CPxplorer

https://github.com/WBS-TW/CPxplorer

A platform-independent tool for rapid quantification and harmonization of polychlorinated alkanes data

CPxplorer

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WORKFLOW

1

CREATE A TARGET LIST

CPion

CPion is an online app that allows exploring mass spectral interferences and creating a target ion list, which excludes the interfering m/z values

2

INTEGRATE THE DATA

Skyline

Skyline is open-source software that allows integrating the mass spectrometric data based on the target list created by CPion and does not necessitate prior data conversion

3

QUANTIFY THE DATA

CPquant

CPquant is an online app that allows quantifying the integration results from Skyline using the homologue deconvolution method

CONTACT INFORMATION:

idoia.beloqui.ezquer@liu.se
thanh.wang@liu.se

CPion

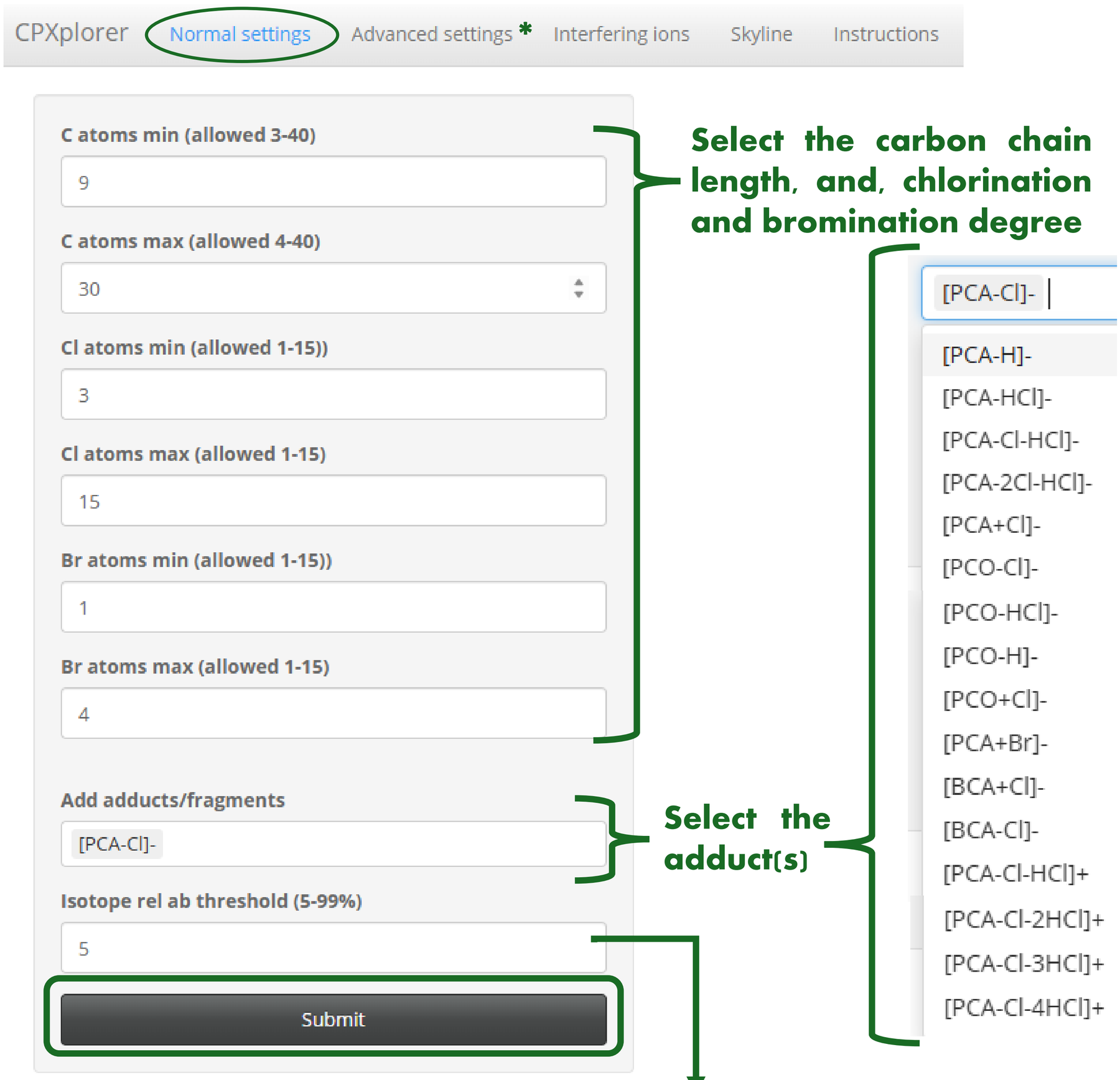
CPxplorer

CREATE A TARGET LIST

CPion is a module part of the CPxplorer package. It consists of an online app that can be found at https://github.com/WBS-TW/CPxplorer. It operates on the browser, so it does not require to be downloaded.

CPion is a tool for exploring mass spectral interferences of compounds that are present at chlorinated paraffin (CP) mixtures, including polychlorinated alkanes (PCAs), brominated alkanes (BCAs), polychlorinated olefins (PCOs) and PCA- transformation products.

The user can select the compounds that want to be investigated at the initial window of CPion, "Normal setting":



Select the carbon chain length, and, chlorination and bromination degree

Select the adduct(s)

CALCULATE THE INTERFERENCES

Only the m/z values that have a relative abundance above the threshold will be generated

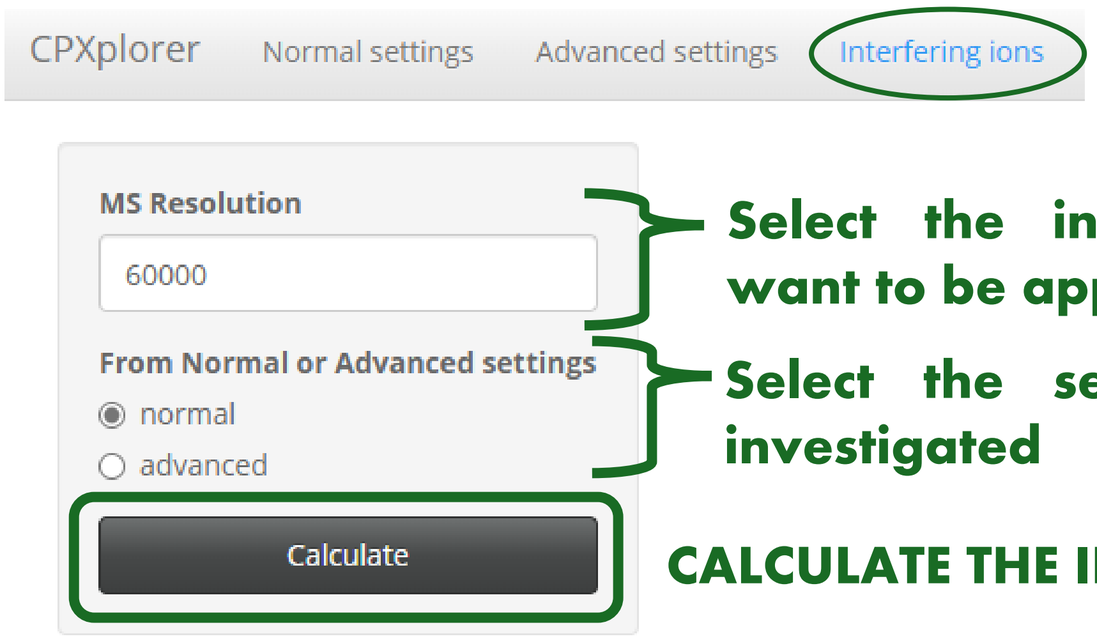
*** BCAs and PCA transformation products can be generated at the "Advanced setting" window**

CPion

CPxplorer

CREATE A TARGET LIST

The ions that fit the selected parameters will be generated and compiled in a table at the "Normal settings" window. The interferences between the m/z values can be investigated at the "Interfering ions" window:

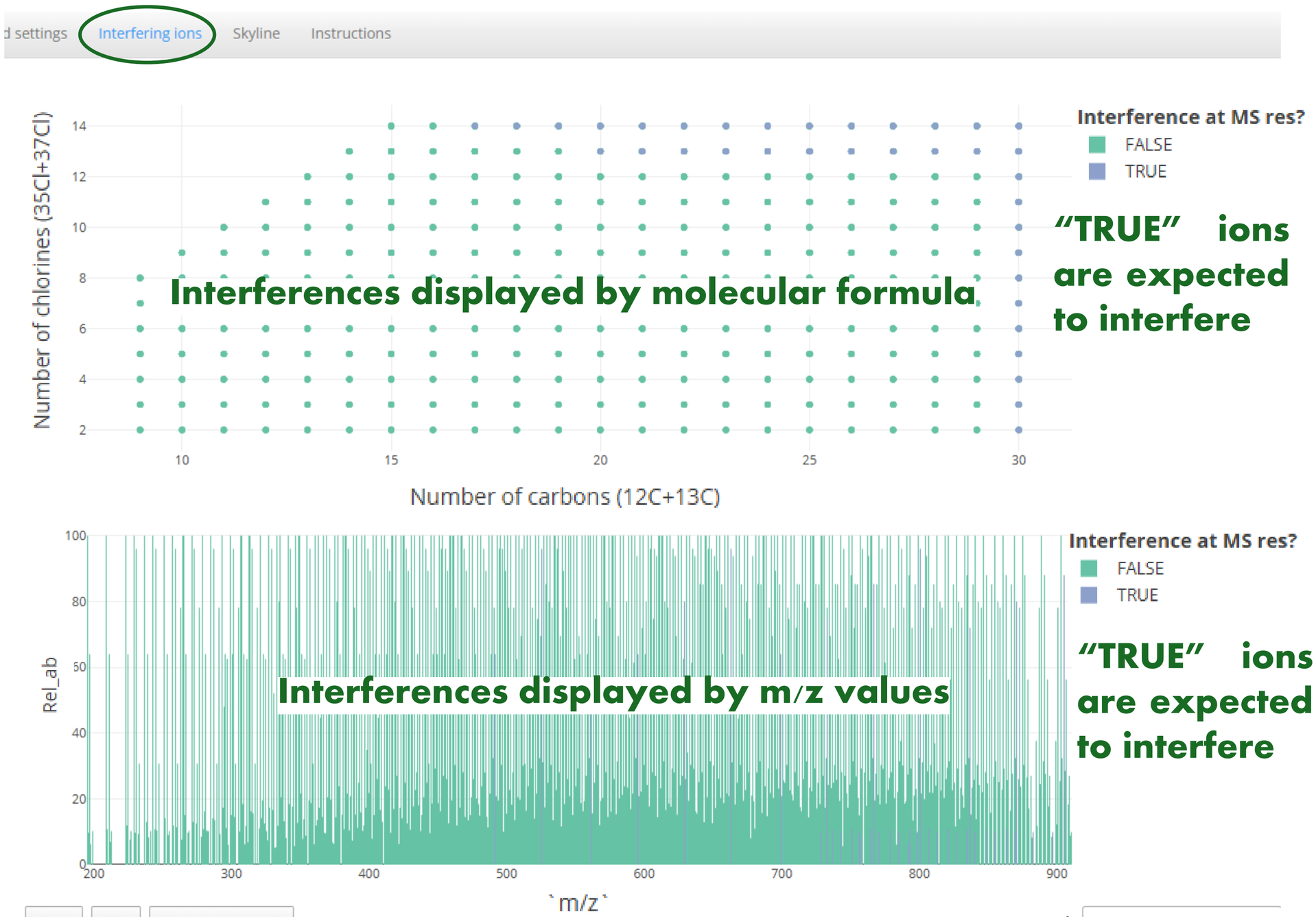


Select the instrumental resolution that want to be applied

Select the settings that want to be investigated

CALCULATE THE INTERFERENCES

The interferences will be displayed in interactive graphs and table:



"TRUE" ions are expected to interfere

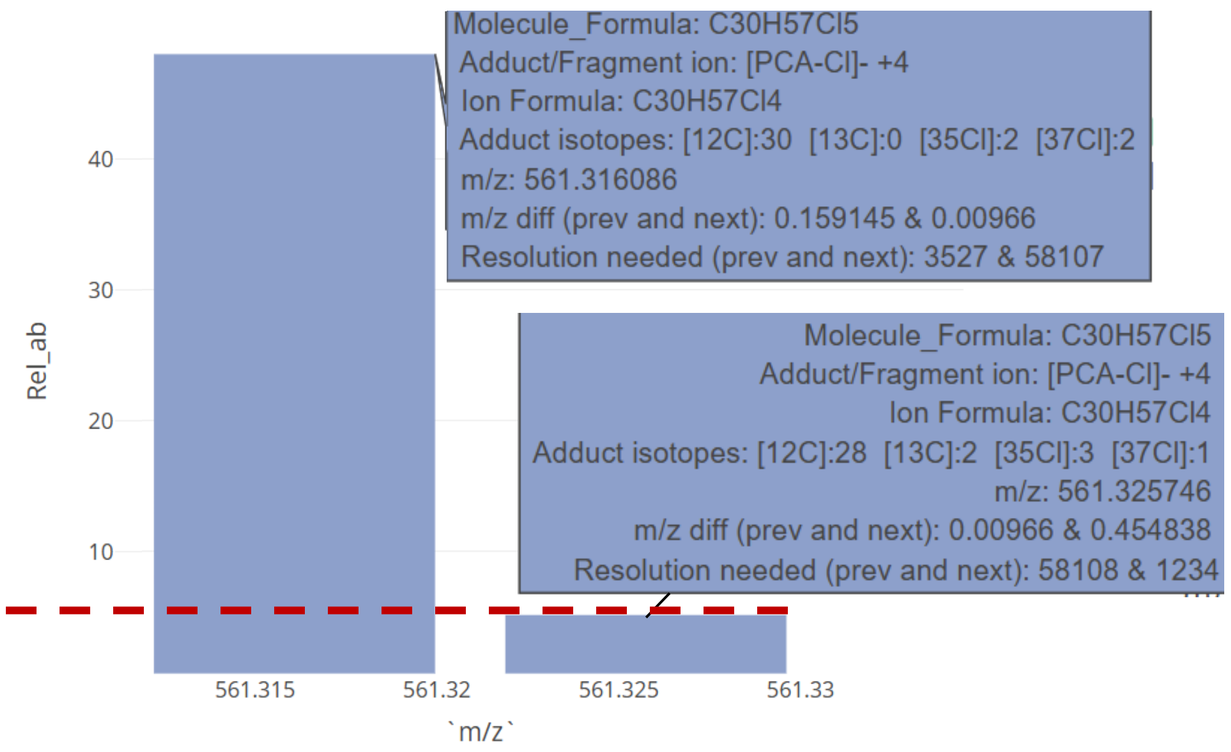
"TRUE" ions are expected to interfere

Molecule_Formula	Halo_perc	Charge	Adduct	Adduct_Formula	Isotopologue	Isotope_Formula	m/z	Rel_ab
C9H17Cl3	46	-1	[PCA-Cl]-	C9H17Cl2		[12C]9[13C]0[1H]17[2H]0[35Cl]2[37Cl]0	195.07128	100
C9H17Cl3	46	-1	[PCA-Cl]- +1	C9H17Cl2	+1	[12C]8[13C]1[1H]17[2H]0[35Cl]2[37Cl]0	196.074634	9.7
C9H17Cl3	46	-1	[PCA-Cl]- +2	C9H17Cl2	+2	[12C]8[13C]1[1H]17[2H]0[35Cl]1[37Cl]1	197.068329	64
C9H17Cl3	46	-1	[PCA-Cl]- +3	C9H17Cl2	+3	[12C]8[13C]1[1H]17[2H]0[35Cl]1[37Cl]1	198.071684	6.2
C9H17Cl3	46	-1	[PCA-Cl]- +4	C9H17Cl2	+4	[12C]9[13C]0[1H]17[2H]0[35Cl]0[37Cl]2	199.065379	10.2

Compilation of all ions and their interferences

CREATE A TARGET LIST

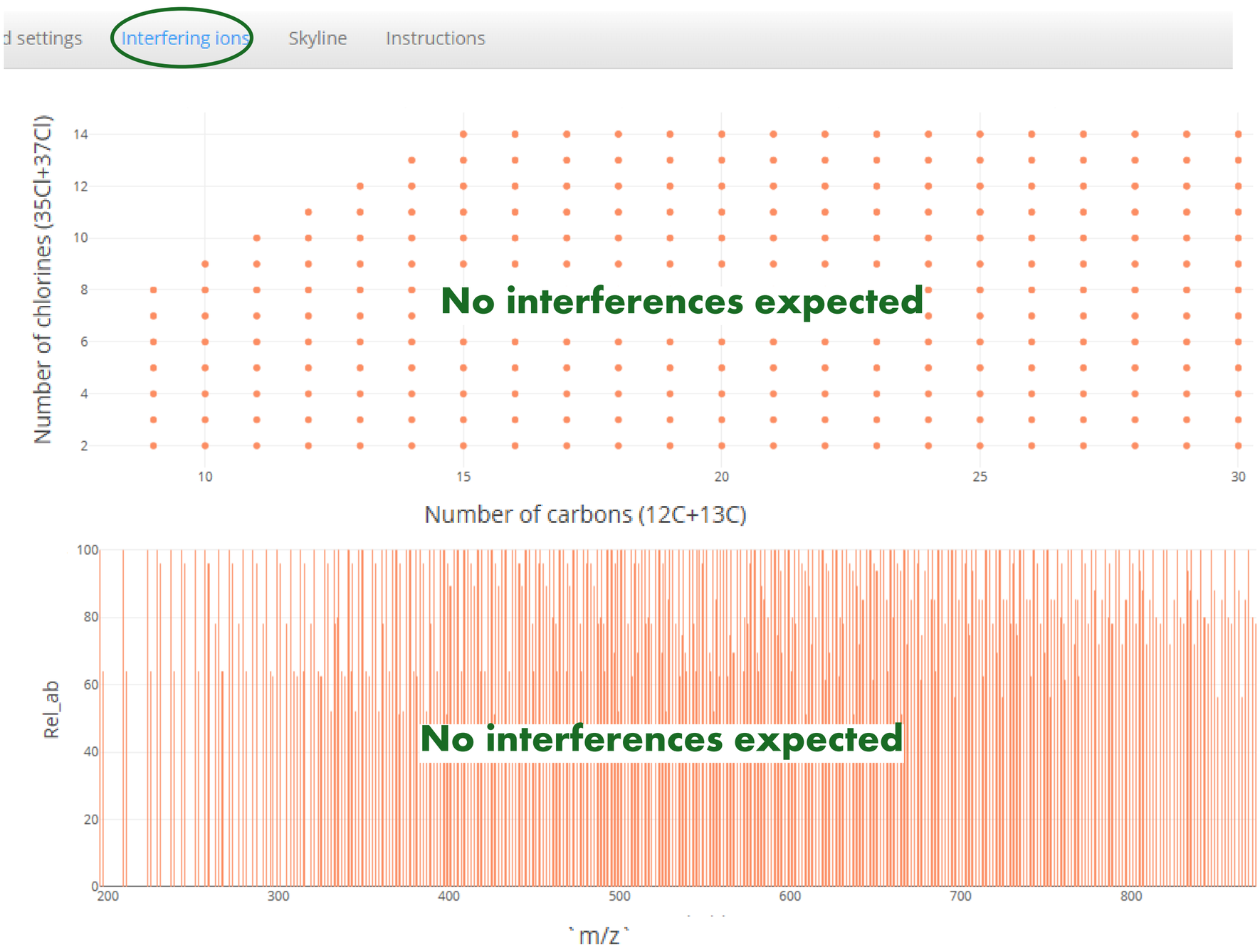
The graphs can be zoomed in and more information about the interfering ions will be displayed. This allows the user to understand the interferences better and select the target ions. As explained in the following example:



Some of the $[C_{30}H_{57}Cl_4\text{-}Cl]^-$ +4 isotopes were spotted to interfere, however, the ion with m/z 562.325746 has a relative abundance below 10, and therefore setting the threshold above it would exclude this ion from the target list

The user can then return to the "Normal setting" window, set a higher relative abundance threshold, calculate the ions above it, and investigate their interferences.

The user can change the relative abundance threshold until finding the one where no ions are expected to interfere and at least three isotopomers are considered for each molecular formula:

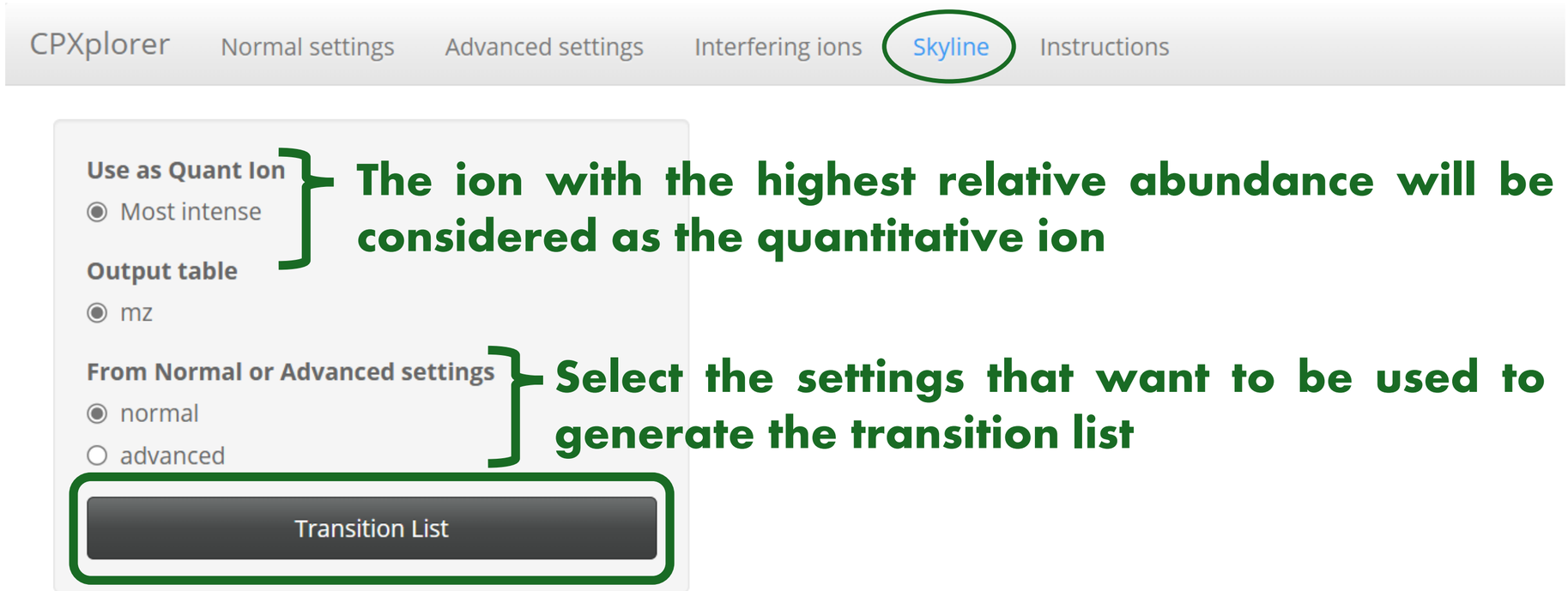


CPion

CPxplorer

CREATE A TARGET LIST

Once the user has selected the target ions, the target list can be created at the "Skyline" window.



GENERATE THE TARGET LIST

The target list can be modified by the user, and it can exported to Excel or CSV files.

Excel | CSV | Column visibility ▼ | Search:

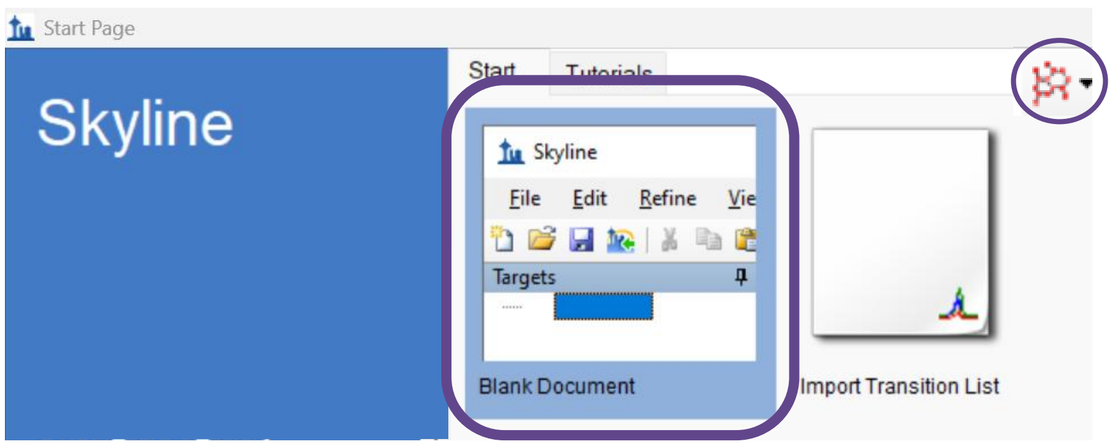
Molecule List Name	Molecule Name	Precursor Charge	Label Type	Precursor m/z	Explicit Retention Time	Explicit Retention Time Window	Note
All	All	All		All	All	All	All
PCA-C11	C11H19Cl5	-1	Qual	296.02209			[PCA-Cl]- -
PCA-C11	C11H19Cl5	-1	Qual	297.015785			[PCA-Cl]- -
PCA-C11	C11H18Cl6	-1	Qual	324.985663			[PCA-Cl]-
PCA-C11	C11H18Cl6	-1	Qual	325.989018			[PCA-Cl]- -
PCA-C11	C11H18Cl6	-1	Quan	326.982713			[PCA-Cl]- -
PCA-C11	C11H18Cl6	-1	Qual	327.986067			[PCA-Cl]- -
PCA-C11	C11H18Cl6	-1	Qual	328.979762			[PCA-Cl]- -
PCA-C11	C11H18Cl6	-1	Qual	329.983117			[PCA-Cl]- -
PCA-C11	C11H18Cl6	-1	Qual	330.976812			[PCA-Cl]- -
PCA-C11	C11H17Cl7	-1	Qual	358.94669			[PCA-Cl]-

The list including the target compounds is named "Transition list" in Skyline it will be used to extract the ion chromatograms from the raw data in the next step.

INTEGRATE THE DATA

The next step of the CPxplorer workflow evolves in Skyline, an open-source Windows client software for treating mass spectrometric data. Skyline can be freely downloaded at the following link: https://skyline.ms/project/home/software/skyline/begin.view

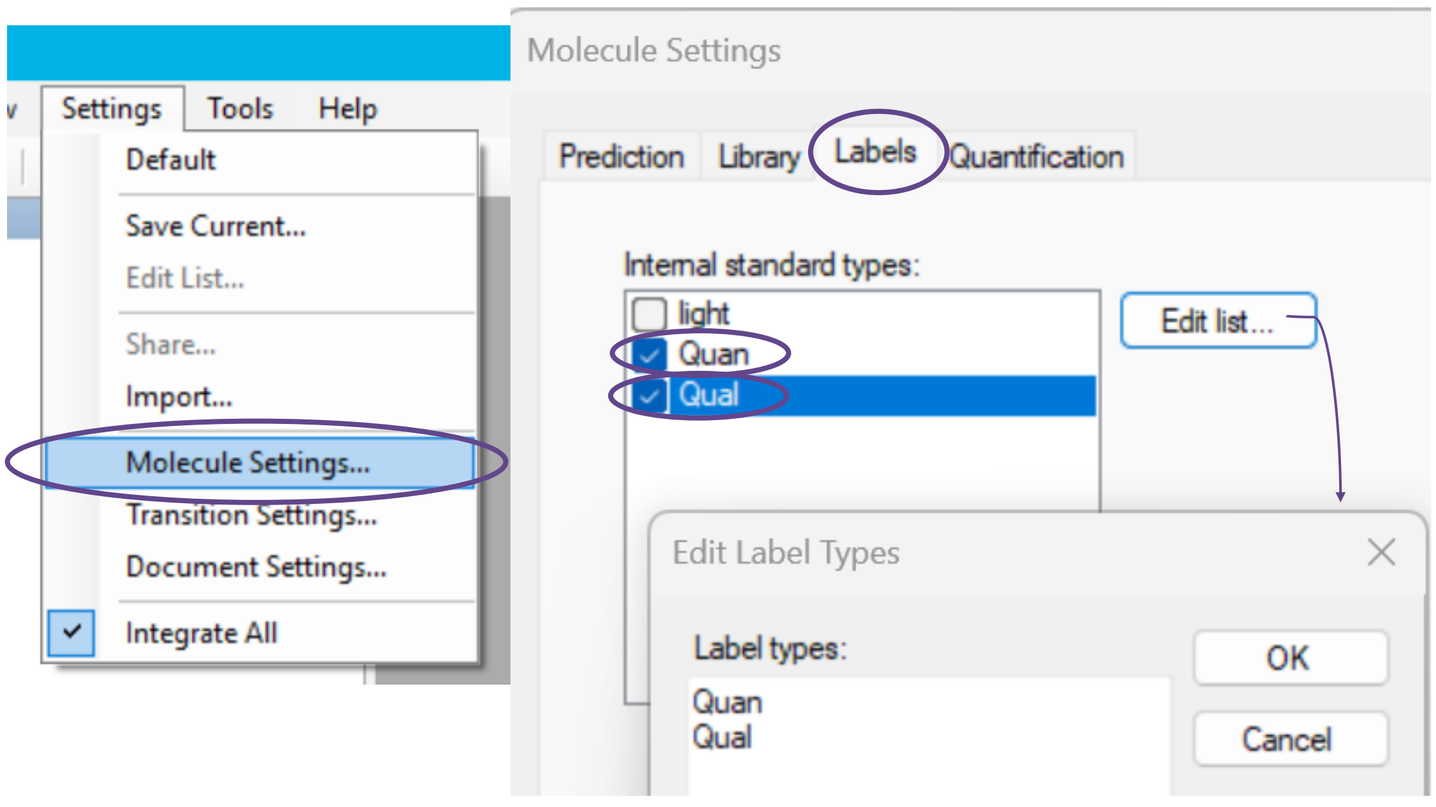
Since Skyline can be used for the analysis of proteomics and small molecules, the first step is to set the software interface to "Moleclule interface". Then a Blank Document can be created:



Set the software interface to "Moleclule interface"

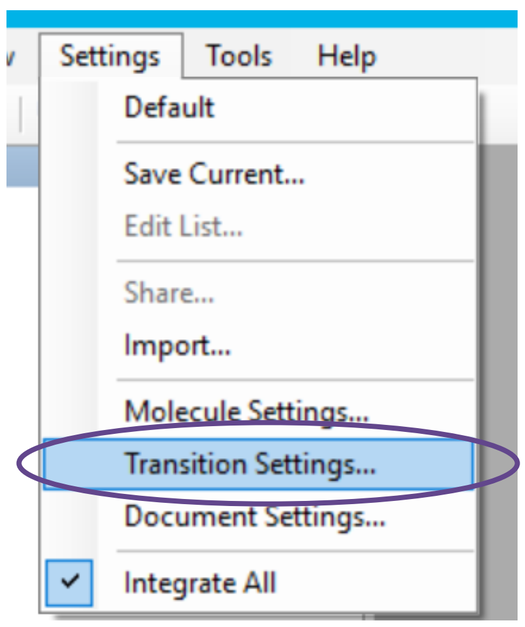
Create a Blank Document

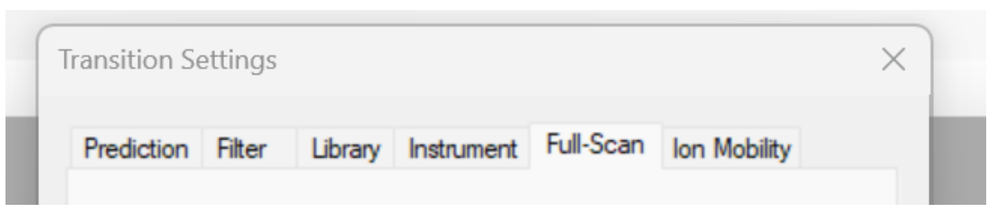
Prior to updating the data into Skyline, the settings for extracting the data must be selected. The "Molecule setting" offers the possibility of labeling the ions and identifying which one is the quantitative one.



Select the labels "Quan" and "Qual", if they do not appear on the display, "Edit list" and write the desired labels

In the "Transition setting" window the user can select the instrumental parameters that were used to acquire the data.



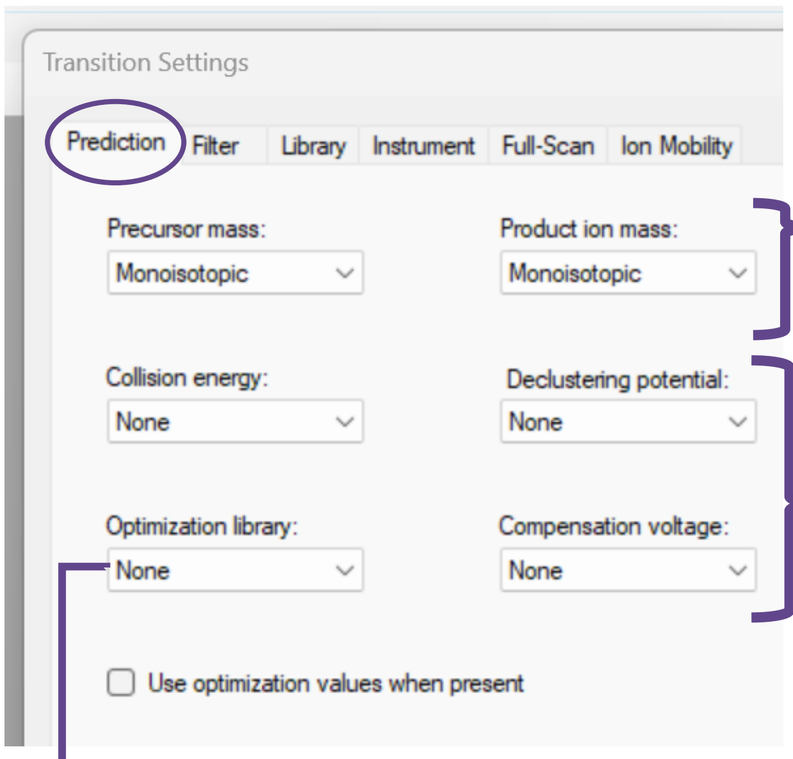


The different "Transition Settings" (Prediction, Filter, Library, Instrument, Full-scan, and Ion Mobility) will be explained in the following sections

Skyline

CPxplorer

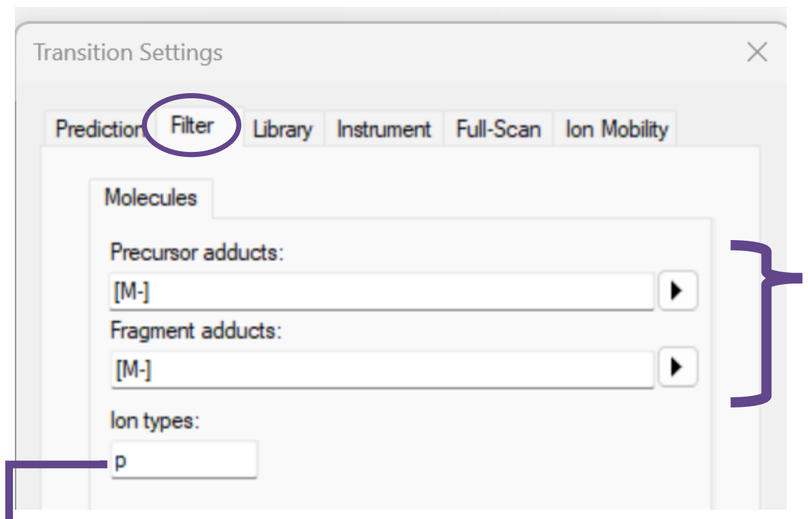
INTEGRATE THE DATA



Not available library

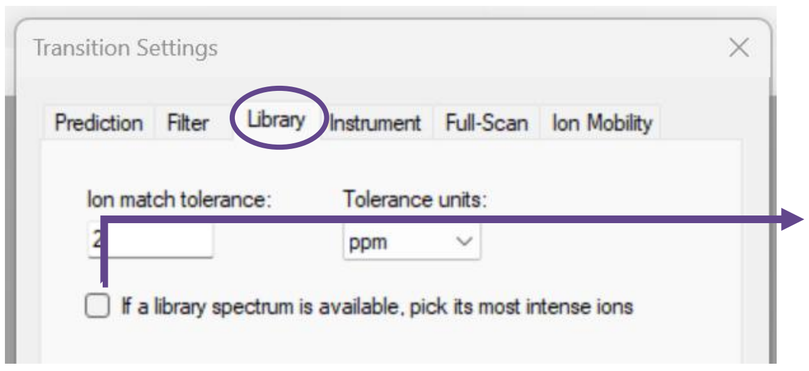
The transition list generated by CPion contains several ions corresponding to different isotopes of each molecule. Therefore, the "precursor mass" and "product ion mass" can be both set as "monoisotopic"

CP mixtures are analyzed only using MS_1 data. Consequently, the "Collision energy", "Declustering potential", and "Compensation voltage" can be set as "None".

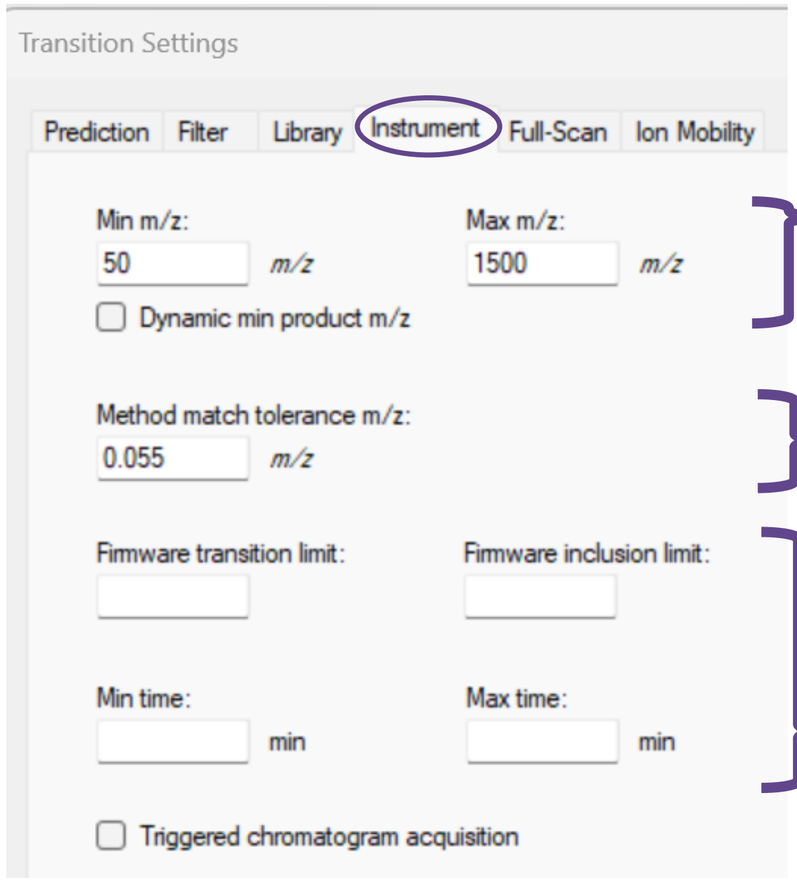


The adducts were already selected in CPion, and they are included in the transition list. So, the "Precursor" and "Fragment" adducts can be set as [M-] and [M+] when applying a negative and positive ionization technique, respectively.

"Ion types" can be set as "p" (parent) when only MS_1 data is used



If a spectral library is not used keep the library box unselected

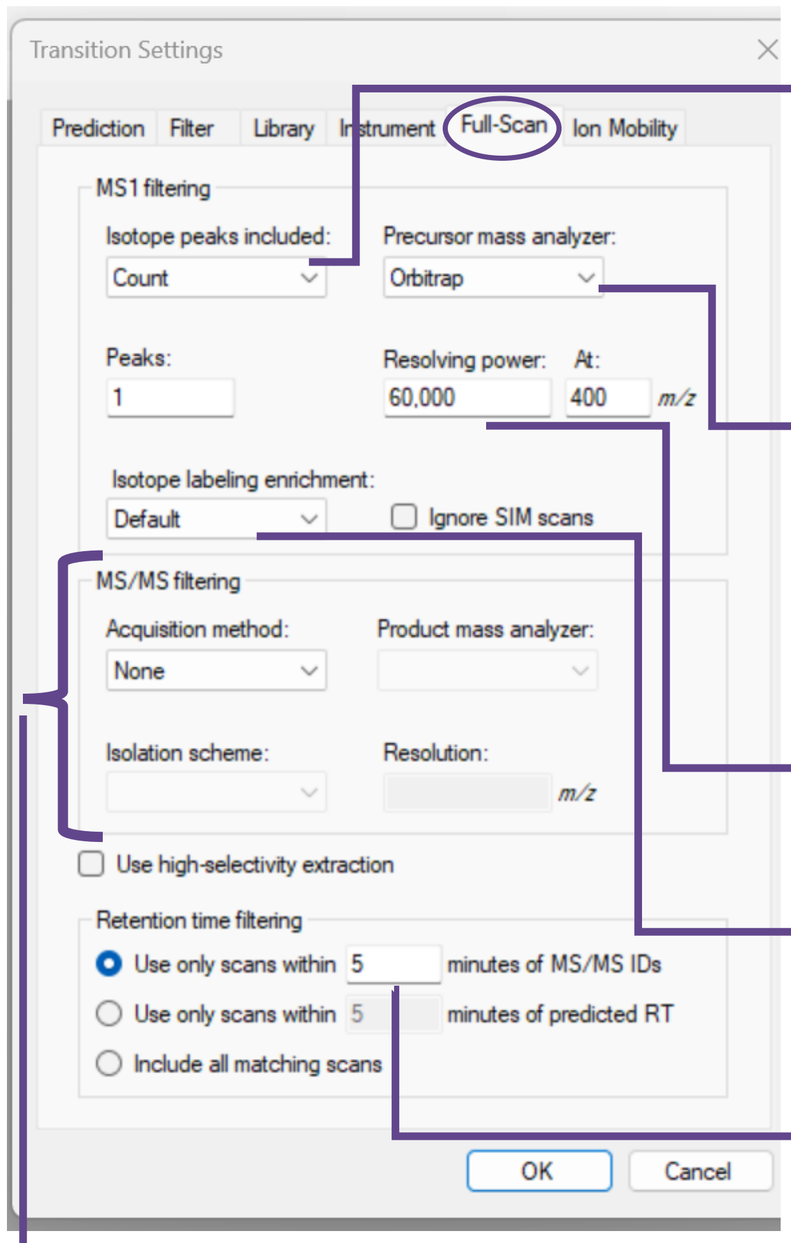


m/z range from the scan used for acquiring the data

Mass error acceptancy when extracting the chromatograms. 0.005 and 0.010 are recommended for Orbitrap and qToF data, respectively

The firmware is not needed when using MS_1 data solely. It is a multiplexing strategy for data-independent acquisition (DIA)-based mass spectrometry data,

INTEGRATE THE DATA



The "Count" option can be selected, to get a specific value for each "Peaks"

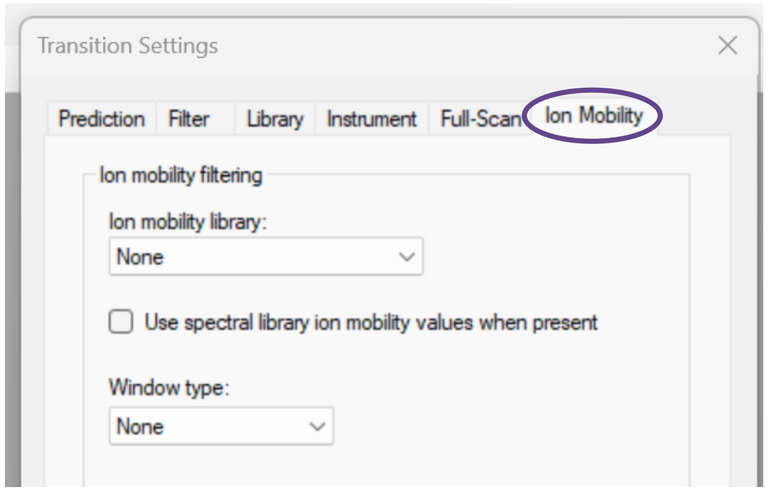
Selecting the instrument used will prompt Skyline to extract chromatograms from profile data, summing all profile intensities across an m/z range within the specified mass resolution

The instrumental resolution used for acquiring the data

The isotope labeling can be set as default because it was already indicated in the "Molecule setting"

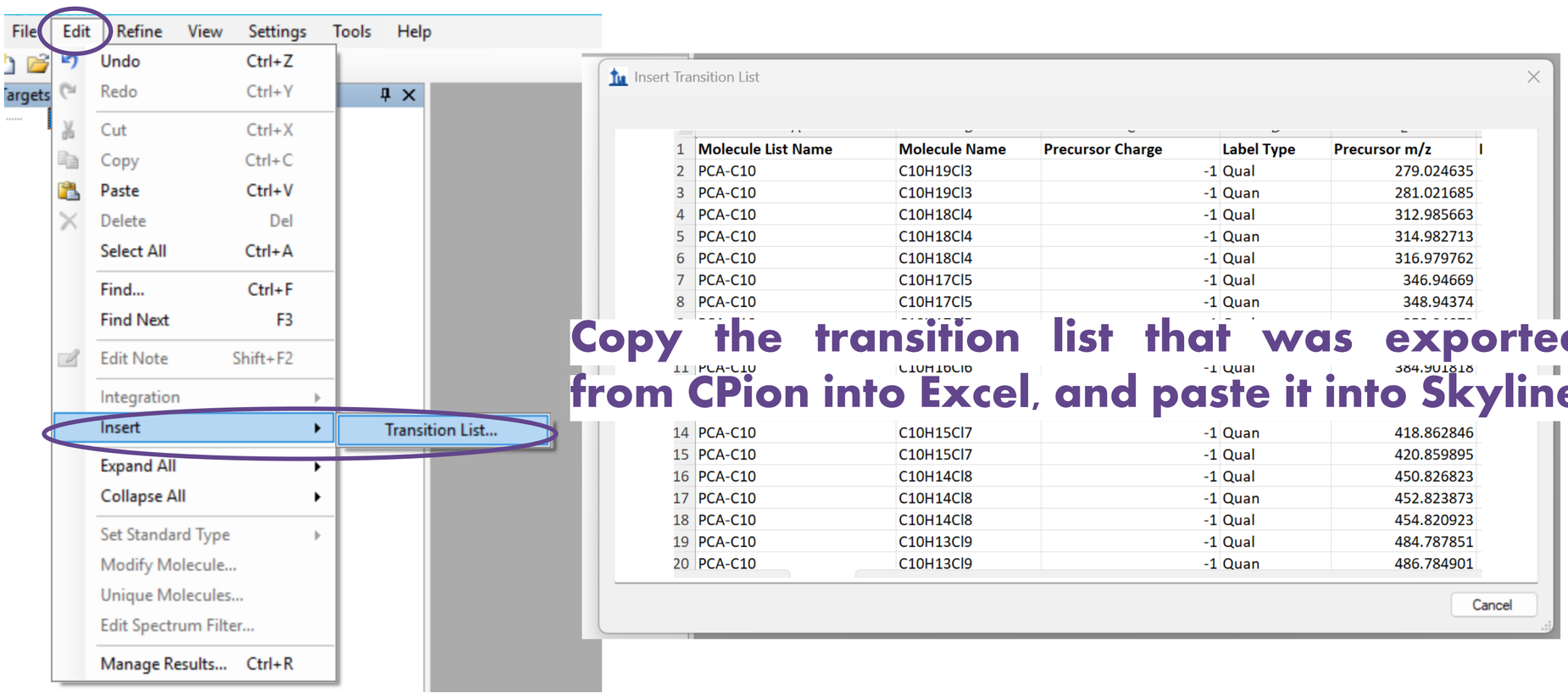
Set a broad window, ex.: 5 min

When only MS_1 data is used the "MS/MS filtering" can be set as "none"



If the data also includes an ion mobility dimension, the library and window type can be indicated

Once the settings are introduced, the transition list can be imported



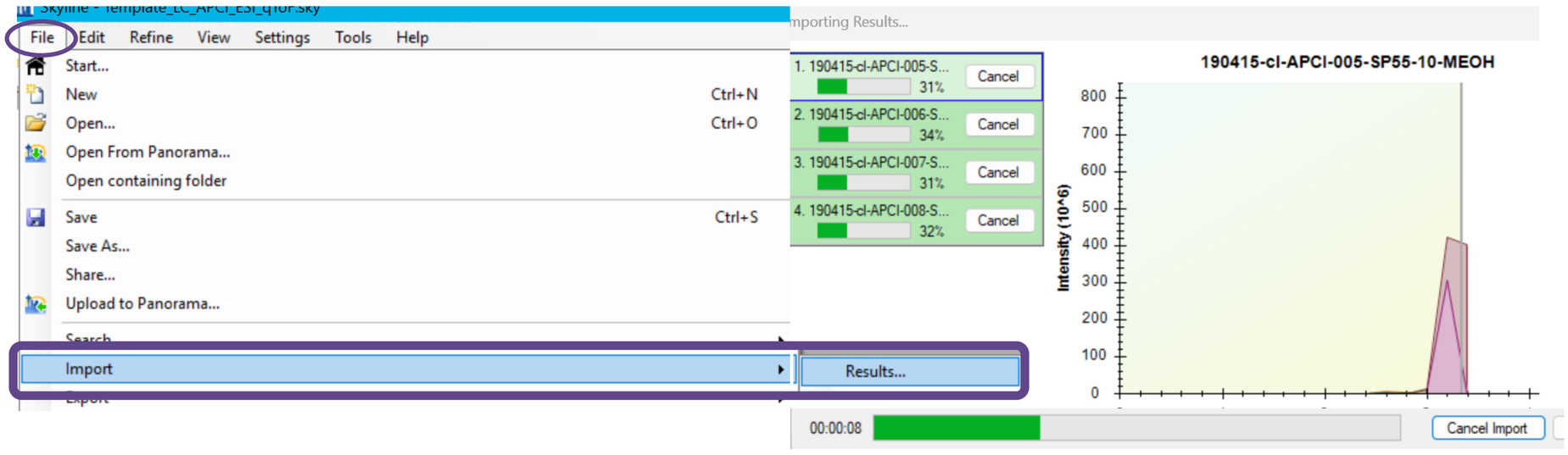
Copy the transition list that was exported from CPion into Excel, and paste it into Skyline

Skyline

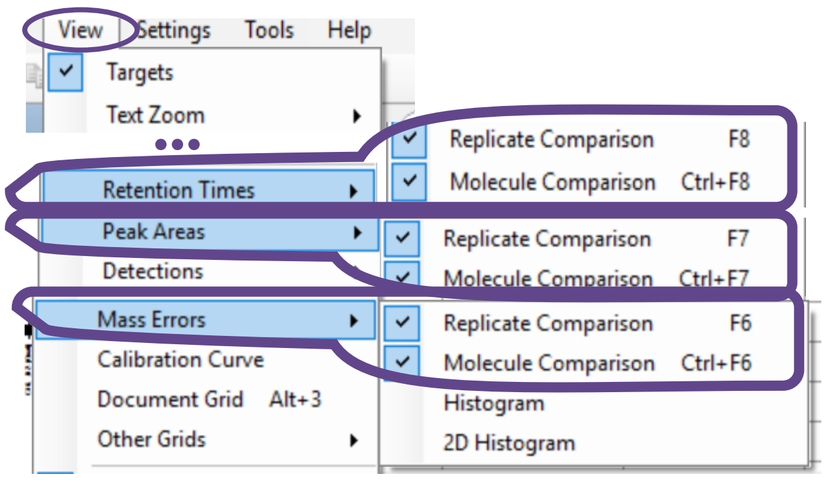
CPxplorer

INTEGRATE THE DATA

Once the settings are set, the method can be saved and the raw data imported. Skyline supports .d, .wiff, .qgd, .raw, and .mzml file extensions from the vendors Agilent and Bruker, Sciex, Shimadzu, and, Thermo and Waters without prior data conersion, respectively.

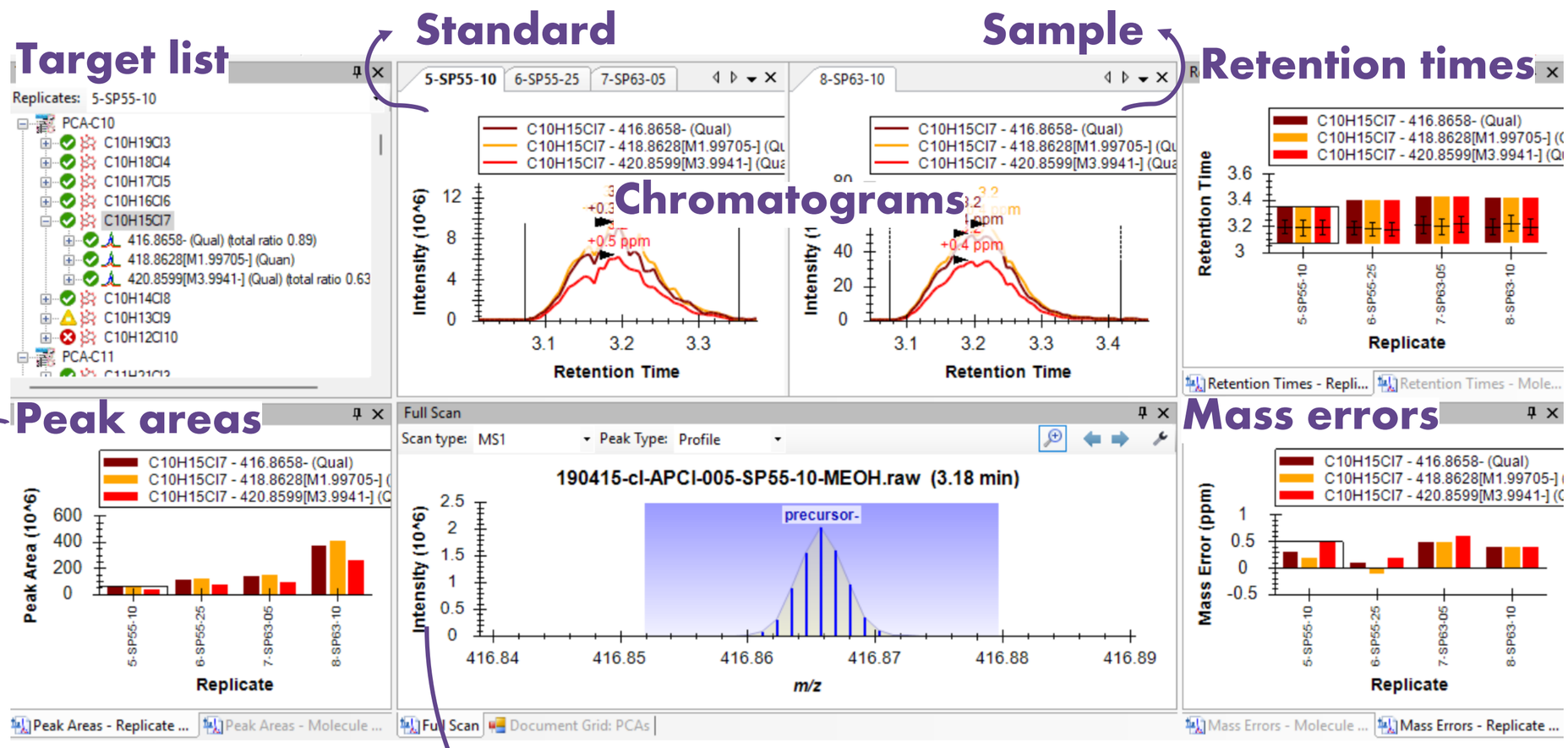


For the peak integration, the CPxplorer workflow advises verifying several parameters, including retention time, isotopic distribution, mass error, and m/z profile data distribution. Therefore, several panels are recommended to display in Skyline



The multiparameter display can be selected at the window "View". The "Replicate Comparison" option displays the same Molecule for the different samples; while the "Molecule Comparison" shows all the molecules in all the samples

The display should look like the following:



It shows the isotopic distribution

The profile data will be displayed when clicking on the chromatogram of one of the ions

Skyline

CPxplorer

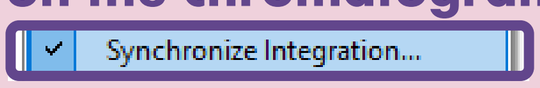
INTEGRATE THE DATA

Once the Skyline display is ready, the peaks can be integrated: The chromatograms of the different targets can be displayed by clicking on them in the transition list, and the peaks are integrated by selecting the start and end point of the chromatogram in the retention time axis. The following criteria is advised for selecting the data:

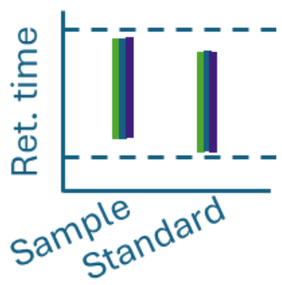
Example:

$C_{18}H_{31}Cl_7$: target m/z
- 528.9910 (qual)
- 530.9880 (quan)
- 532.9851 (qual)

Synchronize the integration of several files by right-clicking on the chromatogram:

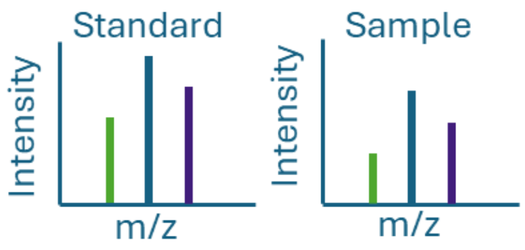


- Retention time:



Same retention time range for the standard and the sample

- Peak area:



Same isotopic distribution for the standard and the sample

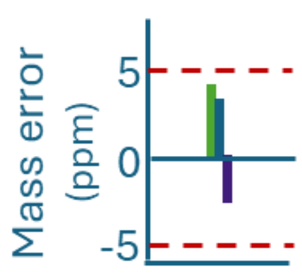
- MS profile data:

MS profile data showing Gaussian distribution

- Chromatograms:

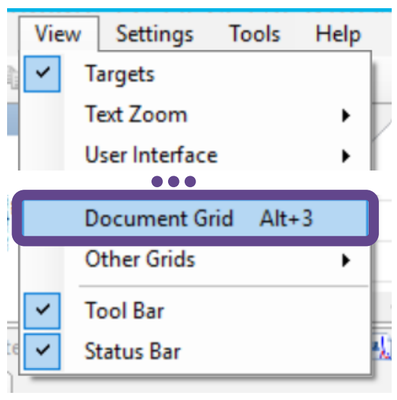
Same peak shape for all isotopomers, the "quan" and "qual" ions

- Mass errors:

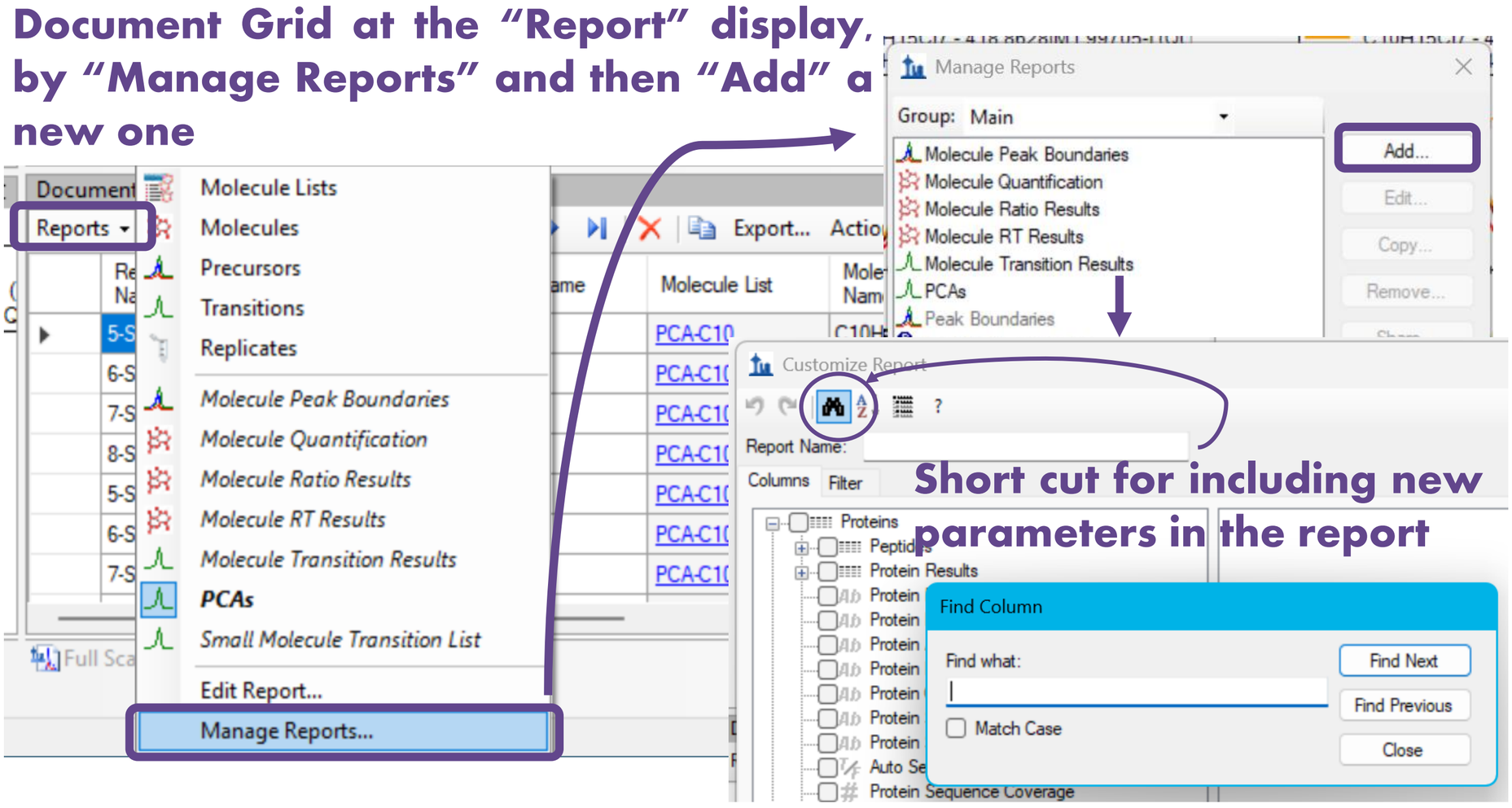


For Orbitrap data less than ±5 ppm, and for qToF ±10 ppm

The integrated data is compiled in the "Document Grid", this is a report that can be edited by the user while treating the data simultaneously. It can be displayed in the "View" window:



A new report can be created in the Document Grid at the "Report" display, by "Manage Reports" and then "Add" a new one



Short cut for including new parameters in the report

Skyline

CPxplorer

INTEGRATE THE DATA

In order to be able to integrate the report of Skyline into CPquant, the next step of CPxplorer, some parameters need to be included in the document Grid (Skyline report):

-Replicate Name: This column will show the name of each file

-Sample Type: This column must be filled in by the user. It indicates the type of file the data corresponds to, such as a sample ("Unknown"), standard ("Standard"), or blank ("Blank"), etc.

-Batch Name: This column is used by CPquant to identify which files belong to the same standard and to know what type of standard they are. The batch name should indicate the chain length of the homologues present in the mixture (ex.: C10, C11, C12, C10-13, C14-17 etc.). The different files that belong to the same standard must have the same letter as a suffix. For instance, all the files belonging to the standard mixture SCCPs 51.5% Cl will have the Batch Name as "C10-13_A", and the ones from SCCPs 55.5% Cl will have "C10-13_B".

-Molecule List: This column groups the homologues by chain length

-Molecule: This column shows the homologues

-Area: The integrated peak area

-Analyte Concentration: This column shows the concentration of the different standard mixtures. It must be indicated by the user

-Mass error PPM: The mass error of the measured m/z value to the theoretical one

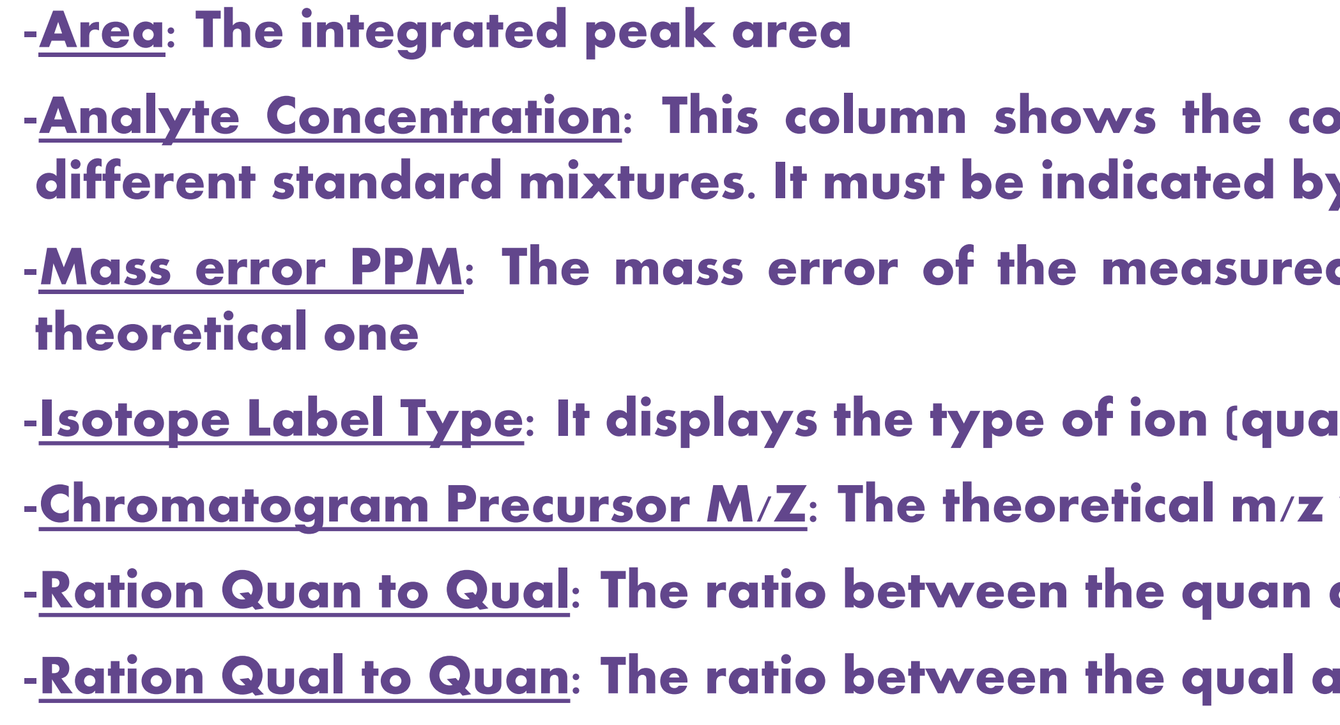
-Isotope Label Type: It displays the type of ion (quan/ qual)

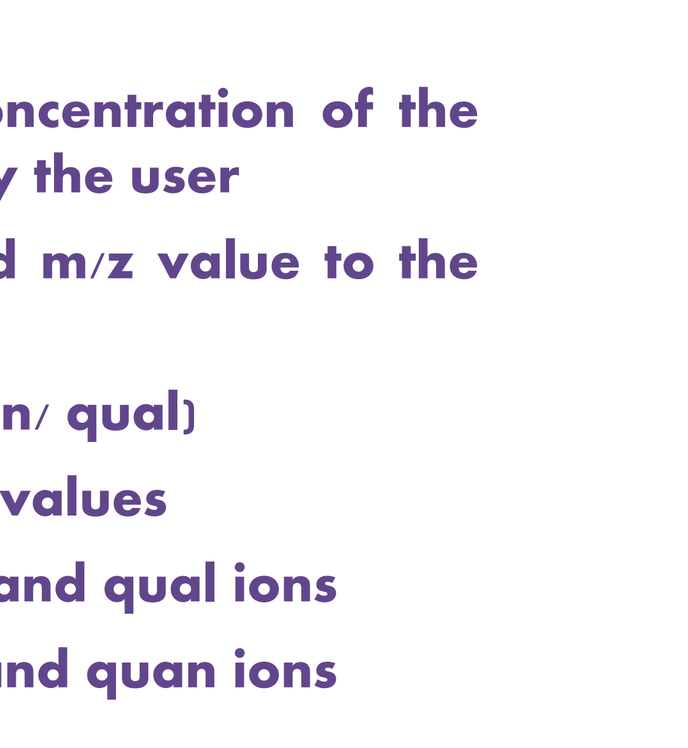
-Chromatogram Precursor M/Z: The theoretical m/z values

-Ration Quan to Qual: The ratio between the quan and qual ions

-Ration Qual to Quan: The ratio between the qual and quan ions

Once the report is finished, it can be Exported to Excel and used in the next module of CPxplorer: CPquant.





Select the report that was previously created in the Document Grid

CPquant

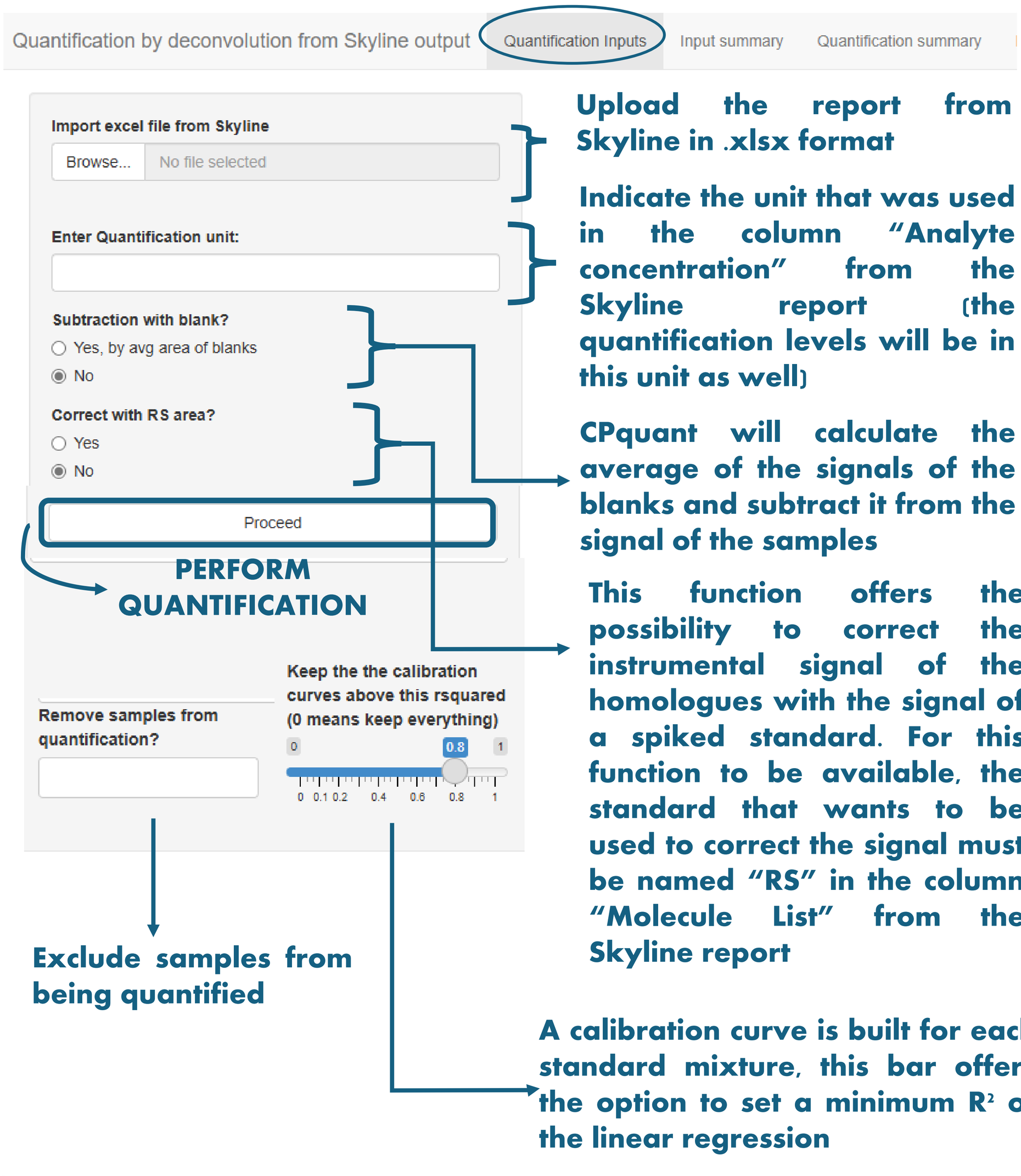
CPxplorer

QUANTIFY THE DATA

CPquant is a module in the CPxplorer package. It consists of an online app that can be found at https://github.com/WBS-TW/CPxplorer. It operates in a browser, so it does not require downloading.

CPquant is a tool for performing automatized quantification of PCAs. It is compatible with the report exported from Skyline and it does not require data adaptation.

CPquant performs the homologue deconvolution proposed by Bogdal et al., (2015)[1]. Briefly, CPquant executes non-negative least squares regression to combine various standards and match them with the PCA pattern found in the sample. Note that CPquant performs the deconvolution on the "quan" ions from the Skyline input.



Upload the report from Skyline in .xlsx format

Indicate the unit that was used in the column "Analyte concentration" from the Skyline report (the quantification levels will be in this unit as well)

CPquant will calculate the average of the signals of the blanks and subtract it from the signal of the samples

This function offers the possibility to correct the instrumental signal of the homologues with the signal of a spiked standard. For this function to be available, the standard that wants to be used to correct the signal must be named "RS" in the column "Molecule List" from the Skyline report

Exclude samples from being quantified

A calibration curve is built for each standard mixture, this bar offers the option to set a minimum R^2 of the linear regression

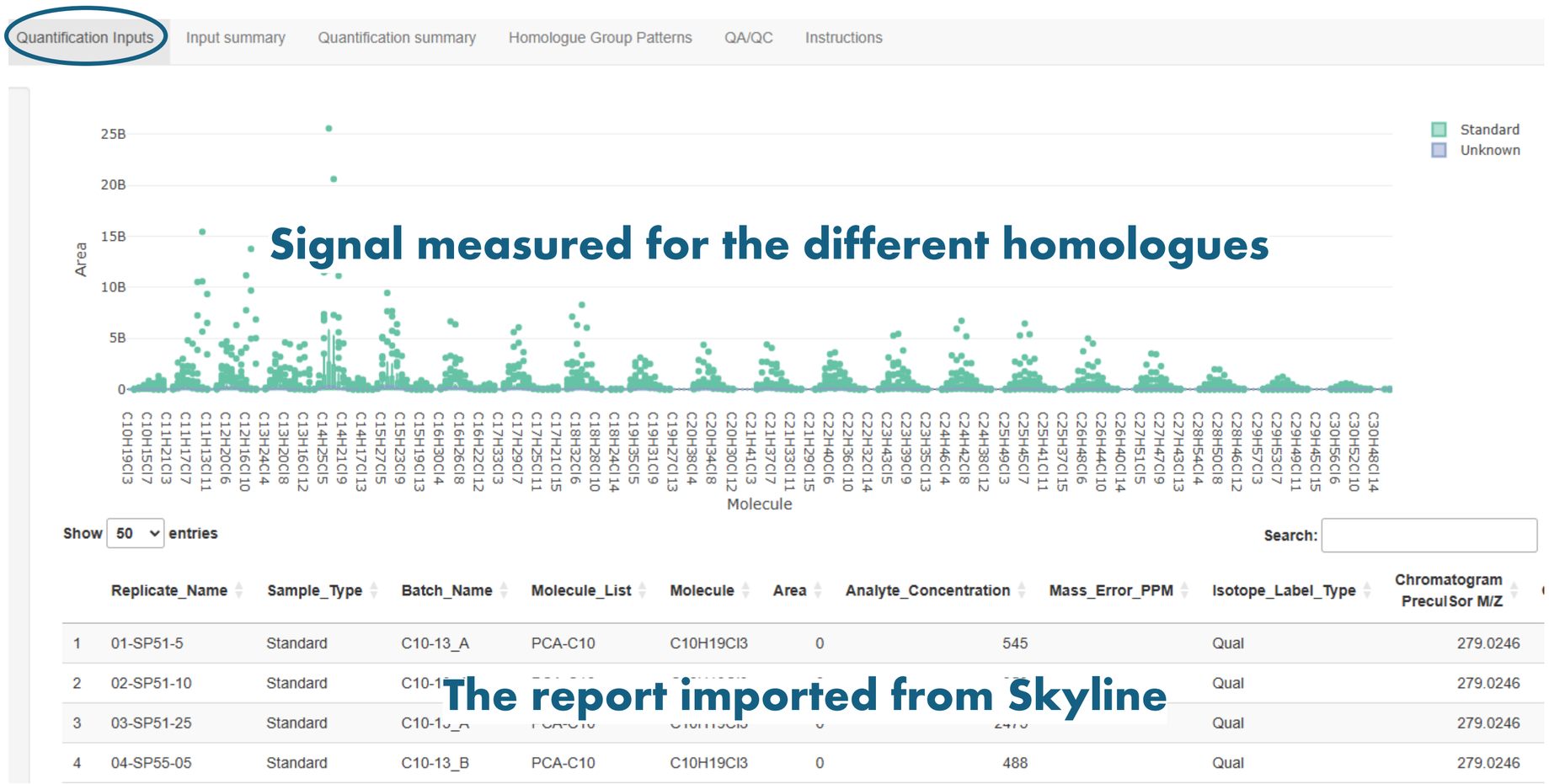
[1]Bogdal, Christian, Tomas Alsberg, Pascal S. Diefenbacher, Matthew Macleod, and Urs Berger. 2015. "Fast Quantification of Chlorinated Paraffins in Environmental Samples by Direct Injection High-Resolution Mass Spectrometry with Pattern Deconvolution." *Analytical Chemistry* 87(5):2852–60. doi: 10.1021/ac504444d.

CPquant

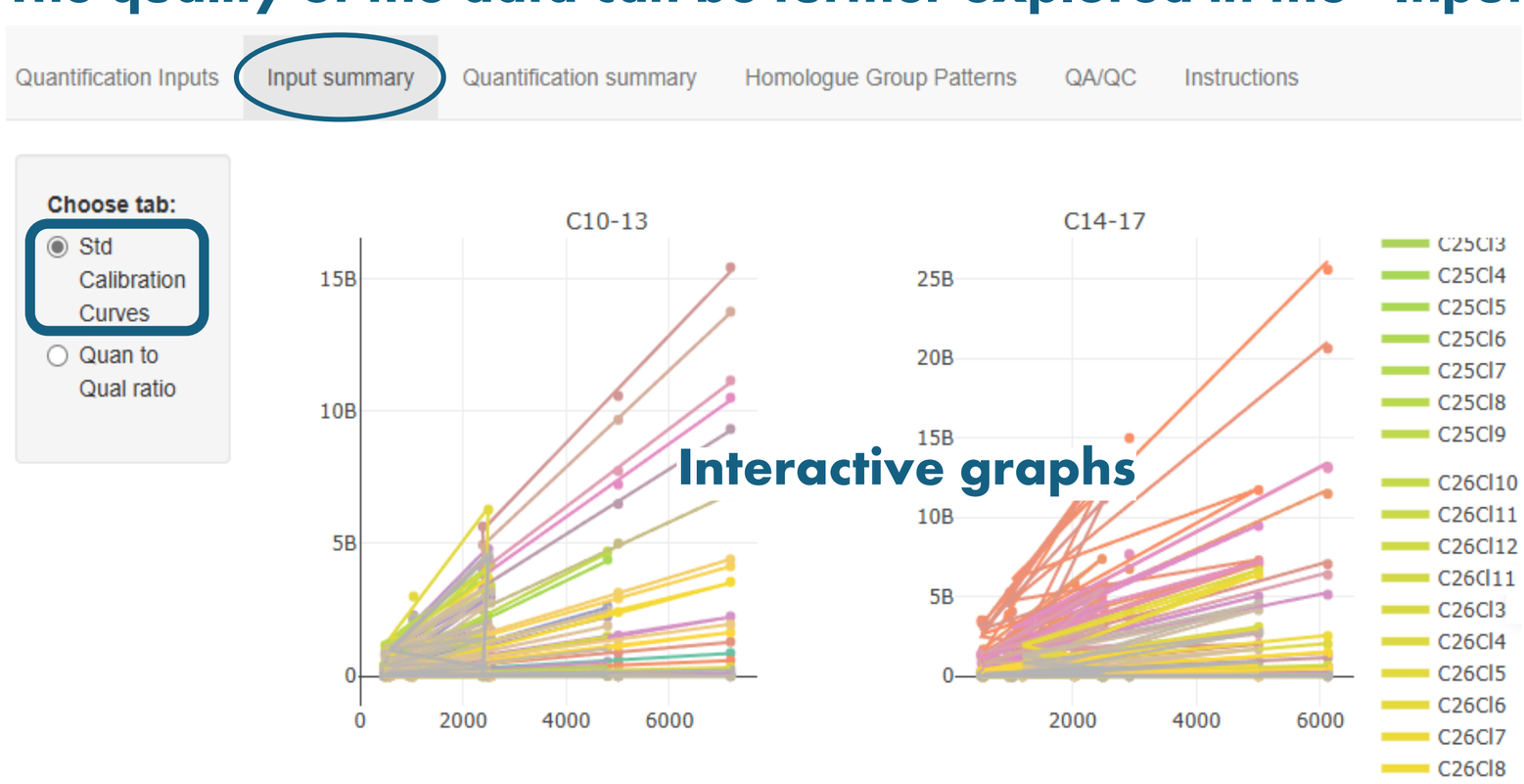
CPxplorer

QUANTIFY THE DATA

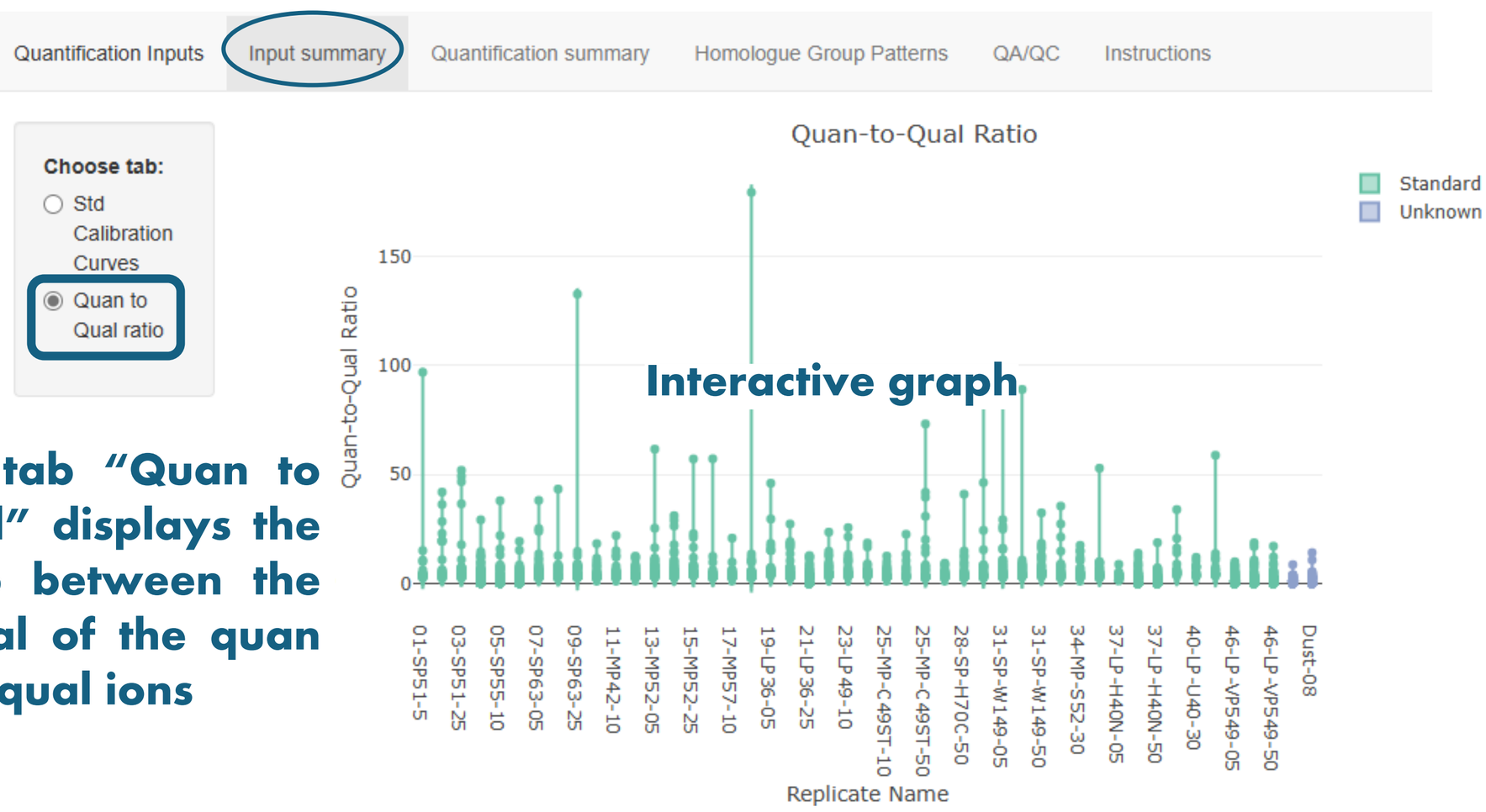
The inputted data can be explored in the "Quantification Inputs" window. Here the data is displayed in an interactive graph and table:



The quality of the data can be further explored in the "Input Summary"



The tab "Std Calibration Curves" displays the calibration curves of the different standards for each homologue



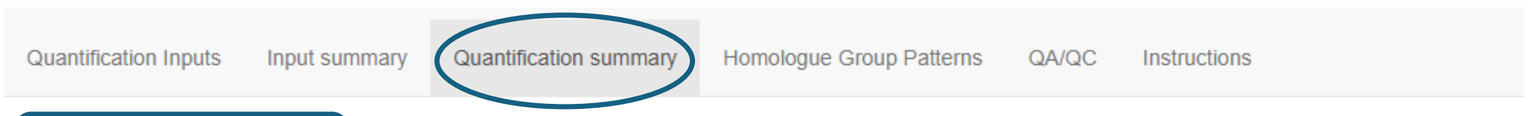
The tab "Quan to Qual" displays the ratio between the signal of the quan and qual ions

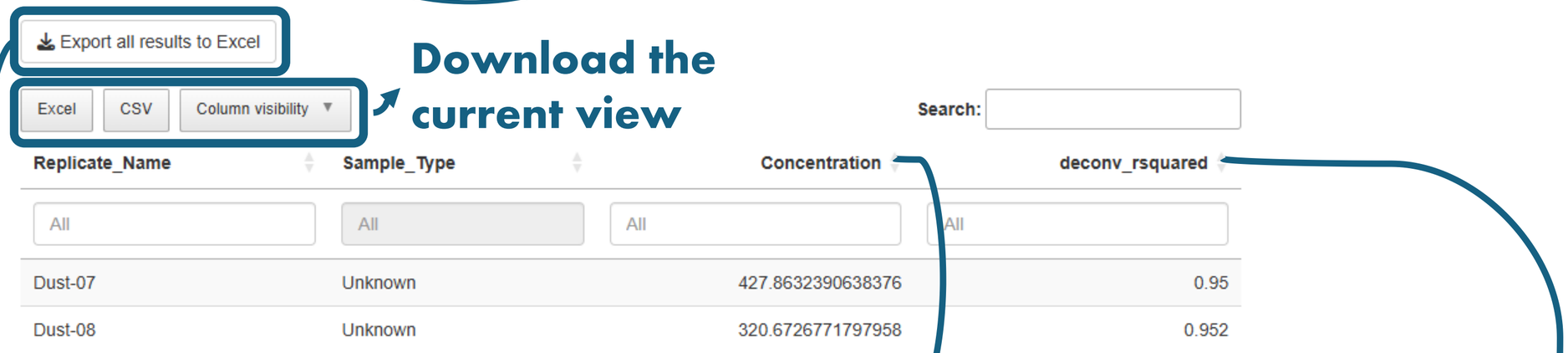
CPquant

CPxplorer

QUANTIFY THE DATA

The quantification results can be found in the "Quantification Summary", more detailed information is displayed in the "Homologue Group Patterns" window.



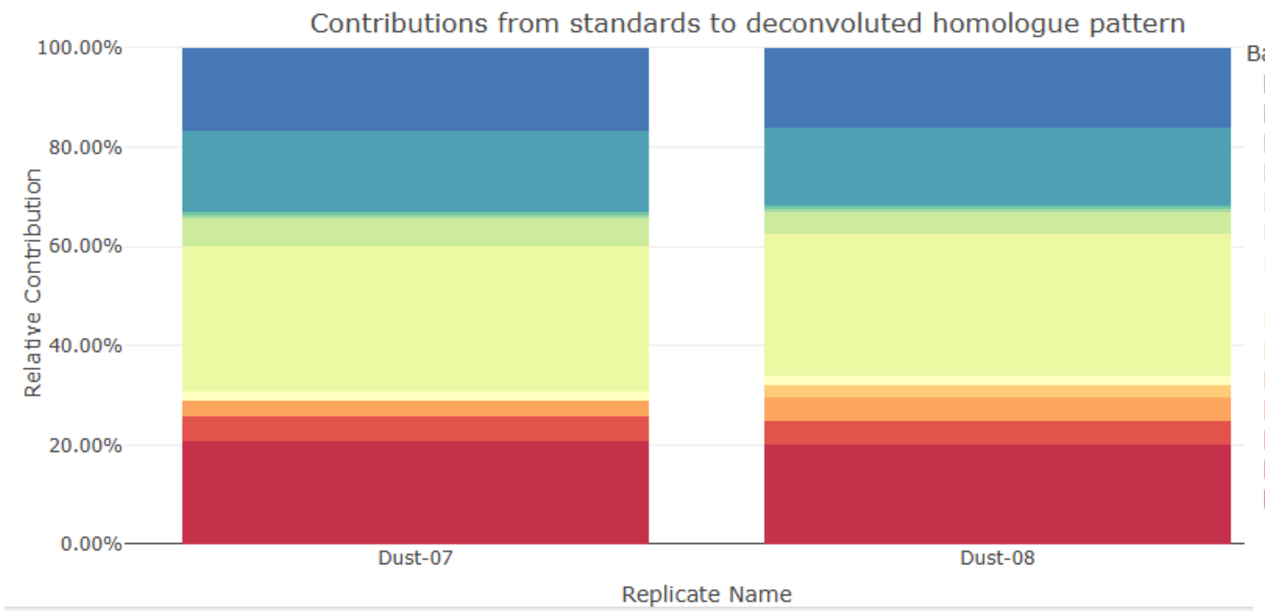


Download the current view

Download the full quantification results:
- **SumPCAs**
- **Deconvolution fit**
- **Homologue concentration**

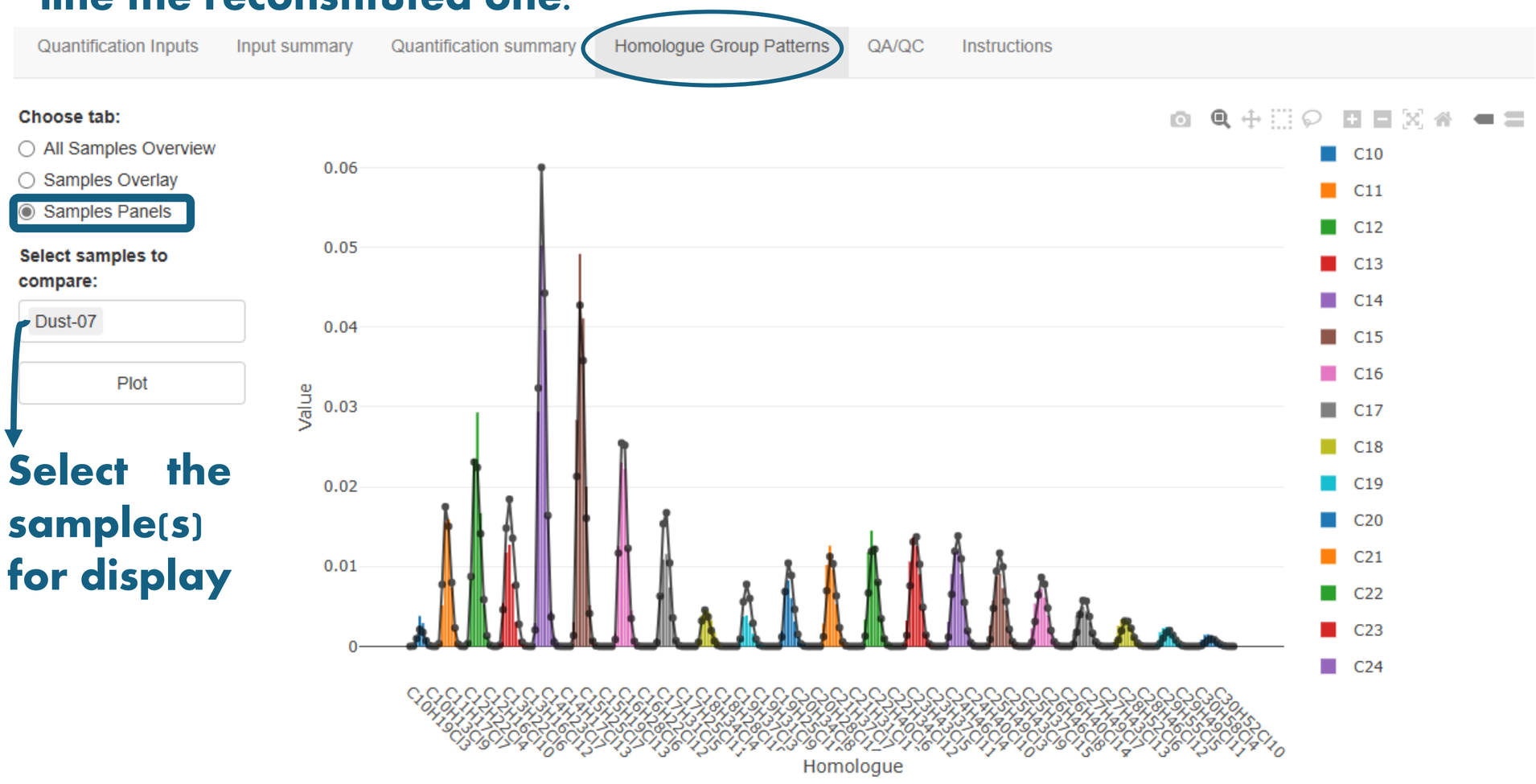
SumPCAs

It shows how well the reconstituted pattern fits the one in the sample, where 1 would mean a perfect fit



The graph displays visually the contribution of each standard for building the reconstituted pattern

A visual comparison between the reconstituted and measured patterns can be displayed at the "Homologue Group Patterns" where the bar plot shows the measured relative distribution and the black line the reconstituted one:

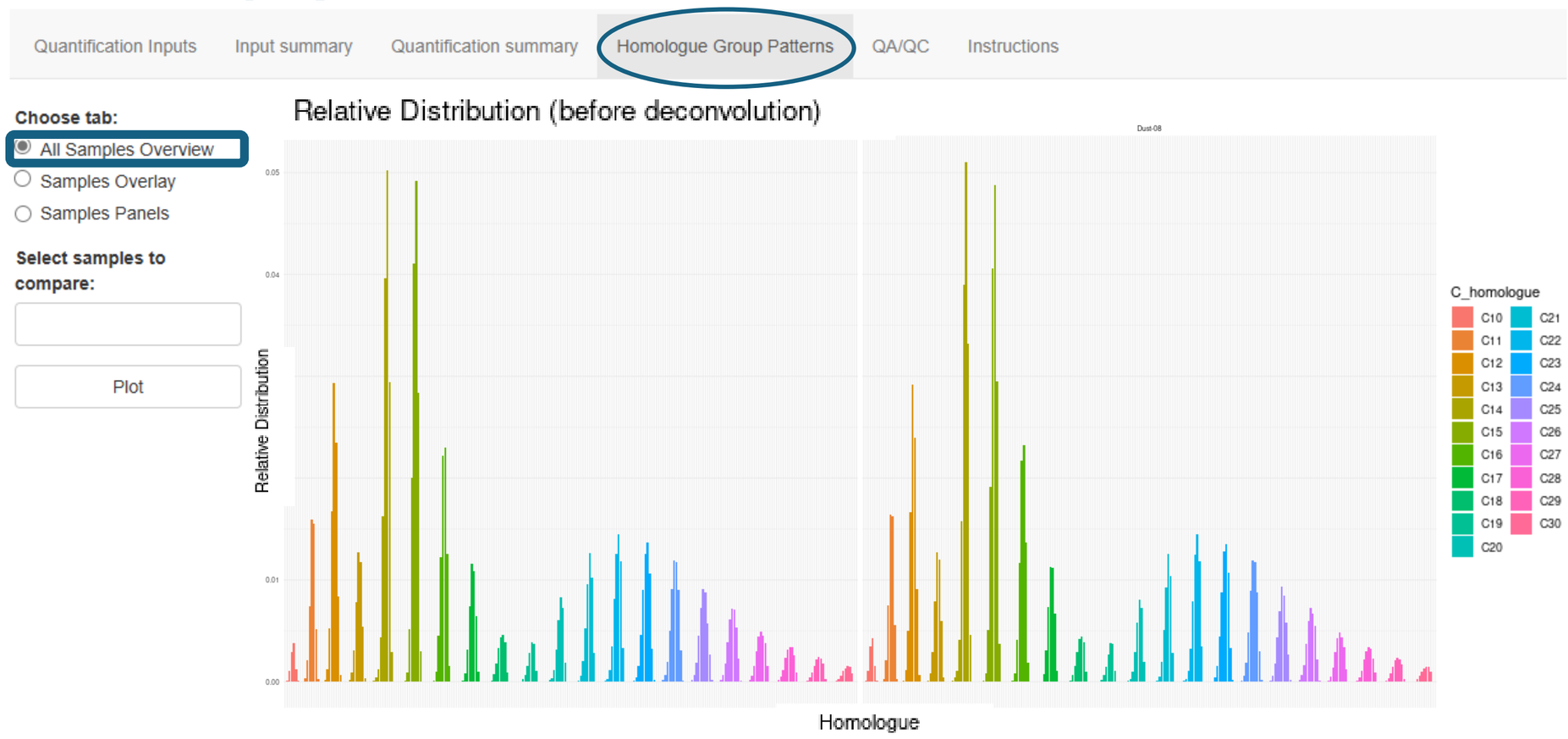


Select the sample(s) for display

QUANTIFY THE DATA

Several displays of the homologue patterns can be found in the "Homologue Group Patterns" window.

The inputted relative distribution of the homologues in all the samples can be displayed at:



The reconstituted relative distribution of the homologues in all the samples can be displayed at:

Select the sample(s) for display

The display shows several samples simultaneously and facilitates the comparison between replicates

CPxplorer

https://github.com/WBS-TW/CPxplorer

OVERVIEW

CPxplorer

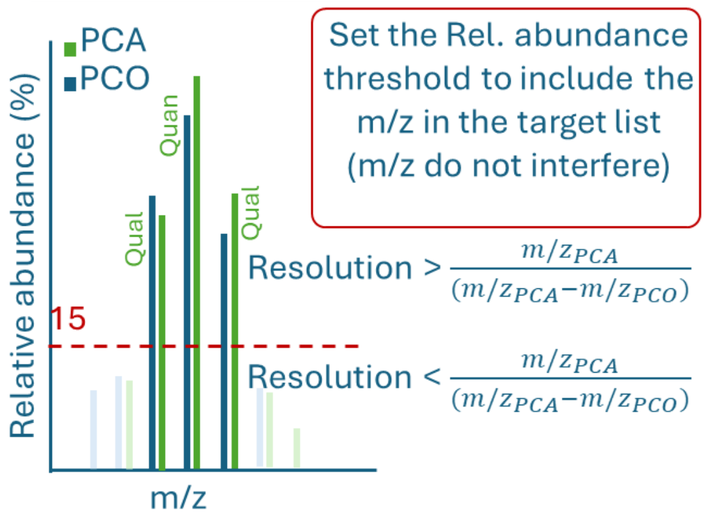
CPion

Input parameters

Number of C	10-30
Number of Cl	5-15
Adduct	$[M+Cl]^-$
Resolution	60 000
Molecule(s)	PCA/O
Rel. abundance	15

Centroided m/z values

Study mass spectral interferences



Set the Rel. abundance threshold to include the m/z in the target list (m/z do not interfere)

$$\text{Resolution} > \frac{m/z_{PCA}}{(m/z_{PCA} - m/z_{PCO})}$$

$$\text{Resolution} < \frac{m/z_{PCA}}{(m/z_{PCA} - m/z_{PCO})}$$

Create the target list

Export the transition list

Ion	m/z	Label type
PCA + Cl-	528.9910	Qual
PCA + Cl-	530.9880	Quan
PCA + Cl-	532.9851	Qual

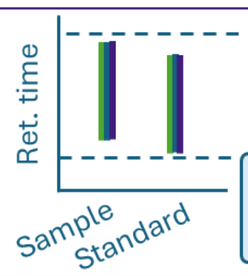
Skyline

Import the transition list into Skyline

$C_{18}H_{31}Cl_7$: target m/z

- 528.9910 (qual)
- 530.9880 (quan)
- 532.9851 (qual)

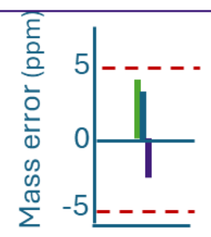
Same ret. time range



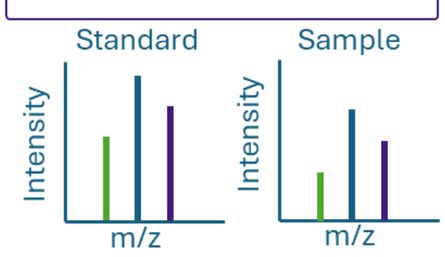
Integrate the data

Same peak shape for all isotopomers

Mass error < ±5 ppm



Same isotopic distribution



Export the signals report from Skyline

File	Molecule	Signal	Type	Batch	Conc (ng)
DustR1	C18Cl7	38943	Sample		
L36_1	C18Cl7	26768	Standard	L-A	345
DustB	C18Cl7	04536	Blank		

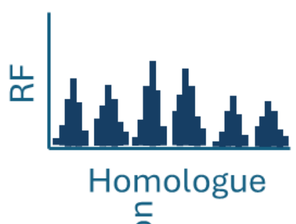
CPquant

Import the report from Skyline into CPquant

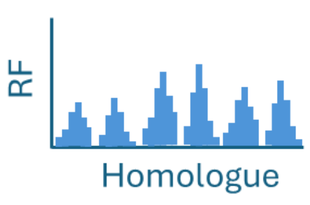
Molecule	m/z	Label type
C18Cl7	528.9910	Qual
C18Cl7	530.9880	Quan
C18Cl7	532.9851	Qual

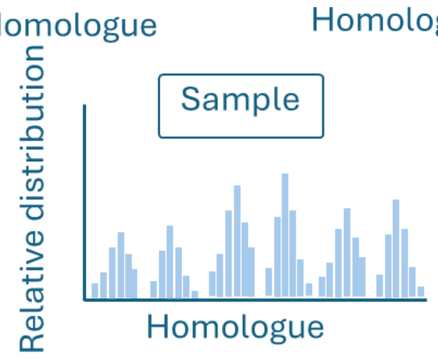
Filter the data for the signals of the quan isomers

Standard A

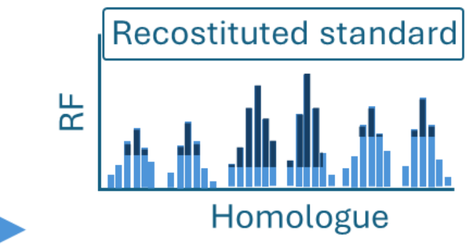


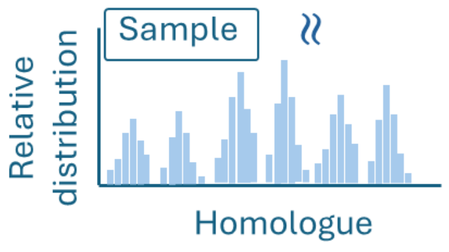
Standard B





Perform nnls to calculate the contribution of each standard





Quantify the unknown

Sample	Molecule	Conc (ng)
DustR1	C18Cl7	250
DustR2	C18Cl7	245
DustR3	C18Cl7	255

CONTACT INFORMATION:

idoia.beloqui.ezquer@liu.se
thanh.wang@liu.se

GOOD LUCK WITH THE DATA ANALYSIS!

Please contact our team if any questions

CPxplorer

https://github.com/WBS-TW/CPxplorer

A platform-independent tool for rapid quantification and harmonization of polychlorinated alkanes data

Idoia Beloki Ezker

Ph.D. student

idoia.beloqui.ezquer@liu.se

Thanh wang

Professor

thanh.wang@liu.se

Department of Physics, Chemistry and Biology (IFM), Linköping University, Sweden