+02
7.62E+01	3.92E+00	1.75E+04	5.44E+06	1.87E+09	8.85E+02	2.71E+05	

Equal

A=	3.54E+10	1.00E+08	3.03E+05	B =	4.95E+06	X =	**-3.446E-05**	a
	1.00E+08	3.03E+05	1.08E+03		1.52E+04		**6.242E-02**	b
	3.03E+05	1.08E+03	7.00E+00		5.52E+01		**-2.531E-01**	c
A^{-1} =	7.18E-10	-2.81E-07	1.23E-05					
	-2.81E-07	1.17E-04	-5.94E-03				**0.9994**	R^2
	1.23E-05	-5.94E-03	5.28E-01					

Weighted

A =	1.87E+09	5.44E+06	1.75E+04	B =	2.71E+05	X =	**-3.267E-05**	a
	5.44E+06	1.75E+04	7.62E+01		8.85E+02		**6.167E-02**	b
	1.75E+04	7.62E+01	9.60E-01		3.92E+00		**-2.114E-01**	c
A^{-1} =	8.57E-09	-3.03E-06	8.45E-05					
	-3.03E-06	1.16E-03	-3.69E-02				**0.9992**	R^2
	8.45E-05	-3.69E-02	2.43E+00					

Standard Residual	y-yhat	Target	w (1/x^1/2) Calculated	yhat	Bias	Equal y-yhat	Calculated	Response	Bias
0.0530	0.0097	10.000	**10.159**	4.02E-01	**1.59%**	0.0441	**10.714**	4.12E-01	**7.14%**
0.6435	0.1181	20.000	**21.958**	1.01E+00	**9.79%**	0.1455	**22.387**	1.13E+00	**11.93%**
-1.5534	-0.2851	50.000	**45.132**	2.79E+00	**-9.74%**	-0.2763	**45.327**	2.51E+00	**-9.35%**
-0.0239	-0.0044	100.000	**99.920**	5.63E+00	**-0.08%**	-0.0196	**99.647**	5.62E+00	**-0.35%**
1.5296	0.2807	200.000	**205.798**	1.08E+01	**2.90%**	0.2444	**205.044**	1.11E+01	**2.52%**
-0.9058	-0.1662	300.000	**296.061**	1.53E+01	**-1.31%**	-0.1877	**295.520**	1.52E+01	**-1.49%**
0.1118	0.0205	400.000	**400.577**	1.92E+01	**0.14%**	0.0496	**401.426**	1.93E+01	**0.36%**
					25.55%				33.15%

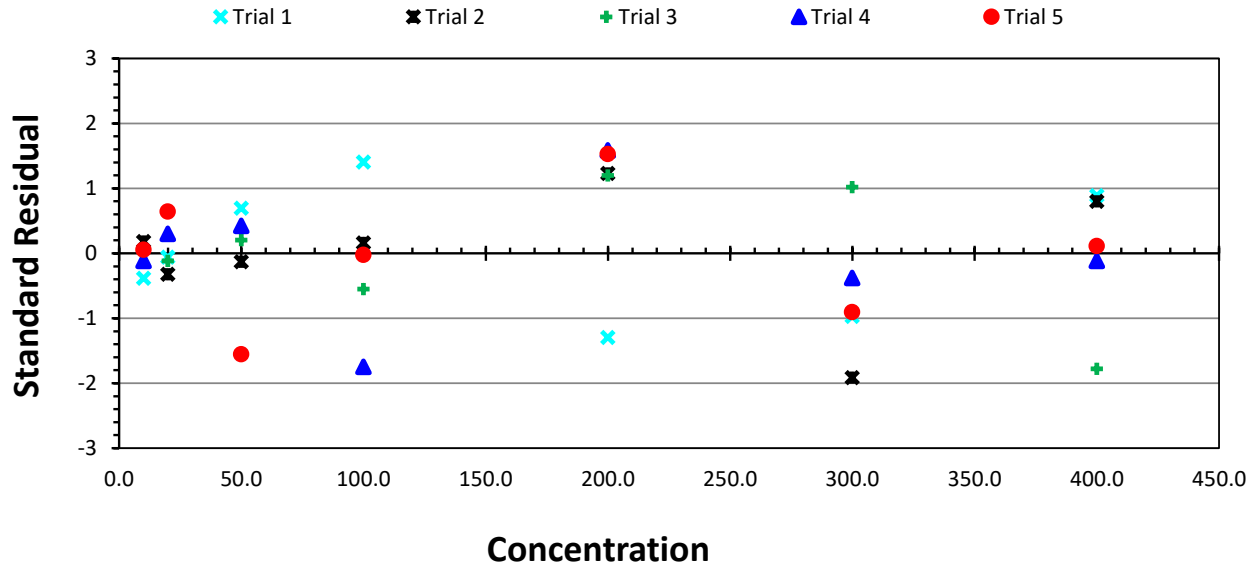
BZDG1 in Blood

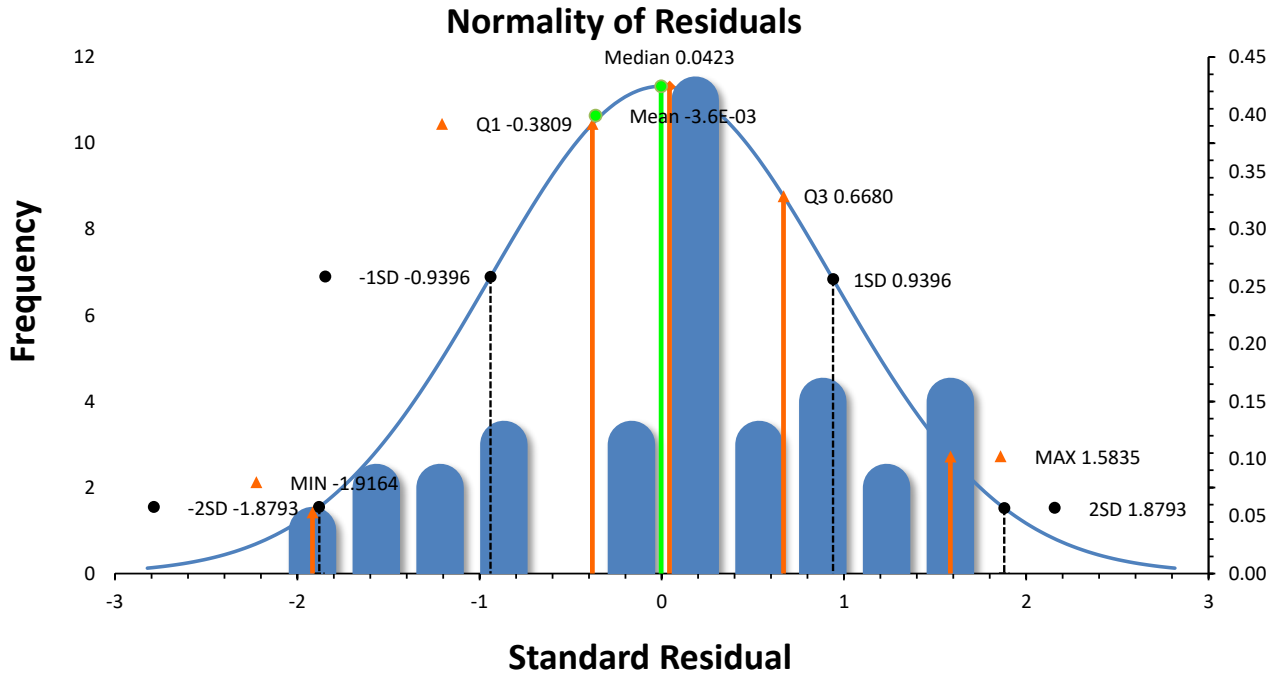
Drug: α-OH-Midazolam

Unit: ng/mL

Calibration Model: Quadratic $(1/x^{1/2})$

Toxicologist(s): JD1 JD2 JD3 JD4 JD5 **Instrument:** LC-MS/MS

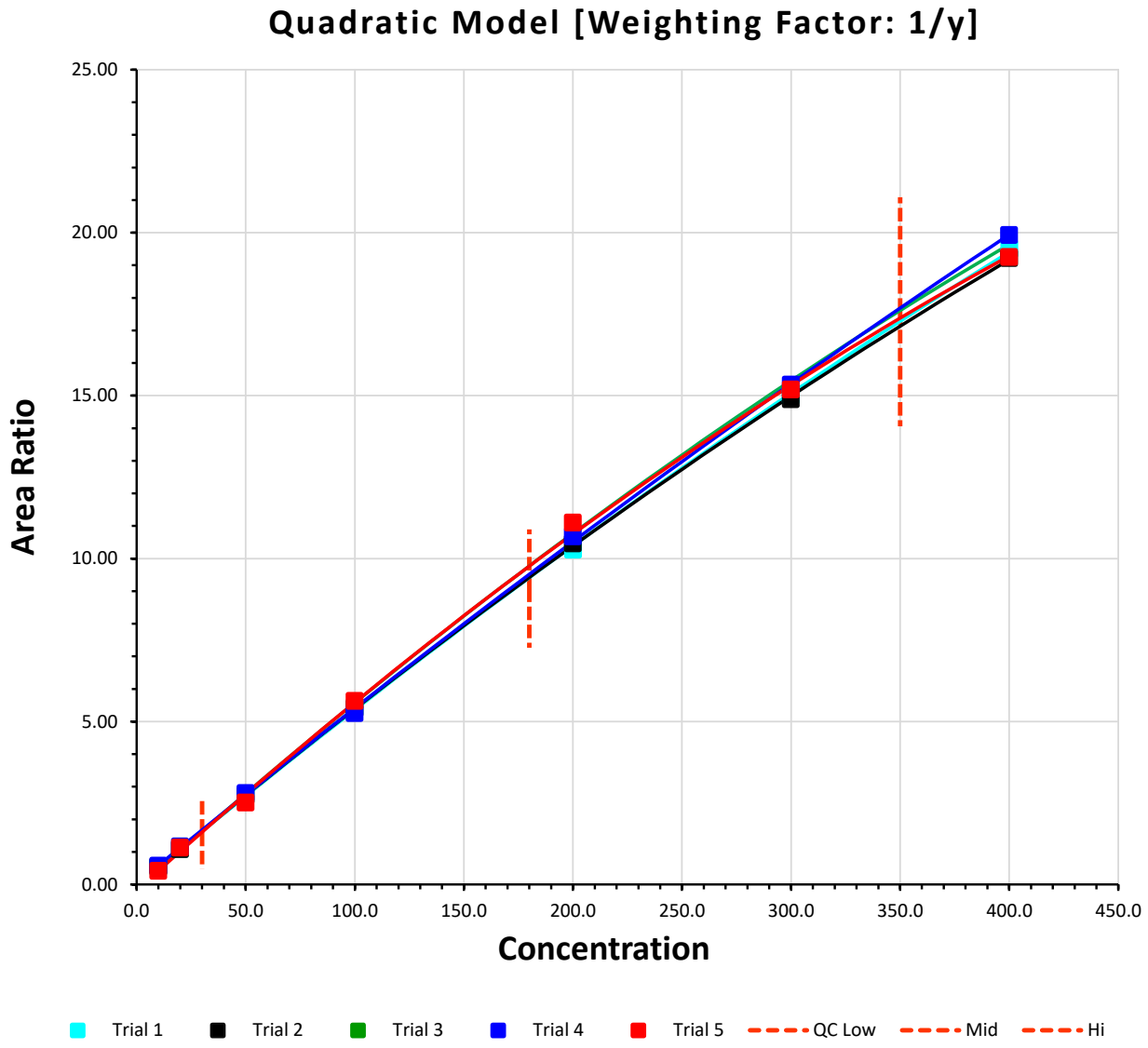




BZDG1 in Blood

Drug: α-OH-Midazolam

Unit: ng/mL

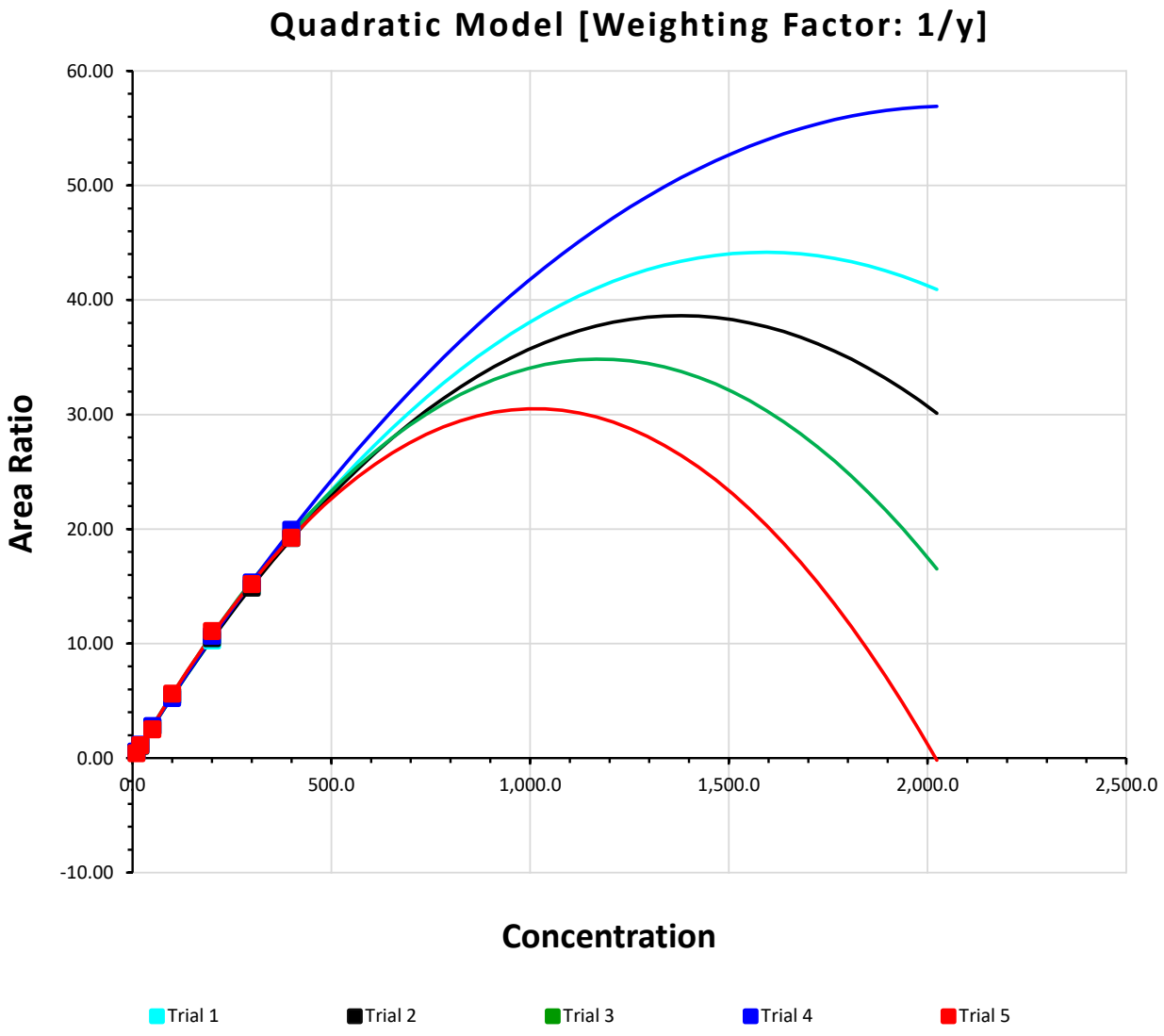


	a		b			c	R^2
Trial 1	-1.745E-05	X^2 +	5.554E-02	X	+	-1.954E-02	0.9999
Trial 2	-2.038E-05	X^2 +	5.613E-02	X	+	-2.014E-02	1.0000
Trial 3	-2.537E-05	X^2 +	5.957E-02	X	+	-1.281E-01	1.0000
Trial 4	-1.334E-05	X^2 +	5.511E-02	X	+	3.206E-02	0.9998
Trial 5	-2.998E-05	X^2 +	6.067E-02	X	+	-1.844E-01	0.9988

BZDG1 in Blood

Drug: α-OH-Midazolam

Unit: ng/mL



		Symmetry, h [Concentration]	Vertex, k [Area Ratio]	Focus [Area Ratio]		Directrix [Area Ratio]
Trial 1	x=	1.59E+03	4.42E+01	-1.43E+04	y=	1.44E+04
Trial 2	x=	1.38E+03	3.86E+01	-1.22E+04	y=	1.23E+04
Trial 3	x=	1.17E+03	3.48E+01	-9.82E+03	y=	9.89E+03
Trial 4	x=	2.06E+03	5.69E+01	-1.87E+04	y=	1.88E+04
Trial 5	x=	1.01E+03	3.05E+01	-8.31E+03	y=	8.37E+03

Drug: α-OH-Midazolam
Unit: ng/mL

Trial 1	x	y	w (1/y)	x^2	x^3	x^4	xy	x^2y	$(y-ybar)^2$
L1	10.000	5.23E-01	1.91E+00	1.00E+02	1.00E+03	1.00E+04	5.23E+00	5.23E+01	5.29E+01
L2	20.000	1.09E+00	9.18E-01	4.00E+02	8.00E+03	1.60E+05	2.18E+01	4.36E+02	4.50E+01
L3	50.000	2.76E+00	3.63E-01	2.50E+03	1.25E+05	6.25E+06	1.38E+02	6.89E+03	2.54E+01
L4	100.000	5.43E+00	1.84E-01	1.00E+04	1.00E+06	1.00E+08	5.43E+02	5.43E+04	5.59E+00
L5	200.000	1.03E+01	9.73E-02	4.00E+04	8.00E+06	1.60E+09	2.05E+03	4.11E+05	6.15E+00
L6	300.000	1.50E+01	6.67E-02	9.00E+04	2.70E+07	8.10E+09	4.50E+03	1.35E+06	5.18E+01
L7	400.000	1.95E+01	5.13E-02	1.60E+05	6.40E+07	2.56E+10	7.80E+03	3.12E+06	1.37E+02
7	1.08E+03	5.46E+01	3.59E+00	3.03E+05	1.00E+08	3.54E+10	1.51E+04	4.94E+06	3.24E+02

wx	wy	wx^2	wx^3	wx^4	wxy	wx^2y	$(y-ybarw)^2$
1.91E+01	1.00E+00	1.91E+02	1.91E+03	1.91E+04	1.00E+01	1.00E+02	2.03E+00
1.84E+01	1.00E+00	3.67E+02	7.34E+03	1.47E+05	2.00E+01	4.00E+02	7.39E-01
1.81E+01	1.00E+00	9.07E+02	4.54E+04	2.27E+06	5.00E+01	2.50E+03	6.50E-01
1.84E+01	1.00E+00	1.84E+03	1.84E+05	1.84E+07	1.00E+02	1.00E+04	1.21E+01
1.95E+01	1.00E+00	3.89E+03	7.79E+05	1.56E+08	2.00E+02	4.00E+04	6.93E+01
2.00E+01	1.00E+00	6.00E+03	1.80E+06	5.40E+08	3.00E+02	9.00E+04	1.70E+02
2.05E+01	1.00E+00	8.21E+03	3.28E+06	1.31E+09	4.00E+02	1.60E+05	3.08E+02
1.34E+02	7.00E+00	2.14E+04	6.10E+06	2.03E+09	1.08E+03	3.03E+05	

Equal

A=	3.54E+10	1.00E+08	3.03E+05	B =	4.94E+06	X =	**-1.443E-05**	**a**
	1.00E+08	3.03E+05	1.08E+03		1.51E+04		**5.435E-02**	**b**
	3.03E+05	1.08E+03	7.00E+00		5.46E+01		**3.426E-02**	**c**
A^{-1} =	7.18E-10	-2.81E-07	1.23E-05					
	-2.81E-07	1.17E-04	-5.94E-03				**0.9999**	R^2
	1.23E-05	-5.94E-03	5.28E-01					

Weighted

A =	2.03E+09	6.10E+06	2.14E+04	B =	3.03E+05	X =	**-1.745E-05**	**a**
	6.10E+06	2.14E+04	1.34E+02		1.08E+03		**5.554E-02**	**b**
	2.14E+04	1.34E+02	3.59E+00		7.00E+00		**-1.954E-02**	**c**
A^{-1} =	5.29E-09	-1.71E-06	3.22E-05					
	-1.71E-06	6.13E-04	-1.27E-02				**0.9999**	R^2
	3.22E-05	-1.27E-02	5.60E-01					

Standard Residual	y-yhat	Target	w (1/y) Calculated	yhat	Bias	Equal y-yhat	Calculated	Response	Bias
-0.1438	-0.0110	10.000	**9.801**	5.34E-01	**-1.99%**	-0.0532	**9.017**	5.23E-01	**-9.83%**
0.0676	0.0052	20.000	**20.094**	1.08E+00	**0.47%**	-0.0260	**19.516**	1.09E+00	**-2.42%**
0.5394	0.0412	50.000	**50.765**	2.71E+00	**1.53%**	0.0394	**50.745**	2.76E+00	**1.49%**
0.9346	0.0713	100.000	**101.371**	5.36E+00	**1.37%**	0.1065	**102.071**	5.43E+00	**2.07%**
-1.5297	-0.1168	200.000	**197.598**	1.04E+01	**-1.20%**	-0.0529	**198.911**	1.03E+01	**-0.54%**
-1.0157	-0.0775	300.000	**298.281**	1.51E+01	**-0.57%**	-0.0454	**299.006**	1.50E+01	**-0.33%**
1.1996	0.0916	400.000	**402.204**	1.94E+01	**0.55%**	0.0316	**400.739**	1.95E+01	**0.18%**
					7.69%				16.87%

Drug: α-OH-Midazolam
Unit: ng/mL

Trial 2	x	y	w (1/y)	x^2	x^3	x^4	xy	x^2y	$(y-ybar)^2$
L1	10.000	5.48E-01	1.83E+00	1.00E+02	1.00E+03	1.00E+04	5.48E+00	5.48E+01	5.20E+01
L2	20.000	1.08E+00	9.29E-01	4.00E+02	8.00E+03	1.60E+05	2.15E+01	4.31E+02	4.46E+01
L3	50.000	2.73E+00	3.66E-01	2.50E+03	1.25E+05	6.25E+06	1.36E+02	6.82E+03	2.53E+01
L4	100.000	5.40E+00	1.85E-01	1.00E+04	1.00E+06	1.00E+08	5.40E+02	5.40E+04	5.57E+00
L5	200.000	1.05E+01	9.56E-02	4.00E+04	8.00E+06	1.60E+09	2.09E+03	4.18E+05	7.29E+00
L6	300.000	1.49E+01	6.72E-02	9.00E+04	2.70E+07	8.10E+09	4.47E+03	1.34E+06	5.08E+01
L7	400.000	1.92E+01	5.20E-02	1.60E+05	6.40E+07	2.56E+10	7.69E+03	3.07E+06	1.31E+02
7	1.08E+03	5.43E+01	3.52E+00	3.03E+05	1.00E+08	3.54E+10	1.49E+04	4.89E+06	3.17E+02

wx	wy	wx^2	wx^3	wx^4	wxy	wx^2y	$(y-ybarw)^2$
1.83E+01	1.00E+00	1.83E+02	1.83E+03	1.83E+04	1.00E+01	1.00E+02	2.07E+00
1.86E+01	1.00E+00	3.72E+02	7.43E+03	1.49E+05	2.00E+01	4.00E+02	8.30E-01
1.83E+01	1.00E+00	9.16E+02	4.58E+04	2.29E+06	5.00E+01	2.50E+03	5.49E-01
1.85E+01	1.00E+00	1.85E+03	1.85E+05	1.85E+07	1.00E+02	1.00E+04	1.16E+01
1.91E+01	1.00E+00	3.82E+03	7.65E+05	1.53E+08	2.00E+02	4.00E+04	7.18E+01
2.02E+01	1.00E+00	6.05E+03	1.81E+06	5.44E+08	3.00E+02	9.00E+04	1.66E+02
2.08E+01	1.00E+00	8.33E+03	3.33E+06	1.33E+09	4.00E+02	1.60E+05	2.97E+02
1.34E+02	7.00E+00	2.15E+04	6.15E+06	2.05E+09	1.08E+03	3.03E+05	

Equal

A=	3.54E+10	1.00E+08	3.03E+05	B =	4.89E+06	X =	**-2.019E-05**	a
	1.00E+08	3.03E+05	1.08E+03		1.49E+04		**5.606E-02**	b
	3.03E+05	1.08E+03	7.00E+00		5.43E+01		**-1.647E-02**	c
A^{-1} =	7.18E-10	-2.81E-07	1.23E-05					
	-2.81E-07	1.17E-04	-5.94E-03				**0.9999**	R^2
	1.23E-05	-5.94E-03	5.28E-01					

Weighted

A =	2.05E+09	6.15E+06	2.15E+04	B =	3.03E+05	X =	**-2.038E-05**	a
	6.15E+06	2.15E+04	1.34E+02		1.08E+03		**5.613E-02**	b
	2.15E+04	1.34E+02	3.52E+00		7.00E+00		**-2.014E-02**	c
A^{-1} =	5.29E-09	-1.71E-06	3.28E-05					
	-1.71E-06	6.17E-04	-1.30E-02				**1.0000**	R^2
	3.28E-05	-1.30E-02	5.76E-01					

Standard Residual	y-yhat	Target	w (1/y) Calculated	yhat	Bias	Equal y-yhat	Calculated	Response	Bias
0.1606	0.0085	10.000	10.152	5.39E-01	1.52%	0.0056	**10.100**	5.48E-01	1.00%
-0.3393	-0.0179	20.000	19.676	1.09E+00	-1.62%	-0.0201	**19.636**	1.08E+00	-1.82%
-0.1284	-0.0068	50.000	49.875	2.74E+00	-0.25%	-0.0071	**49.869**	2.73E+00	-0.26%
0.1850	0.0098	100.000	100.188	5.39E+00	0.19%	0.0118	**100.228**	5.40E+00	0.23%
1.2721	0.0671	200.000	201.400	1.04E+01	0.70%	0.0711	**201.482**	1.05E+01	0.74%
-1.8943	-0.1000	300.000	297.725	1.50E+01	-0.76%	-0.0981	**297.770**	1.49E+01	-0.74%
0.7757	0.0409	400.000	401.028	1.92E+01	0.26%	0.0368	**400.923**	1.92E+01	0.23%
					5.29%				5.02%

Drug: α-OH-Midazolam
Unit: ng/mL

Trial 3	x	y	w (1/y)	x^2	x^3	x^4	xy	x^2y	$(y-ybar)^2$
L1	10.000	4.70E-01	2.13E+00	1.00E+02	1.00E+03	1.00E+04	4.70E+00	4.70E+01	5.62E+01
L2	20.000	1.05E+00	9.56E-01	4.00E+02	8.00E+03	1.60E+05	2.09E+01	4.18E+02	4.79E+01
L3	50.000	2.79E+00	3.59E-01	2.50E+03	1.25E+05	6.25E+06	1.39E+02	6.97E+03	2.68E+01
L4	100.000	5.52E+00	1.81E-01	1.00E+04	1.00E+06	1.00E+08	5.52E+02	5.52E+04	5.97E+00
L5	200.000	1.08E+01	9.24E-02	4.00E+04	8.00E+06	1.60E+09	2.16E+03	4.33E+05	8.14E+00
L6	300.000	1.55E+01	6.44E-02	9.00E+04	2.70E+07	8.10E+09	4.66E+03	1.40E+06	5.71E+01
L7	400.000	1.96E+01	5.11E-02	1.60E+05	6.40E+07	2.56E+10	7.83E+03	3.13E+06	1.35E+02
7	1.08E+03	5.57E+01	3.83E+00	3.03E+05	1.00E+08	3.54E+10	1.54E+04	5.03E+06	3.37E+02

wx	wy	wx^2	wx^3	wx^4	wxy	wx^2y	$(y-ybarw)^2$
2.13E+01	1.00E+00	2.13E+02	2.13E+03	2.13E+04	1.00E+01	1.00E+02	1.84E+00
1.91E+01	1.00E+00	3.82E+02	7.65E+03	1.53E+05	2.00E+01	4.00E+02	6.10E-01
1.79E+01	1.00E+00	8.97E+02	4.48E+04	2.24E+06	5.00E+01	2.50E+03	9.25E-01
1.81E+01	1.00E+00	1.81E+03	1.81E+05	1.81E+07	1.00E+02	1.00E+04	1.36E+01
1.85E+01	1.00E+00	3.70E+03	7.40E+05	1.48E+08	2.00E+02	4.00E+04	8.08E+01
1.93E+01	1.00E+00	5.80E+03	1.74E+06	5.22E+08	3.00E+02	9.00E+04	1.87E+02
2.04E+01	1.00E+00	8.17E+03	3.27E+06	1.31E+09	4.00E+02	1.60E+05	3.15E+02
1.35E+02	7.00E+00	2.10E+04	5.98E+06	2.00E+09	1.08E+03	3.03E+05	

Equal

A=	3.54E+10	1.00E+08	3.03E+05	B =	5.03E+06	X =	**-2.686E-05**	**a**
	1.00E+08	3.03E+05	1.08E+03		1.54E+04		**6.016E-02**	**b**
	3.03E+05	1.08E+03	7.00E+00		5.57E+01		**-1.547E-01**	**c**
A^{-1} =	7.18E-10	-2.81E-07	1.23E-05					
	-2.81E-07	1.17E-04	-5.94E-03				**1.0000**	R^2
	1.23E-05	-5.94E-03	5.28E-01					

Weighted

A =	2.00E+09	5.98E+06	2.10E+04	B =	3.03E+05	X =	**-2.537E-05**	**a**
	5.98E+06	2.10E+04	1.35E+02		1.08E+03		**5.957E-02**	**b**
	2.10E+04	1.35E+02	3.83E+00		7.00E+00		**-1.281E-01**	**c**
A^{-1} =	5.33E-09	-1.72E-06	3.13E-05					
	-1.72E-06	6.18E-04	-1.23E-02				**1.0000**	R^2
	3.13E-05	-1.23E-02	5.21E-01					

Standard Residual	y-yhat	Target	w (1/y) Calculated	yhat	Bias	Equal y-yhat	Calculated	Response	Bias
0.1107	0.0049	10.000	**10.083**	4.65E-01	**0.83%**	0.0257	**10.431**	4.70E-01	**4.31%**
-0.1635	-0.0072	20.000	**19.877**	1.05E+00	**-0.62%**	0.0081	**20.137**	1.05E+00	**0.69%**
0.0385	0.0017	50.000	**50.030**	2.79E+00	**0.06%**	0.0024	**50.042**	2.79E+00	**0.08%**
-1.2144	-0.0536	100.000	**99.016**	5.57E+00	**-0.98%**	-0.0713	**98.699**	5.52E+00	**-1.30%**
1.0693	0.0472	200.000	**200.956**	1.08E+01	**0.48%**	0.0151	**200.306**	1.08E+01	**0.15%**
1.3826	0.0611	300.000	**301.378**	1.55E+01	**0.46%**	0.0444	**301.008**	1.55E+01	**0.34%**
-1.1957	-0.0528	400.000	**398.656**	1.96E+01	**-0.34%**	-0.0243	**399.371**	1.96E+01	**-0.16%**
					3.76%				7.02%

Drug: α-OH-Midazolam
Unit: ng/mL

Trial 4	x	y	w (1/y)	x^2	x^3	x^4	xy	x^2y	$(y-ybar)^2$
L1	10.000	5.70E-01	1.76E+00	1.00E+02	1.00E+03	1.00E+04	5.70E+00	5.70E+01	5.46E+01
L2	20.000	1.16E+00	8.64E-01	4.00E+02	8.00E+03	1.60E+05	2.31E+01	4.63E+02	4.63E+01
L3	50.000	2.80E+00	3.58E-01	2.50E+03	1.25E+05	6.25E+06	1.40E+02	6.99E+03	2.66E+01
L4	100.000	5.25E+00	1.90E-01	1.00E+04	1.00E+06	1.00E+08	5.25E+02	5.25E+04	7.32E+00
L5	200.000	1.07E+01	9.37E-02	4.00E+04	8.00E+06	1.60E+09	2.14E+03	4.27E+05	7.39E+00
L6	300.000	1.53E+01	6.52E-02	9.00E+04	2.70E+07	8.10E+09	4.60E+03	1.38E+06	5.44E+01
L7	400.000	1.99E+01	5.02E-02	1.60E+05	6.40E+07	2.56E+10	7.97E+03	3.19E+06	1.43E+02
7	1.08E+03	5.57E+01	3.38E+00	3.03E+05	1.00E+08	3.54E+10	1.54E+04	5.05E+06	3.40E+02

wx	wy	wx^2	wx^3	wx^4	wxy	wx^2y	$(y-ybarw)^2$
1.76E+01	1.00E+00	1.76E+02	1.76E+03	1.76E+04	1.00E+01	1.00E+02	2.26E+00
1.73E+01	1.00E+00	3.46E+02	6.91E+03	1.38E+05	2.00E+01	4.00E+02	8.39E-01
1.79E+01	1.00E+00	8.94E+02	4.47E+04	2.23E+06	5.00E+01	2.50E+03	5.24E-01
1.90E+01	1.00E+00	1.90E+03	1.90E+05	1.90E+07	1.00E+02	1.00E+04	1.01E+01
1.87E+01	1.00E+00	3.75E+03	7.49E+05	1.50E+08	2.00E+02	4.00E+04	7.40E+01
1.96E+01	1.00E+00	5.87E+03	1.76E+06	5.28E+08	3.00E+02	9.00E+04	1.76E+02
2.01E+01	1.00E+00	8.03E+03	3.21E+06	1.28E+09	4.00E+02	1.60E+05	3.19E+02
1.30E+02	7.00E+00	2.10E+04	5.97E+06	1.98E+09	1.08E+03	3.03E+05	

Equal

A=	3.54E+10	1.00E+08	3.03E+05	B =	5.05E+06	X =	**-1.435E-05**	a
	1.00E+08	3.03E+05	1.08E+03		1.54E+04		**5.550E-02**	b
	3.03E+05	1.08E+03	7.00E+00		5.57E+01		**1.614E-02**	c
A^{-1} =	7.18E-10	-2.81E-07	1.23E-05					
	-2.81E-07	1.17E-04	-5.94E-03				**0.9998**	R^2
	1.23E-05	-5.94E-03	5.28E-01					

Weighted

A =	1.98E+09	5.97E+06	2.10E+04	B =	3.03E+05	X =	**-1.334E-05**	a
	5.97E+06	2.10E+04	1.30E+02		1.08E+03		**5.511E-02**	b
	2.10E+04	1.30E+02	3.38E+00		7.00E+00		**3.206E-02**	c
A^{-1} =	5.37E-09	-1.74E-06	3.36E-05					
	-1.74E-06	6.24E-04	-1.33E-02				**0.9998**	R^2
	3.36E-05	-1.33E-02	5.99E-01					

Standard Residual	w (1/y) y-yhat	Target	Calculated	yhat	Bias	Equal y-yhat	Calculated	Response	Bias
-0.1292	-0.0122	10.000	9.778	5.82E-01	-2.22%	-0.0001	9.999	5.70E-01	-0.01%
0.2974	0.0280	20.000	20.514	1.13E+00	2.57%	0.0365	20.665	1.16E+00	3.32%
0.4527	0.0427	50.000	50.793	2.75E+00	1.59%	0.0415	50.767	2.80E+00	1.53%
-1.6624	-0.1567	100.000	97.015	5.41E+00	-2.98%	-0.1699	96.774	5.25E+00	-3.23%
1.6663	0.1570	200.000	203.157	1.05E+01	1.58%	0.1346	202.706	1.07E+01	1.35%
-0.3324	-0.0313	300.000	299.335	1.54E+01	-0.22%	-0.0429	299.085	1.53E+01	-0.30%
-0.2007	-0.0189	400.000	399.575	1.99E+01	-0.11%	0.0004	400.009	1.99E+01	0.00%
					11.27%				9.76%

Drug: α-OH-Midazolam
Unit: ng/mL

Trial 5	x	y	w (1/y)	x^2	x^3	x^4	xy	x^2y	$(y-ybar)^2$
L1	10.000	4.12E-01	2.43E+00	1.00E+02	1.00E+03	1.00E+04	4.12E+00	4.12E+01	5.59E+01
L2	20.000	1.13E+00	8.87E-01	4.00E+02	8.00E+03	1.60E+05	2.25E+01	4.51E+02	4.57E+01
L3	50.000	2.51E+00	3.99E-01	2.50E+03	1.25E+05	6.25E+06	1.25E+02	6.26E+03	2.89E+01
L4	100.000	5.62E+00	1.78E-01	1.00E+04	1.00E+06	1.00E+08	5.62E+02	5.62E+04	5.11E+00
L5	200.000	1.11E+01	9.01E-02	4.00E+04	8.00E+06	1.60E+09	2.22E+03	4.44E+05	1.03E+01
L6	300.000	1.52E+01	6.59E-02	9.00E+04	2.70E+07	8.10E+09	4.55E+03	1.37E+06	5.33E+01
L7	400.000	1.93E+01	5.19E-02	1.60E+05	6.40E+07	2.56E+10	7.70E+03	3.08E+06	1.29E+02
7	1.08E+03	5.52E+01	4.10E+00	3.03E+05	1.00E+08	3.54E+10	1.52E+04	4.95E+06	3.28E+02

wx	wy	wx^2	wx^3	wx^4	wxy	wx^2y	$(y-ybarw)^2$
2.43E+01	1.00E+00	2.43E+02	2.43E+03	2.43E+04	1.00E+01	1.00E+02	1.68E+00
1.77E+01	1.00E+00	3.55E+02	7.10E+03	1.42E+05	2.00E+01	4.00E+02	3.36E-01
2.00E+01	1.00E+00	9.98E+02	4.99E+04	2.49E+06	5.00E+01	2.50E+03	6.37E-01
1.78E+01	1.00E+00	1.78E+03	1.78E+05	1.78E+07	1.00E+02	1.00E+04	1.53E+01
1.80E+01	1.00E+00	3.60E+03	7.21E+05	1.44E+08	2.00E+02	4.00E+04	8.82E+01
1.98E+01	1.00E+00	5.93E+03	1.78E+06	5.33E+08	3.00E+02	9.00E+04	1.82E+02
2.08E+01	1.00E+00	8.31E+03	3.32E+06	1.33E+09	4.00E+02	1.60E+05	3.08E+02
1.38E+02	7.00E+00	2.12E+04	6.06E+06	2.03E+09	1.08E+03	3.03E+05	

Equal

A=	3.54E+10	1.00E+08	3.03E+05	B =	4.95E+06	X =	**-3.446E-05**	**a**
	1.00E+08	3.03E+05	1.08E+03		1.52E+04		**6.242E-02**	**b**
	3.03E+05	1.08E+03	7.00E+00		5.52E+01		**-2.531E-01**	**c**
A^{-1} =	7.18E-10	-2.81E-07	1.23E-05					
	-2.81E-07	1.17E-04	-5.94E-03				**0.9994**	**R^2**
	1.23E-05	-5.94E-03	5.28E-01					

Weighted

A =	2.03E+09	6.06E+06	2.12E+04	B =	3.03E+05	X =	**-2.998E-05**	**a**
	6.06E+06	2.12E+04	1.38E+02		1.08E+03		**6.067E-02**	**b**
	2.12E+04	1.38E+02	4.10E+00		7.00E+00		**-1.844E-01**	**c**
A^{-1} =	5.21E-09	-1.68E-06	2.98E-05					
	-1.68E-06	6.04E-04	-1.17E-02				**0.9988**	**R^2**
	2.98E-05	-1.17E-02	4.83E-01					

Standard Residual	y-yhat	Target	w (1/y) Calculated	yhat	Bias	Equal y-yhat	Calculated	Response	Bias
-0.0389	-0.0075	10.000	**9.874**	4.19E-01	**-1.26%**	0.0441	**10.714**	4.12E-01	**7.14%**
0.5679	0.1100	20.000	**21.852**	1.02E+00	**9.26%**	0.1455	**22.387**	1.13E+00	**11.93%**
-1.3872	-0.2687	50.000	**45.352**	2.77E+00	**-9.30%**	-0.2763	**45.327**	2.51E+00	**-9.35%**
0.2161	0.0419	100.000	**100.766**	5.58E+00	**0.77%**	-0.0196	**99.647**	5.62E+00	**-0.35%**
1.7887	0.3465	200.000	**207.150**	1.08E+01	**3.58%**	0.2444	**205.044**	1.11E+01	**2.52%**
-0.6943	-0.1345	300.000	**296.856**	1.53E+01	**-1.05%**	-0.1877	**295.520**	1.52E+01	**-1.49%**
-0.1835	-0.0355	400.000	**399.032**	1.93E+01	**-0.24%**	0.0496	**401.426**	1.93E+01	**0.36%**
					25.44%				33.15%

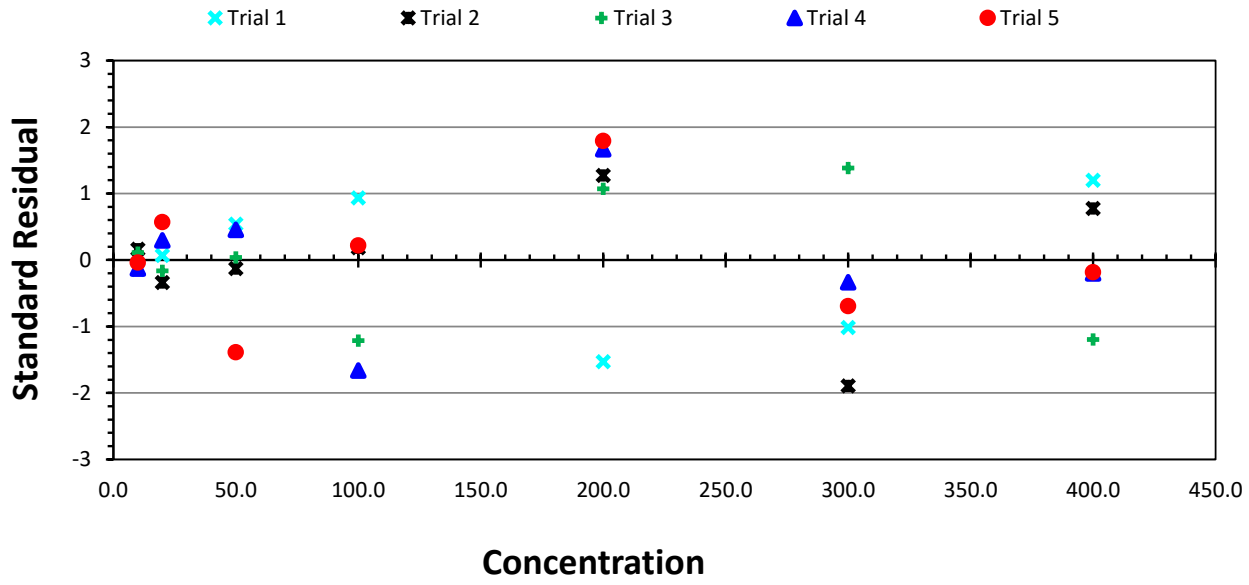
BZDG1 in Blood

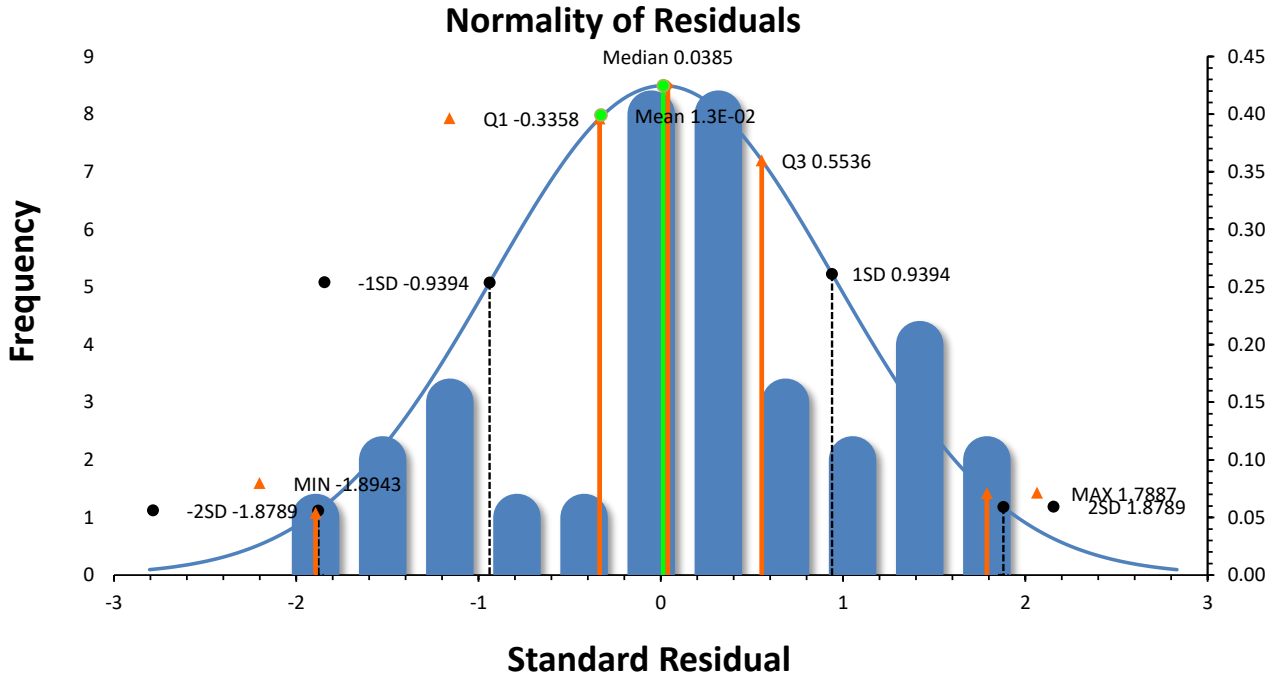
Drug: α-OH-Midazolam

Unit: ng/mL

Calibration Model: Quadratic (1/y)

Toxicologist(s): JD1 JD2 JD3 JD4 JD5 **Instrument:** LC-MS/MS

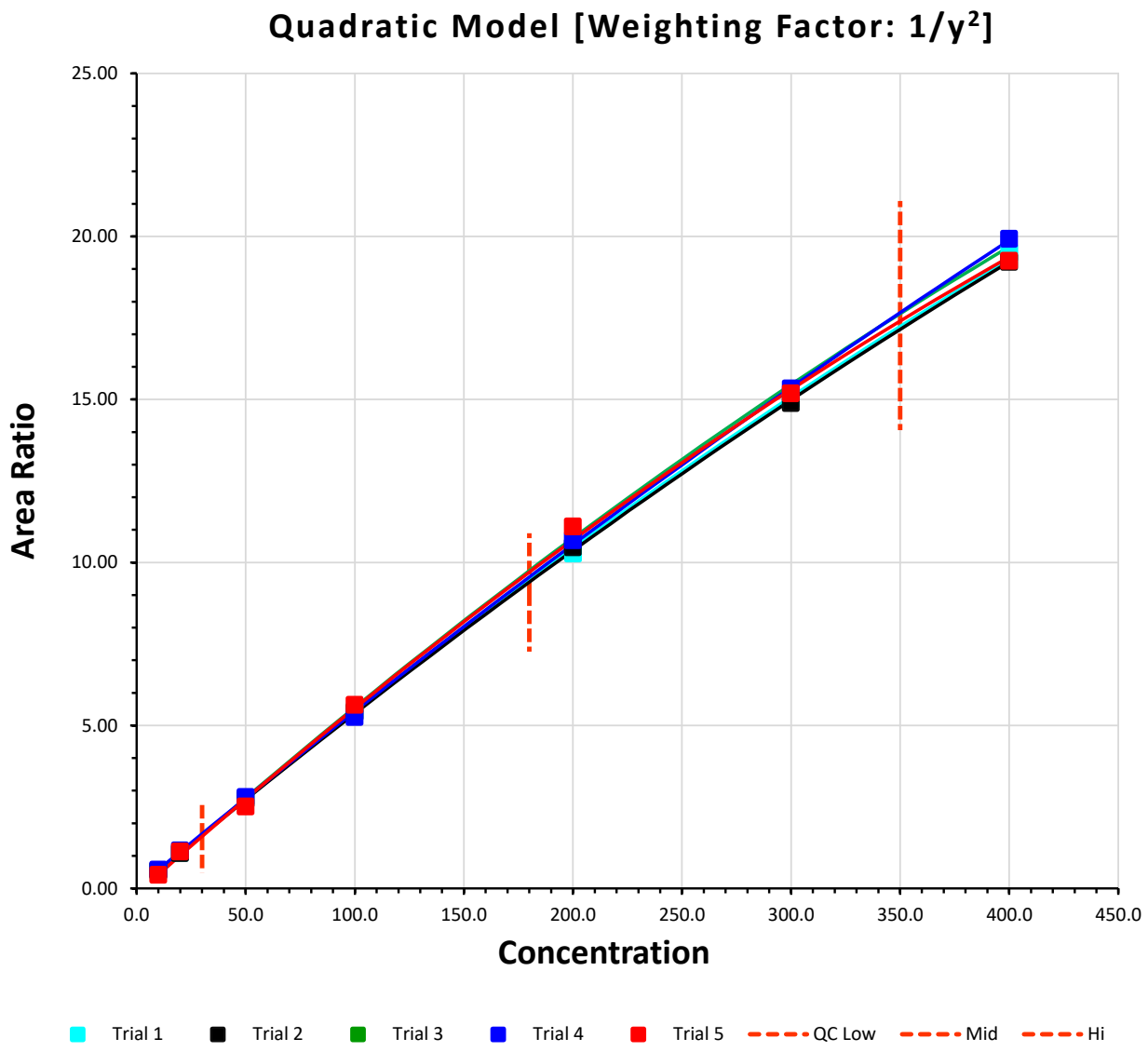




BZDG1 in Blood

Drug: α-OH-Midazolam

Unit: ng/mL

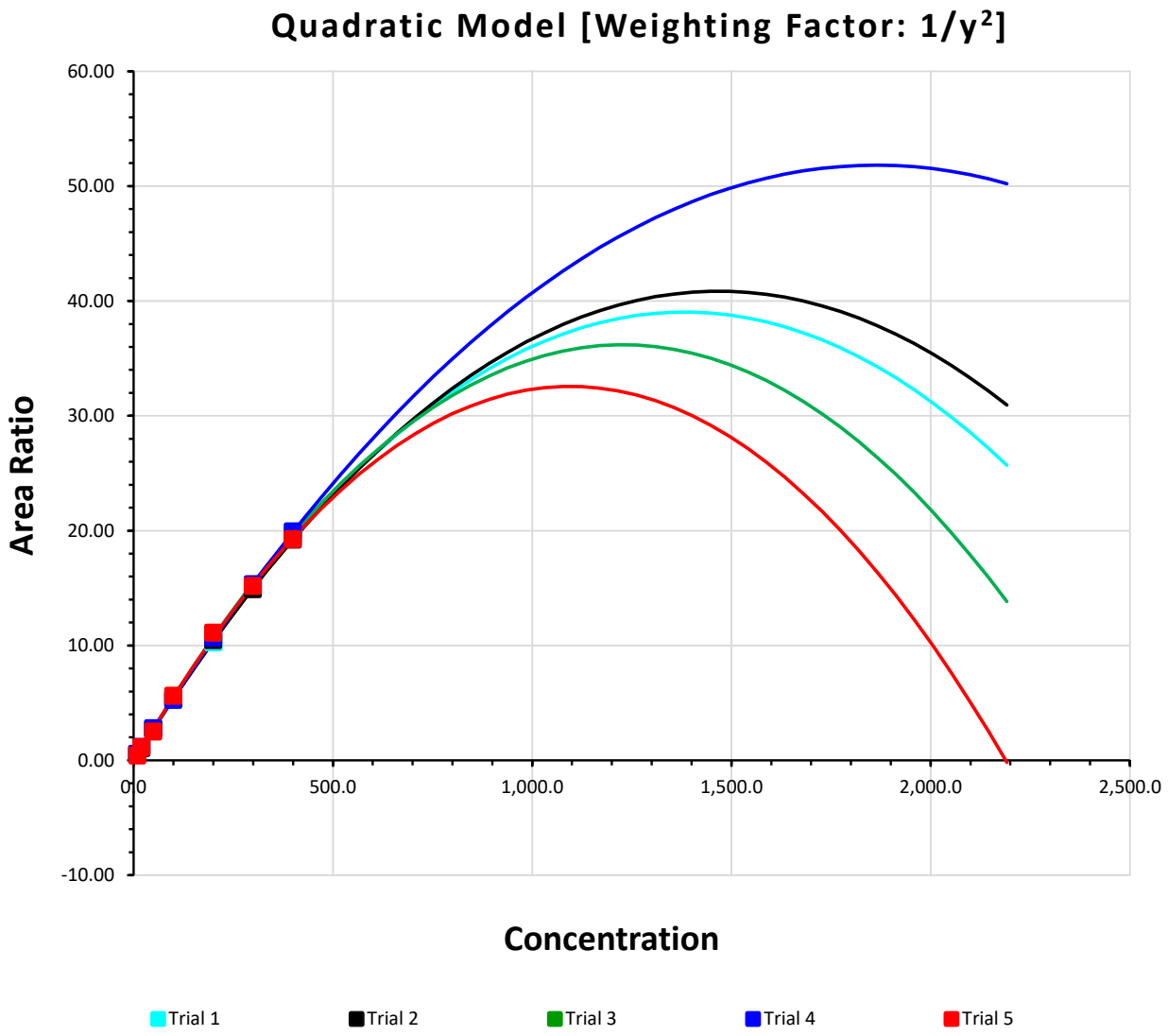


	a		b			c	R^2
Trial 1	-2.043E-05	X^2 +	5.651E-02	X	+	-3.817E-02	0.9999
Trial 2	-1.896E-05	X^2 +	5.566E-02	X	+	-1.115E-02	0.9999
Trial 3	-2.408E-05	X^2 +	5.914E-02	X	+	-1.203E-01	1.0000
Trial 4	-1.493E-05	X^2 +	5.562E-02	X	+	2.147E-02	0.9995
Trial 5	-2.727E-05	X^2 +	5.976E-02	X	+	-1.733E-01	0.9951

BZDG1 in Blood

Drug: α-OH-Midazolam

Unit: ng/mL



		Symmetry, h [Concentration]	Vertex, k [Area Ratio]	Focus [Area Ratio]		Directrix [Area Ratio]
Trial 1	x=	1.38E+03	3.90E+01	-1.22E+04	y=	1.23E+04
Trial 2	x=	1.47E+03	4.09E+01	-1.31E+04	y=	1.32E+04
Trial 3	x=	1.23E+03	3.62E+01	-1.03E+04	y=	1.04E+04
Trial 4	x=	1.86E+03	5.18E+01	-1.67E+04	y=	1.68E+04
Trial 5	x=	1.10E+03	3.26E+01	-9.13E+03	y=	9.20E+03

Drug: α-OH-Midazolam
Unit: ng/mL

Trial 1	x	y	w ($1/y^2$)	x^2	x^3	x^4	xy	x^2y	$(y\text{-}ybar)^2$
L1	10.000	5.23E-01	3.65E+00	1.00E+02	1.00E+03	1.00E+04	5.23E+00	5.23E+01	5.29E+01
L2	20.000	1.09E+00	8.43E-01	4.00E+02	8.00E+03	1.60E+05	2.18E+01	4.36E+02	4.50E+01
L3	50.000	2.76E+00	1.32E-01	2.50E+03	1.25E+05	6.25E+06	1.38E+02	6.89E+03	2.54E+01
L4	100.000	5.43E+00	3.39E-02	1.00E+04	1.00E+06	1.00E+08	5.43E+02	5.43E+04	5.59E+00
L5	200.000	1.03E+01	9.47E-03	4.00E+04	8.00E+06	1.60E+09	2.05E+03	4.11E+05	6.15E+00
L6	300.000	1.50E+01	4.45E-03	9.00E+04	2.70E+07	8.10E+09	4.50E+03	1.35E+06	5.18E+01
L7	400.000	1.95E+01	2.63E-03	1.60E+05	6.40E+07	2.56E+10	7.80E+03	3.12E+06	1.37E+02
7	1.08E+03	5.46E+01	4.68E+00	3.03E+05	1.00E+08	3.54E+10	1.51E+04	4.94E+06	3.24E+02

wx	wy	wx^2	wx^3	wx^4	wxy	wx^2y	$(y\text{-}ybarw)^2$
3.65E+01	1.91E+00	3.65E+02	3.65E+03	3.65E+04	1.91E+01	1.91E+02	5.98E-02
1.69E+01	9.18E-01	3.37E+02	6.74E+03	1.35E+05	1.84E+01	3.67E+02	1.04E-01
6.59E+00	3.63E-01	3.29E+02	1.65E+04	8.23E+05	1.81E+01	9.07E+02	3.95E+00
3.39E+00	1.84E-01	3.39E+02	3.39E+04	3.39E+06	1.84E+01	1.84E+03	2.17E+01
1.89E+00	9.73E-02	3.79E+02	7.58E+04	1.52E+07	1.95E+01	3.89E+03	9.04E+01
1.33E+00	6.67E-02	4.00E+02	1.20E+05	3.60E+07	2.00E+01	6.00E+03	2.02E+02
1.05E+00	5.13E-02	4.21E+02	1.68E+05	6.74E+07	2.05E+01	8.21E+03	3.51E+02
6.76E+01	3.59E+00	2.57E+03	4.25E+05	1.23E+08	1.34E+02	2.14E+04	

Equal

A=	3.54E+10	1.00E+08	3.03E+05	B =	4.94E+06	X =	**-1.443E-05**	**a**
	1.00E+08	3.03E+05	1.08E+03		1.51E+04		**5.435E-02**	**b**
	3.03E+05	1.08E+03	7.00E+00		5.46E+01		**3.426E-02**	**c**
A^{-1} =	7.18E-10	-2.81E-07	1.23E-05					
	-2.81E-07	1.17E-04	-5.94E-03				**0.9999**	R^2
	1.23E-05	-5.94E-03	5.28E-01					

Weighted

A =	1.23E+08	4.25E+05	2.57E+03	B =	2.14E+04	X =	**-2.043E-05**	**a**
	4.25E+05	2.57E+03	6.76E+01		1.34E+02		**5.651E-02**	**b**
	2.57E+03	6.76E+01	4.68E+00		3.59E+00		**-3.817E-02**	**c**
A^{-1} =	3.69E-08	-9.00E-06	1.10E-04					
	-9.00E-06	2.82E-03	-3.58E-02				**0.9999**	R^2
	1.10E-04	-3.58E-02	6.71E-01					

Standard Residual	y-yhat	Target	w ($1/y^2$) Calculated	yhat	Bias	Equal y-yhat	Calculated	Response	Bias
-0.0155	-0.0018	10.000	9.969	5.25E-01	-0.31%	-0.0532	**9.017**	5.23E-01	-9.83%
0.0491	0.0056	20.000	20.100	1.08E+00	0.50%	-0.0260	**19.516**	1.09E+00	-2.42%
0.1650	0.0187	50.000	50.343	2.74E+00	0.69%	0.0394	**50.745**	2.76E+00	1.49%
0.1998	0.0226	100.000	100.432	5.41E+00	0.43%	0.1065	**102.071**	5.43E+00	2.07%
-1.5295	-0.1732	200.000	196.422	1.04E+01	-1.79%	-0.0529	**198.911**	1.03E+01	-0.54%
-0.7255	-0.0822	300.000	298.145	1.51E+01	-0.62%	-0.0454	**299.006**	1.50E+01	-0.33%
1.7511	0.1983	400.000	404.950	1.93E+01	1.24%	0.0316	**400.739**	1.95E+01	0.18%
					5.57%				16.87%

Drug: α-OH-Midazolam
Unit: ng/mL

Trial 2	x	y	w (1/y²)	x²	x³	x⁴	xy	x²y	(y-ybar)²
L1	10.000	5.48E-01	3.33E+00	1.00E+02	1.00E+03	1.00E+04	5.48E+00	5.48E+01	5.20E+01
L2	20.000	1.08E+00	8.63E-01	4.00E+02	8.00E+03	1.60E+05	2.15E+01	4.31E+02	4.46E+01
L3	50.000	2.73E+00	1.34E-01	2.50E+03	1.25E+05	6.25E+06	1.36E+02	6.82E+03	2.53E+01
L4	100.000	5.40E+00	3.43E-02	1.00E+04	1.00E+06	1.00E+08	5.40E+02	5.40E+04	5.57E+00
L5	200.000	1.05E+01	9.14E-03	4.00E+04	8.00E+06	1.60E+09	2.09E+03	4.18E+05	7.29E+00
L6	300.000	1.49E+01	4.51E-03	9.00E+04	2.70E+07	8.10E+09	4.47E+03	1.34E+06	5.08E+01
L7	400.000	1.92E+01	2.71E-03	1.60E+05	6.40E+07	2.56E+10	7.69E+03	3.07E+06	1.31E+02
7	1.08E+03	5.43E+01	4.38E+00	3.03E+05	1.00E+08	3.54E+10	1.49E+04	4.89E+06	3.17E+02

wx	wy	wx²	wx³	wx⁴	wxy	wx²y	(y-ybarw)²
3.33E+01	1.83E+00	3.33E+02	3.33E+03	3.33E+04	1.83E+01	1.83E+02	6.55E-02
1.73E+01	9.29E-01	3.45E+02	6.90E+03	1.38E+05	1.86E+01	3.72E+02	7.45E-02
6.71E+00	3.66E-01	3.36E+02	1.68E+04	8.39E+05	1.83E+01	9.16E+02	3.71E+00
3.43E+00	1.85E-01	3.43E+02	3.43E+04	3.43E+06	1.85E+01	1.85E+03	2.11E+01
1.83E+00	9.56E-02	3.66E+02	7.31E+04	1.46E+07	1.91E+01	3.82E+03	9.32E+01
1.35E+00	6.72E-02	4.06E+02	1.22E+05	3.66E+07	2.02E+01	6.05E+03	1.98E+02
1.08E+00	5.20E-02	4.33E+02	1.73E+05	6.93E+07	2.08E+01	8.33E+03	3.39E+02
6.50E+01	3.52E+00	2.56E+03	4.30E+05	1.25E+08	1.34E+02	2.15E+04	

Equal

A=	3.54E+10	1.00E+08	3.03E+05	B =	4.89E+06	X =	**-2.019E-05**	**a**
	1.00E+08	3.03E+05	1.08E+03		1.49E+04		**5.606E-02**	**b**
	3.03E+05	1.08E+03	7.00E+00		5.43E+01		**-1.647E-02**	**c**
A^{-1} =	7.18E-10	-2.81E-07	1.23E-05					
	-2.81E-07	1.17E-04	-5.94E-03				**0.9999**	**R^2**
	1.23E-05	-5.94E-03	5.28E-01					

Weighted

A =	1.25E+08	4.30E+05	2.56E+03	B =	2.15E+04	X =	**-1.896E-05**	**a**
	4.30E+05	2.56E+03	6.50E+01		1.34E+02		**5.566E-02**	**b**
	2.56E+03	6.50E+01	4.38E+00		3.52E+00		**-1.115E-02**	**c**
A^{-1} =	3.64E-08	-8.92E-06	1.11E-04					
	-8.92E-06	2.81E-03	-3.65E-02				**0.9999**	**R^2**
	1.11E-04	-3.65E-02	7.05E-01					

Standard Residual	y-yhat	Target	w (1/y²) Calculated	yhat	Bias	Equal y-yhat	Calculated	Response	Bias
0.0703	0.0040	10.000	**10.073**	5.44E-01	**0.73%**	0.0056	**10.100**	5.48E-01	**1.00%**
-0.3142	-0.0181	20.000	**19.671**	1.09E+00	**-1.65%**	-0.0201	**19.636**	1.08E+00	**-1.82%**
0.0723	0.0042	50.000	**50.077**	2.72E+00	**0.15%**	-0.0071	**49.869**	2.73E+00	**-0.26%**
0.5823	0.0335	100.000	**100.646**	5.37E+00	**0.65%**	0.0118	**100.228**	5.40E+00	**0.23%**
1.6527	0.0951	200.000	**201.979**	1.04E+01	**0.99%**	0.0711	**201.482**	1.05E+01	**0.74%**
-1.6757	-0.0964	300.000	**297.826**	1.50E+01	**-0.72%**	-0.0981	**297.770**	1.49E+01	**-0.74%**
-0.1449	-0.0083	400.000	**399.794**	1.92E+01	**-0.05%**	0.0368	**400.923**	1.92E+01	**0.23%**
					4.94%				5.02%

Drug: α-OH-Midazolam
Unit: ng/mL

Trial 3	x	y	w (1/y²)	x²	x³	x⁴	xy	x²y	(y-ybar)²
L1	10.000	4.70E-01	4.53E+00	1.00E+02	1.00E+03	1.00E+04	4.70E+00	4.70E+01	5.62E+01
L2	20.000	1.05E+00	9.14E-01	4.00E+02	8.00E+03	1.60E+05	2.09E+01	4.18E+02	4.79E+01
L3	50.000	2.79E+00	1.29E-01	2.50E+03	1.25E+05	6.25E+06	1.39E+02	6.97E+03	2.68E+01
L4	100.000	5.52E+00	3.28E-02	1.00E+04	1.00E+06	1.00E+08	5.52E+02	5.52E+04	5.97E+00
L5	200.000	1.08E+01	8.55E-03	4.00E+04	8.00E+06	1.60E+09	2.16E+03	4.33E+05	8.14E+00
L6	300.000	1.55E+01	4.15E-03	9.00E+04	2.70E+07	8.10E+09	4.66E+03	1.40E+06	5.71E+01
L7	400.000	1.96E+01	2.61E-03	1.60E+05	6.40E+07	2.56E+10	7.83E+03	3.13E+06	1.35E+02
7	1.08E+03	5.57E+01	5.62E+00	3.03E+05	1.00E+08	3.54E+10	1.54E+04	5.03E+06	3.37E+02

wx	wy	wx²	wx³	wx⁴	wxy	wx²y	(y-ybarw)²
4.53E+01	2.13E+00	4.53E+02	4.53E+03	4.53E+04	2.13E+01	2.13E+02	4.49E-02
1.83E+01	9.56E-01	3.66E+02	7.31E+03	1.46E+05	1.91E+01	3.82E+02	1.32E-01
6.43E+00	3.59E-01	3.22E+02	1.61E+04	8.04E+05	1.79E+01	8.97E+02	4.44E+00
3.28E+00	1.81E-01	3.28E+02	3.28E+04	3.28E+06	1.81E+01	1.81E+03	2.34E+01
1.71E+00	9.24E-02	3.42E+02	6.84E+04	1.37E+07	1.85E+01	3.70E+03	1.03E+02
1.25E+00	6.44E-02	3.74E+02	1.12E+05	3.36E+07	1.93E+01	5.80E+03	2.20E+02
1.04E+00	5.11E-02	4.17E+02	1.67E+05	6.67E+07	2.04E+01	8.17E+03	3.57E+02
7.73E+01	3.83E+00	2.60E+03	4.08E+05	1.18E+08	1.35E+02	2.10E+04	

Equal

A=	3.54E+10	1.00E+08	3.03E+05	B =	5.03E+06	X =	**-2.686E-05**	a
	1.00E+08	3.03E+05	1.08E+03		1.54E+04		**6.016E-02**	b
	3.03E+05	1.08E+03	7.00E+00		5.57E+01		**-1.547E-01**	c
A^{-1} =	7.18E-10	-2.81E-07	1.23E-05					
	-2.81E-07	1.17E-04	-5.94E-03				**1.0000**	R^2
	1.23E-05	-5.94E-03	5.28E-01					

Weighted

A =	1.18E+08	4.08E+05	2.60E+03	B =	2.10E+04	X =	**-2.408E-05**	a
	4.08E+05	2.60E+03	7.73E+01		1.35E+02		**5.914E-02**	b
	2.60E+03	7.73E+01	5.62E+00		3.83E+00		**-1.203E-01**	c
A^{-1} =	3.70E-08	-8.96E-06	1.06E-04					
	-8.96E-06	2.82E-03	-3.46E-02				**1.0000**	R^2
	1.06E-04	-3.46E-02	6.05E-01					

Standard Residual	y-yhat	Target	w (1/y²) Calculated	yhat	Bias	Equal y-yhat	Calculated	Response	Bias
0.0204	0.0012	10.000	10.020	4.69E-01	0.20%	0.0257	**10.431**	4.70E-01	4.31%
-0.1224	-0.0071	20.000	19.878	1.05E+00	-0.61%	0.0081	**20.137**	1.05E+00	0.69%
0.2055	0.0119	50.000	50.209	2.78E+00	0.42%	0.0024	**50.042**	2.79E+00	0.08%
-0.5518	-0.0319	100.000	99.413	5.55E+00	-0.59%	-0.0713	**98.699**	5.52E+00	-1.30%
1.2597	0.0728	200.000	201.472	1.07E+01	0.74%	0.0151	**200.306**	1.08E+01	0.15%
1.1199	0.0647	300.000	301.449	1.55E+01	0.48%	0.0444	**301.008**	1.55E+01	0.34%
-1.6751	-0.0968	400.000	397.576	1.97E+01	-0.61%	-0.0243	**399.371**	1.96E+01	-0.16%
					3.64%				7.02%

Drug: α-OH-Midazolam
Unit: ng/mL

Trial 4	x	y	w (1/y²)	x²	x³	x⁴	xy	x²y	(y-ybar)²
L1	10.000	5.70E-01	3.08E+00	1.00E+02	1.00E+03	1.00E+04	5.70E+00	5.70E+01	5.46E+01
L2	20.000	1.16E+00	7.47E-01	4.00E+02	8.00E+03	1.60E+05	2.31E+01	4.63E+02	4.63E+01
L3	50.000	2.80E+00	1.28E-01	2.50E+03	1.25E+05	6.25E+06	1.40E+02	6.99E+03	2.66E+01
L4	100.000	5.25E+00	3.62E-02	1.00E+04	1.00E+06	1.00E+08	5.25E+02	5.25E+04	7.32E+00
L5	200.000	1.07E+01	8.77E-03	4.00E+04	8.00E+06	1.60E+09	2.14E+03	4.27E+05	7.39E+00
L6	300.000	1.53E+01	4.25E-03	9.00E+04	2.70E+07	8.10E+09	4.60E+03	1.38E+06	5.44E+01
L7	400.000	1.99E+01	2.52E-03	1.60E+05	6.40E+07	2.56E+10	7.97E+03	3.19E+06	1.43E+02
7	1.08E+03	5.57E+01	4.01E+00	3.03E+05	1.00E+08	3.54E+10	1.54E+04	5.05E+06	3.40E+02

wx	wy	wx²	wx³	wx⁴	wxy	wx²y	(y-ybarw)²
3.08E+01	1.76E+00	3.08E+02	3.08E+03	3.08E+04	1.76E+01	1.76E+02	7.44E-02
1.49E+01	8.64E-01	2.99E+02	5.98E+03	1.20E+05	1.73E+01	3.46E+02	9.89E-02
6.39E+00	3.58E-01	3.20E+02	1.60E+04	7.99E+05	1.79E+01	8.94E+02	3.82E+00
3.62E+00	1.90E-01	3.62E+02	3.62E+04	3.62E+06	1.90E+01	1.90E+03	1.95E+01
1.75E+00	9.37E-02	3.51E+02	7.02E+04	1.40E+07	1.87E+01	3.75E+03	9.67E+01
1.28E+00	6.52E-02	3.83E+02	1.15E+05	3.45E+07	1.96E+01	5.87E+03	2.10E+02
1.01E+00	5.02E-02	4.03E+02	1.61E+05	6.45E+07	2.01E+01	8.03E+03	3.64E+02
5.98E+01	3.38E+00	2.43E+03	4.08E+05	1.18E+08	1.30E+02	2.10E+04	

Equal

A=	3.54E+10	1.00E+08	3.03E+05	B =	5.05E+06	X =	-1.435E-05	a
	1.00E+08	3.03E+05	1.08E+03		1.54E+04		5.550E-02	b
	3.03E+05	1.08E+03	7.00E+00		5.57E+01		1.614E-02	c
A^{-1} =	7.18E-10	-2.81E-07	1.23E-05					
	-2.81E-07	1.17E-04	-5.94E-03				0.9998	R^2
	1.23E-05	-5.94E-03	5.28E-01					

Weighted

A =	1.18E+08	4.08E+05	2.43E+03	B =	2.10E+04	X =	-1.493E-05	a
	4.08E+05	2.43E+03	5.98E+01		1.30E+02		5.562E-02	b
	2.43E+03	5.98E+01	4.01E+00		3.38E+00		2.147E-02	c
A^{-1} =	3.82E-08	-9.26E-06	1.15E-04					
	-9.26E-06	2.89E-03	-3.76E-02				0.9995	R^2
	1.15E-04	-3.76E-02	7.41E-01					

Standard Residual	y-yhat	Target	w (1/y²) Calculated	yhat	Bias	Equal y-yhat	Calculated	Response	Bias
-0.0691	-0.0066	10.000	9.882	5.76E-01	-1.18%	-0.0001	9.999	5.70E-01	-0.01%
0.3056	0.0290	20.000	20.527	1.13E+00	2.63%	0.0365	20.665	1.16E+00	3.32%
0.3328	0.0316	50.000	50.583	2.77E+00	1.17%	0.0415	50.767	2.80E+00	1.53%
-1.9144	-0.1815	100.000	96.555	5.43E+00	-3.45%	-0.1699	96.774	5.25E+00	-3.23%
1.3543	0.1284	200.000	202.588	1.05E+01	1.29%	0.1346	202.706	1.07E+01	1.35%
-0.3366	-0.0319	300.000	299.316	1.54E+01	-0.23%	-0.0429	299.085	1.53E+01	-0.30%
0.4243	0.0402	400.000	400.921	1.99E+01	0.23%	0.0004	400.009	1.99E+01	0.00%
					10.18%				9.76%

Drug: α-OH-Midazolam
Unit: ng/mL

Trial 5	x	y	w (1/y²)	x²	x³	x⁴	xy	x²y	(y-ybar)²
L1	10.000	4.12E-01	5.90E+00	1.00E+02	1.00E+03	1.00E+04	4.12E+00	4.12E+01	5.59E+01
L2	20.000	1.13E+00	7.87E-01	4.00E+02	8.00E+03	1.60E+05	2.25E+01	4.51E+02	4.57E+01
L3	50.000	2.51E+00	1.59E-01	2.50E+03	1.25E+05	6.25E+06	1.25E+02	6.26E+03	2.89E+01
L4	100.000	5.62E+00	3.16E-02	1.00E+04	1.00E+06	1.00E+08	5.62E+02	5.62E+04	5.11E+00
L5	200.000	1.11E+01	8.12E-03	4.00E+04	8.00E+06	1.60E+09	2.22E+03	4.44E+05	1.03E+01
L6	300.000	1.52E+01	4.34E-03	9.00E+04	2.70E+07	8.10E+09	4.55E+03	1.37E+06	5.33E+01
L7	400.000	1.93E+01	2.70E-03	1.60E+05	6.40E+07	2.56E+10	7.70E+03	3.08E+06	1.29E+02
7	1.08E+03	5.52E+01	6.89E+00	3.03E+05	1.00E+08	3.54E+10	1.52E+04	4.95E+06	3.28E+02

wx	wy	wx²	wx³	wx⁴	wxy	wx²y	(y-ybarw)²
5.90E+01	2.43E+00	5.90E+02	5.90E+03	5.90E+04	2.43E+01	2.43E+02	3.36E-02
1.57E+01	8.87E-01	3.15E+02	6.30E+03	1.26E+05	1.77E+01	3.55E+02	2.83E-01
7.97E+00	3.99E-01	3.98E+02	1.99E+04	9.96E+05	2.00E+01	9.98E+02	3.65E+00
3.16E+00	1.78E-01	3.16E+02	3.16E+04	3.16E+06	1.78E+01	1.78E+03	2.53E+01
1.62E+00	9.01E-02	3.25E+02	6.50E+04	1.30E+07	1.80E+01	3.60E+03	1.10E+02
1.30E+00	6.59E-02	3.90E+02	1.17E+05	3.51E+07	1.98E+01	5.93E+03	2.13E+02
1.08E+00	5.19E-02	4.32E+02	1.73E+05	6.91E+07	2.08E+01	8.31E+03	3.48E+02
8.99E+01	4.10E+00	2.77E+03	4.19E+05	1.22E+08	1.38E+02	2.12E+04	

Equal

A=	3.54E+10	1.00E+08	3.03E+05	B =	4.95E+06	X =	**-3.446E-05**	a
	1.00E+08	3.03E+05	1.08E+03		1.52E+04		**6.242E-02**	b
	3.03E+05	1.08E+03	7.00E+00		5.52E+01		**-2.531E-01**	c
A^{-1} =	7.18E-10	-2.81E-07	1.23E-05					
	-2.81E-07	1.17E-04	-5.94E-03				**0.9994**	R^2
	1.23E-05	-5.94E-03	5.28E-01					

Weighted

A =	1.22E+08	4.19E+05	2.77E+03	B =	2.12E+04	X =	**-2.727E-05**	a
	4.19E+05	2.77E+03	8.99E+01		1.38E+02		**5.976E-02**	b
	2.77E+03	8.99E+01	6.89E+00		4.10E+00		**-1.733E-01**	c
A^{-1} =	3.48E-08	-8.35E-06	9.50E-05					
	-8.35E-06	2.63E-03	-3.10E-02				**0.9951**	R^2
	9.50E-05	-3.10E-02	5.11E-01					

Standard Residual	y-yhat	Target	w (1/y²) Calculated	yhat	Bias	Equal y-yhat	Calculated	Response	Bias
-0.0464	-0.0099	10.000	**9.833**	4.22E-01	**-1.67%**	0.0441	**10.714**	4.12E-01	**7.14%**
0.5439	0.1159	20.000	**21.978**	1.01E+00	**9.89%**	0.1455	**22.387**	1.13E+00	**11.93%**
-1.1325	-0.2413	50.000	**45.777**	2.75E+00	**-8.45%**	-0.2763	**45.327**	2.51E+00	**-9.35%**
0.4419	0.0942	100.000	**101.736**	5.53E+00	**1.74%**	-0.0196	**99.647**	5.62E+00	**-0.35%**
1.9149	0.4081	200.000	**208.393**	1.07E+01	**4.20%**	0.2444	**205.044**	1.11E+01	**2.52%**
-0.5527	-0.1178	300.000	**297.290**	1.53E+01	**-0.90%**	-0.1877	**295.520**	1.52E+01	**-1.49%**
-0.5532	-0.1179	400.000	**396.900**	1.94E+01	**-0.78%**	0.0496	**401.426**	1.93E+01	**0.36%**
					27.61%				33.15%

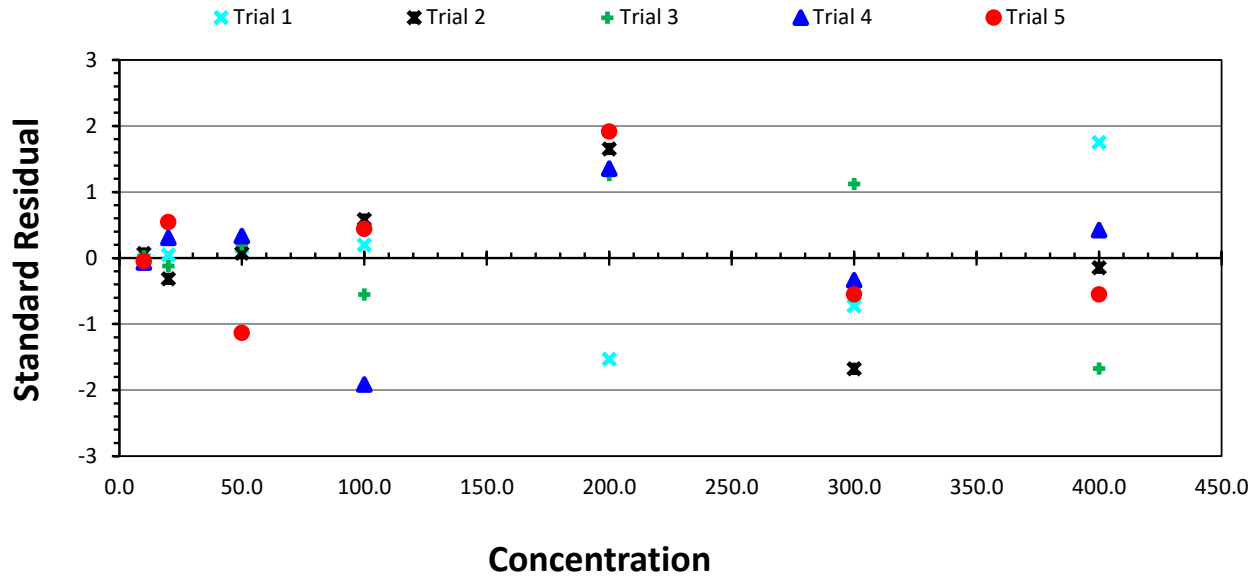
BZDG1 in Blood

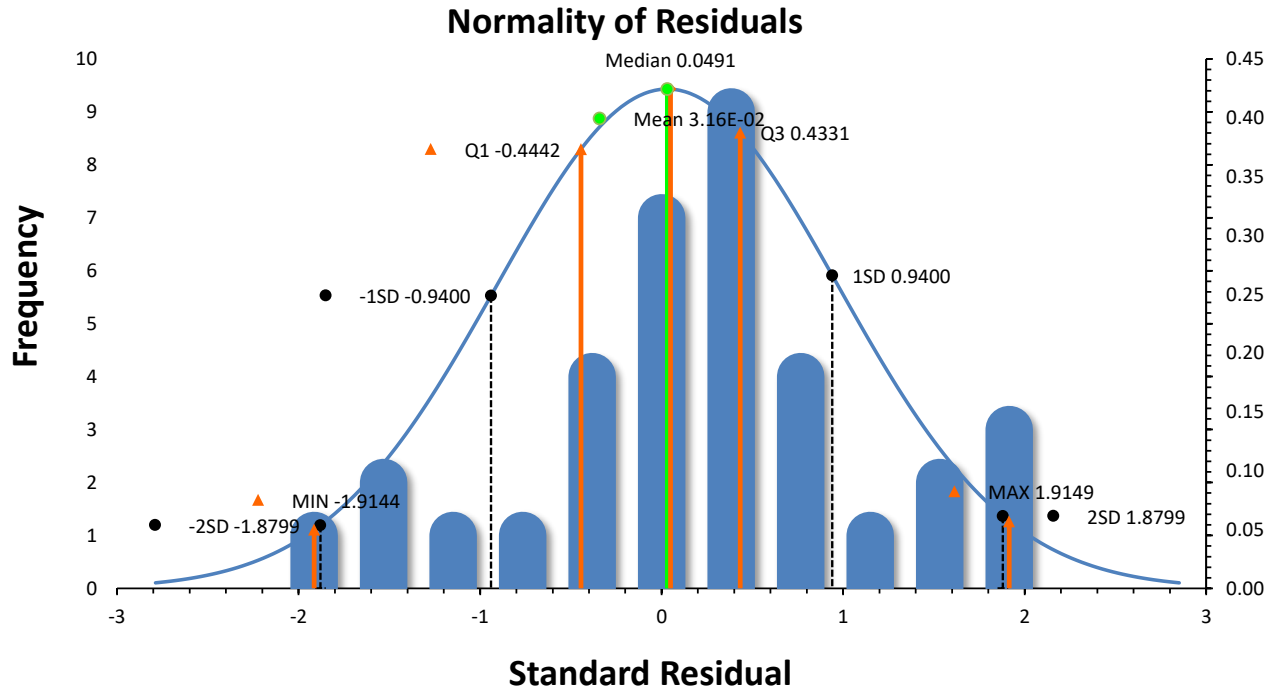
Drug: α-OH-Midazolam

Unit: ng/mL

Calibration Model: Quadratic $(1/y^2)$

Toxicologist(s): JD1 JD2 JD3 JD4 JD5 **Instrument:** LC-MS/MS

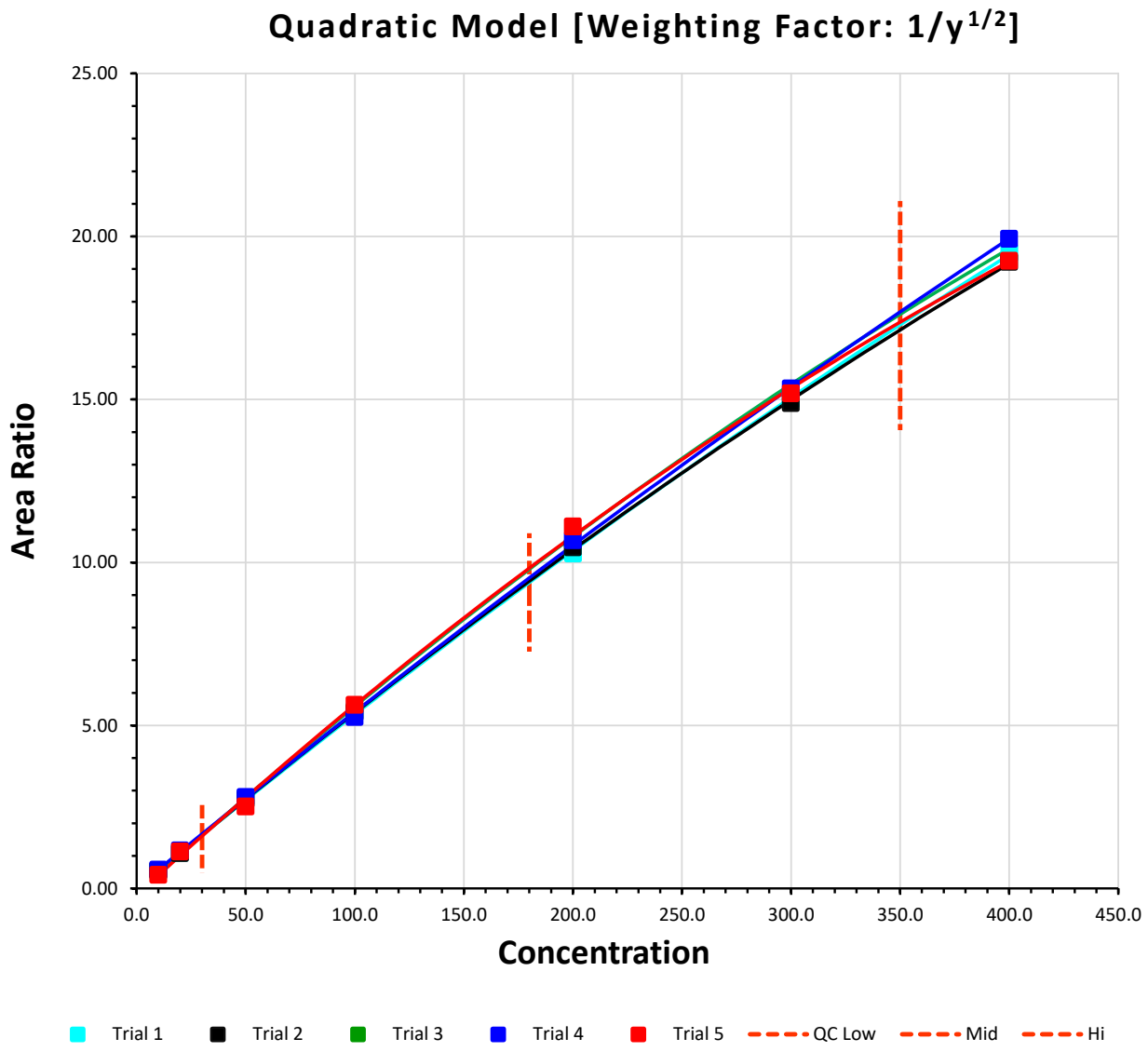




BZDG1 in Blood

Drug: α-OH-Midazolam

Unit: ng/mL

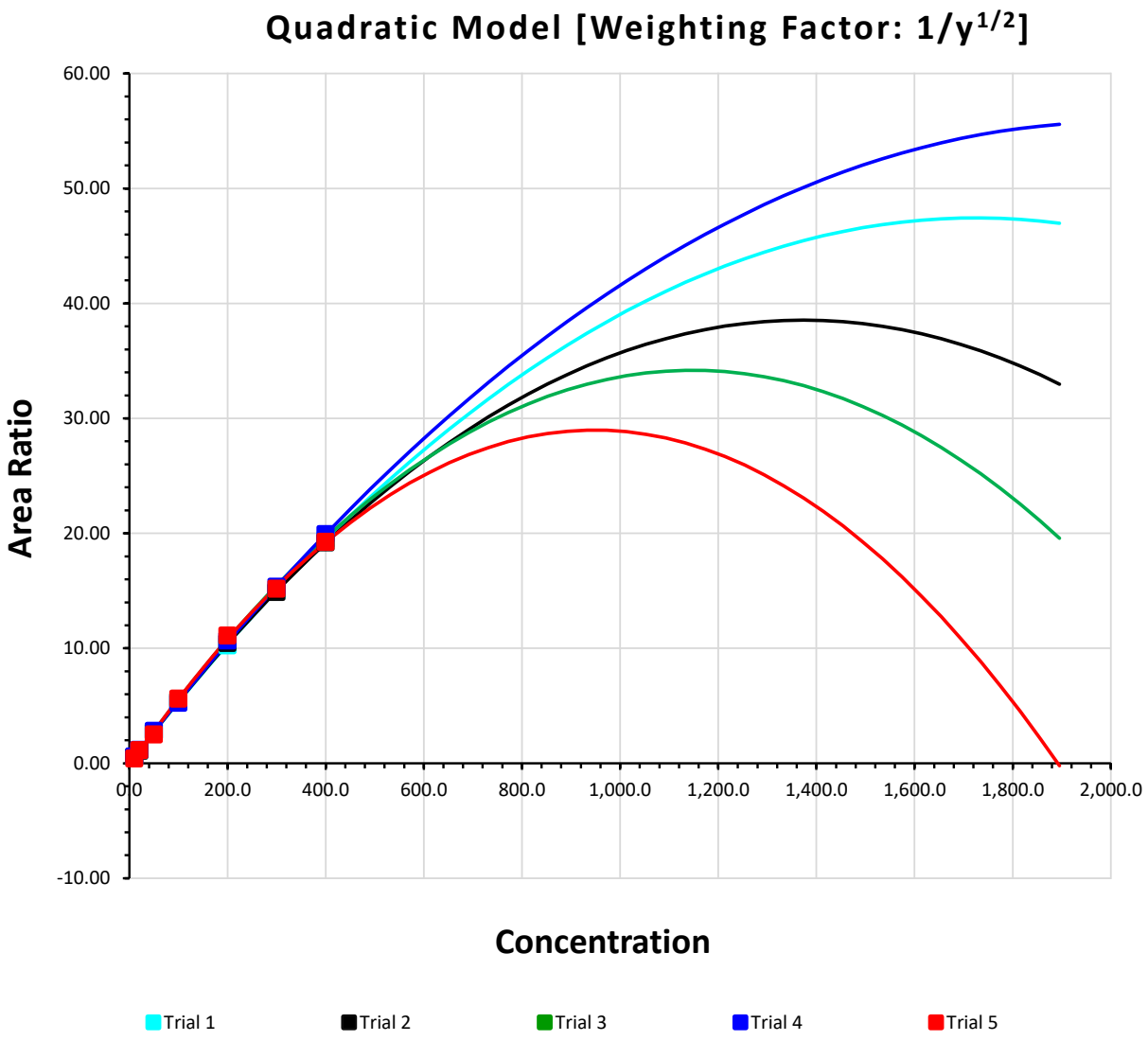


	a		b			c	R^2
Trial 1	-1.592E-05	X^2 +	5.497E-02	X	+	2.318E-04	0.9999
Trial 2	-2.044E-05	X^2 +	5.616E-02	X	+	-2.147E-02	1.0000
Trial 3	-2.608E-05	X^2 +	5.983E-02	X	+	-1.370E-01	1.0000
Trial 4	-1.368E-05	X^2 +	5.523E-02	X	+	2.929E-02	0.9998
Trial 5	-3.250E-05	X^2 +	6.161E-02	X	+	-2.118E-01	0.9993

BZDG1 in Blood

Drug: α-OH-Midazolam

Unit: ng/mL



		Symmetry, h [Concentration]	Vertex, k [Area Ratio]	Focus [Area Ratio]		Directrix [Area Ratio]
Trial 1	x=	1.73E+03	4.74E+01	-1.57E+04	y=	1.57E+04
Trial 2	x=	1.37E+03	3.85E+01	-1.22E+04	y=	1.23E+04
Trial 3	x=	1.15E+03	3.42E+01	-9.55E+03	y=	9.62E+03
Trial 4	x=	2.02E+03	5.58E+01	-1.82E+04	y=	1.83E+04
Trial 5	x=	9.48E+02	2.90E+01	-7.66E+03	y=	7.72E+03

Drug: α-OH-Midazolam
Unit: ng/mL

Trial 1	x	y	w ($1/y^{1/2}$)	x^2	x^3	x^4	xy	x^2y	$(y\text{-}ybar)^2$
L1	10.000	5.23E-01	1.38E+00	1.00E+02	1.00E+03	1.00E+04	5.23E+00	5.23E+01	5.29E+01
L2	20.000	1.09E+00	9.58E-01	4.00E+02	8.00E+03	1.60E+05	2.18E+01	4.36E+02	4.50E+01
L3	50.000	2.76E+00	6.02E-01	2.50E+03	1.25E+05	6.25E+06	1.38E+02	6.89E+03	2.54E+01
L4	100.000	5.43E+00	4.29E-01	1.00E+04	1.00E+06	1.00E+08	5.43E+02	5.43E+04	5.59E+00
L5	200.000	1.03E+01	3.12E-01	4.00E+04	8.00E+06	1.60E+09	2.05E+03	4.11E+05	6.15E+00
L6	300.000	1.50E+01	2.58E-01	9.00E+04	2.70E+07	8.10E+09	4.50E+03	1.35E+06	5.18E+01
L7	400.000	1.95E+01	2.26E-01	1.60E+05	6.40E+07	2.56E+10	7.80E+03	3.12E+06	1.37E+02
7	1.08E+03	5.46E+01	4.17E+00	3.03E+05	1.00E+08	3.54E+10	1.51E+04	4.94E+06	3.24E+02

wx	wy	wx^2	wx^3	wx^4	wxy	wx^2y	$(y\text{-}ybarw)^2$
1.38E+01	7.23E-01	1.38E+02	1.38E+03	1.38E+04	7.23E+00	7.23E+01	1.31E+01
1.92E+01	1.04E+00	3.83E+02	7.66E+03	1.53E+05	2.09E+01	4.18E+02	9.29E+00
3.01E+01	1.66E+00	1.51E+03	7.53E+04	3.77E+06	8.30E+01	4.15E+03	1.91E+00
4.29E+01	2.33E+00	4.29E+03	4.29E+05	4.29E+07	2.33E+02	2.33E+04	1.67E+00
6.24E+01	3.21E+00	1.25E+04	2.50E+06	4.99E+08	6.41E+02	1.28E+05	3.76E+01
7.75E+01	3.87E+00	2.32E+04	6.97E+06	2.09E+09	1.16E+03	3.49E+05	1.18E+02
9.06E+01	4.42E+00	3.62E+04	1.45E+07	5.80E+09	1.77E+03	7.06E+05	2.36E+02
3.36E+02	1.73E+01	7.83E+04	2.45E+07	8.44E+09	3.91E+03	1.21E+06	

Equal

A=	3.54E+10	1.00E+08	3.03E+05	B =	4.94E+06	X =	**-1.443E-05**	a
	1.00E+08	3.03E+05	1.08E+03		1.51E+04		**5.435E-02**	b
	3.03E+05	1.08E+03	7.00E+00		5.46E+01		**3.426E-02**	c
A^{-1} =	7.18E-10	-2.81E-07	1.23E-05					
	-2.81E-07	1.17E-04	-5.94E-03				**0.9999**	R^2
	1.23E-05	-5.94E-03	5.28E-01					

Weighted

A =	8.44E+09	2.45E+07	7.83E+04	B =	1.21E+06	X =	**-1.592E-05**	a
	2.45E+07	7.83E+04	3.36E+02		3.91E+03		**5.497E-02**	b
	7.83E+04	3.36E+02	4.17E+00		1.73E+01		**2.318E-04**	c
A^{-1} =	1.94E-09	-6.89E-07	1.92E-05					
	-6.89E-07	2.64E-04	-8.40E-03				**0.9999**	R^2
	1.92E-05	-8.40E-03	5.57E-01					

Standard Residual	y-yhat	Target	w ($1/y^{1/2}$) Calculated	yhat	Bias	Equal y-yhat	Calculated	Response	Bias
-0.3860	-0.0252	10.000	9.539	5.48E-01	-4.61%	-0.0532	**9.017**	5.23E-01	-9.83%
-0.0580	-0.0038	20.000	19.930	1.09E+00	-0.35%	-0.0260	**19.516**	1.09E+00	-2.42%
0.7079	0.0462	50.000	50.865	2.71E+00	1.73%	0.0394	**50.745**	2.76E+00	1.49%
1.4333	0.0935	100.000	101.807	5.34E+00	1.81%	0.1065	**102.071**	5.43E+00	2.07%
-1.2753	-0.0832	200.000	198.289	1.04E+01	-0.86%	-0.0529	**198.911**	1.03E+01	-0.54%
-0.9675	-0.0631	300.000	298.611	1.51E+01	-0.46%	-0.0454	**299.006**	1.50E+01	-0.33%
0.8627	0.0563	400.000	401.333	1.94E+01	0.33%	0.0316	**400.739**	1.95E+01	0.18%
					10.14%				16.87%

Drug: α-OH-Midazolam
Unit: ng/mL

Trial 2	x	y	w ($1/y^{1/2}$)	x^2	x^3	x^4	xy	x^2y	$(y\text{-}ybar)^2$
L1	10.000	5.48E-01	1.35E+00	1.00E+02	1.00E+03	1.00E+04	5.48E+00	5.48E+01	5.20E+01
L2	20.000	1.08E+00	9.64E-01	4.00E+02	8.00E+03	1.60E+05	2.15E+01	4.31E+02	4.46E+01
L3	50.000	2.73E+00	6.05E-01	2.50E+03	1.25E+05	6.25E+06	1.36E+02	6.82E+03	2.53E+01
L4	100.000	5.40E+00	4.30E-01	1.00E+04	1.00E+06	1.00E+08	5.40E+02	5.40E+04	5.57E+00
L5	200.000	1.05E+01	3.09E-01	4.00E+04	8.00E+06	1.60E+09	2.09E+03	4.18E+05	7.29E+00
L6	300.000	1.49E+01	2.59E-01	9.00E+04	2.70E+07	8.10E+09	4.47E+03	1.34E+06	5.08E+01
L7	400.000	1.92E+01	2.28E-01	1.60E+05	6.40E+07	2.56E+10	7.69E+03	3.07E+06	1.31E+02
7	1.08E+03	5.43E+01	4.15E+00	3.03E+05	1.00E+08	3.54E+10	1.49E+04	4.89E+06	3.17E+02

wx	wy	wx^2	wx^3	wx^4	wxy	wx^2y	$(y\text{-}ybarw)^2$
1.35E+01	7.40E-01	1.35E+02	1.35E+03	1.35E+04	7.40E+00	7.40E+01	1.30E+01
1.93E+01	1.04E+00	3.86E+02	7.71E+03	1.54E+05	2.08E+01	4.15E+02	9.47E+00
3.03E+01	1.65E+00	1.51E+03	7.57E+04	3.78E+06	8.26E+01	4.13E+03	2.03E+00
4.30E+01	2.32E+00	4.30E+03	4.30E+05	4.30E+07	2.32E+02	2.32E+04	1.55E+00
6.18E+01	3.23E+00	1.24E+04	2.47E+06	4.95E+08	6.47E+02	1.29E+05	3.97E+01
7.78E+01	3.86E+00	2.33E+04	7.00E+06	2.10E+09	1.16E+03	3.47E+05	1.15E+02
9.13E+01	4.38E+00	3.65E+04	1.46E+07	5.84E+09	1.75E+03	7.01E+05	2.27E+02
3.37E+02	1.72E+01	7.85E+04	2.46E+07	8.48E+09	3.90E+03	1.21E+06	

Equal

A=	3.54E+10	1.00E+08	3.03E+05	B =	4.89E+06	X =	**-2.019E-05**	a
	1.00E+08	3.03E+05	1.08E+03		1.49E+04		**5.606E-02**	b
	3.03E+05	1.08E+03	7.00E+00		5.43E+01		**-1.647E-02**	c
A^{-1} =	7.18E-10	-2.81E-07	1.23E-05					
	-2.81E-07	1.17E-04	-5.94E-03				**0.9999**	R^2
	1.23E-05	-5.94E-03	5.28E-01					

Weighted

A =	8.48E+09	2.46E+07	7.85E+04	B =	1.21E+06	X =	**-2.044E-05**	a
	2.46E+07	7.85E+04	3.37E+02		3.90E+03		**5.616E-02**	b
	7.85E+04	3.37E+02	4.15E+00		1.72E+01		**-2.147E-02**	c
A^{-1} =	1.94E-09	-6.92E-07	1.94E-05					
	-6.92E-07	2.66E-04	-8.49E-03				**1.0000**	R^2
	1.94E-05	-8.49E-03	5.64E-01					

Standard Residual	y-yhat	Target	w ($1/y^{1/2}$) Calculated	yhat	Bias	Equal y-yhat	Calculated	Response	Bias
0.1808	0.0096	10.000	10.171	5.38E-01	1.71%	0.0056	**10.100**	5.48E-01	1.00%
-0.3231	-0.0171	20.000	19.692	1.09E+00	-1.54%	-0.0201	**19.636**	1.08E+00	-1.82%
-0.1249	-0.0066	50.000	49.878	2.74E+00	-0.24%	-0.0071	**49.869**	2.73E+00	-0.26%
0.1720	0.0091	100.000	100.175	5.39E+00	0.17%	0.0118	**100.228**	5.40E+00	0.23%
1.2432	0.0657	200.000	201.370	1.04E+01	0.68%	0.0711	**201.482**	1.05E+01	0.74%
-1.9110	-0.1010	300.000	297.702	1.50E+01	-0.77%	-0.0981	**297.770**	1.49E+01	-0.74%
0.7876	0.0416	400.000	401.046	1.92E+01	0.26%	0.0368	**400.923**	1.92E+01	0.23%
					5.39%				5.02%

Drug: α-OH-Midazolam
Unit: ng/mL

Trial 3	x	y	w ($1/y^{1/2}$)	x^2	x^3	x^4	xy	x^2y	$(y-ybar)^2$
L1	10.000	4.70E-01	1.46E+00	1.00E+02	1.00E+03	1.00E+04	4.70E+00	4.70E+01	5.62E+01
L2	20.000	1.05E+00	9.78E-01	4.00E+02	8.00E+03	1.60E+05	2.09E+01	4.18E+02	4.79E+01
L3	50.000	2.79E+00	5.99E-01	2.50E+03	1.25E+05	6.25E+06	1.39E+02	6.97E+03	2.68E+01
L4	100.000	5.52E+00	4.26E-01	1.00E+04	1.00E+06	1.00E+08	5.52E+02	5.52E+04	5.97E+00
L5	200.000	1.08E+01	3.04E-01	4.00E+04	8.00E+06	1.60E+09	2.16E+03	4.33E+05	8.14E+00
L6	300.000	1.55E+01	2.54E-01	9.00E+04	2.70E+07	8.10E+09	4.66E+03	1.40E+06	5.71E+01
L7	400.000	1.96E+01	2.26E-01	1.60E+05	6.40E+07	2.56E+10	7.83E+03	3.13E+06	1.35E+02
7	1.08E+03	5.57E+01	4.24E+00	3.03E+05	1.00E+08	3.54E+10	1.54E+04	5.03E+06	3.37E+02

wx	wy	wx^2	wx^3	wx^4	wxy	wx^2y	$(y-ybarw)^2$
1.46E+01	6.85E-01	1.46E+02	1.46E+03	1.46E+04	6.85E+00	6.85E+01	1.31E+01
1.96E+01	1.02E+00	3.91E+02	7.82E+03	1.56E+05	2.05E+01	4.09E+02	9.30E+00
2.99E+01	1.67E+00	1.50E+03	7.49E+04	3.74E+06	8.35E+01	4.17E+03	1.71E+00
4.26E+01	2.35E+00	4.26E+03	4.26E+05	4.26E+07	2.35E+02	2.35E+04	2.03E+00
6.08E+01	3.29E+00	1.22E+04	2.43E+06	4.86E+08	6.58E+02	1.32E+05	4.52E+01
7.62E+01	3.94E+00	2.28E+04	6.85E+06	2.06E+09	1.18E+03	3.55E+05	1.31E+02
9.04E+01	4.43E+00	3.62E+04	1.45E+07	5.78E+09	1.77E+03	7.08E+05	2.40E+02
3.34E+02	1.74E+01	7.74E+04	2.43E+07	8.37E+09	3.96E+03	1.22E+06	

Equal

A=	3.54E+10	1.00E+08	3.03E+05	B =	5.03E+06	X =	**-2.686E-05**	**a**
	1.00E+08	3.03E+05	1.08E+03		1.54E+04		**6.016E-02**	**b**
	3.03E+05	1.08E+03	7.00E+00		5.57E+01		**-1.547E-01**	**c**
A^{-1} =	7.18E-10	-2.81E-07	1.23E-05					
	-2.81E-07	1.17E-04	-5.94E-03				**1.0000**	R^2
	1.23E-05	-5.94E-03	5.28E-01					

Weighted

A =	8.37E+09	2.43E+07	7.74E+04	B =	1.22E+06	X =	**-2.608E-05**	**a**
	2.43E+07	7.74E+04	3.34E+02		3.96E+03		**5.983E-02**	**b**
	7.74E+04	3.34E+02	4.24E+00		1.74E+01		**-1.370E-01**	**c**
A^{-1} =	1.95E-09	-6.91E-07	1.89E-05					
	-6.91E-07	2.65E-04	-8.23E-03				**1.0000**	R^2
	1.89E-05	-8.23E-03	5.39E-01					

Standard Residual	y-yhat	Target	w ($1/y^{1/2}$) Calculated	yhat	Bias	**Equal** y-yhat	Calculated	Response	Bias
0.2813	0.0112	10.000	**10.189**	4.59E-01	**1.89%**	0.0257	**10.431**	4.70E-01	**4.31%**
-0.0843	-0.0034	20.000	**19.943**	1.05E+00	**-0.29%**	0.0081	**20.137**	1.05E+00	**0.69%**
-0.0232	-0.0009	50.000	**49.984**	2.79E+00	**-0.03%**	0.0024	**50.042**	2.79E+00	**0.08%**
-1.6137	-0.0642	100.000	**98.824**	5.59E+00	**-1.18%**	-0.0713	**98.699**	5.52E+00	**-1.30%**
0.7859	0.0313	200.000	**200.634**	1.08E+01	**0.32%**	0.0151	**200.306**	1.08E+01	**0.15%**
1.3545	0.0539	300.000	**301.221**	1.55E+01	**0.41%**	0.0444	**301.008**	1.55E+01	**0.34%**
-0.9296	-0.0370	400.000	**399.051**	1.96E+01	**-0.24%**	-0.0243	**399.371**	1.96E+01	**-0.16%**
					4.34%				7.02%

Drug: α-OH-Midazolam
Unit: ng/mL

Trial 4	x	y	w ($1/y^{1/2}$)	x^2	x^3	x^4	xy	x^2y	$(y\text{-}ybar)^2$
L1	10.000	5.70E-01	1.32E+00	1.00E+02	1.00E+03	1.00E+04	5.70E+00	5.70E+01	5.46E+01
L2	20.000	1.16E+00	9.30E-01	4.00E+02	8.00E+03	1.60E+05	2.31E+01	4.63E+02	4.63E+01
L3	50.000	2.80E+00	5.98E-01	2.50E+03	1.25E+05	6.25E+06	1.40E+02	6.99E+03	2.66E+01
L4	100.000	5.25E+00	4.36E-01	1.00E+04	1.00E+06	1.00E+08	5.25E+02	5.25E+04	7.32E+00
L5	200.000	1.07E+01	3.06E-01	4.00E+04	8.00E+06	1.60E+09	2.14E+03	4.27E+05	7.39E+00
L6	300.000	1.53E+01	2.55E-01	9.00E+04	2.70E+07	8.10E+09	4.60E+03	1.38E+06	5.44E+01
L7	400.000	1.99E+01	2.24E-01	1.60E+05	6.40E+07	2.56E+10	7.97E+03	3.19E+06	1.43E+02
7	1.08E+03	5.57E+01	4.07E+00	3.03E+05	1.00E+08	3.54E+10	1.54E+04	5.05E+06	3.40E+02

wx	wy	wx^2	wx^3	wx^4	wxy	wx^2y	$(y\text{-}ybarw)^2$
1.32E+01	7.55E-01	1.32E+02	1.32E+03	1.32E+04	7.55E+00	7.55E+01	1.38E+01
1.86E+01	1.08E+00	3.72E+02	7.44E+03	1.49E+05	2.15E+01	4.30E+02	9.76E+00
2.99E+01	1.67E+00	1.49E+03	7.47E+04	3.74E+06	8.36E+01	4.18E+03	2.20E+00
4.36E+01	2.29E+00	4.36E+03	4.36E+05	4.36E+07	2.29E+02	2.29E+04	9.45E-01
6.12E+01	3.27E+00	1.22E+04	2.45E+06	4.90E+08	6.54E+02	1.31E+05	4.09E+01
7.66E+01	3.92E+00	2.30E+04	6.90E+06	2.07E+09	1.17E+03	3.52E+05	1.22E+02
8.96E+01	4.46E+00	3.58E+04	1.43E+07	5.74E+09	1.79E+03	7.14E+05	2.45E+02
3.33E+02	1.74E+01	7.74E+04	2.42E+07	8.34E+09	3.96E+03	1.22E+06	

Equal

A=	3.54E+10	1.00E+08	3.03E+05	B =	5.05E+06	X =	**-1.435E-05**	**a**
	1.00E+08	3.03E+05	1.08E+03		1.54E+04		**5.550E-02**	**b**
	3.03E+05	1.08E+03	7.00E+00		5.57E+01		**1.614E-02**	**c**
A^{-1} =	7.18E-10	-2.81E-07	1.23E-05					
	-2.81E-07	1.17E-04	-5.94E-03				**0.9998**	R^2
	1.23E-05	-5.94E-03	5.28E-01					

Weighted

A =	8.34E+09	2.42E+07	7.74E+04	B =	1.22E+06	X =	**-1.368E-05**	**a**
	2.42E+07	7.74E+04	3.33E+02		3.96E+03		**5.523E-02**	**b**
	7.74E+04	3.33E+02	4.07E+00		1.74E+01		**2.929E-02**	**c**
A^{-1} =	1.96E-09	-6.98E-07	1.97E-05					
	-6.98E-07	2.68E-04	-8.64E-03				**0.9998**	R^2
	1.97E-05	-8.64E-03	5.77E-01					

Standard Residual	y-yhat	Target	w ($1/y^{1/2}$) Calculated	yhat	Bias	**Equal** y-yhat	Calculated	Response	Bias
-0.1130	-0.0106	10.000	9.808	5.80E-01	-1.92%	-0.0001	**9.999**	5.70E-01	-0.01%
0.3049	0.0285	20.000	20.522	1.13E+00	2.61%	0.0365	**20.665**	1.16E+00	3.32%
0.4303	0.0403	50.000	50.747	2.76E+00	1.49%	0.0415	**50.767**	2.80E+00	1.53%
-1.7377	-0.1626	100.000	96.906	5.42E+00	-3.09%	-0.1699	**96.774**	5.25E+00	-3.23%
1.5935	0.1491	200.000	202.999	1.05E+01	1.50%	0.1346	**202.706**	1.07E+01	1.35%
-0.3699	-0.0346	300.000	299.264	1.54E+01	-0.25%	-0.0429	**299.085**	1.53E+01	-0.30%
-0.1162	-0.0109	400.000	399.755	1.99E+01	-0.06%	0.0004	**400.009**	1.99E+01	0.00%
					10.93%				9.76%

Drug: α-OH-Midazolam
Unit: ng/mL

Trial 5	x	y	w ($1/y^{1/2}$)	x^2	x^3	x^4	xy	x^2y	$(y\text{-}ybar)^2$
L1	10.000	4.12E-01	1.56E+00	1.00E+02	1.00E+03	1.00E+04	4.12E+00	4.12E+01	5.59E+01
L2	20.000	1.13E+00	9.42E-01	4.00E+02	8.00E+03	1.60E+05	2.25E+01	4.51E+02	4.57E+01
L3	50.000	2.51E+00	6.32E-01	2.50E+03	1.25E+05	6.25E+06	1.25E+02	6.26E+03	2.89E+01
L4	100.000	5.62E+00	4.22E-01	1.00E+04	1.00E+06	1.00E+08	5.62E+02	5.62E+04	5.11E+00
L5	200.000	1.11E+01	3.00E-01	4.00E+04	8.00E+06	1.60E+09	2.22E+03	4.44E+05	1.03E+01
L6	300.000	1.52E+01	2.57E-01	9.00E+04	2.70E+07	8.10E+09	4.55E+03	1.37E+06	5.33E+01
L7	400.000	1.93E+01	2.28E-01	1.60E+05	6.40E+07	2.56E+10	7.70E+03	3.08E+06	1.29E+02
7	1.08E+03	5.52E+01	4.34E+00	3.03E+05	1.00E+08	3.54E+10	1.52E+04	4.95E+06	3.28E+02

wx	wy	wx^2	wx^3	wx^4	wxy	wx^2y	$(y\text{-}ybarw)^2$
1.56E+01	6.42E-01	1.56E+02	1.56E+03	1.56E+04	6.42E+00	6.42E+01	1.27E+01
1.88E+01	1.06E+00	3.77E+02	7.54E+03	1.51E+05	2.12E+01	4.25E+02	8.15E+00
3.16E+01	1.58E+00	1.58E+03	7.90E+04	3.95E+06	7.91E+01	3.96E+03	2.18E+00
4.22E+01	2.37E+00	4.22E+03	4.22E+05	4.22E+07	2.37E+02	2.37E+04	2.70E+00
6.00E+01	3.33E+00	1.20E+04	2.40E+06	4.80E+08	6.66E+02	1.33E+05	5.06E+01
7.70E+01	3.90E+00	2.31E+04	6.93E+06	2.08E+09	1.17E+03	3.51E+05	1.25E+02
9.12E+01	4.39E+00	3.65E+04	1.46E+07	5.83E+09	1.76E+03	7.02E+05	2.33E+02
3.36E+02	1.73E+01	7.79E+04	2.44E+07	8.44E+09	3.93E+03	1.21E+06	

Equal

A=	3.54E+10	1.00E+08	3.03E+05	B =	4.95E+06	X =	**-3.446E-05**	**a**
	1.00E+08	3.03E+05	1.08E+03		1.52E+04		**6.242E-02**	**b**
	3.03E+05	1.08E+03	7.00E+00		5.52E+01		**-2.531E-01**	**c**
A^{-1} =	7.18E-10	-2.81E-07	1.23E-05					
	-2.81E-07	1.17E-04	-5.94E-03				**0.9994**	R^2
	1.23E-05	-5.94E-03	5.28E-01					

Weighted

A =	8.44E+09	2.44E+07	7.79E+04	B =	1.21E+06	X =	**-3.250E-05**	**a**
	2.44E+07	7.79E+04	3.36E+02		3.93E+03		**6.161E-02**	**b**
	7.79E+04	3.36E+02	4.34E+00		1.73E+01		**-2.118E-01**	**c**
A^{-1} =	1.93E-09	-6.86E-07	1.85E-05					
	-6.86E-07	2.63E-04	-8.05E-03				**0.9993**	R^2
	1.85E-05	-8.05E-03	5.23E-01					

Standard Residual	y-yhat	Target	w ($1/y^{1/2}$) Calculated	yhat	Bias	**Equal** y-yhat	Calculated	Response	Bias
0.0580	0.0107	10.000	**10.175**	4.01E-01	**1.75%**	0.0441	**10.714**	4.12E-01	**7.14%**
0.6503	0.1196	20.000	**21.985**	1.01E+00	**9.92%**	0.1455	**22.387**	1.13E+00	**11.93%**
-1.5342	-0.2821	50.000	**45.179**	2.79E+00	**-9.64%**	-0.2763	**45.327**	2.51E+00	**-9.35%**
0.0021	0.0004	100.000	**100.007**	5.62E+00	**0.01%**	-0.0196	**99.647**	5.62E+00	**-0.35%**
1.5591	0.2867	200.000	**205.921**	1.08E+01	**2.96%**	0.2444	**205.044**	1.11E+01	**2.52%**
-0.8824	-0.1622	300.000	**296.158**	1.53E+01	**-1.28%**	-0.1877	**295.520**	1.52E+01	**-1.49%**
0.1047	0.0193	400.000	**400.541**	1.92E+01	**0.14%**	0.0496	**401.426**	1.93E+01	**0.36%**
					25.70%				33.15%

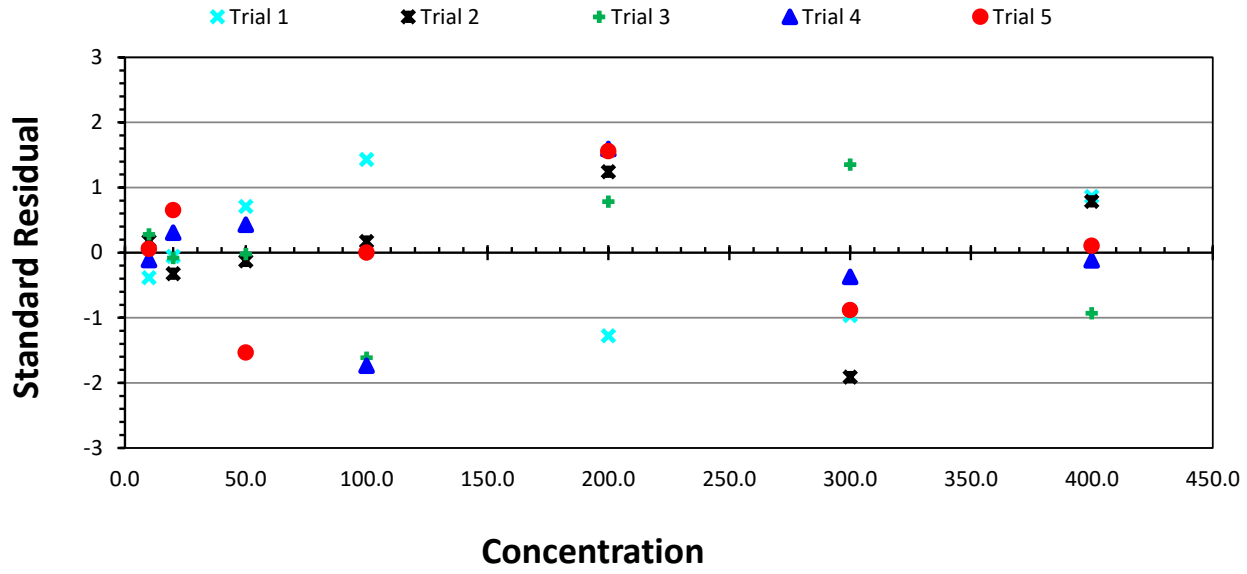
BZDG1 in Blood

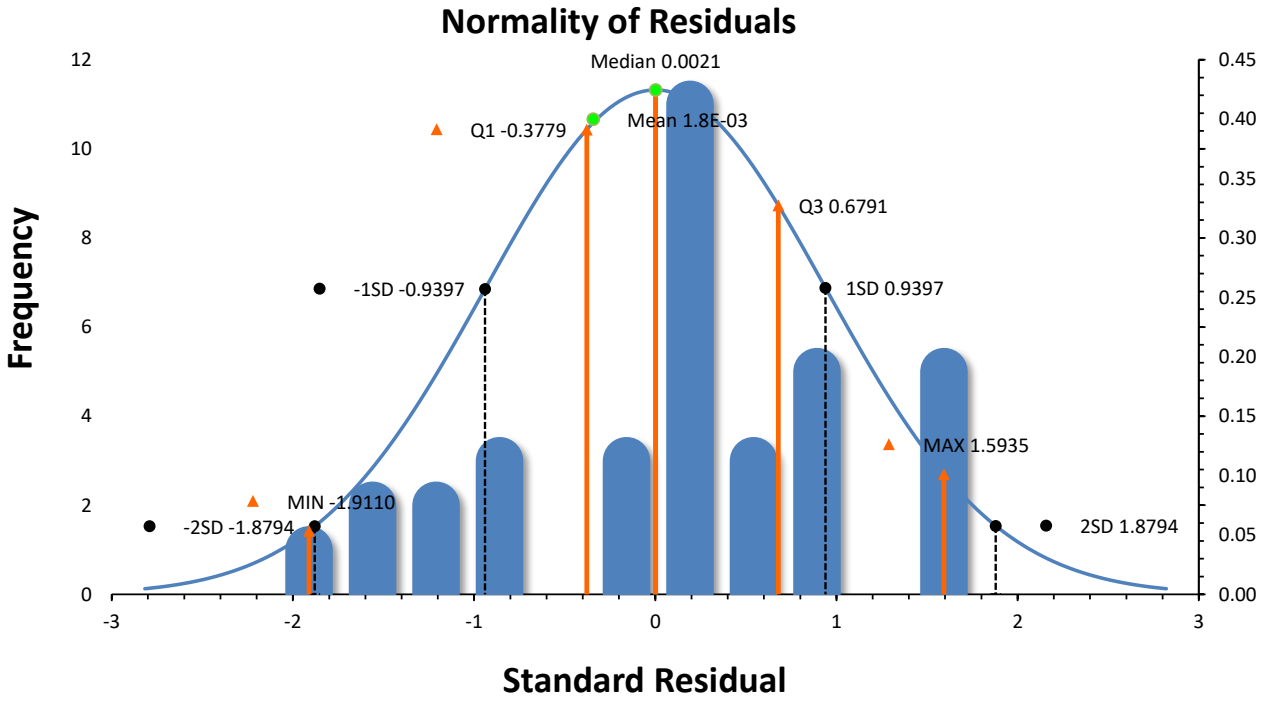
Drug: α-OH-Midazolam

Unit: ng/mL

Calibration Model: Quadratic $(1/y^{1/2})$

Toxicologist(s): JD1 JD2 JD3 JD4 JD5 **Instrument:** LC-MS/MS





BZDG1 in Blood

Drug: α-OH-Midazolam

Unit: ng/mL

Limit of Detection (LOD) (1 of 1)

Toxicologist(s): JD1 JD2 JD3 JD4 JD5 **Instrument:** LC-MS/MS

Estimated LOD from Linear Calibration Curves

	Slope						y-intercept					
Weight	**1/x**	**$1/x^2$**	**$1/x^{1/2}$**	**1/y**	**$1/y^2$**	**$1/y^{1/2}$**	**1/x**	**$1/x^2$**	**$1/x^{1/2}$**	**1/y**	**$1/y^2$**	**$1/y^{1/2}$**
Trial 1	0.050	0.052	0.049	0.050	0.052	0.049	0.087	0.020	0.158	0.087	0.023	0.158
Trial 2	0.050	0.051	0.049	0.050	0.051	0.049	0.104	0.043	0.180	0.106	0.047	0.183
Trial 3	0.051	0.054	0.051	0.051	0.053	0.051	0.026	-0.052	0.120	0.021	-0.051	0.115
Trial 4	0.051	0.052	0.050	0.051	0.052	0.050	0.113	0.064	0.165	0.116	0.066	0.167
Trial 5	0.051	0.054	0.050	0.051	0.053	0.050	0.002	-0.089	0.111	-0.013	-0.099	0.099
Average	**0.051**	**0.052**	**0.050**	**0.051**	**0.052**	**0.050**						
SD							**0.050**	**0.065**	**0.030**	**0.056**	**0.070**	**0.036**
LOD	**3.232**	**4.087**	**1.980**	**3.685**	**4.409**	**2.352**						

Estimated LOD Using Reference Materials

5.000	**Trial 1**	**Trial 2**	**Trial 3**	**Trial 4**	**Trial 5**	**Calculated Mean**
Replicate 1	4.900	5.000	6.400	3.800	7.300	**5.367**
Replicate 2	5.400	4.700	6.500	4.200	6.400	**Bias**
Replicate 3	4.500	4.500	6.800	3.300	6.800	**7.3%**

BZDG1 in Blood

Drug: **α-OH-Midazolam**

Unit: **ng/mL**

Limit of Quantitation (LLOQ) Bias and Precision (1 of 1)

Toxicologist(s):	JD1	JD2	JD3	JD4	JD5	LC-MS/MS
L1	**Concentration**					
10.000 ng/mL	**Trial 1**	**Trial 2**	**Trial 3**	**Trial 4**	**Trial 5**	**Calculated Mean**
Replicate 1	9.800	10.200	10.100	9.800	9.800	**10.220**
Replicate 2	8.800	9.600	9.500	9.900	12.600	**Bias**
Replicate 3	9.900	10.500	10.900	9.800	12.100	**2.2%**

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*	*SS*
Trial 1	3	2.85E+01	9.50E+00	3.70E-01	7.40E-01
Trial 2	3	3.03E+01	1.01E+01	2.10E-01	4.20E-01
Trial 3	3	3.05E+01	1.02E+01	4.93E-01	9.87E-01
Trial 4	3	2.95E+01	9.83E+00	3.33E-03	6.67E-03
Trial 5	3	3.45E+01	1.15E+01	2.23E+00	4.46E+00

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	6.97E+00	4	1.74E+00	2.64E+00	9.75E-02	3.48E+00
Within Groups	6.61E+00	10	6.61E-01			
Total	1.36E+01	14				

Within Run CV (%)	**8.0%**
Between Run CV (%)	**9.9%**

BZDG1 in Blood

Drug: α-OH-Midazolam

Unit: ng/mL

QC Bias (1 of 1)

Toxicologist(s):	JD1	JD2	JD3	JD4	JD5	LC-MS/MS

Low QC		yhat1						
30.000	ng/mL	1.59E+00	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Calculated Mean
	Replicate 1		31.000	29.800	29.500	30.700	30.700	30.313
	Replicate 2		30.900	29.800	29.400	30.200	30.800	Bias
	Replicate 3		31.300	29.900	28.400	30.400	31.900	1.0%

Mid QC		yhat1						
180.000	ng/mL	9.08E+00	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Calculated Mean
	Replicate 1		186.500	180.100	172.600	180.600	184.000	181.620
	Replicate 2		189.600	183.000	177.100	174.400	183.300	Bias
	Replicate 3		188.100	182.400	167.600	180.500	194.500	0.9%

High QC		yhat1						
350.000	ng/mL	1.76E+01	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Calculated Mean
	Replicate 1		353.600	356.300	325.100	353.200	379.700	352.900
	Replicate 2		350.600	355.500	338.200	360.900	369.200	Bias
	Replicate 3		358.200	358.200	345.700	342.300	346.800	0.8%

Replicate Number:	3
Number of Trials:	5

BZDG1 in Blood

Drug: **α-OH-Midazolam**

Unit: **ng/mL**

QC Precision (1 of 3)

Toxicologist(s):	**JD1**	**JD2**	**JD3**	**JD4**	**JD5**	**LC-MS/MS**

Low QC **30.000 ng/mL**

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*	*SS*
Trial 1	3	9.32E+01	3.11E+01	4.33E-02	8.67E-02
Trial 2	3	8.95E+01	2.98E+01	3.33E-03	6.67E-03
Trial 3	3	8.73E+01	2.91E+01	3.70E-01	7.40E-01
Trial 4	3	9.13E+01	3.04E+01	6.33E-02	1.27E-01
Trial 5	3	9.34E+01	3.11E+01	4.43E-01	8.87E-01

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	8.87E+00	4	2.22E+00	1.20E+01	7.80E-04	3.48E+00
Within Groups	1.85E+00	10	1.85E-01			
Total	1.07E+01	14				

Within Run CV (%)	**1.4%**
Between Run CV (%)	**3.1%**

BZDG1 in Blood

Drug: **α-OH-Midazolam**

Unit: **ng/mL**

QC Precision (2 of 3)

Toxicologist(s):	**JD1**	**JD2**	**JD3**	**JD4**	**JD5**	**LC-MS/MS**

Mid QC **180.000 ng/mL**

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*	*SS*
Trial 1	3	5.64E+02	1.88E+02	2.40E+00	4.81E+00
Trial 2	3	5.46E+02	1.82E+02	2.34E+00	4.69E+00
Trial 3	3	5.17E+02	1.72E+02	2.26E+01	4.52E+01
Trial 4	3	5.36E+02	1.79E+02	1.26E+01	2.52E+01
Trial 5	3	5.62E+02	1.87E+02	3.94E+01	7.87E+01

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	5.03E+02	4	1.26E+02	7.93E+00	3.81E-03	3.48E+00
Within Groups	1.59E+02	10	1.59E+01			
Total	6.61E+02	14				

Within Run CV (%)	**2.2%**
Between Run CV (%)	**4.0%**

BZDG1 in Blood

Drug: **α-OH-Midazolam**

Unit: **ng/mL**

QC Precision (3 of 3)

Toxicologist(s):	**JD1**	**JD2**	**JD3**	**JD4**	**JD5**	**LC-MS/MS**

High QC **350.000 ng/mL**

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*	*SS*
Trial 1	3	1.06E+03	3.54E+02	1.47E+01	2.93E+01
Trial 2	3	1.07E+03	3.57E+02	1.92E+00	3.85E+00
Trial 3	3	1.01E+03	3.36E+02	1.09E+02	2.17E+02
Trial 4	3	1.06E+03	3.52E+02	8.73E+01	1.75E+02
Trial 5	3	1.10E+03	3.65E+02	2.82E+02	5.65E+02

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	1.33E+03	4	3.32E+02	3.35E+00	5.49E-02	3.48E+00
Within Groups	9.90E+02	10	9.90E+01			
Total	2.32E+03	14				

Within Run CV (%)	**2.8%**
Between Run CV (%)	**3.8%**

BZDG1 in Blood

Drug: α-OH-Midazolam

Carryover (1 of 1)

Toxicologist(s):	JD1	JD2	JD3	JD4	JD5	LC-MS/MS

L7 Concentration: 400 ng/mL	X 1 Concentration in Blank	Maximum	
Trial 1		N/A	ng/mL
Trial 2			
Trial 3			
Trial 4			
Trial 5			

L7 Concentration: 800 ng/mL	X 2 Concentration in Blank	Maximum	
Trial 1		N/A	ng/mL
Trial 2			
Trial 3			
Trial 4			
Trial 5			

L7 Concentration: 4,000 ng/mL	X 10 Concentration in Blank	Maximum	
Trial 1		N/A	ng/mL
Trial 2			
Trial 3			
Trial 4			
Trial 5			

BZDG1 in Blood

Drug: α-OH-Midazolam

Recovery (1 of 1)

Toxicologist(s):	JD1	JD2	JD3	JD4	JD5	LC-MS/MS

Low QC

30.000 ng/mL	Extracted Drug Response		Unextracted Drug Response	
Trial 1	8,345,769		7,180,171	
Trial 2	8,294,080		7,198,075	
Trial 3	8,294,080		7,601,969	
Trial 4	4,675,987		7,796,902	
Trial 5	5,344,348		8,836,580	
Average	6,990,853		7,722,739	
Standard Deviation	1,823,608		676,458	

% Recovery Low QC **90.52%**

High QC

350.000 ng/mL	Extracted Drug Response		Unextracted Drug Response	
Trial 1	89,875,481		84,074,299	
Trial 2	92,894,936		87,470,681	
Trial 3	87,470,681		90,657,910	
Trial 4	32,373,621		90,815,976	
Trial 5	20,942,331		92,592,643	
Average	64,711,410		89,122,302	
Standard Deviation	35,024,959		3,371,946	

% Recovery Hi QC **72.61%**

BZDG1 in Blood

Drug: α-OH-Midazolam

Unit: ng/mL

Dilution Integrity Bias (1 of 1)

Toxicologist(s):	JD1	JD2	JD3	JD4	JD5	LC-MS/MS

Dilution 250 x 2

500 ng/mL	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Calculated Mean
Replicate 1	505.800	504.400	492.600	510.200	475.200	505.293
Replicate 2	524.000	534.400	491.200	508.000	474.400	Bias
Replicate 3	486.000	532.000	491.600	544.400	505.200	1.1%

Dilution 100 x 5

500 ng/mL	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Calculated Mean
Replicate 1	497.500	516.000	492.000	483.000	402.500	497.200
Replicate 2	495.500	515.500	467.000	559.000	431.000	Bias
Replicate 3	495.500	534.000	462.500	553.500	553.500	-0.6%

Dilution 50 x 10

500 ng/mL	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Calculated Mean
Replicate 1	476.000	502.000	472.000	475.000	426.000	480.133
Replicate 2	499.000	515.000	464.000	528.000	373.000	Bias
Replicate 3	476.000	522.000	460.000	524.000	490.000	-4.0%

BZDG1 in Blood

Drug: **α-OH-Midazolam**

Unit: **ng/mL**

Dilution Integrity Precision (1 of 3)

Toxicologist(s):	**JD1**	**JD2**	**JD3**	**JD4**	**JD5**	**LC-MS/MS**

Dilution	**250**	**x**	**2**	**500**

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*	*SS*
Trial 1	3	1.52E+03	5.05E+02	3.61E+02	7.22E+02
Trial 2	3	1.57E+03	5.24E+02	2.78E+02	5.56E+02
Trial 3	3	1.48E+03	4.92E+02	5.20E-01	1.04E+00
Trial 4	3	1.56E+03	5.21E+02	4.17E+02	8.33E+02
Trial 5	3	1.45E+03	4.85E+02	3.08E+02	6.16E+02

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	3.52E+03	4	8.81E+02	3.23E+00	6.05E-02	3.48E+00
Within Groups	2.73E+03	10	2.73E+02			
Total	6.25E+03	14				

Within Run CV (%)	**3.3%**
Between Run CV (%)	**4.3%**

BZDG1 in Blood

Drug: **α-OH-Midazolam**

Unit: **ng/mL**

Dilution Integrity Precision (2 of 3)

Toxicologist(s):	**JD1**	**JD2**	**JD3**	**JD4**	**JD5**	**LC-MS/MS**

Dilution	**100**	**x**	**5**	**500**

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*	*SS*
Trial 1	3	1.49E+03	4.96E+02	1.33E+00	2.67E+00
Trial 2	3	1.57E+03	5.22E+02	1.11E+02	2.22E+02
Trial 3	3	1.42E+03	4.74E+02	2.53E+02	5.05E+02
Trial 4	3	1.60E+03	5.32E+02	1.80E+03	3.59E+03
Trial 5	3	1.39E+03	4.62E+02	6.44E+03	1.29E+04

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	1.07E+04	4	2.68E+03	1.56E+00	2.59E-01	3.48E+00
Within Groups	1.72E+04	10	1.72E+03			
Total	2.79E+04	14				

Within Run CV (%)	**8.3%**
Between Run CV (%)	**9.1%**

BZDG1 in Blood

Drug: **α-OH-Midazolam**

Unit: **ng/mL**

Dilution Integrity Precision (3 of 3)

Toxicologist(s):	**JD1**	**JD2**	**JD3**	**JD4**	**JD5**	**LC-MS/MS**

Dilution	**50**	**x**	**10**	**500**

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*	*SS*
Trial 1	3	1.45E+03	4.84E+02	1.76E+02	3.53E+02
Trial 2	3	1.54E+03	5.13E+02	1.03E+02	2.06E+02
Trial 3	3	1.40E+03	4.65E+02	3.73E+01	7.47E+01
Trial 4	3	1.53E+03	5.09E+02	8.71E+02	1.74E+03
Trial 5	3	1.29E+03	4.30E+02	3.43E+03	6.86E+03

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	1.41E+04	4	3.52E+03	3.81E+00	3.93E-02	3.48E+00
Within Groups	9.24E+03	10	9.24E+02			
Total	2.33E+04	14				

Within Run CV (%)	**6.3%**
Between Run CV (%)	**8.8%**

BZDG1 in Blood

Drug: α-OH-Midazolam

Ionization Suppression/Enhancement (1 of 1)

Toxicologist	JD1	JD2	JD3	JD4	JD5
Internal Standard:		α-OH-Midazolam-D4			

LOW QC Unextracted

	α-OH-Midazolam	α-OH-Midazolam-D4
Low 1	8,970,447	5,527,904
Low 2	8,730,878	5,285,225
Low 3	7,438,622	4,470,273
Low 4	7,455,624	4,758,398
Low 5	6,768,373	4,508,613
Low 6	7,180,171	4,638,773
Low 7	7,198,075	4,388,594
Low 8	7,601,969	4,278,642
Low 9	7,796,902	3,646,091
Low 10	8,836,580	4,333,291
RSD%	**10%**	**12%**
Avg.	**7,797,764**	**4,583,580**

LOW QC Extracted

	α-OH-Midazolam	α-OH-Midazolam-D4
Low 1	8,674,466	5,154,626
Low 2	8,495,097	5,043,079
Low 3	8,520,461	5,381,449
Low 4	9,418,618	5,745,680
Low 5	7,973,954	5,116,616
Low 6	8,345,769	5,039,058
Low 7	8,294,080	4,860,322
Low 8	8,294,080	4,860,322
Low 9	4,675,987	2,432,241
Low 10	5,344,348	3,119,051
RSD%	**20%**	**22%**
Avg.	**7,803,686**	**4,675,244**

High QC Unextracted

	α-OH-Midazolam	α-OH-Midazolam-D4
High 1	101,868,578	6,119,240
High 2	98,987,356	6,070,741
High 3	85,255,888	4,979,862
High 4	92,557,552	5,592,604
High 5	84,585,546	5,010,736
High 6	84,074,299	5,112,114
High 7	87,470,681	5,166,586
High 8	90,657,910	5,322,856
High 9	90,815,976	4,880,288
High 10	92,592,643	5,694,694
RSD%	**7%**	**8%**
Avg.	**90,886,643**	**5,394,972**

High QC Extracted

	α-OH-Midazolam	α-OH-Midazolam-D4
High 1	95,462,544	5,414,497
High 2	89,713,188	5,228,998
High 3	95,351,751	5,530,179
High 4	99,138,370	5,281,658
High 5	85,181,140	5,013,393
High 6	89,875,481	5,259,909
High 7	92,894,936	5,150,896
High 8	87,470,681	5,166,586
High 9	32,373,621	1,938,910
High 10	20,942,331	1,198,674
RSD%	**35%**	**35%**
Avg.	**78,840,404**	**4,518,370**

% Ionization Low QC:	**0.08%**
% Ionization ISTD Low QC:	**2.00%**
% Ionization High QC:	**-13.25%**
% Ionization ISTD High QC:	**-16.25%**

BZDG1 in Blood

Drug: **α-OH-Midazolam**

Processed Sample Stability

Toxicologist	JD1	JD2	JD3	JD4	JD5
Internal Standard:		α-OH-Midazolam-D4			
Acceptable Area Loss in %:					

	Low QC		High QC	
Time (hr)	α-OH-Midazolam	α-OH-Midazolam-D4	α-OH-Midazolam	α-OH-Midazolam-D4