Journal of Pharmaceutical and Biomedical Analysis 49 (2009) 221–226

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

Modeling the effects of storage temperature excursions on shelf life

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ARTICLE INFO

Article history:
Received 29 April 2008
Received in revised form 17 October 2008
Accepted 20 October 2008
Available online 5 November 2008

Keywords:
Stability
Accelerated stability
Arrhenius model
Shelf life
Degradation

ABSTRACT

Excursions from storage condition requirements may affect product performance and stability. The effects of temperature excursion on stability depend on the amount of time that a product is subjected to these conditions, temperature level, and activation energy. Both time at elevated temperature and the temperature level can be directly measured, while activation energy needs to be estimated from the accelerated stability tests. Coulter Clenz® reagent degradation information is used to demonstrate the effects of temperature excursions. The stability of the product is affected by any excursion, but Coulter Clenz® will not lose all of its stability for excursion of up to 30 days at 35 °C and 20 days at 40 °C. Temperature excursion for up to 20 days at 40 °C will reduce the stability of a product that has activation energy in the range of 26–30 kcal mol^{-1} approximately by 5–7 months. Products with lower activation energy will have a significantly lower reduction in stability. The effects of excursions on shelf life performance are less severe when lower level of risk is implemented to establish the claimed shelf life. The proposed model can effectively predict temperature excursion if used within the scope of a product performance and its characteristics.

1. Introduction

Products must retain their original properties and functionality during storage as defined by the manufacturer's specifications. Products in storage may change as they age but they are considered to be stable as long as their characteristics remain with the specifications. The period of time that a product is stable at the recommended storage conditions is referred to as the shelf life, while the change of the performance as it ages is called degradation [1,2]. Degradation is defined in terms of loss of activity or/and decrease of performance with age and follows a specific pattern depending on the kinetics of the chemical reaction. The amount of change in a unit of time is called the degradation rate. This depends on the required activation energy for the chemical reaction and is product specific [1,2].

Stability tests are used to establish shelf life of a product. Accelerated stability tests are preferred in industry since stability and shelf life estimates are obtained in a shorter period of time and the time to market of a product is reduced [3,4]. Products usually degrade faster when they are subjected to elevated stress conditions like temperature, humidity, radiation, etc. Temperature versus degradation rate explained by the Arrhenius equation is probably the most common relationship used for stability studies. Statistical modeling of accelerated data is well publicized in literature. The development of likelihood estimation approaches in mixed models [2,3] and the availability of statistical software for performing calculations has facilitated the accommodation of different random effects associated with product performance in the models [5]. The planning of stability tests in time and space is a balancing act between the uncertainty of measuring the characteristic(s) of the product, number of lots and replicates, required tolerance on the final estimates, and practical issues of conducting the tests [6]. Design for accelerated testing that account for measurement uncertainty and other product specifics are also available [7,8].

Excursions from storage condition requirements specified by the manufacturer may affect product performance and stability. Temperature is a common excursion in practice. In this paper we will refer to as elevated temperature, a temperature level that exceeds the storage temperature specification by the manufacturer. The effect of temperature excursion on stability depends on the amount of time that a product is subjected to these conditions, temperature level, and activation energy (nature of the product). Both time at elevated temperature and the temperature level can be directly measured while activation energy needs to be estimated from the accelerated stability tests [2,4]. The objective of this paper is to provide a model for estimating stability after the occurrence of temperature excursions using the information on the degradation rate and activation energy of the product obtain from the accelerated stability testing. In addition, experimental stability data will be used to demonstrate the model.

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0731-7085/$ – see front matter
doi:10.1016/j.jpba.2008.10.029

2. Materials and methods

2.1. Model

General model for a first-order degradation reaction is expressed as

$$Y = \alpha\ \exp(-\delta t) + \varepsilon \quad (1)$$

where, Y is the measured result, α is the result at time zero, δ is degradation rate, t is time ($t > 0$), and ε is the experimental error. Error is a pooled estimate of residuals at each time points considering that variances are homogenous. The degradation at an elevated stress condition will be accelerated by the following acceleration factor, calculated as the ratio of the degradation rate at the elevated stress condition, δ_e, to the degradation rate at storage condition, δ_s,

$$\lambda = \frac{\delta_e}{\delta_s} \quad (2)$$

Using the Arrhenius relationship [1,2], the acceleration factor for temperature can be expressed as

$$\lambda = \exp\left[\frac{E_a}{0.00199}\left(\frac{1}{T_s} - \frac{1}{T_e}\right)\right] \quad (3)$$

where E_a is the activation energy (kcal mol^{-1}), and T_s and T_e correspond to the storage and elevated temperatures respectively. Temperatures are expressed in Kelvin (K) = °C + 273. A product in storage that undergoes an excursion at T_e will increase its degradation rate by λ during the time of the excursion. The degradation of this product is expressed as

$$Y = \alpha\ \exp(-\delta_s t_1)\ \exp(-\delta_e t_2)\ \exp(-\delta_s t_3) \quad (4)$$

where, t_1 is the age of the product at the time of excursion, t_2 is the duration of excursion, and t_3 is time after excursion. The maximum amount of time that product can be at elevated temperature before it fails is

$$t_{(2)} = \frac{\ln(\text{crit}) - \ln(\alpha\ ) + \delta_s t_1}{-\delta_e} \quad (5)$$

Consequently, the stability of a product that failed during the excursion is

$$\text{Stability} = t_1 + t_{(2\,\max)} \quad (6)$$

Stability of a product that did not fail during the excursion is

$$\text{Stability} = \frac{\ln(\text{crit}) - \ln(\alpha) + \delta_s t_1 + \delta_e t_2}{-\delta_s} + t_1 + t_2 \quad (7)$$

In both (5) and (7) crit represents a critical level where the essential performance characteristics of the product are within the specification.

2.2. Experiments

Accelerated stability tests were design to evaluate degradation and shelf life of Coulter Clenz®, which is a cleaning agent that is aspirated and circulated during the shutdown cycle of a Beckman Coulter hematology instrument. Coulter Clenz® reagent is reformulated with a replacement antimicrobial to render the reagent formaldehyde free and is recommended to be stored at a temperature of 25 °C with a shelf life of 1 year. The performance of the product is monitored by the level of enzyme activity which tends to decrease as product ages. Three different lots of Coulter Clenz® reagent were stored at three elevated temperatures 40, 45, and 55 °C. The CD-02 enzyme activity was determined and a ratio that corresponds to CD-02 enzyme activity at a particular time versus time zero is calculated. This ratio should be >70% for the product to perform within specification. The degradation of the product follows a first order reaction kinetics and the degradation rate is positively related to temperature according to the Arrhenius relationship modeled in (1) and (3). Coulter Clenz® is considered stable as long as $Y > 0.7$, where Y is the ratio of enzyme activity and crit = 0.7. This period of time can be calculated as

$$\text{Stability} = \frac{\ln(0.7) - \ln(\alpha)}{-\delta} \quad (8)$$

Degradation of this product will be affected by temperature excursions as described in (4) and (7) can be used to calculate stability. A maximum likelihood approach is used to estimate the parameters of (4) and (7) using the level of enzyme activities data collected in accelerated stability testing. Delta method based on the Taylor series of the first derivatives of the function can be used to obtain the approximate error of stability. We used the procedure NLMIXED of SAS® 9.1.3 (SAS Institute, Cary, NC) statistical program to analyze the data [9].

3. Results

3.1. Degradation pattern

Estimates of degradation patterns of Coulter Clenz® at three elevated temperatures are given in Table 1 and shown graphically in Fig. 1. Intercepts that represent a starting point of the performance are similar in different temperatures and are not statistically different from each other. Product performance degrades in time with different rates according to temperatures (Table 1 and Fig. 1). Degradation rates increase from 0.0048 at 40 °C to 0.0117 at 45 °C, and to almost 10 times higher at 0.0440 at 55 °C. The confidence intervals of these estimates do not overlap indicating that the increases of degradation rates at temperatures are statistically significant as well. Degradation patterns at each elevated temperature followed a first order kinetics (exponential pattern) linearly related to temperature as shown in Fig. 1. This validates the use of the Arrhenius

Table 1
Degradation parameters at elevated temperatures.

Temperature	Source	Estimate	S.E.	Lower	Upper
55 °C	Intercept	0.9992	0.0244	0.9485	1.0498
	Degradation rate	0.0440	0.0053	0.0329	0.0550
	Error	0.0020	0.0006	0.0007	0.0033
45 °C	Intercept	0.9942	0.0109	0.9719	1.0166
	Degradation rate	0.0117	0.0006	0.0103	0.0130
	Error	0.0007	0.0002	0.0003	0.0012
40 °C	Intercept	0.9951	0.0117	0.9702	1.0201
	Degradation rate	0.0048	0.0005	0.0037	0.0059
	Error	0.0003	0.0001	0.0001	0.0006

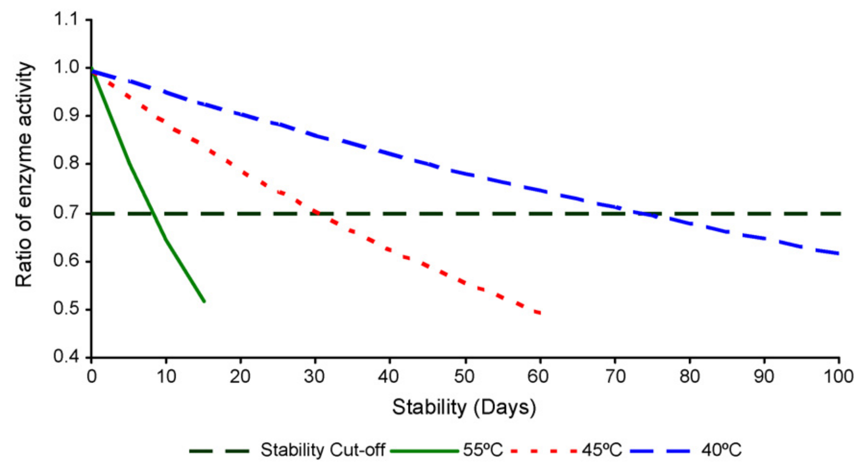

Fig. 1. Degradation trends at elevated temperatures. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

Table 2
Degradation parameters at storage temperature (25 °C).

Source	Estimate	S.E.	Lower	Upper
Intercept	0.9989	0.0046	0.9898	1.0080
Degradation rate	0.0006	0.0001	0.0004	0.0007
Activation energy	28.15	0.79	26.58	29.73
Error	0.0008	0.0001	0.0006	0.0011

Table 3
Estimates of stability.

Temperature	Days	S.E.	Lower	Upper
55 °C	8.1	0.6	6.9	9.3
45 °C	30.1	1.0	28.0	32.3
40 °C	73.2	5.6	61.3	85.1
25 °C	616.4	62.6	492.2	740.6

relationship for modeling the prediction of degradation at storage temperature.

Degradation estimates at storage temperature are shown in Table 2 while degradation pattern along with its confidence limits is shown in Fig. 2. A 95% confidence is used to calculate limits. Degradation rate at storage is estimated to be 0.0006 with upper 95% confidence limit of 0.0007. This is significantly lower that the degradation rate at 40 °C of 0.0048 with a confidence interval of 0.0037–0.0059 (Table 1). The estimated activation energy for Coulter Clenz® is 28.15 kcal mol^{-1}, with 95% confidence limits from 26.58 to 29.73 kcal mol^{-1}. These values are slightly higher in comparison to the range of 10–20 kcal mol^{-1} reported for some pharmaceutical analytes [10].

The effects of degradation rates at different temperatures are evident in the estimates of stabilities shown in Table 3. Coulter Clenz® will perform within specification for about 8.1 days at 55 °C in comparison to 73.2 days at 40 °C. Confidence intervals for stabilities at different temperatures do not overlap indicating statistical differences. Coulter Clenz® is expected to perform within specification for about 616.4 days at the recommended storage temperature of 25 °C. Error of estimated stability at 25 °C is greater than the errors at elevated temperatures since no data are collected at this temperature, while the degradation pattern is estimated using data from the elevated temperatures and the Arrhenius relationship.

3.2. Risk in establishing shelf life

There is always a risk that a product with accepted performance will fail before its claimed shelf life when delivered to the consumer. Shelf life of a product is estimated from real time or accelerated stability tests [3]. The point estimate of shelf life has a certain error that is related to the uncertainty of measurement and experimental design implemented in stability testing [6]. To reduce risk associated with this estimation it is required that shelf life be determined at least as the lower limit of confidence interval of the estimate [11,12]. In addition, manufacturers sometimes choose to allow for some extra time between the estimated and claimed shelf life. This

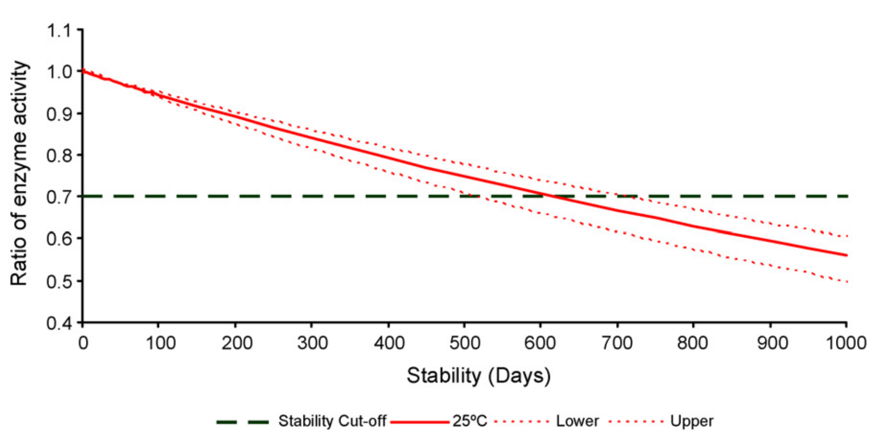

Fig. 2. Degradation at storage temperature (25 °C).

Table 4
Consumer's risk and claimed shelf life for a product with estimated shelf life of 600 days.

Error of estimated shelf life (%)	Manufacturer's tolerance (%)	Claimed shelf life (days)	Consumer's risk (%)
10%	0%	540	2.500%
	5%	510	0.164%
	10%	480	0.004%
	15%	450	0.000%
	20%	420	0.000%
	25%	390	0.000%
20%	0%	480	2.500%
	5%	450	0.714%
	10%	420	0.164%
	15%	390	0.030%
	20%	360	0.004%
	25%	330	0.001%

Table 5
The effects of temperature and duration of excursion of stability.

Excursion temperature (°C)	Duration of excursion (days)	Stability after excursion (days)	Stability loss due to excursion (days)
30	10	605	12
35	10	580	37
40	10	529	87
45	10	428	188
30	20	593	24
35	20	543	73
40	20	442	175
45	20	240	376
30	30	581	36
35	30	506	110
40	30	355	262
45	30	126	490

is referred to as the manufacturer's tolerance and is aimed at reducing the overall risk.

Let us consider a hypothetical product that went through stability testing. The estimated shelf life from the data collected during testing is 600 days with an error of ±60 days (10%). The lower limit of this estimate is 540 days. Using the recommended 95% confidence level [11,12] and the assumption that data are normally distributed there is a 2.5% chance that the shelf life would be greater than 660 days and a 2.5% chance that the shelf life would be smaller than 540 days. Thus, by default, the risk is 2.5% when only the lower limit is used to determine shelf life and the manufacturer's tolerance is 0%. Risk will consequently be reduced when manufacturer allows for some tolerance, going practically down to zero when this tolerance is up to 15%. Calculated risks for two levels of error of estimated shelf life and different manufacturer's tolerances are shown in Table 4. When error is 10% and the manufacturer's tolerance is 0%, the claimed shelf life of the product would only be 60 days less than the estimated shelf life, while in case when manufacturer's tolerance is 20% the claimed shelf life of the product will be reduced by another 120 days, for a total of 180 days less than the estimated shelf life. Risk is also positively related to the error of estimation when the manufacturer's tolerance is greater than zero. Risk at manufacturer's tolerance of 10% is at an almost negligible level of 0.004% for a 10% error in comparison to 0.164% for 20% error (Table 4).

The claimed shelf life of Coulter Clenz® is 360 days and the calculated risk based on the results shown in Table 3 is 0.002%. Calculations using the accelerated stability testing results also indicate that the error of estimated shelf life is 20.1% and the manufacturer's tolerance is 21.4%.

3.3. Temperature excursions

Temperature is an excursion factor that may affect stability by means of the level of elevated temperatures and the duration of elevated temperatures. The simulated effects of these combinations on stability of Coulter Clenz® are shown in Table 5. In all cases, the age of the product at time of excursions is considered to be 100 days, activation energy is 28.15 kcal mol^{-1} and the degradation rate at storage condition is 0.00058 (Table 2). Product will perform within the intended specification when the estimated stability after excursion is greater than the claimed shelf life of 360 days or when the days of stability loss due to excursion plus product age at time of the excursion (100 days) is smaller than 360 days. The stability of the product is affected by any excursion; however with the exception of the elevated temperature of 45 °C product performance will be not affected for durations of up to 20 days. In addition, Coulter Clenz® will not lose all of its stability for excursion of 30 days at 30 and 35 °C.

Graphical representation of estimated shelf life, claimed shelf life, and stability after two different excursions are shown in Figs. 3 and 4. Product performance will not be affected during its shelf life as long as the estimated stability after the excursion is greater that the claimed shelf life as shown in Fig. 3. Product performance will be affected by excursion in Fig. 4 and its shelf life should be reduced by 5 days. Changes in the degradation pattern of Coulter Clenz® stored at different stressed conditions in comparison to the recommended normal conditions are shown in Fig. 5. It is evident from this figure that temperature excursions accelerate degradation and reduce stability.

All the above simulations are based on the activation energy of Coulter Clenz® estimated from the data collected during the accelerated stability testing. Activation energy is a characteristic of a

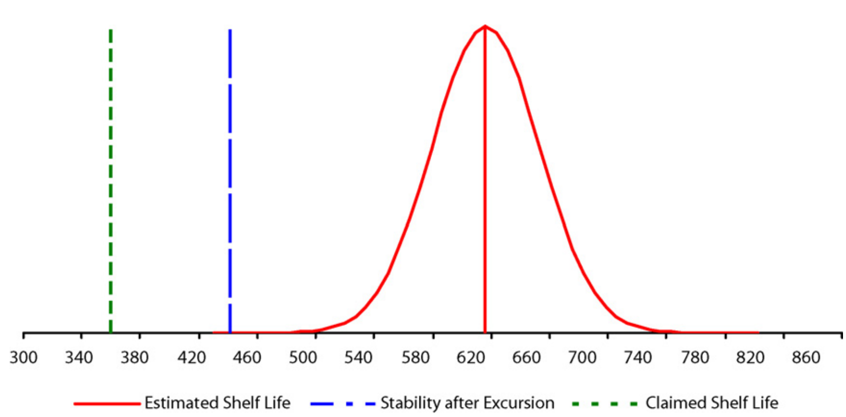

Fig. 3. The effects of excursion temperature = 40 °C and duration of excursion = 20 days.

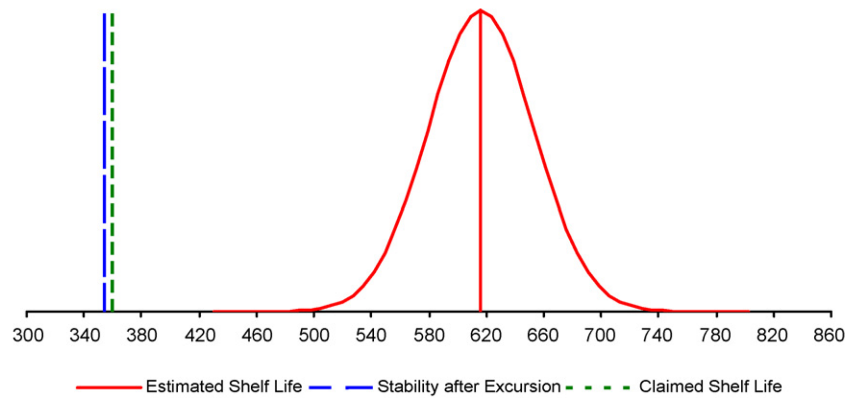

Fig. 4. The effects of excursion temperature = 40 °C and duration of excursion = 30 days.

specific product that determines the acceleration of the degradation rate at elevated temperature. The same temperature excursion may have different effect on the stability of different product based on their activation energy. The effects of different activation energies on stability for different combination of elevated temperatures are simulated and graphically shown in Fig. 6. The effects of temperature and activation energy are synergetic. There is a difference of about 100 days in stability between temperatures of 30 and 45 °C for an excursion of 10 days in a product that has activation energy of 15 kcal mol^{-1} while this difference goes up to about 450 days for the same excursion in a product with activation energy of 30 kcal mol^{-1}.

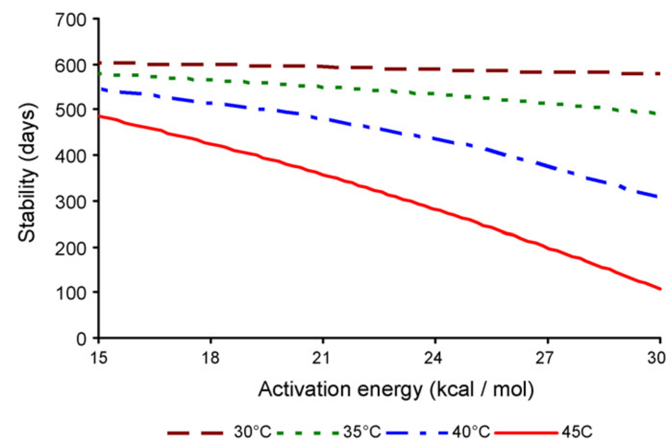

Fig. 6. Relationship between activation energy, temperature, and stability for an excursion of 10 days.

4. Discussion

Information on several characteristics of the product is required in order to use the model for prediction of the effects of temperature excursions that we are proposing in this paper. It is essential that product should degrade according to zero or first-order reaction and degradation rate should depend on temperature as described by Arrhenius relationship. Violations of these conditions are not uncommon in practice and need to be considered specifically when predictions of temperature excursions are concerned. In addition, information on degradation rate and activation energy has to be available. Experience, related products or/and stability tests can be used for this purpose. Degradation rate can be estimated from either real time or accelerated stability testing, but the later one is needed to estimate activation energy and determine the acceleration factor. Ranges of activation energy for families of products can also be used to predict the effects of excursions based on the worst

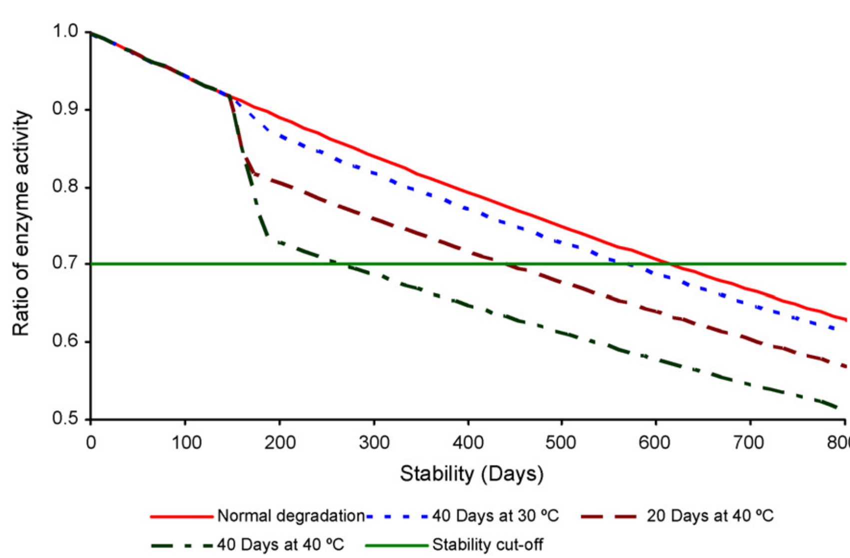

Fig. 5. Normal degradation at storage temperature and degradation at different stressed conditions. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

case scenario. Temperature excursion for up to 20 days at 40 °C will reduce the stability of a product that has an activation energy in the range of 26–30 kcal mol^{-1} by approximately 5–7 months. Products with lower activation energy will have a significantly lower reduction.

The effects of excursions on shelf life performance are less severe when a lower level of risk is implemented to establish the claimed shelf life. The acceptable level of risk is a balanced decision based on the combination of product specific characteristics and manufacturer's tolerance. Each product performance exhibits some inherent uncertainty associated with the random variability of processes used in the manufacturing of that product. This uncertainty affects the estimated stability as well, but the degree of uncertainty can be reduced by controlling the manufacturing process variability and appropriate designing of stability tests. However, since this uncertainty cannot be totally eliminated it is required to be expressed in terms of the 95% confidence lower limit of the stability estimate. Manufacturer's tolerance on the other hand is related to marketing, manufacturing, storage, distribution and other practical decisions that follow a product from the production line to the customer's hands. Basically it implies that product is short dated, and consequently has to be discretionally implemented since it may reduce the claimed shelf life to unacceptable levels.

A product can be subject to additional stress factors during storage or/and distribution that can augment the effects of excursions. All these factors need to be mutually considered when a decision on the shelf life of the affected product has to be made. Sometimes temperature excursions are recurrent during storage but their effects can be considered as additive if the recurrence has happened within a relative short period of time. The summation of time periods of different recurrences will constitute the duration of excursion (t_2) to be used in Eq. (4) of our proposed model.

In conclusion, the proposed model can effectively predict temperature excursion if used within the scope of a product performance and its characteristics. Currently, we are also working to expand this model to products that exhibit higher order reaction degradation kinetics and to accommodate simultaneous excursion effects.

References

[1] S.P. King, M. Kung, H. Fung, J. Pharm. Sci. 73 (1984) 185–190.
[2] R. Magari, J. Pharm. Sci. 91 (2002) 893–899.
[3] W. Nelson, Accelerated Testing: Statistical Models, Test Plans and Data Analysis, John Wiley & Sons, Inc., New York, 1990.
[4] W.Q. Meeker, L.A. Escobar, C.J. Lu, Technometrics 40 (1998) 89–99.
[5] J. Shao, S.C. Chow, Biometrics 50 (1994) 753–763.
[6] R. Magari, J. Pharm. Biomed. Anal. 45 (2007) 171–175.
[7] T.D. Lin, C.W. Chen, J. Biopharm. Stat. 13 (2003) 337–354.
[8] M. Boulanger, L.A. Escobar, Technometrics 36 (1994) 260–272.
[9] SAS Institute, Stat User's Guide, Version 9.1.3 Service Pack 2, SAS Institute, Cary, NC, 2003.
[10] G. Anderson, M. Scott, Clin. Chem. 37 (1991) 398–429.
[11] FDA. Center for Drug and Biologics, Guidelines for submitting documentation for the stability of human drugs and biologics, Rockville, MD, 1987.
[12] Guidance for Industry. ICH. Evaluation for stability data, International Conference on Harmonization, Q1E, Rockville, MD, 2003.