MSInspector - QA targeted MS data for CPTAC assay portal

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The CPTAC Assay Portal is a repository of highly characterized targeted proteomics assays. MSInspector is a tool to evaluate the quality and completeness of data in the five experiments outlined by the CPTAC assay characterization guidance document. These experiments are designed to characterize the linear range, limits of detection, repeatability, selectivity, stability, and reproducibility of targeted proteomics assays. Successful use of the MSInspector tool will ensure a smooth upload of data to the portal.

This tutorial will guide you through the installation of MSInspector and the implementation of Quality Assurance (QA) on a sample Skyline file. In order to use MSInspector with Skyline, it must be installed through the Skyline external tools framework. This is because MSInspector requires the use of custom annotations in Skyline based on the predefined Skyline template for individual experiment. The underlying processing uses Python programming scripts and related Python modules which are automatically installed as part of the MSInspector external tool installation. You will also need to download and install R (v3.5.2 or higher is recommended) and the executable R file, “Rscript.exe,” which is also described below. Finally, the tutorial contains a sample Skyline file for testing the tool is working properly and highlighting its application.

Getting Started

To start this tutorial, download MSInspector_tutorial.zip from https://skyline.ms/files/home/software/Skyline/tools/_tool_MSInspector_1.2/MSInspector_tutorial.zip.

Extract the files to a folder on your computer. This will create a new folder: MSInspector_tutorial

The zip file contains the following files:
MSInspector_ResponseCurve_test.sky.zip: Contains a Skyline document with sample data for Experiment 1 in MSInspector.
MSInspector_Repeatability_test.sky.zip: Contains a Skyline document with sample data for Experiment 2 in MSInspector.
MSInspector_Selectivity_test.sky.zip: Contains a Skyline document with sample data for Experiment 3 in MSInspector.
MSInspector_Stability_test.sky.zip: Contains a Skyline document with sample data for Experiment 4 in MSInspector.
MSInspector_EndogenousReproducibility_test.sky.zip: Contains a Skyline document with sample data for Experiment 5 in MSInspector.

To open the Skyline document, perform the following steps:

- Open Skyline.
- On the **File** menu, click **Open**.

- Set the **file type** in the **File name** field to be “Skyline Files (*.sky, *.sky.zip)”’.
- Navigate to the *.sky.zip file in the folder you created and open it.
- Alternatively, you can also extract the *.sky.zip file first and then open the *.sky file directly.

Installing MSInspector as an External Tool in Skyline

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

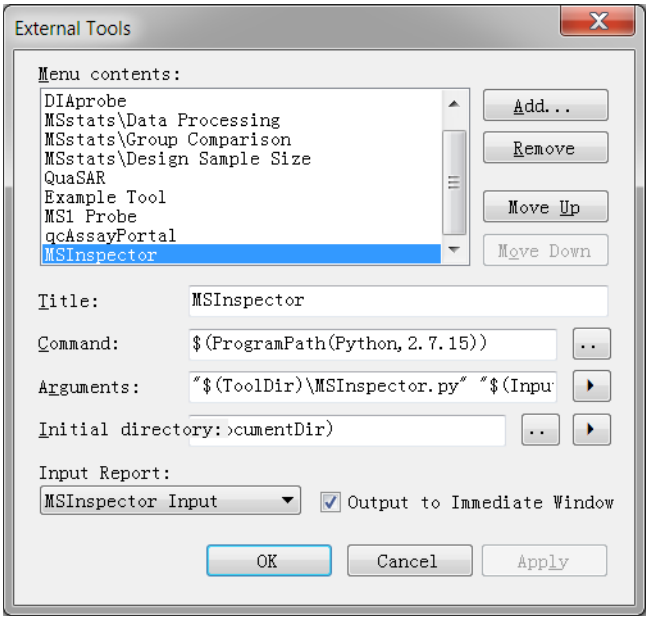
To add MSInspector as an external tool, perform the following steps in Skyline:

- On the **Tools** menu, click **External Tools** to bring up the **External Tools** form.
- Click the **Add** button and select **From File…** from the menu that appears.
- Navigate to the location of the MSInspector_1.1.zip file in the folder you created and select it.
- Click **Open**.

Skyline will now guide you through the process of installing MSInspector as an external tool. This process may require you to install the Python programming environment (currently Python 2.7, 32-bit will be installed even if a 64-bit computer is used due to Python compatibility aspects), as well as a number of Python extension packages, including ‘pandas’ and ‘Jinja2’.

- If you do not have the specified version of Python 2.7 installed on your machine, wait for Python 2.7.15 to download. When the download is completed, you will be asked to install Python onto your computer. Complete the Python installation process by working through the Python installer wizard.
- Once the Python installation is completed (or Python was found in the computer registry from a previous install), Skyline will locate the Python package manager Pip on the local machine and install zip or tar.gz packages of ‘pandas’ and ‘Jinja2’using the pip install <package>.

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



Menu contents:

DIAprobe
MSstats\Data Processing
MSstats\Group Comparison
MSstats\Design Sample Size
QuaSAR
Example Tool
MS1 Probe
qcAssayPortal
MSInspector

Title: MSInspector

Command: $(ProgramPath(Python, 2.7.15))

Arguments: "$(ToolDir)\MSInspector.py" "$(Inpu

Initial directory: cumentDir)

Input Report: MSInspector Input

☑ Output to Immediate Window

! Important notes and troubleshooting regarding installation of Python and its packages:

- If the computer has a preinstalled instance of Python2.7 (version < 2.7.15 is allowed), check whether pip is installed or not. If pip is not installed, please install it by referring to https://pip.pypa.io/en/stable/installing/.
- From the command line prompt, check whether the packages of pandas and Jinja2 are installed by running "pip freeze". If they are not installed, run "pip install pandas==0.21.0 Jinja2==2.9.6" to install them. Check the version of the package of numpy by running "pip show numpy". If the version is lower than 1.13.0, update numpy by running "pip install numpy --upgrade".
- If you come across the error message "Microsoft Visual C++ 9.0 is required" when installing the Python packages, please install Microsoft Visual C++ Compiler for Python 2.7 from https://www.microsoft.com/en-us/download/details.aspx?id=44266
- Click **..** button on the right of the **Command** label and choose **Edit Macro…** to set the path of Python.
- Click the **OK** button on the **External Tools** form.

The built-in R scripts are called for data visualization and statistical calculation. First, download and install R v3.5.2 from: https://cran.r-project.org/bin/windows/base/old/3.5.2/R-3.5.2-win.exe, considering Skyline is a Windows application. After installation, the executable R file, "Rscript.exe," will be found in the default directory – "C:\Program Files\R\R-3.5.2\bin\Rscript.exe". Next, in the R console install the following packages by running: install.packages(c("Cairo", "evaluate", "reshape2", "stringr", "plyr", "MASS", "ggplot2", "dplyr")).

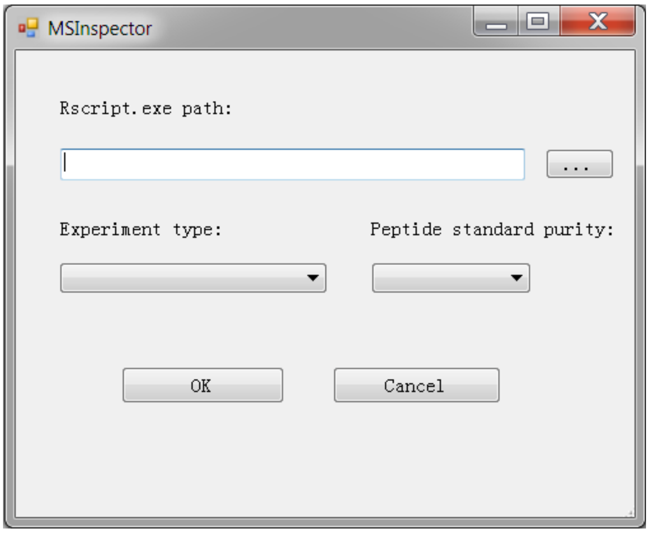
Check the installed ggplot2 version by running: packageVersion("ggplot2"). If the version is >= 3.3.0, uninstall ggplot2 by running: remove.packages("ggplot2"), then install ggplot2 version 3.1.0 in R console by running: install.packages("http://cran.r-project.org/src/contrib/Archive/ggplot2/ggplot2_3.1.0.tar.gz", repos = NULL, type = "source")

Using the **MSInspector** Tool to generate a QA report

The next section will guide you through application of MSInspector to check a characterization document.

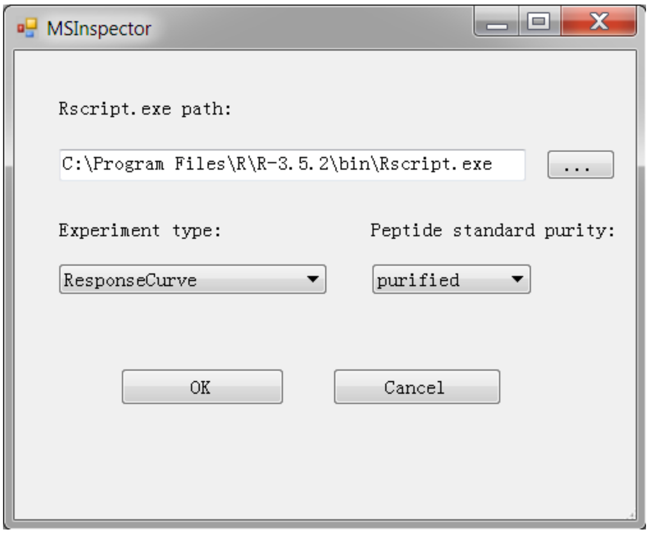
- Annotate the data according to the Skyline templates based on the experiment type. (Please refer to the document named Assay_Portal_User_Manual_V2.pdf)
- In Skyline, from the **Tools** Menu, select the **MSInspector** option.

The following **MSInspector** form will appear:



- For **Rscript.exe path** navigate to the location of Rscript.exe.
- Choose an option in **Experiment type**.
- Choose an option in **Peptide standard purity**.

If you open MSInspector_ResponseCurve_test.sky.zip, your MSInspector form should look as follows:

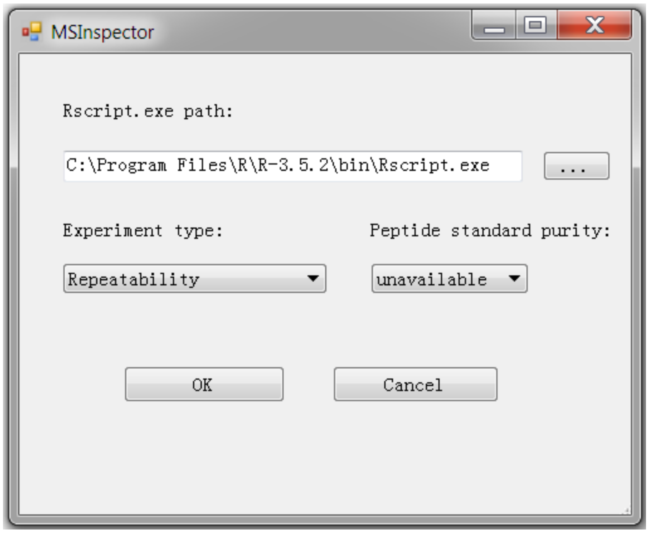


Rscript.exe path: C:\Program Files\R\R-3.5.2\bin\Rscript.exe

Experiment type: ResponseCurve

Peptide standard purity: purified

If you open MSInspector_Repeatability_test.sky.zip, your MSInspector form should look as follows:

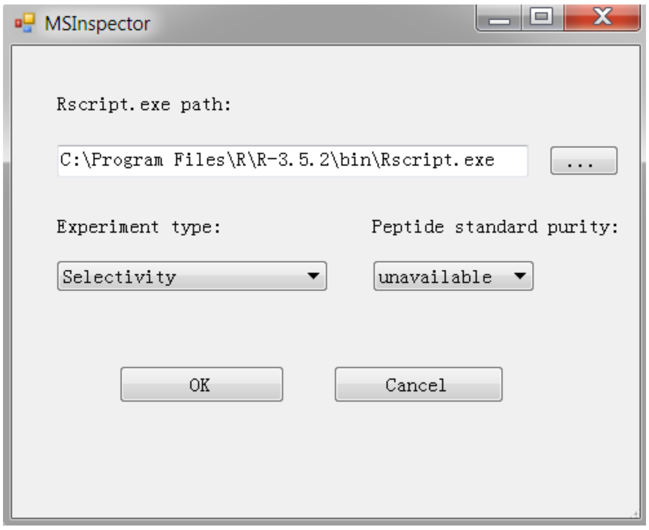


Rscript.exe path: C:\Program Files\R\R-3.5.2\bin\Rscript.exe

Experiment type: Repeatability

Peptide standard purity: unavailable

If you open MSInspector_Selectivity_test.sky.zip, your MSInspector form should look as follows:

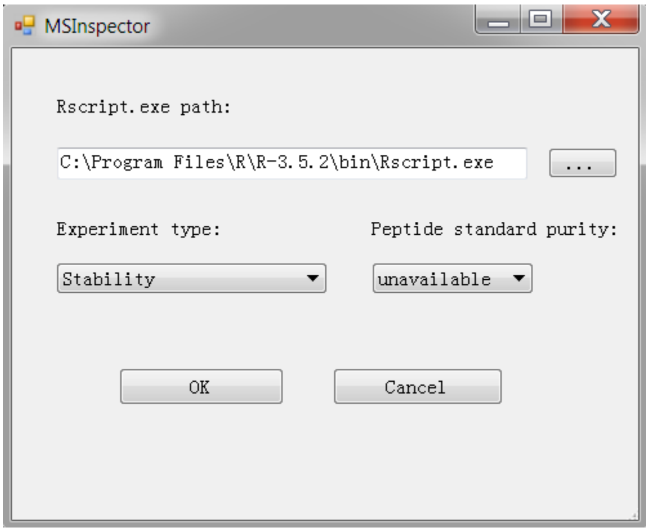


Rscript.exe path: C:\Program Files\R\R-3.5.2\bin\Rscript.exe

Experiment type: Selectivity

Peptide standard purity: unavailable

If you open MSInspector_Stability_test.sky.zip, your MSInspector form should look as follows:

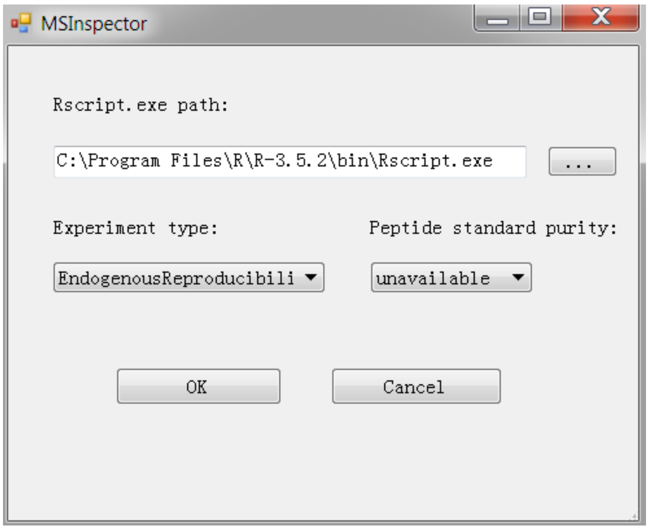


Rscript.exe path: C:\Program Files\R\R-3.5.2\bin\Rscript.exe

Experiment type: Stability

Peptide standard purity: unavailable

If you open MSInspector_EndogenousReproducibility_test.sky.zip, your MSInspector form should look as follows:



Rscript.exe path: C:\Program Files\R\R-3.5.2\bin\Rscript.exe

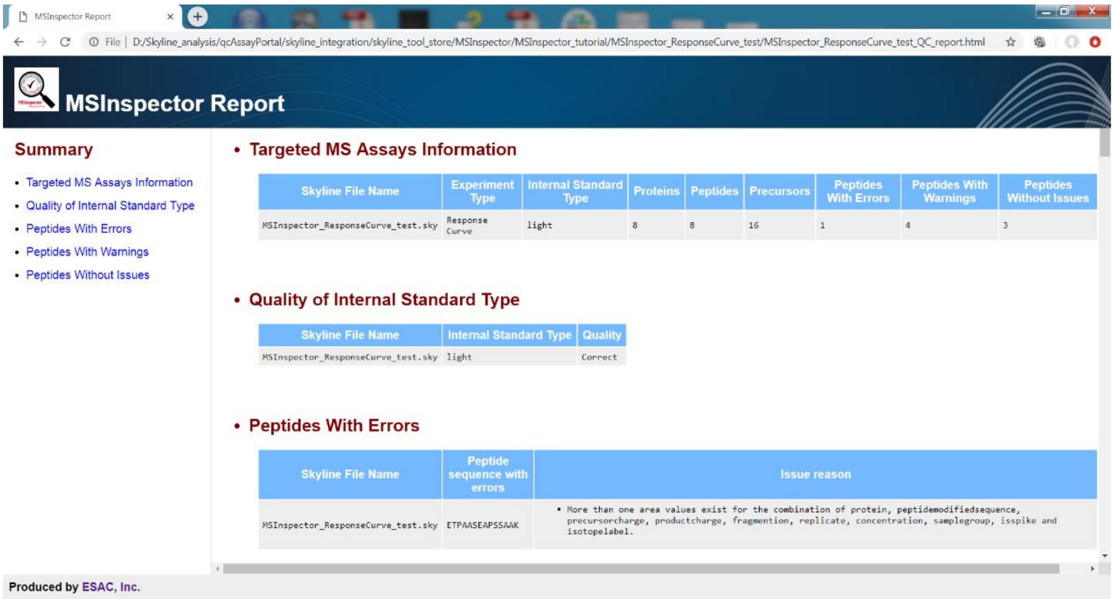
Experiment type: EndogenousReproducibili

Peptide standard purity: unavailable

- Click the **OK** button.

The MSInspector tool will now perform QA. 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 run is completed, switch to the newly created subdirectories named MSInspector_ResponseCurve_test, MSInspector_Repeatability_test, MSInspector_Selectivity_test, MSInspector_Stability_test and MSInspector_EndogenousReproducibility_test. The QA reports are generated there.

In the subdirectory of MSInspector_ResponseCurve_test, the *.html file should look as follows:



Targeted MS Assays Information

Skyline File Name	Experiment Type	Internal Standard Type	Proteins	Peptides	Precursors	Peptides With Errors	Peptides With Warnings	Peptides Without Issues
MSInspector_ResponseCurve_test.sky	Response Curve	light	8	8	16	1	4	3

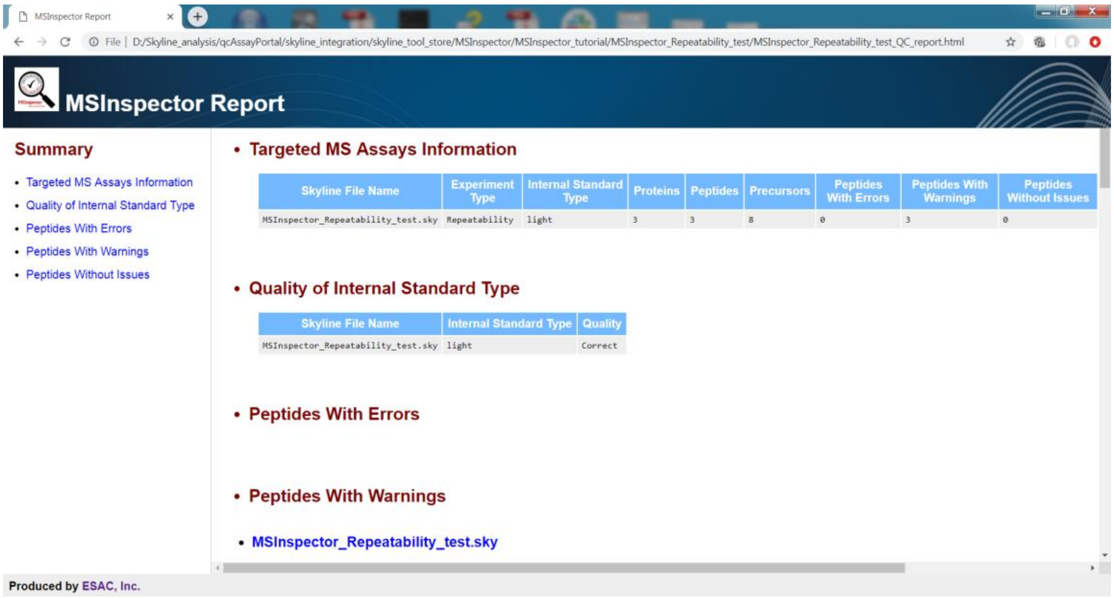
Quality of Internal Standard Type

Skyline File Name	Internal Standard Type	Quality
MSInspector_ResponseCurve_test.sky	light	Correct

Peptides With Errors

Skyline File Name	Peptide sequence with errors	Issue reason
MSInspector_ResponseCurve_test.sky	ETPAASEAPSSAAK	• More than one area values exist for the combination of protein, peptidemodifiedsequence, precursorcharge, productcharge, fragmention, replicate, concentration, samplegroup, isspike and isotopelabel.

In the subdirectory of MSInspector_Repeatability_test, the *.html file should look as follows:



D:/Skyline_analysis/qcAssayPortal/skyline_integration/skyline_tool_store/MSInspector/MSInspector_tutorial/MSInspector_Repeatability_test/MSInspector_Repeatability_test_QC_report.html

MSInspector Report

Summary

- Targeted MS Assays Information
- Quality of Internal Standard Type
- Peptides With Errors
- Peptides With Warnings
- Peptides Without Issues

- **Targeted MS Assays Information**

Skyline File Name	Experiment Type	Internal Standard Type	Proteins	Peptides	Precursors	Peptides With Errors	Peptides With Warnings	Peptides Without Issues
MSInspector_Repeatability_test.sky	Repeatability	light	3	3	8	0	3	0

- **Quality of Internal Standard Type**

Skyline File Name	Internal Standard Type	Quality
MSInspector_Repeatability_test.sky	light	Correct

- **Peptides With Errors**

- **Peptides With Warnings**

- **MSInspector_Repeatability_test.sky**

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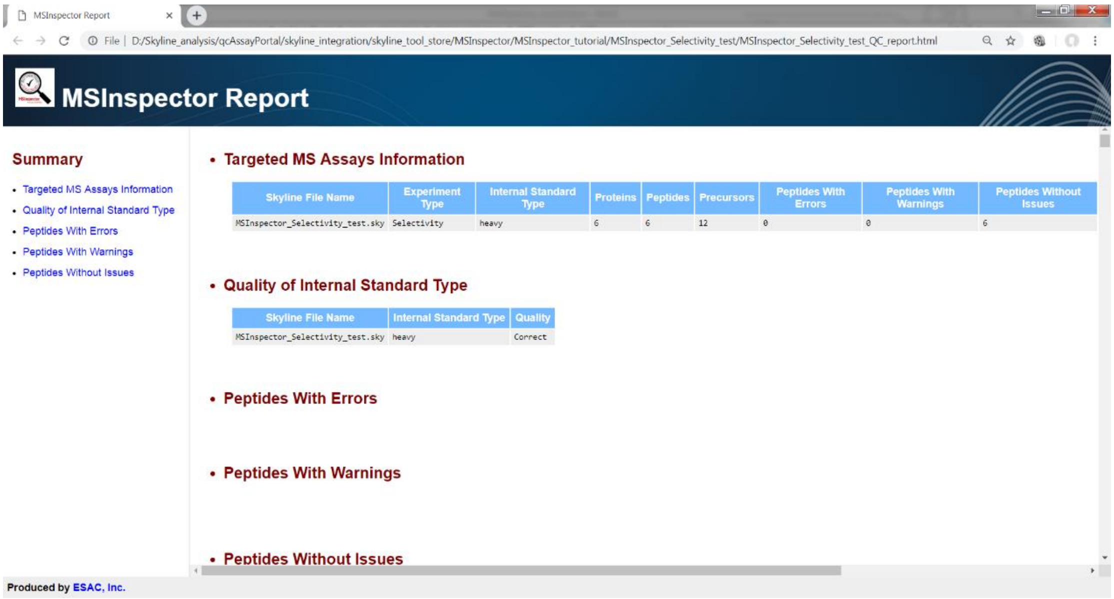
In the subdirectory of MSInspector_Selectivity_test, the *.html file should look as follows:



D:/Skyline_analysis/qcAssayPortal/skyline_integration/skyline_tool_store/MSInspector/MSInspector_tutorial/MSInspector_Selectivity_test/MSInspector_Selectivity_test_QC_report.html

MSInspector Report

Summary

- Targeted MS Assays Information
- Quality of Internal Standard Type
- Peptides With Errors
- Peptides With Warnings
- Peptides Without Issues

- **Targeted MS Assays Information**

Skyline File Name	Experiment Type	Internal Standard Type	Proteins	Peptides	Precursors	Peptides With Errors	Peptides With Warnings	Peptides Without Issues
MSInspector_Selectivity_test.sky	Selectivity	heavy	6	6	12	0	0	6

- **Quality of Internal Standard Type**

Skyline File Name	Internal Standard Type	Quality
MSInspector_Selectivity_test.sky	heavy	Correct

- **Peptides With Errors**

- **Peptides With Warnings**

- **Peptides Without Issues**

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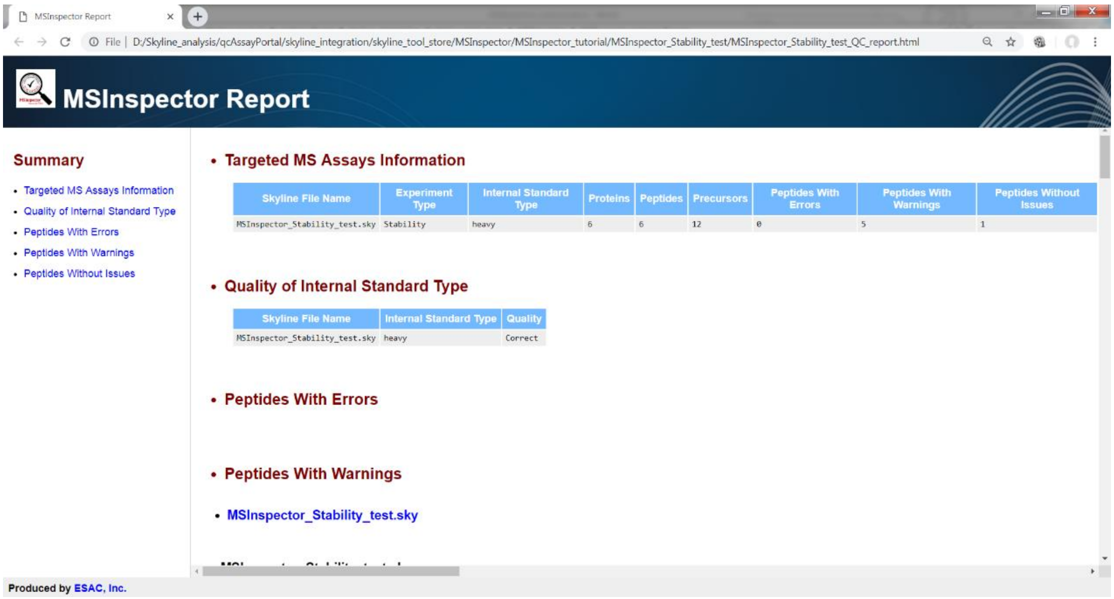
In the subdirectory of MSInspector_Stability_test, the *.html file should look as follows:



MSInspector Report

Summary

- Targeted MS Assays Information
- Quality of Internal Standard Type
- Peptides With Errors
- Peptides With Warnings
- Peptides Without Issues

- **Targeted MS Assays Information**

Skyline File Name	Experiment Type	Internal Standard Type	Proteins	Peptides	Precursors	Peptides With Errors	Peptides With Warnings	Peptides Without Issues
MSInspector_Stability_test.sky	Stability	heavy	6	6	12	0	5	1

- **Quality of Internal Standard Type**

Skyline File Name	Internal Standard Type	Quality
MSInspector_Stability_test.sky	heavy	Correct

- **Peptides With Errors**
- **Peptides With Warnings**
  - **MSInspector_Stability_test.sky**

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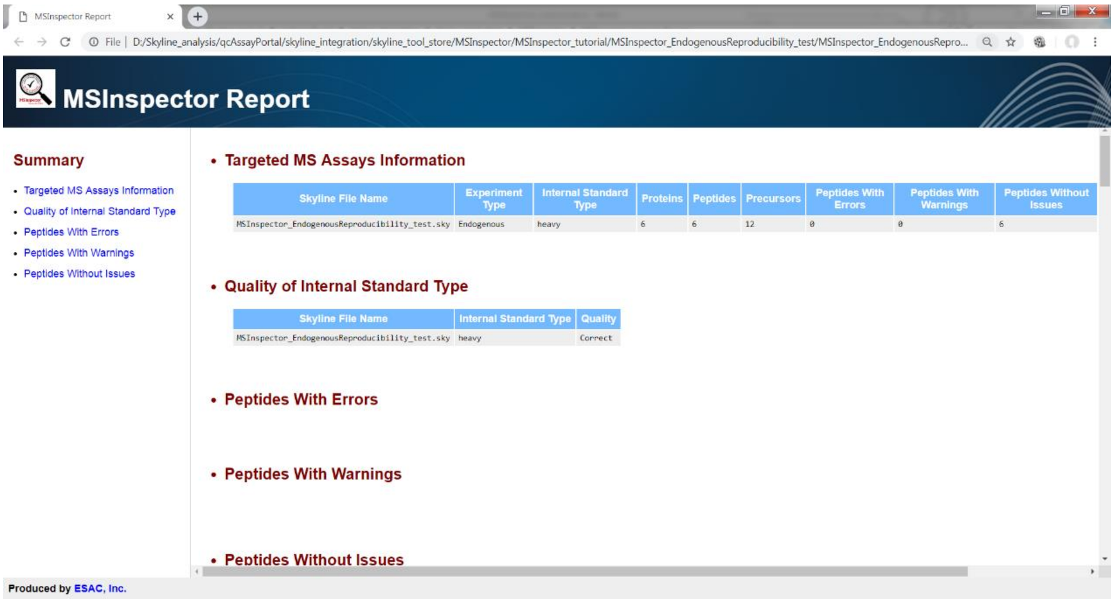
In the subdirectory of MSInspector_ EndogenousReproducibility_test, the *.html file should look as follows:



MSInspector Report

Summary

- Targeted MS Assays Information
- Quality of Internal Standard Type
- Peptides With Errors
- Peptides With Warnings
- Peptides Without Issues

- **Targeted MS Assays Information**

Skyline File Name	Experiment Type	Internal Standard Type	Proteins	Peptides	Precursors	Peptides With Errors	Peptides With Warnings	Peptides Without Issues
MSInspector_EndogenousReproducibility_test.sky	Endogenous	heavy	6	6	12	0	0	6

- **Quality of Internal Standard Type**

Skyline File Name	Internal Standard Type	Quality
MSInspector_EndogenousReproducibility_test.sky	heavy	Correct

- **Peptides With Errors**
- **Peptides With Warnings**
- **Peptides Without Issues**

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For the details of the issue (including error and warning) messages and the solutions to the issues, please refer to the document named **issue_categories.pdf**.

For reference, the work flow of MSInspector is shown in **workflow.pdf**.