Mestrelab Research

# Gears Chrom Calibration 1.0

STARTING GUIDE


Document Number

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# COPYRIGHT

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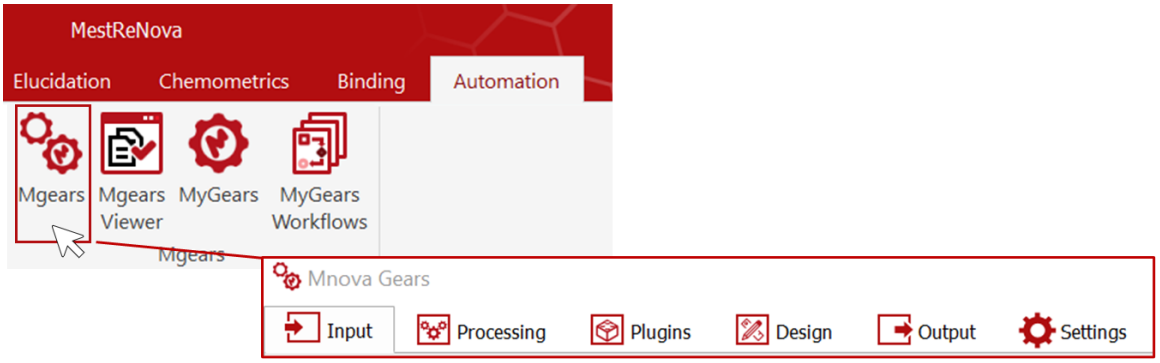
Generating a calibration curve is a standard and routine process when performing quantitative chromatography. With Gears Chrom Cal, this process has been fully automated to allow you work on different analytes at the same time and calculate calibration curves quickly and efficiently in a single, simple run.

In this document, we will go through the different configuration steps right to the output and result revision to help you get started with Chrom Cal.

# 1. The workflow

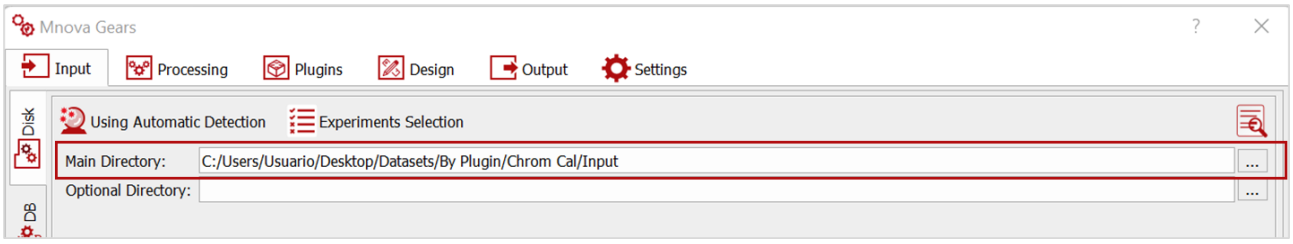
Launch Mgears from the Mnova **Automation** ribbon. The dialog with the usual six tabs will open.




## 1.1. Input

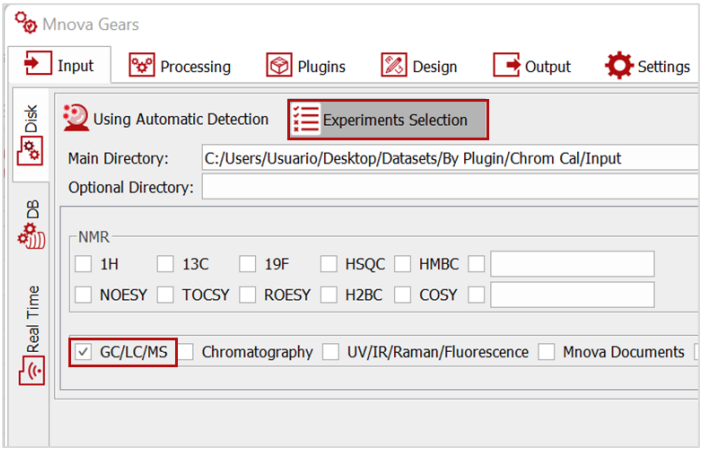
Chrom Cal analysis follows the general Mnova Gears workflow based on its five-step setup, the first step being the choice of input data. Mgears can read data from your **Disk**, **Database**, or from **Real-Time** acquisition. In this guide, we will work with data from disk directories *(please refer to the Mnova Gears manual for further detail about other input types).*

Click on the ... button and select the data files to upload as your **Main Directory**.

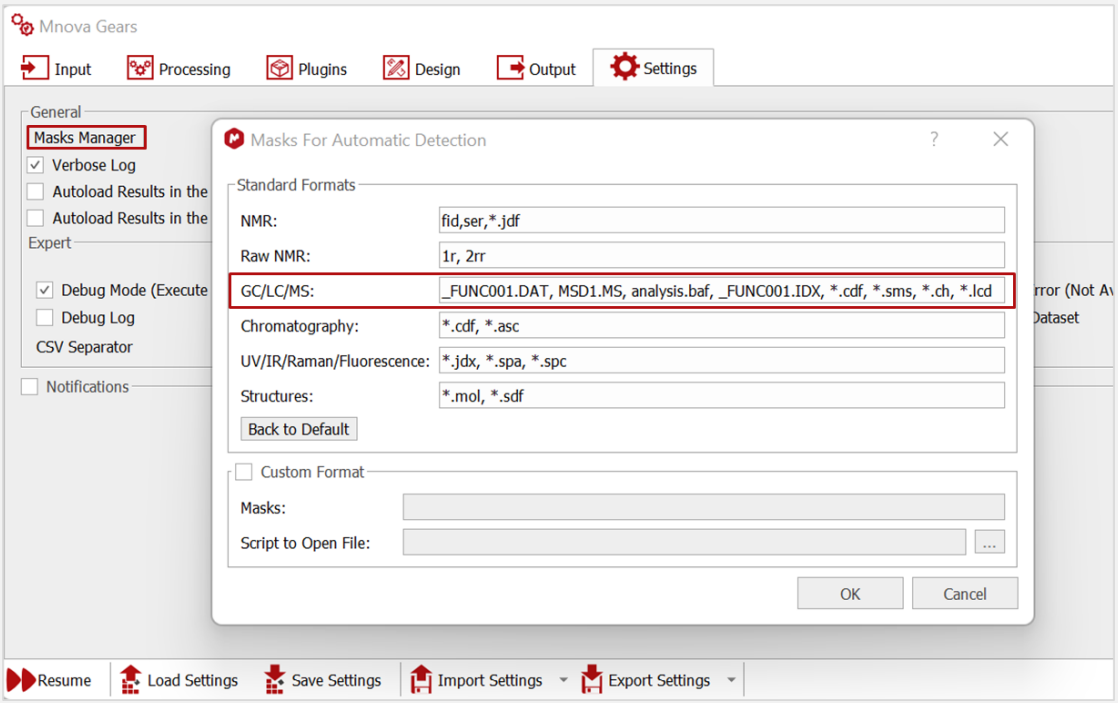



Use the **Automatic Detection** mode to detect you input files.

When using **Automatic Detection,** if your data folder contains different types of data files, it is recommended that **Experiment selection** is used in order to restrict detection to GC/LC/MS and avoid analysis of other undesired files. In this case, you must select the **GC/LC/MS** experiment type, as shown below.

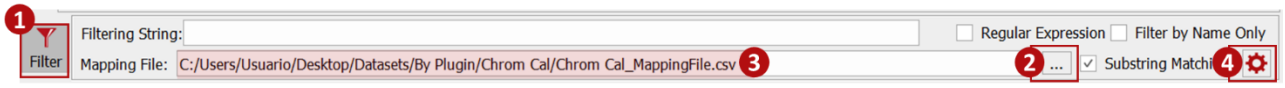



**Important!** When using **Automatic Detection**, make sure your data file extensions are included in the **Mask Manager** under the **Settings** tab so that Mgears can recognize the appropriate files correctly. Otherwise, manually add your standard files extension or custom file formats and press **OK**.




With Chrom Cal, the use of a **Mapping file** is mandatory to provide the necessary input information (i.e., compound information and concentrations) that allows the grouping and evaluation of the calibration curve of each compound in the samples. It also serves to provide the compound and concentration information for the reference samples used for cross-validation of the calibration curves.

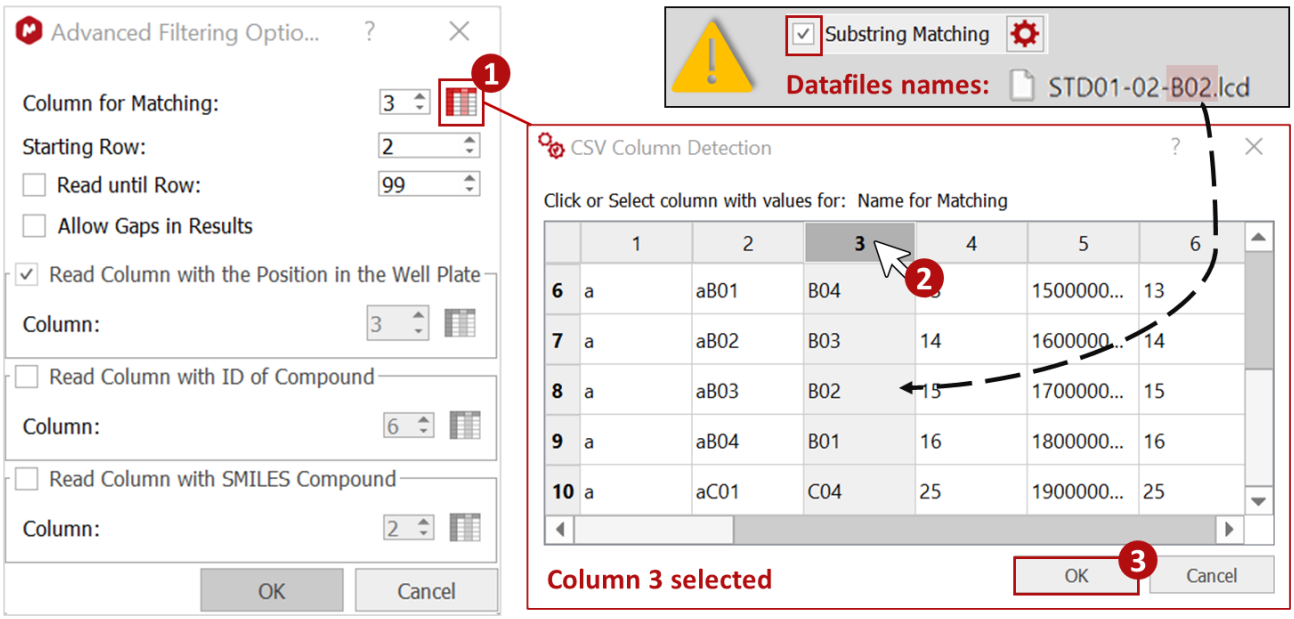
To add and use a **Mapping file**, click on the **Filter** icon at the bottom left-hand side of the Mgears dialog. The two available filtering options (**Filtering String** and **Mapping File**) will appear. Click on ... and upload your **Mapping File** (in either .txt or .csv format) with the data and parameters you want to work with. Then, click on ⚙ to set up the parameters to read the mapping file. The **Advanced Filtering Options** dialog will open.




In the **Advanced Filtering Options** dialog, you must set the column with the datafile names for matching.

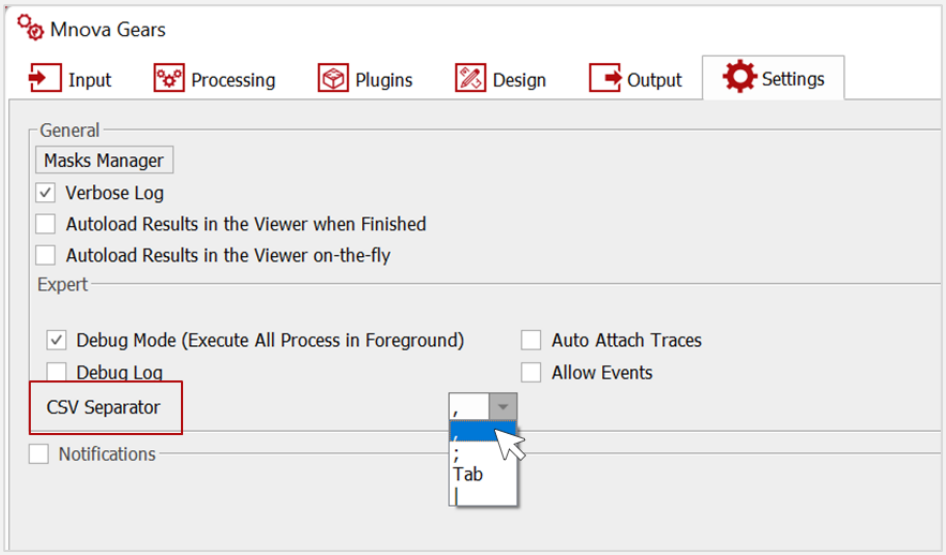
⚠ In our example, the **Well plate** position included in the file names (and not the whole file name) will be used for matching. For this to work correctly, the **Substring Matching** option must be enabled.

To configure matching columns, you can either indicate the number of the column directly by clicking on this box 1, or open the assistant to visualize the CSV and select the corresponding column, as shown in the image below. Click on **OK** to save the selection.

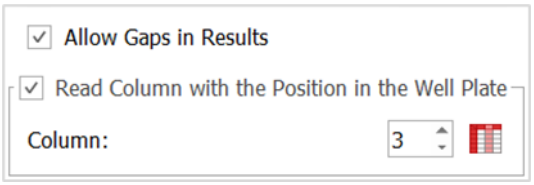



**Note.** In the **CSV Column Detection** dialog, only the first 10 rows of the filtering file will be loaded.

**Important!** Remember that in order to be correctly read by Mgears, the CSV separator (comma, semicolon, tab, or vertical line) should be configured in the **Settings** tab (in this case, the CSV is using a comma as a separator, therefore we select the comma in the **Settings**).

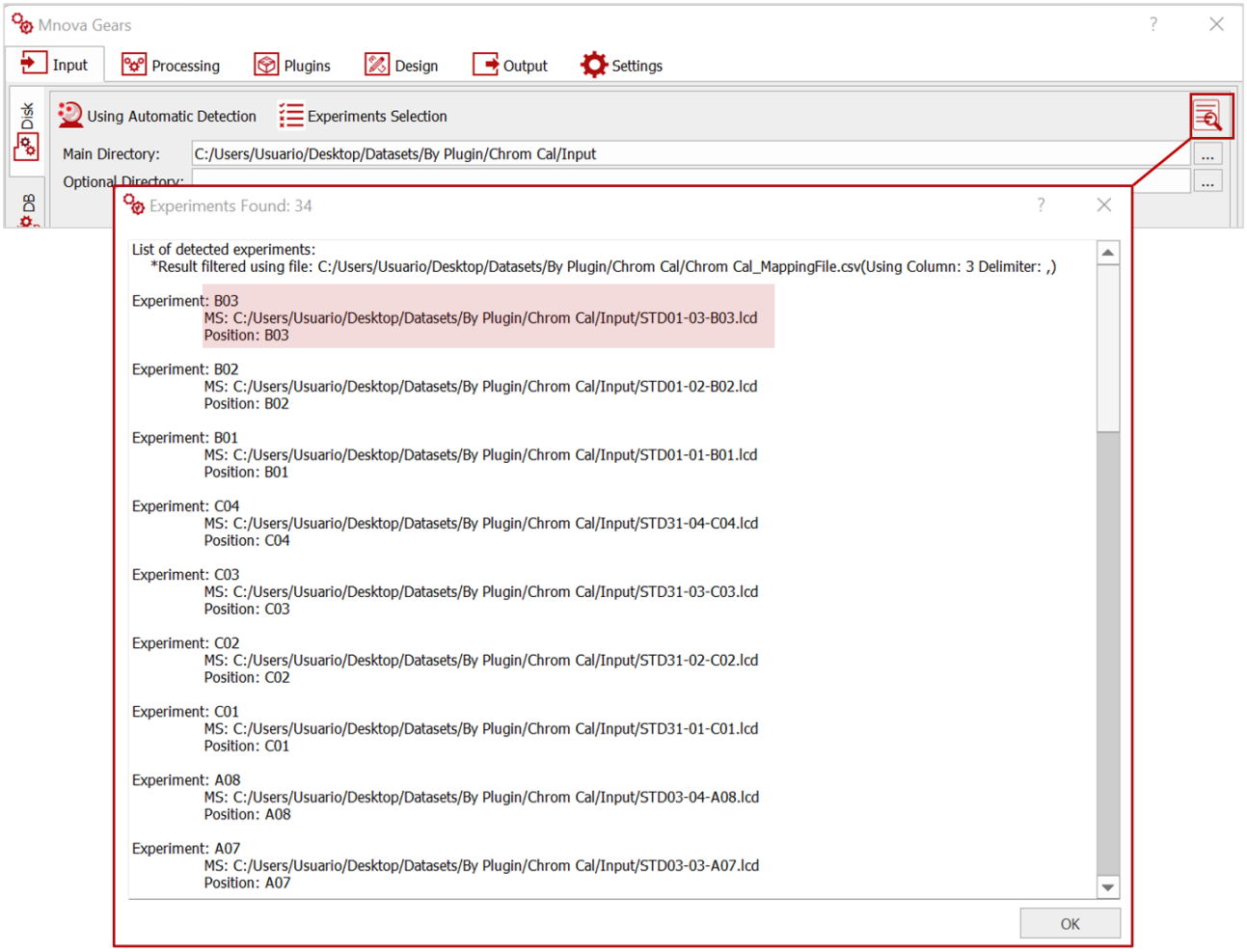



If needed, you can set the **Starting Row** at which matching should start and decide whether to **Read Until** a specific row in the CSV. You can also match the **well plate position** so that the results will be displayed in the Result Viewer according to their actual well plate position.




Once you've completed configurating your filtering options, click on **OK**.

You can now perform an automatic inspection of the selected directory by clicking on  to verify that your input files will be found and filtered correctly. As you can see, the MS data files and sample positions are correctly detected.



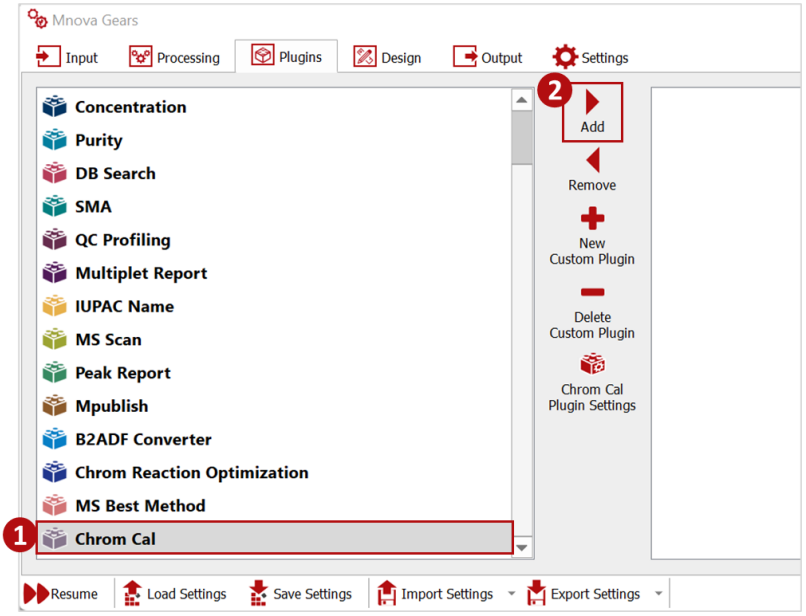

**Watch out!** Depending on the operating system you have on your computer, Mnova may support different dataset format types. You can check the MS supported formats on our website.

## 1.2. Processing

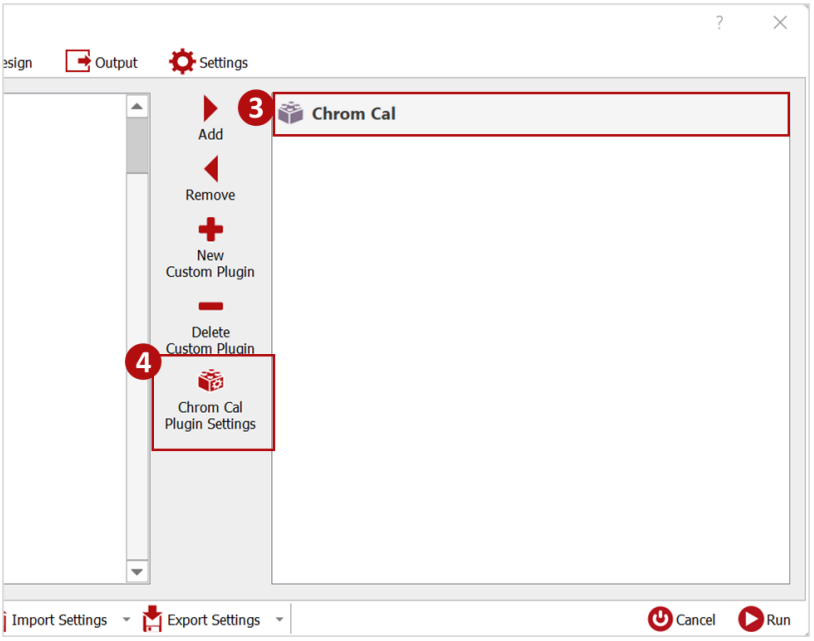
In the **Processing** tab, you can upload a processing script to apply your own customized processing settings *(please refer to the Mnova Gears manual for more details about this option).*

## 1.3. Plugins

In the **Plugins** section, select and add the Chrom Cal plugin.

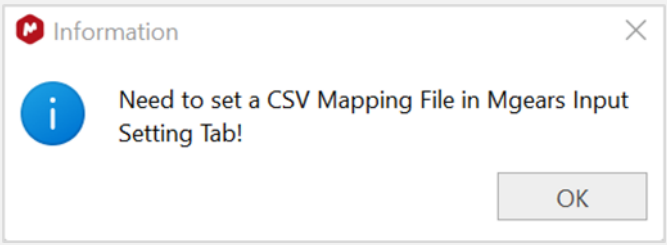



Then, click on **Chrom Cal Plugin Settings** to configure your analysis and report parameters.




A dialog with four tabs should appear.

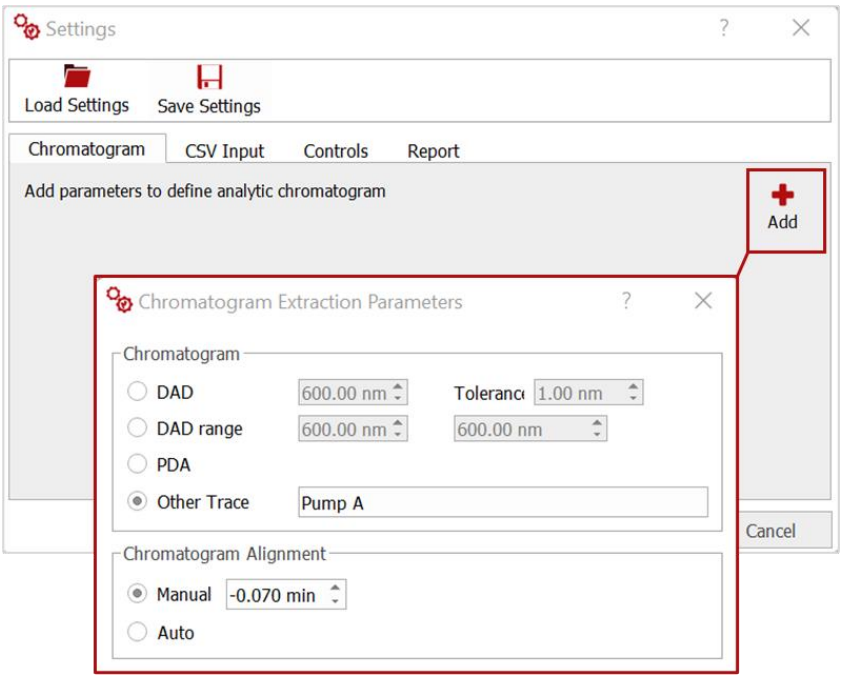
**Note.** At this point, if a **Mapping File** is not configured in the **Input** tab, the following message will appear to remind you to add it.



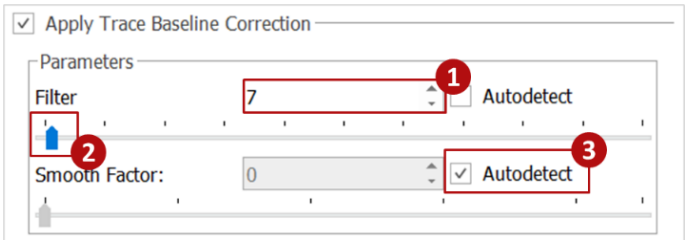

### 1.3.1. The Chromatogram tab

In the **Chromatogram** tab, click on **Add** to configure the chromatogram extraction preferences. A dialog will open.

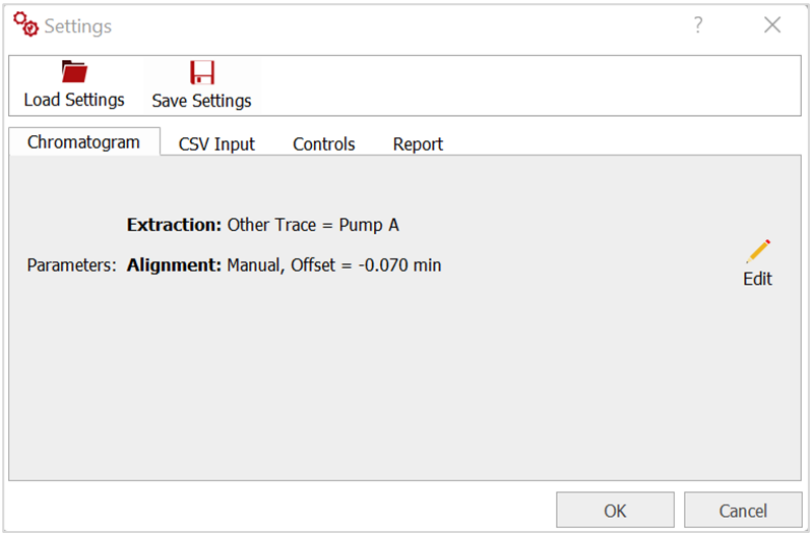
Select the analytical trace type (DAD, DAD range, PDA, or other trace) for chromatogram extraction. Then, choose whether to align the chromatograms automatically or manually; we recommend the use of a manual alignment. In such case, the value introduced will be added to the chromatogram in order to align it with the TIC. *(Please refer to this article to see how you can calculate the time-shift required to align chromatograms manually with Mnova.)*




In this tab, it is also possible to **Apply Trace Baseline Correction**. The **Folder** and **Smooth Factor** can be set manually by: (1) typing the value, (2) using the cursor, or automatically by (3) autodetection.



Once the **Chromatogram tab** is configured, the chosen parameters are displayed in the main **Settings** window, and the **Add** button is replaced by an **Edit** button.




### 1.3.2. The CSV Input tab

In the **CSV Input tab**, Mgears will automatically detect the **Mapping file** and will allow you to configure matching columns with the input information required for the analysis. You must match:

- The **Compound Id** with the column containing the name/number/ID of the analyte. This will allow grouping and evaluation of the datapoints for each analyte separately to generate the corresponding calibration curve. If the column is set to "zero", all datafiles will be evaluated together and a single calibration curve will be obtained.

**Column 7**

| D | E | F | G | H | I | J |
|---|---|---|---|---|---|---|
| Pos | Tube BC | ELN ID | Compound ID | Vol (uL) | Conc ( mM) | Parent MW |
| 1 | 1.1E+09 | 1 | Compound_357 | 30 | 3.333333333 | 259 |
| 2 | 1.2E+09 | 2 | Compound_357 | 30 | 1.111111111 | 259 |
| 3 | 1.3E+09 | 3 | Compound_357 | 30 | 0.37037037 | 259 |
| 4 | 1.4E+09 | 4 | Compound_357 | 30 | 0.12345679 | 259 |
| 13 | 1.5E+09 | 13 | Compound_148 | 30 | 3.333333333 | 608 |
| 14 | 1.6E+09 | 14 | Compound_148 | 30 | 1.111111111 | 608 |
| 15 | 1.7E+09 | 15 | Compound_148 | 30 | 0.37037037 | 608 |
| 16 | 1.8E+09 | 16 | Compound_148 | 30 | 0.12345679 | 608 |
| 25 | 1.9E+09 | 25 | Compound_505 | 30 | 3.333333333 | 343 |
| 26 | 2E+09 | 26 | Compound_505 | 30 | 1.1111111 | |

Compound Id: 7

- The **Molecular Formula** and/or **Monoisotopic Mass** to allow correct peak assignment to the different analytes.

**Column 11** **13**

| I | J | K | L | M | N | O |
|---|---|---|---|---|---|---|
| Conc ( mM) | Parent MW | Full MW | Parent MIW | Full Formula | Salt | Proje |
| 3.333333333 | 259 | 295.81 | 259 | C16H21NO2 | HCl | |
| 1.111111111 | 259 | 295.81 | 259 | C16H21NO2 | HCl | |
| 0.37037037 | 259 | 295.81 | 259 | C16H21NO2 | HCl | |
| 0.12345679 | 259 | 295.81 | 259 | C16H21NO2 | HCl | |
| 3.333333333 | 608 | 608.69 | 608 | C33H40N2O9 | Parent | |
| 1.111111111 | 608 | 608.69 | 608 | C33H40N2O9 | Parent | |
| 0.37037037 | 608 | 608.69 | 608 | C33H40N2O9 | Parent | |
| 0.12345679 | 608 | 608.69 | 608 | C33H40N2O9 | Parent | |
| 3.333333333 | 343 | 343 | 343 | C20H29 | | |

Molecular Formula: 13
Monoisotopic Mass: 11

- The samples' **Concentrations**.

Column20

| Q | R | S | T |
|---|---|---|---|
| ndor | ;hed Mass | Validation | Conc(mg/mL) |
| YYY | 4.13 | | 0.986033333 |
| YYY | 4.13 | | 0.328677778 |
| YYY | 4.13 | | 0.109559259 |
| YYY | 4.13 | | 0.036519753 |
| YYY | 5.84 | | 2.028966666 |
| YYY | 5.84 | | 0.676322222 |
| YYY | 5.84 | | 0.225440741 |
| YYY | 5.84 | | 0.075146914 |
| YYY | 3.59 | | 1.143333333 |

Concentration: 20

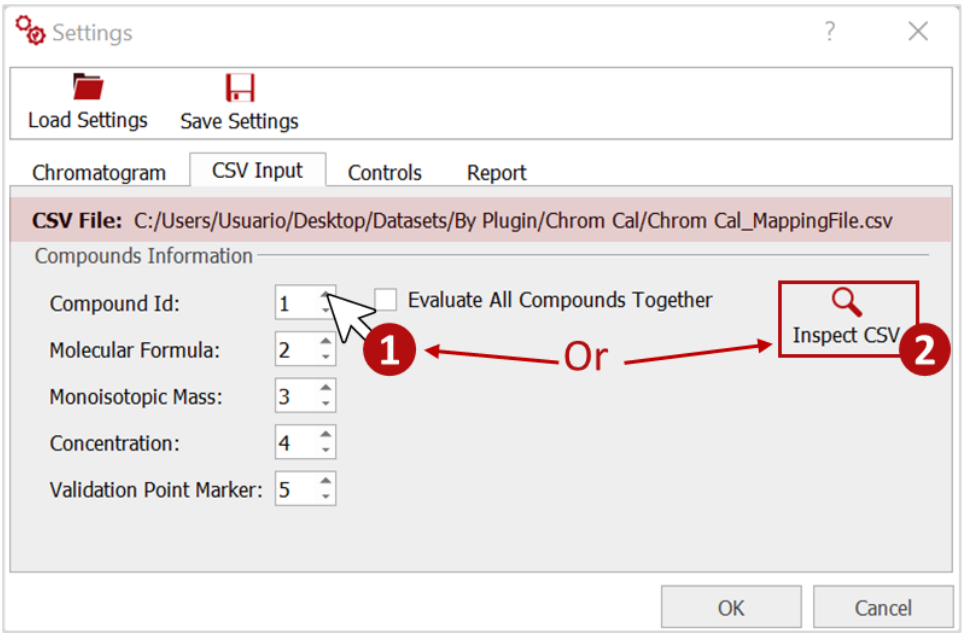
- **Validation Point Marker** for result validation, if cross-validation is required. The column matched to this parameter must contain a "Y" or "YES" in the rows with the validation samples.

Column 19

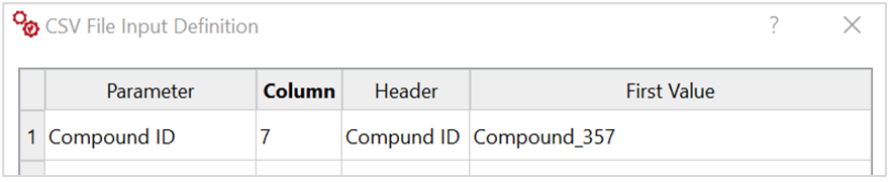
| G | H | I | J | K | L | M | N | O | P | Q | R | S | T |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Compound ID | Vol (uL) | Conc ( mM) | Parent MW | Full MW | 'arent MIV | Full Formula | Salt | Project | Chemist | Vendor | ;hed Mass | Validation | onc(mg/mL |
| Compound_505 | 30 | 0.37037037 | 343 | 343 | 343 | C20H29N3O2 | Parent | | XXXX | YYYY | 3.59 | Y | 0.127037 |
| Compound_357 | 30 | 3.333333333 | 259 | 295.81 | 259 | C16H21NO2 | HCl | | XXXX | YYYY | 4.13 | Y | 0.986033 |
| Compound_148 | 30 | 1.111111111 | 608 | 608.69 | 608 | C33H40N2O9 | Parent | | | | | Y | 0.676322 |

Validation Point Marker: 19

To configure matching columns, you can either indicate the number of the column directly by clicking on this box 1, or click on **Inspect CSV** to define input columns using the CSV assistant.

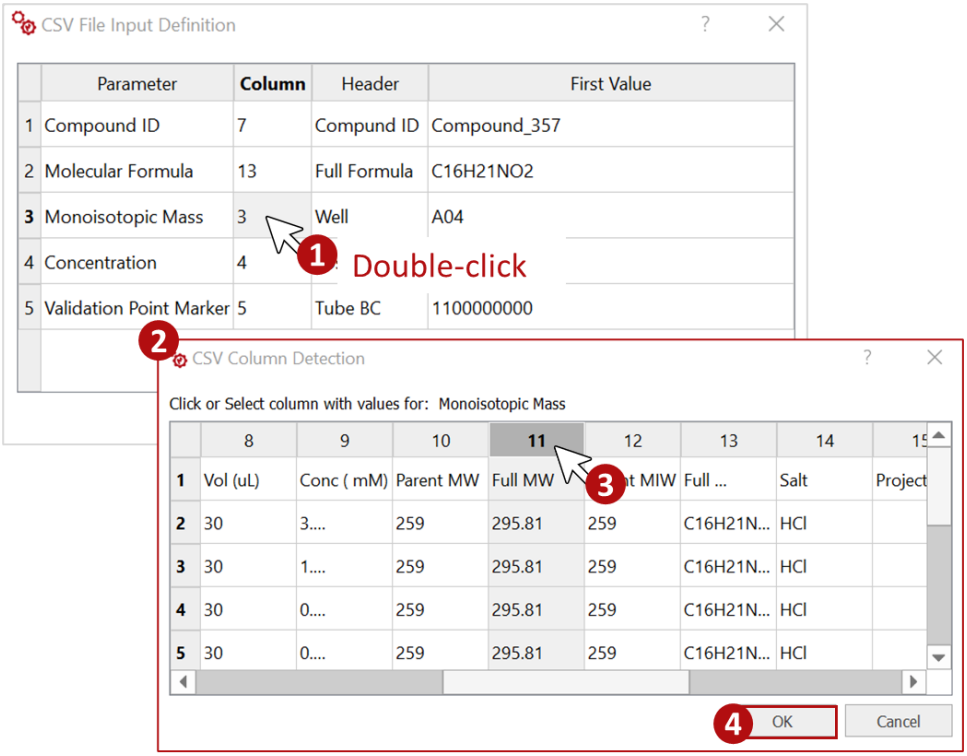



When using the **Inspect CSV** option, a **CSV File Input Definition** dialog will open with a table display of the **Parameters**, their assigned **Column**, column **Header**, and the **First Value** found in the file.

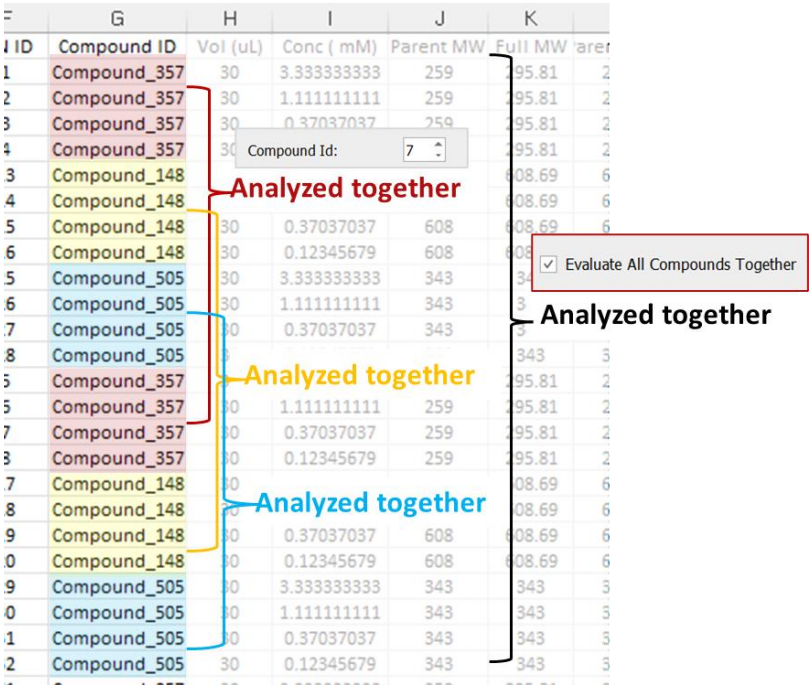



| | Parameter | Column | Header | First Value |
|---|---|---|---|---|
| 1 | Compound ID | 7 | Compund ID | Compound_357 |

Double-click on a "**Column**" cell to open the **CSV Column Detection** dialog and visualize the CSV, then select the column corresponding to the parameter and click **OK** to save the selection.




In the **CSV input** tab, you have also an option to **Evaluate All Compounds Together**. This option is interesting if a universal detector such as CAD is used, and when enabled, a calibration curve with all the compounds is generated in addition to the separate analyte calibration curves.



### 1.3.3. The Controls tab

In the **Controls** tab, result acceptance criteria and cross-validation rules can be defined, and control flags can be configured to receive a color warning when a result does not fit the configured parameters. The available controls include:

- **Accepted R2:** If enabled, a threshold must be defined. A **Pass** ✓/**Fail** ✖ message will be displayed with the calibration result for the **R2** value.

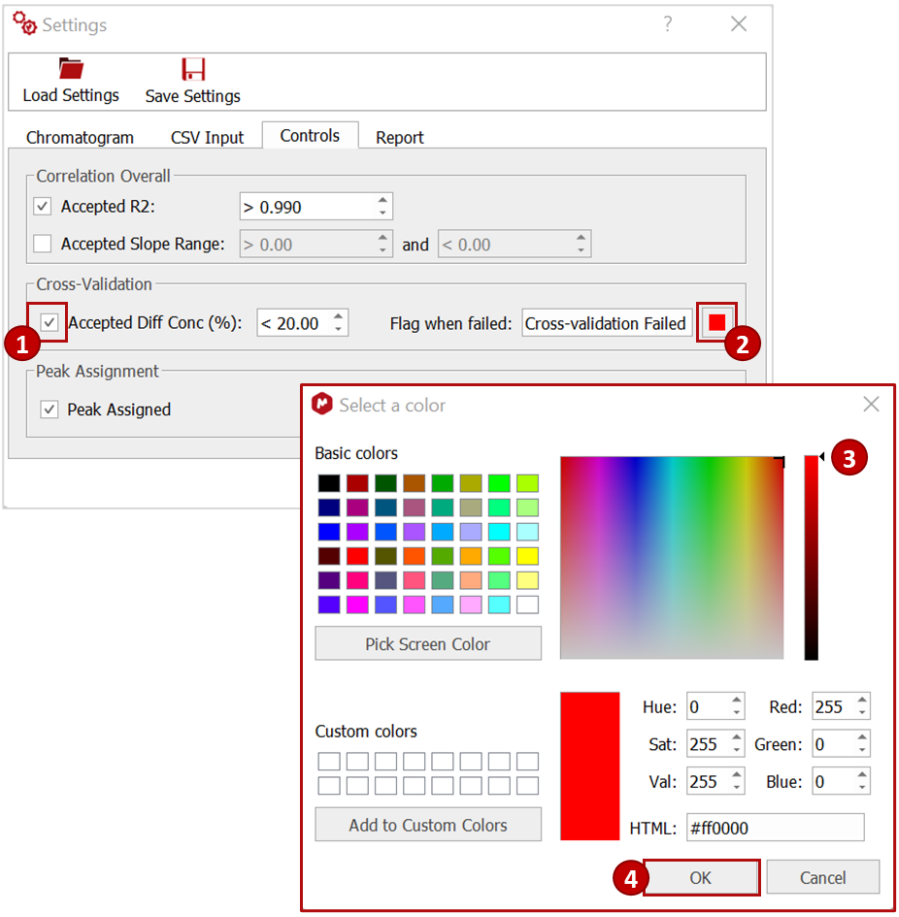
- **Accepted Slope Range:** If enabled, a slope range must be defined. A **Pass** ✓/**Fail** ✖ message will be displayed with the calibration result for the **Slope** value.

- **Accepted Diff Conc (%):** If enabled, a threshold must be defined. A flag is obtained when the variation of the concentration (C) (calculated in percentage as $[C_{Calculated}-C_{Expected}]/C_{Expected}$ ) exceeds the accepted threshold. The flag color and text can be customized.

  Note that for this control to work, a **Validation Point Marker** must be duly defined in the **CSV Input tab.**

- **Peak assigned:** If enabled, a flag will be obtained when no peaks are detected for a component. The flag color and text can be customized.

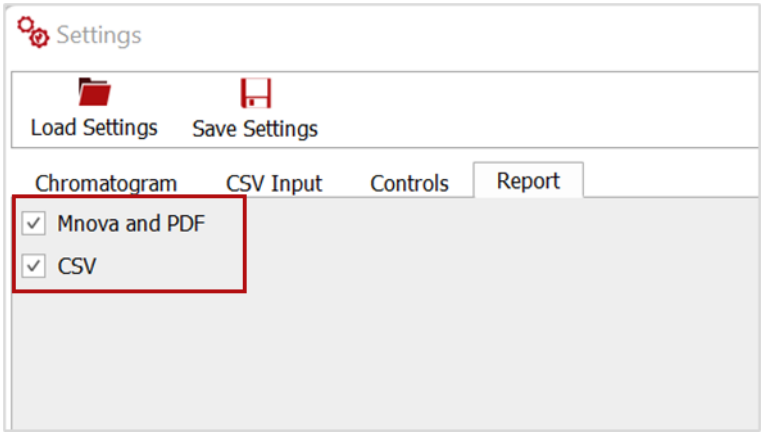
Enable the option of interest, then click on the color selection button and choose the flag color. Click on **OK** to save your preferences.




Finalize your plugin settings setup by pressing **OK** and move to the next step.

### 1.3.4. The Report tab

In the **Report** tab, choose the report formats you wish to obtain for your analysis results. **Mnova**, **PDF**, **CSV** reports can all be generated.



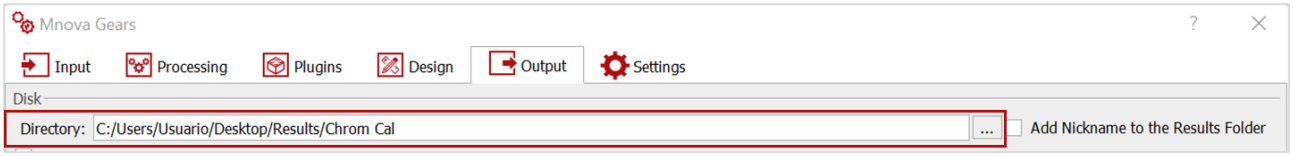

## 1.4. Design

In the **Design** tab, it is possible to load an Mnova layout template or use a custom script to produce a final customized report.

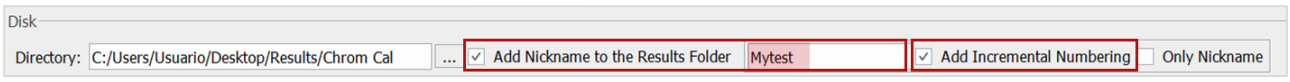
In this analysis, we are not going to use custom designs. The results will be laid out using the default configuration.

## 1.5. Output

Choose a directory to which Mgears can save the results of the analysis.




Optionally, enable the **Add Nickname to the Results Folder** and type the nickname of your choice. You can also **Add Incremental Numbering** after the chosen nickname.



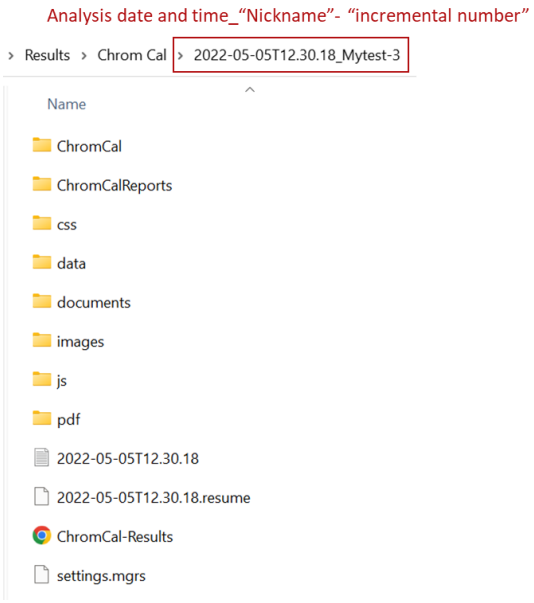

Enable the Mnova document creation to be able to view/review the spectra along with the results in the **Mgears Result Viewer** (if not enabled, when loading the results in the Mgears Result Viewer, only the data and Calibration curves will be visible, with no spectra).

You can also choose to create PDF reports with the individual results or save the results to a database *(please refer to the Mnova Gears manual for more details on the output configuration options).*

Now that everything is set, click on Run to start the analysis.

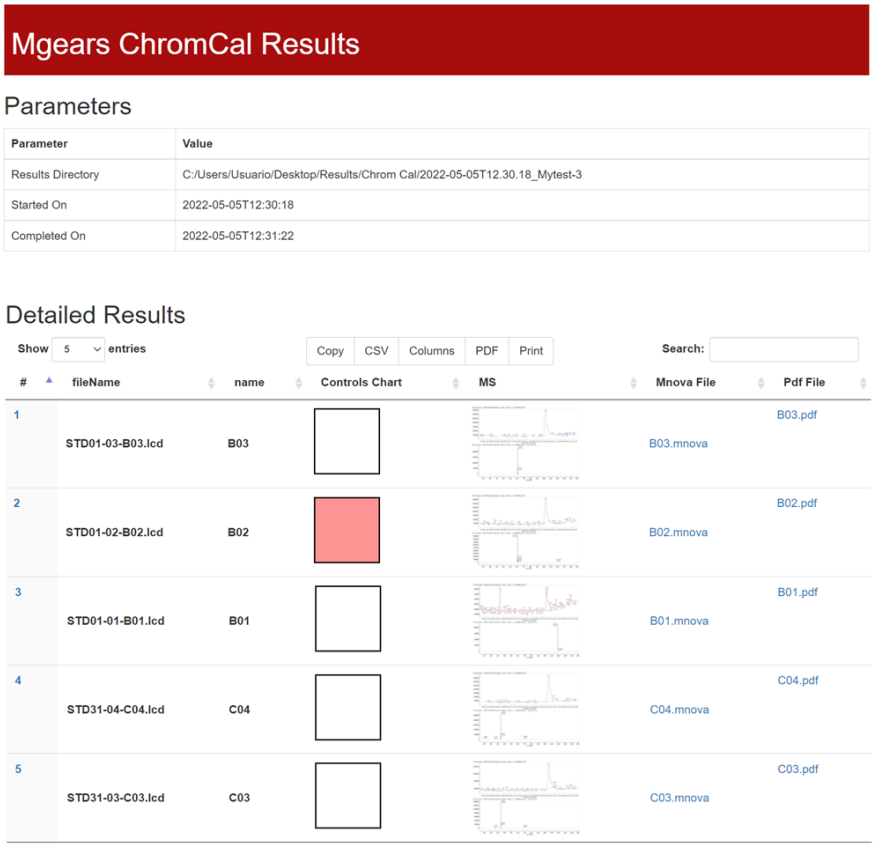
# 2. The Output Folder

The analysis results obtained are saved in the directory selected in the **Output** tab and is named according to the date and time stamp at which the execution was carried out, in addition to the nickname and number added.



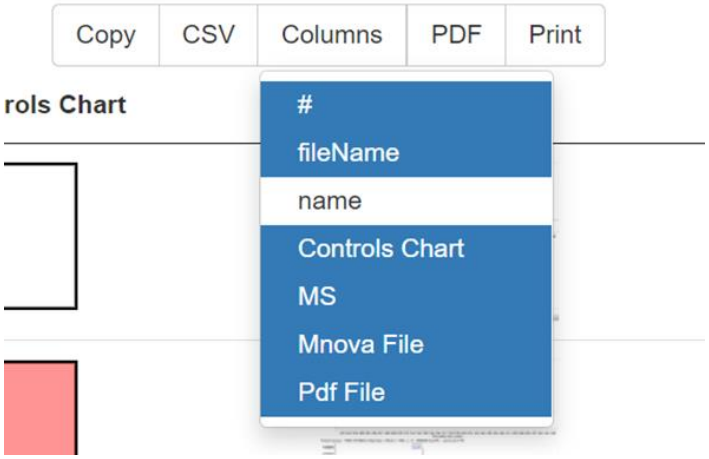

## 2.1. The HTML file

In the HTML report, an overview of the results is available. Each sample is reported in a row with **Controls charts**, a preview of the **MS spectrum**, and hyperlinks to the **Mnova** and **PDF** files generated.



You can change the display of the columns and adapt them to your preferences. Click on **Columns** and select/unselect the parameters you wish to display/hide.

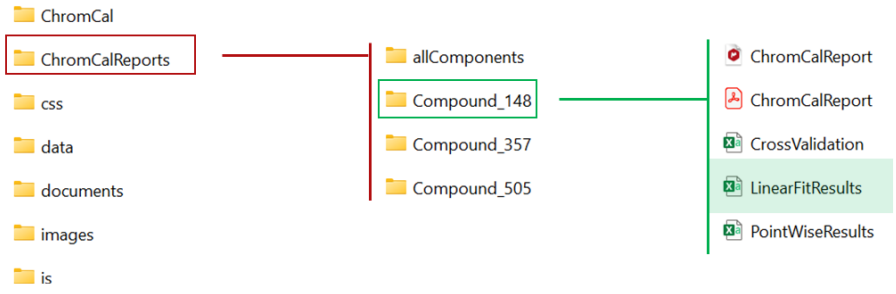
You can also **Copy**, **Print**, or save those results into **CSV** or **PDF** formats.




## 2.2. The CSV reports

Three types of CSV reports are obtained for each calibration analyte when the **CSV** option is checked in the Reports tab of the settings. These reports are saved under a separate sub-folder for each analyte under the "ChromCalReports" folder:

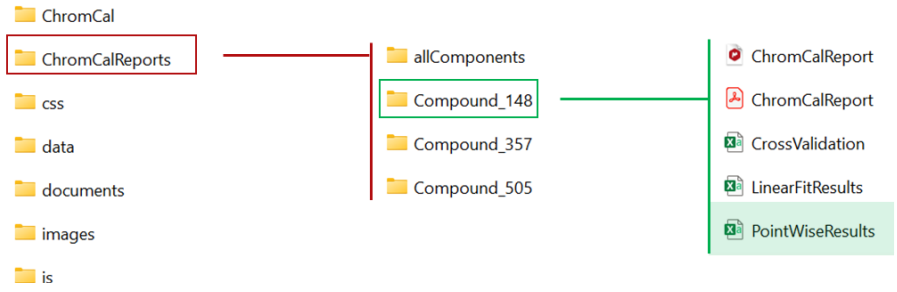
- The **LinearFitResults** reports the calibration curve parameters.




**LinearFitResults**

| | A | B | C | D |
|---|---|---|---|---|
| 1 | Slope | Intercept | R-Square | Validation R-Square |
| 2 | 640565.1 | 41360.54923 | 0.984563711 | NO |

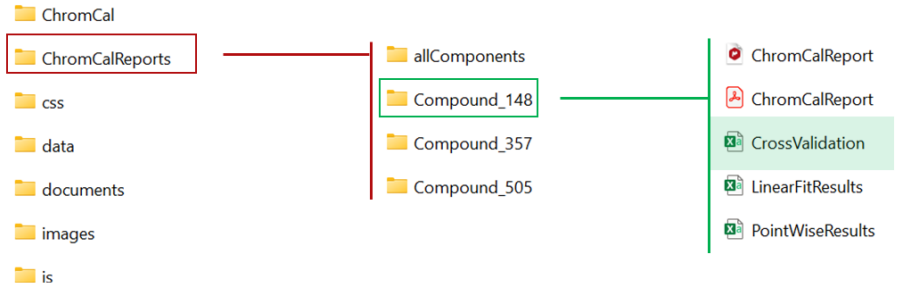
- The **PointWiseResults** reports the results for each data file included in the calibration of the compound.




**PointWiseResults**

| | A | B | C | D | E |
|---|---|---|---|---|---|
| 1 | File Name | Peak Area | Concentration | Estimated Peak Area | Area Diff ^2 |
| 2 | STD01-03-B03.lcd | 569114.2342 | 0.676322222 | 474588.9467 | 8935029969 |
| 3 | STD01-02-B02.lcd | - | 0.225440741 | - | - |
| 4 | STD01-01-B01.lcd | 55016 | 0.075146914 | 89497.03813 | 1188941990 |
| 5 | STD01-04-B08.lcd | 1348666 | 2.028966666 | 1341045.742 | 58068336.66 |
| 6 | STD01-03-B07.lcd | 569114.2342 | 0.676322222 | 474588.9467 | 8935029969 |
| 7 | STD01-02-B06.lcd | - | 0.225440741 | - | - |
| 8 | STD01-01-B05.lcd | 55016 | 0.075146914 | 89497.03813 | 1188941990 |
| 9 | STD01-04-G04.lcd | 1280416 | 2.028966666 | 1341045.742 | 3675965578 |
| 10 | STD01-02-G02.lcd | 118691 | 0.225440741 | 185770.0153 | 4499594290 |
| 11 | STD01-01-G01.lcd | - | 0.075146914 | - | - |

- The **CrossValidation** reports are obtained only when Cross-validation points are configured.

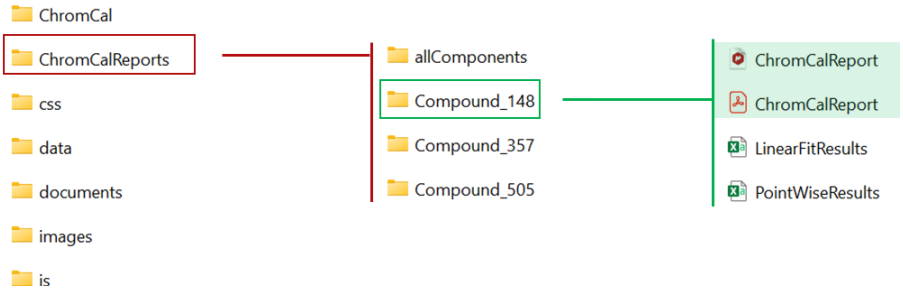



**CrossValidation**

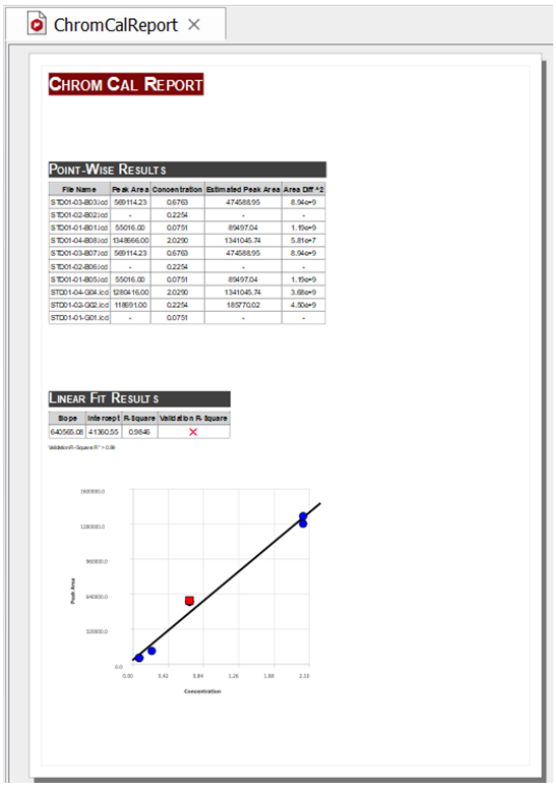
| | A | B | C | D | E | F | G |
|---|---|---|---|---|---|---|---|
| 1 | File Name | Peak Area | Estimated Concentration | Expected Concentration | % Conc Diff | Validation | Flag |
| 2 | STD01-03-G03.lcd | 579811.2 | 0.840586958 | 0.676322222 | 24.28794001 | NO | Cross-validation Failed |

## 2.3. The Mnova and PDF reports

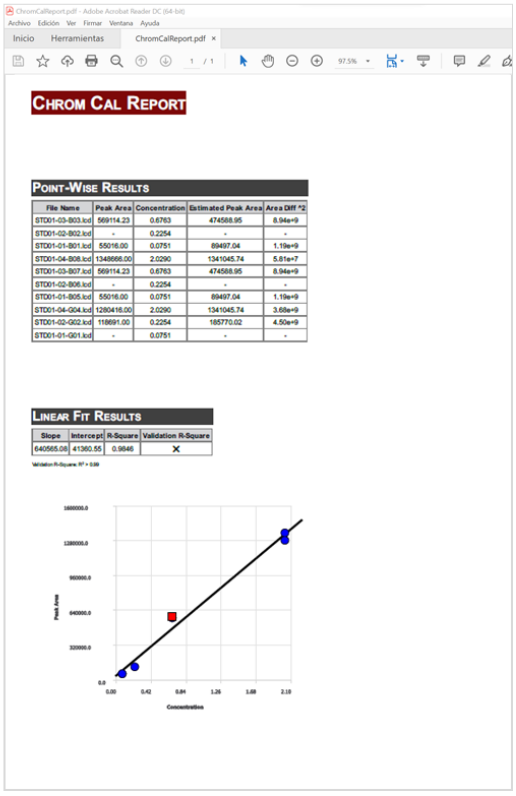
Both Mnova and PDF reports are obtained for each calibration analyte and saved under the corresponding sub-folder when the **Mnova and PDF** option is checked in the Reports tab of the settings:



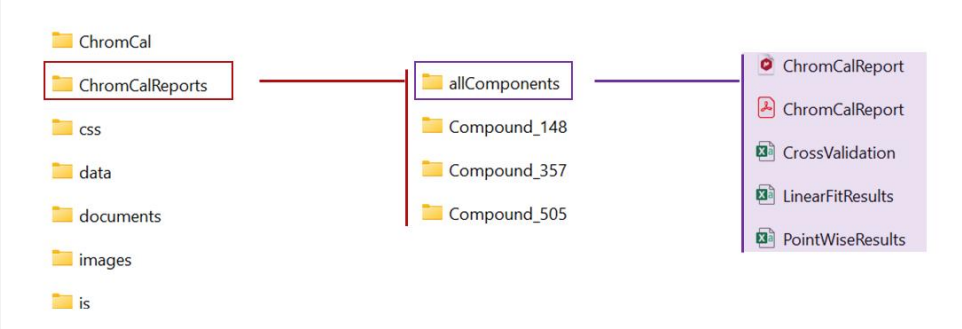

**Mnova**




**PDF**




**Note.** When the Evaluate All Compounds Together option is enabled, **CSV**, **Mnova** and **PDF** reports for the evaluation, with all the available data points together, are saved in a sub-folder named “allComponents”.



## 2.4. Other output

- A “Chrom Cal” directory is created containing the analysis output (JSON files) with data and calibration curves that will be loaded and reviewed in the **Mgears Result Viewer**. These files can also be used as input in downstream applications that need to import the calibration parameters, e.g., Chrom Reaction Optimization.
- A “documents” directory, containing the output individual Mnova files for each data file (unless Mgears is configured to save Mnova files in another location).
- A log file of the execution.
- A copy of the settings used in the current evaluation.
- A resume file of the steps followed in the execution.
- A CSS folder, a data folder, a JS folder, and an images folder.

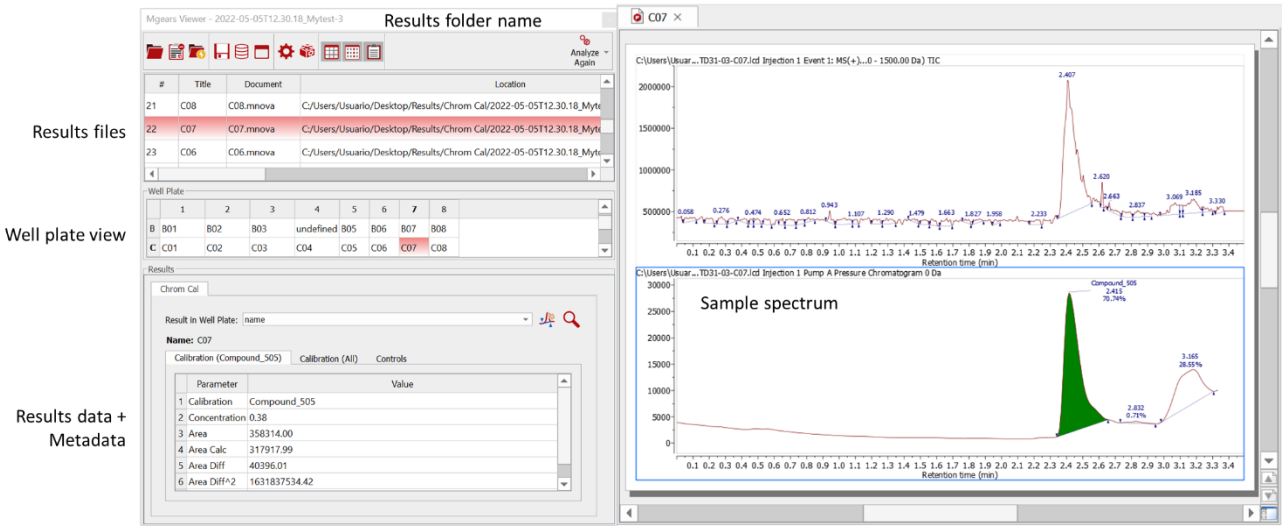
# 3. Mnova Gears Results Viewer

Chrom Calibration has two different – but interconnected – result viewers, the **Mgears Viewer** and the **Chrom Cal Viewer**, to display individual calibration curve results.

Open the **Mgears Viewer** from the Mnova **Automation** tab, then click on  to open your analysis results. When the experiment is open, both **Mgears** and **Chrom Cal Viewers** are launched automatically.

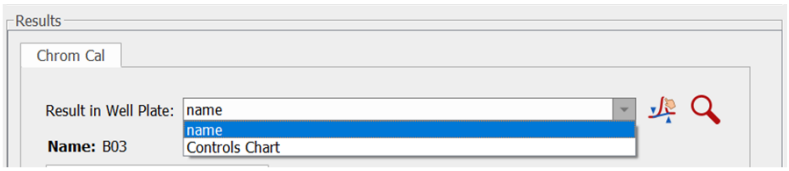
# 3.1. The Mgears viewer

In the **Mgears Viewer** you can see the names of the dataset files, their positions in the well plate, and any associated numerical results. Click on a specific dataset to view the corresponding results and spectrum in Mnova.

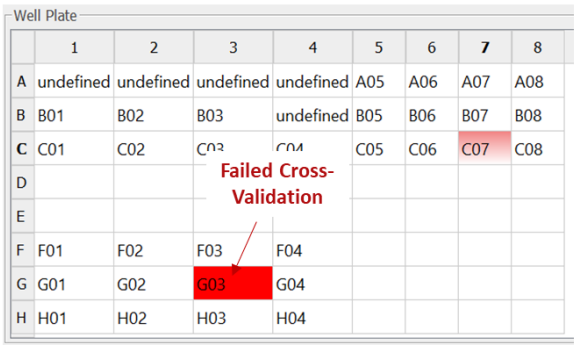



## 3.1.1. The Well plate view

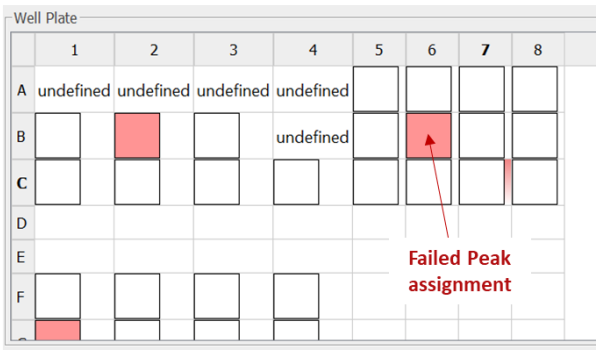
The well plate can show either data file **Names** or **Controls charts**. You can change the display by selecting the corresponding option for the **Result in Well Plate** under the **Results** section.




**File names**



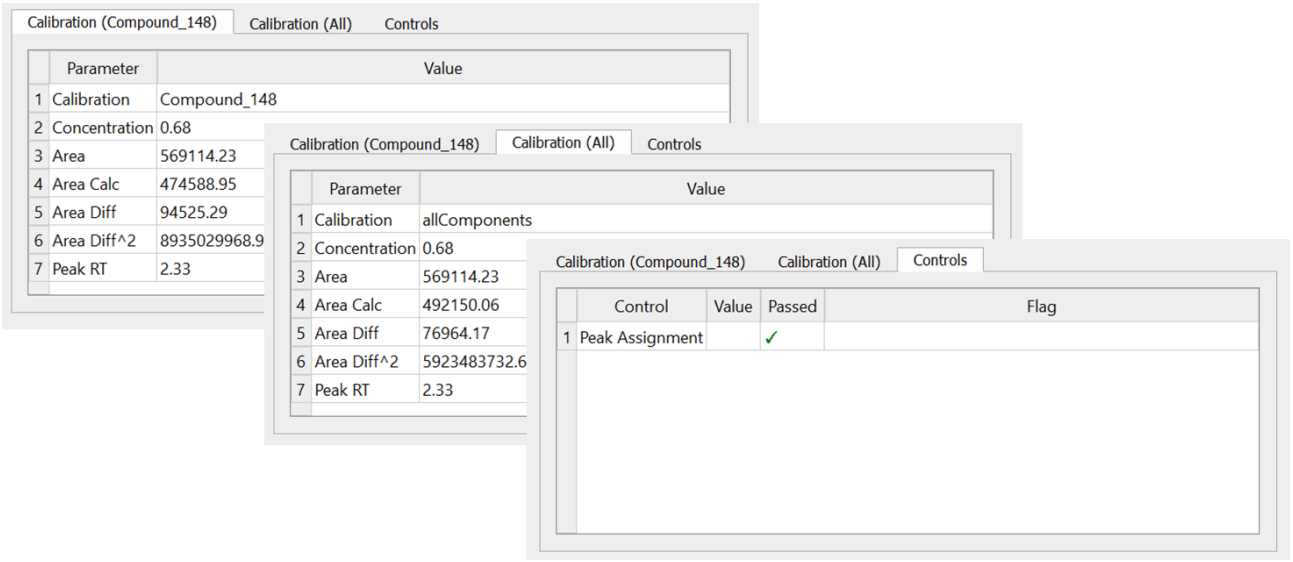

**Controls charts**



### 3.1.2. The Results section

The **Results** section can include up to three tabs with information on:

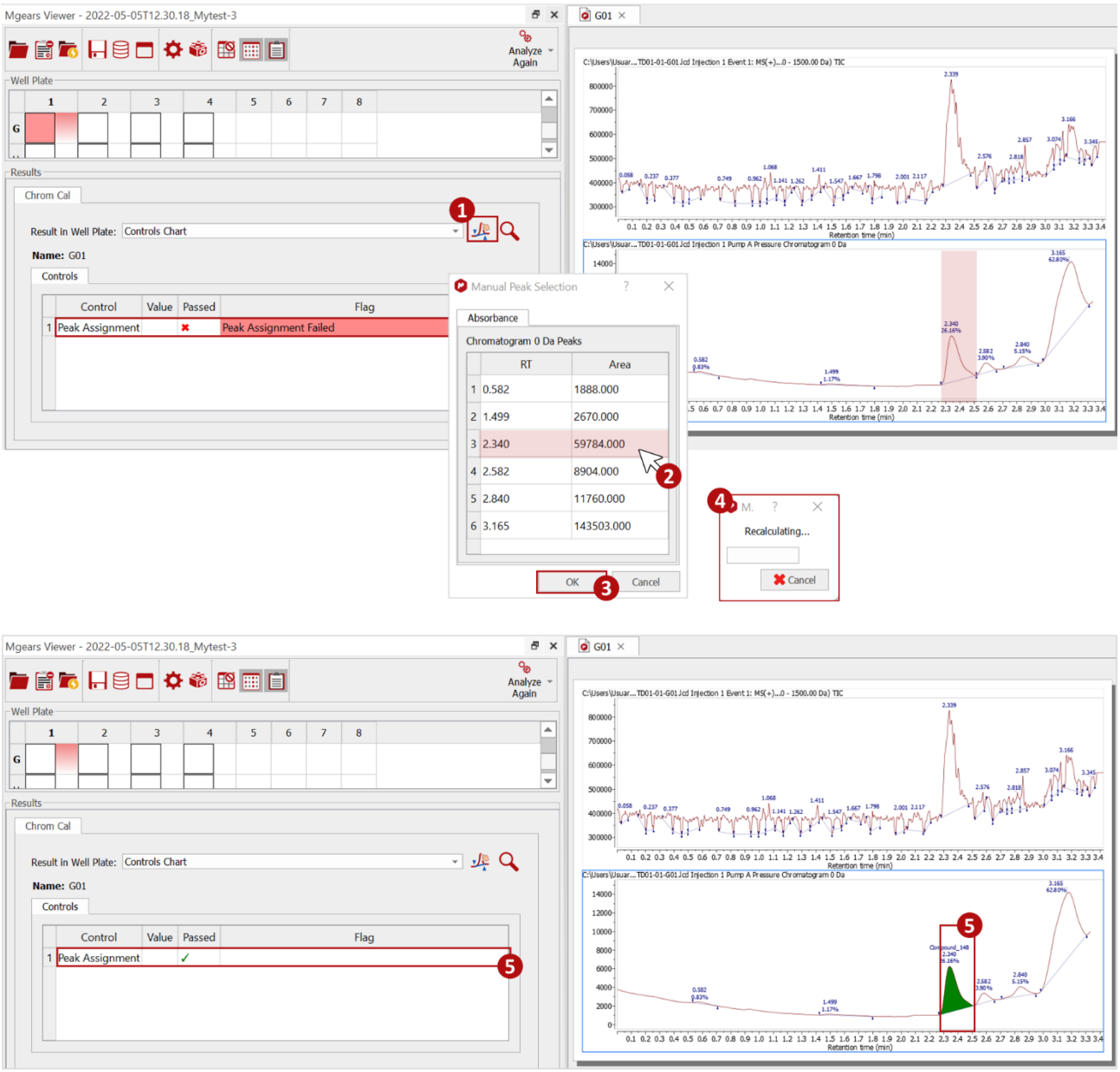
- Peak-specific results relative to the datafile in question under the **Calibration** tabs; when the Evaluate All Compounds Together option is enabled, two **Calibration** tabs are available, one for the analyte evaluation **(Compound_148)** and another for the overall evaluation **(All).**
- **Controls**, as defined in the Controls tab of the settings. This tab is only visible if at least one analysis control is enabled.




### 3.1.3. Assigning peak/Modifying peak assignment

Not only you can visualize analysis results with the **Mgears viewer**, but also you can assign peaks to samples that failed peak assignment, or modify peak assignment manually and update your results.

To do so, open your sample of interest (in this example, we are going to assign a peak to a sample that failed automatic assignment), then click on the  button. A **Manual Peak Selection** dialog will open to allow you choose a peak in the spectrum and assign it to your compound. Select **peak** and press **OK**. Mgears will automatically recalculate and update the results (including output files and reports) for the selected well, as can be seen in the image below.







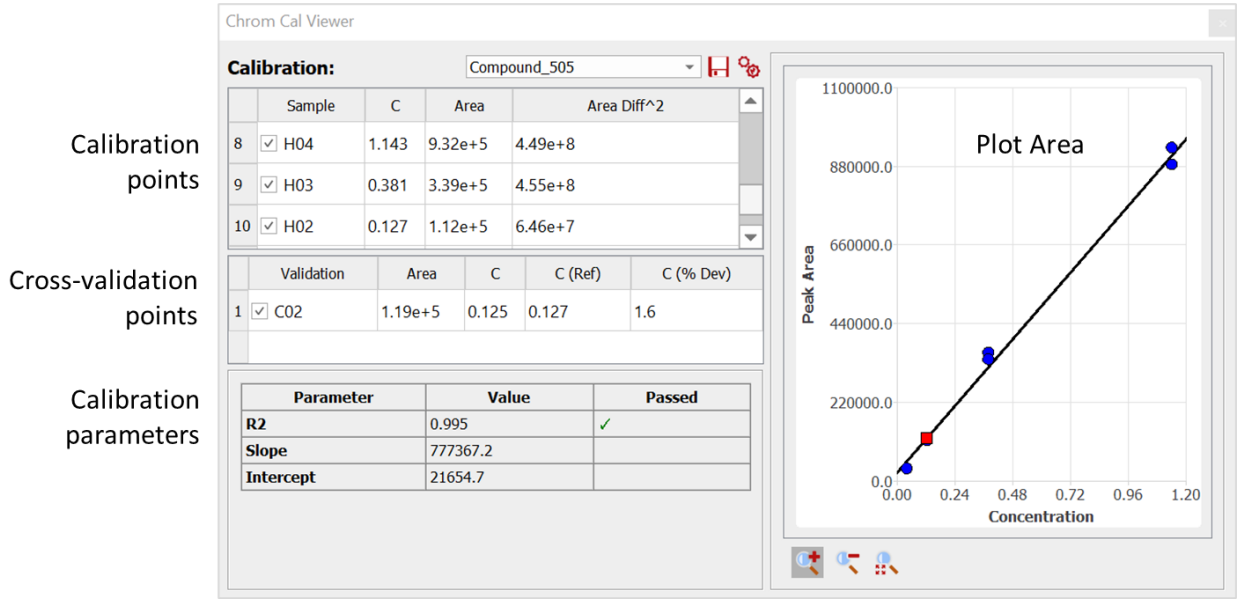
You can also manually edit the spectrum peaks using the Mnova tools. In this case, it will be necessary to press the **Analyze again** button in the **Mgears Viewer** to update the results according to your changes.

**Note.** When modifying peak assignment manually, the selection of the peak in the different calibration curves is not affected. Therefore, it may be necessary to review the calibration result in the **Chrom Cal viewer** and update peak selection (to include or exclude it), as explained in section 3.2.4.

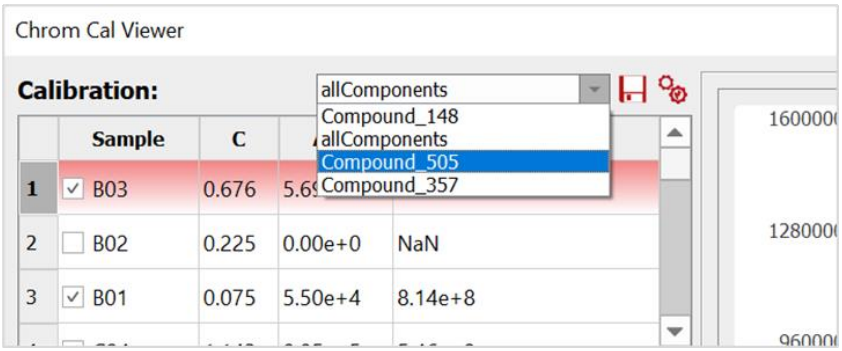
If at any point you close the Chrom Cal Viewer, it is possible to open it again by clicking on the icon in the **Results** section.

## 3.2. The Chrom Cal viewer

In the **Chrom Cal Viewer,** you can view the calibration results (calibration and cross-validation points, calibration parameters, and plot) for each analyte.

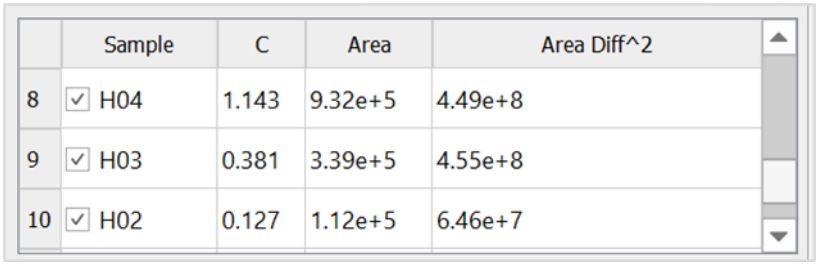



You can select the results to display from the **Calibration** options list.




### 3.2.1. The Calibration and Cross-validation points

For each calibration, the individual samples are listed in the calibration points table with their **Concentration**, **Peak Area**, and **Area Diff^2** (indicating the level of deviation of a point from the calibration curve).



The cross-validation samples are also listed in a separate table with their **Peak Area**, **Concentration**, **Reference Concentration**, and the **Percentage of Deviation** in the concentration value.

| | Validation | Area | C | C (Ref) | C (% Dev) |
|---|---|---|---|---|---|
| 1 | ☑ C02 | 1.19e+5 | 0.125 | 0.127 | 1.6 |

Selected samples ☑ are included in the global calibration result and plot, whereas unselected samples ☐ are excluded from the global result and plot.

### 3.2.2. The Calibration parameters

For each calibration, the **R2**, **Slope**, and **Intercept** values are displayed in a table with the test result (if configured in the Controls tab), as can be seen below:

**Compound_505**

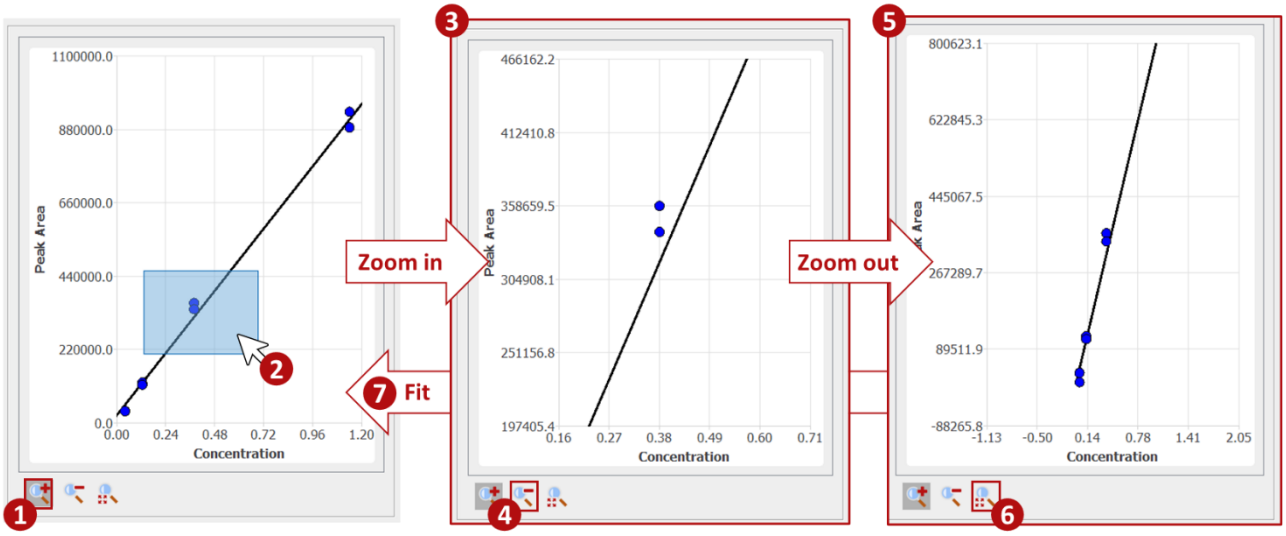
| Parameter | Value | Passed |
|---|---|---|
| R2 | 0.995 | ✓ |
| Slope | 777367.2 | |
| Intercept | 21654.7 | |

**Compound_148**

| Parameter | Value | Passed |
|---|---|---|
| R2 | 0.986 | ✖ |
| Slope | 645493.3 | |
| Intercept | 33139.0 | |

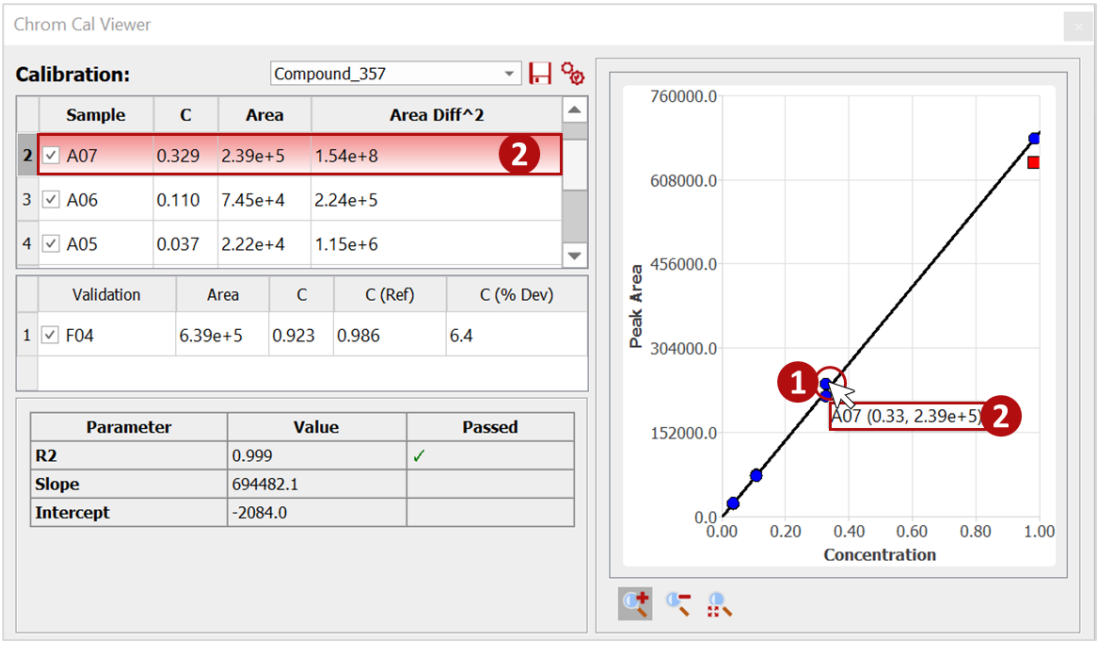
### 3.2.3. The Plot Area

For each calibration, the different samples' **Concentrations** and **Peak areas** are plotted, and the fitted calibration curve is represented as can be seen in the example below. **Zoom in** and **Zoom out** tools are made available to be able to inspect the graph with more flexibility. This button will reset the calibration graph to its original size fitting to the plot area.



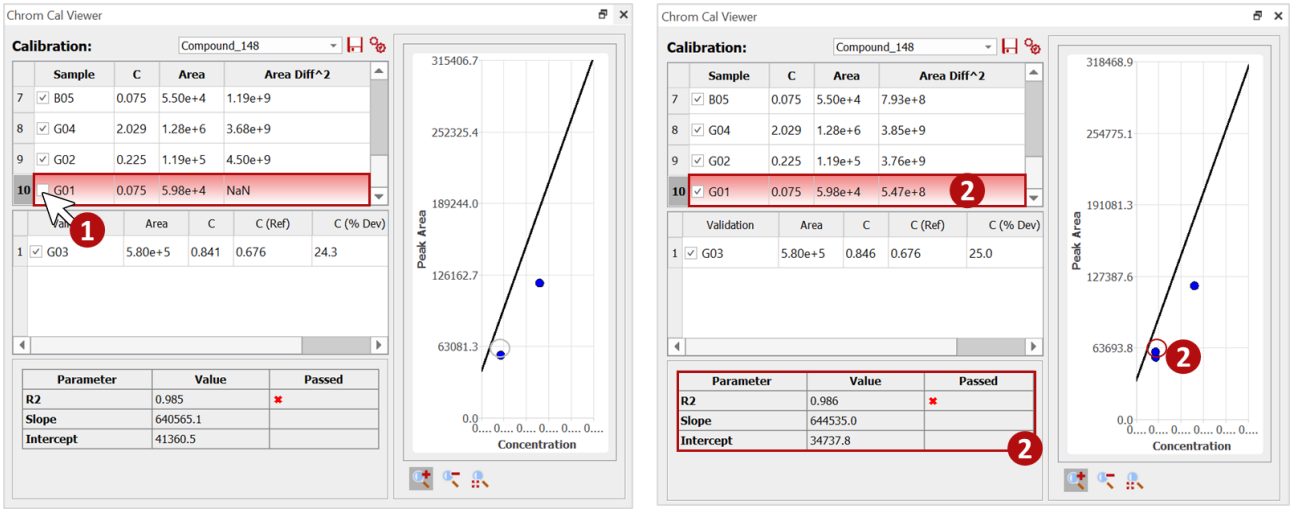
When hovering the cursor over either a calibration or cross-validation point in the plot:

- a tool tip with the name of the sample or validation point, the concentration, and peak area will be displayed: “Name (Concentration, Area)”; and,
- the corresponding row in the results table will be highlighted.




### 3.2.4. Updating the calibration/cross-validation results

It is possible to update the calibration results by selecting/unselecting samples to include/exclude them from the analysis. Chrom Cal will automatically update the results in the viewer as well as in the output files and reports.




If manual changes are introduced to the spectra using the Mnova tools the **Analyze again** button in the **Chrom Cal Viewer** should be used to save the updated results to the output folder and reports.

***For more details on the Mnova Gears options, please refer to the Mnova Gears manual.***