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Automation and applications of the tolerance limit method in estimating meat withdrawal periods for veterinary drugs

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ABSTRACT

A program was written in R to facilitate the implementation of the tolerance limit method (TLM) for establishing regulatory withdrawal times for limiting drug residues in meat, milk, and eggs. The developed computer source code can use pharmacokinetic and regulatory data to calculate the drug withdrawal period according to United States Food and Drug Administration (U.S. FDA) guidelines. The code called the "Withdrawal Time Calculator (WTC)" applied this TLM method to meat samples. The program was tested with the data provided by the U.S. FDA guidance and other published data collected from in vivo studies. Additional algorithm validation data were flunixin and sulfamethazine liver concentration data from peer-reviewed publications generated by our laboratory. This manuscript reports the withdrawal period results from testing the developed WTC code. Moreover, the source code for the WTC contains a data removal algorithm, constructed according to U.S. FDA data elimination recommendations if the user chooses. The power of the WTC is that it bypasses the use of multiple platforms typically required to perform the TLM, including standard commercial spreadsheet software (i.e., Microsoft Excel) and Statistical Analysis System (SAS) while providing speed and usability. This novel program provides a platform to calculate a withdrawal period recommendation for any drug in any class of animal for various regulatory body standards and could be very helpful in cases of extra-label drug use in food animals.

1. Introduction

The United States' Food and Drug Administration (U.S. FDA) requires that drug concentrations in animal tissues intended for human consumption reach or fall below a defined concentration before slaughter or milking. These concentrations as defined by the U.S. FDA are known as the tolerance and are specific for each drug and tissue. In countries other than the U.S., these defined concentrations are called maximum residue limits (MRL). The time taken to reach the tolerance following administration of a drug at a labeled dose is called a withdrawal time (WDT) (FDA, 2006). In the U.S. FDA's publication, *Guidance for Industry No. 3 – Guidance For Establishing A Withdrawal Period* (2006, 2016), the U.S. FDA provides a method for calculating the drug withdrawal time called the Tolerance Limit Method (TLM). Applying the TLM can be a difficult task, typically requiring multiple software computer programs to perform multiple calculations to reach a valid withdrawal time. A statistical program is needed to estimate statistical parameters for each tested withdrawal time and test its validity (measured against the limit of detection (LOD) or the tolerance limit or maximum residue limit (MRL) applied to the drug in question.

Under the Animal Medicinal Drug Use Clarification Act of 1994 (AMDUCA, 1994), veterinarians are allowed to use drugs in food-producing animals in an extra-label manner, provided that an appropriately extended withdrawal time is established (Martin-Jiménez et al., 2002). In addition to the TLM, other methods have been used to determine an extended withdrawal time, including the use of half-life multipliers (number of half-lives contained within the withdrawal time) as well as the withdrawal-period estimator algorithm (Gehring et al., 2004b; Martin-Jiménez et al., 2002). Furthermore, non-parametric methods for specific drugs and physiological based pharmacokinetic modeling and population based kinetic modeling can also be used to calculate safe withdrawal times (Buur et al., 2006; Gehring et al., 2004a; Mason et al., 2015; Wu et al., 2012). Calculating drug withdrawal times for labeled use of drugs in food producing species is crucial to the U.S. FDA for enforcing regulation, and the TLM is used by the pharmaceutical industry, and regulatory export officials.

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It is of interest to simplify the computation according to the U.S. FDA guidance and at the same time be flexible to estimate withdrawal periods in jurisdictions outside of the U.S. Thus, a program called the "Withdrawal Time Calculator" (WTC) was written in R language (R Core Team, 2015) that imports data of target drug concentrations over time and calculates a withdrawal period by applying various statistical tests to the data according to U.S. FDA's guidance. The significance of the WTC is that it automates the TLM calculation while providing the tools and flexibility for drug sponsors to follow U.S. FDA recommendations. While our proposed calculator is focused on US FDA guidance, the calculator can also be used with the European Union (EU) guidance although EU guidance differs from U.S. FDA guidance.

2. Specification of withdrawal time calculator

In this section of the manuscript we first describe the U.S. FDA method as described in their guidance document for calculating a withdrawal period and then describe our developed WTC and its many applications and flexibility.

2.1. U.S. FDA tolerance limit method (TLM)

Withdrawal times are calculated using methods devised by *Guidance For Establishing A Withdrawal Period* (FDA, 2006 and 2016). The U.S. FDA recommends a slaughter withdrawal be calculated from at least 20 animals, with at least 5 animals be slaughtered at 4 separate time points during the expected elimination phase of the drug. Observations of individual animals from the depletion study are assumed to be independent. Additionally, the U.S. FDA suggests that there should be equal numbers of animals slaughtered at each time point. Our proposed WTC allows for the calculation with different number of animals for simulation purposes or according to the above U.S. FDA guidance for regulatory submissions.

In the U.S FDA's guidance, the tolerance limit method (TLM) calculation can be constructed in five major steps. The WTC described in the next section replicates the steps briefly described below.

In the first step, the U.S. FDA advises that the data be linear and independent data for each animal, if any data is outside of the elimination phase or below the limit of detection (LOD) of the drug assay, it should be removed and if a time point along the elimination phase does not contain at least 3 concentrations above the target tolerance of the target drug, that time point should be discarded. Moreover, a minimum of 3 distinct time points is needed to estimate a valid withdrawal time.

In the second step, a Bartlett's test is conducted to ensure that the included data is homoscedastic. That is, determine whether the variances at each slaughter time are constant.

The third step is a one-way ANOVA used to determine the log-linearity of the data. The linear model in the fourth step assumes that the transformed sample data follows a linear trend. Therefore, the third step is used to verify this assumption. A p-value is reported, and a significance level of 0.25 ($P < 0.25$) is used to test the results of the ANOVA relative to the null hypothesis. If the p-value is less than 0.25, the result is judged to be statistically significant and the null hypothesis is rejected. Thus, the linearity of the data is suspect and the U.S. FDA suggests finding and removing points that decrease the linearity of the fit. This data removal can have significant consequences as evaluated and discussed later in this paper. The reader should also refer back to the first step where data from redistribution phases should not be included in the withdrawal calculation.

The fourth step applies a linear fit to the log-transformed data. Based on the linear model used, coefficients including the slope (A), intercept (C_0), and additional calculations are computed. These calculations include the residual mean square (RMS), and residual degrees of freedom ($N - 2$), where N is the number of animals. Furthermore, the time points of data collection (e.g. "Days" column in Table 1) are stored in an array $\{T_1, T_2 \ldots T_N\} = \{T_i\}$. The mean of $\{T_i\}$ is calculated

Table 1
Simulated drug tissue concentration – time data from FDA Guidance (2006) document.

Days	Concentration (PPB)
3	27.9
3	31.5
3	26.6
3	36.9
3	32.9
5	19.8
5	22.5
5	26.6
5	19.8
5	30.6
7	17.1
7	18
7	11.3
7	31.5
7	13.5
10	13.5
10	12.2
10	10.8
10	10.8
10	5
14	3.6
14	5.4
14	6.8
14	5.4
14	7.2

($\langle\{T_i\}\rangle$). For every possible withdrawal time (t, within a range specified) additional calculations are required since TLM assumes a non-central t distribution be applied to the population at each possible withdrawal time. These additional values include the 95th percentile, Q (0.95, df = N − 2, d(t)), for that non-central t-distribution and its corresponding non-centrality parameter, d(t), based on US regulations (FDA, 2006). The non-centrality parameter, d(t), is Z/W(t), where Z is the 99th percentile of the standard distribution and,

$$W(t) = \left[(1/N) + (t - \langle\{Ti\}\rangle)^2 \Big/ \sum_{i=1}^{N} (\{Ti\} - \langle\{Ti\}\rangle)^2 \right]^{1/2} \quad (1)$$

Log-concentrations for each time point, C(t), are calculated with the following equation

$$C(t) = C_0 + A * t + Q(0.95, d(t), df = N-2) * W(t) * \sqrt{RMS} \quad (2)$$

In the fifth step, possible withdrawal times are used to calculate a value of the exponential concentration function modeled by the U.S. FDA TLM. The values of the concentration predicted at the provided times are compared to the target tolerance. The time with a corresponding concentration less than or equal to the specified tolerance is then reported as the withdrawal time.

2.2. WTC inputs

The developed WTC is designed to follow the U.S. FDA method as described above for all five (5) steps and this code scheme is depicted in Fig. 1. The WTC provides tools to enable the user to follow these U.S. FDA recommendations. It should be noted that for the Bartlett's test that the U.S. FDA does not specify the significance level used to determine the results of the Bartlett's test. Instead, the U.S. FDA considers the p-value from its calculation ($P = 0.234$) non-significant (FDA, 2006). Due to the lack of clarity on this issue, our WTC uses the standard ($P > 0.05$) to gauge for non-significance. Failure of the Bartlett's test should bring doubt to the withdrawal period calculation. Regardless, the WTC will still compute a withdrawal time, and the onus is on the user of the WTC to determine the validity of the output.

The code for the WTC uses a file of type .CSV (comma separated value). The file must have the form shown in Tables 1–3. Properties of