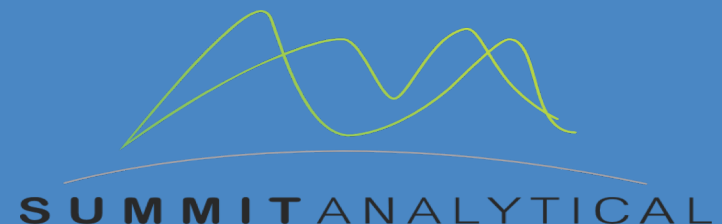


Methods for Handling Concentration Values Below the Limit of Quantification in PK Studies

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June 3-6 2018*

Paper DH05 (Data Handling Stream)



- What is the true value of concentrations reported as Below the Limit of Quantification?
- What is BLOQ? Why is it reported in the first place? How is it related to the Lower Limit of Quantitation?
- Do concentrations reported as BLOQ have a numerical value that can be used for Pharmacokinetic Analysis?
- What are the regulatory considerations for samples reported as BLOQ

There is no clear consensus within the community on how BLOQ values should be handled in Pharmacokinetic Analysis

Nick Holford notes two avoidable sources of bias with BLOQ concentration values:

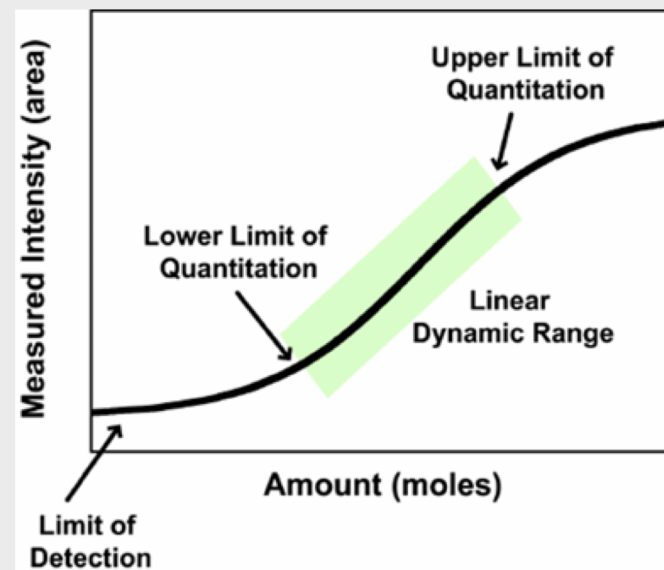
- Do not let your analytical chemists fail to give you the measurements they made that are below BLQ. There is no reason not to use these values. It is just silliness that chemists fail to give you the measurements because of an arbitrary cut off that has no real meaning for pharmacokinetic analysis. Omitting these values will always cause bias.
- Substituting zero for the values that the chemist hides from you is even worse than only using values that not BLQ. Any value after a measurable value is certainly not zero. It may not be easily measured but it is not zero. Assuming it is zero is certain to be wrong.

FDA Guidance (2013): Bioanalytical Method Validation

Lower Limit of Quantification (LLOQ): The lowest standard on the calibration curve should be accepted as the LLOQ if the following conditions are met:

Upper Limit of Quantification (ULOQ): The highest standard will define the ULOQ of an analytical method.

From the Guidance.....*"Concentrations in unknown samples should not be extrapolated below the LLOQ or above the ULOQ of the standard curve. Instead, the standard curve should be extended and revalidated, or samples with higher concentration should be diluted and reanalyzed. Concentrations below the LLOQ should be reported as zeros."*



- The measured concentration value is always the best estimate, regardless of whether it is above or below LOQ.
- Replacing it with any other value will always be worse than using the actual measured value.
- Remember, values below LOQ are not invalid (a common misconception) - they are simply likely to have more bias than an arbitrary, pre-defined threshold.

Senn, et al (2011) stated *“A common but not necessarily logical requirement in drug development is that a ‘limit of quantitation’ be set for chemical assays and that observations that fall below the limit should not be treated as real data but should be labelled as below the limit and set aside for special treatment.”*

Beal Method	Description of Method
M1	Discard BLQ data and estimate using remaining values as if they came from a full distribution.
M2	Discard BLQ data and estimate by treating the remaining values as forming a 'truncated' sample. The likelihood of all remaining samples is calculated conditional on the value being greater than the LLOQ.
M3	Ignore any actual values of the BLQ data and estimate by treating the sample as a whole as one in which BLQ values are censored. The likelihood of the BLQ sample assumes that the value is less than the LLOQ.
M4	Estimate as in M3 but add an additional constraint that all BLQ values must be positive. The likelihood of all values is conditional on their being greater than zero with the additional constraint for BLQ values that they are less than the LLOQ.
M5	Impute BLQ data by $LLOQ/2$ and estimate as if all the values were real.
M6	When measurements are taken for a given individual over time, impute as for M5 for the first BLQ measurement and discard all subsequent BLQ data.
M7	Impute BLQ values by zero and estimate as if all the values were real.

In your SAP or PK Analysis Plan:

The bioanalytical assay for <analyte> an LLOQ assay sensitivity of <value, units>. Values assayed below this limit will be identified as BLOQ in the data reported by the bioanalytical laboratory. In addition, the bioanalytical laboratory will also report the extrapolated concentration value for concentrations identified as BLOQ. Concentrations that are not detected will be identified as “Not Detected” and recorded as BLOQ.

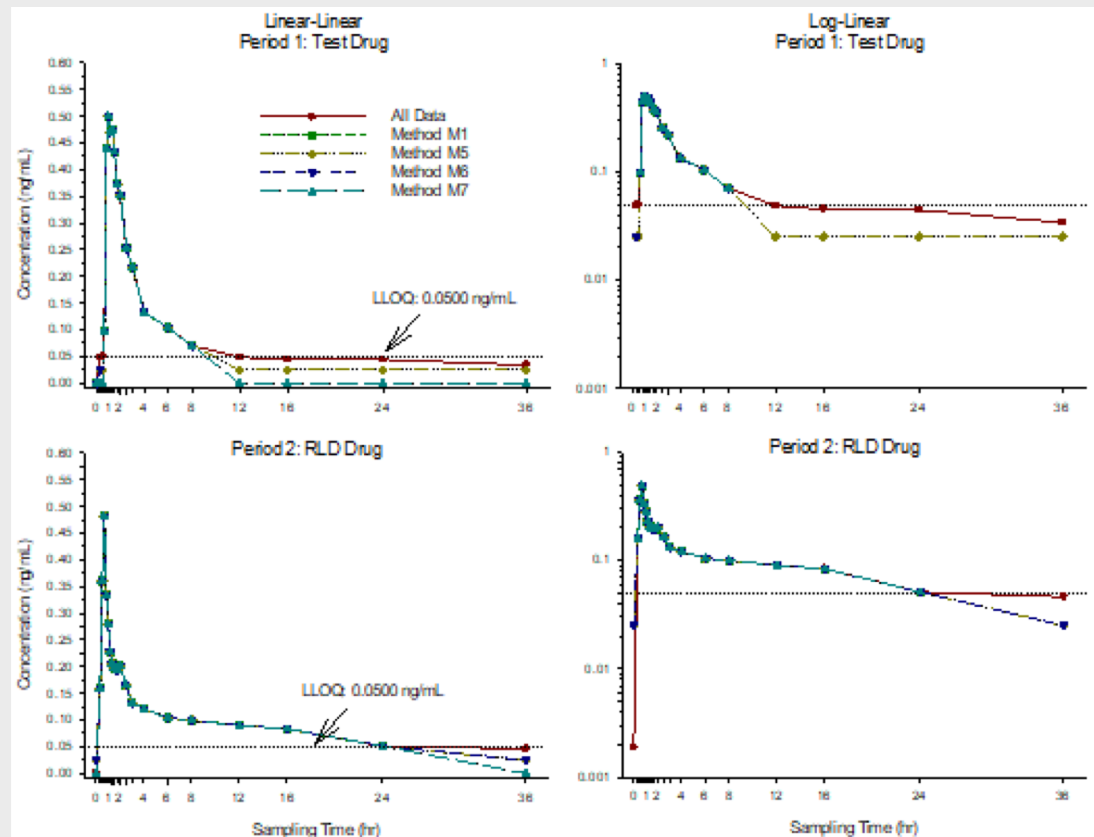
In your SAP or PK Analysis Plan (continued):

For the pharmacokinetic analysis, calculation of mean concentration profiles, and individual concentration vs time plots, values reported as BLOQ will be handled as follows:

- A concentration that is BLOQ is assigned a value of zero if it occurs in a profile after dosing at time zero and before the first measurable concentration.
- If a BLOQ value occurs after a measurable concentration in a profile and is followed by a value above the lower limit of quantification, then the BLOQ is treated as missing data, and the time point is ignored in the computations.
- If a BLOQ value occurs at the end of the collection interval (after the last quantifiable concentration) it is treated as missing data.
- If two BLOQ values occur in succession after C_{max}, the profile is deemed to have terminated at the first BLOQ value and any subsequent concentrations and time points are omitted from pharmacokinetic calculations.

Imputation of BLOQ Concentration Data: A Single Subject Profile

In a 2-period cross over study to evaluate the PK of a drug that has been formulated as both an immediate release (IR) and extended release (ER) formulation consider the impact of the various methods for handling BLOQ samples on the concentration profile for a single subject



Sample Subject Data: Period 1

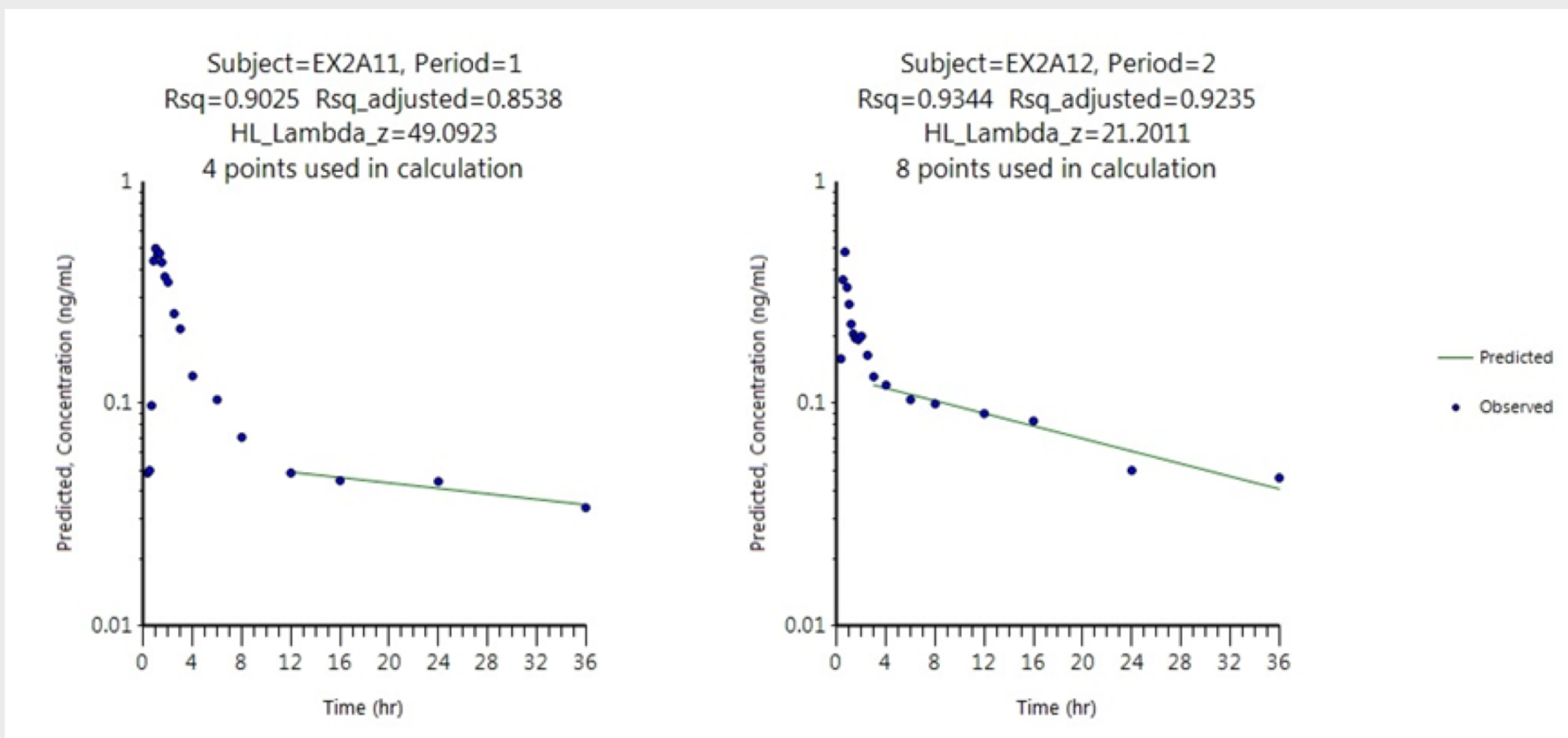
				Imputation Methods for BLOQ				
Period	Time (hrs)	Reported Concentration (ng/mL)	Actual Concentration (ng/mL)	Beal Method M1	Beal Method M5	Beal Method M6	Beal Method M7	Paper
1	Predose (0)	BLQ <0.0500	Not Detected	0	0	0	0	0
	0.33	BLQ <0.0500	0.0486	.	0.0250	0.0250	0.0000	0
	0.50	BLQ <0.0500	0.0499	.	0.0250	.	0.0000	0.000
	0.66	0.0978	0.0978	0.0978	0.0978	0.0978	0.0978	0.0978
	0.83	0.4400	0.4400	0.4400	0.4400	0.4400	0.4400	0.4400
	1	0.5000	0.5000	0.5000	0.5000	0.5000	0.5000	0.5000
	1.16	0.4710	0.4710	0.4710	0.4710	0.4710	0.4710	0.4710
	1.33	0.4750	0.4750	0.4750	0.4750	0.4750	0.4750	0.4750
	1.5	0.4330	0.4330	0.4330	0.4330	0.4330	0.4330	0.4330
	1.75	0.3730	0.3730	0.3730	0.3730	0.3730	0.3730	0.3730
	2	0.3520	0.3520	0.3520	0.3520	0.3520	0.3520	0.3520
	2.5	0.2540	0.2540	0.2540	0.2540	0.2540	0.2540	0.2540
	3	0.2170	0.2170	0.2170	0.2170	0.2170	0.2170	0.2170
	4	0.1330	0.1330	0.1330	0.1330	0.1330	0.1330	0.1330
	6	0.1040	0.1040	0.1040	0.1040	0.1040	0.1040	0.1040
	8	0.0704	0.0704	0.0704	0.0704	0.0704	0.0704	0.0704
	12	BLQ<0.0500	0.0486	.	0.0250	.	0.0000	.
	16	BLQ<0.0500	0.0449	.	0.0250	.	0.0000	.
	24	BLQ<0.0500	0.0445	.	0.0250	.	0.0000	.
	36	BLQ<0.0500	0.0339	.	0.0250	.	0.0000	.

Sample Subject Data: Period 2

				Imputation Methods for BLOQ				
Period	Time (hrs)	Reported Concentration (ng/mL)	Actual Concentration (ng/mL)	Beal Method M1	Beal Method M5	Beal Method M6	Beal Method M7	Paper
2	Predose (0)	BLQ<0.0500	0.0019	.	0.0250	0.0250	0	0
	0.33	0.1590	0.1590	0.1590	0.1590	0.1590	0.1590	0.1590
	0.50	0.3610	0.3610	0.3610	0.3610	0.3610	0.3610	0.3610
	0.66	0.4820	0.4820	0.4820	0.4820	0.4820	0.4820	0.4820
	0.83	0.3340	0.3340	0.3340	0.3340	0.3340	0.3340	0.3340
	1	0.2800	0.2800	0.2800	0.2800	0.2800	0.2800	0.2800
	1.16	0.2280	0.2280	0.2280	0.2280	0.2280	0.2280	0.2280
	1.33	0.2060	0.2060	0.2060	0.2060	0.2060	0.2060	0.2060
	1.5	0.1970	0.1970	0.1970	0.1970	0.1970	0.1970	0.1970
	1.75	0.1940	0.1940	0.1940	0.1940	0.1940	0.1940	0.1940
	2	0.2010	0.2010	0.2010	0.2010	0.2010	0.2010	0.2010
	2.5	0.1650	0.1650	0.1650	0.1650	0.1650	0.1650	0.1650
	3	0.1320	0.1320	0.1320	0.1320	0.1320	0.1320	0.1320
	4	0.1210	0.1210	0.1210	0.1210	0.1210	0.1210	0.1210
	6	0.1040	0.1040	0.1040	0.1040	0.1040	0.1040	0.1040
	8	0.0996	0.0996	0.0996	0.0996	0.0996	0.0996	0.0996
	12	0.0901	0.0901	0.0901	0.0901	0.0901	0.0901	0.0901
	16	0.0834	0.0834	0.0834	0.0834	0.0834	0.0834	0.0834
	24	BLQ<0.0500	0.0499	.	0.0250	0.0250	0.0000	.
	36	BLQ<0.0500	0.0462	.	0.0250	.	0	.

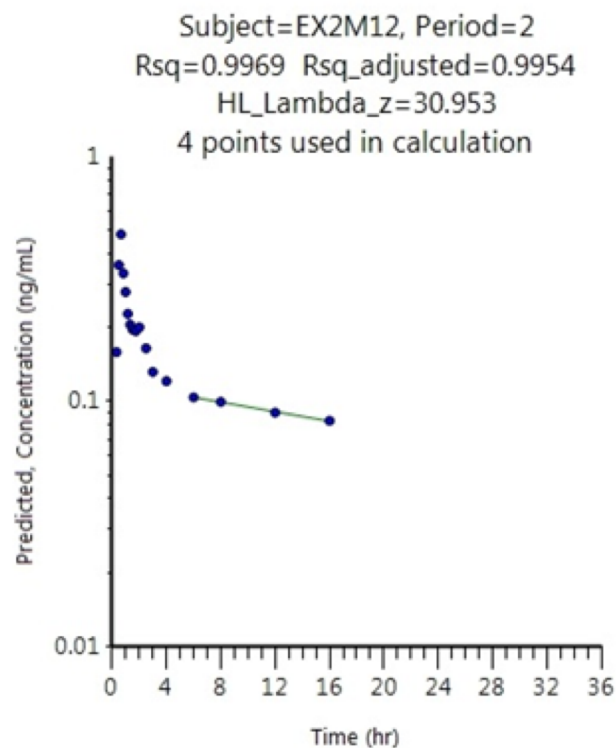
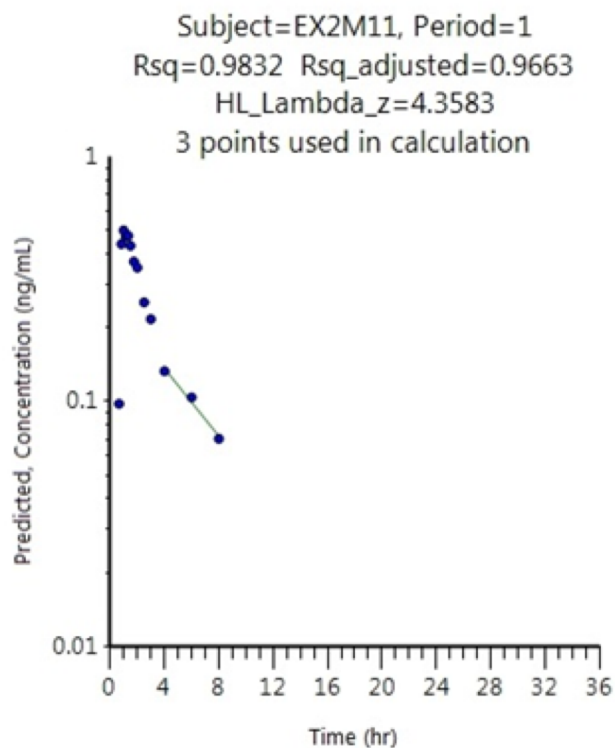
Actual Concentration Data (Periods 1 and 2), Values reported as BLOQ included in analysis

All concentration data included in analysis, observed concentrations reported as BLOQ are included.

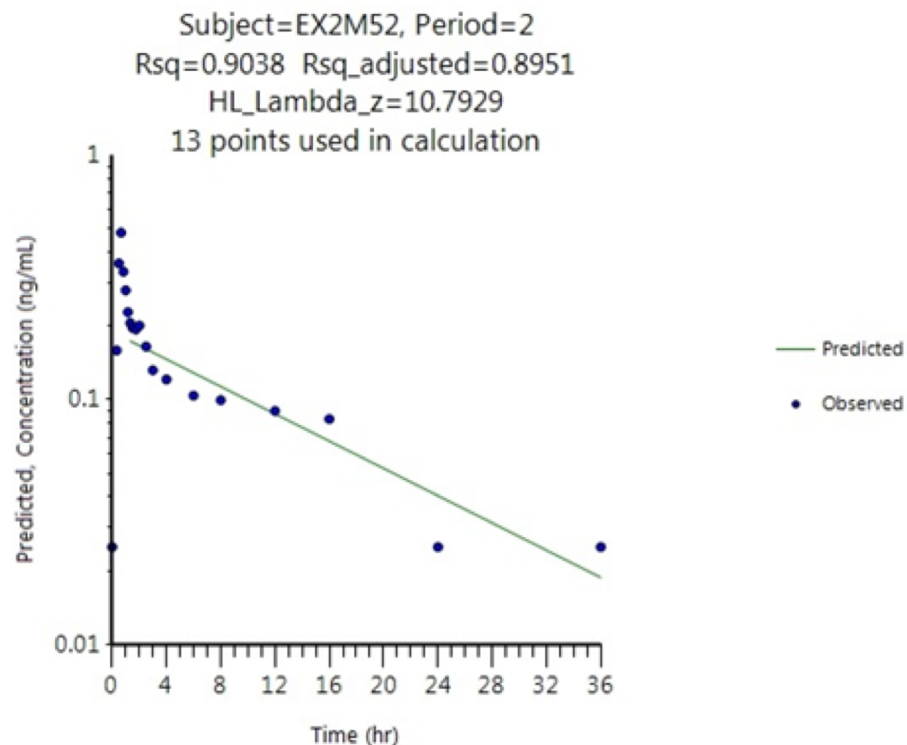
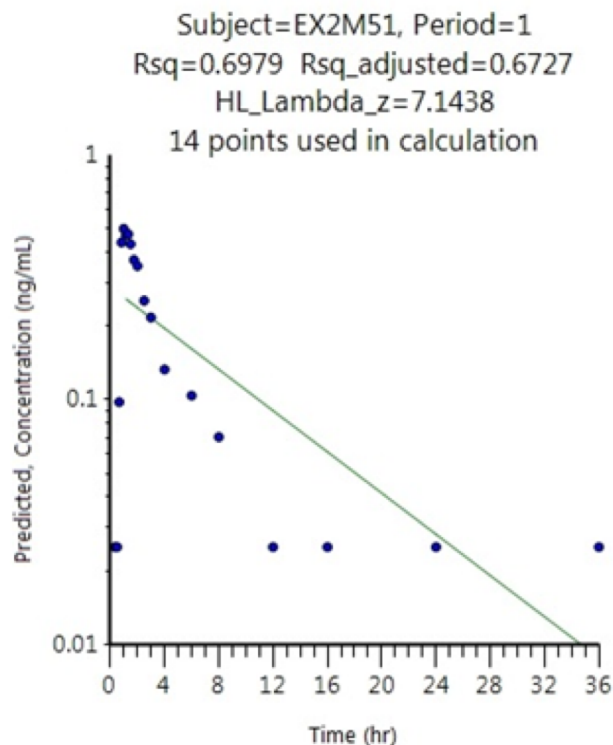


M1

Discard BLQ data and estimate using remaining values as if they came from a full distribution.



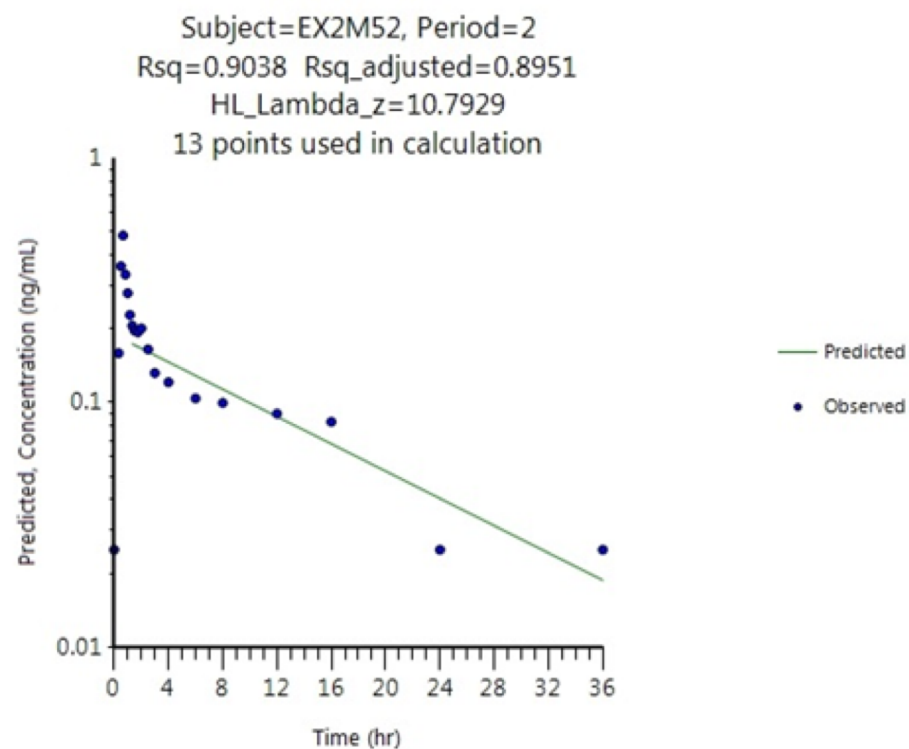
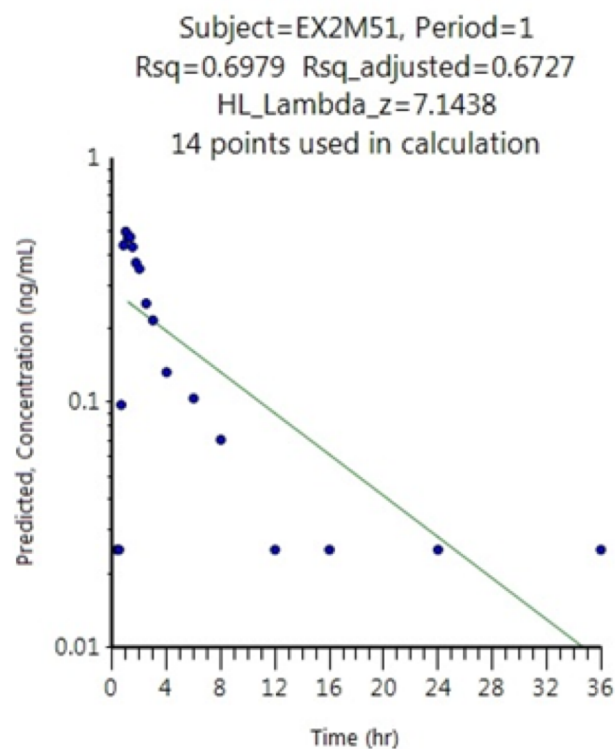
M5 Impute BLQ data by LLOQ/2 and estimate as if all the values were real.



Beal Method M6 BLOQ Imputation (Periods 1 and 2)

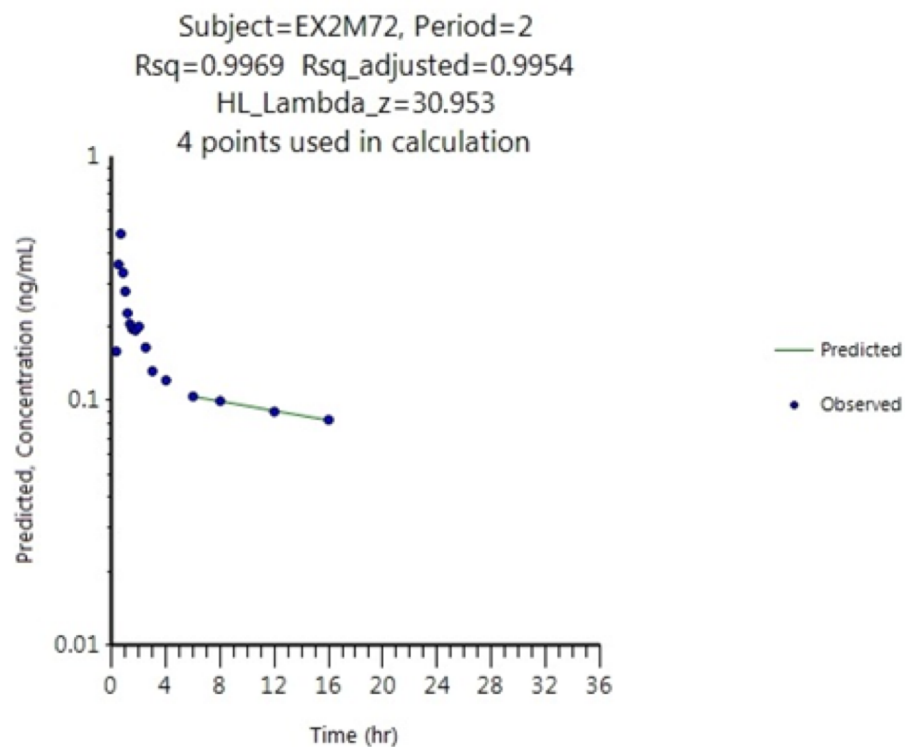
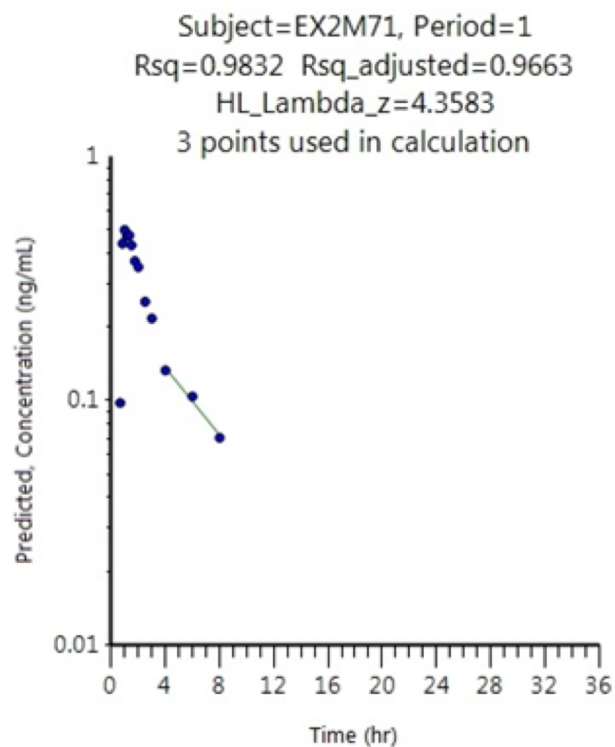
M6

When measurements are taken for a given individual over time, impute as for M5 for the first BLQ measurement and discard all subsequent BLQ data.



Beal Method M7 BLOQ Imputation (Periods 1 and 2)

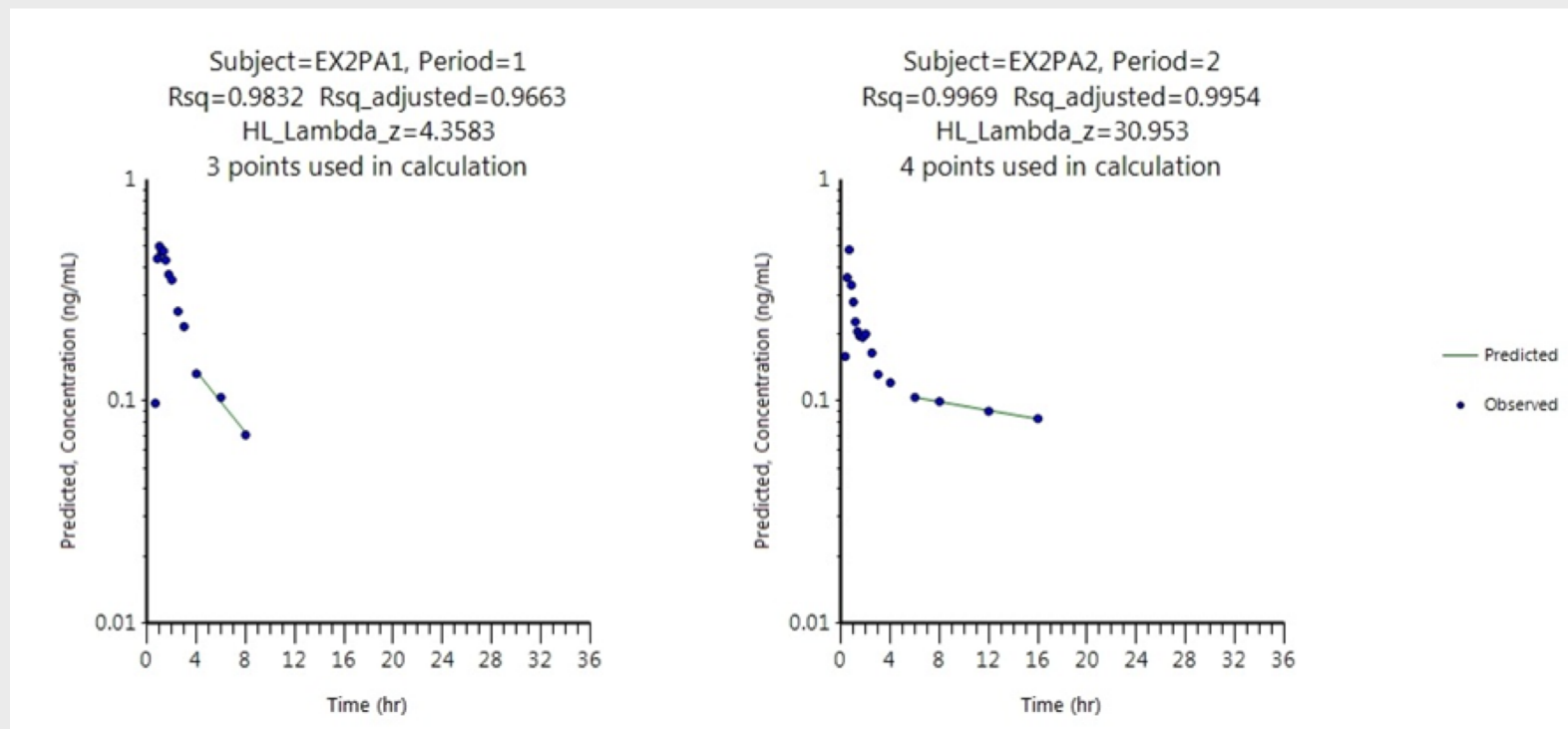
M7 Impute BLQ values by zero and estimate as if all the values were real.



Blended method (Periods 1 and 2), Values reported as BLOQ included in analysis

A concentration that is BLOQ is assigned a value of zero if it occurs in a profile after dosing at time zero and before the first measurable concentration.

- If a BLOQ value occurs after a measurable concentration in a profile and is followed by a value above the lower limit of quantification, then the BLOQ is treated as missing data, and the time point is ignored in the computations.
- If a BLOQ value occurs at the end of the collection interval (after the last quantifiable concentration) it is treated as missing data.
- If two BLOQ values occur in succession after C_{max}, the profile is deemed to have terminated at the first BLOQ value and any subsequent concentrations and time points are omitted from pharmacokinetic calculations.



PK Parameter Estimate Impacts

Imputation Method	Per	No Points Lambda_z	Lambda_z	T _{1/2}	T _{max}	C _{max}	T _{last}	C _{last}	AUC _{0-last}	AUC _{0-inf}	AUC _{0-inf} Percent Extrapol
All Data	1	4	0.0141	49.09	1	0.5000	36	0.0339	2.6891	5.0901	47.17
M1	1	3	0.1590	4.36	1	0.5000	8	0.0704	1.4402	1.8828	23.51
M5	1	14	0.0970	7.14	1	0.5000	36	0.0250	2.2169	2.4746	10.41
M6	1	3	0.1590	4.36	1	0.5000	8	0.0704	1.4323	1.8750	23.61
M7	1	3	0.1590	4.36	1	0.5000	8	0.0704	1.4157	1.8584	23.82
Paper	1	3	0.1590	4.36	1	0.5000	8	0.0704	1.4157	1.8584	23.82
All Data	2	8	0.0327	21.20	0.66	0.4820	36	0.0462	3.0268	4.4399	31.83
M1	2	4	0.0224	30.95	0.66	0.4820	16	0.0834	1.9167	5.6410	66.02
M5	2	13	0.0642	10.79	0.66	0.4820	36	0.0250	2.6544	3.0437	12.79
M6	2	13	0.0811	8.55	0.66	0.4820	24	0.0250	2.3544	2.6628	11.58
M7	2	4	0.0224	30.95	0.66	0.4820	16	0.0834	1.9167	5.6410	66.02
Paper	2	4	0.0224	30.95	0.66	0.4820	16	0.0834	1.9167	5.6410	66.02

- Ignoring BLQ values or replacing them with 0 will inevitably cause biased estimates of PK parameters.
- Using the actual measured value with an appropriate statistical model to account for the residual error will always be better.
- The method proposed in our paper that sets BLOQ values prior to C_{max} to zero and to missing at the end of the concentration curve is a pragmatic and simple method for imputation of these values and can be used in concert with reporting all data for sensitivity. (Using features from Beal 2001)
- Estimating using Beal's Maximum likelihood methods (M3, M4) are good alternatives.

Completing the PK parameter estimates with all concentration data and with imputed BLOQ values should be done in each PK analysis.

The impact of BLOQ values on a subject's concentration profile is both a function of the analyte assay sensitivity and precision, and the number of reported BLOQ samples over the sampling interval.

- A reasonable conclusion is that more BLOQ values will lead to greater biased PK parameter estimates.

More work needs to be completed with simulations on the impact of increasing numbers of BLOQ samples over extended sampling schemas.

QUESTIONS

