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Stability shelf life estimation using linear regression models

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ABSTRACT

The purpose of a drug product stability study is to elucidate the behavior of its quality attributes over time under the influence of different environmental conditions such as temperature, humidity, light, etc. and to define the retest period, the shelf-life as well as the suitable storage conditions for the drug. This paper presents data referring to the product degradation of three batches of a drug in the course of one year storage. The shelf life estimation is carried out using linear regression models. Poolability of the different batches data required for the application of the overall regression model is discussed.

Keywords:stability, shelf life, drug product, linear regression models.

INTRODUCTION

Stability of a drug substance or product can be defined [1] as the capacity to remain within the established specifications, i.e. to maintain its identity, strength, quality and purity throughout the retest or expiration dating periods. Physical, chemical, and microbiological data is generated as a function of time and storage conditions (e.g. temperature and relative humidity [RH]). The goal of a stability study is not only to characterize the degradation of the drug product, but also to establish the shelf life period (or expiration date) of the medicine product for all future batches. The stability of the drug products is one of the most important characteristics for the patient and for manufacturer. A stability program is created when a new drug product is advanced. The design of the product stability study should be based on the nature of the product, the properties of the drug substance and its dosage form. The purpose of the stability testing is to show the behavior of the drug product quality attributes over time under the influence of different environmental conditions such as temperature, humidity, light, etc. and to define the retest period, the shelf-life of the product and the suitable storage conditions. The choice of the test conditions is defined by the climatic conditions in the different regions of the EC, Japan, the United States, etc. Several types of stability testing are defined: long term tests (25 ± 2ºC; 60 ± 5 % RH), long term tests (30 ± 2ºC; 75 ± 5 % RH), intermediate tests (30 ± 2ºC; 65 ± 5 % RH) and accelerated tests (40 ± 2ºC; 75 ± 5 % RH).

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The shelf life is defined [2] as the time period during which a drug product is expected to remain within the approved shelf life specification, provided that it is stored under the conditions defined on the container label. This definition can be applied to mean and individual units, current and future batches. An appropriate approach to retest the period or to estimate the shelf life is to analyze a quantitative attribute (e.g. an assay, degradation products) by determining the earliest time at which the 95 percent confidence limit for the mean intersects the acceptance criterion proposed in case of a sample of at least three batches.

The treating of several batches requires to combine (or pool) the data from the different batches if the batch-to-batch variation is small. Then an overall shelf-life estimate can be found. In order to do this, the batch similarity should be initially tested. The batch-to-batch variation test has to be performed at a level of significance of 0.25 [3]. The retest periods or shelf lives for individual batches in the stability study can be estimated also by using individual intercepts, individual slopes and the pooled mean square error calculated on the ground of all batches. The shortest estimate among the batches should be chosen as the retest period or shelf life for all batches.

The degradation [4] for continuous assay results in stability analysis. For example the potency expressed in percent of the label claim can be described by the following linear model:

$$y_{ij} = \beta_i' x_{ij} + e_{ij,} \quad i=1, \ldots, k; \; j = 1, \ldots, n$$

where i is the index for the batch, x_{ij} is a $p \times 1$ vector of nonrandom covariates of the form $(1, t_{ij}, \omega_{ij})'$, $(1, t_{ij}, t_{ij}\omega_{ij})'$ or $(1, t_{ij}, \omega_{ij}, t_{ij}\omega_{ij})'$, t_{ij} is the j-th time point for the i-th batch, ω_{ij} is the j-th value of a vector of nonrandom covariates (e.g. a package type and strength), β_i is an unknown $p \times I$ vector of parameters, while e_{ij} are independent random errors (in observing y_{ij}'s) which are assumed to be identically normally distributed as $N(0, \sigma_e^2)$. When $\beta_i = b$ for all i, then there is no batch-to-batch variation. In such a case model (1) reduces to a fixed effects linear regression model.

The present communication shows how data referring to degradation of three batches of a drug product in the course of one year storage is used to estimate the shelf life using linear regression models. The poolability of the different batches data required for the application of the overall regression model is discussed.

Case study

For the purpose of our investigation data referring to the degradation of three batches of a drug product in the course of 12 months is used. The tests are carried out each 3 months. The data is given in Table 1.

Table 1. Drug product degradation data for three batches over a period of 12 months.

№	Month	Batch	Assay (%)
1	0	1	100
2	0	2	99
3	0	3	98
4	3	1	97
5	3	2	98
6	3	3	98
7	6	1	98
8	6	2	95
9	6	3	96
10	9	1	95
11	9	2	93
12	9	3	95
13	12	1	95
14	12	2	92
15	12	3	95

The expiration date (shelf life) is determined as the time up to which the product remains stable when stored under the storage conditions recommended. Thus, an expiration date is the date beyond which it is predicted that the product may no longer retain fitness for use. If the product is not stored in accordance with the manufacturer's instructions, then the product may be expected to degrade more rapidly.

The shelf life estimate is determined by the earliest time at which the 95 % confidence interval of the mean response of the limiting characteristic stability intersects the acceptance limit (labeled claim of potency > 95 %) when batches are treated as fixed effects. During this time the product, if stored appropriately as per the manufacturer's instructions, will remain fit for use.

Each batch is considered individually at the beginning of the procedure (see Fig. 1).

The shelf lives are determined from the data obtained from the one year storage studies. Linear regression equations are estimated for each batch and their correspondence is tested. All estimated models have significant multiple correlation coefficients (R). The estimated batch regression models and shelf lives are summarized in Table 2.

The results obtained for the potency stability data fit, the standard errors, their confidence and prediction limits (CL and PL) are listed in Table 3 (they correspond to the observations pointed out in Table 1).

The tables and Fig. 2 show that the lower prediction limit is more conservative (lower) than the 95 % confidence interval for the mean (red dashed line) and reflects the scatter of the individual values about the fitted line. The acceptable shelf life from a regulatory point of view can be determined from the lower confidence interval limit (shelf life = 5.4). If the out-of-specification risk is close to the end of the shelf life, the latter can be defined by predicting the interval limits (shelf life = 3.2).

Testing of batches data poolability

Before pooling the data from several batches to estimate the retest period or the shelf life, a preliminary statistical test should be performed

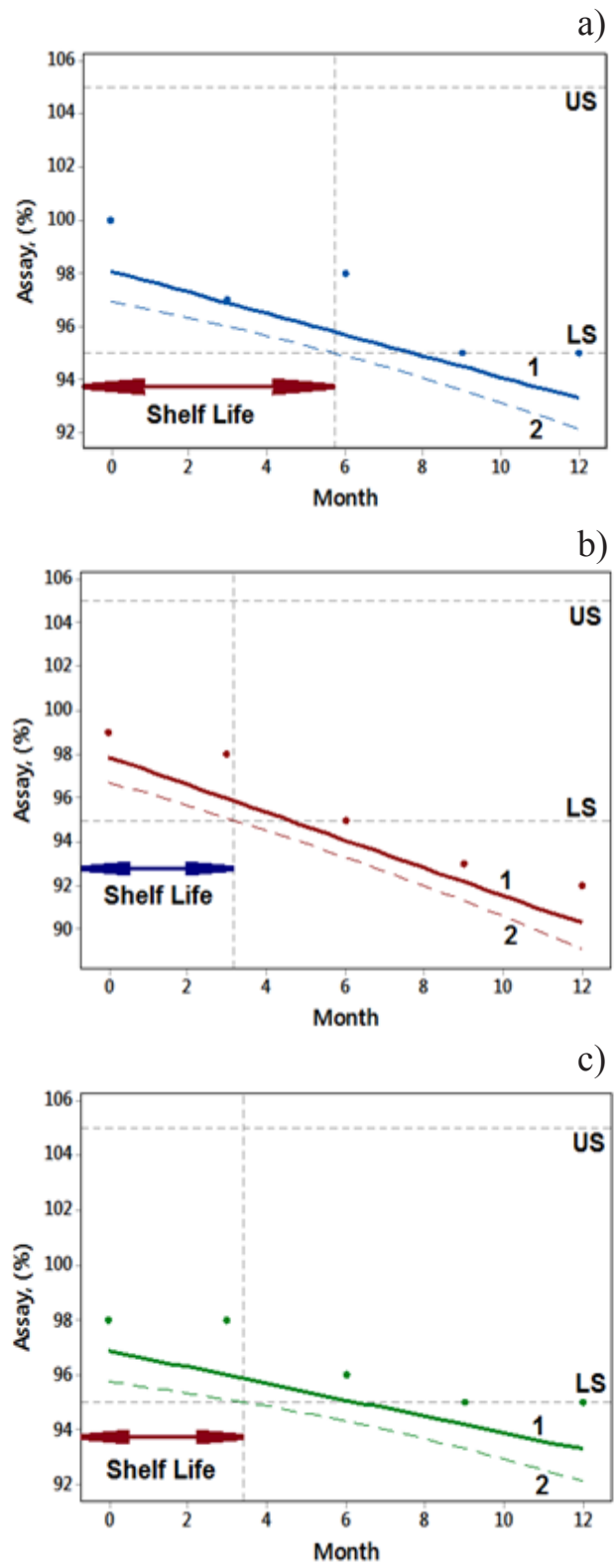

Fig. 1. Estimation of individual shelf lives: a) batch 1, b) batch 2, c) batch 3. (LS – lower specification, US – upper specification, 1 – 5 percentile, 2 – 95 % lower bound).

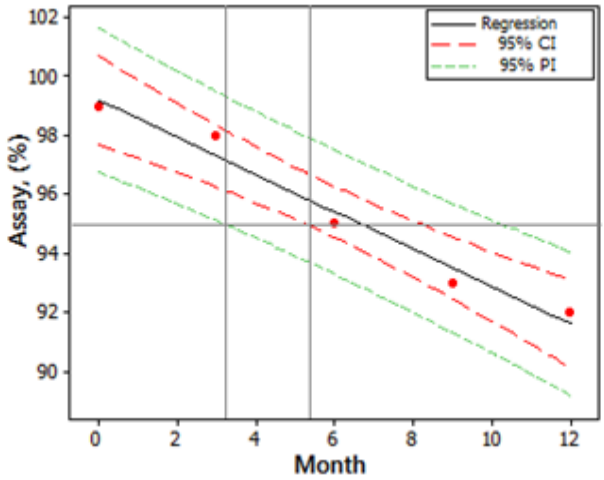

Fig. 2. Linear regression model (for batch 2) - solid line, experimental data, 95% confidence interval and 95 % predicted interval.

to determine whether the regression lines from different batches have a common slope and a common time-zero intercept [4, 5]. There are four possible analytical results: the slopes and the intercepts are identical, the slopes are identical but the intercepts are different, the slopes are different, while the intercepts are identical, the slopes and the intercepts are different. The combining (pooling) of the data from all batches is acceptable only when the slopes and the intercepts are identical. A difference in the slopes can be attributed to a variation of the storage conditions, while a difference in the intercepts is referred to the batches – e.g. an unequal initial drug concentration.

A covariance (ANCOVA) analysis treating time as a covariate can be implemented to test the differences of the slopes and intercepts of the regression lines referring to the different batches. Each of these tests should be conducted using a significance level of 0.25. This is done to compensate for the expected low power of the design due to the relatively limited sample size in a typical formal stability study. If the test rejects the hypothesis of slopes equality (i.e. if there is a significant difference in slopes among batches), it is not considered appropriate to combine the data from all batches.

Table 4 provides the results obtained from the variance analysis. The p-value associated with the separate slopes test (Source = Month*Batch) is 0.054620. This value is less than 0.25, which is an evidence for different slopes among the batches. The p-value associated with the separate intercepts test (Source = Batch) is 0.383240. In this case it is greater than 0.25 and hence there is no evidence for significantly different intercepts.

The estimated regression model for all batches

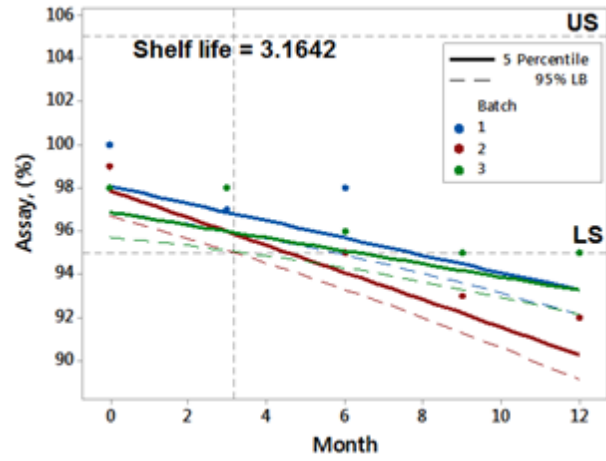

Fig. 3. Stability shelf life for the drug product.

Table 2. Linear regression models and shelf lives.

Batch	Linear regression model	R^2	Shelf Life
1	$\hat{y}_1 = 99{,}4 - 0{,}400\ t$	80%	5.74302
2	$\hat{y}_2 = 99.2 - 0.633\ t$	97%	3.16423
3	$\hat{y}_3 = 98{,}2 - 0.300\ t$	88%	3.39299

Table 3. Estimated fits for potency stability data, the standard errors and their confidence and prediction limits (CL and PL).

№	$\hat{y}$	SE_{fit}	Lower CL	Upper CL	Lower PL	Upper PL
1	99.4	0.621825	97.9933	100.807	97.1029	101.697
2	99.2	0.621825	97.7933	100.607	96.9029	101.497
3	98.2	0.621825	96.7933	99.607	95.9029	100.497
4	98.2	0.439697	97.2053	99.195	96.1294	100.271
5	97.3	0.439697	96.3053	98.295	95.2294	99.371
6	97.3	0.439697	96.3053	98.295	95.2294	99.371
7	97.0	0.359011	96.1879	97.812	95.0107	98.989
8	95.4	0.359011	94.5879	96.212	93.4107	97.389
9	96.4	0.359011	95.5879	97.212	94.4107	98.389
10	95.8	0.439697	94.8053	96.795	93.7294	97.871
11	93.5	0.439697	92.5053	94.495	91.4294	95.571
12	95.5	0.439697	94.5053	96.495	93.4294	97.571
13	94.6	0.621825	93.1933	96.007	92.3029	96.897
14	91.6	0.621825	90.1933	93.007	89.3029	93.897
15	94.6	0.621825	93.1933	96.007	92.3029	96.897

is as follows:

$$\hat{y} = 98.9333 - 0.44444\ t$$

with significant coefficients and a squared multiple correlation coefficient $R_2 = 84.40\%$. Nevertheless, it is not suggested to use the model because the data referring to the different batches cannot be combined (or pooled) due to the proved significant batch-to-batch variation.

The linearized data from all batches can be individually analyzed to define the differences between the intercepts and the slopes of all batches and the overall linear regression model. Statistical t-tests are applied to determine the significance of the slope or intercept differences in this case. The results obtained are shown in Table 5. The results are identical when F-tests are applied.

The linearized all batches data can be analyzed two by two to define the differences between the intercepts and the slopes. The results obtained are listed in Table 6.

It is seen that the intercepts differences in case of a significance level of 0.25 are essential when

Table 4. Analysis of variance.

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Regression	5	65.1333	65.1333	13.0267	20.2138	0.000119
Month	1	53.3333	53.3333	53.3333	82.7586	0.000008
Batch	2	6.5333	1.3778	0.6889	1.0690	0.383240
Month*Batch	2	5.2667	5.2667	2.6333	4.0862	0.054620
Error	9	5.8000	5.8000	0.6444		
Total	14	70.9333				

Table 5. T-tests for the differences between the intercepts and the slopes of all batches and the overall linear regression model.

Batches	Intercept			Slope		
	Difference	T	p	Difference	T	p
1-model	0.47	0.550	0.602	0.044443	0.385	0.714
2-model	0.27	0.569	0.590	-0.18889	-2.959	0.025
3-model	-0.7333	-1.563	0.169	0.144443	2.263	0.064

Table 6. T-tests for the differences between the intercepts and the slopes between all batches two by two.

Batches	Intercept			Slope		
	Difference	T	p	Difference	T	p
1-2	0.1000	0.206	0.843	0.1167	1.769	0.127
1-3	0.600	1.238	0.262	-0.050	-0.758	0.477
2-3	0.5000	1.508	0.182	-0.1667	-3.693	0.010

the linearized data from the third batch and the model as well as those referring to batch 2 and batch 3 are compared. The slopes differences are also considerable when the linearized data referring to batch 2 and the model, batch 3 and the model, batch 1 and batch 2, batch 2 and batch 3 is juxtaposed.

The analysis performed leads to the conclusion that it is not appropriate to combine the data of all batches. The worst shelf life (the minimum

batch expiration time) value estimated individually is obtained for the new drug product (Fig. 3). It should be accepted equal to 3 months (3.1642) in this case.

CONCLUSIONS

The general practice is to grant initially two-year expiry time for new drugs. It is based on a satisfactory year long-term under full stability testing conditions and 6 months accelerated stability data. The expiry date for the third and subsequent years is allowed only if real-time data for this period is provided. Most pharmaceutical products are characterized by only one shelf life. However, in some cases a product may have two shelf lives - one shelf life, for example of 2 years for the product stored under the dry conditions and a second shelf life, for example 2 days for the product when it has been reconstituted or the package is open.

Data on drug product in the course of one year storage is implemented for shelf life estimation using linear regression models. Three drug batches were used. The poolability of all data available is discussed. A covariance analysis is applied to determine whether the regression lines referring to the different batches have a common slope and a common time-zero intercept. The shelf life accepted for the new drug product is estimated to be equal to 3 months (3.1642) based on the worst value of the shelf life (the minimum batch expiration time) estimated individually. The reason for the different slopes of the individual regression lines is probably connected with the variation in the storage conditions.

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