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Statistical Estimation in Accelerated Stability Testing of Drug Products

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ABSTRACT

An initial expiration dating period (EDP) is required by the Bureau of Food and Drug for registration of a new drug product prior to marketing. Since long-term stability testing may take several years, the initial EDP is determined in the laboratory under accelerated conditions. The classical approach involves estimating the degradation rate constants at different elevated temperatures and using Arrhenius equation to predict the degradation rate constant at normal storage condition, *e.g.*, 30° and 75% relative humidity. Unfortunately, the initial EDP is often unreliable due to large standard error.

This paper presents a single unified statistical model, which combined the first-order reaction kinetic model and Arrhenius equation. Using nonlinear regression analysis, estimates were obtained of the initial EDP, initial potency, frequency factor, energy of activation, and degradation rate constant. The standard errors of these estimates were much smaller since the degrees of freedom were based on the total number of observations in the experiment rather than just on the number of elevated temperatures.

Also discussed in this paper are the calculations of release limit for quality control of the production batch; excess amount of drug substance needed to compensate for manufacturing loss and inherent instability of the active moiety; and control limits for monitoring the degradation behavior of the drug product during long-term stability testing.

1. INTRODUCTION

The Philippine Bureau of Food and Drug (BFAD) requires that labels on marketed drug products must have expiration date. This is to assure the customers that the drug maintains its safety, identity, strength, quality or purity until the expiration date if the recommended storage conditions are met (BFAD, 1974). The proposed stability testing guideline (BFAD, 1993) provides guidance on the design, conduct, evaluation, and reporting of stability studies in the Philippines. It was adapted from the United States Food and Drug Administration stability guideline for human drugs and biologics (U.S. FDA, 1987), which is essentially the same as the International Committee on Harmonization Tripartite Guideline (ICH, 1993). This new guideline sets out the stability testing requirements for registration application within three countries; namely, U.S.A., European Community, and Japan.

It will take at least 2 years to generate stability data under normal storage conditions for a drug product with an expiration dating period (EDP) of 24 months. Since long-term stability testing could delay registration prior to marketing of a new drug product, the practice is to determine the initial EDP in the laboratory under accelerated conditions, which will take only a few months instead of years. Samples of the drug product are randomly allocated to different elevated temperatures. Then, at each elevated temperature, the drug concentration (% potency) is assayed at several withdrawal time points. The degradation rate constant at normal storage conditions, e.g., 30°*C* and 75% relative humidity, is extrapolated from the estimates of degradation rate constants obtained at elevated temperatures, and subsequently used to predict the initial EDP.

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The degradation rate constant is a parameter of the *zero-*, *first-*, or *second-*order reaction kinetic model, which describes the relationship between the drug concentration versus time at each elevated temperature. This paper will consider only the *first-order* reaction kinetic model since it is often assumed in drug product stability testing especially when the order is unknown. In a first-order reaction, the degradation rate of the drug product is proportional to the concentration C at time t, *i.e.*,

$$dC/dt = - kC \tag{1}$$

where k is the degradation rate constant. Integrating the rate equation (1) gives

$$C = C_0 \, exp[-kt] \tag{2}$$

where C_0 is the initial concentration at t = 0 and *exp*[ ] denotes exponential function. The linearized form of equation (2) is given by

$$ln[C] = ln[C_0] - kt \tag{3}$$

and *ln*[ ] denotes natural logarithm function.

The relationship between the degradation rate constant and temperature can be described by the Arrhenius equation

$$k = A \, exp[-E/RT] \tag{4}$$

where A is the frequency factor, E is the energy of activation, R is the gas constant, and T is absolute temperature in degrees Kelvin. The linearized form of equation (4) is given by

$$ln\,[k] = \alpha + \beta/T \tag{5}$$

where $\alpha = ln\ A$ and $\beta = -E/R$.

The problem of fitting the first-order reaction kinetic model and Arrhenius equation to accelerated stability data in the context of predicting the initial EDP has been widely discussed by several authors. Nash (1987) provides a brief review while a comprehensive statistical discussion is given by Davies (1981). The classical approach was to employ simple linear regression analysis using equations (3) and (5). The first to consider a nonlinear approach using equations (2) and (4) were Carstensen and Su (1971). They employed the Gauss-Newton iterative technique with a calculating machine, which was a tedious task. King et al (1984) combined the two equations into a single statistical model and used a nonlinear estimation program developed for pharmacokinetic modelling by the Upjohn Company in U.S.A. This paper follows the same approach but employs a widely available statistical software package for estimating the parameters of the nonlinear model.

The goal of this paper is to develop a unified statistical model based on the *first-order* reaction kinetic model and Arrhenius equation not only to predict the initial EDP based on the lower 95% confidence limit but also to obtain valid estimates with standard errors of the initial potency, frequency factor, energy of activation, and degradation rate constant. These estimates are employed in the calculation of *release limit* for quality control of the production batch , *excess amount of drug substance* needed to compensate for manufacturing

loss and inherent instability of the active moiety, and *control limits* for monitoring the degradation behavior of the drug product during long-term stability testing.

2. THE CLASSICAL APPROACH

The classical approach employs simple linear regression analysis in two steps. In the first step, an error term ε_1 is added to equation (3):

$$ln[C] = ln[C_0] - kt + \varepsilon_1. \quad (6)$$

Equation (6) is fitted by ordinary least squares to concentration C and time t at each elevated temperature. Then, the estimates of the degradation rate constants are obtained.

In the second step, an error term ε_2 is added to equation (5):

$$ln[k] = \alpha + \beta/T + \varepsilon_2 \quad (7)$$

Equation (7) is fitted by ordinary least squares to the estimates of the degradation rate constant k and temperature T. Then, the degradation rate constant at normal storage conditions is extrapolated from the fitted regression line.

Let T* denote the temperature at normal storage conditons, *e.g.*, 303°*K* (equivalent to 30°*C*). The extrapolated mean degradation rate constant, k*, is

$$k^* = exp(\hat{\alpha} + \hat{\beta}/T^*) \quad (8)$$

and its estimated standard error is given by

$$se(k^*) = exp[\sqrt{MSE\ \{1/n + (Z^* - \bar{Z})^2 / \Sigma(Z_i - \bar{Z})^2\}}] \quad (9)$$

where ^ denotes the estimates of the parameters of the Arrhenius equation, $Z = 1/T$, $\bar{Z}$ is the mean of Z, and MSE is the mean square error with n-2 degrees of freedom. The predicted initial EDP is given by

$$EDP = -ln[L/\hat{C}_0]/k^* \quad (10)$$

where L is the lower specification limit of the product. Usually k* is replaced by its upper 95% confidence limit (Carstensen, 1990).

Shown in Table 1 is a typical accelerated stability testing data at 3 elevated temperatures.

Table 1
Accelerated Stability Testing Data
(% Potency)

t (weeks)	40°C	50°C	60°C
0	100.8	100.8	100.8
4	100.7	100.3	100.0
8	100.0	100.0	99.8
12	99.8	99.6	99.0
16	99.4	98.7	98.2
24	99.2		

The data were analyzed using regression analysis in Lotus 123 for Windows (1993). The following are the estimates of the initial concentration at t = 0 and degradation rate constants with their standard errors (enclosed in parentheses) at each elevated temperature T (°Centigrades + 273).

T°K	C_o,%	k (10^{-4}), $week^{-1}$
313	100.7655	7.32
	(1.0014)	(1.05)
323	100.8633	12.28
	(1.0015)	(1.52)
333	100.8045	15.58
	(1.0015)	(1.52)

The analysis of variance and estimates of the parameter of Arrhenius equation are given below.

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Prob>F
Model	1	0.28739	0.28739	26.328	0.1225
Error	1	0.01092	0.01092		
C Total	2	0.29830			

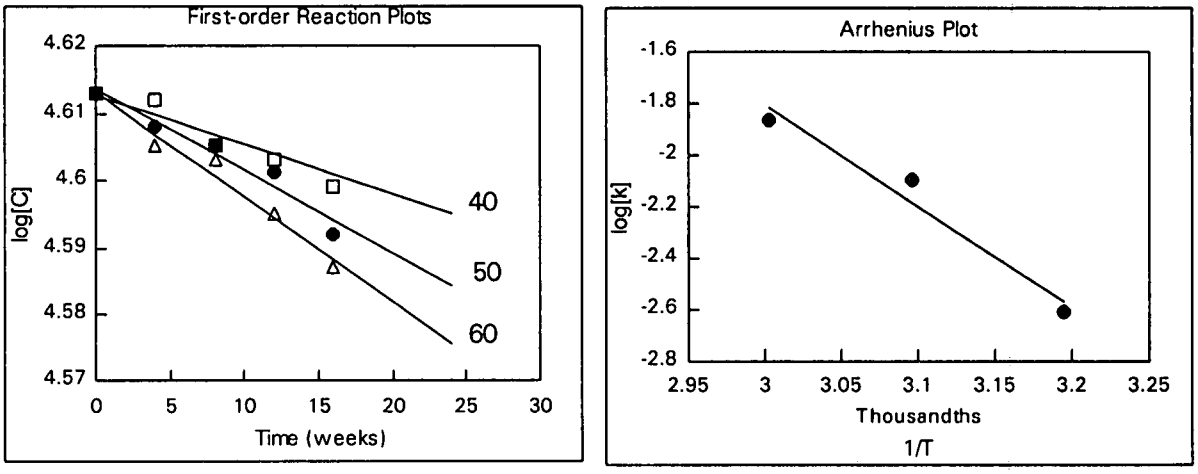
Variable	DF	Parameter Estimate	Standard Error	T for H0: Parameter=0	Prob > |T|
INTERCEPT	1	5.44260	2.38585	2.281	0.2630
SLOPE	1	-3950.37471	769.89218	-5.131	0.1225

The fitted Arrhenius equation is $\hat{ln}[k] = 5.442601 - 3950.37471/T$. Using equations (6) and (7) for T = 303°*K*, the extrapolated mean value of $ln[k]$ is -7.59493 and its standard error is 0.16711. The 95% confidence limits of $ln[k]$ are $-7.59493 \pm 12.706(0.167109)$ or -.71822 and -5.47166. Summarized below are the extrapolated values of k and predicted initial EDP.

	k (% per week)	EDP (weeks)
Lower 95% confidence limit:	6.01770×10^{-5}	985
Predicted mean value	5.02990×10^{-4}	118
Upper 95% confidence limit:	4.20425×10^{-3}	14

Shown in Figure 1 are the plots of the fitted regression lines for the 3 elevated temperatures and the Arrhenius equation.

Figure 1
Plots of data and fitted regression lines of the first-order reaction kinetic model at 40°, 50°, and 60°C and the Arrhenius equation using the classical approach



3. THE UNIFIED APPROACH

The unified approach employs nonlinear regression analysis. Substituting (4) in (2) and adding an error term ε gives

$$C = C_o/\exp[-t \exp(\alpha + \beta/T)] + \varepsilon \qquad (11)$$

which does not involve k. Equation (11) is nonlinear in the parameters C_o, α, and β.

Suppose that at normal conditions the temperature is denoted by T* and the degradation rate constant by k*. The time t* for the concentration to reach the lower specification limit, L, of the product is

$$t^* = - \ln [L/C_o]/k^* \qquad (12)$$

and the frequency factor which is approximately a constant is

$$A = \ln k^* - \beta /T^* \qquad (13)$$

Substituting (12) and (13) in (11) gives

$$C = L \exp[k^*t^*]/\exp[-tk^* \exp(\beta \{1/T - 1/T^*\})] + \varepsilon \qquad (14)$$

which does not involve C_o and A. Equation (14) is nonlinear in the parameters k*, t*, and β.

The parameters of equations (11) and (14) can easily be estimated using any computer software package with nonlinear regression analysis. SPSS nonlinear regression (SPSS, 1993) was used since partial derivatives are not required. The analysis of variance and estimates of the parameters with their asymptotic standard errors and 95% confidence limits are summarized below.

Analysis of Variance

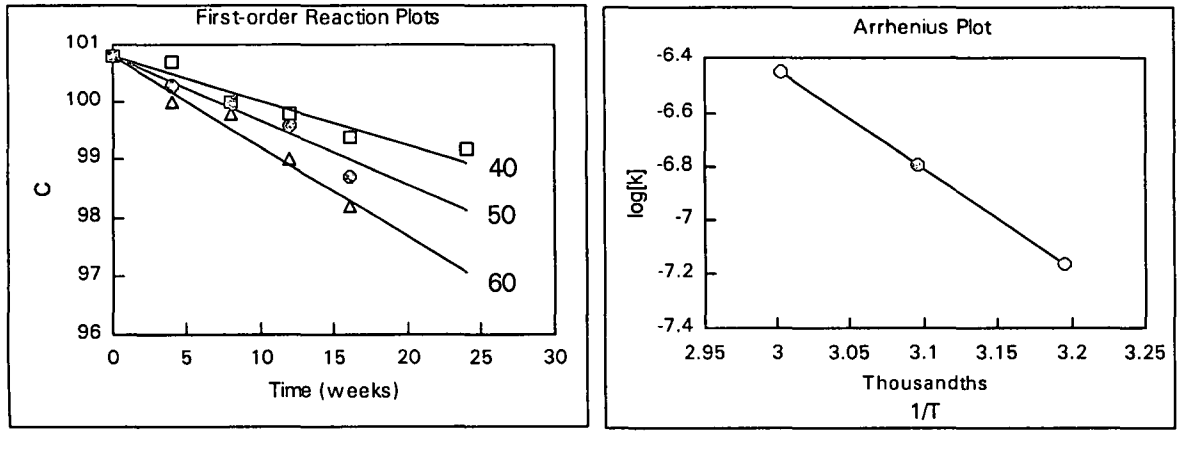
Source	DF	Sum of Squares	Mean Square
Regression	3	159429.25454	53143.08485
Residual	13	.41546	.03196
Uncorrected Total	16	159429.67000	
(Corrected Total)	15	9.14438	

R squared = 1 - Residual SS / Corrected SS = .95457

Parameter	Estimate	Asymptotic Std. Error	Asymptotic 95 % Confidence Interval Lower	Upper
α	4.69402	1.43672	1.59018	7.79785
β	-3711.77600	470.24691	-4727.68269	-2695.86931
C_0 ,%	100.80169	.07656	100.63630	100.96709
k*(10^{-4}),%/week	5.22927	.68682	3.74547	6.71306
t*,week	113.35867	14.12854	82.83582	143.88153

All parameter estimates have standard errors. The estimate of initial potency is 100.8% $\pm$ 0.08%, the upper 95% confidence limit of k* is 6.71 x 10^{-4}% per week, and the predicted initial EDP based on the lower 95% confidence limit is 82.8 weeks or 21 months. The reason why the U.S. FDA and ICH guidelines require that the EDP be based on the lower 95% confidence limit is to provide high level of confidence that the average percent potency will be within specification up to the end of the expiration period. On the other hand, if the EDP is simply based on the estimate of t*, then one may only be 50% confident that the batch average is within specification at the expiration date of the product.

Figure 2
Plots of data and fitted regression curves of the first-order reaction kinetic model at 40°, 50°, and 60°C and the Arrhenius equation using the unified approach



The estimate of the frequency factor A is exp(4.69402) = 109.29165 with s.e. = 4.20687. On the other hand, the estimate of the energy of activation E is - (3711.77600*R) = 7.375299 Kcal/deg/mol, where the gas constant R = 1.987 x 10^{-3} Kcal/deg/mol. Its 95%

confidence limits are 5.3466 and 9.3939 Kcal/deg/mol. Comparison of calculated versus known E values for the drug compound could indicate whether the assumed order of reaction is valid (Connors et al., 1986).

Shown in Figure 2 are the plots of the fitted regression lines for the 3 elevated temperatures and the Arrhenius equation.

4. APPLICATIONS

Let the minimum required EDP based on the lower 95% confidence limit be denoted by EDP'. Then,

$$t^* = EDP' + t_{0.025}\, se(t^*) \tag{15}$$

where $t_{0.025}$ is Student's t-value at 0.05 level of significance with n-2 degrees of freedom, and se(t*) is the standard error of t*.

The release limit, R, is defined as the potency at release of the batch by Quality Control, initial time t = 0, so that there is 95% confidence that the potency will meet the EDP (Peace, 1988). Using equation (12), the release limit is

$$R = \hat{C}_0 \exp(-\hat{k}^* t^*) \tag{16}$$

and the amount of *excess drug substance* needed at t = 0 is

$$\Delta = R - \hat{C}_0 \tag{17}$$

In the example, the predicted initial EDP is only 21 months. Suppose that the minimum required EDP for registration is 24 months or 96 weeks, then

$$t^* = 96 + 2.160(14.12854)$$

$$= 126.5 \text{ months}$$

The release limit should be $R = 100.8\, exp[(5.22927 \times 10^{-4})(126.5)]$

$$= 107.7\%$$

Therefore, the *excess drug substance* needed at t = 0 is

$$\Delta = 107.7 - 100.8 \approx 7\%$$

A long-term stability testing usually follows immediately an accelerated stability study. The upper and lower limits of the trend control chart for monitoring the degradation of the drug product are given by

$$UCL_C = \hat{C}_t + 3se(\hat{C}_0) \tag{18}$$

$$LCL_C = \hat{C}_t - 3se(\hat{C}_0) \tag{19}$$

where $\hat{C}_t = \hat{C}_o \exp(-\hat{k}*t)$ and $se(\hat{C}_o)$ is the standard error of the initial potency. The upper and lower control limits of the range control chart are given by

$$UCL_R = D_4 \bar{R} \quad (20)$$

$$LCL_R = 0 \quad (21)$$

where D_4 is a factor for the R chart, and the mean range, $\bar{R}$, is estimated using the following relation

$$\bar{R} = d_2 \, se(\hat{C}_o) \quad (22)$$

The factor d_2 is a function of the number of observations in the subgroup. If the potency assays are in duplicate, then $d_2 = 1.128$ and D4 = 3.267 (Grant and Leavenworth, 1995).

To illustrate the construction of the trend and range control charts, consider the long-term stability data shown in Table 2.

Table 2
Long-term Stability Data
(% Potency)

t (months)	C_1	C_2	$\bar{C}$	$\bar{R}$
0	100.72	100.85	100.79	0.13
3	100.18	100.00	100.09	0.18
6	99.50	99.40	99.45	0.10
9	98.90	99.10	99.00	0.20
12	98.30	98.28	98.29	0.02
18	97.10	96.92	97.01	0.18
24	95.60	95.55	95.58	0.05
30	94.70	94.60	94.65	0.10
36	93.50	93.10	93.30	0.40

The extrapolated degradation rate constant is 5.22927 x 10^{-4} % per week or 20.91708 x 10^{-4} % per month, the estimted initial potency is 100.80% with standard error of 0.07657%. Therefore, the upper and lower control limits for the trend control chart are given by the curves:

$$UCL_C = 100.80\exp(-0.0002091708t) + 0.22971$$

$$LCL_C = 100.80\exp(-0.0002091708t) - 0.22971$$

and the upper and lower control limits of the range control chart are

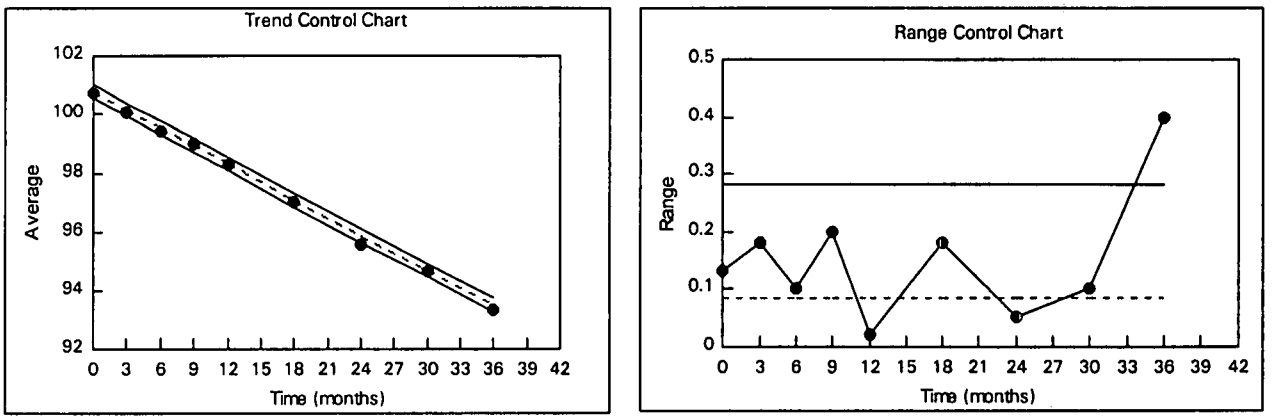
$$UCL_R = 3.267\ (0.08636)$$

$$= 0.2821$$

$$LCL_R = 0$$

Shown in Figure 3 below are the trend and range control charts for the data in Table 2.

Figure 3
Plots of long-term stability data on the trend and range control charts



5. DISCUSSION

The classical approach is widely used in the pharmaceutical industry because the calculations can easily be performed with a calculator. However, it has several disadvantages.

1. There are two error terms ε_1 and ε_2, which are additive to *ln*[C] in equation (4) and *ln*[k] in equation (5), respectively. However, there should be only one error term $\varepsilon \sim N(0,\sigma^2)$, due to the same assay and sampling errors, and additive to concentration C.
2. Equation (4) gives different estimates of the initial concentration at different elevated temperatures. Since the samples are from the same batch, there should be only one estimate of initial concentration at t = 0.
3. The degrees of freedom of the mean square error for equation (5) is usually very small because it is determined solely by the number of elevated temperatures rather than the number of observations.
4. The extrapolated mean degradation rate constant has large standard error due to small degrees of freedom and consequently the predicted initial EDP is often unreliable.
5. The standard errors of the estimates of frequency factor, energy of activation, and EDP cannot be easily calculated.

On the other hand, the unified approach has not been widely used in the pharmaceutical industry because of two problems: (1) nonlinear estimation requires an efficient computer software package, and (2) good initial estimates of the model parameters must be available or else, problem of convergence may occur. But, there are now computer software packages that can efficiently perform nonlinear regression analysis without partial derivatives and programming. The required input are only the data and model statement. Parameter estimates obtained by the classical approach can be used to initialize the nonlinear estimation. Some of the advantages of the unified approach are:

1. Only one error term $\varepsilon \sim N(0,\sigma^2)$ is used which is additive to the concentration C.
2. The analysis of variance is the same whether equation (9) or (12) is used.
3. The degree of freedom of the mean square error considers the total number of observations in the experiment and not the number of elevated temperatures used.

4. Prediction of initial EDP based on the lower 95% confidence limit can be obtained directly.
5. All estimates of model parameters have standard errors and 95% confidence limits can be calculated.
6. The release limit and the amount of excess drug or overage needed during compounding can easily be calculated.
7. Trend and range control charts can be constructed for monitoring the degradation pattern of the drug product during a long-term stability testing.

6. CONCLUSION AND RECOMMENDATION

The classical approach is popular because the steps are intuitively simple requiring only an electronic calculator to obtain estimates of the degradation rate constants at different elevated temperature and fitting of Arrhenius equation. Also, most pharmaceutical researchers have some knowledge of simple regression analysis. It provides them with confidence in performing the statistical analysis even if the estimates are not reliable due to the lack of standard errors.

Although the unified approach involves additional computational efforts and an efficient computer software package, 95% confidence limits for the estimates of model parameters are provided. Therefore, the reliability of the estimates can be determined. It is desirable to have a highly reliable prediction of initial EDP because of its effect on product quality. The customer is ultimately the victim if the drug product is no longer potent before its expiration date.

In conclusion, a regulatory agency like BFAD should require pharmaceutical companies to employ an approach that will provide reliable prediction of initial EDP. The author recommends the unified approach.

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