

SCALiR Tutorial

This quick (~5-10 minute) tutorial helps users understand different features of SCALiR and how to visualize and customize data generated by the app. Instructions are also available on the main page of the web-based app.

External standard curves

Step 1. Create a new folder on your device to store the sample data upload files. Open the SCALiR web application and on the left-hand side of the screen, click on the link to the standards concentration file. Repeat this step for the three peaklist data files (two for Mint data and one for Maven data).

NOTE: the “¼” symbol denotes “μ” in the units column of the standards concentration file for CSV file format.

1) Please upload standards concentrations file

[Click here to download an example of the standards concentrations file](#)

[Click here to download an example of the internal standards concentrations file](#)

Upload standards concentrations file

Drag and drop file here
Limit 200MB per file

Browse files


2) Please upload the peaklist data file. Data from Maven or Mint are accepted. Data files may be in .csv or .xlsx format.

[Click here to download an example of the Mint dense peaklist file](#)

[Click here to download an example of the Mint full peaklist file](#)

[Click here to download an example of the Maven peaklist file](#)

[Click here to download an example of the](#)



An APP for computing concentrations using standard curves. V1.0.0

check out this [link](#) for the source code

Click here to see instructions

Close

or download the tutorial for better explanation

Close

Please upload your standards concentrations file to start

No peaklist datafile has been uploaded

There are no results to show

Step 2. On the web application, upload the standards concentration file and the “SCALiR_Maven_Peaklist_sample” file. The data will now pop up on the screen. Scroll down and select “Maven” in the program selection menu. The default option for slope fitting is “Fixed fit” and you should see the links to the standard curve parameters and concentration datafiles pop up below.

NOTE: Concentration values are calculated based on the concentration of analyte injected into the LC-MS system. If applicable, users must manually calculate the final concentration of the analyte in the sample based on the dilution factor used during sample preparation.

The screenshot displays the SCALiR web application interface. On the left, a sidebar contains instructions for uploading peaklist data files, mentioning that data from Maven or Mint are accepted in .csv or .xlsx format. It includes links to download example files for Mint dense, Mint full, Maven, and internal standards. Below the instructions is a file upload area with a 'Browse files' button. Two files are listed at the top: 'SCALiR_Standards_Conce...' (0.7KB) and 'SCALiR_Maven_Peaklist_s...' (2.4KB). The main content area features a button to 'Click here to display/hide your peak_list table'. Below this is a dropdown menu for selecting the program used for generating the peaklist data, with 'Mint' selected and 'Maven' highlighted. Another dropdown menu for 'intensity measurement' shows 'peak_max' as the selected option. At the bottom, a message states 'Data uploading or parameter settings incomplete' and 'There are no results to show'.

SCALiR_Standards_Conce... X
0.7KB

2) Please upload the peaklist data file. Data from Maven or Mint are accepted. Data files may be in .csv or .xlsx format.

[Click here to download an example of the Mint dense peaklist file](#)

[Click here to download an example of the Mint full peaklist file](#)

[Click here to download an example of the Maven peaklist file](#)

[Click here to download an example of the internal standards peaklist file](#)

upload the data file..

Drag and drop file here
Limit 200MB per file

Browse files

SCALiR_Maven_Peaklist_s... X
2.4KB

Click here to display/hide your peak_list table

Select the program used for generating the peaklist data

Mint

Mint

Maven

Please select the intensity measurement.. peak_max will be used as the default value

intensity measurement

peak_max

Data uploading or parameter settings incomplete

There are no results to show

Step 3. You can click on the highlighted links to download these files. Scroll down to the bottom of the page. The app is displaying the default metabolite. Click on the compound menu and select “Fumarate”. If necessary, you can now manually change the axis labels on the displayed log-log graph from the units you provided in the concentration upload file.

The standard curves have been fitted

[Click here to display/hide the standard curve parameters table](#)

[Click here to download your standard curve parameters table](#)

[Click here to display/hide the concentration data table](#)

[Click here to download your concentration data](#)

Log-log plot visualization:

Description: This plot shows the x- and y-axes in the log scale, with the axis title

select the compound D-Glucose 6-Phosphate will be used by default

Fumarate

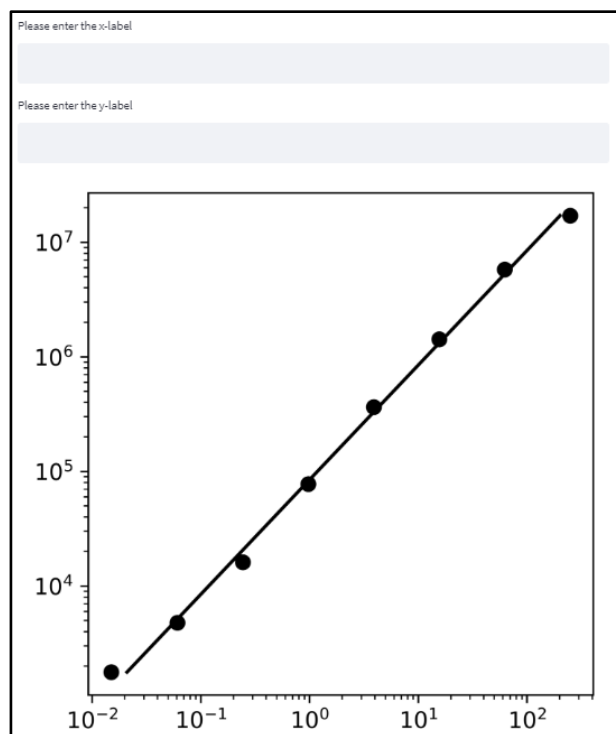
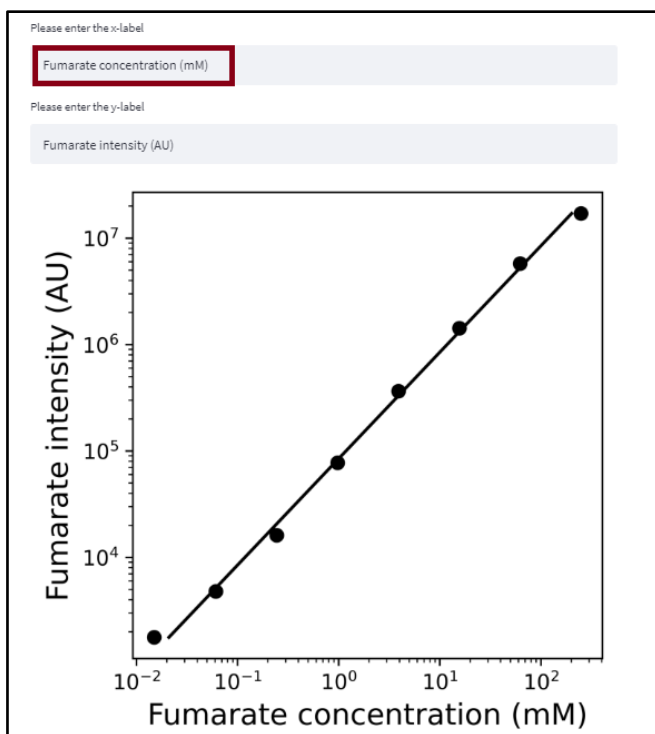
Please enter the x-label

Fumarate concentration (μM)

Please enter the y-label

Fumarate intensity (AU)

Step 4. Change the x-axis label to “Fumarate concentration (mM)” and click enter. If you would like to leave the axis labels blank, delete all text in the box and click enter. To download the plot, right-click and select “Save as.”.



Step 5. Refresh the browser. Upload the sample standards concentrations and the “SCALiR_MINT Peaklist_Full_Results_sample” files. The default program and table type are Mint and full results, so you do not need to change those but can select “peak_area” under the intensity measurement menu. Also, select “Interval fit” under the line of best fit menu. Change the slider bar to 0.90 – 1.10 from the recommended interval of 0.85 to 1.15. SCALiR will re-run the algorithm and your new results will pop up below as before so you can download the data files as well as standard curve visualizations. To use the “SCALiR_MINT_Peaklist_Dense_Peak_Max_sample” file, select “dense peak_max” for the type of table used (peak max is the only intensity measurement available for this file type).

Select the program used for generating the peaklist data

Mint

Indicate the type of table used, see Mint documentation for details

full results

Please select the intensity measurement.. peak_max will be used as the default value
intensity measurement

peak_area

Select the flexibility for your line of best fit

Interval fit – bounds for slope values can be defined. The interval 0.85-1.15 is recommended

interval
Select a range of values

0.90

1.10

0.00

2.00

	0
0	0.9000
1	1.1000

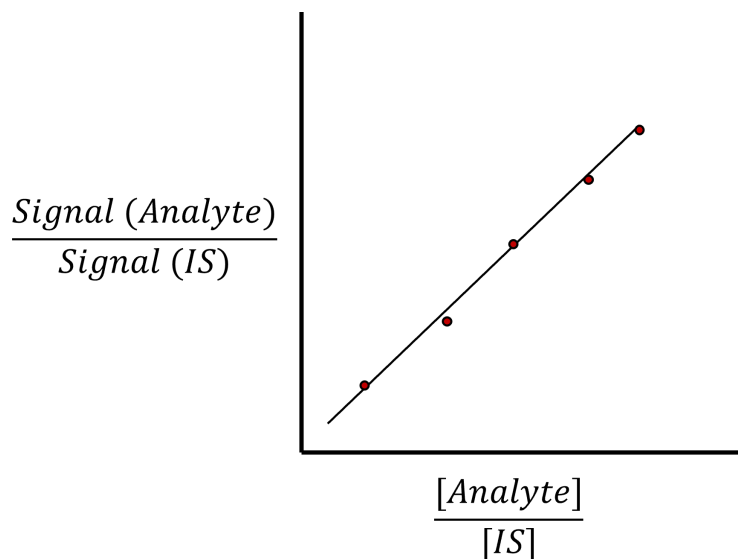
Internal standard curves

In the case where matrix effects are a concern for the analysis, users may wish to spike both their standards and samples with a set concentration of an internal standard (IS). This is usually an isotope labeled version of the analyte of interest. If users wish to use internal standard(s) in their analysis, they can calculate the concentration and response ratios for the analyte compared to the IS and upload those values to SCALiR.

Internal standards concentrations file: Uploaded values are the concentration ratios calculated by taking the ratio of the concentration of the analyte to the concentration of the IS. Users must also provide the concentration of the IS that was injected onto the LC-MS system for SCALiR to automatically calculate the concentration of the analyte.

Internal standards peaklist data file: Uploaded values are the response ratio of the signal intensity of the analyte to the signal intensity of the IS.

This will result in a standard curve with the concentration ratio on the x-axis and response ratio on the y-axis:



SCALiR uses the same algorithm to calculate the line of best fit through the data provided as concentration ratios and response ratios as for external standard curves, but uses one additional step to calculate the analyte concentration based on the IS concentration. The user uploads the concentration of the IS in the internal standard concentration file so that SCALiR can automatically calculate the concentration of the analyte:

$$\text{Line of best fit equation: } y = m * x + b \quad (1)$$

$$\text{Reported concentration of analyte: } [Analyte] = x * [IS] \quad (2)$$

When users upload the internal standard concentration file and internal standard peaklist file, the data are automatically processed. Users can then choose the fitting flexibility, download standard curve parameters and concentration data files, manually change the log-log plot axis units if necessary, and visualize/download the log-log plots for specified analytes.

The standard curves have been fitted

[Click here to display/hide the standard curve parameters table](#)

[Click here to download your standard curve parameters table](#)

[Click here to display/hide the concentration data table](#)

[Click here to download your concentration data](#)

Log-log plot visualization:

Description: This plot shows the x- and y-axes in the log scale, with the axis tic

select the compound Aspartate will be used by default

Aspartate

Please enter the x-label

Aspartate concentration (mM)

Please enter the y-label

Aspartate response ratio

