CITRULLIA – QUICK GUIDE

Citrullination identification software

Manual version 1.0

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Content

Installation

To install Citrullia:

1. Go to https://github.com/Citrullia-SDU/Citrullia.
2. Download the .MSI or the .EXE-install file.
   NB: Users at locations with restrictive security policies on computers such as universities and research facilities may encounter problems with the MSI-installation file. Therefore, the EXE-installation file should be used
3. Follow the installation guide
4. Congratulations. Citrullia has been installed.

To open Citrullia for users mentioned in step 2, it may be necessary to run Citrullia as an administrator.

Requirements

System requirements

- .NET Framework 4.7.2 (Can be downloaded from https://dotnet.microsoft.com/download/dotnet-framework/net472. Download the Runtime version and not the Dev pack)
- Windows 10 64-bit (Programmed and tested. It might be working on Windows 7, but it has not been tested).
- Screen with minimum size of 1600x1100. (Dual screens are recommended).

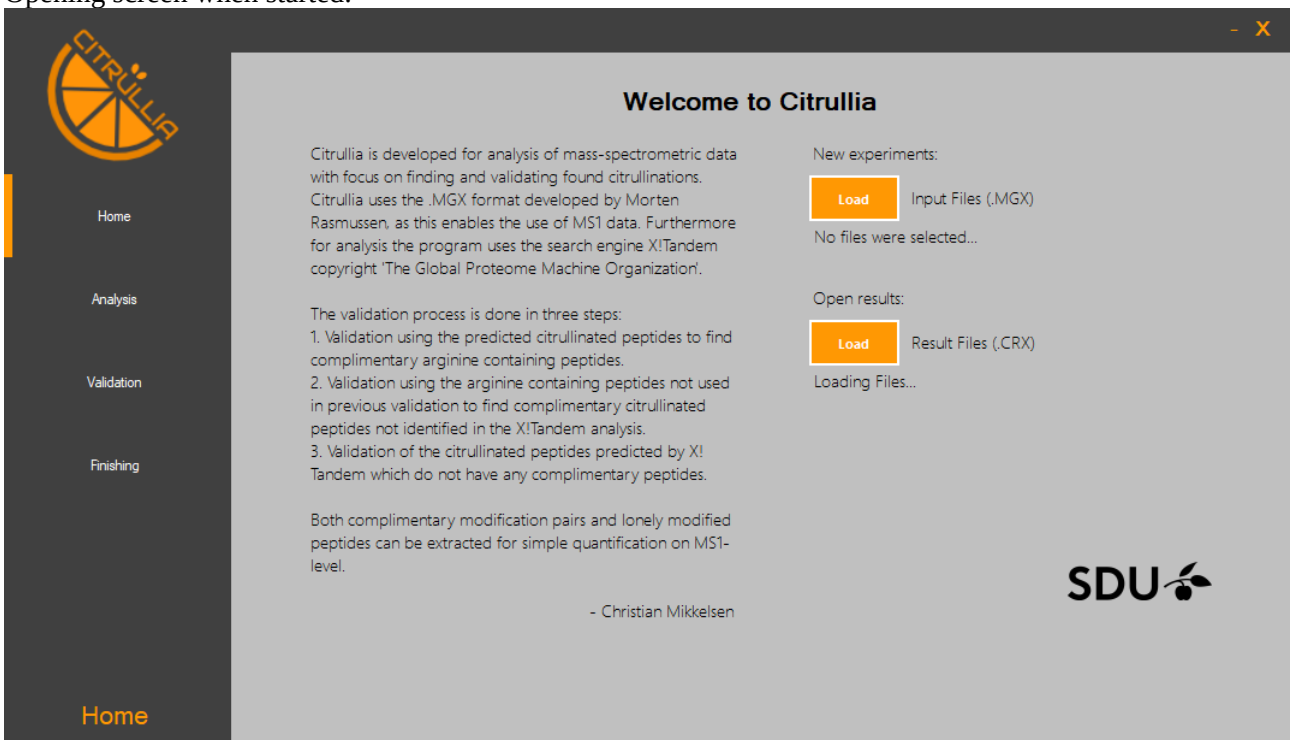
File requirements

- Input file: The input file must a Mascot Generic Extended (MGX)-file. This is a custom format that contains additional information such as parent mass and MS1 spectra. To create these files MassAI must be used (http://www.massai.dk/).
- Sequence file: A FASTA formatted file containing a list of protein entries with label and protein sequence to be searched . The sequence file can be generated from UniProt etc.

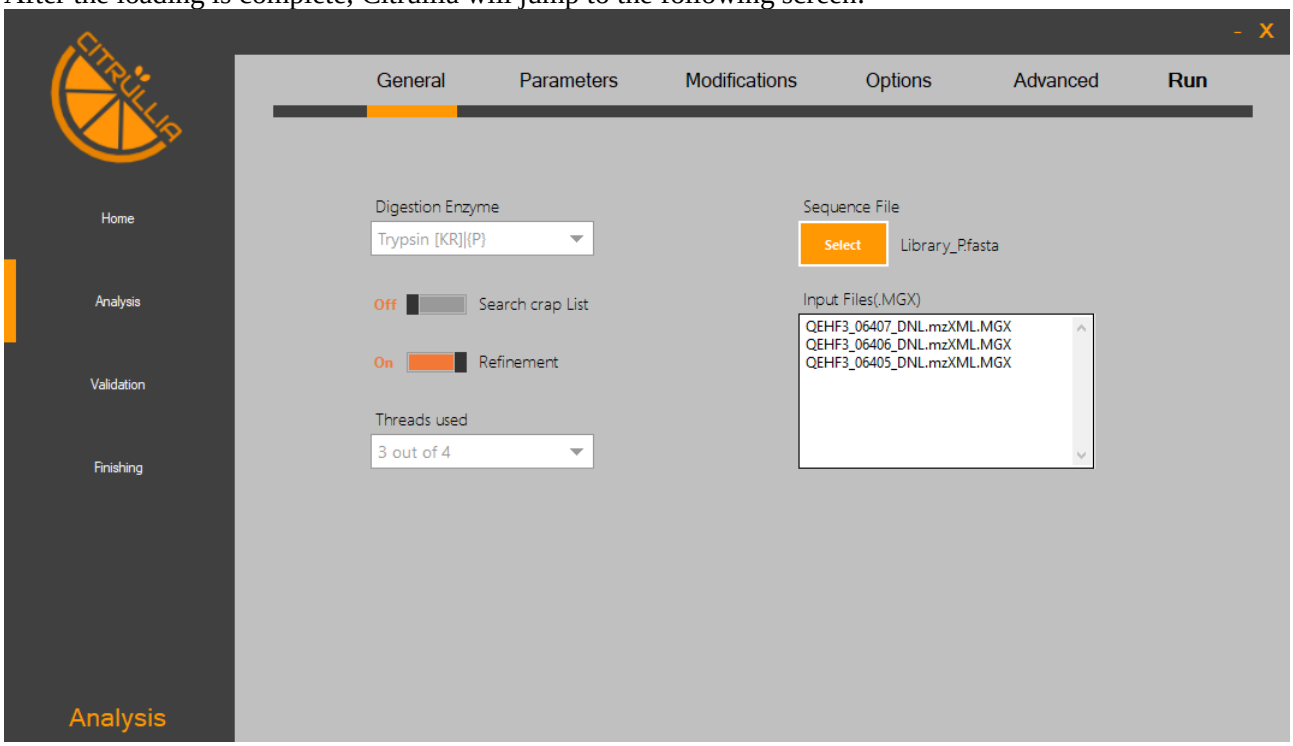
NB: Please refer to the full guide for information about the settings.

Quick guide (For first time users)

Opening screen when started:

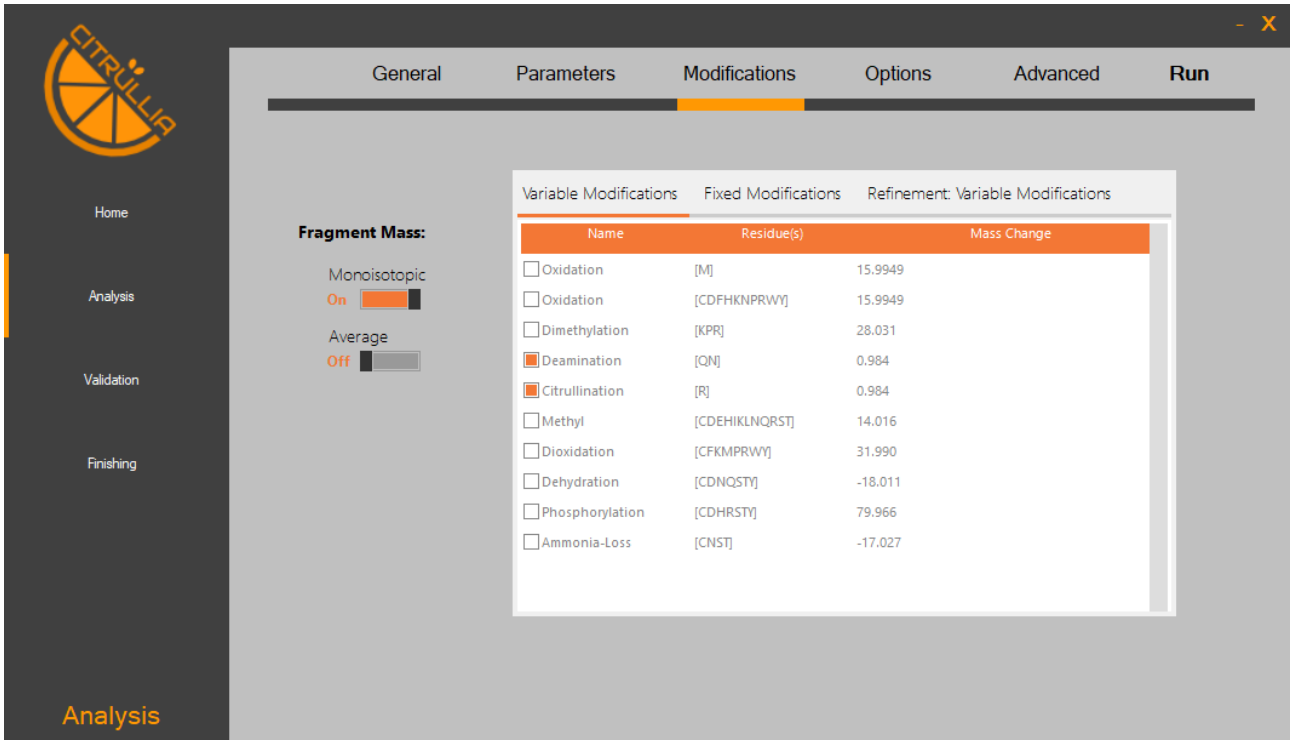


Click on the Load-button under New experiments. A dialog box will pop up prompting for the selection of the files.
Select the MGX-files and click OK. Citrullia will load the files.
After the loading is complete, Citrullia will jump to the following screen:



Select the digestion enzyme used to digest the sample
Select the sequence file

Then click on modifications:

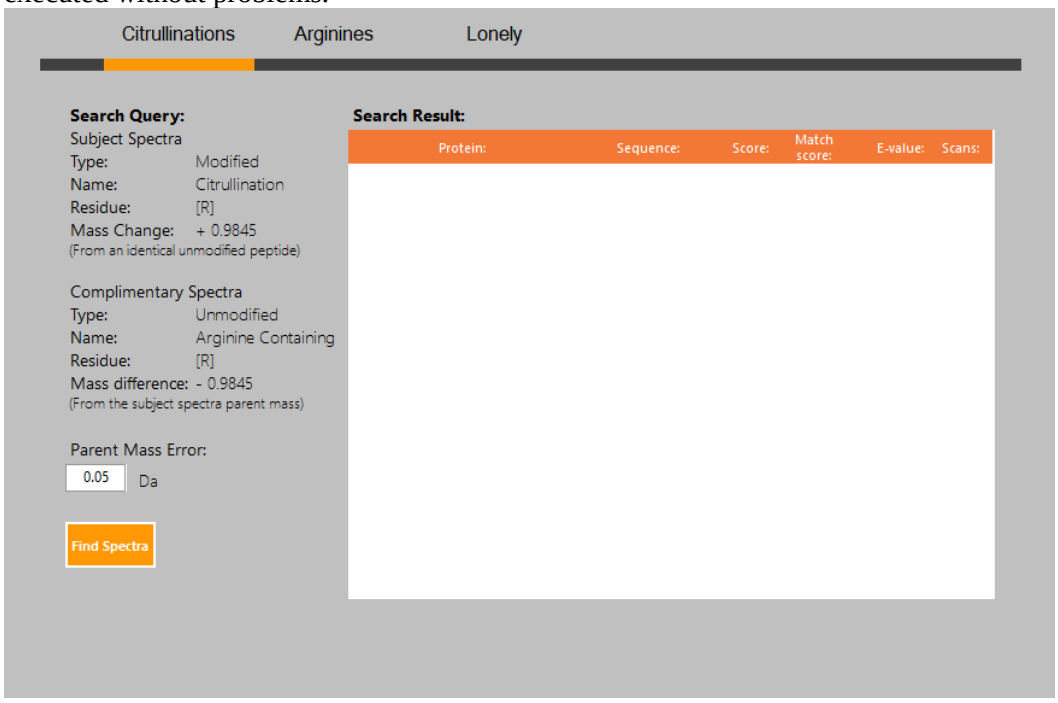


Select Citrullination of Arginine and Deamination of Asparagine and Glutamine by the checking the appropriate box.
The selection of deamination is essential to reduce the number of false positives.
If there are any fixed modifications, click on the Fixed modifications tab and select the modifications.

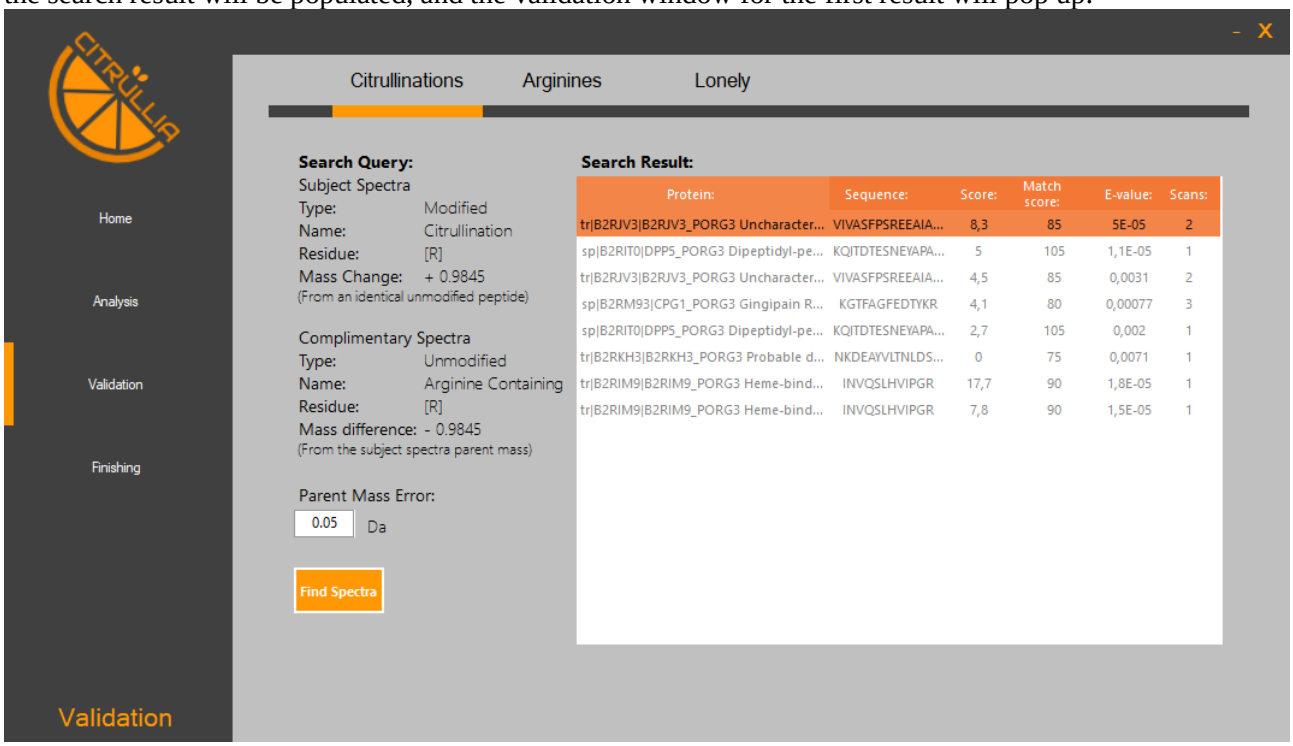
If X!Tandem should account for missed cleavage sites, go to the Options tab and add them.

Search

Afterwards, click Run. The command window will open and show the program of X!Tandem. This will happen for each selected file. If no dialog box pops up and the following window is shown, X!Tandem executed without problems.

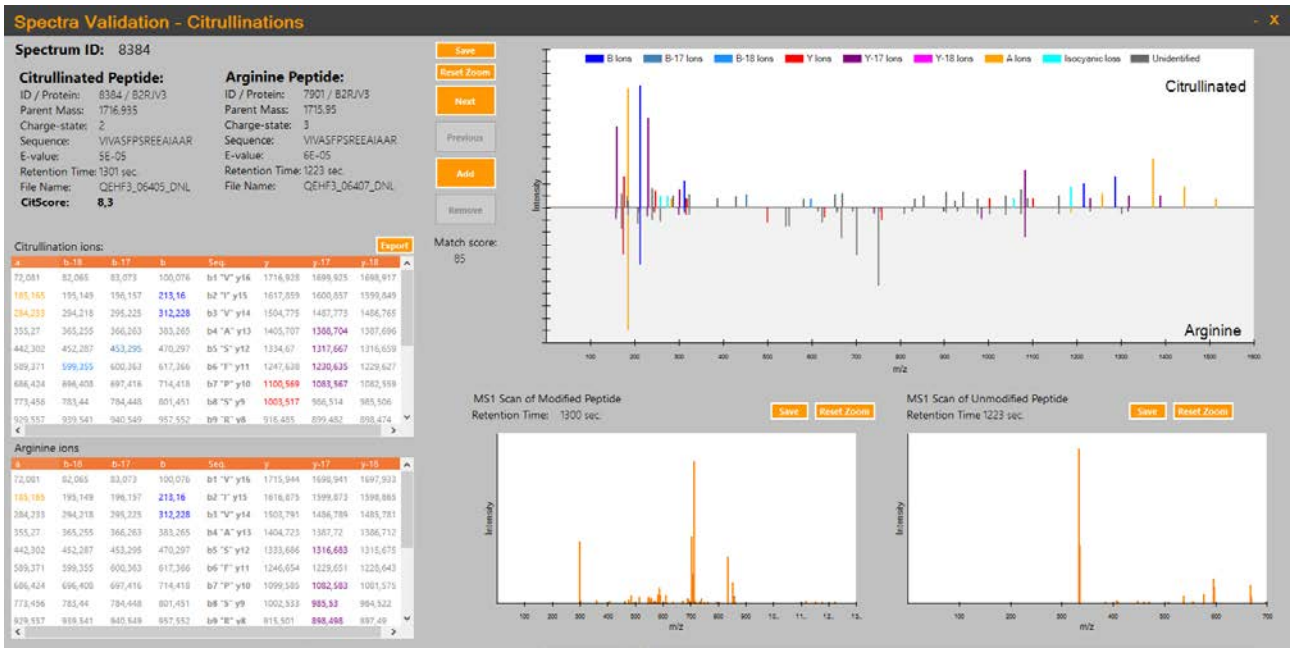


Click on Find Spectra. Citrullia will try to find citrullinations, which will take a little while. When finished the search result will be populated, and the validation window for the first result will pop up:



Validation

The validation window:



In the upper left corner, the information for the citrullination and arginine spectra can be seen.
Bellow it, the ions found are shown for both the citrullinated and the arginine spectra. These can be exported to an Excel spreadsheet by selecting the Export-button.
In the upper right corner, the two MS2 spectra can be seen. The upper spectrum is the citrullinated and the lower is the arginine spectrum. The key to the colours can be seen above the citrullinated spectra.

Below the MS2 spectra, the scan for the MS1 level are shown. A normal peak is orange, while the precursor is blue and possible isocyanic loss (only in the citrullinated MS1 scan) is marked in cyan.
For all three spectra it is possible to zoom in on the chart by marking it with the mouse. This zoom can be reset by clicking the Reset zoom-button. The spectra can be saved to a TIFF-file by selecting the Save-button.
Moreover, it is possible to move between the complementary spectra by clicking the Next and previous button.
To add or remove the validation pair for quantification, click on the Add or Remove button. The possibility of moving between the spectra is disabled by adding the spectra to quantification.

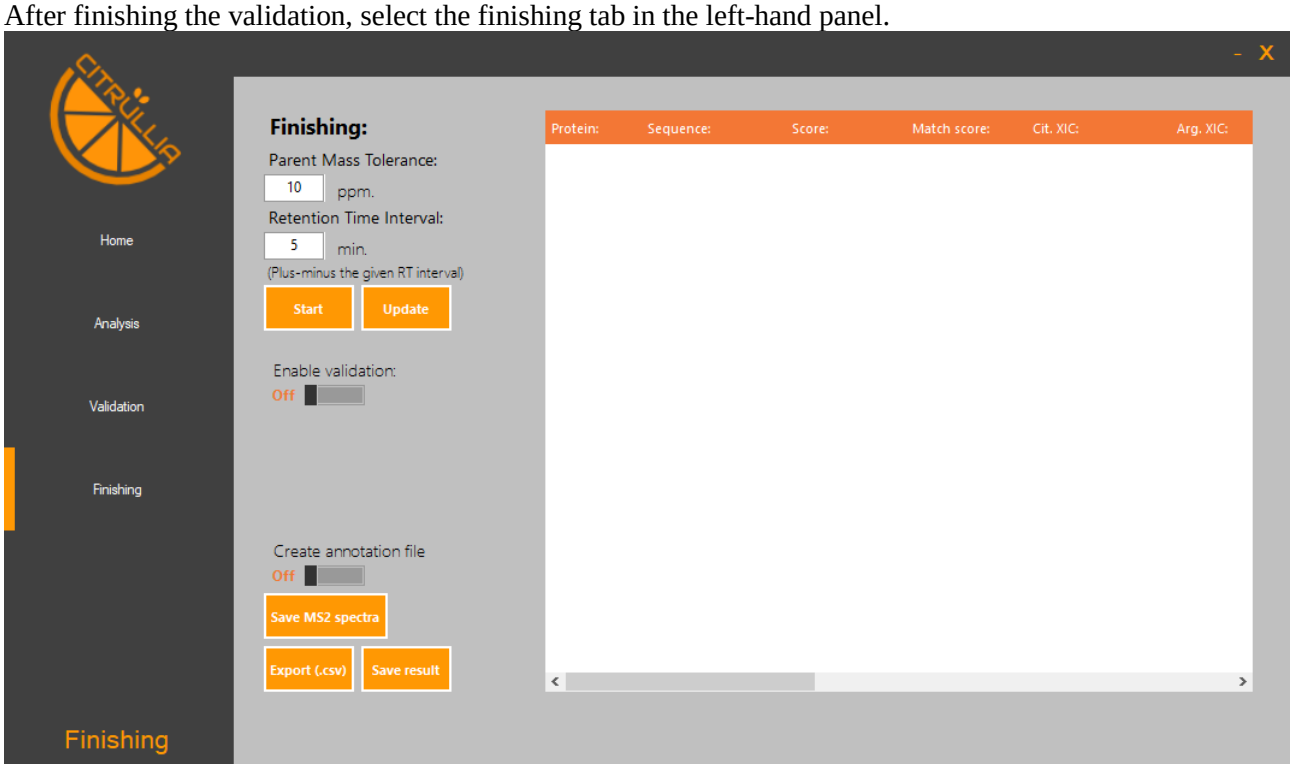
In the cases, where Citrullia cannot pair an arginine spectrum with a citrullinated spectrum, the citrullinated spectrum can be created as an “Lone Citrullination” result.

The principle is the same for Arginine- and Lone Citrullination-validation. However, the validation window for Lone Citrullination looks different, as it only displays a single spectrum.

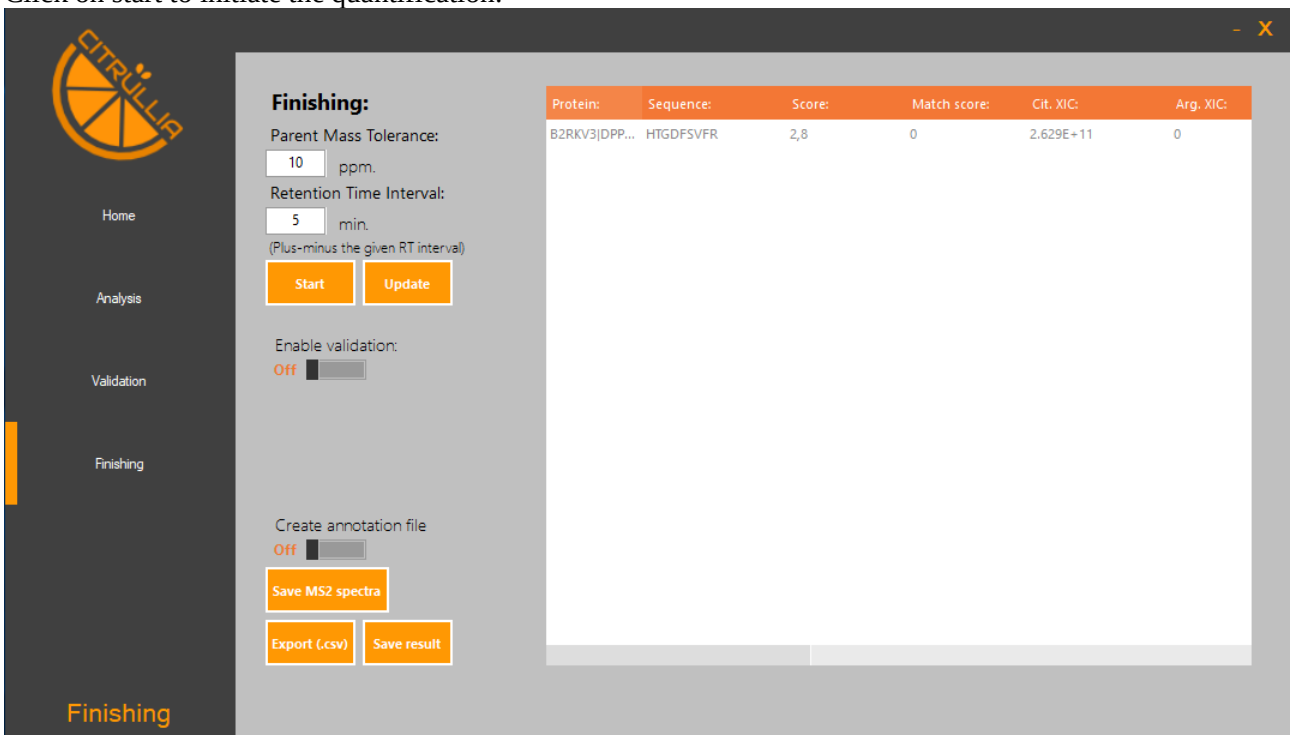
If no results are found for any of the validation types, try to go back and check that the settings are as they should be. It is possible that no results are found in the citrullination validation. However, results should be found in the arginine validation.

Quantification

After finishing the validation, select the finishing tab in the left-hand panel.



Click on start to initiate the quantification:



In the quantification step, Citrullia will calculate the extracted ion chromatogram for the spectra and a citrullination percentage.

From here it is possible to look at the spectra again by selecting the “enable validation”-option. This will open a window like the one in the validation step.

It is also possible to save the quantified annotated spectra to a folder along with a file containing the m/z-values and the ion type for all the spectra (if “Create annotation” is selected).
The quantification data can also be exported to an CSV-file by selecting the Export (.csv)-button.

By selecting the “Save result” you can save the X!Tandem files along the MGX-files. This saves time by bypassing the running of X!Tandem in the future.