Statistical Techniques for Testing Batch to Batch Variability and Accelerated Testing

by

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ABSTRACT

The purpose of stability studies is to determine the shelf life of drug products. The period between the date of manufacture and expiration date of a given product is known as the shelf life, and consequently the shelf life indicates the period of time during which the consumer can expect the product to be safe and effective. A minimum of three batches is required to perform stability studies. If batch-to-batch variability is small then the batches can be analyzed as one batch, assuming that they come from a single population. This result is determined by testing the batches for equality of slopes and intercepts of the linear degradation rate. However, if a test shows that the batches cannot be combined, then the expiration date should be based on the minimum of the expiration dates obtained from analyzing each batch separately. This project presents ANCOVA (Analysis of Covariance), and the Indicator Variables method, both methods were used to identify and evaluate with the help of simulation techniques if multiple batches can be considered as coming from the same population. Initially it was thought that Indicator Variables would overcome ANCOVA but the simulation results indicate that ANCOVA is superior to Indicator Variables. It is then recommended to use ANCOVA to test variability between batches.

Accelerated studies were also analyzed in this project. The drug investigated is stored under high temperatures to accelerate the degradation and thus allows the rate constants to be determined in a shorter period of time. Temperature is the most common acceleration factor used because its relationship with drug degradation is characterized by the Arrhenius equation.

RESUMEN

El propósito de los estudios de estabilidad es determinar la vida útil de los productos farmacéuticos. El período comprendido entre la fecha de fabricación y fecha de vencimiento de un determinado producto se conoce como “shelf life”, y por tanto el “shelf life” indica el período de tiempo durante el cual el consumidor puede esperar que el producto sea seguro y eficaz. Un mínimo de tres lotes se requiere para realizar estudios de estabilidad. Si la variabilidad entre lotes es pequeña, entonces los lotes pueden ser analizados como un solo lote, presumiendo que provienen de una sola población. Este resultado se determina mediante pruebas entre los lotes para verificar igualdad de pendientes e interceptos de la línea de razón de degradación. Sin embargo, si la prueba muestra que los lotes no se pueden combinar, entonces la fecha de expiración debe basarse en el mínimo de las fechas de expiración obtenidos del análisis de cada lote por separado. Este proyecto presenta ANCOVA (Análisis de Covarianza) y el método de variables indicadoras, ambos métodos fueron utilizados para identificar y evaluar con la ayuda de técnicas de simulación si varios lotes pueden ser considerados como provenientes de la misma población. Inicialmente se pensó que el método de variables indicadoras iba a superar ANCOVA pero los resultados de la simulación indicaron que ANCOVA es superior al método de variables indicadoras. Así que se recomienda el uso de ANCOVA para probar variabilidad entre lotes.

Estudios acelerados también se analizaron en este proyecto. La droga que se investiga es almacenada a altas temperaturas para acelerar la degradación y por tanto poder determinar las constantes de la reacción en un periodo de tiempo más corto. La temperatura es el factor

de aceleración más comúnmente usado porque su relación con la degradación de la droga se caracteriza con la ecuación de Arrhenius.

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To God and my family…

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Acronyms and Abbreviations

ANCOVA	Analysis of Covariance
ICH	International Conference Harmonization
FDA	Federal Drug Administration
ILC	Initial Label Claim
LC	Label Claim
MS_{slope}	Mean Square Error due to slope
MSE	Mean Square Error
MSE_{int}	Mean Square Error for the intercept effect
MSE_c	Mean Square Error for the complete model
SSE_a	Sum of Square Error based on the aggregated sums
SSE_r	Sum of Square Error for the reduce model
SSE	Sum of Square Error for the complete model

1 INTRODUCTION

Stability is defined as the capacity of a drug substance or drug product to remain within established specifications to maintain its density, strength, quality, and purity throughout the expiration date. Physical, chemical, and microbiological data are observed as a function of time and storage conditions (e.g., temperature and relative humidity). Stability studies are the primary tool used to estimate the shelf life of a drug product. Many protocols have been used for stability testing, but most in the industry are now standardized on the recommendations of the International Conference of Harmonization (ICH). These guidelines were developed as a cooperative effort between regulatory agencies from the United States, Europe and Japan ICH Topic Q1A (R2) (2003).

A complete stability study includes *long-term* and *accelerated studies.* In long term studies the samples of a drug product are stored in controlled room at constant temperature and relative humidity, which is similar to the expected normal environmental conditions. However, in accelerated studies the product is stored under stress conditions such as high temperature and large relative humidity to increase the rate of chemical degradation or physical change. Proper design, implementation, monitoring and evaluation of the studies are crucial for obtaining useful and accurate stability data. Stability studies are linked to the establishment and assurance of safety, quality, and efficacy of the drug product from early phase development through the lifecycle of the drug product.

Stability testing provides evidence that a drug product under the influence of various environmental factors changes with time. Although storage conditions are relatively constant, the

distribution of environmental conditions can vary greatly, especially when a drug product is shipped between various climatic conditions.

The main purpose of this study is to use two statistical techniques to study whether or not a set of samples from different batches can be analyzed as if they come from a single batch. Simulation techniques were used to compare the performance of the methods. Statistical techniques were also used to determine the expiration date for a given drug product. This project also includes the study of accelerated stability methods.

1.1 Justification

The shelf life of a drug product is the time that the average drug characteristic remains within an approved specification after manufacture. The FDA (Federal Drug Administration) and ICH require indication of the expiration date for every drug product on the container label. Since the true shelf life of a drug product is unknown, it has to be estimated in the early stage of drug development, when limited number of stability data is available. Furthermore, both the FDA and ICH requires that the estimated shelf life is smaller than the true shelf-life.

Determining shelf life requires designing a systematic and efficient procedure to conduct the stability studies. The studied methods will rely on statistical tools and will be implemented according to the rules and regulations established by the FDA. Currently many of the available procedures are tedious and time consuming. The Analysis of Covariance is the conventional method used to determine whether or not several batches of drugs can be analyzed as a single batch. The pharmaceutical industry requires a method that can be practical but at the same time robust to determine if multiple batches can be analyzed and grouped as a single batch and

determine the expiration date based on a single batch. This project aims to study which of the two analyzed methods is more suitable for the pharmaceutical industry.

1.2 Literature Review

The purpose of the stability testing of drug substance is to determine for what period of time and under which conditions the drug substance can be stored and transported without relevant changes in quality of drug product. Extensive study and research have been done in order to determine the stability of a drug product. The FDA and the ICH require that pharmaceutical companies present factual evidence supporting the shelf life for drug products, (Ramírez et al. 2008).

The design of a stability study should be based on the behavior and properties of the drug substance and the data generated during clinical formulation studies. Statistical design principles can be applied to reduce the amount of testing required (Fairweather, et. al 1995). Designing a stability study is based on a factorial design of experiments where a systematic procedure is used to determine the effect on the response variable of various factors and factor combinations, (Ramírez et al. 2008). A linear model is used to represent the relationship between the factors and the factor combination with the response variable. The typical factors that affect the stability of a drug product are: drug strength, batch, package, and storage.

When designing a stability study two types of studies are considered: full stability study and reduced stability study. A full stability study is one in which samples for every combination of all factors are tested at the required points in time. A reduced stability study is used when

there are circumstances where it is not possible to obtain the total amount of required samples to perform a full stability study or if there is a desire to reduce the samples that will be analyzed. To perform this type of design two methods are recommended by the FDA; bracketing and matrixing, each one is applicable to different situations. Any full or reduced stability design should have the ability to adequately estimate the shelf life. In the "*Guidance for Industry – Q1D Bracketing and Matrixing: Designs for Stability Testing of New Drug Substances and Products"* (2003), the FDA addresses recommendations on the application of bracketing and matrixing to stability studies conducted in accordance with principles outlined in the ICH guidance ICH Topic Q1A (R2) (2003).

- **Bracketing**

Bracketing is the design of a stability schedule such that only samples on the extremes of certain design factors are tested at all points in time as in a full design. The design assumes that the stability of any intermediate levels is represented by the stability of the extremes.

For example, in Table 1 for a product with three different strengths, tablets at 50, 75 and 100 mg, it may be possible to omit testing of the 75 mg tablets, without affecting the stability study.

Table 1 Example of Factor Combinations for Bracketing of three factors with three levels

Strength		50 mg			75 mg			100 mg		
Batch		1	2	3	1	2	3	1	2	3
Container Size	15 mL	T	T	T				T	T	T
	100 mL									
	500 mL	T	T	T				T	T	T

- **Matrixing**

Matrixing is the design of a stability schedule such that the selected subset of the total number of possible samples for all factor combinations would be tested at specified points in time. The design assumes that the stability of each subset of samples tested represents the stability of all samples at a given time. The differences in the samples for the same drug product should be identified. Examples of a matrixing design at different points in time for a product in two strengths (S1 and S2) are shown in Table 2.

Table 2 Example of Matrixing Design on Time Points for a Product with Strengths.

Time Points (month)			0	3	6	9	12	18	24	36
STRENGTH	S1	Batch 1	T	T		T	T		T	T
		Batch 2	T	T		T	T	T		T
		Batch 3	T		T		T	T		T
	S2	Batch 1	T		T		T		T	T
		Batch 2	T	T		T	T	T		T
		Batch 3	T		T		T		T	T

Lin et al. (1994) investigated the applicability of both, the matrix and bracket design and concluded that the full factorial design is the best design when precision in the estimation of the expiration date is needed, and indicated that matrix designs could be useful for drug products with less variability among different strengths and package types.

In general, two stability studies are conducted to ensure that the drug product is under the required specifications: long term and short term stability analysis. The first one is the stability study under the regular and recommended storage condition for the retest period or shelf life; the product is stored at room temperature and regular humidity conditions. For long term studies,

frequency of testing should be sufficient to establish the stability profile of the drug substance, ICH Topic Q1A (R2) (2003). The second one is an accelerated stability testing study, designed to increase the rate of chemical degradation or physical change of a drug product by using exaggerated storage conditions. The principal objective is not only to determine the chemical reaction kinetics but also to establish a tentative expiration date under the environment of marketing storage conditions. Chemical reaction kinetics can be used to evaluate degradation data at accelerated conditions and predict the drug products assay at normal conditions for periods longer than the proposed shelf life.

There are three steps in the stability analysis presented by Chen (1997) and this methodology will be explored in the development of this project:

1. Collect assay results in different time intervals
2. Select the appropriate model to describe the relation between the measured data and the time intervals.
3. Estimate the expiration date based on the information from all the assayed batches.

Long and short term stability analysis and drug shelf life for a single batch and multiple batches, will be analyzed during the development of the project. All these cases are presented in Ramírez et al. (2008), but with a different scope. To determine the shelf life for a single and multiple batches Ramírez, et al. (2008), introduced and developed the indicator variables method by using a regression analysis. This method had never been used and provides similar results as the covariance analysis. The covariance analysis will be used during the development of this project and Montgomery (2005) gives the general formulation. The covariance analysis and much of the methods, such as the methods for testing poolability based on testing slopes and

intercepts presented by Ruberg and Stegeman (1991) and Chow and Liu (1995) are tedious. The indicator variables method is presented in this project as a simple approach to analyzing poolability of batches.

To calculate the shelf life for a single batch some steps must be performed. These steps are presented and described by Ramírez et al. (2008). First, the assay results are collected and second a regression technique is used to estimate the expected degradation line, and finally the lower confidence bound for the mean degradation rate is calculated to determine the shelf life.

Ramírez et al. (2008), presents a series of steps to analyze the drug shelf life for multiple batches. They developed a test for poolability of batches that tests whether or not the slopes are the same across batches over time. Before using the data from several batches to estimate the shelf life, a preliminary statistical test should be performed to determine whether the regression lines from different batches have a common slope and intercept. The analysis of covariance can be employed using a significance level of 0.25 adopted by the FDA after recommendations of Bancroft (1964). The FDA established that the 0.25 significance level is used to compensate for the expected low power of the design due to the relatively limited sample size in a formal stability study. If the test rejects the hypothesis of equality of slopes, it is not considered appropriate to combine the data from all batches.

It is important to mention that at least three primary batches and preferably more should be tested. To allow for some estimates from batch to batch variability and to test the hypothesis that a single expiration date for all future batches is justifiable, the FDA requires:

a. Batch sampling consideration – at least three or more batches. A single batch does not permit assessment of batch to batch variability, and two may not provide a reliable estimate.
b. Container – container from the batches must be included in the stability study, it is recommended that at least as many containers be sampled at each sampling time.

Sampling time – The FDA recommends each three month for the first year, every 6 months during the second year, and annually thereafter. However if the drug product is expected to degrade rapidly, more frequent sampling may be necessary.

This study is organized as follows. Chapter 2 presents the conventional method to estimate the expiration date based on a single batch. Chapter 3 shows the batch-to-batch variability tests and simulation techniques used to compare the methods. Chapter 4 presents the accelerating testing method, and the estimation of the tentative expiration date. Chapter 5 exhibits some conclusions, recommendations and further work.

2 ESTIMATION OF SHELF LIFE

As indicated earlier, the primary objective of a stability study is not only to examine the degradation of a drug over time, but also to establish an expiration date for the drug product. The shelf life is used to establish the expiration dates for individual batches and should be applicable to all future batches produced by the manufacturing process for the drug product.

2.1.1 Drug Shelf Life for a Single Batch

The shelf life is the time length that a material may be stored without chemical and physical deterioration; i.e., the length of time a product remains with the original product properties. The expiration date on the other hand is the date in which a product expires because the product exceeded the degradation level. The FDA regulation establishes that the expiration date for a drug product consists on determining the time at which the 95% one sided lower confidence interval for the mean degradation curve intersects the lower acceptable specification limit, which is usually adopted by the FDA as 90% LC (label claim). The label claim is a claim in the label to describe ingredients present in the drug at an specific percent. Assuming that the degradation of a drug product decreases linearly with time, the degradation of a drug product can be expressed as:

$$y_i = \alpha + \beta x_i + \varepsilon_i \quad i = 1, \dots, n \tag{1}$$

Where y_i is the percentage of the label claim at a given time x_i for the ith sample. α and β are the regression parameters, α represents the percentage label claim when $x_i = 0$. β is known as the degradation rate, and the product βx_i is the stability loss over time. It is assumed that the random variable ε_i follows a normal distribution with zero mean and constant variance.

2.1.2 Drug Expiration Date Calculation for a Single Batch

The drug expiration date for a single batch exhibits a 95% confidence that the average drug characteristic of the dosage units in the batch is within specifications up to the end of the expiration date. The method for computing the expiration date was presented by Ramírez et al. (2008), and the method is summarized as follows:

The 95% confidence interval can be expressed by the following probability statement:

$$P\left(t \leq t_{0.05,n-2}\right) = 0.95 \tag{2}$$

where $t_{0.05,n-2}$ is the upper five percentile of the t student distribution with $n-2$ degrees of freedom. Assuming that the percentage of the label claim (%LC) follows a normal distribution, the statistic t is defined as

$$t = \frac{z}{\sqrt{\chi_m^2/m}} = \frac{[\hat{y} - (\mu + \beta x)] \Big/ \sqrt{\sigma^2 \left[\frac{1}{n} + \frac{(x-\bar{x})^2}{S_{xx}}\right]}}{\sqrt{\frac{SSE}{[\sigma^2(n-2)]}}} \tag{3}$$

where

$$S_{xx} = \sum_{i=1}^{n} (x - \bar{x})^2 \tag{4}$$

$$SSE = \sum_{i=1}^{n} (y_i - \hat{y})^2 \tag{5}$$

Where SSE is the sum-of-square errors, z is a random variable that follows the standard normal distribution, $\frac{SSE}{[\sigma^2(n-2)]}$ follows a chi-square distribution with $n-2$ degrees of freedom. $\hat{y}$ is the

estimated %LC at time x, a and b are the estimates of the parameters α and β, respectively, and $\bar{x}$ is the average sampling time.

The t statistic was used to eliminate the unknown parameter σ^2. Thus, the equation defining the statistic t (3) can be written as:

$$t = \frac{\hat{y} - (\mu + \beta x)}{S_{\hat{y}}} \tag{6}$$

$$S_{\hat{y}} = \sqrt{MSE\left[\frac{1}{n} + \frac{(x - \bar{x})^2}{S_{xx}}\right]} \tag{7}$$

$$MSE = \frac{SSE}{n - 2} \tag{8}$$

where MSE is the mean-square error.

Thus equation (2) can be now written as

$$P\left(\frac{\hat{y} - (\mu - \beta x)}{S_{\hat{y}}} \leq t_{0.05,n-2}\right) = 0.95 \tag{9}$$

Therefore, the 95% one sided lower confidence bound is

$$L(x) = \hat{y} - t_{0.05,n-2}S_{\hat{y}} = a - bx - t_{0.05,n-2}\sqrt{MSE\left[\frac{1}{n} + \frac{(x - \bar{x})^2}{S_{xx}}\right]} \tag{10}$$

The points where $L(x)$ intersects the acceptable lower specification limit δ can be obtained by finding the roots of the following equation: $\delta - L(x) = 0$. It should be pointed out that this equation can also be written in the following form:

$$f(x) = [\delta - (a + bx)]^2 - t_{0.05,n-2}^2 MSE\left[\frac{1}{n} + \frac{(x - \bar{x})^2}{S_{xx}}\right] = 0 \tag{11}$$

The quadratic equation (11) has two roots and the shelf life can be obtained by calculating the root of the equation that is smaller than the established reference point. The reference point can be defined as follows:

$$x_{ref} = \frac{a - \delta}{-b} \tag{12}$$

2.1.3 Example: Shelf Life Calculation

The label claim results for a batch of drug are presented in Table 3. Based on these results, the shelf life for the batch will be determined using the methodology previously described.

Table 3 Single batch assay results to calculate shelf life.

Time (months)	Label Claim (%)
0	101.4
3	98.7
6	96.5
9	95.4
12	93.6
18	92.3
24	90.1
36	88.9

Given the data on Table 3, the equation for the line to minimize errors is shown in Figure 1. The line represents the label claim (%) as a function of time (months) of the degradation rate from the analyzed drug.

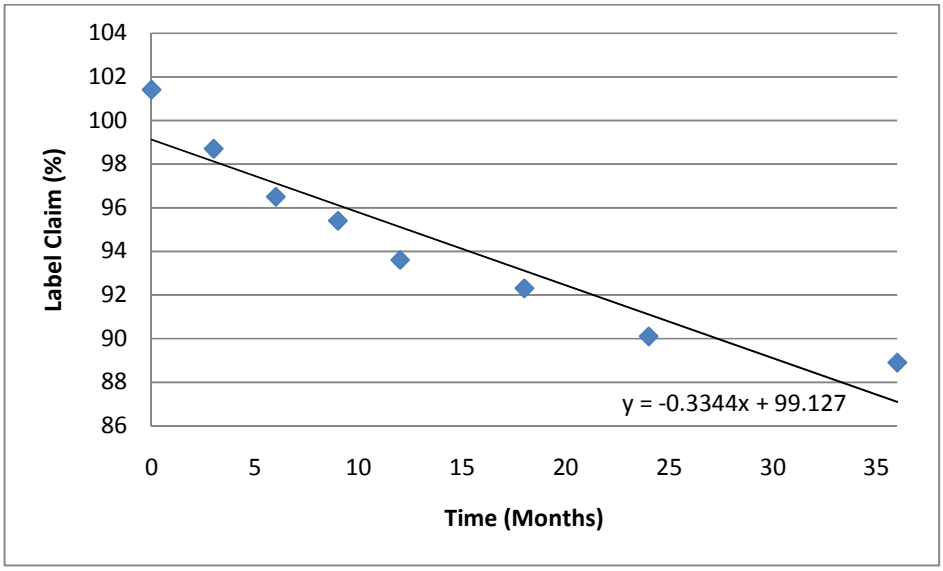

Figure 1 Degradation Line for Single Batch Shelf Life Calculation

Using regression techniques the degradation line has to be calculated as shown in Figure 1. Thus the degradation line may be expressed as:

$$\hat{y} = 99.127 - 0.3344x$$

A computer program to calculate the statistics required to determine the shelf life is presented in Appendix A.2, but a conventional statistical computer program can be used as well to conduct model fitting. The following information can be obtained:

Table 4 Additional Information for Model Fitting

$t_{0.05,6}$	1.943
MSE	2.2713
n	8
$\bar{x}$	13.5
S_{xx}	1008

Thus, using the methodology previously explained for shelf life calculation, the 95% one-sided lower confidence bound for the mean degradation rate is:

$$L(x) = 99.127 - 0.334x - 1.943\sqrt{2.2713\left[{}^{1}\!/_{8} + \frac{(x-13.5)^2}{1008}\right]}$$

Consequently, the shelf life can be obtained using the following equation:

$$f(x) = [90 - (99.127 - 0.3344x)]^2 - 1.943^2(2.2713)\left[{}^1\!/_8 + \frac{(x - 13.5)^2}{1008}\right] = 0 \quad (13)$$

The reference point can be calculated as follows:

$$x_{ref} = \frac{a - \delta}{-b} = \frac{(99.127 - 90)}{0.334} = 27.326$$

Therefore, the shelf life is the root smaller than 27.326. A tool was created using Matlab® and is presented in Appendix A.2. This was used to compute the roots of equation (13). It is important to remark that the equation was considered to be solved using the quadratic equation. However, it was found more convenient to use line search. Thus, to solve the problem, it is equivalent to find *x* such that minimizes the value of *f(x)*. This root can be obtained using the Quasi-Newton Line Search algorithm.(Reklaitis et al. 1983) The algorithm consists of two phases: determination of a direction search and lines search procedures. The Matlab® program requires an initial point, and it is advisable to use the following equation:

$$x(0) = x_{ref} - p \quad (14)$$

In equation (14) p is a positive value that should be estimated using trial and error until the program converges to a local minimum. The range for p goes usually from 0 to 10. For this example the algorithm converges to $x_{root} = 23.202$. The shelf life is the integer part of the root; therefore the expiration date of this specific batch is 23 months.

2.1.4 Minimum Approach for Expiration Date with Multiple Batches

As previously mentioned the FDA guidelines indicate that at least three or more batches should be tested to allow for some estimate of batch-to-batch variation and to test the hypothesis of similarity of slopes and intercepts to justify a single expiration date for all batches. However, if the test results in a rejection of the hypothesis the pooling would not be permitted and the minimum approach is implemented. Minimum approach means that the minimum value of the expiration dates calculated for the batches has to be implemented for all the future batches. This is because the degradation lines of individual batches cannot be considered as they have the same degradation rate. Under these circumstances the FDA guidelines establish that the overall expiration dating period has to guarantee that the product will remain within acceptable limits regardless of the batch from which it came. Thus, the shelf life for each batch is calculated and the expiration dating period is based on the lowest of all shelf lives. This method is known as the minimum approach and it can be expressed as:

$$\min \{x_L(i), \dots, x_L(k)\} \tag{15}$$

where $x_L(i)$ is the shelf life of the i^{th} batch, and k is the total number of batches. Since the minimum of all the expiration dating periods is the shortest shelf life among all batches, this estimate will provide a 95% confidence that the strength of the drug product will remain above the acceptable lower specification limit.

2.1.5 Example: Shelf Life Minimum Approach

A stability study was performed and results are presented in Table 5. Using this information the shelf life of the drug product can be estimated.

Table 5 Assay results for the Shelf life minimum approach example

Time (months)	0	3	6	9	12	18	24
Batch 1	104.76	103.74	102.35	101.85	99.67	98.32	96.98
Batch 2	101.52	99.958	98.15	96.75	96.26	96.12	95.57
Batch 3	105.32	104.36	102.36	100.98	98.34	97.45	95.45

To determine the shelf life, the slopes and intercepts had to be analyzed to test if the degradation rates of the batches are similar. To test similarity of slopes and intercepts the ANCOVA method is used. The first step is to test whether or not the degradation rates for all the batches exhibit similar slope. The following hypothesis should be tested:

$$H_0: \beta_1 = \beta_2 = \beta_3$$

To determine whether or not the hypothesis is rejected the *F* statistic is calculated.

$$F_{slope} = \frac{MS_{slope}}{MSE} = \frac{3.9617}{0.7382} = 5.366$$

$$F_{(0.25,2,15)} = 1.522$$

The null hypothesis is rejected at 0.25 level of significance because $F_{slope} > F_{(0.25,2,15)}$. It is concluded that the slopes of the degradation lines of the batches are different and consequently the minimum approach for the shelf life determination applies. The next step is to calculate the shelf life for each batch. The batch that exhibits the minimum shelf life is the one applied to all the future batches.

The shelf life for the first batch is obtained by finding the root that is smaller than the reference point of the following equation:

$$[90 - (104.54 - 0.3344x)]^2 - 2.015^2(0.2434)\left[\frac{1}{7} + \frac{(x - 10.29)^2}{429.43}\right] = 0$$

The reference point for this equation is:

$$x_{ref} = \frac{a - \delta}{-b} = \frac{104.54 - 90}{0.3344} = 43.48$$

Using $x(0) = x_{ref} - 5$ as the initial point, the root is $x_{root}(1) = 39.17$ and the shelf life for the first batch of the analysis $x_{shelf}(1) = 39\ months$.

The shelf life for the second batch is obtained by finding the root that is smaller than the reference point of the following equation:

$$[90 - (100.16 - 0.2336x)]^2 - 2.015^2(1.2883)\left[\frac{1}{7} + \frac{(x - 10.29)^2}{429.43}\right] = 0$$

The reference point for this equation is:

$$x_{ref} = \frac{a - \delta}{-b} = \frac{100.16 - 90}{0.2336} = 43.49$$

Using $x(0) = x_{ref} - 3$ as the initial point, the root is $x_{root}(2) = 32.41$ and the shelf life for the second batch is: $x_{shelf}(2) = 32\ months$.

Finally, the shelf life for the third batch is obtained by finding the root that is smaller than the reference point of the following equation:

$$[90 - (104.99 - 0.4256x)]^2 - 2.015^2(0.6830)\left[\frac{1}{7} + \frac{(x - 10.29)^2}{429.43}\right] = 0$$

The reference point for this equation is:

$$x_{ref} = \frac{a - \delta}{-b} = \frac{104.99 - 90}{0.4256} = 35.22$$

Using $x(0) = x_{ref} - 4$ as the initial point that accomplishes convergence, the root is $x_{root}(3) = 31.05$ and the shelf life for batch three is: $x_{shelf}(3) = 31\ months$.

Thus, the shelf life that should be applied to all future batches is:

$$\min\{39, 32, 31\} = 31\ months$$

3 STATISTICAL TECHNIQUES FOR TESTING BATCH-TO-BATCH VARIABILITY

This chapter presents a review of how to test similarity between batches, and the different statistical techniques studied during the research. The chapter is organized as follows: first, it covers how the similarity between batches can be concluded when there is equality of slopes and intercepts, followed by a description of the analyzed methods (Analysis of Covariance and Indicator Variables), and finally Monte Carlo simulation is used to evaluate the performance of both methods under different scenarios.

Typically multiple batches are manufactured, and it is desirable to pool the data from the different batches to obtain a single shelf life for all batches. A poolability test determines whether the data from different batches can be combined for an overall estimate of a shelf life.

3.1 Testing Poolability of Batches

The FDA establishes that batch similarity can be assessed by the equality of slopes and intercepts of the individual batches. Assuming that the degradation line decreases linearly with time and it can be represented by:

$$y_{ij} = \alpha_i + \beta_i x_{ij} + \varepsilon_{i\,j} \quad i = 1, \dots, I \; j = 1, \dots, n_i \tag{16}$$

where y_{ij} is the assay results and usually known as the percentage of label claim (%LC) of the i^{th} batch for a drug product sampled at time x_{ij}, j is the number of sampling times for the i^{th} batch, n_i is the total number of sampling times and I is the total number of batches. α_i and β_i are the

intercept and slope of the degradation line for the i^{th} batch, respectively, ε_{ij} is assumed to be random variable, with zero mean and constant variance.

After the test is completed if the batches belong to a single population a model should be derived with a single intercept and slope for the entire population of batches. Thus, a poolability test should be implemented to verify if a single population of batches should be used. To test the poolability of batches two methods will be used and two steps performed with each method. ANCOVA and indicator variables are the two methods used throughout the project, and each method tests equality of slopes and equality of intercepts.

3.2 Analysis of Covariance (ANCOVA)

ANCOVA is a general linear model with one continuous response variable (quantitative) and one or more factor variables (qualitative). ANCOVA is a merger of ANOVA and regression techniques. ANOVA is a method commonly used to in checking the significance and adequacy of the calculated linear regression model. It includes a model, in which the variance is partitioned into components due to different sources of variation. ANOVA is used to test whether or not the means of several groups are all equal (Paulson, 2007). In contrast, ANCOVA tests whether certain factors have an effect on the response variable after removing the variance for which quantitative predictors (covariates) account. The inclusion of covariates can increase statistical power because it accounts for some of the variability. As in any statistical procedure, ANCOVA makes certain assumptions about the variables included in the model. Only if these assumptions are met, at least approximately, ANCOVA will yield valid results. Specifically, ANCOVA, just like ANOVA, assumes that the errors are independently and normally distributed, with mean

zero and constant variance. Further, since ANCOVA is a method based on linear regression, the relationship of the dependent variable to the independent variable(s) must be linear in the parameters.

Before analyzing the data from several batches to estimate shelf life, a preliminary statistical test should be performed to determine whether the regression lines from different batches have a common slope and a common intercept. ANCOVA can be employed, where time is considered the covariate, to test the differences in slopes and intercepts of the regression lines among batches. Each of these tests should be conducted using a significance level of 0.25 to compensate for the expected low power of the design due to the relatively limited sample size in a typical formal stability study.

It is allowed to combine stability data from different batches because batch-to-batch variability can be assessed based on differences in the slope and intercept for the dug degradation line for each batch using ANCOVA. Thus, if the statistical procedure shows evidence that the batches belong to the same population a single shelf life will be determined for all batches. The FDA guidelines also established that batch similarity of the degradation lines can be assessed by the equality of slopes and the equality of intercepts of individual batches.

The sections below estimate the stability analysis for long-term studies. The objective of the sections is to provide an explanation of the statistical tools used to calculate the shelf life for single and multiple batches.

3.2.1 Analysis of Covariance for Testing Similarity of Slopes

Under the assumption that the strength of a drug product decreases linearly with time, the degradation of the strength over time for a batch can be described by the simple linear regression model:

$$y_{ij} = \alpha_i + \beta_i x_{ij} + e_{ij}, \quad i = 1, \dots, I \quad j = 1, \dots, n_i \tag{17}$$

where y_{ij} is the percent label claim at sampling time x_{ij}; α_i is the intercept, which is the value of the percent label claim at the initial time point; β_i is the slope, which is the degradation rate and e_{ij} are i.i.d. normal random variables with mean zero and variance σ^2. The statistical model used most often for comparing I batches is the ANCOVA method given by equation (17).

The ANCOVA method starts determining whether or not the degradation rates for all batches (*I*) behave in a similar way. The following hypothesis should be tested:

$$H_o: \beta_i = \beta_j \; for \; all \; i \neq j \quad i = 1, \dots I \; and \; j = 1, \dots, n_i \tag{18}$$

To be able to test this hypothesis it is required to compute the aggregated sum of squares of the sampling times and the percentage label claim, the cross product between the sampling times and the percentage label claim and the aggregated sum of squares for the batches. This method is also fully described in Chow (1995), Montgomery (1996) and Ramirez et al. (2008). Here you will find the summary of the procedure.

After all the aggregated sum of squared described before were calculated the mean square errors due to slope contribution is defined as follows:

$$MS_{slope} = \frac{SSE_a - SSE}{I - 1} \tag{19}$$

$$MSE = \frac{SSE}{N - 2I} \tag{20}$$

where,

$$N = \sum_{i=1}^{I} n_i \tag{21}$$

and SSE_a is the sum of square errors based on the aggregated sums.

Finally, the F statistics to test the hypothesis can be written as follows:

$$F(slope) = \frac{MS_{slope}}{MSE} \tag{22}$$

The hypothesis is rejected when $F(slope) > F_{0.25,I-1,N-2I}$, where $F_{0.25,I-1,N-2I}$ is the upper percentile for the F distribution with $I - 1$, and $N - 2I$ degrees of freedom. If the hypothesis is not rejected (i.e., the slopes are similar), it can be proceeded with the next step; to test whether or not the intercepts of the batches are similar.

3.2.2 Analysis of Covariance for Testing Similarity of Intercepts

To determine whether the degradation lines are equal, given that the degradation lines from the considered batches have similar slopes, the following hypothesis should be tested:

$$H_o: \alpha_i = \alpha_j \; for \; all \; i \neq j \quad i = 1, \dots, I \; and \; j = 1, \dots, I \tag{23}$$

Since differences in slopes were not identified, the model used for testing differences in slopes (23) reduces to:

$$y_{ij} = \alpha_i + \beta x_{ij} + \varepsilon_{i\,j} \quad i = 1, \dots, I \; j = 1, \dots, n_i \tag{24}$$

The intercepts can be decomposed in two elements: the common intercept and the batch effect. That is $\alpha_i = \alpha + \tau_i$, where $\alpha = \mu - \beta\bar{x}_{ij}$ is the common intercept, and τ_i is the batch effect; μ is the expected value of y_{ij} , and $\bar{x}_{ij}$ is the average of x_{ij}. Thus, y_{ij} can be written as:

$$y_{ij} = \mu + \tau_i + \beta(\bar{x}_{ij}) + \varepsilon_{ij} \tag{25}$$

A model without a batch effect is compared with a model that includes batch effect to be able to measure the intercept effect. The above model includes the batch effect and is a complete model. The model that has no batch effect is known as a reduced model and can be expressed as follows:

$$y_{ij} = \mu + \beta(x_{ij} - \bar{x}_{ij}) + \varepsilon_{ij} \;\; i = 1, \dots, I \;\; j = 1, \dots, n_i \tag{26}$$

To be able to test this hypothesis it is required to compute the sum of squares, cross products and errors for the reduced and complete model. This method is also fully described in Chow (1995), Montgomery (1996) and Ramirez et al. (2008). For more details Montgomery (1996) explains the full method.

The mean square errors for the intercept effect and the mean square errors for the complete model can be written as follows:

$$MSE_{int} = \frac{SSE_r - SSE_c}{I - 1} \tag{27}$$

$$MSE_c = \frac{SSE_c}{N - I - 1} \tag{28}$$

SSE_r is the sum of square errors for the reduced model, and SSE_c is the sum of square errors for the complete model.

Finally, the F statistics to measure whether or not the intercepts are different from batch to batch is given as follows:

$$F(int) = \frac{MSE_{int}}{MSE_c} \tag{29}$$

If the F-value obtained for the intercept $F(int) > F_{0.25,I-1,N-I-1}$; where $F_{0.25,I-1,N-I-1}$ is the upper percentile of the F distribution with $I-1$, and $N-I-1$ degrees of freedom the hypothesis is rejected. Thus if the null hypothesis is not rejected at the 0.25 level of significance, the batches can be considered as if they came from a single population and the shelf life can be computed based on the studies batches. If that is the case, the model reduces to the following expression:

$$y_{ij} = \alpha + \beta x_{ij} + \varepsilon_{ij}\ i = 1, \dots, I\ \ j = 1, \dots, n_i \tag{30}$$

Where α and β are the common intercept and slope, respectively.

If $H_O: \beta_i = \beta_j\ for\ all\ i \neq j$ is not rejected and $H_O: \alpha_i = \alpha_j\ for\ all\ i \neq j$ is rejected, the model reduces to:

$$y_{ij} = \alpha_i + \beta x_{ij} + \varepsilon_{ij}\ i = 1, \dots, I\ \ j = 1, \dots, n_i \tag{31}$$

If a common slope with different intercepts for different batches was obtained, the expiration dating period is computed for each individual batch and the minimum of the expiration dating periods of the individual batches is the expiration dating period of the drug product.

Another alternative may occur if $H_O: \beta_i = \beta_j\ for\ all\ i \neq j$ is rejected, it is then concluded that the batches do not belong to a single population and the shelf life should be computed for each individual batch. The minimum of the expiration periods of the individual batches is the shelf life for the whole population of batches.

The following example illustrated the application of ANCOVA method when testing similarity of intercepts and slopes.

3.2.3 Numerical Example to Test Similarity of Slopes and Intercepts

Table 6 shows the simulated data created to test the hypothesis, similarity of slopes and similarity of intercepts. Figure 2 shows the degradation lines for the simulated data during the 24 months of the analysis.

Table 6 Simulated data used for the numerical example to test similarity of slopes and intercepts

Time	0	3	6	9	12	18	24
1	105.19	104.86	104.79	104.57	104.49	104.35	103.93
2	105.08	104.95	104.75	104.72	104.56	104.36	103.82
3	105.16	104.87	104.71	104.66	104.47	104.35	103.83
4	105.23	104.85	104.64	104.56	104.50	104.47	103.74

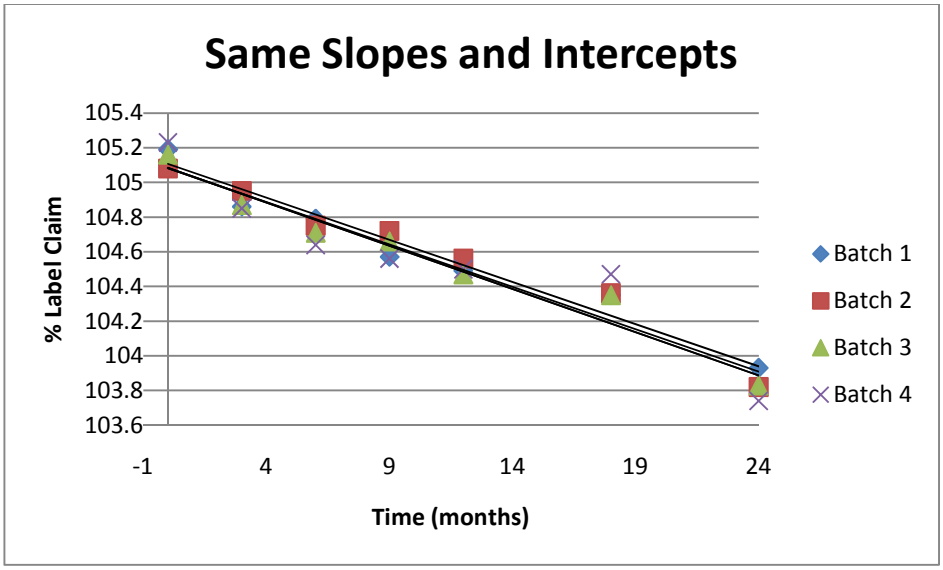

Figure 2 Degradation lines to test similarity of slopes and intercepts.

Results were obtained using a computer program developed to calculate similarity of intercepts and slopes, this program is presented in the Appendix A.3. In the program all the sum of squares from the intercepts and the slopes were calculated. To determine if the degradation lines from the considered batches have similar slopes, the following hypothesis should be tested:

$$H_o: \beta_i = \beta_j \; for \; all \; i \neq j \quad i = 1, \dots, I \; and \; j = 1, \dots, I$$

To determine whether the degradation lines are equal, given that the degradation lines from the considered batches have similar slopes. The following hypothesis should be tested for the intercepts:

$$H_o: \alpha_i = \alpha_j \; for \; all \; i \neq j \quad i = 1, \dots, I \; and \; j = 1, \dots, I$$

Table 7 shows the results obtained from the computer program. The F-value obtained for the slopes equals 0.053, when compared to the critical value obtained for the slopes it is smaller than $(F_{3,20})1.481$. The F-value obtained for the intercepts equals 0.153 resulting in a value smaller than the critical value for the intercepts $(F_{3,23})1.466$. If the hypotheses are not rejected at the 0.25 level of significance, the batches can be considered as if they came from a single population and the shelf life can be computed based on the studied batches.

Table 7 ANCOVA Final Results

$F_{(0.25,3,20)}$	1.481
$F_{(0.25,3,23)}$	1.466
$F_{(slope)}$	0.053
$F_{(intercept)}$	0.153

3.3 Indicator Variables Method

A general analysis of covariance model for a stability design with several batches and packages can be expressed as follows:

$$y_{ijk} = \mu_{ij} + \beta_{ij} x_{ijk} + \varepsilon_{ijk} \quad i = 1, \dots, I \quad j = 1, \dots, J \quad k = 1, \dots, n \tag{32}$$

where y_{ijk} is the response from the k^{th} time point of the i^{th} batch and the j^{th} package of a drug product; x_{ijk} is the sampling time at which the y_{ijk} response was obtained; μ_{ij} is the intercept for the i^{th} batch and the j^{th} package; β_{ij} is the degradation rate for the i^{th} batch and the j^{th} package; ε_{ijk} is the random error and is assumed to be independently and normally distributed with zero mean and constant variance.

The general procedure consists of identifying the model that is associated to a given assay information. Once the correct model is determined, the appropriate procedure should be implemented.

3.3.1 Identification of the Regression Model

A general procedure based on regression analysis, to identify the analysis of covariance model that applies to a given set of assay results was introduced by Ramirez et al (2008). The procedure was called regression model with indicator variables for testing poolability of batches and packages. A simple case will be introduced to facilitate the understanding.

Suppose we have three batches, the corresponding indicator variables model is defined as follows:

$$y_{ij} = \beta_0 + \delta_1 A_1 + \delta_2 A_2 + x_{ij}(\beta_1 + \Delta_1 A_1 + \Delta_2 A_2) + e_{ij} \quad (33)$$

where y_{ij} is the assay result from the j^{th} time point of the i^{th} batch and the x_{ij} is the time at which the assay sample y_{ij} was obtained; A_1 and A_2 are indicator variables that assign a binary code to each one of the factor combinations, β_0 is part of the intercept of the regression line, β_1 is part of the slope of the regression line. The parameters $\delta's$ and $\Delta's$ are estimated from assay data, ε_{ij} is the random error and is assumed to be independently and normally distributed with zero mean and constant variance.

If we represent the batches 1, 2 and, 3 by B_1, B_2 and B_3 respectively. We can use indicator variables to indicate at which batch is assigned the response variable. For example, a response variable for B_1 is represented by $A_1 = 1\ and\ A_2 = 0$. Similarly, a response variable from B_2 is represented by $A_1 = 0\ and\ A_2 = 1$. Also the response variable for B_3 is represented by $A_1 = 0\ and\ A_2 = 0$. Thus, the values of $A_1$and A_2 define the factor combination for each batch. The indicator variables used for each one of the batches are summarized in the table below.

Table 8 Binary Codification for Indicator Variables

	A_1	A_2	
B_1	1	0	y_{1j}
B_2	0	1	y_{2j}
B_3	0	0	y_{3j}

The response variables for batch 1 (B_1) can be written as follows, after replacing the values of the indicator variables presented in Table 8 in model (34):

$$
\begin{aligned}
y_{11} &= \beta_0 + \delta_1 + x_{11}(\beta_1 + \Delta_1) + e_{11} \\
y_{12} &= \beta_0 + \delta_1 + x_{12}(\beta_1 + \Delta_1) + e_{12} \\
&\vdots \\
y_{1n_1} &= \beta_0 + \delta_1 + x_{1n_1}(\beta_1 + \Delta_1) + e_{1n_1} \\
y_{21} &= \beta_0 + \delta_2 + x_{21}(\beta_1 + \Delta_2) + e_{21} \\
y_{22} &= \beta_0 + \delta_2 + x_{22}(\beta_1 + \Delta_2) + e_{22} \\
&\vdots \\
y_{2n_2} &= \beta_0 + \delta_2 + x_{2n_2}(\beta_1 + \Delta_2) + e_{2n_2} \\
y_{31} &= \beta_0 + x_{31}\beta_1 + e_{31} \\
y_{32} &= \beta_0 + x_{32}\beta_1 + e_{32} \\
&\vdots \\
y_{3n_3} &= \beta_0 + x_{3n_3}\beta_1 + e_{3n_3}
\end{aligned}
\tag{34}
$$

where n_i is the number of sampling times in the i^{th} batch. The system of linear equations expressed by (34) represents the response variable from the three batches. The required condition for the three batches to have the same intercept is that $\delta_1 = \delta_2 = 0$. The three batches will have the same slope if $\Delta_1 = \Delta_2 = 0$. Thus, the problem for testing the poolability of batches reduces to fit the regression model (34) and test the following hypothesis: H_1: $\delta_1 = \delta_2 = 0$, and H_2: $\Delta_1 = \Delta_2 = 0$. Therefore if the null hypothesis H_2 is not rejected at the 0.25 significance level it implies that the slopes of the three batches are the same, i.e., $\beta_1 = \beta_2 = \beta_3 = \beta$. Similarly, if the null hypothesis H_1 is not rejected, the intercepts of the three batches are the same, i.e., $\mu_1 = \mu_2 =$

$\mu_3 = \mu$. If that is the case, the shelf life can be calculated using a model with a single intercept and single slope.

3.3.2 Indicator Varibles Model for Two Factors

The indicator variables model to perform a poolability test for two factors (packages and batches) can be expressed as follows (Ramirez et al 2008):

$$\begin{aligned} y_{ijk} = \beta_0 + \beta_1 x_{ijk} + \delta_1 A_1 + \delta_2 A_2 + \cdots + \delta_r A_r + \emptyset_1 C_1 + \emptyset_2 C_2 + \cdots + \emptyset_s C_s \\ + x_{ijk}(\Delta_1 A_1 + \Delta_2 A_2 + \cdots + \Delta_r A_r + \Phi_1 \mathrm{C}_1 + \Phi_2 \mathrm{C}_2 + \cdots + \Phi_\mathrm{s} \mathrm{C}_\mathrm{s} \\ + \omega_{11} \mathrm{A}_1 \mathrm{C}_1 + \omega_{12} \mathrm{A}_1 \mathrm{C}_2 + \cdots + \omega_{\mathrm{rs}} \mathrm{A}_\mathrm{r} \mathrm{C}_\mathrm{s}) + \mathrm{e}_{\mathrm{ijk}} \end{aligned} \quad (35)$$

Where $i = 1, \dots, I$ $j = 1, \dots, J$ and $k = 1, \dots, n_{ij}$, and I represents the number of batches, J represents the number of packages, n_{ij} is the sample size of the i^{th} batch and the j^{th} package; A_i and C_j are indicator variables that are defined in Table 9.

Table 9 Binary Code for factor Combinations

Factor Combinations	A_1	A_2	…	A_r	C_1	C_2	…	C_r	y_{ijk}
B_1P_1	1	0	…	0	1	0	…	0	y_{11k}
B_2P_1	0	1	…	0	1	0	…	0	y_{21k}
⋮	⋮	⋮	⋮	⋮	⋮	⋮	⋮	⋮	⋮
B_rP_1	0	0	…	1	1	0	…	0	y_{r1k}
B_IP_1	0	0	…	0	1	0	…	0	y_{I1k}
B_1P_2	1	0	…	0	0	1	…	0	y_{12k}
B_2P_2	0	1	…	0	0	1	…	0	y_{22k}
⋮	⋮	⋮	⋮	⋮	⋮	⋮	⋮	⋮	⋮
B_rP_2	0	0	…	1	0	1	…	0	y_{r2k}
B_IP_2	0	0	…	0	0	1	…	0	y_{I2k}
⋮	⋮	⋮	⋮	⋮	⋮	⋮	⋮	⋮	⋮
B_1P_J	1	0	…	0	0	0	…	1	y_{1Jk}
B_2P_J	0	1	…	0	0	0	…	1	y_{2Jk}
⋮	⋮	⋮	⋮	⋮	⋮	⋮	⋮	⋮	⋮
B_rP_J	0	0	…	1	0	0	…	1	y_{rJk}
B_IP_J	0	0	…	0	0	0	…	1	y_{IJk}

The first column in Table 10 shows the factor combinations. For example, B_1P_2 represents the first batch and the second package. The subscripts *r* and *s* are defined as follows: $r = I - 1, s = J - 1$.

To perform a test for poolability it requires fitting the regression model with indicator variables to the assay data and testing four possible hypotheses as Chen et. al. (1997), shows and, the FDA implemented. Table 10 shows the hypothesis presented by Chen et. al. (1997) to be tested, the corresponding interpretation to whether or not the hypothesis is accepted (A) or rejected (R).

Table 10 Procedure to identify the analysis of covariance model

Code	Hypothesis	A	R	Interpretation	ANCOVA Model
1 C0: M0a	$H_1: \delta_1 = \delta_2 = \cdots = \delta_r = 0$	X		Intercepts of all batches are the same. $\alpha_{1j} = \alpha_{2j} = \cdots = \alpha_{Ij} = \alpha_j$	$y_{ijk} = \alpha_j + \beta_{ij}x_{ijk} + \varepsilon_{ijk}$
2 C1: M1	$H_2: \Delta_1 = \Delta_2 = \cdots = \Delta_r = 0$ and $\omega_{11} = \omega_{12} = \cdots = \omega_{rs} = 0$	X		Slopes of all batches are the same. $\beta_{1j} = \beta_{2j} = \cdots = \beta_{Ij} = \beta_j$	$y_{ijk} = \alpha_{ij} + \beta_j x_{ijk} + \varepsilon_{ijk}$
2 C1: M3	H_1 and H_2	X		All batches have the same intercept and the same slope. $\alpha_{1j} = \alpha_{2j} = \cdots = \alpha_{Ij} = \alpha_j$ $\beta_{1j} = \beta_{2j} = \cdots = \beta_{Ij} = \beta_j$	$y_{ijk} = \alpha_j + \beta_j x_{ijk} + \varepsilon_{ijk}$
1 C0: M0b	$H_3: \emptyset_1 = \emptyset_2 = \cdots = \emptyset_s = 0$	X		Intercepts of all batches are the same $\alpha_{i1} = \alpha_{i2} = \cdots = \alpha_{iJ} = \alpha_i$	$y_{ijk} = \alpha_i + \beta_{ij}x_{ijk} + \varepsilon_{ijk}$
3 C2: M2	$H_4: \Phi_1 = \Phi_2 = \cdots = \Phi_s = 0, \omega_{11} = \omega_{12} = \cdots = \omega_{rs} = 0$	X		Slopes of all packages are the same $\beta_{i1} = \beta_{i2} = \cdots = \beta_{iJ} = \beta_i$	$y_{ijk} = \alpha_{ij} + \beta_i x_{ijk} + \varepsilon_{ijk}$
3 C2: M4	H_3 and H_4	X		Intercepts and slopes of all packages are the same $\alpha_{i1} = \alpha_{i2} = \cdots = \alpha_{iJ} = \alpha_i$	$y_{ijk} = \alpha_i + \beta_i x_{ijk} + \varepsilon_{ijk}$

				$\beta_{i1} = \beta_{i2} = \cdots = \beta_{iJ} = \beta_i$	
3 C3: M5	H_2 and H_4	X		All batches and all packages have a common slope $\beta_{1j} = \beta_{2j} = \cdots = \beta_{Ij} = \beta_j$ $\beta_{i1} = \beta_{i2} = \cdots = \beta_{iJ} = \beta_i$ and $\beta_i = \beta_j = \beta$	$y_{ijk} = \alpha_{ij} + \beta x_{ijk} + \varepsilon_{ijk}$
4 C3: M6	H_1, H_2 and H_4	X		All batches and packages have common slope, and the intercepts of all batches are the same. $\alpha_{1j} = \alpha_{2j} = \cdots = \alpha_{Ij} = \alpha_j$ $\beta_{1j} = \beta_{2j} = \cdots = \beta_{Ij} = \beta_j$ $\beta_{i1} = \beta_{i2} = \cdots = \beta_{iJ} = \beta_i$ and $\beta_i = \beta_j = \beta$	$y_{ijk} = \alpha_j + \beta x_{ijk} + \varepsilon_{ijk}$
4 C3: M7	H_2, H_3 and H_4	X		All batches and packages have common slope, and the intercepts of all packages are the same. $\alpha_{i1} = \alpha_{i2} = \cdots = \alpha_{iJ} = \alpha_i$ $\beta_{1j} = \beta_{2j} = \cdots = \beta_{Ij} = \beta_j$ $\beta_{i1} = \beta_{i2} = \cdots = \beta_{iJ} = \beta_i$ and $\beta_i = \beta_j = \beta$	$y_{ijk} = \alpha_i + \beta x_{ijk} + \varepsilon_{ijk}$
4 C8: M8	H_1, H_2, H_3 and H_4	X		Slopes and intercepts of all batches and packages are the same. $\alpha_{1j} = \alpha_{2j} = \cdots = \alpha_{Ij} = \alpha_j$ $\alpha_{i1} = \alpha_{i2} = \cdots = \alpha_{iJ} = \alpha_i$ and $\alpha_i = \alpha_j = \alpha$ $\beta_{1j} = \beta_{2j} = \cdots = \beta_{Ij} = \beta_j$ $\beta_{i1} = \beta_{i2} = \cdots = \beta_{iJ} = \beta_i$ and $\beta_i = \beta_j = \beta$	$y_{ijk} = \alpha + \beta x_{ijk} + \varepsilon_{ijk}$
1 C0	H_1, H_2, H_3 and H_4		X	Intercepts and slopes of batches and packages are different.	$y_{ijk} = \alpha_{ij} + \beta_{ij} x_{ijk} + \varepsilon_{ijk}$

Table 10 shows a set of rules presented by Chen et. al. (1997) that can be implemented when computing the shelf life. After the analysis of covariance has been correctly identified the expiration-dating period can be determined using the nine models described next.

1. **C0:** The expiration dating periods are computed separately for each batch/package combination. The minimum of the expiration dating periods of the individual batches (at least 3 batches) from the same package is the expiration dating period of that package.
2. **C1:** The data from the individual batches are combined within each package. For each package:

   M1: Assuming a common slope with different intercepts for different batches, the expiration dating periods are computed for individual batches. The minimum of the expiration dating periods of the individual batches is the expiration dating period of that package.

   M3: Assuming a common slope and a common intercept for all the batches, a single expiration dating period is computed as the expiration dating period of that package.
3. **C2:** The data from the individual packages are combined within each batch:

   M2: For each batch, assuming a common slope with different intercepts for different packages, the expiration dating periods are computed for individual packages. The minimum of the expiration dating periods of the individual batches from the same package is used as the expiration dating period of that package.

   M4: For each batch, assuming a common slope and a common intercept for all packages, a single expiration dating period is computed. The minimum of the

expiration dating periods of the individual batches is used as the expiration dating period for every package.

4. **C3:** The data for all the packages and batches are combined:

   M5: Assuming different intercepts but a common slope for all the batch/package combinations, the expiration dating periods are computed for all individual batch/package combinations. The minimum of the expiration dating periods of the individual batches from the same package is used as the expiration dating period of that package.

   M6: Assuming a common intercept for all the batches with a common slope for the all batch/package combinations, a single expiration dating period is computed for each package to be used as the expiration dating period of that package.

   M7: Assuming a common intercept for all the packages and a common slope for the entire batch package combinations, the expiration dating periods are computed for each batch. The minimum of the individual batches is used as the expiration dating period for every package.

   M8: Assuming a common slope and a common intercept for all the package/batch combinations, a single expiration dating period is computed to be used as the expiration dating period for every package.

3.4 Simulation Results

A simulation exercise was created to study batch-to-batch variability. Three different scenarios were simulated using Monte Carlo simulation techniques. Computer programs to compute ANCOVA and Indicator variables methods are presented in Appendix A.4, and A.5. The first scenario includes changes in the degradation rate, the second scenario includes changes on the initial label claim, and the third scenario includes changes in the degradation rate as well as the initial label claim.

The simulation process consists in creating two components: one deterministic and one stochastic component. The deterministic component represents the drug degradation through time. The drug degradation is represented by: $y = a + bx$ since it is assumed to have a lineal degradation rate. The Initial Label Claim (ILC) is represented in the equation by a, the degradation rate is represented by b, and x is the time (month) in which the analysis was performed; i.e., $x = \{0, 3, 6, 9, 12, 18, 24\}$, finally the concentration of the active ingredient is represented in the equation by y. The Label Claim (LC) was simulated using the following equation $LC = y + e$, where e is the stochastic component and was generated by using a random variable that followed a normal distribution with mean zero and variance 0.04. The variance was selected small enough to avoid interference with degradation rate.

Figure 3 shows the slow degradation rate, and how the label claim varies from 100% to 98% during 24 months.

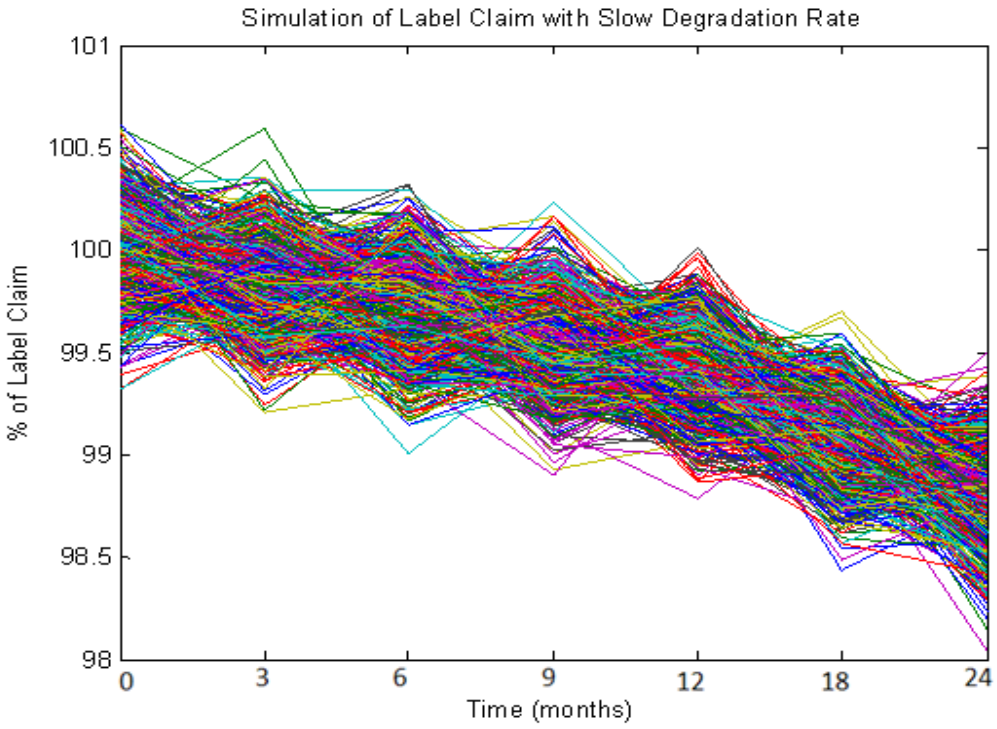

Figure 3 Simulation of 1000 batches with slow degradation rate equal to -0.05, and the initial label claim was 100%

3.4.1 Analysis 1: Changes on degradation rate

The simulation of this scenario includes the development of three populations. The initial label claim in the three populations was established as 100%. The first population was designed to simulate low degradation rate, which was equal to -0.05. The second population simulates a moderate degradation rate and was established equal to -0.2, and the last population represents a fast degradation rate, which was equal to -0.4. The degradation of the drug product was modeled according to real cases in the pharmaceutical industry. Each population contains a thousand of batches. The analysis consists of selecting 2 out of 3 populations and extracts two batches from each selected population. Two batches from each population were randomly selected to create a sample of 4 batches. This process was repeated over a thousand times and results from these

samples were used to validate the statistical methods. It is expected that both of the methods (ANCOVA and indicator variables) detect that the batches exhibit different degradation rates since they are from different populations. Figure 4 shows how the ANCOVA method detected changes in degradation rates with no errors. The continuous line is the statistics for each one of the samples and the horizontal line is the critical value. However, in Figure 5 it is important to point that ANCOVA incorrectly showed that the initial label claim is different since the F-statistics are larger than the critical value (1.466). The ANCOVA method presents 100% false alarm because actuality there were no changes on the initial label claim (ILC). The Indicator Variables method also detected without errors that the degradation rates are different since the statistics are larger than the critical value, as shown in Figure 6. The indicator variable method shows incorrectly that 507 out of 1000 batches have different ILC, as shown in Figure 7. As a result, both methods detected without error the change in the degradation rate. In this scenario the Indicator Variables performed better than ANCOVA, because ANCOVA detected that all the batches analyzed have a change in the ILC when indicator variables shows that only 507 batches have a change in the ILC.

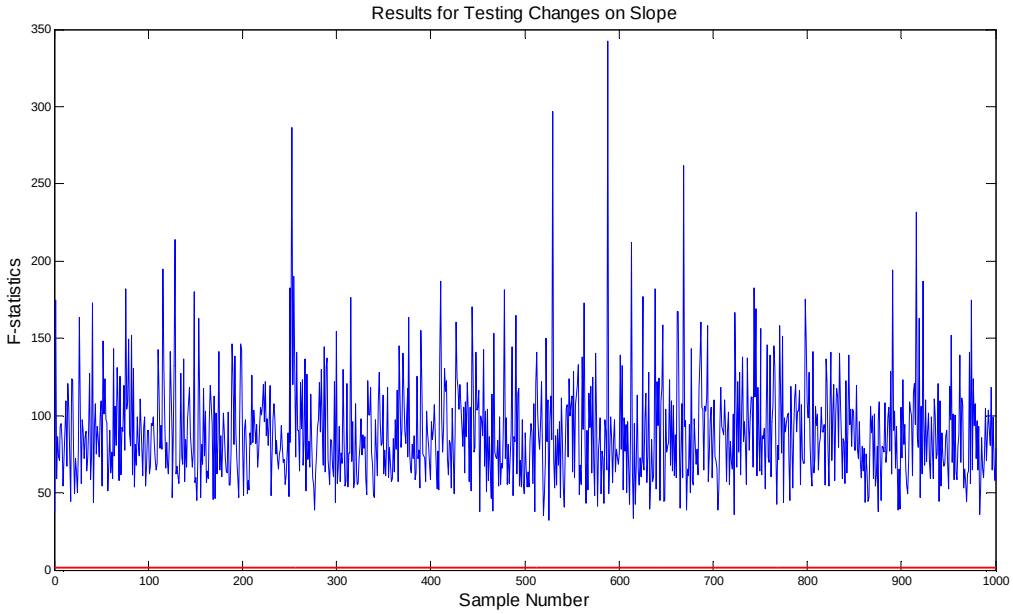

Figure 4 Analysis #1 Results for testing changes on slope for ANCOVA.
Results for testing changes on slope for ANCOVA. The continuous line shows the statistic for testing changes in slope, the red line shows the critical value of the statistic ($F_{3,20}$)=1.48).

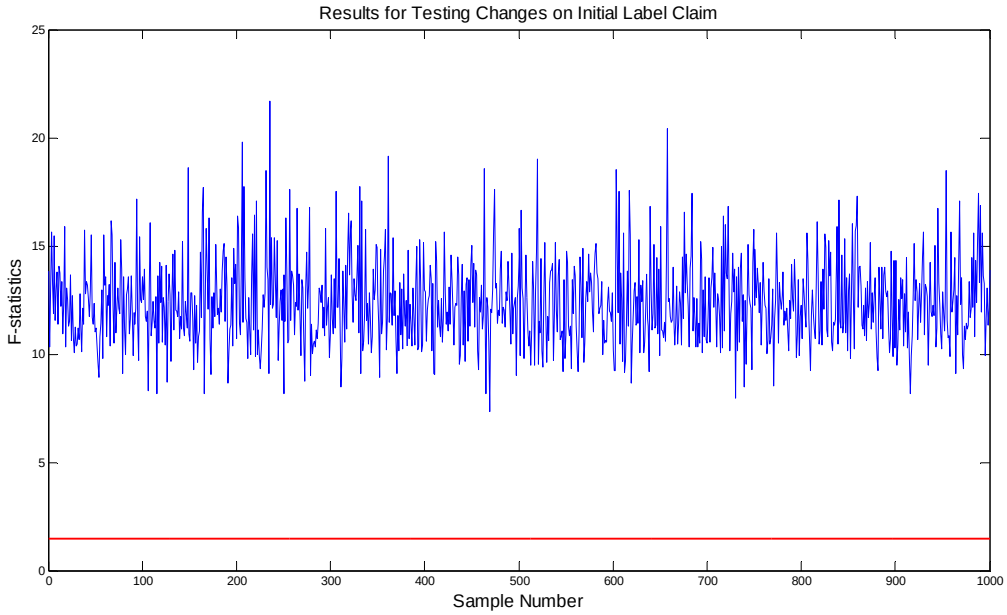

Figure 5 Analysis #1 changes on the Initial Label Claim (ILC), for ANCOVA.
Changes on the Initial Label Claim (ILC), for ANCOVA. All of the batches were incorrectly detected as with changes in the ILC. The horizontal line shows the critical value ($F_{3,23}$=1.46).

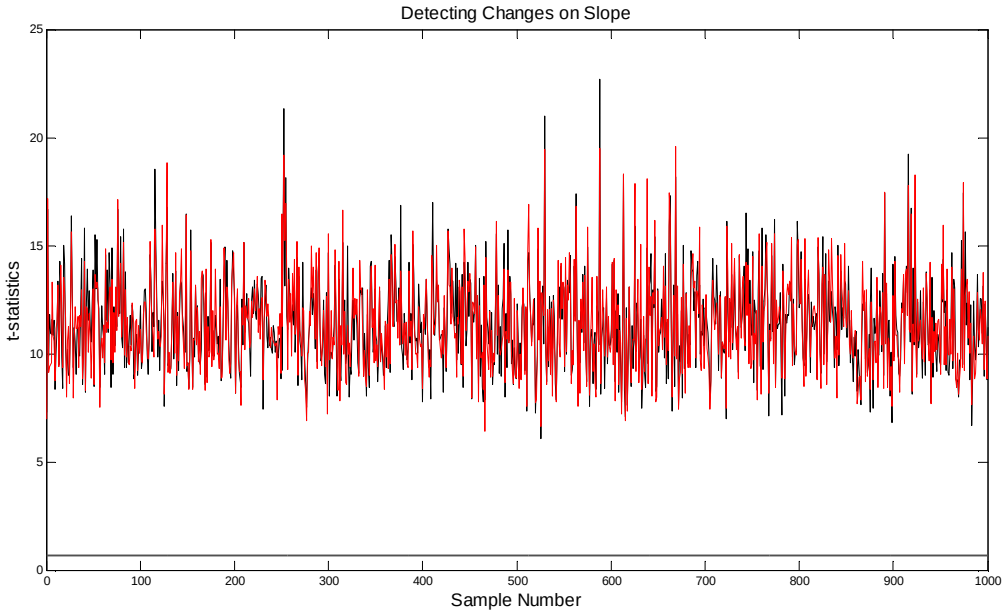

Figure 6 Analysis #1 Detecting the change on slope with indicator variables.

Detecting the change on slope with indicator variables. The continuous line shows the statistics for testing whether or not the batches have the same slope. The horizontal line represents the critical value.

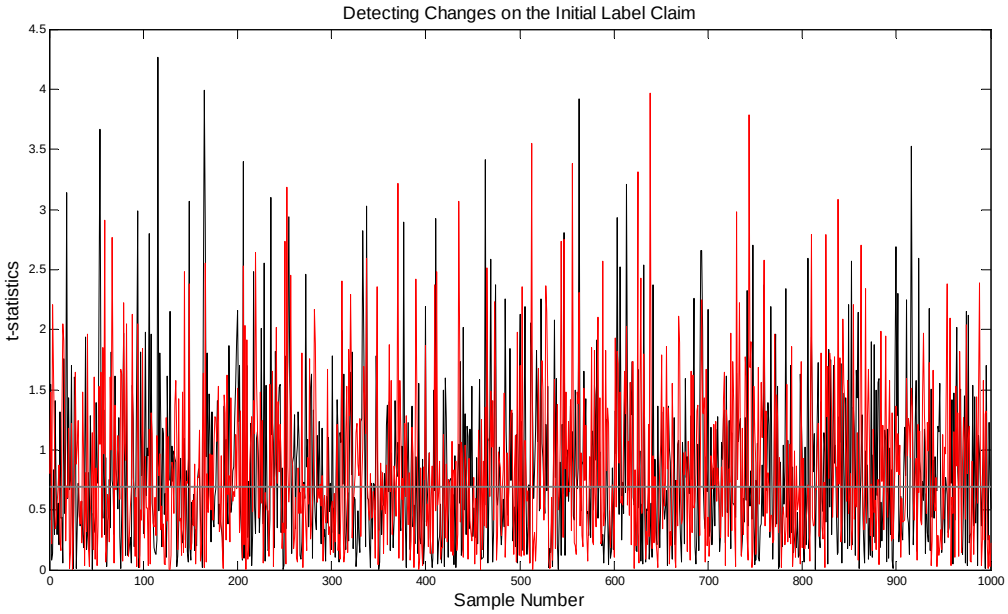

Figure 7 Analysis #1 Detecting changes on the ILC, using Indicator Variables.

Detecting changes on the ILC, the continuous line are the statistics for testing whether or not changes in the intercept were detected. The horizontal line indicates the critical value. This figure indicates that 507 batches show changes on the intercept when in reality no changes have occurred.

3.4.2 Analysis 2: Changes on the initial label claim

The second analysis takes into account changes occurring in the initial label claim and maintaining constant degradation rate. The selected initial label claims were 105%, 100% and 95%. The degradation rate in all simulated cases was selected as moderate degradation rate and equal to -0.2.

The ANCOVA method detects without any error the changes in the intercept as shown in the Figure 8. The critical value to detect changes in the intercept was 1.46. Figure 8 shows that the statistics are greater than the critical value indicating that there is a difference between the intercepts. Figure 9 shows the statistics for testing slopes, the horizontal line indicates the critical value for testing the difference on slope. It is important to show that ANCOVA detected changes and differences in the slope when in reality there were no differences in the slopes. The number of times that incorrectly indicate a change was 260.

The Indicator Variable method detects also without error that the batches have different initial label claim. However, it is important to demonstrate that the method detects changes in the slope for 501 batches when there were no actual changes. Figure 10 shows that the Indicator variable method detected the changes that occurred in the initial label claim. Figure 11 shows the statistics for testing changes on slope and how many times the method incorrectly showed changes on slopes.

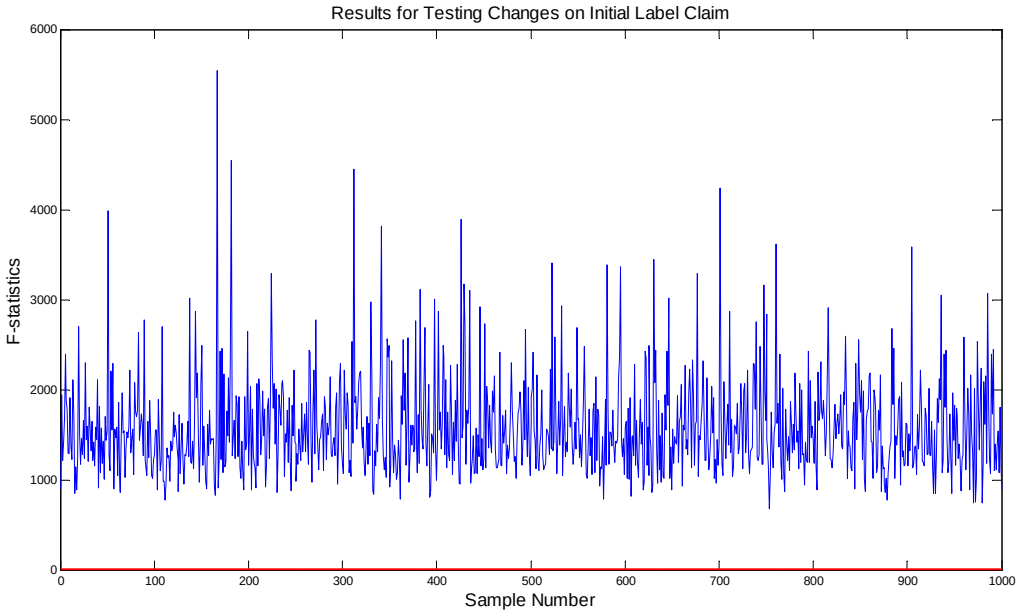

Figure 8 Analysis #2 Results for testing changes on the initial label claim with ANCOVA.
Statistic to measure whether or not there are changes on the initial label claim, the critical value is $F_{3,23}$=1.46. ANCOVA method detected without any error changes in the ILC.

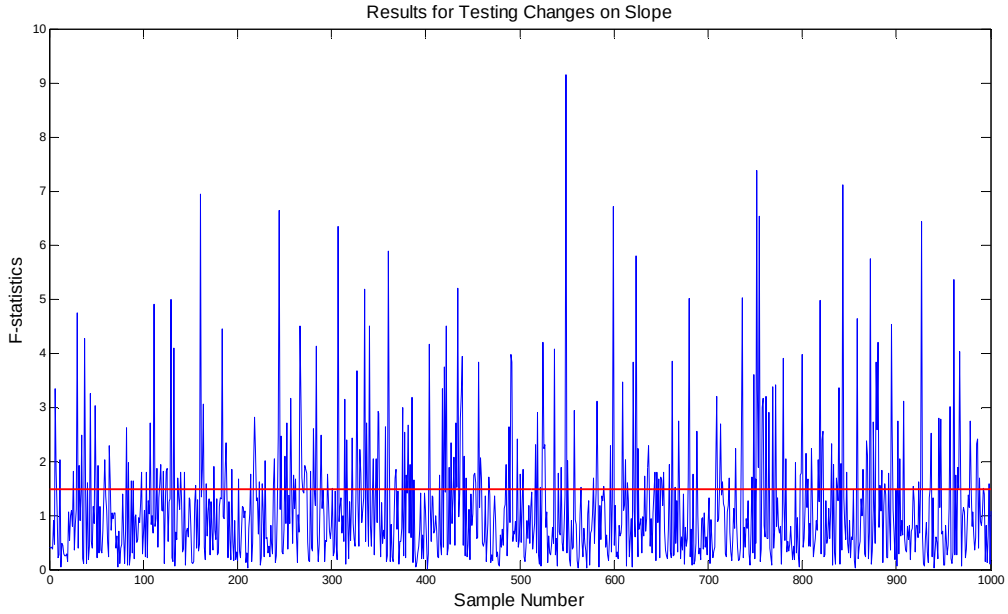

Figure 9 Analysis #2 Results for testing changes on slope with ANCOVA.
Statistic for testing changes on slope, the horizontal line shows the critical value of (F3,20=1.48). There are 260 out of 1000 samples that incorrectly indicate that there are some changes in the slope, when in reality there were no changes in the slope.

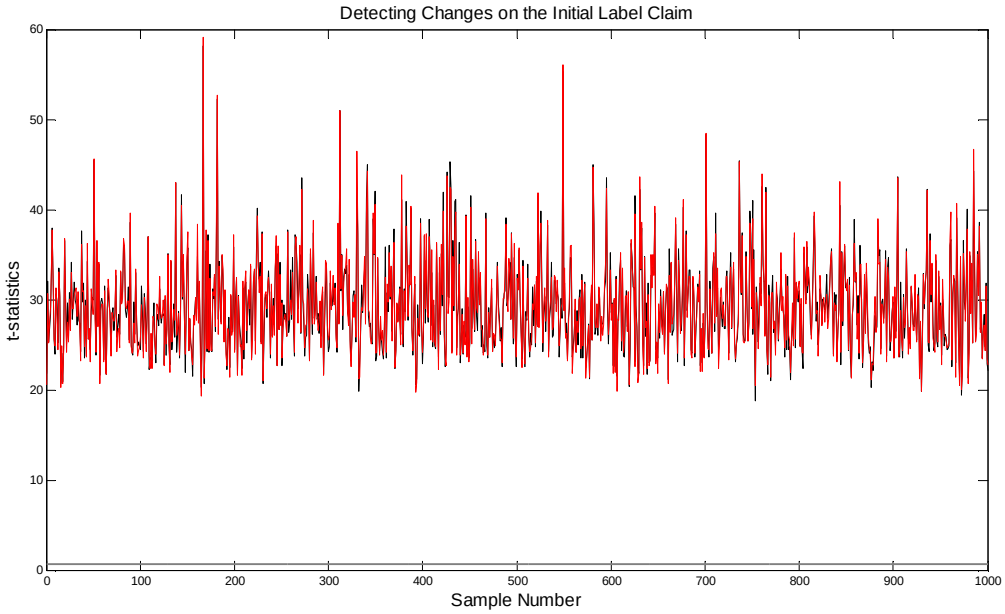

Figure 10 Analysis #2 Changes in the initial label claim with Indicator Variables.
Statistic for testing changes on the intercepts. The Indicator Variables method detected without any error changes in the initial label claim in the 1000 batches.

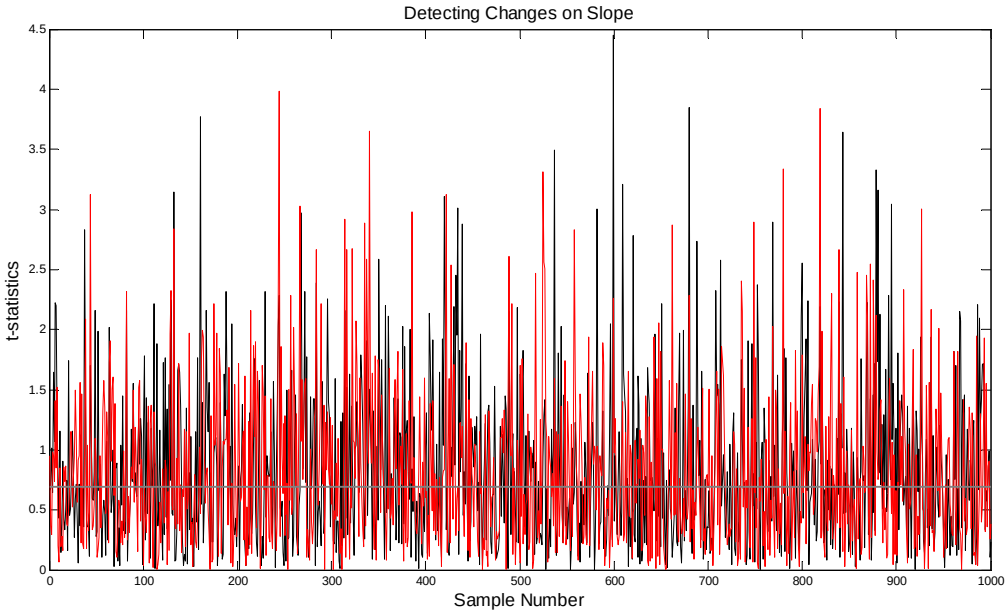

Figure 11 Analysis #2 Changes in the slopes with Indicator Variables.
Statistics for testing changes on slopes. The horizontal line shows the critical value. There are 501 out of 1000 samples that incorrectly indicate that there are some changes in the slope.

3.4.3 Analysis 3: Changes in both, the initial label claim and slope

This scenario consists of implementing changes in both the degradation rate and the initial label claim. The analyzed data have changes on slope: -0.05, -0.2 and -0.4; and also include changes in the initial label claim: 105%, 100% and 95%.

The ANCOVA method detects without any error the changes in the intercept and the slope as it is shown in Figures 12 and 13. The critical value to detect changes in the intercept was $F_{0.25,\ 3,\ 23}=1.46$ and changes in the slopes $F_{0.25,\ 3,\ 30}=1.48$. Figures 12 and 13 show that the statistics are greater than the critical value indicating that there is a difference between the intercepts and the slopes. The Indicator Variable method also detects without error that the batches have different initial label claim and changes on slopes. Figures 14 and 15 show that the statistics are greater than the critical value in both cases, intercepts and slopes. It can be concluded that both methods perform perfectly detecting the change in initial label claim and the change on slope.

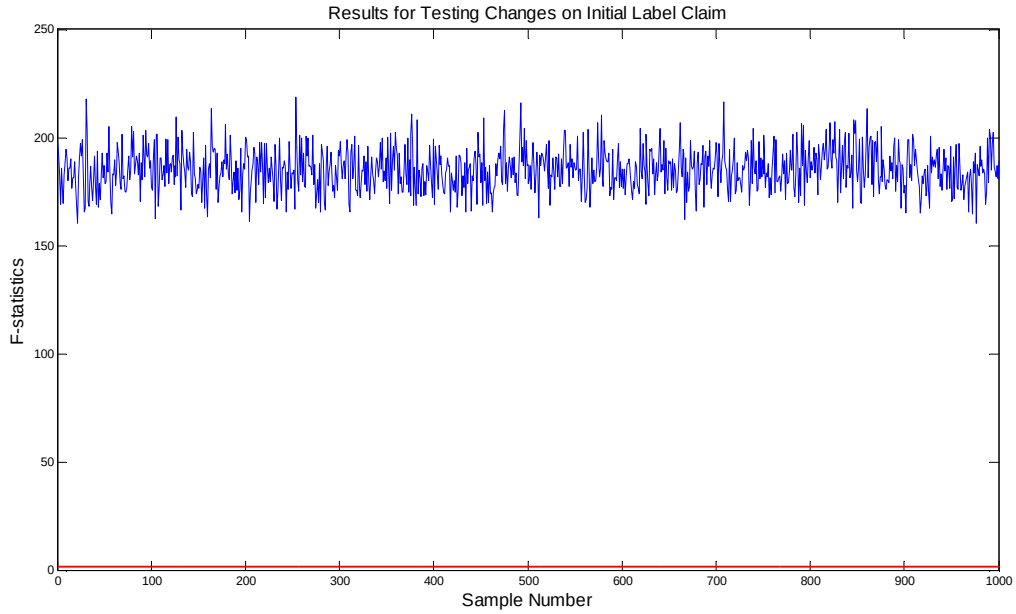

Figure 12 Analysis #3 Results for testing changes on the initial label claim with ANCOVA.
The continuous line shows the statistic for testing changes on the initial label claim, the critical value is 1.46 (F3,23) and is represented by the horizontal line. ANCOVA method detected without any error changes in the initial label claim.

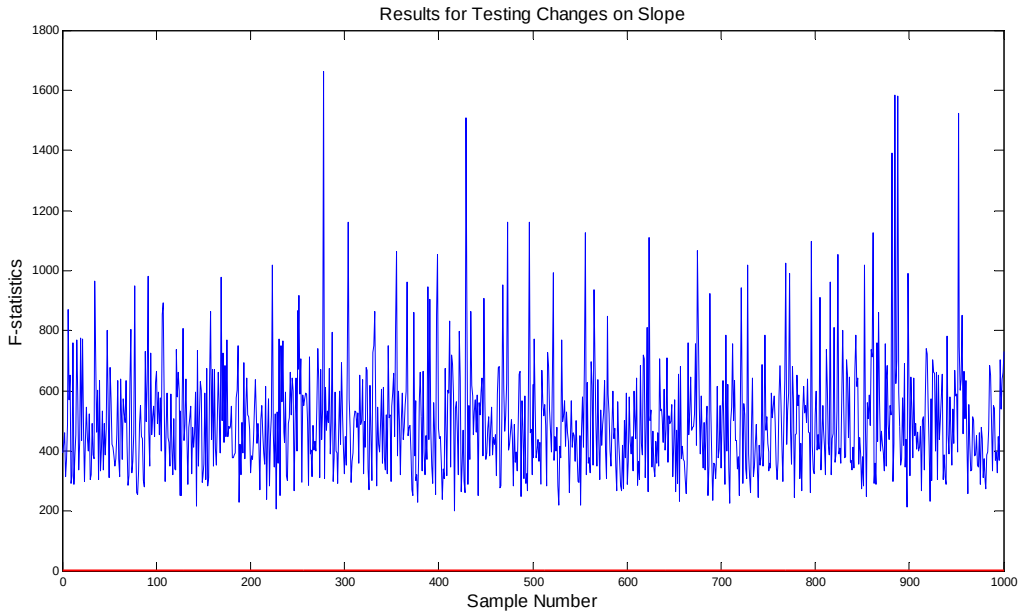

Figure 13 Analysis #3 Results for testing changes on the slopes with ANCOVA.
The continuous line shows the statistic for testing changes on slope, and the horizontal line shows the critical value of 1.48 ($F_{3,20}$). The ANCOVA method detected without any error the changes in the slope.

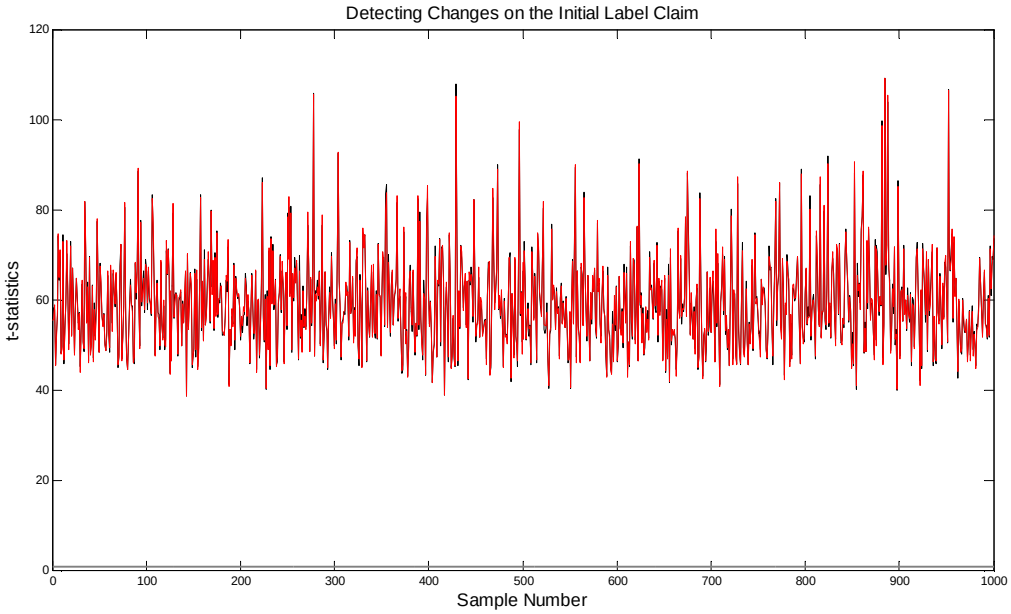

Figure 14 Analysis #3 Detecting changes in the initial label claim with Indicator Variables.
Statistic to measure whether or not there are changes on the initial label claim, the critical value is 0.69. The Indicator Variable method detected without any error changes in the ILC.

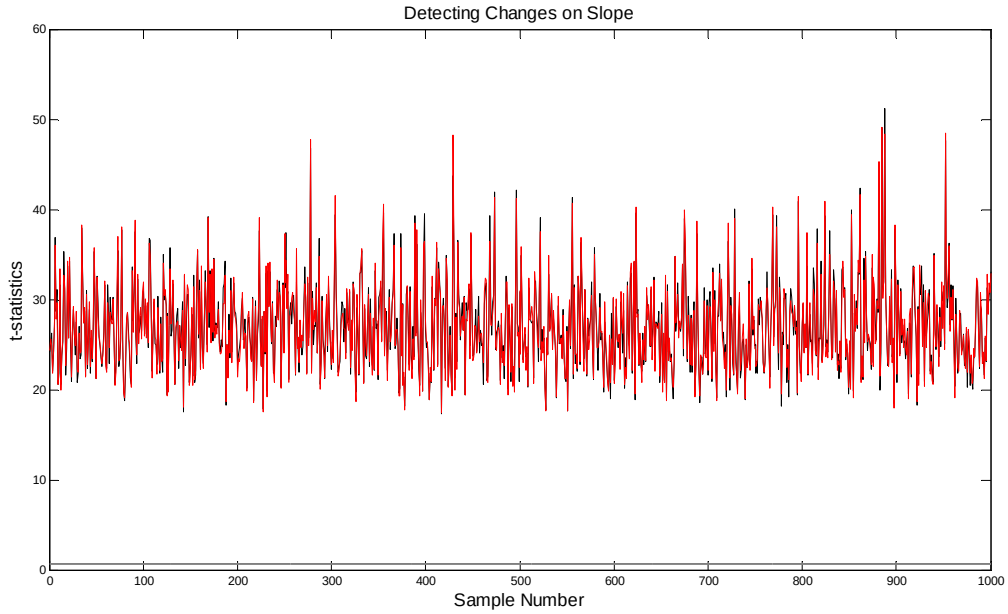

Figure 15 Analysis #3 Detecting changes on slopes with Indicator Variables.
The continuous line shows the statistic for testing changes on slope, and the horizontal broken line shows the critical value of 0.69. The Indicator Variable method detects without any error the changes in the slope.

3.4.4 Analysis 4: Batches are selected from the same population.

The last analysis consists on studying four batches from the same population and testing whether or not the methods confirm that there is no batch-to-batch variability. Thus, if there is no batch-to-batch variability the expiration date can be computed based on a single batch created by including the data from the four batches.

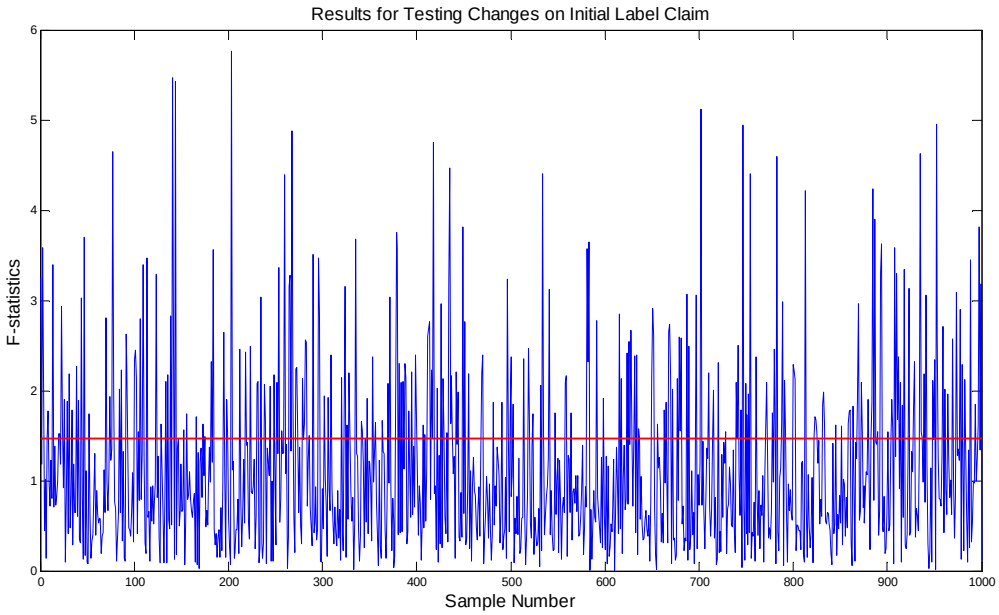

Figure 16 Analysis #4 Results for testing changes on the initial label claim with ANCOVA.
The continuous line shows the statistic for testing changes on the initial label claim, the critical value is 1.46. ANCOVA method detected incorrectly changes in the initial label claim in 276 batches.

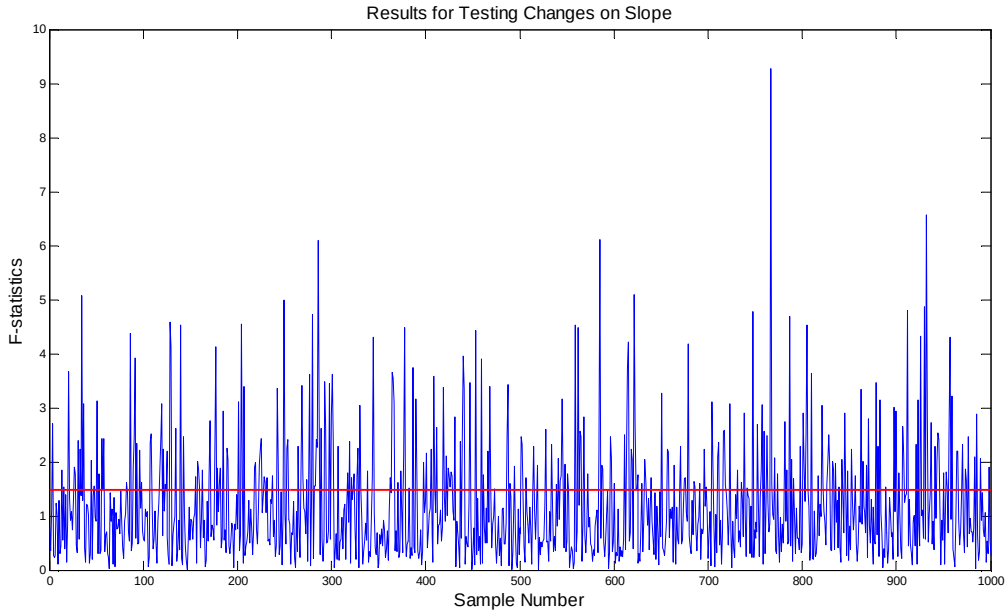

Figure 17 Analysis #4 Results for testing changes on the slope with ANCOVA.
The continuous line shows the statistic for testing changes on slope, and the horizontal line shows the critical value of 1.48. The ANCOVA method detected incorrectly changes in the slope in 294 batches.

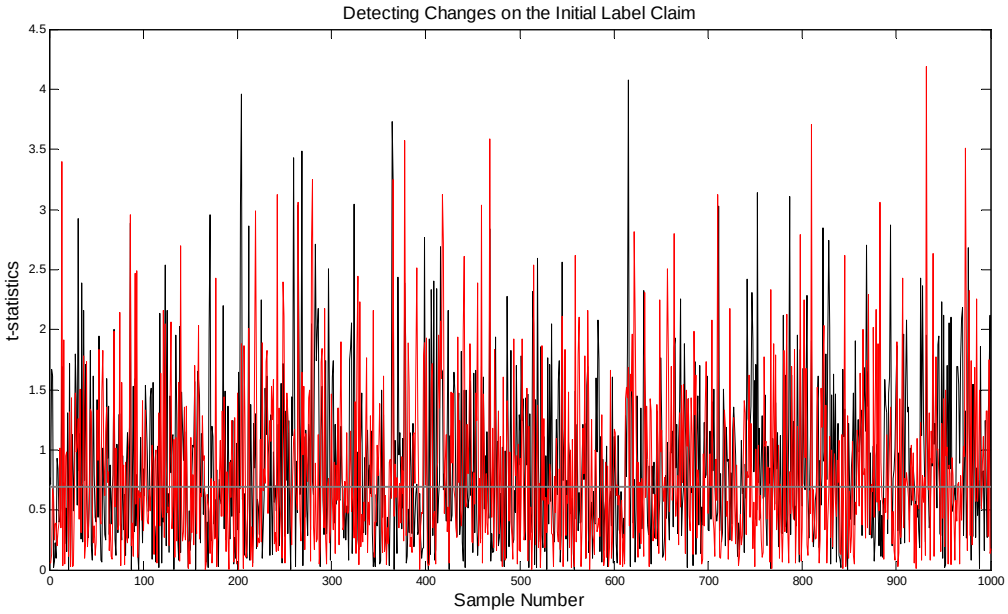

Figure 18 Analysis #4 Detecting changes in the initial label claim with Indicator Variables.
Statistic to measure whether or not there are changes on the initial label claim, the critical value is 0.69. The Indicator Variable method detected incorrectly changes in the initial label claim in 519 batches.

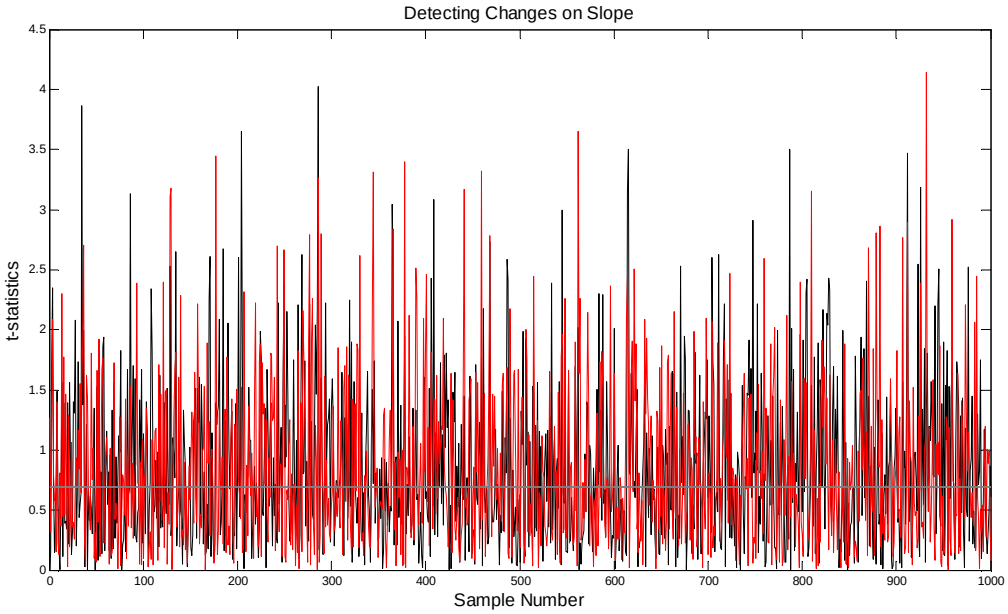

Figure 19 Analysis #4 Detecting changes on the slopes with Indicator Variables.
The continuous line shows the statistic for testing changes on slope, and the horizontal line shows the critical value of 0.69. The Indicator Variable method detects incorrectly changes in the slope in 536 batches.

Figure 16 shows the frequency ANCOVA incorrectly detected changes in the initial label claim of 276 batches. In the graph, it can be observed how some of the statistical values are higher than the critical value. This indicates a change in the initial label claim which is not correct. Figure 17 shows how ANCOVA incorrectly detected changes in slopes in 294 batches.

The Indicator Variables method also detected changes in the initial label claim. Figure 18 shows that the method incorrectly detected changes in the initial label claim of 519 batches. Figure 19 shows changes in the slopes of 536 batches. It is important to clarify or take into consideration that both methods detected changes in the slopes and intercepts when the changes never occurred.

The method of Indicator variables is simpler than ANCOVA and than much of the methods that have been proposed. It is easier to design and, to analyze. This project demonstrated the advantage of simplicity of the method but when specifically compared to ANCOVA, and ANCOVA is the one that presents the lower false alarm. It is recommended to use ANCOVA when detecting changes in slopes and intercepts.

This chapter presented the different statistical techniques used during the study, and the results of a Monte Carlo simulation created to analyze different scenarios. The purpose of the simulation was to evaluate the performance of ANCOVA and Indicator Variables. It was attempted to overcome ANCOVA using the method of Indicator Variables but the simulation results indicate that ANCOVA is superior to Indicator Variables. Indicator Variables tests the hypothesis twice therefore, the possibility of committing error type I is approximately twice using Indicator Variables in comparison with ANCOVA.

Chapter 4 introduces the accelerated testing method and how the accelerated data is used to establish a shelf life.

4 Accelerated Testing

This chapter will focus on the analysis of accelerated data and how to determine the shelf life of a drug under stress conditions. The chapter is organized as follows. First, it discusses the basis of chemical reaction kinetics as well as the reaction order, then the shelf life calculation is described, and finally the simulation results were discussed.

An accelerated testing is a short term stability study and most of the time realized under stress conditions. The main goal of an accelerated stability testing is not only to determine the chemical reaction kinetics but also to establish a tentative expiration date under the environment of marketing storage conditions. The degradation of a drug product is mainly caused by the chemical reaction of the active pharmaceutical ingredient (API) with the excipients or with the environment causing a decrease on the assay results over time (Waterman, 2005). During the entire shelf life, the API of a drug product decreases at an unknown rate.

Under the assumption that the rate of a chemical reaction is a function of temperature and concentration of the reactants present, to induce a change in the reaction rate, a change in temperature of reactants should be implemented.

The primary goal of stability functions is to determine the rates of chemical and physical reactions and their relationships with storage conditions such as temperature, moisture, light, and others. To achieve this goal, accelerated stability testing is usually conducted. Stress conditions are used to accelerate the reaction rate so that significant degradation of the drug product can be observed in a relatively short period of time. The longest possible expiration period is, of course

an economic desirability, and many of the efforts of the stability programs of pharmaceutical companies are geared towards lengthening this period.

In the pharmaceutical industry, the Arrhenius (Nelson, W. 1990) equation is employed to relate the degradation reaction rate and the corresponding temperature. FDA guidelines indicate that the results obtained from accelerated testing can be used only as supportive stability data. Results from accelerated test can be used:

1. To estimate the kinetic parameters for the rates of reactions.
2. To characterize the relationship between degradation and storage conditions.
3. To provide critical information in the design and analysis of long term stability studies under ambient conditions at the planning stage.

4.1 Chemical Reaction Kinetics

Is crucial to discuss basic chemical reaction kinetics because it helps to further understand degradation rates. A chemical reaction is the process in which one or more components are transformed into one or more products. The rate of reaction is the velocity at which the components are being transformed in a chemical reaction. For the following chemical reaction: $B + C \rightarrow D$ the reaction rate can be expressed as (Fogler, S.H. 2005):

$$\frac{dD}{dt} = -k_{n+m}\,[B]^n[C]^m \tag{35}$$

where D is the molar concentration of the component to be studied, k is the rate constant also known as specific reaction rate. The rate of reaction is positive if it refers to a product and negative if it refers to a reactant. Establishing the order is important because the establishing of expiration periods depends to some degree on extrapolating the concentration of the drug beyond the last time point of testing. The reaction order is the sum of exponents $n + m$. In the pharmaceutical industry, the first order reaction is probably the most commonly employed model for describing the decomposition and the degradation of active ingredients of a drug product. The zero order reaction is used occasionally, and the second order reaction is rarely adopted.

4.1.1 The Zero Order Reaction

A zero order reaction has a rate that is independent of the concentration of the reactants. Increasing the concentration of the reactants will not speed up the rate of the reaction. It describes a linear relationship between the drug characteristics and time. According to this, (Fogler, S.H., 2005):

$$\frac{dD}{dt} = -k_0 \tag{36}$$

where $D(t)$ is the concentration at time t and the solution of the differential equations is

$$D = D_0 - k_0 t \tag{37}$$

where D is the molar concentration at any time t, D_0 is the concentration at time zero, k_0 is the rate constant of the zeroth order reaction, and t is the time. It is critical to notice that the temperature has a strong effect on the rate constant. This effect is represented by the Arrhenius equation, since the Arrhenius equation is a physics-based model derived for temperature dependence, it is strongly recommended that the model be used for temperature accelerated test.

The Arrhenius equation (38) gives the dependence of the rate constant (*k*) of chemical reactions on the temperature (*T*) and activation energy *(E*).

$$k = Ae^{-E/RT} \tag{38}$$

where *A* is the frequency factor and has the same units of *k*, *E* is the activation energy (the energy that the molecule must have to participate in the reaction), *R* is the gas constant and T is the absolute temperature in Kelvin (K). The logarithmic expression of the Arrhenius equation is:

$$\ln k = -\frac{E}{RT} + \ln A \tag{39}$$

So, when a reaction has a rate constant which obeys the Arrhenius equation, a plot of ln(*k*) versus T^{-1} gives a straight line, whose slope and intercept can be used to determine *E* and *A*. This type of plot is called an Arrhenius plot that displays the logarithm of kinetic constants plotted against the inverse of the temperature. The Arrhenius plots are often used to analyze the effect of temperature on the rates of chemical reactions. Figure 20, presents an example of the Arrhenius plot.

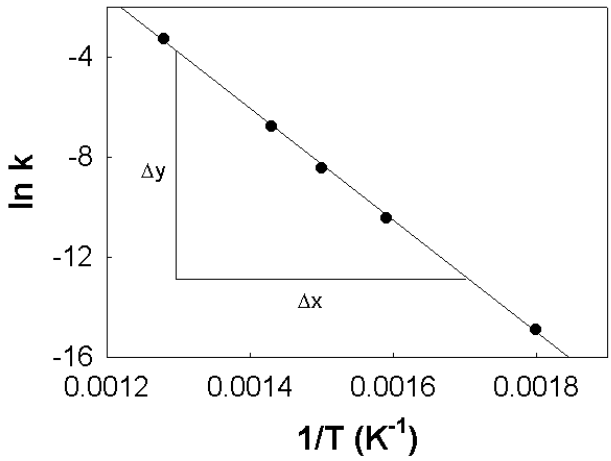

Figure 20 Arrhenius Plot

4.1.1.1 Zero Order Reaction Sample Calculations

Table 11 shows an example of the data for a zero order of reaction. The data is not real and will only be used for demonstration of the sample calculations.

Table 11 Data at normal conditions

t (Months)	Assay 25°C
0	99.6
3	98.2
6	96.9
9	95.7
12	94.4
18	93.7
24	92.2
36	91.5

Table 12 Accelerated Stability Data Zero Order

t (Months)	Assay 30°C	Assay 40°C	Assay 50°C
0	99	99.0	99
2	98	97.1	94.7
4	96.8	95.9	90.3
6	95.5	94.2	87.5
k_o	**-0.585**	**-0.78**	**-1.945**
$\ln(k_o)$	**-0.5361**	**-0.2485**	**0.6653**
1/T	**0.0033**	**0.0032**	**0.0031**

Table 12 presents data at normal, intermediate and, accelerated conditions. All assays are expressed as a percentage of the label claim (%LC) and at 25°C. This data helps in the estimation of the assay values at 25°C (normal conditions) as a function of time.

For a zero order reaction, the original concentrations can be graphed as a function of time. Figure 21 shows the graph for each one of the concentrations. The slope of each one of the lines corresponds to the value of *k* at that specific temperature. The next step, is a plot of *ln(k)* vs. T^{-1}. This plot (Figure 22) is prepared with the values of *k* obtained from each one of the accelerated storage conditions, determine from Figure 21.

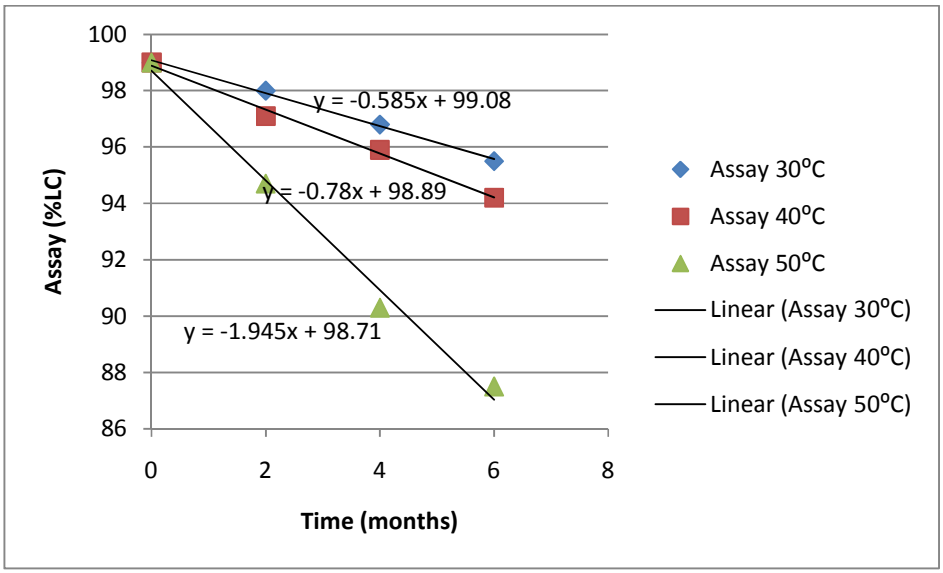

Figure 21 Degradation Assay Data treated as Zero Order of Reaction

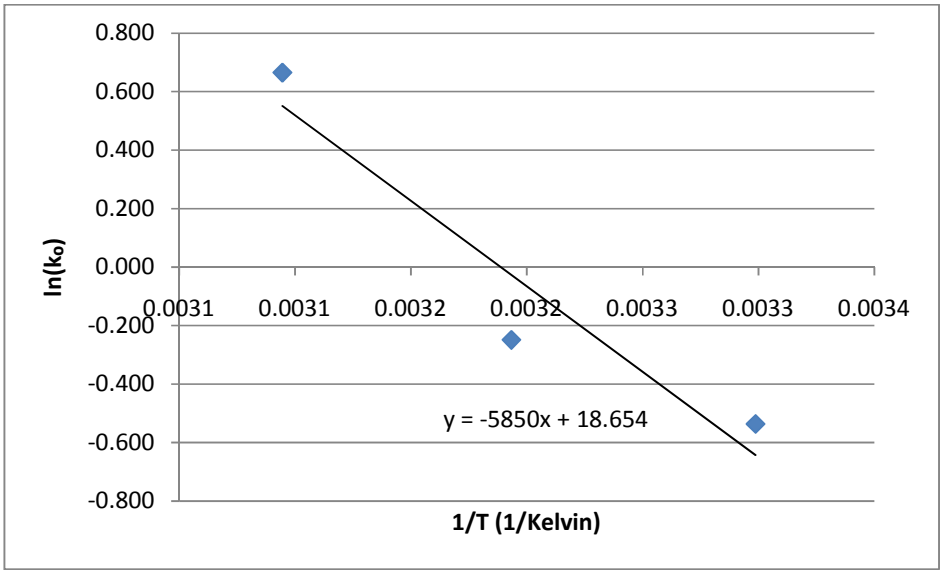

Figure 22 Arrhenius Plot for Zero Order Degradation

Using the Arrhenius equation to predict the drug assay as a function of time, the values of the slope and intercept can be obtained using Figure 21. The rate constant or specific reaction rate (*k)* can be calculated using the data obtained from Figure 22. It shows the Arrhenius plot, the x-axis is the inverse of the temperatures analyzed and the y-axis the natural logarithm of the specific reaction rate.

$$\ln(k_{25}) = \frac{-E}{RT} + \ln A$$

$$\ln(k_{25}) = -5{,}8509\ (\frac{1}{298.15}) + 18.65$$

$$k_{25} = 0.3802\ month^{-1}$$

Once the specific reaction rate (*k*) at 25°C or 298.15 K is calculated, the drug product assay can be predicted using:

$$D = D_0 - k_0 t$$

For example, the predicted concentration obtained at 18 months can be calculated as follows:

$$D(18) = 100 - 0.3802\left(\frac{1}{months}\right)(18\,months)$$

$$D(18) = 93.16$$

Table 13 Predicted Assay for Zero Order of Reaction

Time (months)	Assay	Predicted Zero Order
0	99.6	100.00
3	98.2	98.86
6	96.9	97.72
9	95.7	96.58
12	94.4	95.44
18	93.7	93.16
24	92.2	90.87
36	91.5	86.31

4.1.2 First Order Reaction

The first order reaction equation indicates that a logarithmic transformation of the drug characteristic is a linear function of time.

For the first order reaction, the rate can be expressed as:

$$\frac{dD}{dt} = -k_1 D \tag{40}$$

After solving the differential equation the result is as follows:

$$D = D_0 - D_0 e^{-k_1 t} \tag{41}$$

.

Once, the order of reaction is determined empirically, the relationship between the rate of degradation and the temperature can be characterized using the Arrhenius equation. The Arrhenius equation describes the linear relationship between log (degradation) and the reciprocal of the absolute temperature. A prediction of the expiration date can be then obtained after applying the statistical techniques presented in the previous chapters, as illustrated in section 4.1.4.

4.1.2.1 First Order Reaction Sample Calculations

The data used for these calculations was the same used for the zero order of reaction example. The objective of using the same set of data was to compare the predicted assay values obtained with each one of the orders and to compare and decide which order of reaction performs better.

Table 14 Degradation data treated as First Order Kinetics

t (Months)	Assay 30^0C	Assay 40^0C	Assay 50^0C	ln (Assay 30^0C)	ln (Assay 40^0C)	ln (Assay 50^0C)
0	99	99.0	99	4.595	4.595	4.595
2	98	97.1	94.7	4.585	4.576	4.551
4	96.8	95.9	90.3	4.573	4.563	4.503
6	95.5	94.2	87.5	4.559	4.545	4.472

The analysis was developed assuming that the reaction is of first order (usually the case in real life). The first step is to obtain the natural logarithm of the concentration; these values are presented in Table 14. First order of reaction indicates that a logarithmic transformation of the drug characteristic is a linear function of time, and these values are graphed as a function of time and presented in Figure 23.

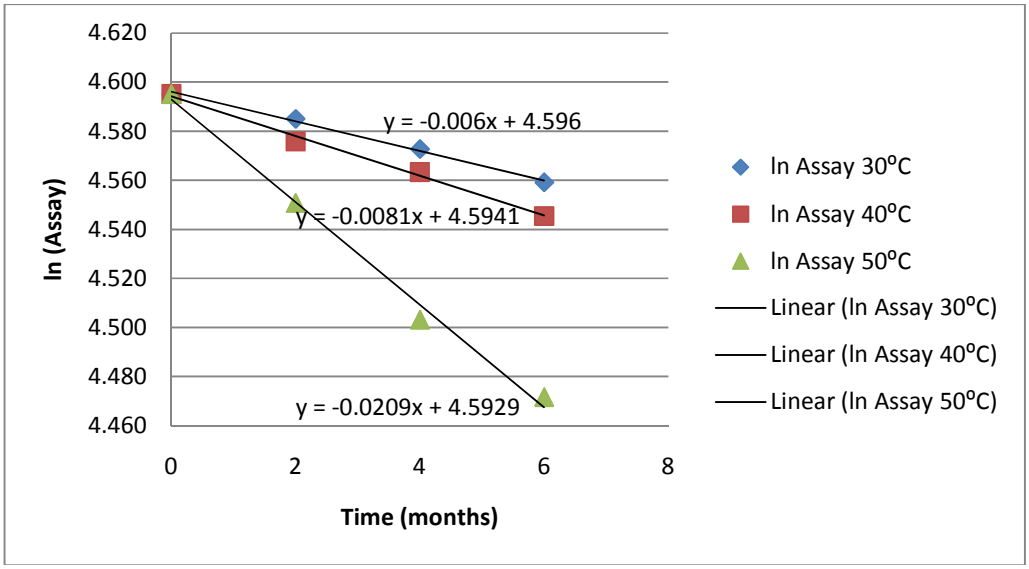

Figure 23 Degradation Data treated as First Order Kinetics

The slope of each line corresponds to the value of the rate constant (*k*), for each one of the temperatures. The next step is to plot *ln (k) vs.* (T^{-1}), using the specific rate values obtained for each of the analyzed temperatures.

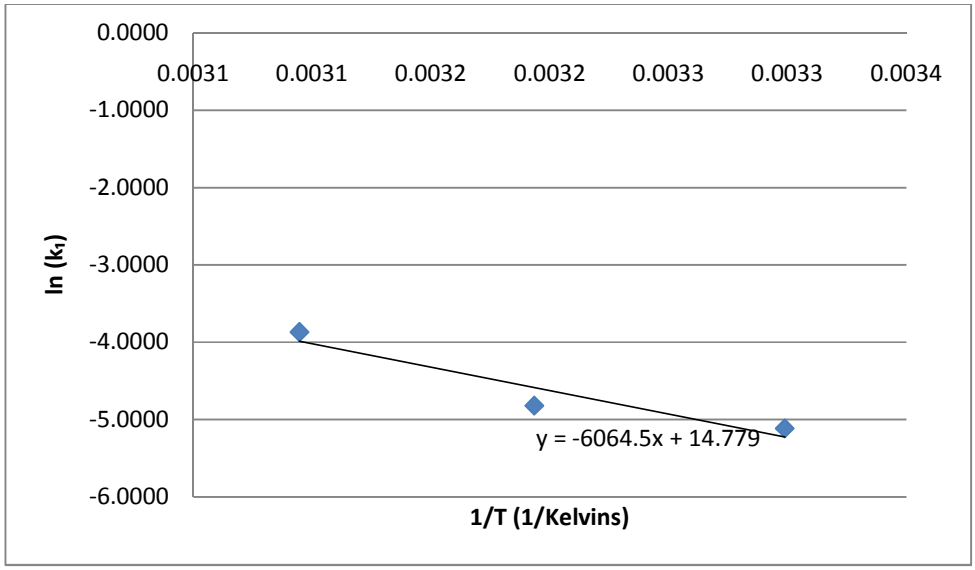

Figure 24 Arrhenius Plot for the First Order Degradation

Using the Arrhenius equation to predict the drug assay as a function of time, the values of the slope and intercept can be obtained using Figure 23. Figure 24 shows the Arrhenius plot for the first order degradation, the rate constant or specific reaction rate (*k)* can be calculated using the data obtained from this graph.

$$\ln(k_{25}) = \frac{-E}{RT} + \ln A$$

$$\ln(k_{25}) = -6{,}064.5(\frac{1}{298.15}) + 14.779$$

$$k_{25} = 0.00384\ month^{-1}$$

Once the specific reaction rate (*k*) at 25°C is calculated, the drug product assay for first order reaction can be predicted using Equation 41.

For example, the predicted concentration obtained at 9 months can be calculated as follows:

$$D(9) = 100 - 100(e^{(-0.00384\ (9))})$$

$$D(18) = 96.599$$

The results for the rest of the predicted assay for first order reaction are presented in Table 15.

Table 15 Predicted Assay for the First Order Reaction Kinetics

t (Months)	Assay 25°C	Predicted 1st order
0	99.6	100
3	98.2	98.853
6	96.9	97.720
9	95.7	96.599
12	94.4	95.492
18	93.7	93.315
24	92.2	91.187
36	91.5	87.076

4.1.3 Comparison of the Zero and the First Order Models

To compare the predictions of both models zero and first order of reaction, Table 16 was prepared. As it was expected, both models provide quite similar results. The first order model gives better results, suggesting that the degradation is actually of first order as it was pointed out before.

Table 16 Predicted assay values using Arrhenius for zero and first order kinetics

t (Months)	Assay 25°C	Predicted Zero Order	Predicted First Order	$Error_o$	$Error_1$
0	99.6	100	100	0.4	0.4
3	98.2	98.86	98.853	0.66	0.653
6	96.9	97.72	97.72	0.82	0.82
9	95.7	96.58	96.599	0.88	0.899
12	94.4	95.44	95.492	1.04	1.092
18	93.7	93.16	93.315	-0.54	-0.385
24	92.2	90.87	91.187	-1.33	-1.013
36	91.5	86.31	87.076	-5.19	-4.424
			SSE	**31.38**	**23.22**

4.1.4 Shelf-Life Calculation for accelerated data

The shelf life can be calculated using the accelerate results. The described regression techniques can also be applied to the predicted assays obtained from accelerate data. The procedure used for the long term stability analysis is also applied to analyze results from accelerate stability analysis, the only change is that this time the predicted assays are included in the calculation.

The quadratic equation to determine the shelf life for the predicted assay values using the zero order reaction kinetics presented in Table 16 and implementing Equation (11) is:

$$[90 - (100 - 0.380x)]^2 - 1.94^2\ (6.99\ \times 10^{-6})\left[\frac{1}{8} + \frac{(x - 13.5)}{1008}\right] = 0$$

The reference point for this equation is $x_{ref} = 26.32$ and the initial point to accomplish convergence is $x_{ref} - 5$. The root for this equation is $x_{root} = 26.30$ and the observed shelf life for the predicted assay values using the zero order of reaction is $x_{shelf}(obs) = 26\ months$.

The quadratic equation for the predicted assay presented in Table 16 and assuming first order reaction kinetics is:

$$[90 - (99.881 - 0.359x)]^2 - 1.94^2\ (0.0094)\left[\frac{1}{8} + \frac{(x - 13.5)}{1008}\right] = 0$$

The reference point for this equation is $x_{ref} = 27.52$ and the initial point to accomplish convergence is $x_{ref} - 3$. The root for this equation is $x_{root} = 27.50$ and the observed shelf life for the predicted assay values for the first order of reaction is $x_{shelf}(obs) = 27\ months$.

The quadratic equation for the assay data at 25°C (Table 16) is:

$$[90 - (98.304 - 0.2244x)]^2 - 1.94^2\ (1.046)\left[\frac{1}{8} + \frac{(x - 13.5)}{1008}\right] = 0$$

The reference point for this equation is $x_{ref} = 37.00$ and the initial point to accomplish convergence is $x_{ref} - 8$. The root for this equation is $x_{root} = 31.17$ and the observed shelf life for the assay data at 25°C is $x_{shelf}(obs) = 31\ months$

In summary, the shelf life from the actual data is: 31 months, the shelf life for the predicted assay using a zero order reaction is 26 months and, the shelf life for a first order reaction is 27 months. Thus, it is again confirmed that in this case the first order reaction analysis is better than the zero order reaction analysis.

4.1.5 Analysis of Simulation Results for Accelerated Testing

One thousand sets of accelerated stability data from 3 batches were generated using Monte Carlo simulation. Data at 25°C was simulated for 0, 3, 6, 9, 12, 18, and 24 months. Assay values were simulated at 5°C and 30°C and at 0, 3, 6 and 9 months, and data at 40°C were also simulated at 0, 3 and 6 months. The accelerated data were analyzed to make predictions of the assay data at 25°C with zero order of reaction and first order of reaction. Table 17 presents an example of the accelerated simulated data, which were used to predict the concentration at 25°C.

Table 17 Example of the accelerated simulated data used to predict the concentration at 25^{0}C

5°C	**0**	**3**	**6**	**9**
	100	99	96	91
	100	97	94	91
	100	99	95	91
30°C	**0**	**3**	**6**	**9**
	100	94	90	84
	100	93	88	84
	100	93	88	83
40°C	**0**	**3**	**6**	
	100	86	82	
	100	84	81	
	100	84	81	

Table 18 presents an extracted example of the predicted data obtained after using the Arrhenius equation to predict the drug concentration at 25°C. It is possible to see from Table 18 that the slopes of the batches are different whereas the intercepts are the same. The intercepts were expected to be the same because an initial concentration of 100 was established in the analyzed data.

Table 18 Predicted assay for 25°C

	Batch 1	Batch 2	Batch 3
0	100.000	100.000	100.000
3	98.514	98.479	98.393
6	97.050	96.981	96.812
9	95.608	95.505	95.256
12	94.188	94.052	93.725
18	91.410	91.212	90.737
24	88.913	88.458	87.285
Slope	**-0.464**	**-0.481**	**-0.524**
Intercept	**99.868**	**99.901**	**99.993**

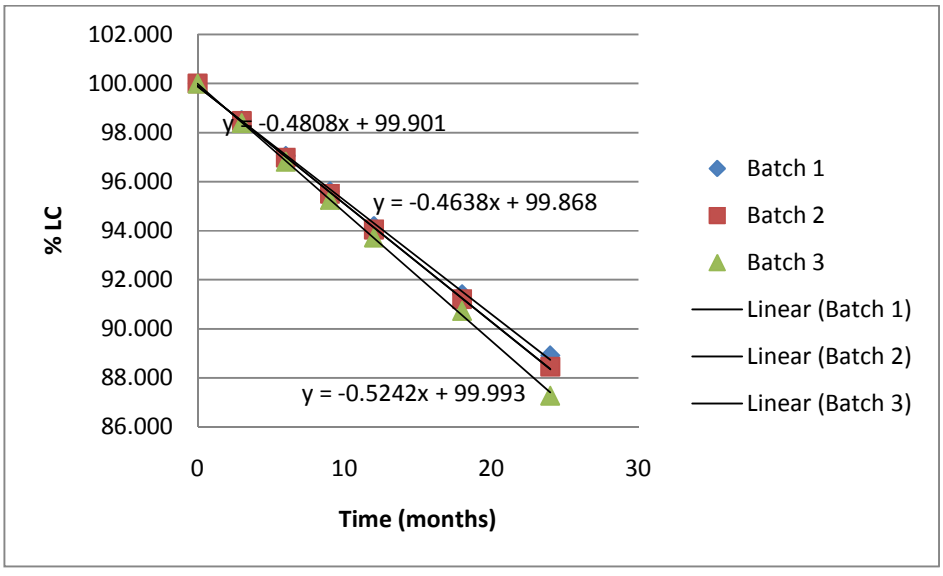

Figure 25 Graph of the predicted assay values from Table 18.

Table 18 and Figure 25 show only a small example of the results obtained from the simulation. At the end, all of the batches analyzed presented different slopes. The two statistical methods presented in previous chapters to detect variability between batches were applied to the simulated data. All the batches were rejected due to the variability in the existing slopes. Table 19, shows the results for the indicator variables method using the data presented in Table 18. The method concludes that the slopes of the batches are different thus, are significant for the analysis, but the intercepts are not significant.

Table 19 Indicator Variables simulation results for accelerated data

Predictor	Coef	SE Coef	T	P
Constant	99.9933	0.0660	1514.93	0.000
B1	-0.524235	0.005105	-102.68	0.000
δ1	-0.12532	0.09335	-1.34	0.199
δ2	-0.09234	0.09335	-0.99	0.338
Δ1_1	0.060448	0.007220	8.37	0.000
Δ2_1	0.043408	0.007220	6.01	0.000

S = 0.105799 R-Sq = 99.9% R-Sq(adj) = 99.9%

Table 20 presents the results for the same predicted data, but analyzed with ANCOVA. The obtained results were not expected, since it was observed a difference in slopes and intercepts. The ANCOVA method shows that the simulated batches belong to different populations since they have different slopes and intercepts. Table 20 shows that both statistics are larger than the corresponding critical values indicating that the hypothesis for equal slopes and equal intercepts were rejected.

Table 20 ANCOVA simulation results for the accelerated data

$\mathbf{F_{(slope)}}$	37.27
$\mathbf{F_{(0.25,2,15)}}$	1.52
$\mathbf{F_{(intercept)}}$	7.76
$\mathbf{F_{(0.25,2,17)}}$	1.51

Both methods conclude that all the batches analyzed during the simulation had large variability. It is possible to conclude that the slopes of the accelerated data look graphically similar at first sight, but the slopes of the batches are statistically different from each other, as was shown in Figure 25 and Tables 19 and 20. The prediction exercise based on the Arrhenius theory is not useful with this type of simulation because it produces predictions with different slopes, although the slopes have been controlled to have a small variation.

Analyzing and comparing the results of the two methods we can observe how sensitive the indicator variables method is as it was able to detect differences in the slopes and the similarity of the intercepts. ANCOVA in contrast determines variability of the intercepts, estimating that the intercepts obtained in the predictions are different when in reality they are not.

The Arrhenius theory is essential to design experiments that lead to a provisional estimate of the expiration date of a drug product. Increasing or decreasing the temperatures is the most common acceleration factor used because its relationship with the drug degradation is characterized by the Arrhenius equation. Accelerated stability studies can be used to support tentative expiration dates in the event that full shelf life studies are not available.

5 CONCLUSIONS AND FUTURE WORK

5.1 Conclusions

This chapter presents conclusions, recommendations, and future work. The first section summarizes the important points of this study and specially the application of regression techniques to analyze stability data.

The aim of this research has been to explore two different techniques to analyze batch-to-batch variability. The first technique used was the analysis of covariance which is currently used by the FDA to determine whether the studied batches came from the same population. The second technique used was the indicator variables method that was developed by Ramirez et al. (2008) to analyze the similarities between slopes and intercepts of batches under study and to determine whether or not the underlying batches come from the same population.

This research study has contributed in several ways. Principally is an actual implementation of the indicator variables method and a complete analysis using real and simulated stability data. Specific contributions to the performance measurement field have been accomplished as follows:

1. We have analyzed two different techniques to test batch-to-batch variability with simulated data.
2. A study of accelerated data was also implemented.

Four scenarios were simulated to study batch-to-batch variability. The first analysis includes changes on the degradation rate, the second analysis considers changes on the initial label claim, the third analysis includes changes in both the initial label claim and in the degradation rate, and the fourth analysis consists of determining whether or not the selected batches from the same population confirm that the batches belong to the same population. Three populations were simulated representing the drug degradation according to the described simulated scenarios. The explored degradation rates were: slow, moderate, and fast degradation rates.

In the first analysis a change in degradation rates was implemented, both methods detected with no errors the changes on the degradation rate of the batches. However, ANCOVA showed incorrectly that the initial label claim was different in all the samples analyzed when in reality there were no changes in the initial label claim of the batches. Indicator Variables on the other hand, detected with no errors the changes on the degradation rate of the batches, but showed incorrectly that the initial label claim was different in some batches. In the second analysis changes in the initial label claim were implemented. Both ANCOVA and indicator variables achieved similar results. The changes in the initial label claim were detected in all of the samples, however, both methods also detected changes in the degradation rate, when in reality there were no changes. The third analysis explored changes in the initial label claim and in the degradation rate of the samples. Both methods detected without any error changes in slopes and intercepts. The fourth and final analysis consists of extracting data from the same population to test for batch-to-batch variability. Therefore, if there is no batch-to-batch variability the expiration date can be calculated based on a single batch created by including the

data from the four batches. The ANCOVA, outperformed the Indicator Variable method in this analysis. Indicator Variable detected incorrectly changes in the initial label claim in 51.9% of the samples and changes in the degradation rate in 53.6%, whereas the ANCOVA method detected incorrectly changes in the initial label claim in 27.6% of the samples and changes in the degradation rate in 29.4%. It was clearly demonstrated that both methods perform similarly during the different analysis, but ANCOVA performed better when data come from the same population.

Results from accelerated data shows that the slopes look similar to the naked eye (graphically), but the slopes of the batches are statistically different from each other. The simulation exercise based on the Arrhenius theory was not useful, because it produces predicted data with different slopes, even if the slopes of the accelerated data were controlled to have small variation. It is important to remark that when analyzing the sensitivity of both methods, the indicator variables method detects a difference in slopes, but did detect differences in intercepts. ANCOVA however, detected variability in both slopes and intercepts. In this case, the indicator variable method surpasses ANCOVA when sensing variability because there were no changes in the intercepts, however ANCOVA did detect an erroneous change in the intercepts.

5.2 Recommendations and Future Work

The current research can be extended to improve the data generation when working with accelerated testing analysis. Other statistical methods such as the method proposed by Chow and Shao (1989) to test batch to batch variation and the method proposed by Chen (1997) to take into account different batches and packages can be explored in future projects.

This project revealed significant information about the two methods analyzed and how they detected changes in slopes and intercepts under different cases. This project introduces for the first time the indicator variable method and describes how it can be applied. Other types of cases and factors may be tested (i.e. packages, strengths), such as data that contain different cases presented every day in pharmaceutical industries. Further studies may focus on the effects of the accelerated data in ANCOVA and indicator variable method.

Overall, this project demonstrates an application and comparison of the indicator variables method versus ANCOVA. Both methods classified the data quite similar giving statistically valid results, it is important to establish that the simulation exercise results presented are meaningful because the data used follows the data obtained in real life scenarios when analyzing drug stability in pharmaceutical industries.

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APPENDIX A.

This appendix contains the computer programs used for this project. Degradation Line Single Batch specifically computes the degradation line for a single batch and save the results to be input into the Shelf Life Estimation program.

A.1 Degradation Line Single Batch

```
%Regression Analysis for a Single Batch
clear all;
clc;
close all;

batch=1;
i=1;
n1(1)=8;    %Batches to be analyzed
N=n1(i);
x=[0 3 6 9 12 18 24 36];      %Dates in which the LC are analyzed
y=[99.6 98.2 96.9 95.7 94.4 93.7 92.2 91.5];    %Label Claim
x1=[x(i,1:n1(i))];
y1=[y(i,1:n1(i))];
x1=x1';
X=[ones(N,1) x1];
Y=y1';
b=inv(X'*X)*X'*Y; % Regression parameters
y_est=X*b;
e=Y-y_est;  % Residuals
SSE=e'*e;  % Sum of squared errors
SST=Y'*Y-N*(mean(Y)^2); % Total sum of squares
SSR=SST-SSE;      % Sum of squared errors due to regression
t=tinv(0.95,N-2);  %T-test
R2=SSR/SST;
MSE=SSE/(N-2);    %Mean Squared Errors
av_x=mean(x1);
Sxx=x1'*x1-N*(av_x)^2;
vec=[b' N MSE av_x Sxx];
save res vec      %Files saved to determine the shelf life
```

Shelf Life Estimation estimates the shelf life for data that have been input after the calculation of a degradation line program.

A.2 Shelf Life Estimation

```
% Shelf Life Estimation
% This program computes the shelf life for data that have been input in
% Degradation Line Single batch & Analysis for slopes and intercepts

clear all;
clc;
close all;
delete it*
display ('1:f=(90-(b0-b1*x))^2-t^2*MSE*[1/n+(x-av_x)^2/Sxx]');
flag_fun=input('Enter the number of the function:   ');
load res
b=vec(1:2); % Data from the files saved
n=vec(3);
MSE=vec(4);
av_x=vec(5);
Sxx=vec(6);
d=-5.5;
reference=(b(1)-90)/(-b(2));
if flag_fun==1;
    x0=(b(1)-90)/(-b(2))+d;         %Initial Point
end
options=optimset('LargeScale','off');
cont=0;
save contador cont
[x, fval, exitflag, output]=fminunc(@(x)
obj_fun_expiration_date(x,flag_fun),x0,options);
% x0 Initial point that the computer program requires (x0=reference-d)
% d is a negative value that should be explored by using trial and error
% x is the shelf life estimated for all the future batches
```

Routine needed for shelf life estimation

```
%Function needed by estimation of shelf life

function f=obj_fun_expiration_date(x,flag_fun)

load contador;
cont=cont+1;
save contador cont;
load res;
b=vec(1:2);
n=vec(3);
MSE=vec(4);
av_x=vec(5);
Sxx=vec(6);
t=tinv(0.95,n-2);
if flag_fun==1;
```

```
    f=abs((90-(b(1)+b(2)*x))^2-(t^2)*MSE*(1/n+(x-av_x)^2/Sxx));
end
save (['it' num2str(cont)],'f','x','flag_fun');
```

This program performs the ANCOVA method to determine whether or not the slopes and intercepts of the degradation lines from the different batches analyzed are similar.

A.3 ANCOVA Method

```
%Analysis of Slopes and Intercepts from several batches
%ANCOVA Method
%Test for similarities (slopes & intercepts)

clear all;
clc;
close all;
batch=3;    %Number of batches to be analyzed

x=[0 3  6   9   12  18  24
0   3   6   9   12  18  24
0   3   6   9   12  18  24];  %Dates

y=[104.76   103.74  102.35  101.85  99.67   98.32   96.98
101.52  99.958  98.15   96.75   96.26   96.12   95.57
105.32  104.36  102.36  100.98  98.34   97.45   95.45];     %Label Claims

n(1)=7; %Label Claim to be analyzed in each batch
n(2)=7;
n(3)=7;

N=sum(n);
%%%%%%%%%%%%%%%%%%%%%%
%Testing the Slopes
%%%%%%%%%%%%%%%%%%%%%%
for i=1:batch;
    sy=0;
    sx=0;
    sxy=0;
    for j=1:n(i);
        sy=sy+y(i,j)^2;
        sx=sx+x(i,j)^2;
        sxy=sxy+x(i,j)*y(i,j);
    end
    Syy(i)=sy-(sum(y(i,1:n(i))))^2/length(y(i,1:n(i)));  %SSE of time
    Sxx(i)=sx-(sum(x(i,1:n(i))))^2/length(x(i,1:n(i)));  %SSE of label claim
    Sxy(i)=sxy-(sum(y(i,1:n(i)))*(sum(x(i,1:n(i))))/length(y(i,1:n(i))));
      %SSE cross product between time and LC
end
SxxW=sum(Sxx);    %SEE within batches
SyyW=sum(Syy);
SxyW=sum(Sxy);
SSEW=SyyW-SxyW^2/SxxW;
for i=1:batch;
    sse(i)=Syy(i)-Sxy(i)^2/Sxx(i);  %Residuals for each batch
end
```

```
SSE=sum(sse);     %Total residuals
SS_slope=SSEW-SSE;      %Residuals for differences on slopes
k=batch-1;
MS_slope=SS_slope/(batch-1);
MSE=SSE/(N-2*batch);
F_slope=MS_slope/MSE;   % F statistic to test if the slopes are similar from
batch to batch
F_slope;
df_num=batch-1;
df_den=N-2*batch;
F_cri=finv(0.75,df_num,df_den);     %Critical F for the slopes

%If the null hypothesis of similarity between slopes is not rejected at 0.25,
the intercepts should be test for similarities.

%%%%%%%%%%%%%%%%%%%%%%%%%
%Testing the Intercepts
%%%%%%%%%%%%%%%%%%%%%%%%%
% The intercepts should be tested given that the degradation lines from the
batches analyzed have similar slopes.
sy=0;
sy1=0;
sx=0;
sx1=0;
sxy=0;
for i=1:batch;
    for j=1:n(i);
        sy=sy+y(i,j)^2;
        sx=sx+x(i,j)^2;
        sxy=sxy+x(i,j)*y(i,j);
    end
    sy1=sy1+sum(y(i,1:n(i)));
    sx1=sx1+sum(x(i,1:n(i)));
end
sy;   % Sum of squares, cross products and errors for the reduced model
SY1=sy1^2/N;
SYY=sy-SY1;
sx;
SX1=sx1^2/N;
SXX=sx-SX1;
sxy;
SXY1=sx1*sy1/N;
SXY=sxy-SXY1;
tx=0; % Sum of squares for the complete model
ty=0;
txy=0;
for i=1:batch;
ty=ty+(sum(y(i,1:n(i))))^2/length(y(i,1:n(i)));
tx=tx+(sum(x(i,1:n(i))))^2/length(x(i,1:n(i)));
txy=txy+(sum(x(i,1:n(i))))*(sum(y(i,1:n(1))))/length(y(i,1:n(i)));
end
my=sy1^2/N;
mx=sx1^2/N;
mxy=sx1*sy1/N;
Tyy=ty-my;
Txx=tx-mx;
Txy=txy-mxy;
```

```
Eyy=SYY-Tyy;
Exx=SXX-Txx;
Exy=SXY-Txy;
SSEr=SYY-SXY^2/SXX;     % Sum of square errors for the reduced model
SSEc=Eyy-Exy^2/Exx;     % Sum of square errors for the complete model
SSb0=SSEr-SSEc
MSb0=SSb0/(batch-1);    % Mean square errors for the intercept effect
MSEc=SSEc/(N-batch-1);  % Mean square errors for the complete model
F_inter=MSb0/MSEc;      % F statistic to test if the intercepts are different
from batch to batch
df_num=batch-1;
df_den=N-batch-1;
F_cri_inter=finv(0.75,df_num,df_den);     %Critical F for the intercepts
```

This program performs the ANCOVA method for the simulation created to test the methods presented in this project.

A.4 Data Simulation Analysis Using ANCOVA

```
clear all;
close all;
clc;

%a=load('data_change_slope.mat');
%a=load('data_change_int.mat');
a=load('data_single_population.mat');%Data to analyze
%a=load('data_change_int_slope.mat');
LC_1=a.P1;  %Label Claim for each population
LC_2=a.P2;
LC_3=a.P3;
lotes_1=a.lt1;    %Random batches choose for the simulation
lotes_2=a.lt2;


P1=LC_1(lotes_1(1,:),:);      %Batches to be analyzed from the 2 populations
P4=LC_1(lotes_2(1,:),:);
P=[P1;P4];
LC=[];
for i=1:1000
P1=LC_1(lotes_1(i,:),:);
P4=LC_1(lotes_2(i,:),:);
LC=[LC;P1;P4];
end

LC=LC(:,[1:7]);                                                    %R
eading the LC inside the folder
batch_time=[0 3 6 9 12 18 24];

length_data=size((LC),1);     %Analyzing the LC from each group of batches
  l=1;
  u=4;
F_slope_all=[];
F_inter_all=[];
shelf_life=[];
raices=[];
batch_LC_all=[];
reference_all=[];
for s=1:length_data/4;
batch_LC=LC(l:u,:);     %Analyzing the data in groups of 4
     l=l+4
     u=u+4
batch_LC_all=[batch_LC_all,batch_LC];
     y=batch_LC;
time=7;
batch=4;
x=repmat(batch_time,batch,1);
```

```
n(1)=time;
n(2)=time;
n(3)=time;
n(4)=time;
N=sum(n);
%%%%%%%%%%%%%%%%%%%%%%
%Testing the Slopes
%%%%%%%%%%%%%%%%%%%%%%
for i=1:batch;
    sy=0;
    sx=0;
    sxy=0;
    for j=1:n(i);
        sy=sy+y(i,j)^2;
        sx=sx+x(i,j)^2;
        sxy=sxy+x(i,j)*y(i,j);
    end

Syy(i)=sy-(sum(y(i,1:n(i))))^2/length(y(i,1:n(i)));  %SSE of time
    Sxx(i)=sx-(sum(x(i,1:n(i))))^2/length(x(i,1:n(i)));  %SSE of label claim
    Sxy(i)=sxy-(sum(y(i,1:n(i)))*(sum(x(i,1:n(i))))/length(y(i,1:n(i))));
      %SSE cross product between time and LC
end
SxxW=sum(Sxx);    %SEE within batches
SxxW=sum(Sxx);
SyyW=sum(Syy);
SxyW=sum(Sxy);
SSEW=SyyW-SxyW^2/SxxW;
for i=1:batch;
    sse(i)=Syy(i)-Sxy(i)^2/Sxx(i);
end
SSE=sum(sse);
SS_slope=SSEW-SSE;
k=batch-1;
MS_slope=SS_slope/(batch-1);
MSE=SSE/(N-2*batch);
F_slope(s)=MS_slope/MSE;      % F statistic to test if the slopes are
different from batch to batch
df_num=batch-1;
df_den=N-2*batch;
F_cri=finv(0.75,df_num,df_den);     % Critical F
statistic  %%%%%%%%%%%%%%%%%%%%%%%%
%Testing the Intercepts
%%%%%%%%%%%%%%%%%%%%%%%%
% The intercepts should be tested given that the degradation lines from the
batches analyzed have similar slopes.

sy=0;
sy1=0;
sx=0;
sx1=0;
sxy=0;
for i=1:batch;
    for j=1:n(i);
        sy=sy+y(i,j)^2;
        sx=sx+x(i,j)^2;
```

```
        sxy=sxy+x(i,j)*y(i,j);
    end
    sy1=sy1+sum(y(i,1:n(i)));
    sx1=sx1+sum(x(i,1:n(i)));
end
sy;   % Sum of squares, cross products and errors for the reduced model
SY1=sy1^2/N;
SYY=sy-SY1;
sx;
SX1=sx1^2/N;
SXX=sx-SX1;
sxy;
SXY1=sx1*sy1/N;
SXY=sxy-SXY1;
tx=0; % Sum of squares for the complete model
ty=0;
txy=0;
for i=1:batch;
ty=ty+(sum(y(i,1:n(i))))^2/length(y(i,1:n(i)));
tx=tx+(sum(x(i,1:n(i))))^2/length(x(i,1:n(i)));
txy=txy+(sum(x(i,1:n(i))))*(sum(y(i,1:n(1))))/length(y(i,1:n(i)));
end
my=sy1^2/N;
mx=sx1^2/N;
mxy=sx1*sy1/N;
Tyy=ty-my;
Txx=tx-mx;
Txy=txy-mxy;
Eyy=SYY-Tyy;
Exx=SXX-Txx;
Exy=SXY-Txy;
SSEr=SYY-SXY^2/SXX;     % Sum of square errors for the reduced model
SSEc=Eyy-Exy^2/Exx;     % Sum of square errors for the complete model

SSb0=SSEr-SSEc
MSb0=SSb0/(batch-1);    % Mean square errors for the intercept effect
MSEc=SSEc/(N-batch-1);  % Mean square errors for the complete model
F_inter=MSb0/MSEc;      % F statistic to test if the intercepts are different
from batch to batch
df_num=batch-1;
df_den=N-batch-1;
F_cri_inter=finv(0.75,df_num,df_den);     % Critical F statistic for the
intercepts
 Equal_slopes_inter=find(F_slope<F_cri & F_inter<F_cri_inter);    % To
determine the batches with similar slopes and intercepts
Equal_slopes_inter=Equal_slopes_inter';
Not_equal_slope=find(F_slope>F_cri);      % To determine the batches with
different slopes
Not_equal_inter=find(F_inter>F_cri_inter);      % To determine the batches
with different intercepts
F_slope_all=[F_slope_all;F_slope(s)];
F_inter_all=[F_inter_all;F_inter(s)];
end
```

This program performs the Indicator Variables method for the simulation created to test the methods presented in this project.

A.5 Data Simulation Analysis Using Indicator Variables

```
clear all;
close all;
clc;
%a=load('data_change_slope.mat');
%a=load('data_change_int.mat');
%a=load('data_change_int_slope.mat');
a=load('data_single_population.mat');
LC_1=a.P1;  %Data available for the simulation (Different populations)
LC_2=a.P2;
LC_3=a.P3;
lotes_1=a.lt1;    %Random batches choose for the simulation
lotes_2=a.lt2;

 % P1=LC_1(lotes_1(1,:),:);
% P4=LC_1(lotes_2(1,:),:);
% P=[P1;P4];
LC=[];
for i=1:1000
P1=LC_1(lotes_1(i,:),:);      %Batches to be analyzed from 2 populations
P4=LC_1(lotes_2(i,:),:);
LC=[LC;P1;P4];    %Mixed Populations
end

LC=LC(:,[1:7]);                                                    %R
ead all the LC inside the folder
batch_time=[0 3 6 9 12 18 24];      %Times analyzed
same_slope=[];
same_inter=[];
p_value_all=[];
P_all=[];
t_all=[];
t0_all=[];

length_data=size((LC),1);     %Length of the data=Length LC
  l=1;
  u=4;
 inter_freq=[];
slope_freq=[];
for s=1:length_data/4;
batch_LC=LC(l:u,:);     %Analyzing each set of 4 batches until the end of
data
     l=l+4
     u=u+4
y=batch_LC;
time=7;                                                             %
Times analyzed
batch=4;
x=repmat(batch_time,batch,1); %Time matrix of the size of the batches
sig_level=0.25;   %Significance Level
%Indicator variables matrix
```

```
V1=[ones(7,2) zeros(7,2) x(1,:)' x(1,:)' zeros(7,2)];
V2=[ones(7,1) zeros(7,1) ones(7,1) zeros(7,1)  x(2,:)' zeros(7,1) x(2,:)'
zeros(7,1)];
V3=[ones(7,1) zeros(7,2) ones(7,1) x(3,:)' zeros(7,2) x(3,:)' ];
V4=[ones(7,1) zeros(7,3) x(4,:)' zeros(7,3)];
V=[V1;V2;V3;V4];
Y=[y(1,:)';y(2,:)';y(3,:)';y(4,:)'];      %LC of the batches
n=size(V);

% Calculating regression parameters
P=((V'*V)^(-1)*V')*Y;   %Parameters values (Least Square Estimates)
VP=V*P;                                                                    %
Fitted Model
C=(V'*V)^-1;                                      %Covariance Matrix
error=Y-V*P;         %Difference between the observation and the fitted value
SSE=error'*error;
% Sum of squares due to errors
SST=sum(Y.^2)-n(1)*mean(Y).^2;
SSR=SST-SSE;
% Sum of squares due to regression
MSR=SSR/(8-1);
MSE=SSE/(28-8);
F(s)=MSR/MSE;
 F_inv=finv(0.75,7,20); %Critical F value
 T_Stat_all=[];

 for i=1:n(2);               %P-values and t-test values for each parameter
    d(i)=sqrt(SSE/(n(1)-length(P))*C(i,i));
    t(i)=abs(P(i)/d(i));
    p_value(i)=2*(1-tcdf(t(i),n(1)-length(P)));
end
t0=tinv(0.75,n(1)-length(P));
p_value_all=[p_value_all;p_value];
P_all=[P_all;P'];
t_all=[t_all,t'];
t0_all=[t0_all,t0'];
end

common_intslope=find((p_value_all(:,2)>0.25 & p_value_all(:,3)>0.25 &
p_value_all(:,4)>0.25)&(p_value_all(:,6)>0.25 & p_value_all(:,7)>0.25 &
p_value_all(:,8)>0.25)); %Finding batches with similar slopes and intercepts
diferente_int=find(p_value_all(:,2)<0.25 | p_value_all(:,3)<0.25 |
p_value_all(:,4)<0.25); %Finding batches with different intercepts
diferente_slope=find(p_value_all(:,6)<0.25 | p_value_all(:,7)<0.25 |
p_value_all(:,8)<0.25); %Finding batches with different slopes
```