StabilityPlus™ Quick User Guide

StabilityPlus is a Microsoft Excel add-in from Biostat Plus, LLC to perform pharmaceutical stability and related statistical analyses, and to generate formal stability analysis reports rapidly and seamlessly.

Performing stability analysis using a general-purpose statistical software can be time consuming and clumsy. Usually, a general-purpose software (SW) does not provide batch specific intercept and slopes directly as StabilityPlus does. Also, a general-purpose software may not directly give the estimated shelf-life. To get individual batch intercepts and slopes in a general-purpose SW, the batch information will need to be coded using dummy variables. To estimate shelf-life, confidence interval plot should be examined, and the shelf life is estimated and recorded manually. StabilityPlus includes the estimated shelf-life in the stability analysis results.

StabilityPlus can perform common slope (CS), separate slopes (SS) and common line (CL) stability regression models; shelf-life estimation per ICH guidelines Q1E and Q1A (**Reference 1** and **Reference 2**), the time value at which the 95% one-sided confidence coincides with the registered specification; and release limit calculations (**Reference 3** and **Appendix A**).

In a SS model, each batch (or lot) is allowed to have its own slope (non-parallel regression lines) and intercept; in CS model, all batches share a same slope (parallel regression lines); in CL model all batches share a same intercept and a same slope. Some attributes are known to change fast initially but the rate of change slows down later and then the overall change somewhat levels off after some time. This behavior has been observed, for example, with residual moisture of lyophilized products (**Reference 4**). This non-linear trend can be modeled by linearizing the rate with respect to fractional power, such as square root, of time. In StabilityPlus, users can perform non-linear regression models by linearizing with respect to fractional power of time (time^(1/k)) or exponential time (-exp(-k*time)). To do these analyses, the user can iteratively set k (> 0) that fits the data well.

Note 1: Between the two transformations StabilityPlus provides, time^(1/k) transformation may be preferred, since it is simpler and adequate. This transformation has some desirable properties. It increases with increasing time, and at time = 0 the transformed value is also 0. Additionally, when k = 1, the transformed values are the same as the original values resulting in a model equivalent to the linear model. This transformation fits the data adequately well. The best value for the parameter k can be reached by trial and error by entering a value in cell AT13 (see **Data Input** below).

StabilityPlus generates formal reports with analysis summary, trend plots and statistical details. The data can be shown as scatter plot, connected plot, or regression line plot. The analyses use live Excel functions, but the functions are hidden from the user to protect the functional integrity of software. They cannot be altered by the user. The built-in integrity checking feature will flag any unlikely event of tampering any function. The analyses must pass functional integrity before preparing a report.

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Since the model calculations are live using Excel spread sheet functions, StabilityPlus serves as a valuable tool to solve problems using what-if type analyses. Built-in regression residuals and approximate assay date calculation and plots can be used as diagnostic tools.

Note 2: Since the calculations are live, it is recommended to turn the Excel AutoSave option off.

Note 3: When Excel is launched by opening an email workbook attachment, StabilityPlus add-in is not activated. Download email attachment and open or launch Excel first and then open the email attachment.

Data Input

To do a stability analysis, data can be entered directly into the data area (**Figure 1**) of analysis worksheets or preferably imported from another analysis or a data worksheet. The data area spans the range A3:AJ64. Batch names are entered in B3:AJ63, time values in A4:A63 and attribute values in B4:AJ63 in the cells corresponding to appropriate batch column and time value row. Optionally, date of manufacture (DoM) of batches can be included in B64:AJ64. The data area and thus StabilityPlus can accommodate up to 35 batches and 60 time point values. The batches can be entered in any of the 35 batch locations in any order and similarly the time values can be entered in any of the 60 time point locations in any order. However, entering batches in chronological DoM and increasing time values is recommended. Any text entered in the data area is ignored in the calculation. Thus, values below the limit of quantitation (LOQ) such as <0.5 can be shown in the report but without using in the calculation. Similarly, out of trend results can be shown but excluded from the analysis by enclosing within square brackets. This text display feature can also be used to show lot specific information such as dosage as text, if lots from multiple dosages are combined in an analysis. If the date of enrolling in stability differs from DoM, this information can be captured in a row. If the time value is a text, the entire row is excluded in the analysis but displayed for information.

In **Figure 1**, the out of trend value 280 for 6-month point for Lot 3 is excluded from the analysis by changing it to a text, i.e., enclosing within square brackets. Texts and blank time point rows appear gray in the data area. Analyses can have multiple rows with the same time value, to indicate replicate results.

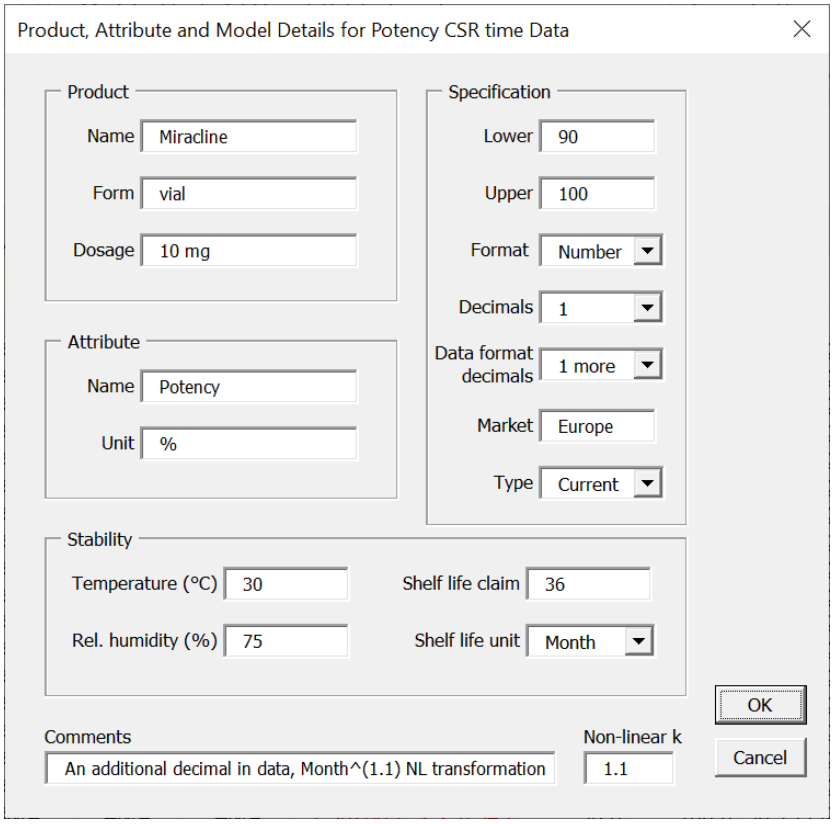
If it is necessary to copy and paste the data manually into the data area, they must be pasted only as value. This is because, integrity check will fail if format is changed.

Other details such as product name, attribute name, unit, storage conditions, etc. can be entered through a dialog as shown in **Figure 2**.

Figure 1. Data area

Any Product Any Attribute 0°C Any Attribute (Any unit)										
Month	**Lot 1**	**Lot 2**	**Lot 3**	**Lot 4**	**Lot 5**	**Lot 6**	**Lot 7**	**Lot 8**	**…**	**Lot 35**
0	100	125	150	175						
3	125	150	175	200						
6	150	175	[280]	225						
9	175	200	225	250						
12	200	225	250	275						
18										
24										
36	any	text	is	ignored						
48										
…										
time 36										
DoM	2Jan15	3Jul15	20May16	11Oct17						

Figure 2. Dialog to enter other analysis details



Data can also be imported from a general data worksheet. Two different formats are allowed. Minimally batch (or lot), stability time (month) and attribute time point results must be provided.

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The first format is a matrix format similar to the data area. Within the rows 1 through 5 of column 1, stability time value heading such as “Month”, “Day”, “Week”, “Time”, etc. must be included. In the same row, batch/lot names should be included in columns 2 through 36. Below this row, time values should be included in column 1 in up to 60 rows. Similarly, attribute time point results should be included in columns 2 through 36, corresponding to the batch/lot and time value as a matrix. Optionally, date of manufacturing of each batch/lot can be included in the next row below the time values. In that case, column 1 that row must say “DoM”.

Note 4: When giving time values in column 1, skipping any rows is not allowed. In fact, a blank for time value would indicate the end of time values and attribute results, unless the optional DoM is included.

The second format is a list where the first row is used as the heading row. Heading for first column must indicate “Batch” or “Lot”. Batch or lot names must appear in first column. Another required heading is for stability time values column such as “Month”. Additional recognizable optional headings are: “Product”, “Form” and “Dosage” for product description; “Temperature, °C “ and “Humidity, %RH” for the storage condition; “Shelf Life Claim” and “Market” for the product; “Unit”, “Lower Spec” and “Upper Spec” for the attribute; and “Comment” for any other information. Value for these columns, if present, are read from the second row. An example data listing is provided in **Figure 3**.

Note 5: In the second format, when data for several batches/lots are present, the first found lot will automatically be selected for a single lot analysis.

Figure 3. A section of an example data worksheet

	A	B	C	D
1	Batch	Month	Attribute	DoM
2	A	0	7.44	25May08
3	A	6	6	25May08
4	A	12	6.57	25May08
5	A	18	6.54	25May08
6	A	24	6.59	25May08
7	A	36	6.53	25May08
8	B	0	7.52	5Jun08
9	B	6	6.49	5Jun08
10	B	12	6.67	5Jun08
11	B	18	6.54	5Jun08
12	B	24	6.57	5Jun08
13	B	36	6.57	5Jun08

As before, the optional information can also be entered through the dialog shown in **Figure 2**.

For non-linear models, the transformation parameter k can also be entered in cell AT13. The best value for the parameter can be reached by trial and error (for minimum standard error of residuals).

Note 6: Time values in the range A68:A79 (A68:A92 for non-linear models) used in prediction can be changed from their default values.

General Settings

Dialog shown in **Figure 4** is used to set some general settings that will used for any future analyses. They can be changed any time.

If a batch does not meet certain the minimum requirements for available data for the batch, the batch name will be highlighted in red. The batch will still be included from the analysis unless it is explicitly excluded by deleting the data or changing the data to text. In the example in **Figure 4**, a batch must have at least 5 points and data at least up to 0.25 times the claimed shelf-life. For shelf-life of 48 months, 0.25 fraction is at least 12 months data.

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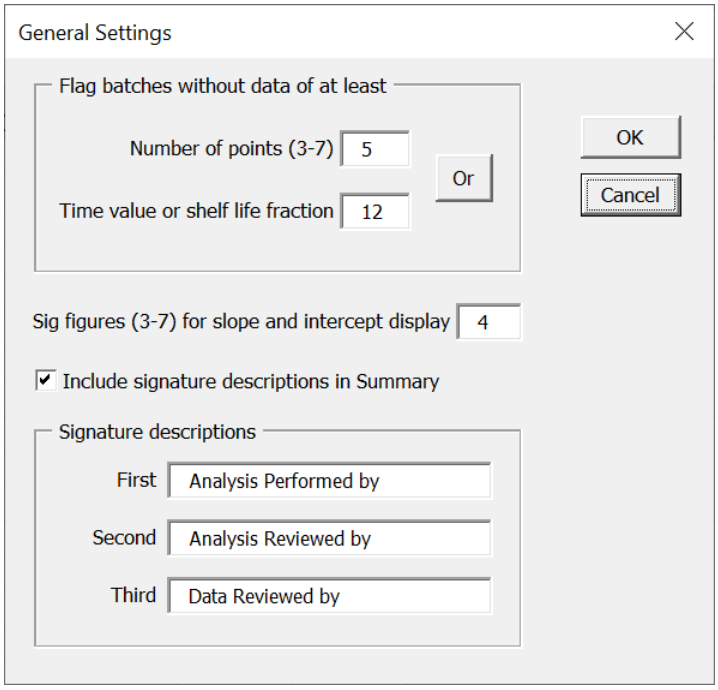
Figure 4. General settings dialog



The finished stability analysis report can have a signature area with up to three signature designations. Number of significant figures for displaying slope(s) and intercept(s) can also be set in general settings.

Model Names

During a stability model creation, the stability data can be imported from another tab of the active workbook or another workbook. The data can also be imported into an existing model or after creating model sheets with built-in data as a placeholder. When data are imported into an existing model, the existing data will be completed replaced by the imported data. If the data are imported from another model, the data description and formats are also imported.

A stability model has two associated tabs for the models that are linear with respect to time; one tab for the data and analysis results, the other for the plot. Non-linear models have two pairs of tabs, one pair for the original time scale and the other for the transformed time scale. Model tabs can be renamed, and descriptive names are recommended. Recommended naming convention for analysis has three parts: short description, model type (CS, SS or SL) and model sheet type (Data or Plot). The short description can include product name, attribute name and any excluded data. When StabilityPlus creates a new model by reading the data from an existing model, it gives a similar name to the new model by replacing the strings CS, SS or CL in the name accordingly respectively for Common Slope, Separate Slopes and Common Line models. If the stability regression model is linearized using a transformation of time, then recommend model type string is such as CSP time for the time scale sheet of power (root of time) transformation, and CSE time for exponential transformation. Similarly, CSP ^time and CSE ^time are recommended for transformed counterparts.

When a model data sheet is renamed, StabilityPlus automatically also updates the stability analysis summary (see below) and, if present, release limits, approximate assay date and residual analyses with the new name. However, the summary should be reviewed before finalizing the stability analysis report.

Stability Analysis Summary

Stability analysis summary serves as the cover page for the analysis report. It can be created anytime. The summary worksheet becomes the first tab of the stability analysis workbook.

Make sure all model names in the summary are correct. To exclude a model from the summary, it is enough to delete the entry of model sheet name from the summary sheet. Any resulting errors in summary due to the erased worksheet name is considered extraneous and will automatically be deleted when finalizing the analysis workbook.

Notes summarizing the analysis results and conclusions can be added to Summary tab. Similarly, if release limit analyses are present, notes can be added to release limit analysis tab(s). A menu option is available to edit the notes and to spell check in Word. After editing in Word, the notes can be copied and pasted to the analysis summary or release limit sheets.

Release Limits Calculations

Details of release limits calculations are provided in **Appendix A**.

Release limits can be calculated by entering the stability analysis worksheet name in the worksheets area (A23:A40) of the Release limits analysis.

Note 7: Calculations in Release limit analysis require some custom functions available only when StabilityPlus is installed in your computer and the Excel analysis workbook resides in a drive mapped to a drive letter. If you see errors, make sure that StabilityPlus is installed and the workbook is saved and resides in a drive mapped to a drive letter (i.e., the workbook is newly created and not saved or resides at an internet cloud location). If the error persists, either of the following will correct the error: a) save and close the analysis workbook and reopen, b) re-enter worksheet names in A23:A40.

In addition to this, Allen-Dukes-Gerger release limits can also be calculated in situ (in the same worksheet as stability analysis) using Additional Functions menu option. Make sure that the registered shelf-life is one of the time values in the range A68:A79 (A68:A92 for non-linear models).

Creating and Working with the Stability Models

StabilityPlus highlights certain results such as batches not having minimum required data, values exceeding specification, significant slopes, shelf-life not met, etc. in red. This can be helpful when reviewing the results and reports. The font color in certain areas is considered integral part of analysis. It is advised not to change font color in the analysis. If data for a lot are missing, you may see errors for the lot. This is normal and does not affect the overall analysis results. This will be cleaned in the report when finalizing the analysis workbook.

Data formats, plot axis scales, plot types (Fitted, Connected or Scatter can) can be changed using model settings and/or plot format dialogs of StabilityPlus. While plot axis scales can be conveniently set using StabilityPlus, they can also be set using regular Excel interface thus maintaining the flexibility. StabilityPlus settings override Excel default settings; manual settings by the user override StabilityPlus settings.

After the summary and release limit notes are completed (please see stability analysis summary), the model can be finalized. When finalizing, StabilityPlus checks the functional integrity of all analyses in the workbook using a proprietary algorithm, cleans up (or hides) extraneous items, and then locks (password protects) the analyses and plots from further change. Passing or failing status from integrity check is prominently displayed for all analyses. If a model did not pass integrity, it might be due to some inadvertent change to functions, model analysis output, or unallowed format change by the user that interferes with the built-in formats used for flagging such as out of specification (OOS) results, batches with limited date, etc. In that case, it is recommended to recreate a new model using the same data, the delete the model that did not pass the check and not change formats.

Note 8: It is not possible to know what change caused the integrity failure.

In the finalized state, page brakes can be added to model worksheets and a report can be generated. Models can be deleted, but to edit an existing model or create a new model, the existing model or the workbook should be changed to unfinished state.

The formal report, a PDF file, includes stability analysis summary, model plots and model results; optionally, the PDF report can have only the summary and plots. The plots can be exported to a Word document allowing them to be ported to any other documents.

References

1. ICH guidelines Q1E Evaluation for Stability Data
2. ICH guidelines Q1A Stability Testing of new Drug Substances and Products
3. Allen PV, Dukes GR, Gerger ME (1991) Determination of Release Limits: A General Methodology. Pharm Res 8(9):1210–1213
4. Vromans H, van Laarhoven JAH (1992) A Study on Water Permeation through Rubber Closures of Injection Vials. Int J of Pharm 79:301-308

Appendix A

For some products such as biopharmaceuticals, the methodology reported in **Reference 3** by Allen, Dukes and Gerger (ADG) can be too limiting due to high analytical variability. The approach proposed here assumes that extreme results observed in a steady state process (process in control) are due to extreme assay result. For an extreme release result, the true value is more likely lie towards the process center than lie further outside. This allows to reduce the uncertainty adjustment outside the release result. As a practical approach, the uncertainty is based on the standard error of mean result as the mean would be calculated from the proposed number of stability time points for a batch, even though at the time of product release only the release result is available. This will be smaller than the standard error of the residuals, i.e., the standard deviation due to analytical method that would allow full uncertainty outside the release result as proposed in **Reference 3**.

For example, if the proposed stability time points are at 0, 6, 12, 18, 24 and 36 months, i.e., 6 points, the standard error of mean is standard error of residuals ÷ √6. This approach can also be thought of the same as ADG method but applying a lower outward uncertainty to the release result as though the number of replicates in release result is more than one, the same number as the proposed number of stability time points for a batch.

Release limit analysis in StabilityPlus calculates release limits using both ADG and the modified method. To provide some helpful insights, StabilityPlus can be asked to calculate release limits based on separate slopes models using the minimum and maximum observed slopes. Formulas used to calculate release limits are summarized below.

For Mean of a Batch in Stability

[1] Stability Change (ADG)

$$\text{spec} - \text{slope} \times \text{life} \pm t_{0.1} \times \sqrt{(\text{s.e. slope} \times \text{life})^2 + (\text{s.e. residual})^2}$$

[2] Stability Change (Conservative release uncertainty)

$$\text{spec} - \text{slope} \times \text{life} \pm t_{0.1} \times \sqrt{(\text{s.e. slope} \times \text{life})^2 + (\text{s.e. residual})^2 \div n}$$

[3] No Stability Change (ADG)

$$\text{spec} \pm t_{0.1} \times \text{pooled sd}$$

[4] No Stability Change (Conservative release uncertainty)

$$\text{spec} \pm t_{0.1} \times \text{pooled sd} \div \sqrt{n}$$

For Individual Time Point of a Batch in Stability

[5] Stability Change (ADG)

$$\text{spec} - \text{slope} \times \text{life} \pm t_{0.1} \times \sqrt{(\text{s.e. slope} \times \text{life})^2 + (\text{s.e. residual})^2 + (\text{s.e. residual})^2}$$

[6] Stability Change (Conservative release uncertainty)

$$\text{spec} - \text{slope} \times \text{life} \pm t_{0.1} \times \sqrt{(\text{s.e. slope} \times \text{life})^2 + (\text{s.e. residual})^2 + (\text{s.e. residual})^2 \div n}$$

[7] No Stability Change (ADG)

$$\text{spec} \pm t_{0.1} \times \sqrt{2} \times \text{pooled sd}$$

[8] No Stability Change (Conservative release uncertainty)

$$\text{spec} \pm t_{0.1} \times \sqrt{1 + 1 \div n} \times \text{pooled sd}$$

Note: For common slope or common line model, slope is the common slope. For separate slopes model, slope is the minimum slope for the lower limit or the maximum slope for the upper limit. Using separate slopes model to calculate release limits is not recommended.

ADG: Allen, Dukes and Gerger, **Reference 3**.

Conservative release uncertainty: Release uncertainty is calculated for the mean of scheduled time points for a batch. This calculation is equivalent to assuming n replicates for release. StabilityPlus ignores release uncertainty altogether if $n = 0$.

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