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Article type : Commentary

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COMMENTARY: THE LOWER LIMIT OF QUANTIFICATION IN PHARMACOKINETIC ANALYSES

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This is the author manuscript accepted for publication and has undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the [Version of Record](#). Please cite this article as [doi: 10.1111/JVP.12778](https://doi.org/10.1111/JVP.12778)

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21 Pharmacokinetic (PK) analyses rely fundamentally on independent measurements of drug
22 concentrations in biological matrices, traditionally using chromatographic analytical methods. These
23 methods have imperfect performance and introduce measurement error to the experiment. Commonly,
24 the measurement error is used to constrain the report of analytical outputs. An important issue in PK
25 is the influence of these analytical constraints on available inferences.

26 A major analytical constraint results from loss of precision at low analyte concentrations, traditionally
27 resolved by defining of a Lower Limit of Quantification (LLOQ) during assay validation
28 (Anonymous, 2005). Although the handling of such data is a matter warranting careful consideration
29 by the researcher (Jusko, 2012), non-arbitrary approaches to managing this constraint are unusual in
30 the veterinary PK literature. In volume 41 (2018) of the *Journal of Veterinary Pharmacology and*
31 *Therapeutics*, 54/61 (89%) of PK manuscripts did not specify either the presence of observations
32 below the LLOQ, or the method used to handle them. Of the remaining 7/61 manuscripts, 6 specified
33 the absence of observations below the LLOQ (Supplementary Material; **table 1**). Unfortunately,
34 methods for appropriate management of data which fall outside analytical performance limits, which
35 have been prominently addressed in the literature for some time (Beal, 2001), have not been widely
36 adopted in veterinary studies.

37 In conventional usage, a limit of quantification (LOQ) is a parameter which describes an upper or
38 lower boundary on analytical observations such that observations with values outside of the boundary
39 are not 'reliable'. It is typical for LLOQ to be set by the analyst based on a signal-to-noise ratio (e.g.
40 10 times the variability of baseline noise) or a measure of precision in the response (e.g. relative
41 standard deviation) (Anonymous, 1994; Anonymous, 2018). It is critical to note that all methods for
42 the selection of LLOQ are arbitrary: the typically selected criteria have no formal justification and
43 have become accepted simply by convention e.g., 10x baseline noise or >20% RSD (Guo et al., 2010;
44 Jelliffe et al., 2015).

45 In analysis of PK data, observations flagged as below the lower limit of quantification (BLLOQ)
46 create special concern because a decision must be made about how to handle them. The aims of any
47 method to handle BLLOQ observations should be to control bias and preserve information. The
48 available options explored or reported include;

- 49 1. replacement with zero;
- 50 2. replacement with an arbitrary value (e.g. $\frac{1}{2}$ LLOQ);

3. discarding from the dataset;
4. treatment as censored (partially observed);
5. use of data BLLOQ without modification.

It has been clearly demonstrated (Beal, 2001; Bergstrand and Karlsson, 2009; Keizer et al., 2015) that Options 1 and 2 are unacceptable because of their adverse effects on parameter estimation. Option 1 is especially problematic as empirical pharmacokinetic models cannot predict concentrations of zero. It is noteworthy that Option 2 has been recommended for determination of with-holding times in residues studies, where its use causes a conservative error; arguably, this is reasonable in risk management. Methods for using BLLOQ data for withholding time determination for milk have been recommended (Henri et al., 2017).

Various sources have recommended Option 3, especially in the presence of small numbers of BLLOQ observations; however, removal of these samples from the dataset ensures the maximum degree of information loss and probably introduces bias (Hing et al., 2001). It is likely that this is the most commonly used approach in veterinary PK studies, but rarely do veterinary PK reports describe the number of BLLOQ samples analysed.

Option 4 has been widely applied in non-linear mixed-effects (NLME) modelling (Bergstrand and Karlsson, 2009; Hing et al., 2001; Yang and Roger, 2010) as it is unbiased and minimizes information loss, though its uptake in regression-based PK studies is limited. In censored regression, the BLLOQ observations are treated as partially observed; their concentration is known not to exceed the LLOQ, but any positive value below the LLOQ is plausible. Estimation of the parameters may be conducted using maximization of the likelihood (Tobin, 1958). This approach has been recommended for veterinary residues studies (Henri J 2017) and has been adopted as a regulatory option (Anonymous 2000b).

General support for Option 5 is recently developing as it is recognised to preserve all available information, including the case where the LOQ is simply ignored (Keizer et al., 2015). Some progress has recently been made in this area in NLME, including techniques that directly incorporate validation data describing the measurement error into the pharmacokinetic analysis (Neely et al., 2012).

The boundary set as the LLOQ is usually determined by an arbitrarily “acceptable” measurement error. However, as error is a feature of all analytical methods and is present in all measurements, the BLLOQ observations are not distinguished by the presence of errors, merely by their relative size. A fundamental and long-standing weakness of PK and related biostatistical practice is that measurement errors are either treated as ‘absent’ ($>LLOQ$) and the observations considered perfectly precise, or ‘intolerable’ ($<LLOQ$) and the observations considered corrupt (Guo et al., 2010). Observations

which may be minimally different in measurement error are resultingly treated fundamentally differently during data analysis. Observations outside of an LOQ are not invalid (Guo et al., 2010). Their validity matches those of the other observations if their analyses were conducted in the same manner as other samples. It is a particular concern if the loss of information occurs based on an arbitrarily set boundary, which may be at or near a concentration of biological relevance.

We contend that to define an analytical LLOQ is an outdated methodology. However, until analytical laboratories cease defining LLOQ and reporting results based on the outdated approach, we are constrained to use data contaminated by this methodology. In the medical pharmacology literature, high-profile arguments have been made to promote an approach wherein quantification limits be completely discarded in favour of reporting actual measurement error, and using statistical techniques that explicitly consider it (Jelliffe et al., 2015; Neely et al., 2012).

Further, we contend that techniques of arbitrary replacement of BLLOQ observations, for example with LLOQ/2 or zero, are never valid and should not be considered acceptable for data analysis.

The options that remain available to the researcher for BLLOQ observations are;

3. discarding from the dataset;
4. treatment as censored (partially observed);
5. use of data BLLOQ without modification.

Although it is probably the most commonly used approach in veterinary PK, Option 3 is undesirable due to introduction of bias. Option 3 remains a reasonable choice only if the arbitrary LLOQ has been demonstrated to be demonstrably below the biologically important concentrations.

The treatment of BLLOQ results as censored (Option 4) is reasonable. This approach is accessible to the data analyst by using maximum likelihood methods for parameter estimation (Lynn, 2001; Pfitzer et al., 2019) and these methods are available in modern statistical or scientific computing software. This approach is certainly preferable to Option 3.

To use Option 5 should be an aspirational goal. Although its availability is currently limited by analytical habits, these can be influenced by an early conversation during assay development and validation. The analyst should determine the measurement error at each point of the standard curve and extend the measured standards (and their RSD) to concentrations that approach the LOD. The pharmacokineticist then has the necessary information to utilize all sample data optimally.

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Author Contributions: AW and TW designed the study and jointly prepared the manuscript. AW conducted the review of previous *Journal of Veterinary Pharmacology and Therapeutics* papers. Both authors read and approved the final manuscript.

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Title:

The lower limit of quantification in pharmacokinetic analyses

Date:

2019-11-01

Citation:

Woodward, A. & Whitem, T. (2019). The lower limit of quantification in pharmacokinetic analyses. *Journal of Veterinary Pharmacology and Therapeutics*, 42 (6), pp.585-587. <https://doi.org/10.1111/jvp.12778>.

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