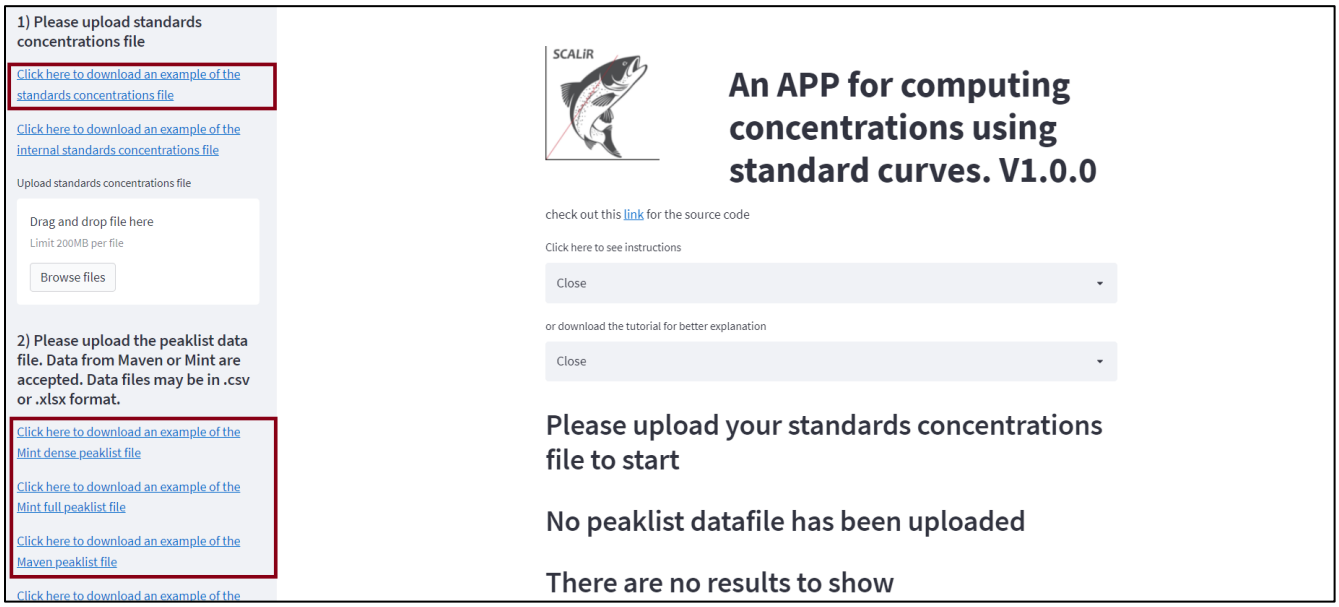
SCALiR Tutorial

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

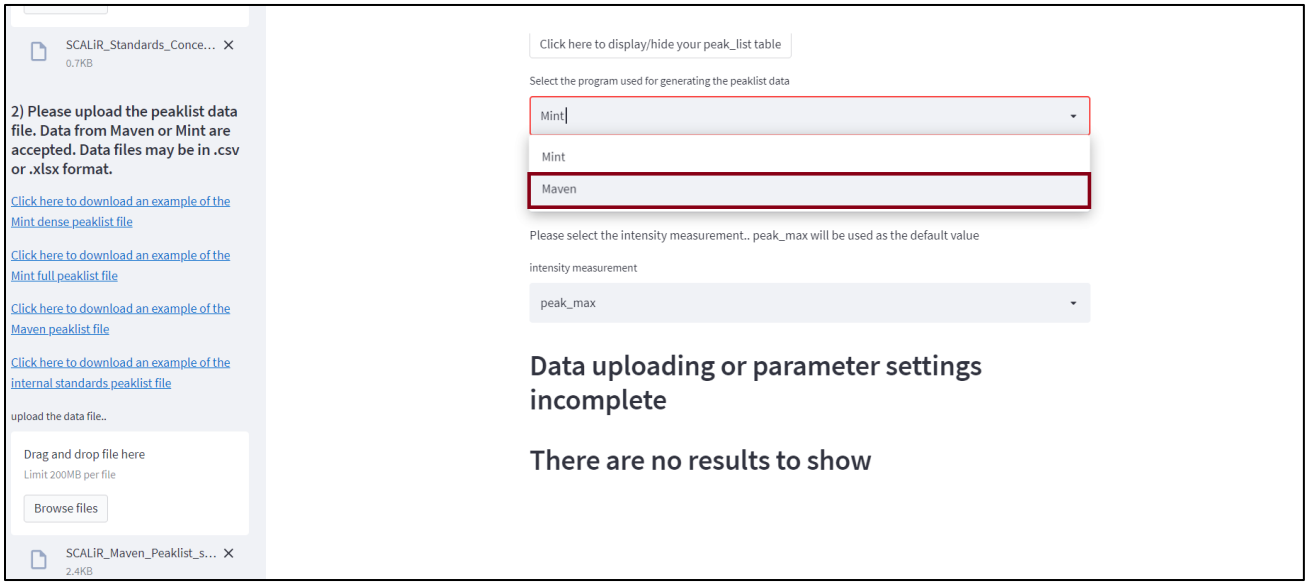
Step 1. Create a new folder on your device to store the sample data upload files. Open the SCALiR web application and on the left-hand side of the screen, click on the link to the standards concentration file. Repeat this step for the three peaklist data files (two for Mint data and one for Maven data).

NOTE: the “Î¼” symbol denotes “µ” in the units column of the standards concentration file for CSV file format.

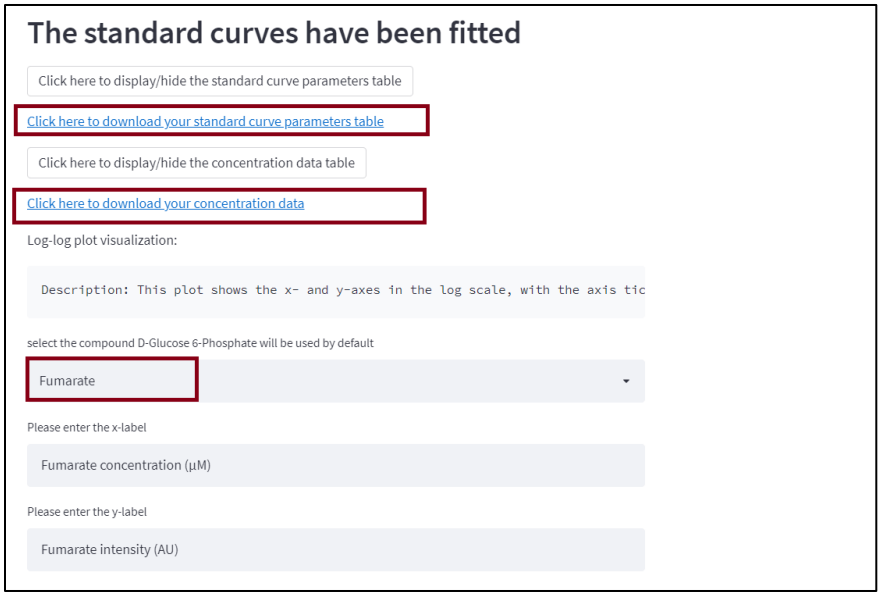


Step 2. On the web application, upload the standards concentration file and the "SCALiR_Maven_Peaklist_sample" file. The data will now pop up on the screen. Scroll down and select "Maven" in the program selection menu. The default option for slope fitting is "Fixed fit" and you should see the links to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

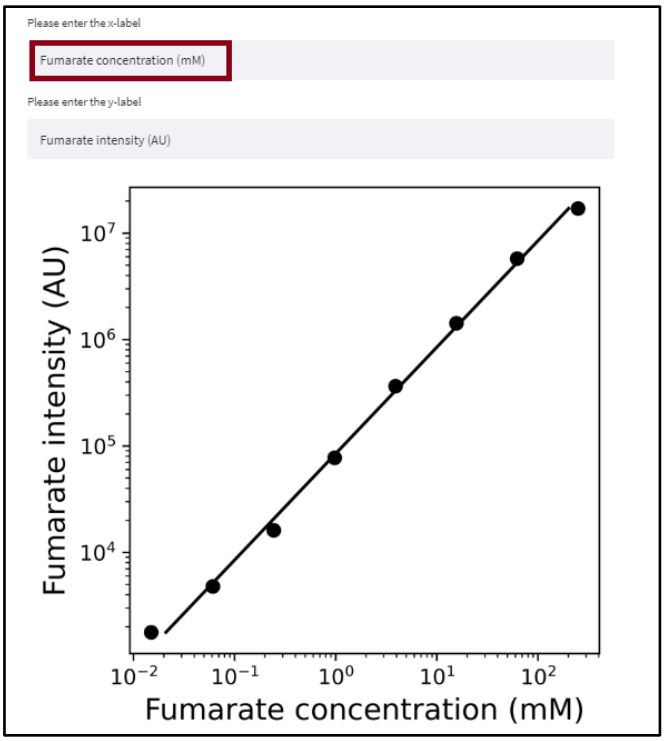
NOTE: Concentration values are calculated based on the concentration of analyte injected into the LC-MS system. If applicable, users must manually calculate the final concentration of the analyte in the sample based on the dilution factor used during sample preparation.

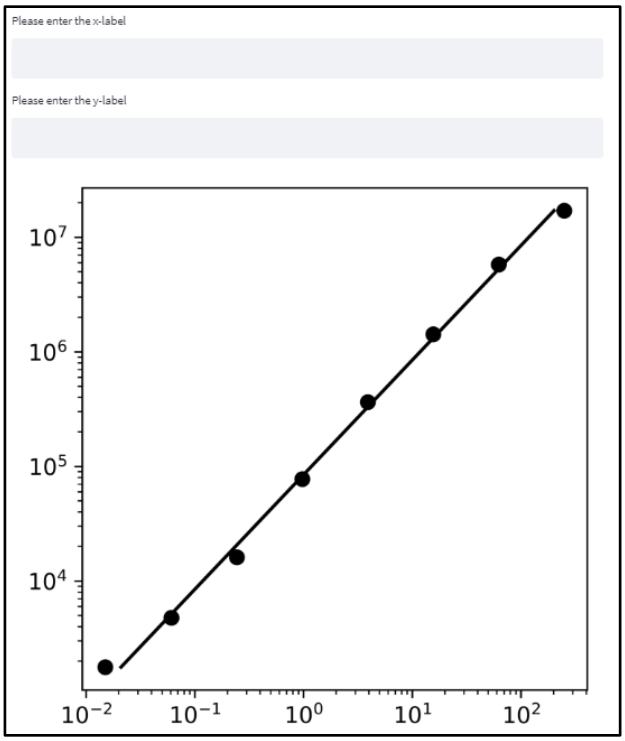


Step 3. You can click on the highlighted links to download these files. Scroll down to the bottom of the page. The app is displaying the default metabolite. Click on the compound menu and select “Fumarate”. If necessary, you can now manually change the axis labels on the displayed log-log graph from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

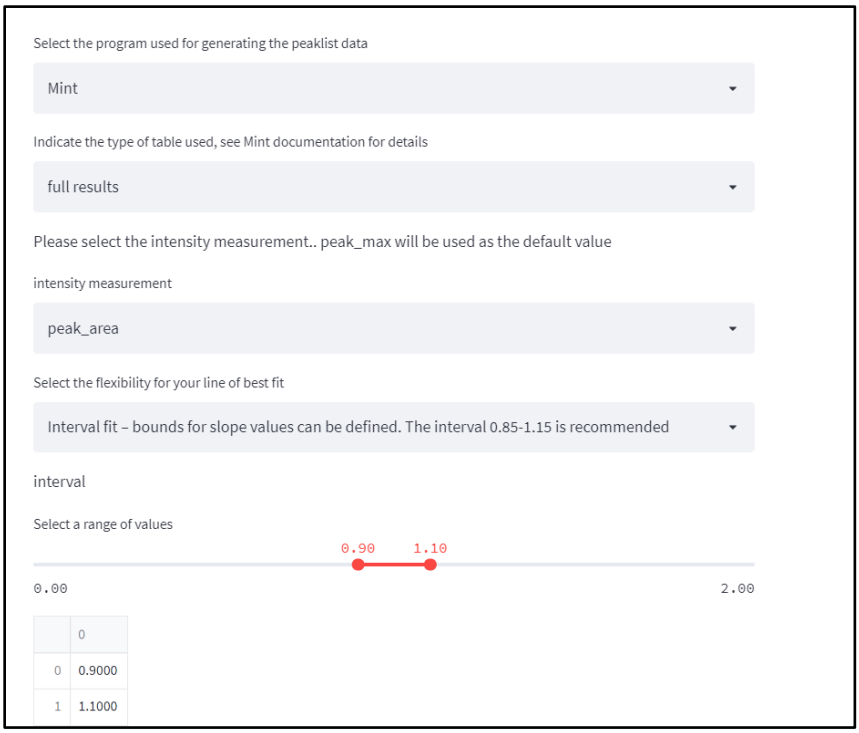


Step 4. Change the x-axis label to “Fumarate concentration (mM)” and click enter. If you would like to leave the axis labels blank, delete all text in the box and click enter. To download the plot, right-click and select “Save as.”.





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).



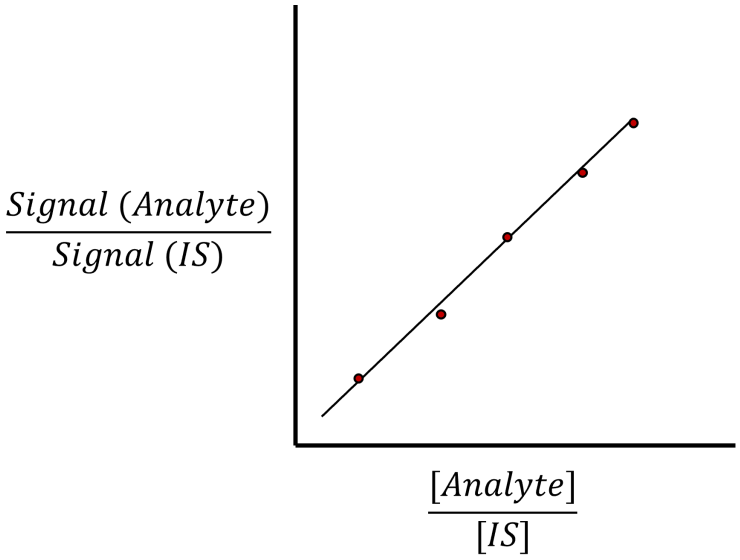
Internal standard curves

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:

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

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

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.