STUDY OF EFFICIENCY TIME OF RECOMBINANT DNA INSULIN VIA ACCELERATED LIFE TESTING AND INTERVAL CENSORING

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▪ ABSTRACT: This paper aims to study the efficiency of recombinant DNA insulin via models for accelerated life tests. The potency loss of these insulin products was evaluated periodically, subject to the conditions of temperature of 8°C, 25°C and 37°C. Insulin samples with potency at less than 100% were considered unfit for consumption, which characterizes the event of interest. Samples suitable for consumption were considered to be censored. The response variable was observed periodically for 736 days. For data analysis, statistical models of stress-response regression were used. The deterministic part of these models is the Arrhenius model because the stress variable is the temperature, while the probabilistic part was comprised of the Exponential, Weibull, and Log-normal models. The techniques of accelerated life tests proved adequate to address the time of potency loss of the insulin for the various temperature levels. The times of occurrence of the events were treated in three different ways, which were compared in this study. First, interval censoring was considered, or only the upper and lower limits of the interval in which the failure occurred were known. Then, the midpoint of this interval was considered as a failure time. Finally, only the lower limit of the interval in which the failure occurred was considered. According to the results, it is concluded that the use of the interval lower limit is more appropriate for estimating the reliability curves, as the estimates are closer to those using interval censoring then using the midpoint of the interval. For the specific case of the recombinant DNA insulin data, it was observed that the Arrhenius-Weibull model and the Arrhenius-lognormal are suitable for adjusting the data. It follows also that the temperature affects the power of the insulin: The higher the temperature are, the lesser the efficiency.

▪ KEYWORDS: Accelerated life tests; stress variable; interval censoring; exact failure times.

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1 Introduction

Reliability models aim to study data from experiments in which the response variable is usually the time of the occurrence of an event of interest (failure time). The following elements are the failure time: the start time, the scale of measurement, and the event of interest.

According to Kalbfleisch and Prentice (2002), the main feature of the data in reliability studies is the presence of censoring: that is, a partial observation of the response. There are three types of censoring: right, left, and interval censoring (MEEKER and ESCOBAR, 1998). Data with interval censoring appear when the failure time cannot be observed, but only an interval in which the failure time is contained can be given (HUANG and WELLNER, 1996 YANG and PAN, 2013). However, some authors suggest that, in case of interval censoring , standard analysis methods may be applied if it is considered that the event of interest occurred in the beginning (LINDSEY and RYAN, 1998), in the end (DUCROCQ, 1999), or in the midpoint of the interval (ODELL *et al.*, 1992).

Regardless of the type of censoring observed to estimate the life time of a product, experiments must be conducted for a long period of time. Thus, to minimize the time and expenses of the experiments, an alternative is to carry out an accelerated life test, which is a methodology widely used, especially in industries in the reliability context. Accelerated life tests, according to Nelson (1990) consist of a variety of methods to shorten the product life or accelerate the degradation of its performance. The goal in this case is to accelerate the failure of components by testing them in extreme environmental conditions and then extrapolate the accelerated test results for regular operating conditions (ZELEN, 1959). Accelerated life tests can be used on various types of materials and products, according to which various performance degradation mechanisms are applied. To ensure that the failure times will occur more quickly, acceleration variables must be carefully chosen to make it sure that the variable actually interferes with the experiment time.

According to Nelson (1972), one of the stress-response relationships frequently applied in practice is the Arrhenius Relationship, which is used in general to relate failure times of a product that was subjected to extreme temperatures. Several researchers have proposed the use of accelerated life tests, including Kötz *et al.* (2010), Kim *et al.* (2010), Pascual (2010), Núñez *et al.* (2011), Chan *et al.* (2011), Zhang *et al.* (2011), Zhao and Xu (2012), Yang and Pan (2013), Espinet-González *et al.* (2014), and Zhang and Wang (2015).

The methodology of accelerated life tests can be used in diverse areas of knowledge. Most recently, we can cite studies done by Kurniadi *et al*. (2017) in which they selected one of the traditional foods of Indonesia, "Nasi Uduk" that is made from rice and cooked with coconut milk and spices. The objective of this research was to determine the shelf life of canned "nasi uduk" and the Arrhenius model was used. Storage temperatures were 35, 45 and 55° C for 35 days. The authors came to the conclusion that the useful life of canned "nasi uduk" was 9.6 months

Chiodo *et al*. (2016) studied a probabilistic battery design method with reference to lithium-ion batteries, based upon battery life and related economical aspects. The authors used the Weibull distribution to estimate the duration of the batteries, while Chang *et al*. (2016) presented a reliability evaluation method of the scroll compressor for an air conditioner system.

Widely used to predict failures in equipment in the electronic industry, accelerated life testing has also been applied in the pharmaceutical industry. In this paper, accelerated life testing is applied to the production of insulin, a drug to treat *diabetes mellitus* (DM), which is a disease that is considered a serious medical and public health problem. DM is characterized by inadequate or no production of insulin, which is, according to Silva *et al.* (2003), a hormone naturally produced by the β cells of the islets of Langerhans of the pancreas. DM has, due to its complications, a high mortality rate (CASTRO and GROSSI, 2007; ARAUJO *et al.*, 2009).

In order to make an analogy of pancreatic functioning, pharmaceutical companies started to provide numerous preparations of human recombinant DNA insulin (JOHNSON, 1983). Such preparations should be given to the needs of each diabetic individual and inappropriate use will result in the loss of their biological functions, resulting in poor control of blood glucose (STACCIARINI *et al.*, 2008). According to the medical and pharmaceutical literature, it is known that, in normal use, the efficiency of insulin is mainly affected by extremes of temperature. Thus, an improper process of storage and preservation of bottles has been a major problem affecting their good stability and preservation. Given the importance and the increase in cases of DM in Brazil and in the world, it is necessary to study the relationship between the time of the power loss of recombinant insulins with the temperature in which the bottles are exposed during the storage and conservation process.

Reliability techniques in the context of accelerated life testing are considered in this paper for modelling data of efficiency loss of recombinant DNA insulin. Details of the models are given in Section 2. Section 3 describes the results obtained. Finally, Section 4 concludes the paper with final remarks and topics for future research in the area.

2 Materials and methods

The data in this study are related to the time until power loss of human DNA recombinant insulin, periodically evaluated in 10 ml bottles, from an insulin manufacturer in the state of Minas Gerais, Brazil. Three batches of the product were selected, 525g, 586G, and 597G. From each batch three bottles were removed and equally distributed in the conditions of 8°C, 25°C, and 37°C temperatures, relative humidity controlled to 70%, upside down, and analyzed according to standard methodologies in certain periods. The storage period of the insulins was measured in days, and the maximum time of 736 days was observed. Unfit for consumption were those insulin samples with power less than 100%, which characterizes the event of interest. Thus, the dependent variable is the time until the samples are deemed unfit for human consumption. Samples suitable for consumption by the end of the observation time were considered to be censored. During this period, the insulin bottles showed power between 100% and 55.2%.

For data analysis, we will use statistical models of stress-response regression in which the deterministic part was composed by the Arrhenius model. According to Nelson (1990), the expression of the time to failure for the Arrhenius model is defined by:

$$T = A\ exp\left[\frac{E}{K\tau}\right] \quad (1)$$

where T is the failure time; $\tau = (°C + 273{,}16)$ is the stress variable (temperature); A is a constant determined by the experiment; E is the activation energy in electron-volts; K is Boltzmann constant, 8.6171×10^{-5}K.

The linear form of Eq. (1) is given by $ln(T) = ln(A) + \frac{E}{K\tau}$, in which $ln(T)$ is the logarithm of the failure time and $ln(A)$ is the logarithm of the constant A. The values of constants A and E are estimated by the experimental data. Thus, the linearized stress-response Arrhenius model is defined mathematically by $ln(T) = \beta_0 + \beta_1 x$, in which $\beta_0 = ln(A)$, $\beta_1 = \frac{E}{K}$, and $x = \tau^{-1}$, in which $\tau = (°C + 273.16)$ is the stress variable, the temperature.

For being the most used in studies involving accelerated life tests, the probabilistic part was composed by the exponential, Weibull, and log-normal models. These models have the function to explain the variability in the time of the insulin efficiency loss to the various levels of temperature and are described as follows.

2.1 Exponential Arrhenius model

The exponential Arrhenius regression model has the following conditions: i) for each level of stress, the failure time T follows the exponential distribution; ii) the model involves only a covariate. The reliability function $R(t)$ of failure time T, subject to the stress variable, temperature $x = \frac{1}{\tau}$, is given by:

$$R(t;x) = exp\left\{-\left(\frac{t}{exp\{\beta_0 + \beta_1 x\}}\right)\right\} = exp\left\{-\frac{t}{\alpha}\right\} \quad (2)$$

in which $\boldsymbol{\alpha} = \exp(\boldsymbol{\beta_0} + \boldsymbol{\beta_1 x}) = \boldsymbol{A} \exp\left[\frac{\boldsymbol{E}}{\boldsymbol{K\tau}}\right]$, $\boldsymbol{\beta_0} = \boldsymbol{ln}(\boldsymbol{A})$, $\boldsymbol{\beta_1} = \frac{\boldsymbol{E}}{\boldsymbol{K}}$, and $\boldsymbol{x} = \boldsymbol{\tau^{-1}}$.

2.2 Weibull-Arrhenius model

The use of the Weibull-Arrhenius regression model requires that the following conditions are met: i) for each level of stress x, the failure time T follows the Weibull distribution $\alpha(x), \delta)$; the logarithm of that time has distribution of the extreme value, that is, $Y = \ln(T)$ has extreme value distribution with location parameter $\ln[\alpha(x)]$ and scale $\sigma = \frac{1}{\delta}$, in which $\alpha = \exp(x'\beta)$; ii) the scale parameter $\sigma = \frac{1}{\delta}$ is the same for all stress levels. The reliability function $R(t)$ of failure time T, conditioned to the stress variable, temperature $x = \frac{1}{\tau}$ is given by:

$$R(t;x) = exp\left\{-\left(\frac{t}{exp\{x'\beta\}}\right)^{\frac{1}{\sigma}}\right\} = exp\left\{-\left(\frac{t}{\alpha}\right)^{\frac{1}{\sigma}}\right\} \quad (3)$$

in which $\boldsymbol{\alpha} = \exp(\boldsymbol{\beta_0} + \boldsymbol{\beta_1 x}) = \boldsymbol{A} \exp\left[\frac{\boldsymbol{E}}{\boldsymbol{K\tau}}\right]$, $\boldsymbol{\beta_0} = \boldsymbol{ln}(\boldsymbol{A})$, $\boldsymbol{\beta_1} = \frac{\boldsymbol{E}}{\boldsymbol{K}}$, and $\boldsymbol{x} = \boldsymbol{\tau^{-1}}$.

2.3 Log-normal Arrhenius model

The log-normal Arrhenius regression model has the following condition: for each stress level, the failure time follows the log-normal distribution; the logarithm of the time follows a normal distribution with mean $\mu(x)$, variance σ^2, and constant standard deviation. The reliability function $R(t)$ of failure time T, conditioned to the stress variable, temperature $x = \frac{1}{\tau}$, is given by:

$$R(t;x) = \Phi\left\{-\left[\frac{ln(t) - \mu(x)}{\sigma}\right]\right\} \quad (4)$$

in which $\mu(x) = \beta_0 + \beta_1 x = ln\left[Aexp\left[\frac{E}{K\tau}\right]\right]$, $\beta_0 = ln(A)$, $\beta_1 = \frac{E}{K}$, $x = \tau^{-1}$, and Φ is the standard normal accumulate distribution function, that is, mean is equal to 0 and standard deviation is equal to 1.

Because the failure times were not observed but only the intervals in which occurred the event of interest, there is a typical case of interval-censored. So in this article we chose to work with the data in the way they were originally collected and also considering that the failure time is known, assuming initially that the event occurred at the midpoint of the range defined interval-censored. Thus, the reliability functions were estimated for different temperatures, by means of non-parametric Kaplan-Meier methods (KAPLAN and MEIER, 1958), when considering the midpoint of the range, and Turnbull method (TURNBULL, 1976), for interval censoring.

The *log-rank* test was used to compare equality between the estimated reliability curves. This test compares the observed number of failures in each group with the expected number of failures under the null hypothesis that the reliability curves are equal (COLOSIMO and GIOLO, 2006).

To estimate the parameters of the models we used the maximum likelihood estimator and the likelihood ratio test was used in choosing the probability model that best fits the data set.

The likelihood function in the case of interval censorship, according to Colosimo and Giolo (2006) is given by $L(\theta) = \prod_{i=1}^{r}[R(l_i|x_i) - R(u_i|x_i)]^{\delta_i}\,[R(l_i|x_i)]^{1-\delta_i}$, being x_i the vector of covariates l_i and u_i their respective lower and upper limits of the time range observed for the i-th subject. If $\delta_i = 1$ if the event occurred in $(L_i, U_i]$, $\delta_i = 0$, if the event occurred in a longer time than the li. Thus, the maximum likelihood estimators are the values of the parameters $\theta = (\beta' s,\ \sigma)$ that maximize the logarithm function of likelihood, i.e., the values that satisfy $U(\theta) = \frac{\partial \log L(\theta)}{\partial \theta} = 0$.

The estimates obtained in times of stress were extrapolated to the normal conditions of use, making it possible to forecast the warranty period and the failure percentage for all insulin bottles that would still be sent to the market. Analyzes of residues were performed in which you can seek evidence of violation of the assumptions made about the regression models accelerated through graphic techniques that use the residues (COLOSIMO and GIOLO, 2006). In this case, Cox-Snell residues were used.

All the routines used to generate the results of this paper were performed with R *software* (R CORE TEAM, 2017) and are available directly from the authors for research and educational purposes.

3 Results and discussion

In an initial exploratory data analysis, results obtained by the nonparametric Kaplan-Meier and Turnbull estimators are presented. In Figure 1, the estimated curves of the reliability function by Kaplan-Meier method may be seen, for different temperatures and considering that the failure time is the midpoint of the interval in which the event occurred.

It can be seen in Figure 1 that the insulin reliability curve falls more rapidly when subjected to a temperature of 37°C, which agrees with the ADA (AMERICAN DIABETES ASSOCIATION, 2004) suggesting that the insulin should not be exposed to temperatures higher than 30°C. That is, temperatures above this value will result in lower effectiveness of insulin times. It is also observed that the curves with temperatures of 8°C to 25°C do not appear to differ from each other.

The results of log-rank test to compare the estimated reliability curves showed no significant differences at the level of significance of 5% between the curves estimated for temperatures of 8°C to 25°C (p-value = 0.495). It is noteworthy that these results are consistent with the suggestions to keep insulins refrigerated at temperatures from 2 to 8°C and/or at ambient temperatures between 15°C and 30°C (GROSSI and PASCALI, 2009).

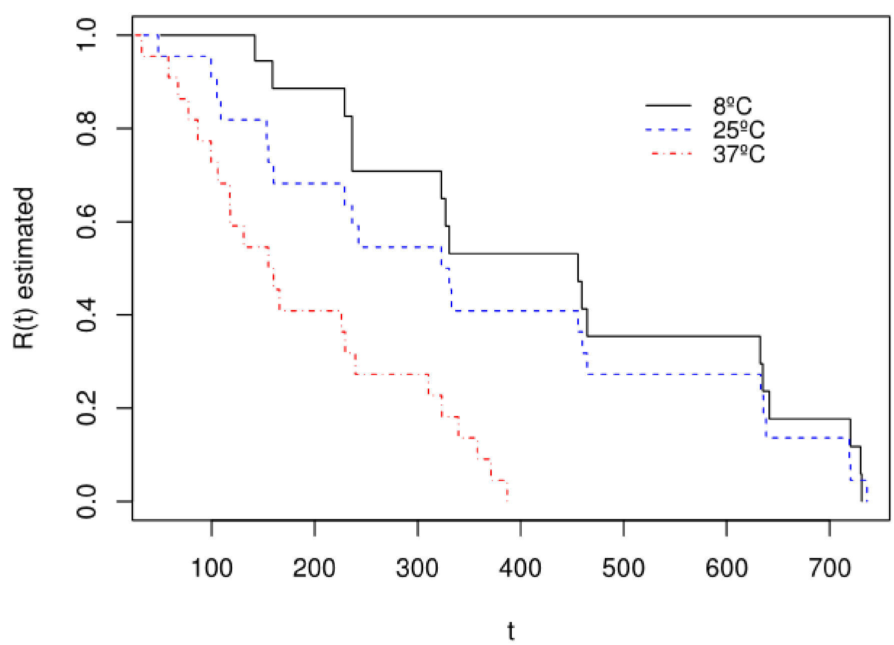

Figure 1 - Estimated reliability curves by Kaplan-Meier for the times at different temperatures.

Additionally, the Turnbull method was applied to the original data, that is, interval-censored, in order to verify whether, in equivalent situations, there are significant differences between the estimates, considering the two situations studied. The curves estimated by the Turnbull method and the Kaplan-Meier method are shown in Figure 2, which shows that, in general, the estimates by Turnbull method show lower values than the estimates obtained by the Kaplan-Meier curves, which used the midpoint of the intervals.

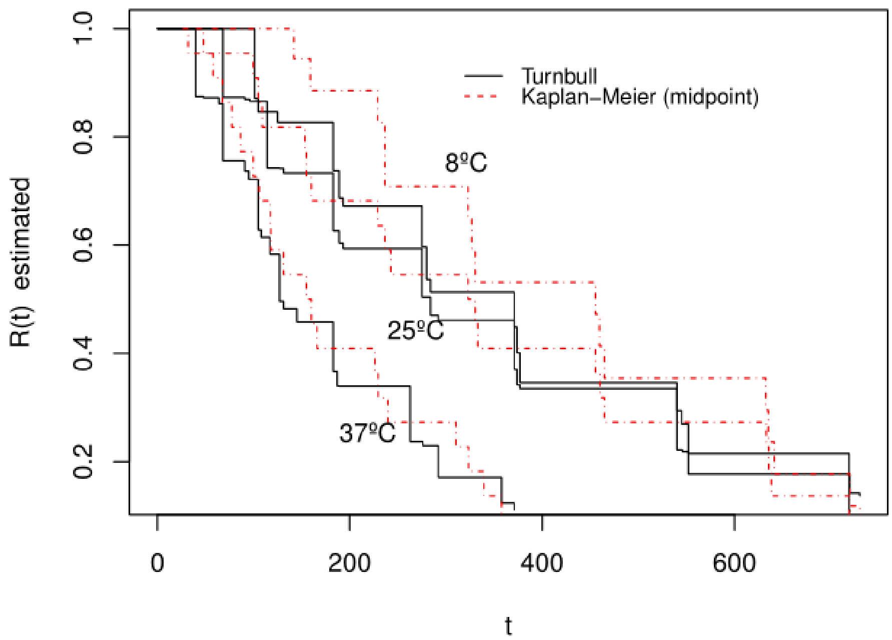

Figure 2 - Estimated reliability curves by Kaplan-Meier, considering the midpoint of the interval, and by Turnbull, for interval censoring.

Confidence limits based on the failure times for the estimated reliability curves were estimated to verify that the curves estimated via Turbull algorithm are within the confidence limits of those estimated through Kaplan-Meier. Figure 3 shows the curves and the estimated confidence limits, from which it can be seen that Turnbull estimates are within the confidence limits of those who consider the midpoint of the interval. However, at the initial time, these estimates were presented closer to the lower limit of confidence, especially at a temperature of 8°C.

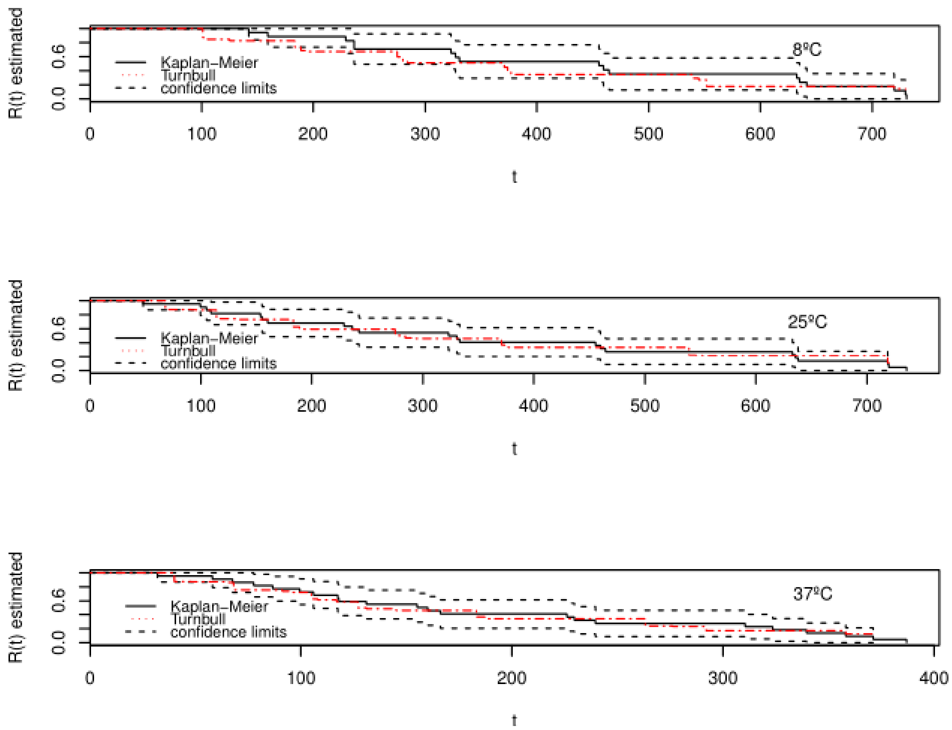

Figure 3 - Estimated reliability curves considering the midpoint of the intervals and their average with the confidence limits.

Based on the presented results is plausible to apply the Kaplan-Meier method considering the lower limit rather than the midpoint of the intervals in which the failure occurred and to compare with the results obtained by the Turnbull method. The curves are shown in Figure 4 from which it is noticed that the curves estimated using both methods for each temperature are very close. The results were different from those suggested by Odell *et al.* (1992), Ducrocq (1999) and Colosimo and Giolo (2006). According to the authors, the differences in the times will be lower when considering the midpoint of the interval, rather than the lower limit of the interval when compared with estimates considering interval censoring.

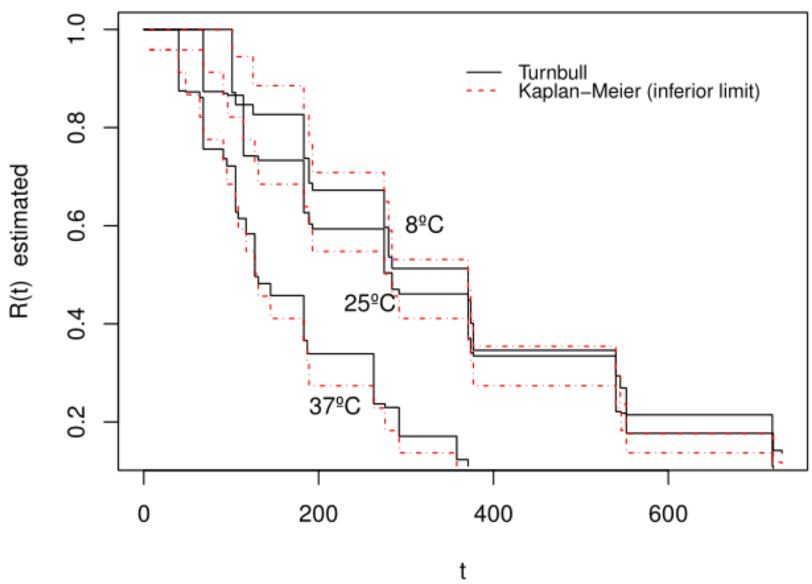

Figure 4 - Estimated reliability curves by Kaplan-Meier and Turnbull methods considering the inferior limit of the intervals.

The results of log-rank test, with the lower limit of the intervals showed no significant differences at the level of significance of 5% between the reliability curves estimated for temperatures of 8°C to 25°C (p-value $=$ 0.498). It can be seen that the results are very similar to those in which it was considering the midpoint of the intervals and it was decided then to adjust regression models for all situations studied here.

3.1 Accelerated regression models for failure times at the interval midpoints

As suggested by Colosimo and Giolo (2006), it was initially ignored the acceleration variable temperature to facilitate the process of choosing the regression model that best fitted the data. Thus, it was performed, initially, the graphical analysis (omitted here), and then reliability functions were estimated via the Kaplan-Meier method for the exponential, Weibull, and log-normal models, as well as the graphics linearization of reliability functions estimated by the proposed models. The results suggest that the exponential distribution is not adequate to adjust the insulin data and the appropriate models are Weibull and log-normal. To confirm the results of the graphical analysis, it performed the test of likelihood ratio, based on Generalized Gamma Distribution. This distribution is used because of Exponential distribution, Weibull and log-normal are individual cases of the Generalized Gama distribution. The results presented a p-value $= 0.23$ for the Weibull model and p-value $= 0.25$ for the log-normal model. Based on these results, it is concluded that these models are suitable for data analysis and thus will make the probability of the stress-response regression models. To study the influence of temperature on failure times the

Weibull and log-normal Arrhenius accelerated regression models were considered. Estimates of the parameters of these models are presented in Table 1.

Table 1 - Estimates of parameters of Weibull and log-normal Arrhenius models considering the midpoint of the intervals

	Arrhenius Weibull regression model		Arrhenius log-normal regression model	
	Value	estimated standard errors	Value	estimated standard errors
intercep ($\hat{\boldsymbol{\beta}}_0$)	7.145	0.424	7.166	0.438
temperature ($\hat{\boldsymbol{\beta}}_1$)	-0.424	0.137	-0.542	0.141
ln(scale ($\hat{\sigma}$))	-0.521	0.098	-0.372	0.089
scale ($\hat{\boldsymbol{\sigma}}$)	0.594	-	0.689	-
form ($\hat{\boldsymbol{\delta}}$)	1.683	-	-	
AIC	807.21		807.10	
Loglik model	-400.60		-400.50	

For both models the estimates of parameter β_1 is negative. This indicates that the larger the value of the lower temperature variable lower is the probability of the power of the insulin to remain efficient. This observation is confirmed by Figure 5, which generated reliability curves considering temperatures of 2°C and 40°C than those in which the data were collected, and maintaining the temperature of 25ºC.

Considering Figure 5, when analyzing the reliability curves estimated by Weibull Arrhenius model for the different temperatures, it is seen that approximately 55% of the insulin, which were subjected to 40ºC temperatures, will be fit for consumption for 200 days whereas the percentage of effective insulins when stored at temperatures of 2°C at 200 days will be around 95%. From the results generated by the log-normal Arrhenius model, we can see that under the same conditions, the results were approximate. For example, at 40ºC, they will still be able to use about 42% of the insulin. To check the suitability of the models a Cox-Snell residue analysis was performed and from the results it could be concluded that they fit satisfactorily. The graphics of the residue can be seen in annex I (Figures 9, 10 e 11).

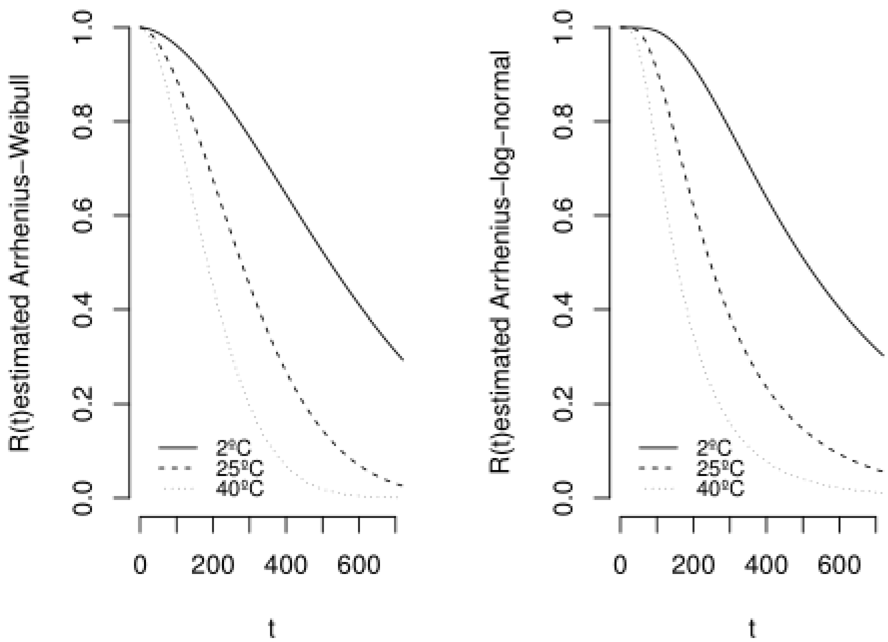

Figure 5 - Estimated reliability curves by Weibull and log-normal Arrhenius accelerated regression models, considering the midpoint of the intervals.

Finally, the results of stress temperatures were extrapolated to the normal conditions of use at room temperature, depending on the region under study. Such quantities are presented in Table 2, from which it is clear that regardless of percentiles and models, the time insulins remain with the power of 100% tends to decrease as the temperature increases. For example, considering the 50th percentile for the Weibull Arrhenius model, we note that the time for 50% of the insulin to be fit for consumption decreases from 467 days at 6°C room temperature, 213 days in room temperature 35°C, or different regions determine different times efficiencies.

Table 2 - Results obtained by Weibull and log-normal Arrhenius models at different temperatures for some percentiles

Arrhenius Weibull regression model			Arrhenius log-normal regression model		
Percentile (%)	Temperature	Time*	Percentile (%)	Temperature	Time*
10	6	158	10	6	190
10	15	121	10	15	140
10	23	98	10	23	108
10	35	72	10	35	76
10	45	57	10	45	57
50	6	467	50	6	452
50	15	360	50	15	333
50	23	289	50	23	258
50	35	213	50	35	180
50	45	168	50	45	136
63	6	574	63	6	563
63	15	443	63	15	417
63	23	356	63	23	323
63	35	262	63	35	226
63	45	207	63	45	171
90	6	932	90	6	1076
90	15	718	90	15	793
90	23	577	90	23	614
90	35	425	90	35	429
90	45	335	90	45	325

*day

3.2 Fit of accelerated regression models, considering the accurate failed time as the starting point of the range

The same techniques seen in (3.1) were applied. Of the graphic methods applied, only the Weibull model proved to be suitable for calibration of data. This result is confirmed by the likelihood ratio test (p value = 0.274). In this case, the Weibull model composes the probabilistic part of the stress-response regression models and to study the influence of temperature at the time of failure, the accelerated regression Arrhenius-Weibull model was used. The estimates of the parameters of this model are shown in table 3.

Table 3 - Estimates for the parameters of the Arrhenius model-Weibull, considering the lower limit of the range

Arrhenius Weibull regression model		
	value	estimated standard errors
Intercep ($\hat{\boldsymbol{\beta}}_0$)	7.039	0.508
temperature ($\hat{\boldsymbol{\beta}}_1$)	-0.445	0.164
ln(scale ($\hat{\sigma}$))	-0.315	0.101
scale ($\hat{\boldsymbol{\sigma}}$)	0.729	-
form ($\hat{\boldsymbol{\delta}}$)	1.370	-
AIC	803.58	
Loglik model	-398.80	

Note that, in the table 3, the estimated value of βˆ1 is negative. This indicates that the higher the value of the variable temperature, the lower the likelihood of the insulin's potency to remain efficient. This observation can be displayed in Figure 6.

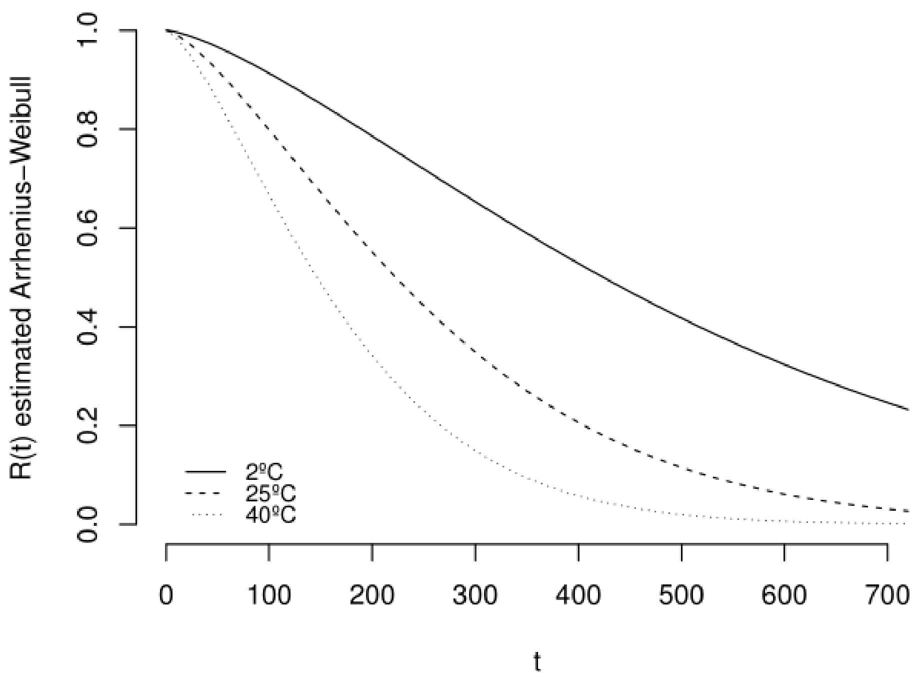

Figure 6 - Reliability curves estimated by Arrhenius -Weibull regression models to NPH human insulin data to different temperatures, considering the starting point of the range.

When analyzing the estimated reliability curves in Figure 6, to different temperatures, it is observed that about 38% of the insulin, which was subjected to temperatures of 40° C, will be fit for consumption (potency equal to 100%) within 200 days, while the percentage of efficient insulin, stored for the same time, will be around 80% when kept at temperatures of 2ºC.

To assess the adequacy of the Arrhenius-Weibull regression model, Cox-Snell residue were considered and through the results, it can be concluded that the model adjusts, in a satisfactory manner, to the failure times of NPH human insulin data, considering the starting point of the range.

We once again observe that the time in which the insulin remains with efficiency equal to 100%, tends to decrease as the temperature increases. Considering the median (50 percentile), note that the time of reliability so that 50% of the insulin is apt for consumption at 6°C, will decrease from 380 to 232 days when subjected to temperatures of 23ºC.

In table 4, we can see the results obtained at different temperatures, which can be considered normal use conditions, depending on where the insulin is stored. We can observe, again, that the time in which the insulin remains with 100% potency, tends to decrease as the temperature increases. Considering the median (50 percentile), note that the time of reliability, so that 50% of the insulin is apt for consumption at 6ºC, it will fall from 380 to 232 days when subjected to temperatures of 23ºC.

Table 4 – Results obtained with the Arrhenius -Weibull model at different temperatures for a couple of percentiles

Arrhenius Weibull regression model					
Percentile(%)	Temperature	Time*	Percentile(%)	Temperature	Time*
5	6	59	63	6	492
5	15	45	63	15	377
5	23	36	63	23	301
5	35	26	63	35	220
5	45	21	63	45	172
10	6	99	90	6	897
10	15	76	90	15	686
10	23	61	90	23	548
10	35	44	90	35	400
10	45	35	90	45	313
50	6	380			
50	15	291			
50	23	232			
50	35	170			
50	45	133			

*day

Considering extrapolations to the normal conditions of use and the temperature of 23º C, Table 5 presents predictions for the insulin data, for both situations of failure time considered.

Table 5 - Results of the predictions obtained using the Arrhenius -Weibull and Arrhenius-lognormal models to both exact failure times, considering the room temperature of 23ºC

	Midpoint of the interval		Inferior limit of the interval
	Arrhenius Weibull regression model	Arrhenius log-normal regression model	Arrhenius Weibull regression model
Average life time (day)	318	412	276
Failure percentage in a year	65	57	73
Warranty period-5% failure (day)	64	85	36
Percentile 10 (day)-median (day)	98	108	61
Percentile 50 (day)	289	258	232
Percentile 90 (day)	577	614	548

One realizes that, in Table 5, the predictions for failure times, given the starting point of the range, resulted in lower values, i.e. a more conservative scenario. It is suggested that manufacturers will opt for this result to prevent consumers from using insulin that does not have proper efficiency. Insulin manufacturers should stipulate, in accordance with the Arrhenius -Weibull model, a warranty period of 36 days, so that only 5% of the produced insulin show a compromised efficiency, when kept at a temperature of 23°C.

3.3 Accelerated regression models for interval censoring

Figures 7 and 8 present the estimated reliability curves via Kaplan-Meier to failed times considered in this article overlapping curves estimated by Turnbull considering interval censoring.

In Figure 7, the estimates (blue and red lines) are closer to estimates that consider the lower limit of the range, especially for the Arrhenius-lognormal model. However, in both charts, after 300 days, the chances of the insulin remaining efficient, suffered a change in both situations. In Figure 8, we observe the same behavior in relation to Figure 7.

The results of this analysis show that it is plausible to use the lower limit of the range to treat the data with interval censorship and thus, the results in stress conditions when extrapolated to the normal conditions of use will be reliable.

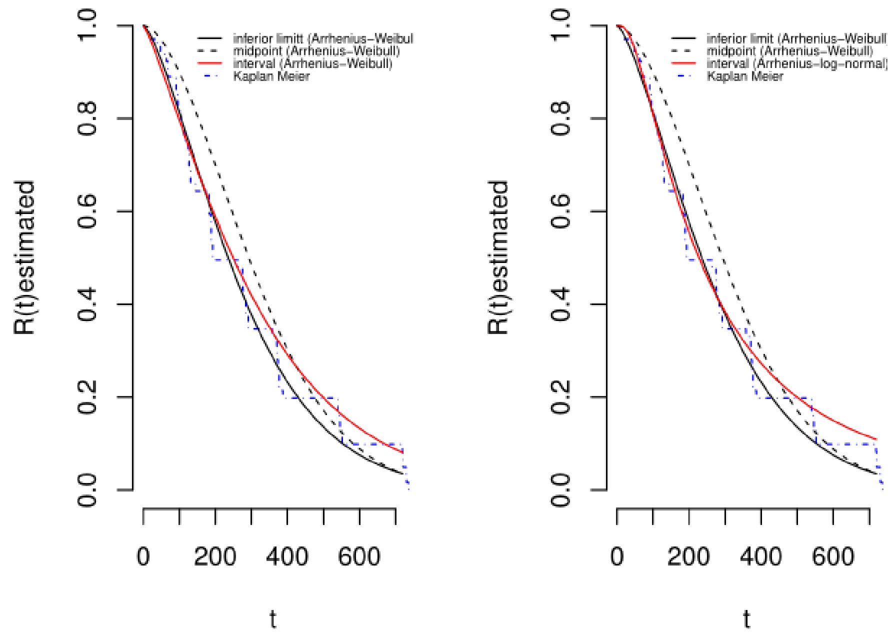

Figure 7- Estimated reliability curves, considering the Arrhenius -Weibull model to the midpoint and lower limit of the range and Arrhenius-Weibull, Arrhenius-log-normal for interval censoring.

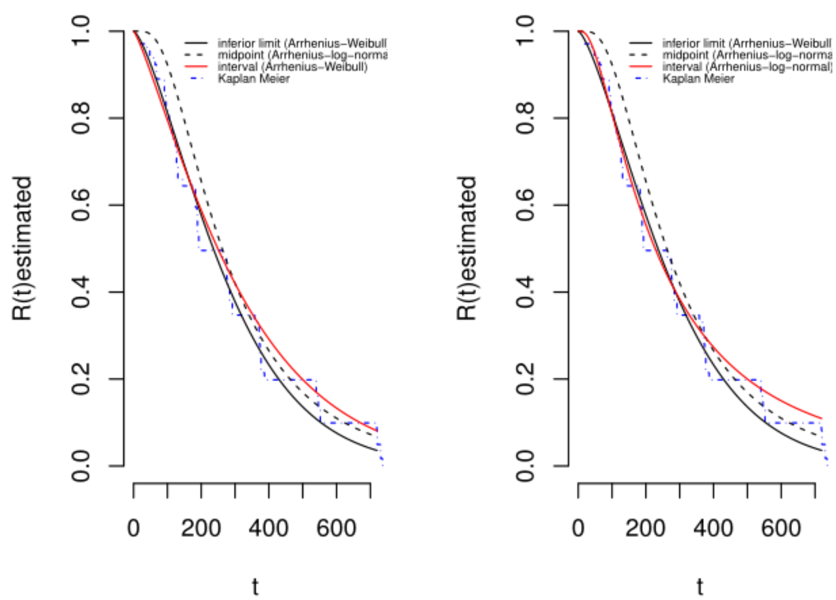

Figure 8 - Estimated reliability curves, considering the Arrhenius-lognormal model for the midpoint, Arrhenius-Weibull for the starting point and Arrhenius-Weibull and Arrhenius-lognormal for the range.

4 Conclusions

The findings that the techniques of accelerated life tests proved adequate to address the efficiency time of recombinant DNA insulin for various temperature levels. The event of interest was treated as interval censoring and also assuming that the failure time is the midpoint of the interval in which the event of interest occurred, and finally, assuming that the upper limit of interval is the exact time to failure. According to the results, it is concluded that the use of the lower limit of the interval is more appropriate for estimating the reliability curves when compared to the use of the midpoint, as it is closer to the results generated using interval censoring. For the specific case of the recombinant DNA insulin data, it was observed that the Weibull and the lognormal Arrhenius models are suitable for the adjustment of the data in the situation that considers the midpoint of the interval. In the case where the failure time was determined by the lower limit of the interval, the Weibull Arrhenius model fits the data in the study. It found also that the temperature affects the efficiency of the insulin. The higher the temperature, the shorter the efficiency duration. The statements regarding the efficiency of insulins time were reliable and secure. Finally, with the results presented, it is concluded that, through models for accelerated life tests, which provide estimates of the product failure times, manufacturers will not only be able to produce insulin with more quality and reliability but they also will offer products with higher efficiency.

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ILAMBWETSI, P. S.; GOUVÊA, G. D. R.; CRUZ, F. R. B.; OLIVEIRA, F. L. P. LEAL, B. G. Study of efficiency time of recombinant dna insulin via accelerated life testing and interval censoring. *Rev. Bras. Biom*., Lavras, v.36, n.1, p.207-229, 2018.

- *RESUMO: Este trabalho tem como objetivo estudar a eficiência da insulina recombinante de DNA via modelos para testes de vida acelerada. A perda de potência destes produtos de insulina foi avaliada periodicamente, sujeita às condições de temperatura de 8°C, 25°C e 37°C. Amostras de insulina com potência inferior a 100% foram consideradas impróprias para consumo, o que caracteriza o evento de interesse. Amostras adequadas para consumo foram consideradas censuradas. A variável resposta foi observada periodicamente por 736 dias. Para análise dos dados, foram utilizados modelos estatísticos de regressão de resposta ao estresse. A parte determinística desses modelos é o modelo de Arrhenius, pois a variável estresse é a temperatura, enquanto a parte probabilística foi composta pelos modelos Exponencial, Weibull e Log-normal. As técnicas de testes de vida acelerada mostraram-se adequadas para abordar o tempo de perda de potência da insulina para os vários níveis de temperatura. Os tempos de ocorrência dos eventos foram tratados de três maneiras diferentes que foram comparadas neste estudo. Primeiro, a censura intervalar foi considerada, ou apenas os limites superior e inferior do intervalo em que a falha ocorreu eram conhecidos. Em seguida, o ponto médio desse intervalo foi considerado como um tempo de falha. Finalmente, apenas o limite inferior do intervalo em que a falha ocorreu foi considerado. De acordo com os resultados, conclui-se que o uso do limite inferior do intervalo é* *mais adequado para estimar as curvas de confiabilidade, pois as estimativas são mais próximas das que utilizam a censura intervalar, utilizando-se então o ponto médio do intervalo. Para o caso específico dos dados de insulina de DNA recombinante, observou-se que o modelo de Arrhenius-Weibull e o Arrhenius-lognormal são adequados para o ajuste dos dados. Segue-se também que a temperatura afeta o poder da insulina: quanto maior a temperatura, menor a eficiência.*

- *PALAVRAS-CHAVE: Testes de vida acelerados; variável de estresse; censura intervalar; tempos exatos de falha.*

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ATTACHMENTS - Residual charts generated for the estimated models.

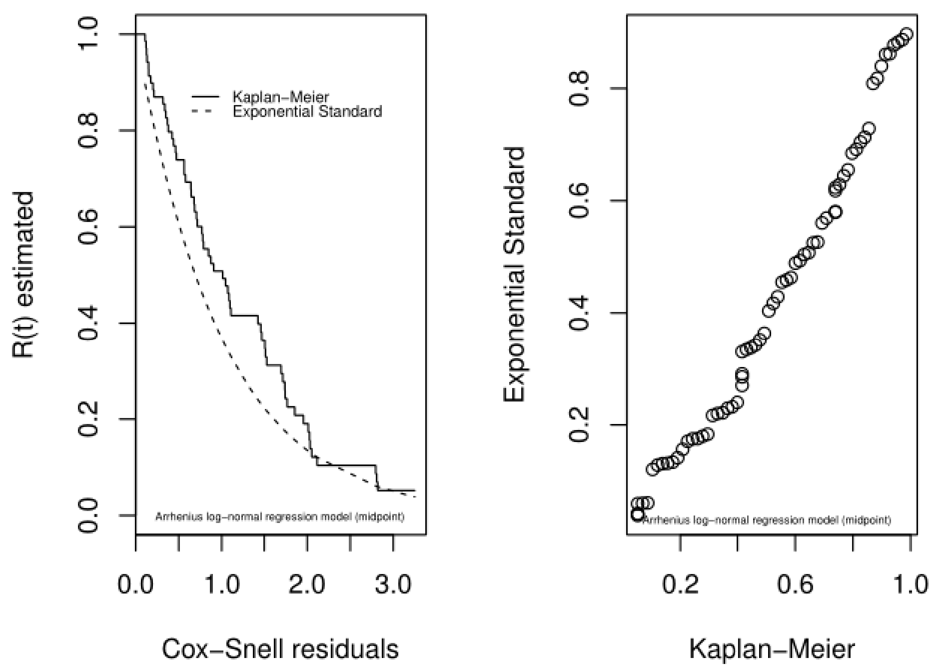

Figure 9 – Cox-Snell residual for the Log-normal model (midpoint).

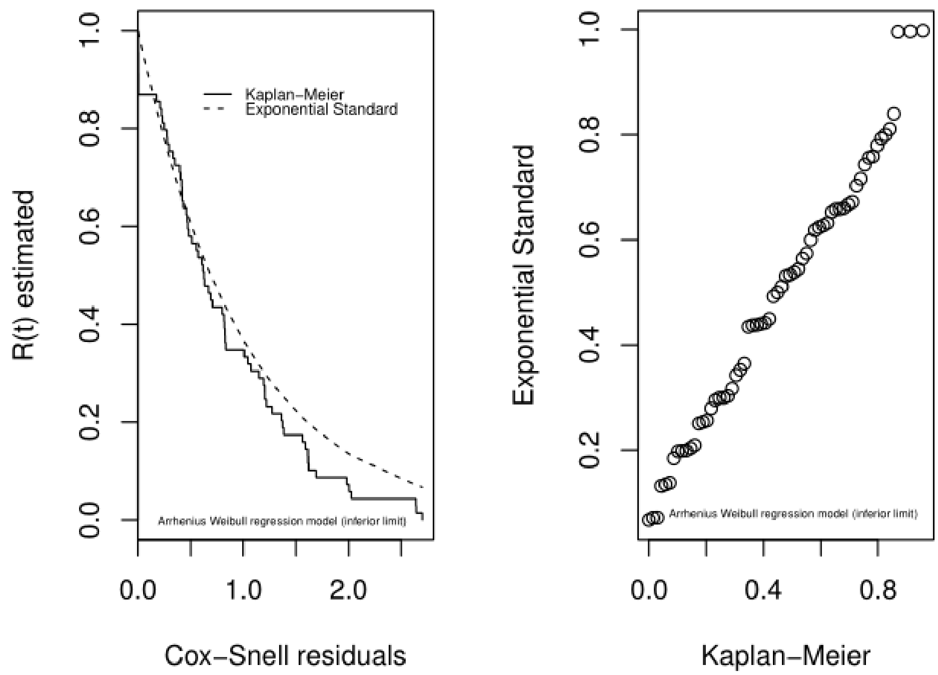

Figure 10 - Cox-Snell residual for the Weibull model (lower limit).

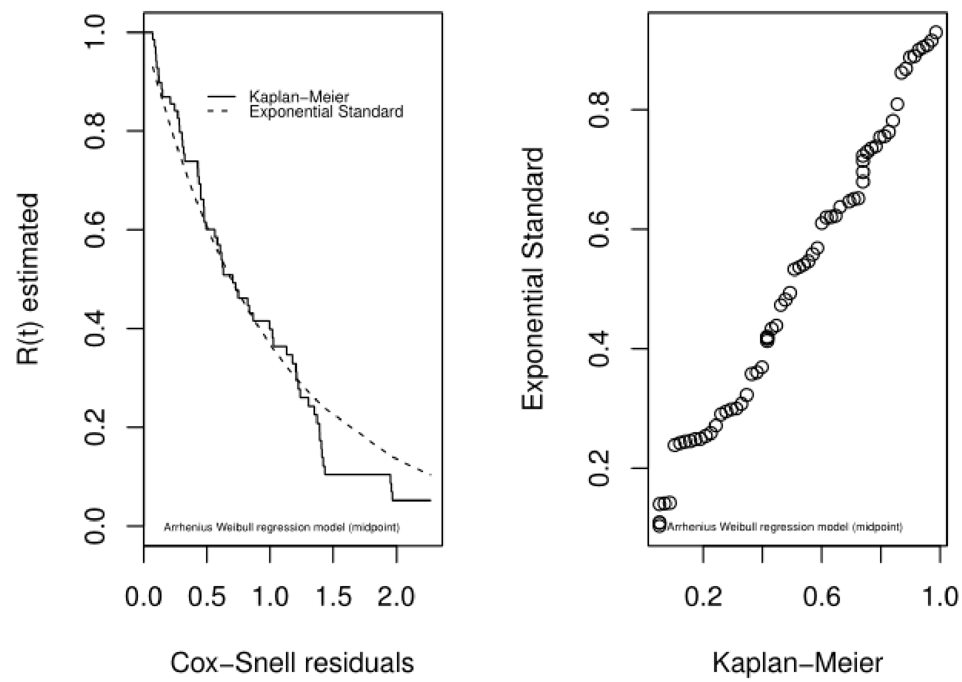

Figure 11 - Cox-Snell residual for the Weibull model (midpoint).