

Analyzing calibration curve data

- [QuickStart > Browse Project > Results or Result Sets tab > right-click data you want to use, and click Review > Review Data Main window > Window > Calibration > Calibration Curve window](#)

When you process calibration standards (if you have entered the proper information), the software generates a calibration curve for each component listed in the Components table of your processing method. Each resulting component calibration curve appears in the Calibration Curve window.

Requirement: View Numerical and Textual Results in Review privilege. Users without this privilege can view only the 2D chromatogram, with the baseline drawn for processed data. Users can still process peaks automatically or manually and the software recalculates the peak results, but the results are not visible.

You can review the following information on the calibration curves:

- Calibration status results - Displays information about the current calibration curve
- Calibration curve plot - Displays the calibration curve
- Points table - Displays the calibration point data used to produce the curve. (The software displays averaged points when the processing method specified average points.)

The points in a calibration curve are generated differently depending on whether you use external or internal standard calibration.

Viewing the calibration status results

The software displays the following information about the calibration results associated with the specified component:

Field	Information for calibration results
Method	Name of the processing method on which the calibration is based.
Date/Time	Date and time at which the calibration curve was generated.
System	Chromatographic system used to acquire the data that was processed to produce the calibration curves.
Channel	Name of the 2D channel that was processed to produce the calibration curves and the data that is quantitated using the curves.
Component	Name of the component for which the calibration curve was made. Select different curve component names from the list. Selecting a different component in the list selects that component in the Components table associated with the processing method and that peak in the current result.
Time (min)	Retention time of the calibration curve.
Equation	Equation of the calibration curve. How the equation is calculated depends on the associated fit type.
R ²	Coefficient of determination value for the calibration curve.
R	Correlation coefficient value for the calibration curve.
Standard Error	Standard error of calibration for the calibration curve.

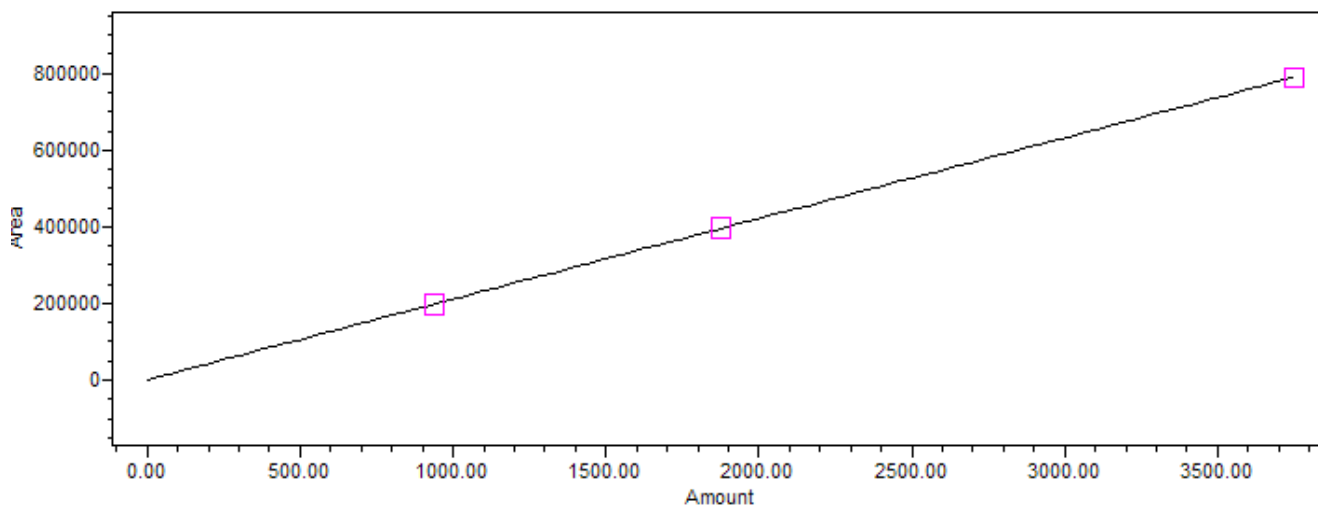
Field	Information for calibration results
RSS	Sum of the squares of the difference between the measured response and the calculated response of the calibration point (Residual Sum of Squares).
RSD	Relative standard deviation of the calibration curve.
RSD(E)	The line relative standard deviation.
Weighting	How the points were weighted when the calibration curve was calculated.
Codes	Informational and/or warning codes that the software encountered while calculating the calibration curve.
Normalized Intercept/Slope	Calculated for Linear and Linear Through Zero curves only.

Viewing the calibration curve plot

The calibration curve displays the response as a function of X value (as specified in the Components table).

External standard

An external standard generates a calibration curve where the detector response (the peak's Y-Value) is plotted as a function of the X value (amount, concentration, or custom field, depending on the selection in the processing method).



Internal standard

An internal standard generates a calibration curve where the response (calculated as the peak's Y-Value divided by the Internal Standard peak's Y value and multiplied by the Internal Standard's X value) is plotted as a function of the component's X value (amount, concentration, or custom field).

$$\text{Response} = \frac{\text{Y Value}}{\text{Y Value}_{\text{IS}}} \times \text{X Value}_{\text{IS}}$$

Equation for the calibration curve

The equation for the calibration curve is based on the fit type you specified in the Component table. There are three calibration curve types:

- Linear – Allows you to select different fits to a single level or multilevel calibration curve, including Linear, response factor, inverse linear, log-log linear, and power curve.
- Non-Linear – Allows you to select different fits to a multilevel calibration curve, including cubic spline, point-to-point, linear, quadratic, cubic, fourth-order, and fifth-order curve fits.
- Forced through Zero – Allows you to select the forced-through-zero options for linear, quadratic, cubic, fourth-order, fifth-order curve fits.

Tip: If required, you can enable weighting of some multilevel fit types in the processing method.

If the slope of an LC calibration curve is zero at any point along the X-axis from 0.0 to the maximum X-value of the points in curve, the LC calibration curve is marked invalid. This means that the Valid column is not checked in the Peak Calibration Curve table of the Results window and a Curve is invalid for quantitation message appears in the Calibration Curve window. Although the software tries to use an invalid calibration curve for quantitation, it may yield incorrect results.

Using custom fields for the X-value

In addition to the selections of Amount and Concentration, Empower can use a custom field for the X-value. You select the X-value field from a list in the Component table of the processing method. Empower displays all real custom fields in addition to amount and concentration. This X-value is used to plot the calibration curve.

Empower calculates the custom field value for calibrated peaks and calculates it using the curve for quantitated peaks. When the X-value is a custom field, Empower uses the Sample Weight and Dilution values (if entered) to scale the amount and concentration for calibrated peaks so that the scaled amount or concentration can be used to calculate the X-value custom field for the standard peaks.

Empower does not use the Sample Weight and Dilution (if entered) to scale the X-value custom field reported for the quantitated peaks. In the Peaks table of the Review Data Main window, the Amount, Concentration, and % Amount fields are blank for all quantitated peaks except for Internal Standard peaks.

The Peak Calibration Curve table of the Results window includes the following three fields to help you assess the state of your calibration curve:

- X-axis displays the field used for X-value of the Curve
- Y-axis displays the field used for Y-value of the Curve
- Points displays the number of points in the Curve

Determining how close the calibration points come to the LC calibration curve

As an indication of how close the calibration points come to the curve (the precision of the data), Empower displays the Line Relative Standard Deviation in the RSD(E) field, at the top of the Calibration Curve window. Empower calculates this field using the following percent relative standard deviation formula:

$$\%RSD(E) = \frac{E}{\bar{y}} (100)$$

where:

E = Standard Error of Calibration

\bar{y} = weighted mean of response of the calibration points

Calculating the normalized intercept/slope value

Empower calculates and displays the Normalized Intercept/Slope ratio for calibrations using the Linear and Linear through Zero fit type (set in the processing method). Empower calculates the normalized intercept/slope value using the following formula:

$$\text{Norm Intercept/Slope} = (A / B) / ((X_{\max} + X_{\min}) / 2)$$

where:

A = intercept of the linear calibration curve

B = the slope of the linear calibration curve

X_{\max} = maximum X Value of the points used to calculate the calibration curve

X_{\min} = minimum X Value of the points used to calculate the calibration curve

Viewing calibration curves

Before you process data, view the calibration curve to determine how close it is to the chosen fit type.

To view calibration curves:


From the Calibration Curve window, click the component whose calibration curve you want to view.

Tip: You can view saved calibration curves in the Curves table view of the Browse Project window.

To view calibration curves associated with a processing method:

1. From Browse Project, click either unprocessed data or results (depending on your use).
2. Click File > Open > Method and select the appropriate processing method from the list.

Alternative: If you use results, a processing method and set of calibration curves is already associated with each result.

3. Click Calibration Curve .
4. Click Options > Select Calibration. A list of calibration curves associated with the current processing method appears.

Restriction: The Select Calibration selection is disabled if the current processing method is named Untitled or if the Calibration Curve window is not active. Open a previously saved processing method (click File > Open > Processing Method) to activate this selection.

5. Select the calibration curve to use, and then click OK.

Result: When there is no current 2D channel, the list of calibration curves is not restricted to a specific

system-channel pair. When there is a current 2D channel, the list is restricted to those curves with the same channel and system as the data.

Requirement: If you select a calibration that is not the most recent one stored in the database, a message box prompts you to either:

- Click OK and open the older calibration
- Click Cancel to return to the list of calibration curves and make another selection

If you select the same calibration that is already open, a message box prompts you to do one of these actions:

- Click OK, and then reopen (or reset) the current calibration
- Click Cancel to return to the list of calibration curves and make another selection

Modifying calibration curves

You can add, delete, or modify calibration the data.

To modify calibration curve data:

1. In the lower pane of the Calibration Curve window, click the Individual Points tab.
2. To modify a point, click either a row number in the Individual Points table or the data point in the displayed calibration curve.

Result: The data point, and its corresponding row in the table are highlighted.



3. Modify the X value or response as needed.

Result: The software automatically applies the new value.

4. To ignore one or more points in the Individual Points table, select the Ignore check box for the desired point(s), and then click Process > Calculate Curve to apply the changes.

Result: The ignored points in the calibration curve appear with an X through them.

5. To display a different component's calibration curve, do one of these actions:

- Select a different component from the Component list
- Click Next Component  or  Previous Component

Saving calibration curve data

If you make changes to your calibration data you will want to save the associated changes to the curves in the database.

Tip: If the calibration curves do not appear in the Curves view of the Browse Project window, click Update to ensure the view contains the latest curve data.

To save modified calibration curve data:

1. From the Calibration Curves window, click File > Save As > Calibration.

Tip: If you are saving calibration curves containing points from different versions of the same method (or from a method before it was saved with a different name), the software informs you that the calibration includes points from standards processed by processing methods other than the current method. Click on one of the following to save your data:

- Save – Saves all calibration points, and the points from the non-current processing methods are labeled as manual points to indicate that the standards were processed with different processing methods than the method with which the calibration is saved.
 - Cancel – Cancels saving changes to the calibration data.
2. To discard changes, click File > Reset > Calibration.

Tip: When you save a calibration curve, the software saves the results for the standards used to generate the calibration curve.

Using older calibration curves

When your current calibration curves do not have points in them (for example, you cleared calibration or you saved changes to a modified processing method and did not copy the curves) you are unable to perform quantitation without recalibrating your standards. You can use older calibration curve data associated with either the current processing method or with an older processing method to process your data.

Older calibration curve data can be from these two sources:

- associated with a processing method (either the current or another processing method)
- linked to results

To use older calibration curves for processing:

1. Depending on the source of the calibration curve data, do one of these actions:
 - To use calibration curves associated with the current processing method, first click Options > Select Calibration, click on the desired calibration, click OK, and then proceed to step 3
 - To use calibration curves associated with another processing method, click File > Open > Method, and then select the processing method
 - To use calibration curves associated with a processed result, click Browse Project > Results, and select the result. Right-click and select Review.

2. Click Calibration Curve .

Result: The software displays the calibration curve and data for the first component.


3. Click File > Save As > Calibration, and then click OK.
4. Save the calibration under a new and unique processing method name, and then click OK.

Using modified calibration curves

If you modified the calibration curve data and recalculated the curve, you can apply the calibration curve changes to the quantitation by reprocessing your unknown samples using the processing method associated with the calibration curve you just modified.

To modified calibration curves to quantitation:

1. Click the 2D channel you want to quantitate.
2. Click File > Open > Processing Method, and then select the processing method associated with the calibration curve you just modified.

3. Click Calibration Curve .

Result: The software displays the calibration curve and data for the first component.

4. Click Integrate .

5. Click Quantitate .

6. Click Results  and review the result.

7. To save the result, either click File > Save > Result or click File > Save > All.

! **Notice:** Do not reprocess your standards. Doing so adds additional points to the curve already created.

Restriction: The Save command is unavailable if the result was quantitated and the processing method and/or the calibration have not been saved. Save the processing method and the calibration, before saving the results.

Requirement: When you save results using Save All (or Save Calibration), and the processing method is untitled, the software prompts you to name it. If the processing method was modified since you generated the result, or if you opened a new method, you cannot save the result. You must save the processing method and recalculate the result.

Tip: In either case, the results are saved in the database as new results. The original results are not affected.

Related Topics

- » [Calculating detector noise and drift](#)
- » [Creating component tables](#)
- » [Modifying peak detection and integration parameters during processing](#)
- » [Optimizing integration](#)
- » [Processing data in the background](#)
- » [Processing data interactively](#)
- » [Using data to develop a processing method](#)
- » [Manually altering integration](#)