

QuaSAR Quantitative Statistics

QuaSAR is a program that aids in the Quantitative Statistical Analysis of Reaction Monitoring Experiments. It was designed to quickly and easily convert processed SRM/MRM-MS data into calibration curves, determine limits of detection and quantification, calculate mean and coefficient of variation for all transitions of each peptide in a set of samples, as well as determine the peptide analyte concentration in unknown samples. The resulting output files, consisting of *.csv tables and *.pdf figures, can be readily used for further statistical analyses or reported as output for reports or publications.

In order to use QuaSAR with Skyline, it must be installed through the Skyline external tools framework. Furthermore, QuaSAR requires the use of custom annotations in Skyline. This tutorial covers the installation of QuaSAR, how to input data for custom annotations, and how to run the tool itself.

Getting Started

To start this tutorial, download the following ZIP file:

<https://skyline.gs.washington.edu/tutorials/QuaSARTutorial.zip>

Extract the files in it to a folder on your computer, like:

C:\Users\brendanx\Documents

This will create a new folder:

C:\Users\brendanx\Documents\QuaSARTutorial

The zip file contains the following files:

QuaSAR_Tutorial.sky: A Skyline document containing sample data for QuaSAR.

QuaSAR_Tutorial.skyd: Contains extracted chromatogram data for the Skyline document.

QuaSAR-1_0.zip: The QuaSAR external tool.

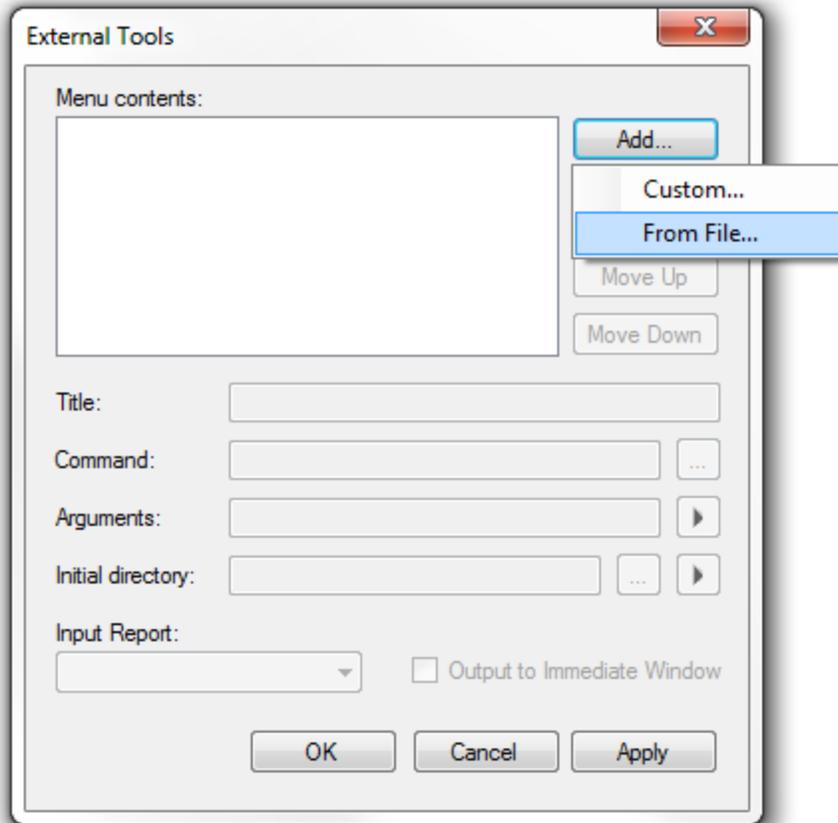
Open the provided file `QuaSAR_Tutorial.sky` in Skyline.

Installing QuaSAR as an External Tool

The Skyline external tools framework supports the direct integration of statistical analysis tools that process the data in a Skyline document.

To add QuaSAR as an external tool, perform the following steps:

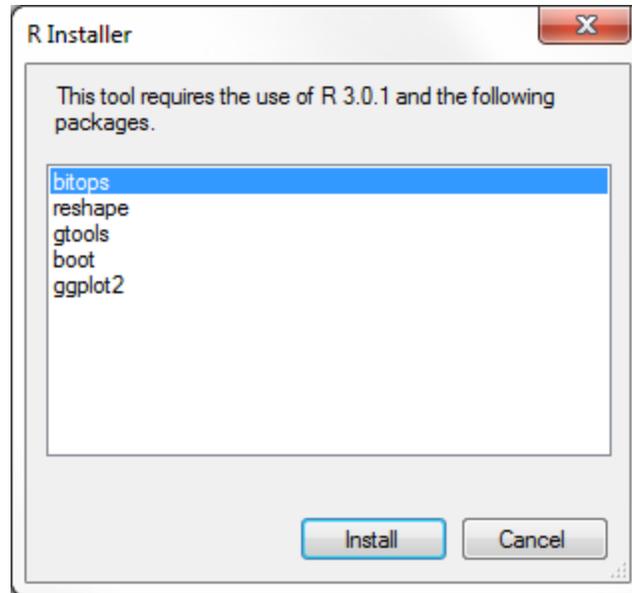
- On the **Tools** menu, click **External Tools** to bring up the **External Tools** form.



Note: Your External Tools menu may already contain some Tools

- Click the **Add** button, and select **From File** from the context menu.
- Navigate to the location of the `QuaSAR-1_0.zip` file in the folder you created and select it.
- Click the **Open** button.

Skyline will now guide you through the process of installing QuaSAR as an external tool. This may require you to install the R statistical programming environment, as well as a number of R packages which are used by QuaSAR to perform its statistical analysis. For example, if you do not have the appropriate version of R installed, the following form will be displayed:

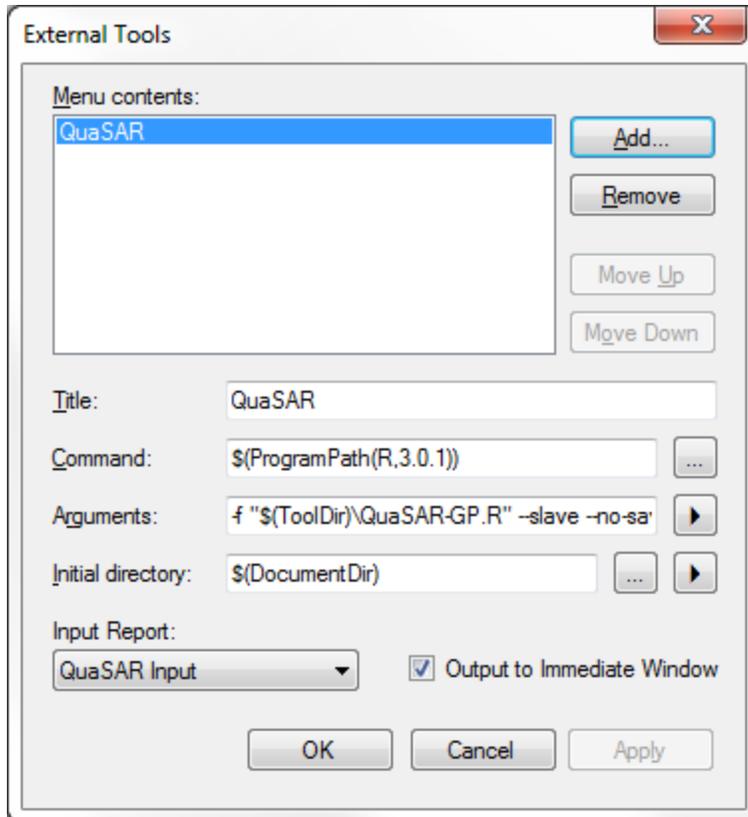


Note: This form may look slightly different as the packages might change and if you already have R 3.0.1 installed.

To complete the installation, performing the following steps:

- On the **R Installer** form, click **Install**.
- If you do not have the specified version of R installed on your machine, wait for R to download. When the download is completed, you will be asked to install R onto your computer. Complete the R installation process by working through the R installer wizard.
- Once the R installation is completed, Skyline will download and install the necessary R packages for QuaSAR. When prompted to run the “SkylineProcessRunner”, click the **Yes** button to install packages.

Once the installation is complete, the QuaSAR tool will appear in the **External Tools** form:



- Click the **OK** button on the **External Tools** form.

Annotating samples with concentration information

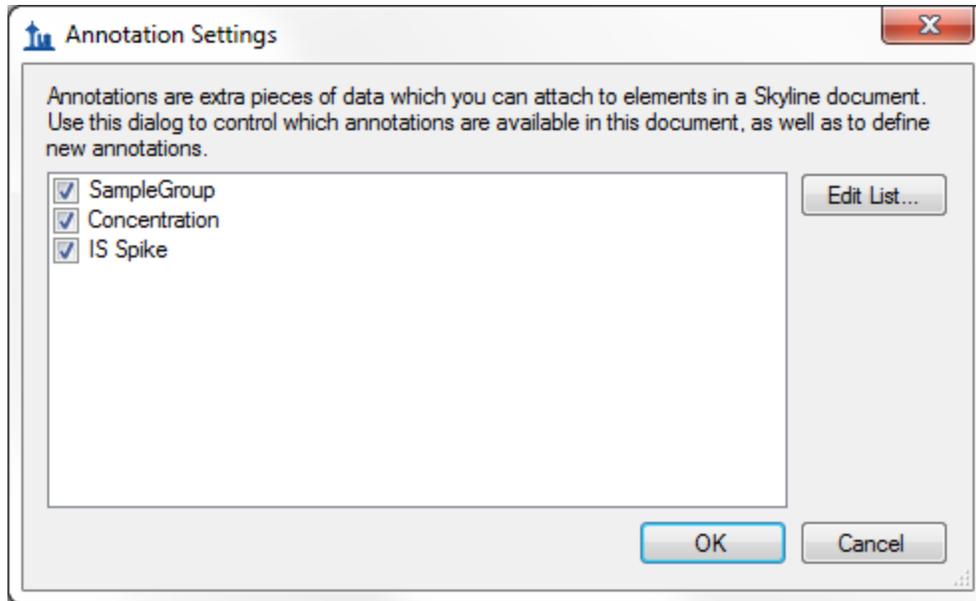
Skyline allows you to associate additional information with the replicates in the document by defining custom annotations. When QuaSAR was installed, it should have defined three custom annotations: **SampleGroup**, **Concentration** and **IS Spike**.

The **Annotation Settings** form will show the list of the annotations that have been defined.

To view the Annotation Settings form, perform the following step:

- On the **Settings** menu, click **Annotations**.

You must check the checkboxes in the list in order to be able to use these annotations in your current Skyline document. The **Annotation Settings** form should look like the one shown below:



- Click the **OK** button.

Editing annotation values in Skyline is done using the Results Grid. To bring up the Results Grid do the following:

- On the **View** menu, click **Results Grid** (Alt-2).

The **Results Grid** will show you chromatogram peak areas and other measured results for the currently selected peptide, or transition. Select a protein to see only the replicate annotations.

Copy the values in the following tables and paste them into the results grid:

SampleGroup	Concentration	IS Spike
A	0	10
B	0.001	10
C	0.004	10
D	0.018	10

E	0.075	10
F	0.316	10
G	1.33	10
H	5.62	10
I	23.71	10
J	100	10

The completed grid should look like this:

Results Grid

	Replicate Name	SampleGroup	Concentration	IS Spike
	9-1_Site56B_A1_...	A	0	10
	9-1_Site56B_A2_...	A	0	10
	9-1_Site56B_A3_...	A	0	10
	9-1_Site56B_A4_...	A	0	10
	9-1_Site56B_B1_...	B	0.001	10
	9-1_Site56B_B2_...	B	0.001	10
	9-1_Site56B_B3_...	B	0.001	10
	9-1_Site56B_B4_...	B	0.001	10
	9-1_Site56B_C1_...	C	0.004	10
	9-1_Site56B_C2_...	C	0.004	10
	9-1_Site56B_C3_...	C	0.004	10
	9-1_Site56B_C4_...	C	0.004	10
	9-1_Site56B_D1...	D	0.018	10
	9-1_Site56B_D2...	D	0.018	10
	9-1_Site56B_D3...	D	0.018	10
	9-1_Site56B_D4...	D	0.018	10
	9-1_Site56B_E1_...	E	0.075	10
	9-1_Site56B_E2_...	E	0.075	10
	9-1_Site56B_E3_...	E	0.075	10
	9-1_Site56B_E4_...	E	0.075	10
	9-1_Site56B_F1_...	F	0.316	10
	9-1_Site56B_F2_...	F	0.316	10
	9-1_Site56B_F3_...	F	0.316	10
	9-1_Site56B_F4_...	F	0.316	10
	9-1_Site56B_G1...	G	1.33	10
	9-1_Site56B_G2...	G	1.33	10
	9-1_Site56B_G3...	G	1.33	10
	9-1_Site56B_G4...	G	1.33	10
	9-1_Site56B_H1...	H	5.62	10
	9-1_Site56B_H2...	H	5.62	10
	9-1_Site56B_H3...	H	5.62	10
	9-1_Site56B_H4...	H	5.62	10
	9-1_Site56B_I1_...	I	23.71	10
	9-1_Site56B_I2_...	I	23.71	10
	9-1_Site56B_I3_...	I	23.71	10
	9-1_Site56B_I4_...	I	23.71	10
	9-1_Site56B_J1_...	J	100	10
	9-1_Site56B_J2_...	J	100	10
	9-1_Site56B_J3_...	J	100	10
▶	9-1_Site56B_J4_...	J	100	10

Record: |< < 40 of 40 > >| Filter:

- Close the **Results Grid** in Skyline by clicking the red X in its upper right corner.

Using QuaSAR

To run a QuaSAR analysis, perform the following steps:

- On the **Tools** menu, click **QuaSAR**.

Skyline will begin exporting the QuaSAR input report. This may take a few minutes. You can track the progress of the report export with the green progress bar in the bottom of the Skyline Window. Once the report is exported, Skyline will display the **QuaSAR** form:

The image shows the QuaSAR dialog box with the following settings:

- Title: (empty)
- Buttons: OK, Cancel
- Tab: Basic
- Generate section:
 - Calibration curves
 - CV table
 - LOD/LOQ table
 - LOD/LOQ comparison
 - Peak area plots
- Options section:
 - Standard present
 - Use PAR for analysis
 - Analyte: light Area
 - Standard: heavy Area
 - Units: fmol/ul
- Buttons: Use Defaults

For this tutorial you will leave the options in their default settings. If you wish to learn more about the many options provided by QuaSAR, you can read the documentation [here](#).

- In the **Title** textbox enter 'Tutorial'.
- Click the **OK** button on the **QuaSAR** form.

The QuaSAR tool will perform its analysis. The output from the tool run is shown in the **Immediate Window**, the textbox docked at the bottom of the main Skyline window. When the tool is completed, use the Windows explorer to navigate to the directory containing the `QuaSAR_Tutorial.sky` file. The directory should now contain a number of output files in .csv and .pdf formats generated by QuaSAR, based on the data contained in the Skyline document.

Parameters

Name	Location	Description
Title	Basic	The title to be displayed on each calibration plot.
Analyte	Basic > Options	User must specify the exact analyte column header name as it appears in the input CSV file. The default is "light Area".
Standard	Basic > Options	User must specify the exact internal standard column header name as it appears in the input CSV file. The default is "heavy Area".
CV table	Basic > Generate	If checked then generate CV (coefficient of variation) table.
Calibration Curves	Basic > Generate	If checked then generate calibration curves.
LOD/LOQ table	Basic > Generate	If checked then generate LOD/LOQ table.
Peak area plots	Basic > Generate	If checked then generate peak area plots with Peak Area units on the y-axis and analyte concentration on the x-axis.
Use PAR for analysis	Basic > Options	If checked then use peak area ratio (PAR) for analysis (instead of concentration) with PAR on the y-axis and analyte concentration on the x-axis.
linear scale	Plots	The maximum value for linear scale in Units.
Max calcurve log scale	Plots	The maximum value for log scale in Units.
Units	Basic > Options	Define units, the default is fmol/ul. The unit will be a label on the output files.
Generate graphs as JPEGs	Plots	Generates and saves JPEGs to directory of the .sky file.
Number of transitions to plot	Plots	Specifies number of transitions to plot.
Perform AuDIT	Additional Tools > AuDIT	If "yes" then perform AuDIT for interference detection.
AuDIT cv threshold	Additional Tools > AuDIT	For AuDIT the threshold for coefficient of variation below which transition is quantification-worthy. Default is 0.2.
Perform endogenous determination	Additional Tools>Endogenous estimation	If checked performs endogenous determination when QuaSAR runs.

Endogenous confidence level	Additional Tools>Endogenous estimation	Default 0.95.
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Output Files

If all options that generate an output are selected then upon successful completion QuaSAR will generate the following output files:

1. Filename with suffix: **-parameters-summary.csv**
A CSV file containing all parameters used by user to generate results
2. Filename with suffix: **-calcurves.pdf**
A PDF file with the calibration plots ordered by peptide sequence
3. Filename with suffix: **-calcurves.csv**
A CSV file with associated calibration plot information
4. Filename with suffix: **-calcurves-peakareas.pdf**
A PDF file with the calibration plots using peak areas instead of concentrations and ordered by peptide sequence
5. Filename with suffix: **-calcurves-peakareas.csv**
A CSV file with associated calibration plot information using peak areas instead of concentrations
6. Filename with suffix: **-by_protein_calcurves.pdf**
A PDF file with the calibration plots ordered by protein name
7. Filename with suffix: **-by_proteins_calcurves-peakareas.pdf**
A PDF file with the calibration plots using peak areas instead of concentrations and ordered by protein name
8. Filename with suffix: **-cvplot.pdf**
A PDF file showing overall assay reproducibility by plotting CV vs. theoretical concentration values.
9. Filename with suffix: **-lodboxplot.pdf**
A PDF file showing distribution of LOD values with a box-whiskers plot.
10. Filename with suffix: **-lod-loq-raw.csv**
A CSV file with lod-loq raw values.
1. Filename with suffix: **-lod-loq-final.csv**
A CSV file with final lod-loq values, which is a subset of the raw file. The lowest LOD and LOQ values are selected for a given peptide.

2. Filename with suffix: **-cvtable.csv**
A CSV file with a CV table for all peptide/sample/transitions.
3. Filename with suffix: **-cvtable-final.csv**
A CSV file with the minimum CV reported in table for all peptide/sample/transitions
4. Filename with suffix: **-audit.csv**
A CSV file with the AuDIT results for all transitions.
5. Filename : **Rplots.pdf**
This is a system generated empty file and needs to be ignored by the user. In a future release of QuaSAR this file will be removed.