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ACCELERATED TEMPERATURE PHARMACEUTICAL PRODUCT STABILITY DETERMINATIONS

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ABSTRACT

A review is given of several calculation methods used to obtain a statistically valid estimate of an expiration date from isothermal accelerated temperature studies. Common problems and how to handle them are described for this class of data. A novel computer generated plot to compare zero, first and second order decay is presented as is a somewhat simplified set of nonlinear equations. An example of the recommended analysis, which meets FDA guidelines, is given which shows that good estimates of the expiration date can be calculated from relatively poor data without knowing the degradation order. A Fortran nonlinear least squares estimation program for the analysis is described.

INTRODUCTION

Accelerated stability testing may be defined as the validated method or methods by which product stability may be predicted by storage of the product under conditions that accelerate change in a defined and predictable manner. Stress conditions that accelerate change fall under the general headings of temperature, light, moisture, agitation, gravity, pH, packaging, and method of manufacture. This paper only covers the effect of modifying temperature without simultaneously varying any of the other conditions. It is limited to an examination of isothermally stressing the product at a small number of fixed temperatures, although nonisothermal stressing is briefly described. (Nelson[1] has a general book on accelerated testing with a chapter on drugs.)

Due to assay variation and the relatively good stability of most pharmaceutical products, often at room temperature a statistically significant degradation rate cannot be gotten and thus a valid forecast of the expiration date cannot be made. Higher temperatures intensify the loss and validated mathematical relationships can be used to predict room temperature decay rates. The objective of accelerated temperature stressing is to obtain a statistically valid estimate of the product's expiration date for FDA submission in less time than would be needed by room temperature stability testing alone.

ARRHENIUS' LAW

$$\text{Rate Constant} = (\text{Frequency})\varepsilon^{-(\text{Activation Energy})/(1.987)(^\circ K)} \quad (1)$$

This expression fits experimental data well over wide temperature ranges and is a good first approximation to the true temperature dependency of a reaction. It implies that a plot of the log of the rate constant against the inverse of the absolute temperature is a straight line. If there is sufficient data, this plot is the easiest way of checking the validity of Arrhenius' Law. The relationship is valid for interpolating a rate constant at a given temperature within the available range of data but extrapolation is generally invalid.

The most common causes for the invalidity of Arrhenius' Law are either that the degradation mechanism changes above a critical temperature or that there are two or more simultaneous decay mechanisms with different rate constants and heats of activation. (Some pharmaceutical products are hydrates which lose their water at a critical temperature transforming them into a less stable form.) If the Arrhenius plots are not straight lines but are monotonic, another empirical or quasi theoretical function could be found which fits the data better. This function could then be algebraically combined with the order equations and the resultant parameters estimated via nonlinear least squares. This approach has been used but it represents a fair amount of effort and would probably not be accepted by the FDA unless one could substantiate the assumed underlying mechanism. A nonmonotonic relationship between the rate constants and the absolute temperature indicates a more serious problem with the data. Possible valid estimates could be gotten via an outlier analysis but the usual recourse would be to exclude all elevated temperature data from the analysis.

ORDER MODELS

Pharmaceutical products decay monotonically with time. A number of empirical models have been employed in the literature to represent this relationship. The usual model is based on reaction order where the rate of disappearance of the pharmaceutical product is proportional to its concentration raised to an order.

$$\delta(\text{Potency})/\delta(\text{Time}) = -(\text{Rate Constant})_{\text{Temp}} \times (\text{Potency})^{\text{order}} \qquad (2)$$

The most common orders employed are 0, 1, and 2 with the order being equal to the number of molecules involved in the rate-determining step of the reaction. Since a true reaction of this type can only take place in solution, solid dosage decay is usually described as pseudo-order. Since more than one drug product and/or reaction mechanism could simultaneously be involved, there is no requirement that the order be integer.

The FDA Guidelines[2] state that the relationship can be adequately depicted by a linear, quadratic or cubic function on an

arithmetic or a logarithmic scale. Linear arithmetic is zero order and linear logarithmic is first order. However, quadratic and cubic functions have the inherent fault that they may not be monotonic.

Unless there is a valid a priori reason to assume otherwise, first order decay is usually assumed. For a relatively small amount of degradation there is no practical difference between zero and first order as can be seen from comparing the Figure 1 potency scales with each other. However, for accelerated determinations of expiration dates, one is trying to maximize decay at the highest temperature employed without violating Arrhenius' Law. It is unreasonable to assume that the rate of degradation is independent of the concentration unless some factor other than heat is controlling the decay. Also, zero order implies that the concentration of the product must eventually become negative which is a physical impossibility. Second order is a more complex equation and plot than first order which can be represented by a simple semi-log plot of potency against time. Perhaps 90% of all product degradations can satisfactorily be represented by a first order model. (Some governing decay mechanisms such as the bimolecular reaction of a minor impurity with the product require some kinetic changes to the equations to make this method work.)

Because of the high degree of variability usually associated with stability data, it is best if the order is determined a priori mechanistically based on the known behavior of the product. If one must determine the order from the stability data alone, one can nonlinearly estimate a non-integer order either as a fourth parameter in the Arrhenius model or as a third parameter for the single temperature model. Statistically, one can nonlinearly fit the three order models and compare the resultant error mean squares. Also, for both of these cases, residual plots can be visually compared. If either the zero or the second order comparison statistic is appreciably better than the first order value, the order in question can be deemed to be best descriptive for the given stability data set. For a single temperature, one gets a straight line if one plots against time: potency for zero

order; log potency for first order; and inverse potency for second order. To make the second order plot comparable (ie, descending) to the other two, one calculates a modified ordinate:

Ordinate= 1/(Minimum Potency) + 1/(Maximum Potency) - 1/Potency (3)

One could make all three plots for all temperatures for which data was collected and visually ascertain the best order. In Figure 1, zero order can be visually ruled out but one cannot visually or statistically distinguish between first and second order.

KINETIC MODELS

Occasionally, one must obtain assays of some decay products because they have quality control limits affecting the expiration date. It may be possible to enhance the estimate of the expiration date by writing differential equations for these products, taking into account that they may also degrade during the study, and nonlinearly solve them simultaneously with (2) and material balance equations[3]. The order of the equations must be assumed a priori, otherwise the various permutations would be untenable. This is not a routine approach, as the statistical analysis must be adapted[4] for each individual case. In some instances it might be worth the extra effort provided that (a) the data is good enough and (b) there are sufficient observations so that appreciably more degrees of freedom than parameters are added to the analysis.

MODEL FITTING

Before the advent of high speed computers, Arrhenius parameters were estimated only via the classical linear two step approach. This method, which is still occasionally employed in the literature, entails using either intrinsic linear regression or a plot to (a) estimate the rate constants at each temperature once the proper order is ascertained and (b) predict or extrapolate the room temperature rate constant and from this, the expiration period of the drug. However, since only the mean rate constants obtained at elevated temperatures are utilized, errors associated with drug content are not included in the analysis. Also, the 95% upper

confidence interval of the predicted room temperature rate may be quite high resulting in a short expiration period. Nash[5] proposed the use of a linear model for zero and first order which he showed to be superior to the conventional one. King et al[6], clearly show that a nonlinear treatment is superior to the classical linear one.

This paper somewhat simplifies King's nonlinear model and computation of the expiration date (ie, (10) → (12) in Appendix A) by combining the Arrhenius and order equations as follows:

Order	Predicted Potency =	
Zero	Initial Potency - Q	(4)
First	Initial Potency × ε^{-Q}	(5)
Second	1/(1/(Initial Potency) + Q)	(6)

$Q=(\text{Rate Constant})\times\text{Months}\times\varepsilon^{[(\text{Activation Heat})\times(ST-BT)/(ST\times BT\times 1.987)]}$

ST and BT are the storage and base temperatures in °K. 1.987 is the universal gas constant in cal/(gm mole)(°K).

The three parameters to be estimated by nonlinear least squares in the above equations are the initial potency, the rate constant at the base temperature, and the heat of activation. Weighting is not used as it is reasonable to assume variance homogeneity over the relatively small range of potencies obtained from the great majority of these studies. In order to force the rate constant to be negative and the heat of activation to be positive, one estimates the exponential of these two parameters. While this rarely improves the fit, it does yield estimates of the parameters which approach zero and thus give an immediate indication that the data is problematical. To enable one to calculate the necessary confidence limits and model discrimination statistics, one needs from the algorithm, besides estimates of the transformed parameters, estimates of their standard deviations and correlation coefficients as well as the mean square error.

Confidence limits on the parameters themselves are obtained by simply (a) multiplying the transformed parameter standard deviations by Student's t with the proper degrees of freedom (Appendix A); (b) adding and subtracting this quantity to the transformed parameter estimate; and (c) taking the inverse

transformation which in this case is simply the logarithm. Confidence limits on functions (eg, predicted potency) estimated from the parameters may be calculated using the standard Taylor expansion as follows: $\sigma_f^2 = (f_{Pi}\sigma_{Pi})^2 + (f_{RC}\sigma_{RC})^2 + (f_{AH}\sigma_{AH})^2 + 2(f_{Pi}f_{RC}Cov_{Pi,RC} + f_{Pi}f_{AH}Cov_{Pi,AH} + f_{RC}f_{AH}Cov_{RC,AH})$ (7)

where f is the desired function of the three transformed parameters (Pi, RC, and AH). f with a subscript is the function differentiated with respect to the subscripted parameter. σ and Cov are the standard deviations and covariances estimated by the utilized nonlinear regression scheme. As an example, the formulas used for calculating the 95% lower one sided confidence limits on the predicted percent of initial potency are given in Appendix A.

For single temperature data, the storage and base temperatures are equal and thus Q = (Rate Constant)×Months. The zero order equation is now linear. The first and second order equations are intrinsically linear in that they can be linearized using simple transformations. The two resultant linearized equations are:

Order	Linearized Equation	
First	ln(Predicted Potency) = ln(Pi) + (Rate Constant)×Months	(8)
Second	1/(Predicted Potency) = Pi + (Rate Constant)×Months	(9)

Linear regression can now estimate the two parameters and their standard errors but, for the first order case, the log of the initial potency is estimated rather than the original parameter.

For the zero order case, it should not make any difference whether the parameters are estimated linearly or nonlinearly. For the other two orders, it is probably more correct to nonlinearly estimate the parameters as it is unlikely that the transformed equations will have constant variance and/or normally distributed residuals. However, the linear method always appears to estimate a longer expiration period. Since the FDA guidelines find this method to be acceptable, it is the method of choice. Parameter, standard error, correlation coefficient, and confidence limit equations are simple and can be found in any standard reference text. Since the units of the three equations differ, the correlation coefficient rather than the mean square error discriminates between orders.

MINIMUM DATA SIZE

One can estimate the three parameters in the Arrhenius relationship from three distinct (ie, at three different conditions as opposed to replicates) data points using simultaneous equations provided one has a minimum of two different times and temperatures. The two parameters for a single temperature can be estimated via simultaneous equations if one has two data points at two different times. When the estimates are obtained via least squares, an additional data point is required to avoid having the statistical estimates divided by zero degrees of freedom. As a practical matter, considering the variation usually found in pharmaceutical assays, one should have at least ten data points to have a reasonable confidence in the predicted expiration date. In view of the frequency one finds extreme assay outliers in some products, one would need at least twenty data points in order to perform a statistical analysis via which one can justifiably reject an extreme outlier. Having less data points may still yield reasonable estimates for good data. However, a risk is run which increases with decreasing data size of getting worthless expiration date estimates which will not be accepted by the FDA.

POSITIVE RATE CONSTANT

For a single temperature for a stable product, it is by no means rare that the statistical estimation will yield a positive rate constant as does the 37°C. data in the example. This occasionally, by chance, may even be statistically significant. In this case, one takes the mean and standard deviation of all of the stability assays irrespective of decay time. One uses this to generate 95% lower confidence limits to justify an expiration period of at least the longest degradation period in the data set.

This is much less likely to occur with a data set containing data at multiple temperatures. When this occurs, it usually is an indication of problems with the data set. One should thus analyze the base temperature data alone and if the rate constant is still positive, one should proceed as in the single temperature case.

NEGATIVE HEAT OF ACTIVATION

This is an extreme case of the Arrhenius relationship being invalid. It could be due to a single outlier in a scanty data set. It is more likely to be caused by aberrant data for one or more elevated temperatures resulting in either a nonmonotonic or inverse relationship between temperature and rate constant. Usually one must discard all elevated temperature data from the analysis.

OUTLIERS

There are cases in pharmaceutical stability data where dropping an extreme outlier will change unacceptable parameter estimates to acceptable ones or discernibly increase the predicted expiration period for a given batch. Some statistical tests, plots and/or analyses would be required to justify the dropping of outliers to the FDA when no physical cause can be found. Suspected outliers can easiest be visualized from a plot of the data. However, note that an improper model can result in apparent outliers. In Figure 1, the 70°C. data point appears to be an outlier for the zero order model while it is fit very well by the other two orders. One should also examine the residuals from the model fit. This allows one to easily identify a single outlier. Multiple outliers may compensate for one another and not correspond to the largest residuals in the data set. Multiple outliers will usually result in poor model fits with either physically impossible parameter estimates or unacceptably wide confidence intervals. Virtually no work has been done on handling outliers in nonlinear estimation. However, provided that there is enough data and the residuals approximate a known statistical distribution, there are a myriad of statistical tests available for flagging a single outlier as well as some of more dubious applicability for flagging multiple ones[7]. The removal of an outlier may not appreciably affect the parameter and error estimates. This can be studied with the aid of one of a number of published influence statistics (eg, Cook's distance). Also, when one has enough replicates at an experimental condition, they should be analyzed for the presence of outliers.

NONISOTHERMAL METHODS

These consist of heating the product with a temperature profile that need not be accurately controlled but it is vital that the temperature and potency be precisely determined as a function of time. The same underlying models are assumed but the mathematical treatment is appreciably more complex than herein described[8]. Also, this method is better suited for solutions rather than for solid dosage forms due to (a) problems associated with uneven heat transfer through a solid and (b) the difficulties associated with automatically assaying a solid and measuring its temperature as opposed to doing the same thing with a liquid. However, if applicable, it has the advantages that (a) heat of activation and rate constants can be determined in a single experiment; (b) less degradation is required to ascertain order than in the isothermal case; and (c) the experiment can be terminated at any point provided sufficient data has been amassed. Automated data gathering and analysis allows one to get updated estimates of the desired parameters as each data point is garnered. Instrumentation to accomplish this is available for parenterals[9].

EXPERIMENTAL DESIGN

If one knows the pseudo-order of the decay and the critical temperature up to which Arrhenius' Law holds, the optimum design would be a four point one. It consists of the initial sample and the longest feasible storage time allowing one to get an adequately rapid estimate of the expiration date at three temperatures: (a) base, (b) slightly less than critical, and (c) as well as their log mean. These four conditions should be independently replicated as many times as cost and assay restraints permit. For a single temperature, the optimum design would be a three point one with major replication emphasis being placed on the initial condition and at the longest feasible storage point. Another time should be run in the middle. In both cases, earlier samples should be frozen upon being removed from the stability study and all samples assayed together to minimize assay variation. Norwood[10] gives a statistical technique for handling assay variation for a single temperature.

Generally, one does not know that the order equation(s) and/or Arrhenius' Law hold and one wants to run a design which will check their validity. A good design from a statistical viewpoint would be to sample initially as well as at three times at each of the above three temperatures and independently replicate each condition three or four times. This would permit one to perform a statistical linearity test at each temperature to allow one to validate order and the applicability of Arrhenius' Law. One could eliminate the two middle times at the base temperature as for most pharmaceutical products, insufficient degradation occurs there to allow one to evaluate linearity within a reasonable time period.

Since, the Arrhenius model has not been completely accepted by the FDA, current protocols call for taking a relatively large number of samples at the base temperature which is not very efficient for yielding good rapid estimates. Assaying all samples at one time minimizes the assay variation, but one cannot obtain an early preliminary estimate of the expiration date. Also, in case anything goes wrong, all data may be lost and one may need to start from scratch. Haynes et al[11], give a more sophisticated sequential apportionment solution to the experimental design problem. Also, Davis and Flaherty[12] of the FDA state that data collected only at 45°C and 75% relative humidity (to consider the adverse affect of moisture), can yield an admissible expiration date assuming first order kinetics and a conservative estimate of the activation heat of 20 Kcal/Mole. This is clearly simpler than anything else given in this paper but it is questionable whether the FDA will accept expiration dates calculated by this method despite its source.

PROGRAM

A fairly sophisticated interactive Fortran program has been written to fit data to equations (4) → (6) using nonlinear least squares. If data is collected at one temperature, equations (8) and (9) are also fit via linear least squares. The program has been extensively debugged and tested on a VAX. It recognizes and handles

bad data and there are no known aborts or system error messages. Minor modifications would be needed to get it to run on another computer. The source code for the plot can easily be removed if TELLAGRAF is not available. Instructions, a sample output including the example, and further details are available from the author.

The designed paginated output contains for the three orders: (a) the three parameters and their standard errors, correlation coefficients and confidence limits; (b) the statistical Appendix B test(s) for homogeneity for up to 25 batches; (c) Arrhenius and isothermally (ie, linearly) estimated rate constants at the data collection temperatures ($\leq$9); (d) estimated potency at a specified base temperature on an as is basis, % of label claim and % of estimated initial potency with 95% lower one sided confidence limits on the latter; (e) mean % overage needed to meet a given % of label claim at the base temperature; and (f) months at base temperature required to reach a given % of initial potency. Finally it summarizes the error mean squares and fitting problems for the three orders. It then prints the observed and predicted data as well as the residuals for each batch and their combined data. It also produces a TELLAGRAF source file for a plot akin to Figure 1 for either the best fit or a given order for all of the data. This file is optionally plotted on a LN03 laser printer. Figure 1 comparisons require computerized plots with equal length ordinates and cannot be hand plotted. Unlike raw data where the units differ, slopes can be compared between orders. Also, differences in fits, outliers and estimated expiration dates, are readily apparent.

EXAMPLE

Time, temperature, and potency data for a batch of injection solution is given in Table 1 and plotted in Figure 1 as a percent of the predicted initial potency. The five data points at the base temperature (ie, ovals) are quite variable. If one uses an intrinsic linear regression to calculate the expiration date, and defines it as the number of months where the one sided 95% lower confidence limit is equal to 90% of the predicted initial potency, the expiration ranges between 7 and 10 months for the three orders.

TABLE 1: Predicted Potencies and Residuals for the Three Order Equations and Raw Data

Months	Temp °C	Potencies				Residuals		
		Data	P(0)	P(1)	P(2)	P(0)	P(1)	P(2)
.1	23	3.40	3.287	3.268	3.269	.113	.132	.131
3.0		3.09	3.257	3.262	3.262	-.167	-.172	-.172
6.0		3.45	3.227	3.256	3.256	.223	.194	.194
9.0		3.19	3.197	3.249	3.249	-.007	-.059	-.059
12.0		3.10	3.167	3.243	3.243	-.067	-.143	-.143
3.0	37	3.01	3.222	3.228	3.227	-.212	-.218	-.217
6.0		3.30	3.157	3.188	3.187	.143	.112	.113
1.0	42	3.28	3.259	3.243	3.243	.021	.037	.037
2.0		3.29	3.231	3.218	3.217	.059	.072	.073
4.0		3.05	3.175	3.169	3.167	-.125	-.119	-.117
.5	56	3.18	3.260	3.203	3.202	-.080	-.023	-.022
1.0		3.41	3.232	3.139	3.137	.178	.271	.273
2.0		2.99	3.176	3.015	3.016	-.186	-.025	-.026
.5	70	2.92	3.235	2.980	2.984	-.315	-.060	-.064

When one uses all of the data, the zero order model poorly predicts the two highest temperatures as exemplified by the single 70°C. data point in the lower left corner. This yields a fit that is appreciably worse than that for the other two orders and in a predicted expiration date where the thick solid line crosses the dashed 90% line of 9.5 months. The other two plots can be seen to be almost identical to one another and, as can be seen from Table 2, as is their fit. They yield a predicted expiration date of 33.1 and 31.7 months for the first and second order, respectively. The 33.1 months would be the value to report as the residual mean square is slightly better for the first order model which should be chosen as long as the two mean squares are not significantly

TABLE 2: Computed Parameters from the Nonlinear Fit

Order	Parameters Pi	Rate Constant	AH	10,000 Mean Square	Isothermally Estimated (-)RC 23	37	42	56	Correlation Pix RC	Pix AH	RCx AH
0	3.288	.01004	10075	35.57	.0056	-.0322	.0276	.0562	.716	.14	-.43
1	3.268	.00064	24377	27.04	.0051	-.0307	.0262	.0540	.714	-.61	-.97
2	3.269	.00020	24312	27.13	.0047	-.0292	.0248	.0519	.718	-.61	-.97

different. It is appreciably better than that predicted from the relatively scanty, highly variable 12 month base temperature data and thus provides a good testimonial for this analysis method.

The linearizing transformations described earlier, yield three virtually identical ordinates for the Figure 1 percent of initial predicted potency plots due to the relatively small range of the data. This illustrates the difficulty in using graphics or even regression to ascertain the best order unless a sufficient amount of degradation occurs at the highest temperature. The figures most often quoted in the literature as to what constitutes sufficient degradation are 50% for a designed Arrhenius study and 20% for a nonisothermal study. However, as can be seen from this example, a statistically acceptable estimate of the expiration date can be gotten from far less degradation without knowing the best order.

As can be seen from the isothermally estimated rate constants in Table 2 and the rectangles in Figure 1, the data at 37°C. and three months would appear to be an outlier, as it has the second largest residual and results in a positive rate constant for that temperature when coupled with the potency at six months which assayed above the predicted initial potency. Also, the potency at 56°C. and one month appears to be an extreme outlier as it has the largest residual and second highest assay. While dropping these two data points would improve the fit, there is no justification for removing two out of 14 data points on purely statistical grounds.

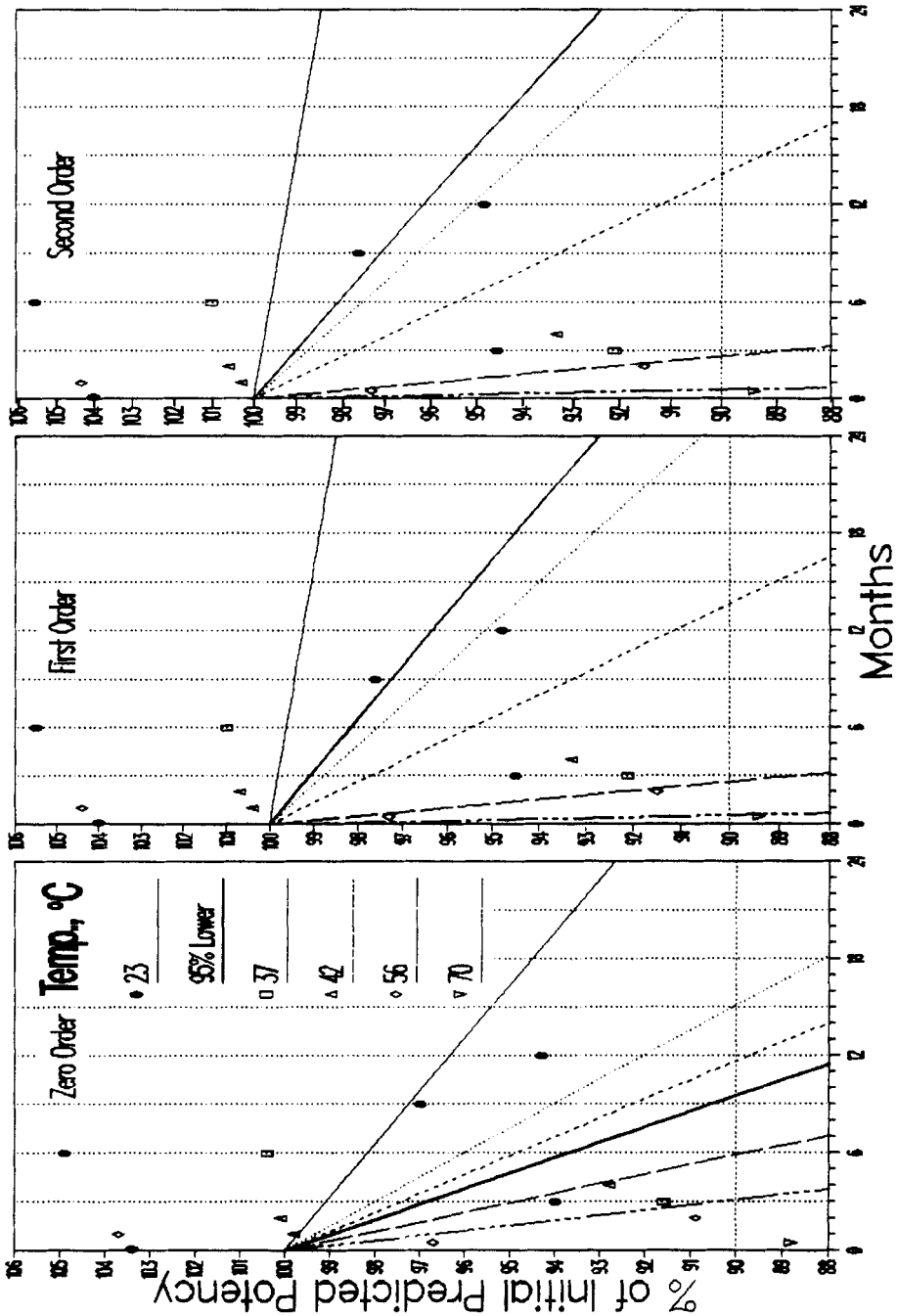

FIGURE 1

This product has a label claim of 3 and to take its 9% overage into account, one calculates its expiration date as the months where the one sided 95% lower confidence limit is equal to the following modified percent of the predicted initial potency:

90 x (Label Claim) + (Initial Predicted Potency)

For the first order model this is 90 x 3 + 3.268 = 82.62%

This yields a predicted expiration date of 58.4 months.

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APPENDIX A: PREDICTED POTENCY AND STANDARD DEVIATION (σ) FORMULAS

When fitting (4) → (6), define the base temperature as the one at which one wants to calculate the predicted potencies and their standard deviations. Then none of these equations will involve the heat of activation or the base temperature. Also, if one calculates σ of the percent of the initial predicted potency, initial potency drops out from the most commonly employed first order model.

Pi = (Initial Potency)/(Label Claim)

(Dividing by the label claim, stabilizes the nonlinear fit by estimating a transformed initial potency in the vicinity of one.)

RC = Log(Rate Constant)

G = Months $\times \varepsilon^{-RC}$

V = Correlation$_{Pi,RC} \times 2 \times \sigma_{Pi} \times \sigma_{RC} = 2 \times Cov_{Pi,RC}$

t = one sided t statistic with degrees of freedom of the number of data points minus (a) 2 for a single temperature or (b) 3 for an Arrhenius estimation.

Zero Order: Predicted = Pi - G

% of Initial = $100 \times (1 - G/Pi)$

$$\sigma = G \times 100/Pi \times [(\sigma_{Pi}/Pi)^2 + \sigma_{RC}^2 - V/Pi]^{1/2} \quad (10)$$

$$\text{Expiration Date} = (Pi - .9)/\varepsilon^{-RC}/(1 - t[(\sigma_{Pi}/Pi)^2 + \sigma_{RC}^2 - V/Pi]^{1/2})$$

First Order: Predicted = $Pi \times \varepsilon^{-G}$ % of Initial = $100 \times \varepsilon^{-G}$

$$\sigma = G \times 100 \times \sigma_{RC} \times \varepsilon^{-G} \quad (11)$$

Second Order: Predicted = $1/(1/Pi + G)$

% of Initial = $100/(1 + Pi \times G)$

$$\sigma = G \times 100/(1 + Pi \times G)^2 \times [\sigma_{Pi}^2 + (Pi \times \sigma_{RC})^2 + V \times Pi]^{1/2} \quad (12)$$

(10) → (12) come from applying (7) to the % of initial model. 95% lower one sided confidence limits on the predicted value is

simply the predicted value less t multiplied by σ. The expiration date is calculated by equating the 95% lower one sided confidence limits on the predicted percent of the transformed initial potency to 90+(Estimated Pi). For first order it must be solved for iteratively and for second order it is a root of a rather messy quadratic equation. If there is a statistically significant difference between the initial potencies of the combined batches, use the actual overage rather than the estimated initial potency.

APPENDIX B: COMBINING PARAMETER ESTIMATES ACROSS BATCHES

The FDA guidelines require that a minimum of three batches be adequately randomly sampled and tested to evaluate the hypothesis that a single expiration dating period for all future batches produced by the manufacturing process for the drug product is justifiable. They require that the probability of there being a statistically significant difference between the rate constants of the batches in question be less than 75%. The guidelines can be met by first performing a global test of homogeneity which tests whether or not there is a statistically significant difference between the batches among the three estimated parameters taken as a whole. If the calculated significance probability of this test is less than 25% then individual tests should be performed for the three parameters. The batches can be legitimately combined as long as the calculated significance probability obtained for the rate constants is greater than 25%.

The formulas for the two tests are:

$$F_{Global} = [\ (\text{Error Mean Square})_c \times (\Sigma n_i - \eta_c - WD)\ ] \times (\Sigma n_i - \Sigma \eta_i) / (\Sigma \eta_i - \eta_c)/WD$$

with $\Sigma \eta_i - \eta_c$ and $\Sigma n_i - \Sigma \eta_i$ degrees of freedom.

$$F_{Parameter} = [\Sigma P_i^2 - (\Sigma P_i)^2/\Sigma n_i][\Sigma n_i - \Sigma \eta_i]/\Sigma \sigma_i^2/(\Sigma n_i - 1)$$

with $\Sigma n_i - 1$ and $\Sigma n_i - \Sigma \eta_i$ degrees of freedom.

Subscripts: i: i^{th} batch

c: combined over all batches

n_i: the number of data points for the i^{th} batch

η_i: parameters estimated for the i^{th} batch (ie, 2 or 3)

η_c: parameters estimated for combined batches (ie, 2 or 3)

WD: $\Sigma(n_i - F_i)(\text{Error Mean Square})_i$

P_i: the parameter in question for the i^{th} batch

σ_i: the standard deviation of P_i

The significance level for the above F tests can be obtained by looking the values up in a F table. Subroutines were written to calculate the probabilities associated with F as well as the Student's t associated with a given probability for the program.