

ASAPprime® Software Technical Notes

Low Degradant Calculations

ASAPprime® Low Conversion Module

The shelf-life of a product can be estimated in a short timeframe using accelerated conditions. A mathematical model is built based on the humidity-modified Arrhenius equation and the results are extrapolated down to storage conditions. A good model requires a good estimate of the time to reach the specification limit at each condition. Very stable products may show little or no conversion in the short time used, preventing a reliable model from being obtained. However, even in these cases, a lower limit of the shelf-life at storage conditions can be estimated.

The Low Conversion tool is a separate calculational domain in ASAPprime® that provides a conservative probability of passing when all conditions show conversions (degradation or loss of potency) that are below a threshold. This calculation is meant to consider a worst-case situation where change above a noise threshold was not observed during an ASAP study. For illustration, we will consider an example with the data shown in Table 1 having a specification limit of 0.20% and limit of detection, LOD, of 0.02%, where the accelerated aging condition was 80°C/60%RH and the long-term storage condition is 25°C/60%RH for two years. The numerical calculations in each section are worked out for this example in green.

Table 1. Data set used as an example.

Time (days)	%Degradant
0	0.04, 0.02, 0.03
7	0.01
21	0.05

Determining when Low Conversion Module is Appropriate

The Tool first runs a check on the data in the degradation table to determine if indeed all conditions can be considered as being low conversion. ASAPprime® uses the criterion that each measured value must be distinguishable from the mean. This is done by the following steps:

- Take the average amount of degradant or potency for any time points that have repeats. In the example, the control has three repeats with an average degradant value of 0.03%.
- Take the average value of all points at a condition, using the average value for any points having repeats. In this case, the overall average is 0.03%.
- The standard deviation, SD, at the control (zero time) is determined. This will be the greater of the LOD, the SD from repeat values (student T), or the SD (as set by the user) divided by the square root of the number of repeats. In this case, that value corresponds to the LOD of 0.02%. This method of calculating SD is used for all points in ASAPprime® because the number of repeats used is not generally sufficiently high to be considered a reliable for calculation of a true distribution.

- The absolute value of the difference between the value at each timepoint and the average value at that condition is determined. This value must be less than 1.645 times the SD at the control for the condition to be considered “low conversion” (i.e., there is only a 10% chance that a random measurement will be outside this range). In this example, the threshold equals $1.645 \times 0.02 = 0.033$. The three data points, using the average of the control as a single point, show deviations from the overall average of 0.00, 0.02 and 0.02, which is below the threshold value.
- If all conditions show low conversion, the program will bring up the Low Conversion dialog, as will occur for this example data set. Otherwise, the user is informed that the dialog is not appropriate; i.e., that some conditions show statistically significant conversions. In some cases, a condition may show data not considered Low Conversion, but falls into the category of “Bad Data” which may not be appropriate for building a model.

Limiting (Minimum) Isoconversion Time

A limiting isoconversion time, $t_{iso-min}$, can be calculated at each condition based on the idea that any signal for change is hidden in the noise when the low conversion threshold is not exceeded. The $t_{iso-min}$ is calculated using only the initial and longest time points used at each condition:

- The conversion value at the initial time is set to one SD below the mean at that condition based on the SD for the control.
- The conversion value at the longest time for that condition is set to one SD above the mean, also based on the SD of the control.
- From these two points, a limiting slope line can be calculated, as shown in Figure 1.

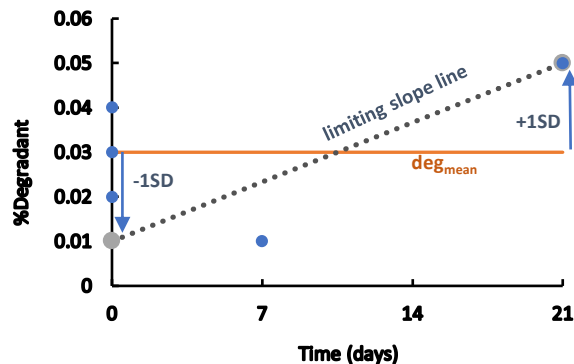


Figure 1. Example showing how the limiting slope line is determined with the low conversion algorithm.

- We can define $t_{iso-min}$ based on the intersection of the limiting slope line with the specification limit (deg_{spec}) as



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shown in Figure 2, where t_{max} is the longest timepoint at a given stress condition:

$$t_{iso-min} = \frac{t_{max}[(deg_{spec} - deg_{mean}) + SD]}{2SD}$$

$$= 21 \text{ days } (0.20\% - 0.03\% + 0.02\%) / (2 * 0.02\%)$$

$$= 99.75 \text{ days}$$

The process is repeated at each stress condition. The true isoconversion time is very likely to be much longer than these limiting values.

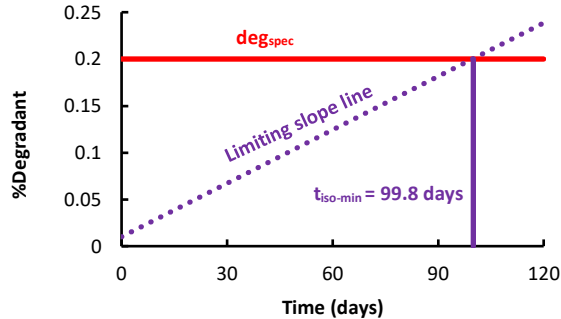


Figure 2. Illustration of determination of $t_{iso-min}$ from the example data set.

Calculation of Uncertainty

Having set a limiting value for the isoconversion times at each condition, the low conversion module in ASAPprime® treats these times as being the centers of normal distributions, as would be done had the isoconversion times been determined by fit to data. When there is sufficient conversion to estimate isoconversion, error bars are assigned in ASAPprime® using an extrema method. In the low conversion case, the conversion values are considered indistinguishable because the values are of the same order of magnitude as the estimated SD. Because the conversion is low, extensive extrapolation is required to reach the specification limit. The error bars resulting from this extrapolation are very sensitive to the exact time points used, which is problematic if we consider the data to be resulting from noise. To provide more consistency, the error bars at each condition are calculated using the SD of the control rather than an error in the fit. Each condition contains the same number of time points as in the original experiment, equally spaced from zero to the longest time.

The confidence interval, CI, for each $t_{iso-min}$ is determined by the following process:

- For each condition, all n time points (t_i) are evenly spaced from zero to the longest time point at that condition, t_{max} (independent of the actual time points used), with the mean time being designated t_{mean} , and SD representing the SD of the control:

$$CI = SD \sqrt{\frac{1}{n} + \frac{(t_{iso-min} - t_{mean})^2}{\sum_{i=1}^n (t_i - t_{mean})^2}}$$

$$= 0.02\% \sqrt{\frac{1}{3} + \frac{(99.8 - 10.5 \text{ days})^2}{(0 - 10.5 \text{ days})^2 + (10.5 - 10.5 \text{ days})^2 + (21 - 10.5 \text{ days})^2}}$$

$$= 0.121\%$$

- We can determine the probability that the actual conversion is as fast as indicated by the limiting slope line. Our threshold for considering a data set as being low conversion has a cut-off of a p-value of 0.2. This means that only 10% of the time, the data set results from random scatter about the limiting slope line (when the slope is positive). We therefore set a 10% value at the isoconversion time ($t_{iso-min}$) for the probability of the true isoconversion time being this value or lower. An adjusted SD at $t_{iso-min}$, SD_{adj} , is calculated based on where 90% of the values for a normal distribution have lower conversions based on the calculated CI at $t_{iso-min}$. For a normal distribution, 1.282 times the SD corresponds to a one-sided distribution of 90% probability:

$$SD_{adj} = \frac{1.282CI}{\frac{2t_{iso-limiting}}{t_{max}} - 1}$$

$$= \frac{1.282 * 0.121\%}{\frac{2 * 99.8 \text{ days}}{21 \text{ days}} - 1} = 0.018\%$$

- A new line can be drawn defining the limits around deg_{mean} ; i.e., $-SD_{adj}$ at the origin and $+SD_{adj}$ at t_{max} . The intersection of this new line with the specification limit is the adjusted isoconversion time, $t_{iso-adj}$, Figure 3.

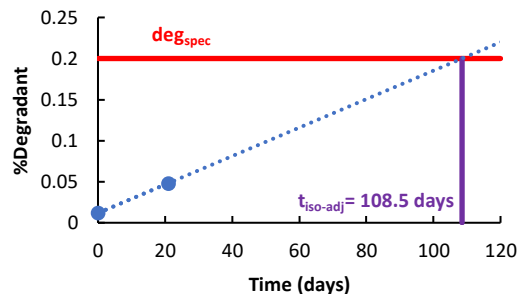


Figure 3. Adjusting the isoconversion time based on the confidence interval.

The adjusted isoconversion time is calculated in the following equation:

$$t_{iso-adj} = \frac{t_{max}(deg_{spec} - deg_{mean} + SD_{adj})}{2SD_{adj}}$$



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$$= \frac{21 \text{ days}(0.20\% - 0.03\% + 0.018\%)}{2 * 0.018\%} = 108.5 \text{ days}$$

- d) A new distribution is defined centered at $t_{iso-adj}$ with the final confidence interval, CI_{final} , recalculated at that point, again assuming an even distribution of the points:

$$CI_{final} = SD \sqrt{\frac{1}{n} + \frac{(t_{iso-adj} - t_{mean})^2}{\sum_{i=1}^n (t_i - t_{mean})^2}}$$

$$= 0.02\% \sqrt{\frac{1}{3} + \frac{(108.5 \text{ days} - 10.5 \text{ days})^2}{(-10.5 \text{ days})^2 + (0 \text{ days})^2 + (10.5 \text{ days})^2}}$$

$$= 0.133\%$$

Propagation to the Storage Condition

Once $t_{iso-adj}$ and the CI_{final} have been determined, an estimate of the shelf-life and probability of passing at storage conditions must be made. When ASAPprime® builds an Arrhenius model, the shelf-life can be determined from the humidity modified Arrhenius equation:

$$\ln k = \ln A - \frac{E_a}{RT} + B \cdot RH$$

The temperature (T) and humidity (RH) are for the storage conditions. If the Arrhenius parameters were known, then the shelf-life based on the isoconversion time at each condition could be determined by extrapolation using the equation:

$$shelflife_{Ts,RHs} = t_{iso-adj_{Ta,RHa}} e^{\left(\frac{E_a}{R}\right)\left(\frac{1}{T_s} - \frac{1}{T_a}\right) - B(RH_s - RH_a)}$$

The subscript *s* refers to the storage condition and the subscript *a* refers to the accelerated condition. The condition that yielded the longest shelf-life would be the one used for the calculation of probability of passing, since if the shelf-life were actually shorter, conversion should have been seen at that condition.

Since the scenario we are dealing with does not provide sufficient fitting data to estimate Arrhenius parameters, an alternative is to use historical data. Since the goal is to obtain a reasonable limit on the shortest shelf-life that might be obtained from the data, Arrhenius parameters at the ends of the distributions obtained from prior studies are used. Normally, one extrapolates to lower temperature, so an activation showing little temperature dependence, i.e., a low activation energy, is appropriate. The default in the program is 11.5 kcal/mol (48.1 kJ/mol), which corresponds to where 95% of products studied to date show greater activation energies. The humidity at the accelerated conditions might be higher or lower than storage conditions. No B value is assumed when extrapolating down (no humidity dependence), while a B value of 0.1 is used when extrapolating up (high humidity dependence). Users have the option of assigning different values for the assumed activation energy and B values, which admittedly are

conservative on top of the other conservative assumptions in these calculations. To adjust these parameters, from the main menu bar, Options → Isoconversion → Low Conversion. The choice of 11.5 kcal/mol (48 kJ/mol) for E_a is very conservative, which is compounded with other conservative assumptions used in the calculations. It should be noted that the usual guideline recommendations, ignoring any RH impact, suggest that 6 months at 40°C is equivalent to 2 years at 25°C. This corresponds to an activation energy of 17 kcal/mol (71 kJ/mol).

ASAPprime® uses CI_{final} to calculate the distribution of isoconversion times around $t_{iso-adj}$. The values can be converted to rates at the accelerated conditions then propagated to the storage conditions using the Arrhenius parameters assigned. For each time element, an amount of degradant (or loss of potency) is calculated at the mean and desired confidence interval. The program can also use these data to calculate the probability of passing as done with ASAPprime when a model is built. For this example, the probability of passing at two years is 99.6% (Figure 4).

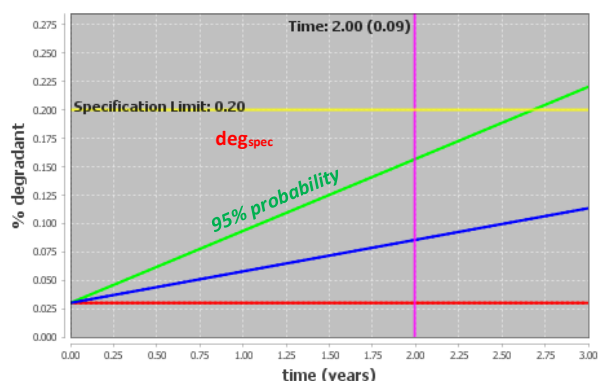


Figure 4. Degradant curve based on the low conversion module with the example data set.

The value of the time to failure will increase when longer storage times are tested at higher temperature stress conditions. It will also increase with decreasing SD at the initial point; however, the lower SD (LOD) can result in the program not considering the data as “Low Conversion.” In many cases, the SD can be decreased by better resolution (LOD for degradation), or more repeats for potency.

Conclusion

ASAPprime® incorporates a methodology for assigning a minimum shelf-life when accelerated conditions do not show statistically significant changes. This methodology is not a stability model and therefore is not meant to be predictive. Instead, the calculations should enable users to have confidence, where appropriate, that a product will pass a stability specification at an assigned shelf-life.

