

Skyline Batch

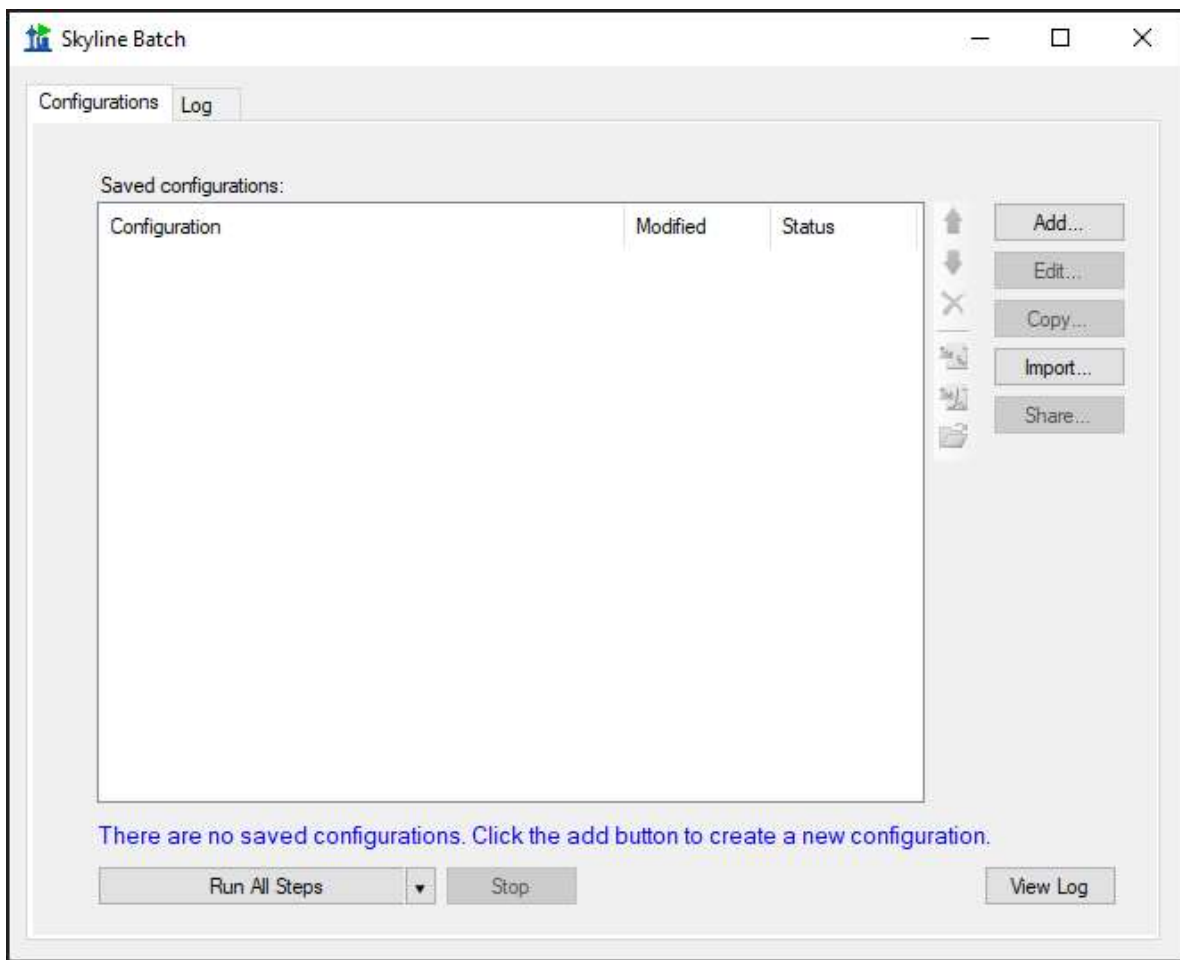
Skyline Batch is a user-friendly tool to run a simple skyline workflow on many files at a time. When run, it uses an invisible instance of Skyline to do the following:

- Step 1: Copy a specified skyline template file
- Step 2: Import data into this new file
- Step 3: Export reports using the imported data
- Step 4: Run R scripts on these reports

STARTUP

Skyline Batch requires an installation of Skyline (or Skyline-Daily).

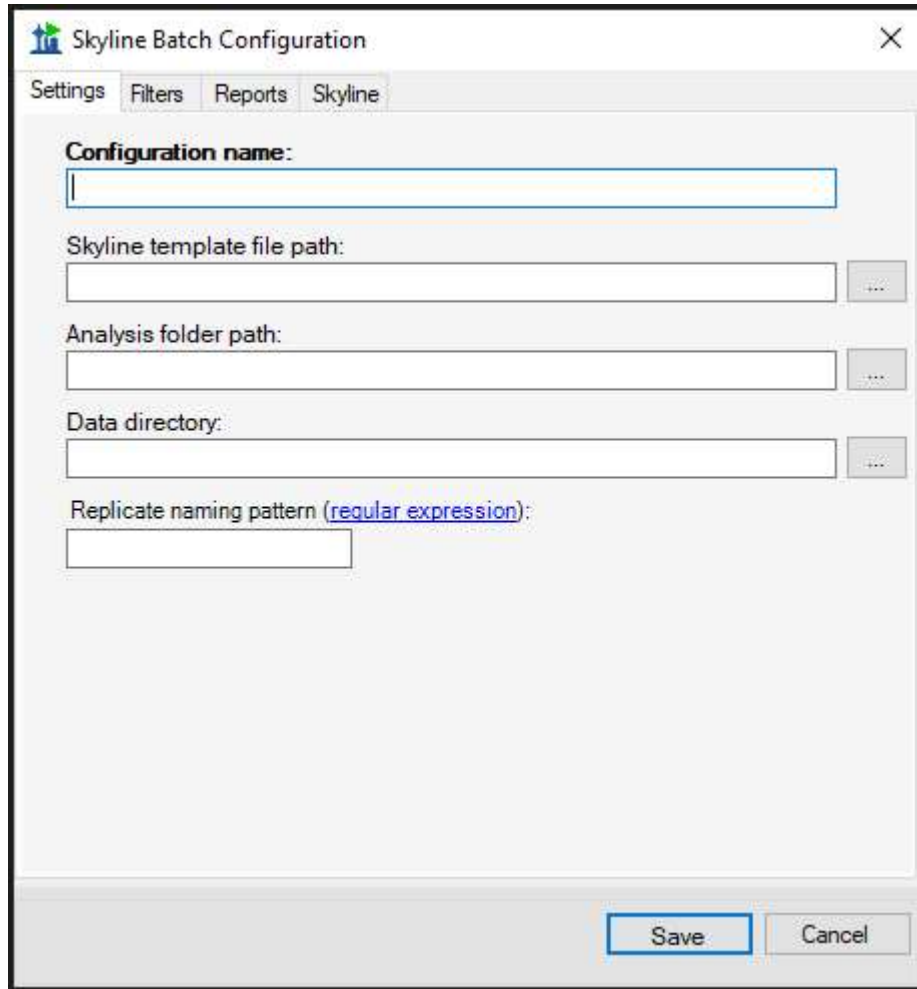
On startup, you will see the main window.



To start using Skyline Batch, create a new configuration by clicking the **Add** button in the **Configurations** tab.

CONFIGURATION SETTINGS

In the **Skyline Batch Configuration** form enter the appropriate settings in the **Settings** tab.



The screenshot shows the 'Skyline Batch Configuration' dialog box with the 'Settings' tab selected. The dialog has a title bar with the Skyline logo and a close button. Below the title bar are four tabs: 'Settings', 'Filters', 'Reports', and 'Skyline'. The 'Settings' tab contains the following fields:

- Configuration name:** A text input field.
- Skyline template file path:** A text input field with a browse button (three dots) to its right.
- Analysis folder path:** A text input field with a browse button (three dots) to its right.
- Data directory:** A text input field with a browse button (three dots) to its right.
- Replicate naming pattern (regular expression):** A text input field.

At the bottom right of the dialog are two buttons: 'Save' and 'Cancel'.

Configuration name: This can be the name of the project or study for which data is being acquired. Must be unique.

Skyline template file path: This is the path to the template Skyline document that will be copied to the analysis folder and used to import data into.

Analysis folder path: The path to the local folder in which the copied template document and exported reports will be stored. For Skyline Batch to find the assay library for the template file, the anal

Data directory: The folder containing data to import into the copied template document

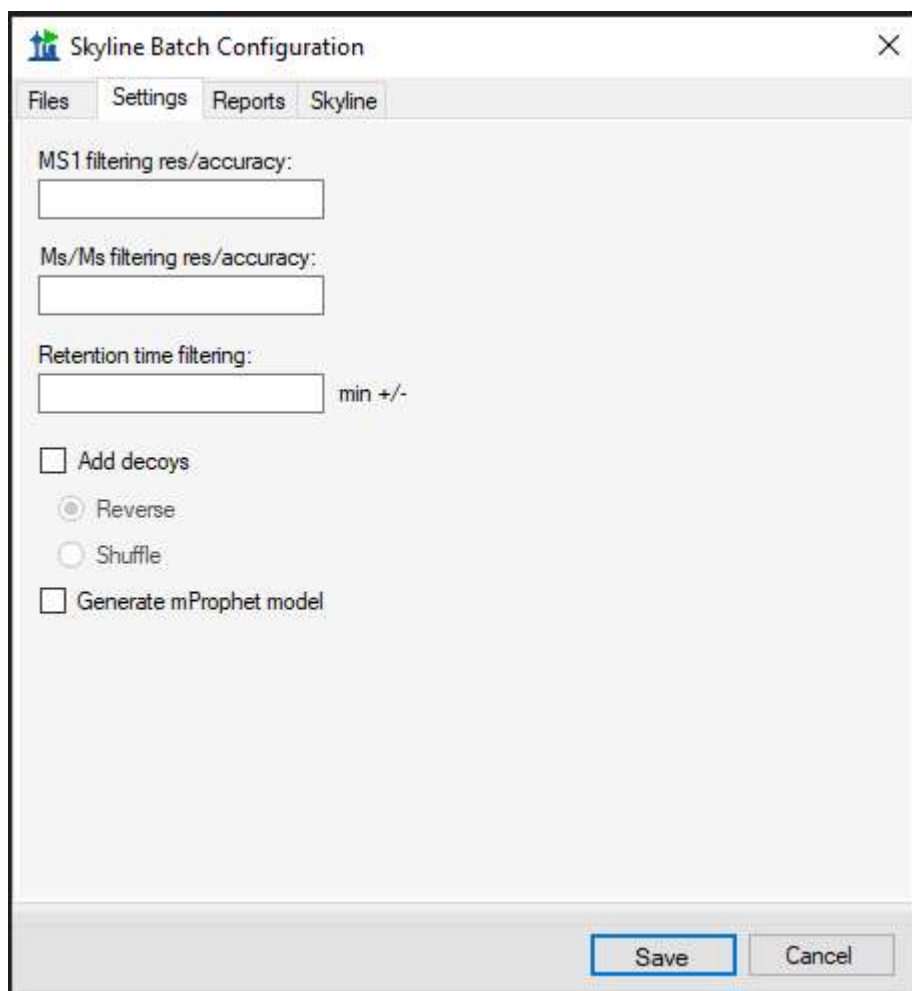
Replicate naming pattern (optional): A regular expression from which the first group will be used to name replicates when importing.

Note: For Skyline Batch to find the assay library, the Skyline Template file must be in the parent hierarchy of the analysis folder. (ie: If my Analysis folder is

“C:\Users\myaccount\Documents\Analysis”, then my template file must be in “users,” “myaccount,” or “Documents”)

CONFIGURATION FILTERS

In the **Filters** tab, various changes to the Skyline document can be specified.



The screenshot shows the 'Skyline Batch Configuration' dialog box with the 'Skyline' tab selected. The dialog has four tabs: 'Files', 'Settings', 'Reports', and 'Skyline'. The 'Skyline' tab contains the following configuration options:

- MS1 filtering res/accuracy: [text input field]
- Ms/Ms filtering res/accuracy: [text input field]
- Retention time filtering: [text input field] min +/-
- Add decoys
- Reverse
- Shuffle
- Generate mProphet model

At the bottom right of the dialog are 'Save' and 'Cancel' buttons.

MS1 filtering res/accuracy (optional): Resolving power of the precursor mass analyzer. If the mass analyzer is 'Centroided' the value is in PPM.

Ms/Ms filtering res/accuracy (optional): Resolving power of the product mass analyzer. If the mass analyzer is 'Centroided' the value is in PPM.

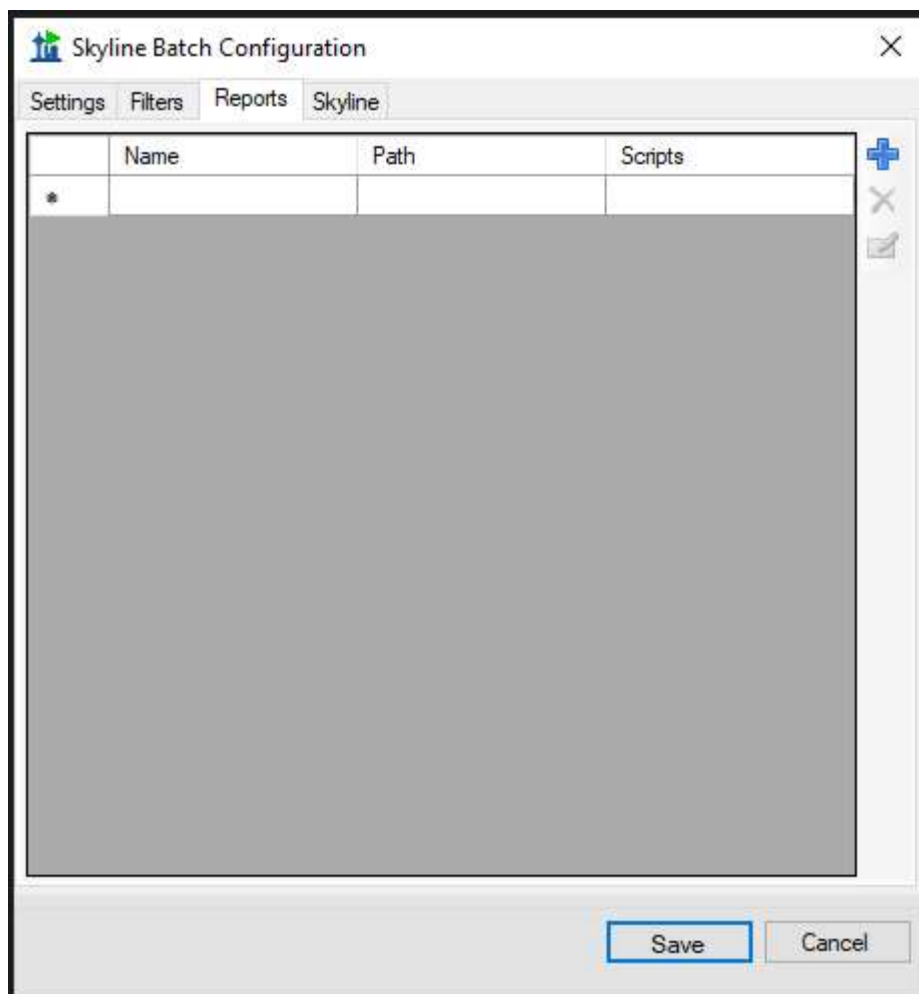
Retention time filtering (optional): The number of minutes on either side of the predicted time or MS/MS IDs, i.e. \pm minutes.

Add decoys: Add decoys to the template document for reintegrate model generation with mProphet. Added decoys are shuffled or reversed based on selection.

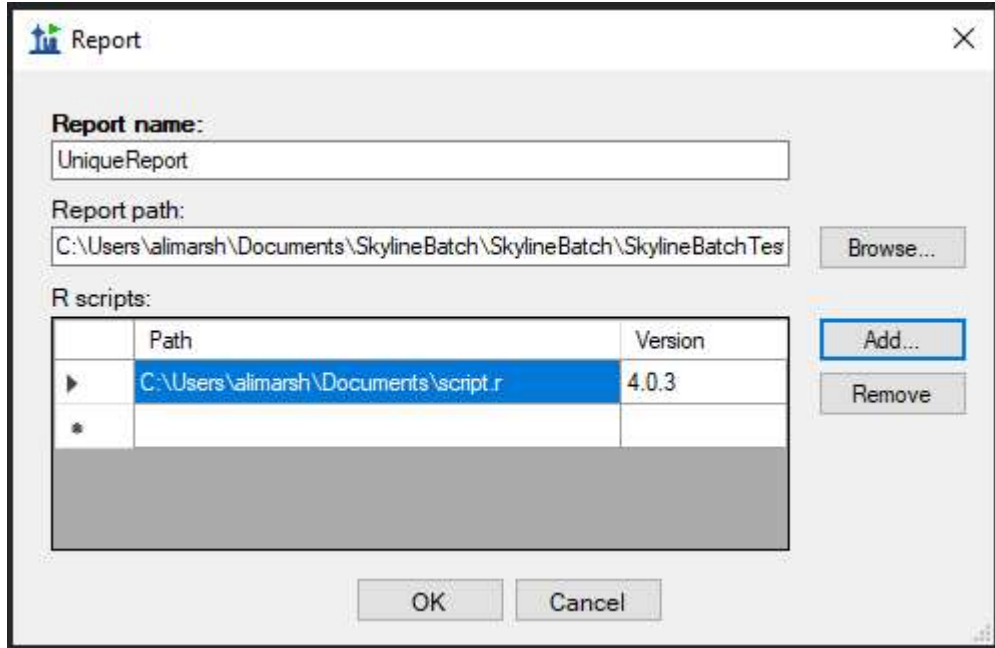
Generate mProphet model: causes a new model to be created, using the mProphet algorithm with all available scores for the results found in the document. Existing manually integrated peaks will be overwritten with peaks chosen by the reintegration model.

CONFIGURATION REPORTS

In the **Reports** tab, a list of reports can be created. If no reports are included for a configuration, Skyline Batch will skip steps 3 and 4 when this configuration is run.



To add a report, click the blue + icon on the right and fill in the appropriate information.

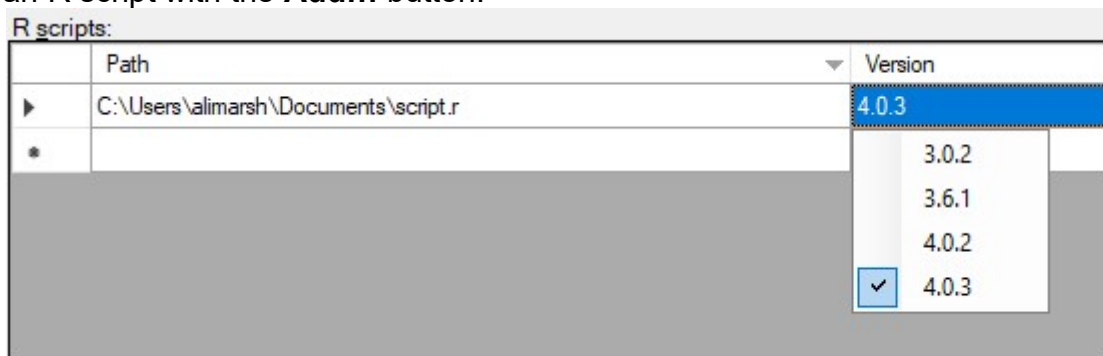


Report name: The name of the report to be exported. This MUST correspond to the name of the report when used in Skyline, which may differ from the file name. To find the name of a report, open the skyr file in Notepad and look for: *name="report name here"*.

Report path: The path to the skyr file.

R scripts: The list of R scripts to run on this report after it has been exported to a csv file. You must have R installed in C:\Program Files to add an R script.

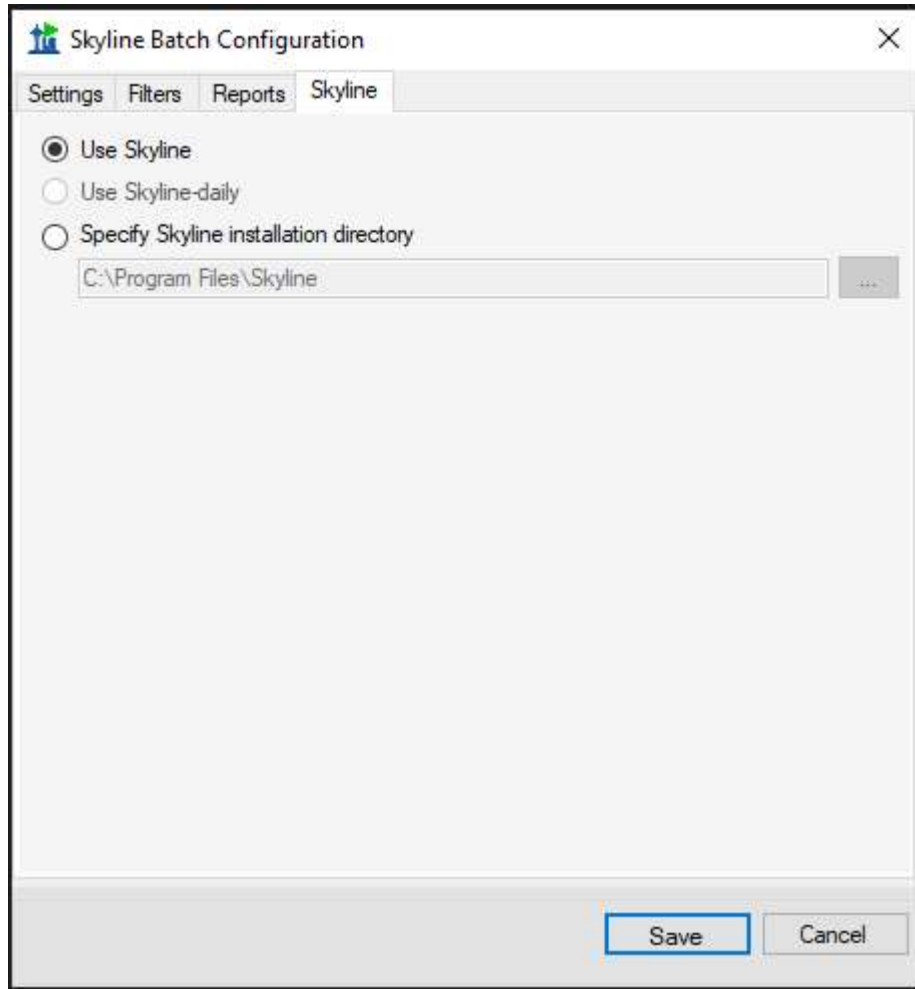
Add an R script with the **Add...** button.



Skyline Batch defaults to running all R scripts on the most recent downloaded version of R, but if you want to specify a different version this can be done by clicking in the **Version** column of the table to see a drop-down menu of all installed R versions, then selecting the desired version.

Click **OK** to add the report.

SKYLINE SETTINGS



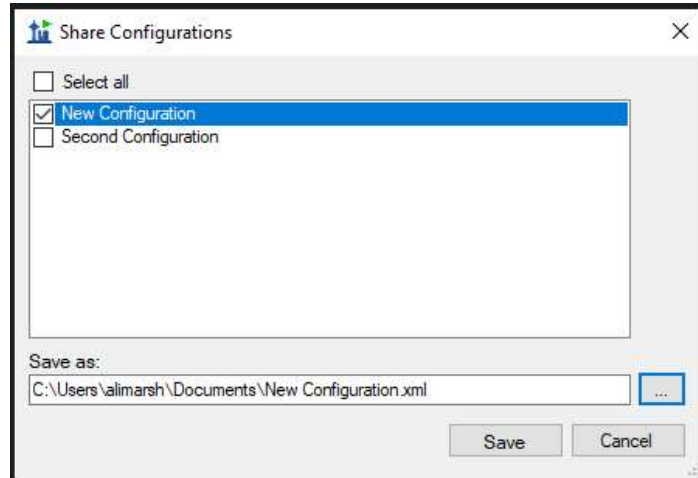
The image shows a screenshot of the "Skyline Batch Configuration" dialog box, specifically the "Skyline" tab. The dialog has a title bar with the application icon and the text "Skyline Batch Configuration" and a close button (X). Below the title bar are four tabs: "Settings", "Filters", "Reports", and "Skyline". The "Skyline" tab is active. Inside the tab, there are three radio button options: "Use Skyline" (which is selected), "Use Skyline-daily", and "Specify Skyline installation directory". Below the "Specify Skyline installation directory" option is a text input field containing the path "C:\Program Files\Skyline" and a browse button (three dots). At the bottom right of the dialog are two buttons: "Save" and "Cancel".

In the **Skyline** tab of the **Skyline Batch Configuration** form, you can specify which installation of skyline this configuration will use when run. If you do not have an administrative or web-based installation of Skyline or Skyline-daily, the corresponding option will be grayed out. You can always specify a path to a Skyline (or Skyline-daily) installation somewhere else on your computer.

Click **Save** to add the configuration to the list and return to the main window.

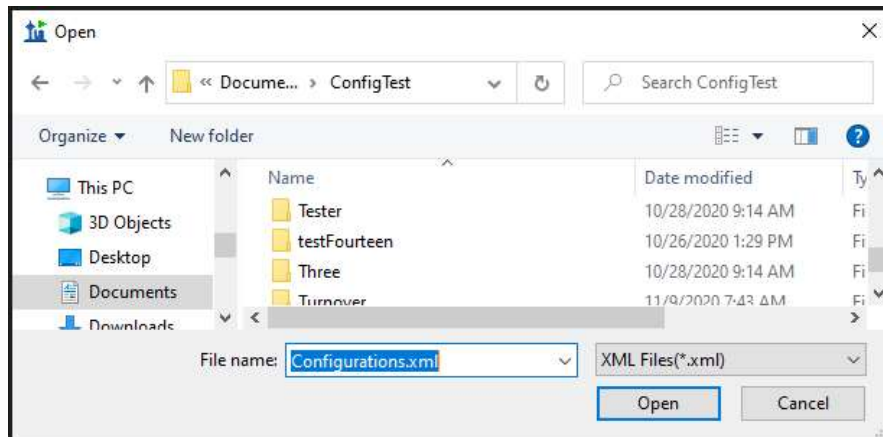
SHARING CONFIGURATIONS

To save some or all of your existing configurations to an xml file, click the **Share...** button in the main window.

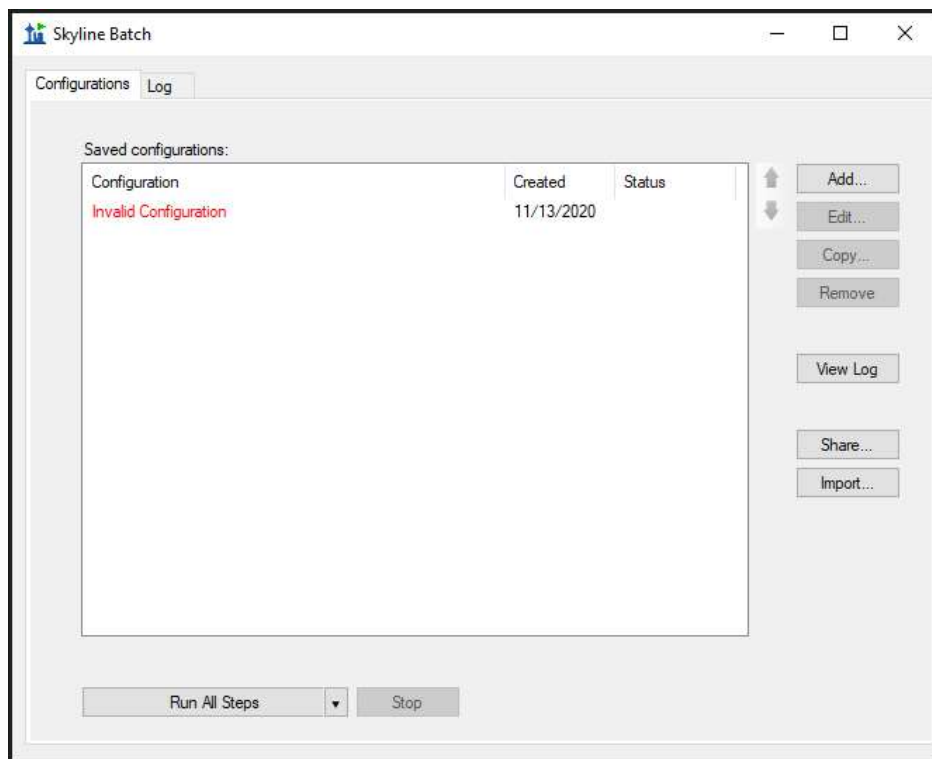


In the Share Configurations Form, you can select configurations you wish to save and specify a file path. Then click **Save**.

The configurations in the resulting file can be imported using the **Import...** button and specifying the path to the file you wish to import from.

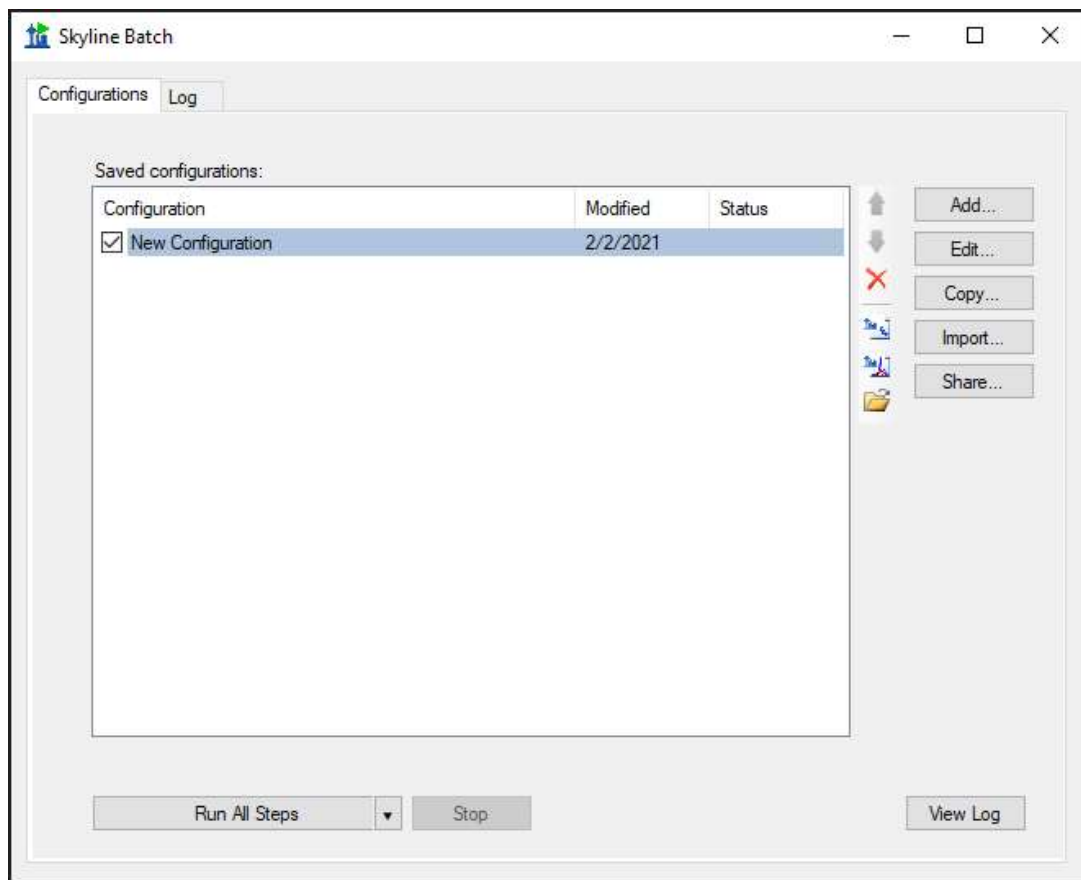


INVALID CONFIGURATIONS



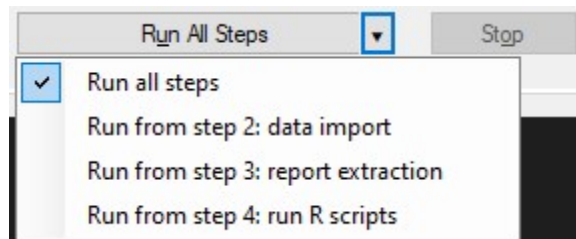
Invalid configurations may occur if files in the configuration do not exist. They will appear in red, and will prevent any configuration from running. When you **Edit** an invalid configuration, the **Configuration Set Up Manager** will guide you through the process of fixing it.

RUNNING CONFIGURATIONS



To run a configuration, check the checkbox next to it, then click the **Run All Steps** button.

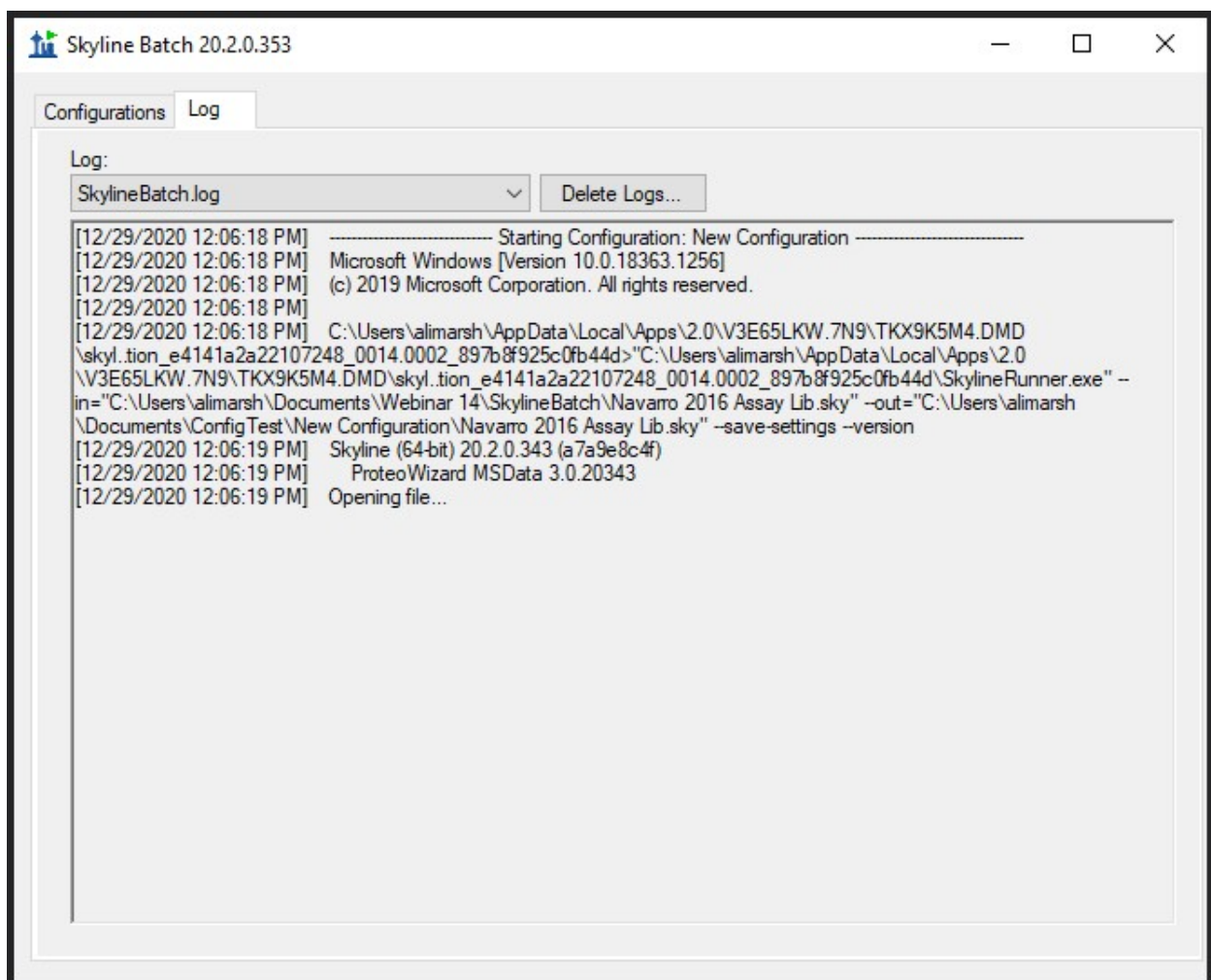
The **Run All Steps** button will run steps 1-4 on every checked configuration in the list from top to bottom. The downward-facing triangle on the righthand side of the **Run All Steps** button enables the user to start from a later step when running the configurations.



When the **Run All Steps** button is clicked, the program switches to the **Log** tab to display the progress of the current run. Returning to the **Configurations** tab, the status of all configurations is displayed, and will show if they are Running, Waiting, Finished, Cancelled, or had an Error. Stop the configurations from running by clicking the **Stop** button. If any configurations are running when Skyline Batch is closed, they will be cancelled.

LOGGING

This is the view of the **Log** tab immediately after the configurations have started running.



The log will display all output from running the configurations, including which configuration is currently running, what step it's on, and the progress of that step. Logs of previous runs can be viewed by clicking the **Log:** dropdown menu. Previous logs can be managed with the **Delete Logs...** button.

ACCESSING RESULTS

When a configuration has finished running, the resulting skyline document and exported reports can be found in its analysis folder. To open the analysis folder, click on the configuration in the main window. Then click on the Open Folder icon in the toolbar.

