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Hands-on training for ALEXYS users

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Introduction

This hands-on training is intended for end-users of ALEXYS® LC-ECD Analyzers, who want to get familiar with the CLARITY chromatography software, which is used for control, data-acquisition and data processing.

This hands-on document contains three cases:

- CASE 1: setting up method files and a sequence table
- CASE 2: process/reprocess and reporting of data
- CASE 3: how to set-up a system configuration from scratch (Advanced)

CASE 1 and 2 are recommended to get familiar with daily actions, and CASE 3 is intended to understand how Clarity is organized. This document is aimed to serve as a manual for ALEXYS users. All the steps are highlighted using a few example chromatograms, but they are of course generally applicable.

Requirements

Personal Computer

- Windows NT based Operating System (Windows 10 or 11)

Software

- Clarity (version 10) installed

Hardware

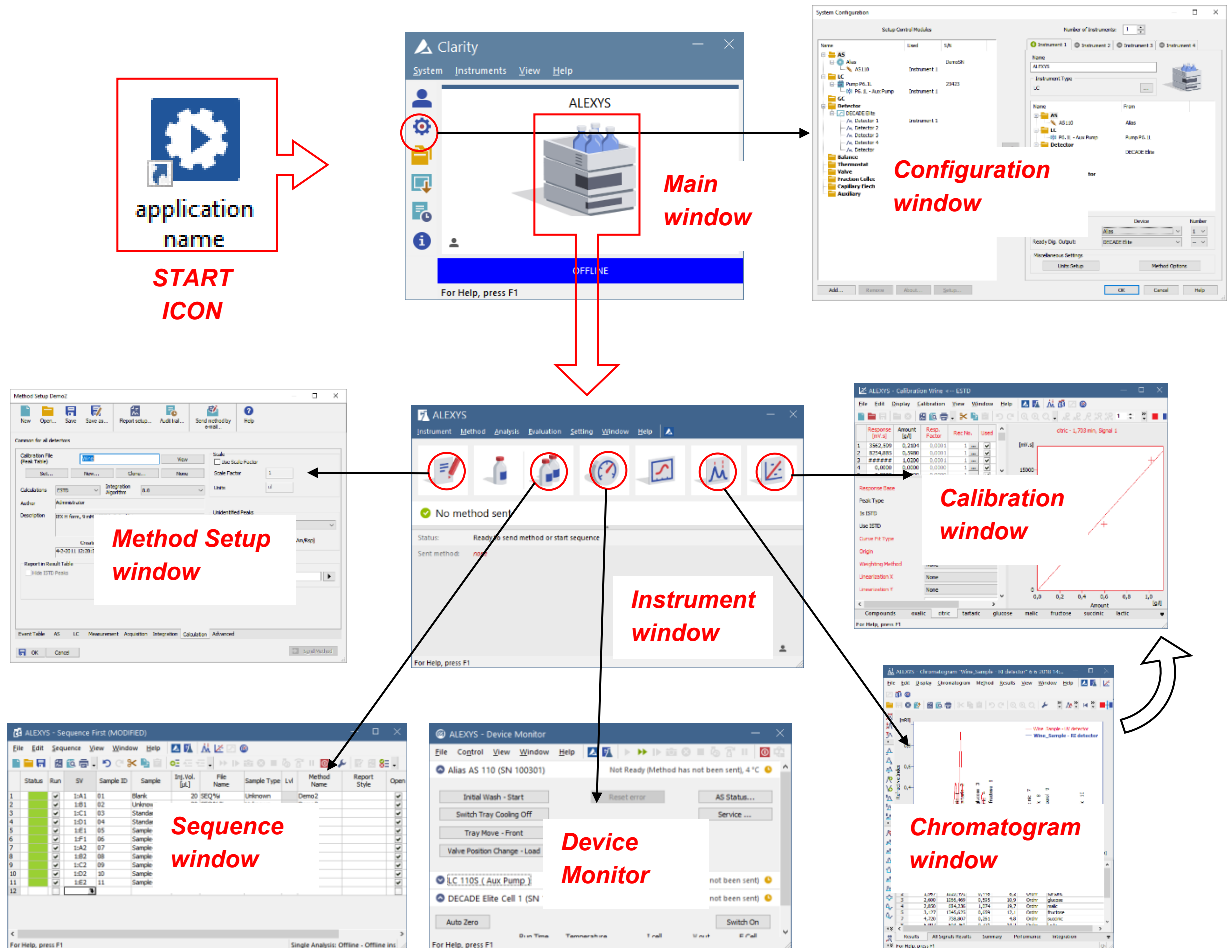
- A Clarity license dongle; one of either types:
    - acquisition (p/n 195.0035)
    - off-line (pn. 195.0C59)

Example chromatograms

- Install the feature ‘Always install’ and ‘Hands-on session’ from the installation-program *Clarity analyzers from Antec_10-20xx.exe*. This will install a shortcut on the desktop and two sets of example chromatograms into the project folders C:\Clarity\DataFiles\CASE1 and C:\Clarity\DataFiles\CASE2.

Overview chart of Clarity windows

As reference material, the schematics below shows the names of the different Clarity windows.



CHAPTER 1

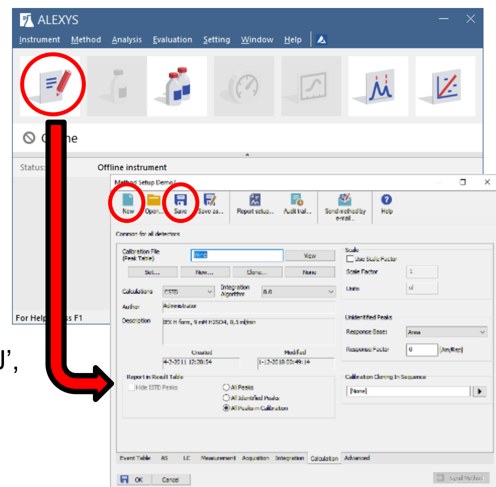
CASE 1: setting up method files & a sample queue

Goal of the following instructions is to get familiar with:
- setting up an instrument method
- setting up a sample queue.

The method and sample queue should result in automated peak identification and quantification of various components in a set of samples.

Step 1 Start Clarity software and select/make the project

1. Start up the Clarity software program and log-in on the Project **'CASE1'**, which contains a chromatogram as a training subject (alternatively, make your own project and make your own test chromatogram).



Step 2 Create a new method

2. Open the Method Setup window using the icon in the Instrument Window.
3. Create a new method (use 'New' button).
4. Save the method with the name 'yymmdd_INJ', where yymmdd is the date of today.

Step 3 Setting the method parameters

Have a printout of the method that you want to program, or use the example of the settings here to follow along

5. Doublecheck that the method that you are about to modify is the one you just named 'yymmdd_INJ', (see the text displayed in the top rim of the Method Setup window).

DECADE Elite detector settings

6. In the Method Setup window, activate the tab 'Acquisition'.
7. Set the detector parameters to Pulse mode (or DC mode if there is only 1 potential in your particular method), and include an autozero at the start of the run:

   Main:
   - ☐ Mode -> Pulse
   - ☐ Pulse settings:

E1	0.1	[V]	t1	0.4	[s]			
E2	-2	[V]	t2	0.02	[s]			
E3	-0.6	[V]	t3	0.01	[s]	ts	200	[ms]
E4	-0.1	[V]	t4	0.07	[s]			
E5	0	[V]	t5	0	[s]			

Output:

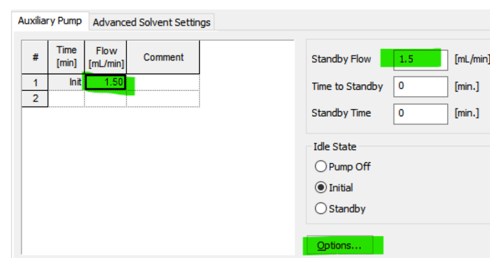
- ☐ Compensation: on
- ☐ Range: 2 µA/V
- ☐ filter setting: off

Time table:

- ☐ at t=0.10 min program a Sensor auto-zero (Active).

P6.1L pump

8. In the Method Setup window, activate the tab ‘LC’.
9. Configure the pump to run at a specific flow rate, to stay at that flow rate when ready.
   - ☐ Initial flow rate: 1.5 mL/min
   - ☐ Stand by flow rate: 1.5 mL/min
   - ☐ ‘Options’:
     - Min Pressure: 150 bar (to prevent running dry)
     - Max pressure: 250 bar for regular HPLC, or 550 bar for UHPLC systems.
     - Max pressure for set flow: 250 bar for regular HPLC, or 550 bar for UHPLC systems.



AS6.1L autosampler

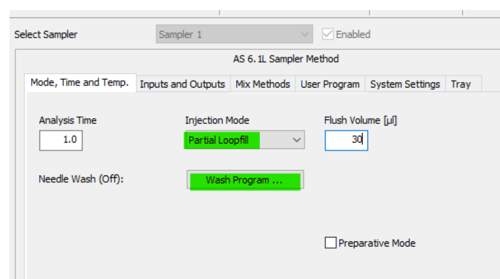
10. In the Method Setup window, activate the tab ‘AS’.
11. Configure the sub-tabs to match the hardware installed in the autosampler, and select the partial loop fill injection method:

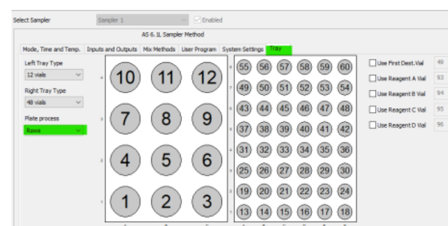
    Mode, time and Temp:
    - ☐ Injection method: Partial loop fill
    - ☐ Flush volume: 30 µL (=2x needle volume)

    Wash Program:
    - ☐ Needle wash: ON
    - ☐ Standard, 2 times

    System settings:
    - ☐ Syringe volume: 250 or 500 µL
    - ☐ Loop volume: 20 uL
    - ☐ Syringe speed: Low - 3

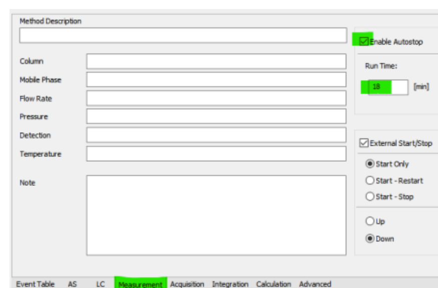
    Tray:
    - ☐ 48-vials tray on left position, and tray with 12 vials on right position





Run time

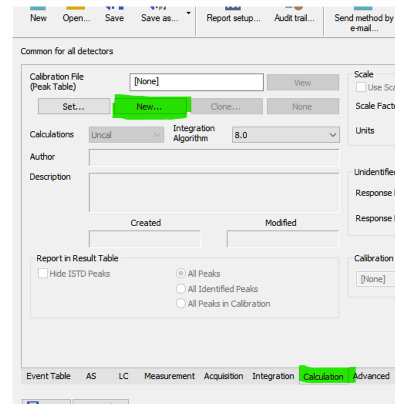
12. In the Method Setup window, activate the tab ‘Measurement’:
13. Configure the run time of the analysis:
    - ☐ Enable Autostop
    - ☐ Run time: 18 minutes.
    - ☐ External start/Stop: Start only



Tip:

In principle, the rest of the fields in the left side of this tab can be used to fill in all relevant method parameters that would be beneficial for reference purpose (column type, mobile phase composition, etc.). This information will then be stored with each chromatogram.

Post-run calculation settings



14. In the Method Setup window, activate the tab ‘Calculation’.
15. Configure the link to the calibration file:
    - ☐ Click the ‘New’ button
    - ☐ Name the new Calibration file ‘Calibration_yymmdd’, where yymmdd is todays date format.

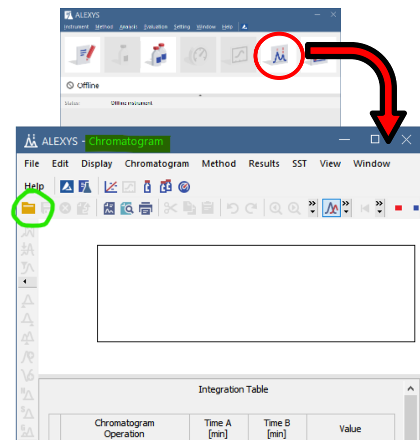
Tip:
We advise to rename calibration files every day using the ‘**Clone’** button. This is a very handy short-cut key that opens, clears, saves, and sets the new file all with one click.

16. Close the Method Setup window with the OK/Safe button

All the hardware method settings are now programmed. In principle, the method is now ready to be used to run a test injection. In the next step, the integration parameters and peak name recognition are optimized on the basis of a test chromatogram.

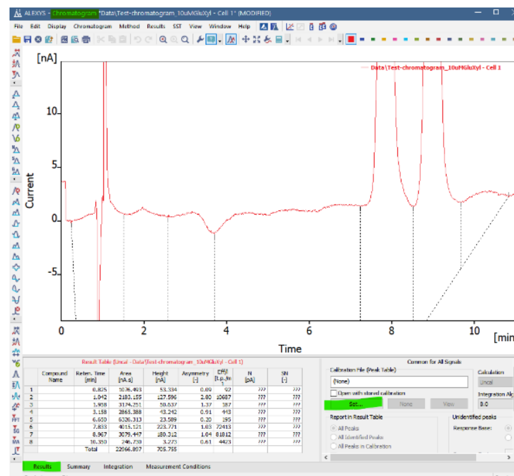
Step 4 Optimizing the peak recognition

The calibration file ‘Calibration_yymmdd’ was just made, but it does not yet contain any parameters. Also, the integration parameters for post-run peak recognition are not optimized yet for the method. Both need to be optimized on the basis of the test injection.



A test chromatogram is available for training purpose:

17. Open the ‘Chromatogram Window’.
18. Open the Data folder with the yellow folder icon or through the menu (‘File/Open chromatogram’) and select the test chromatogram.
19. Activate the Results tab, and manually link the calibration file ‘Calibration_yymmdd’ using the button ‘Set’.

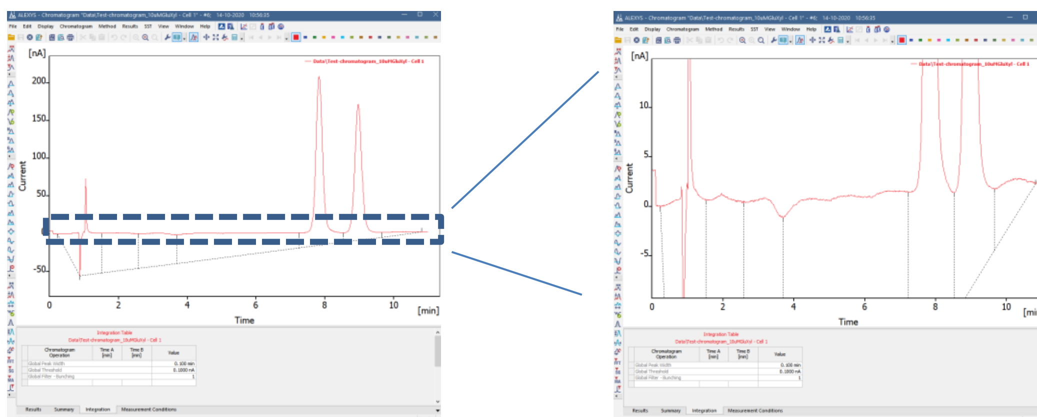


Optimizing general integration parameters

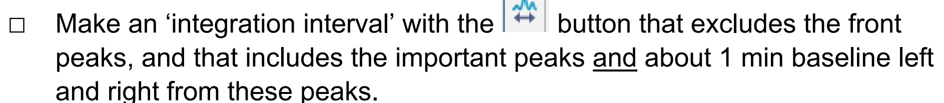
The test chromatogram will be used to develop a set of optimized integration parameters, which can then be used in the analysis method to be standard applied to each newly measured chromatogram:

Note: do not use the buttons that apply to single peaks when developing a general integration table, as the list of parameters should work on any new chromatogram, not only this one.

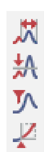
20. Zoom in on the baseline of the whole chromatogram (click/pull a box in the shape of the example below) to clearly see where the start and end marks will be set.



21. Activate the tab 'Integration' and set the *Integration Algorithm* to 'Wave': this already improves the peak recognition and integration.
22. Use the buttons displayed on the left side of the Chromatogram Window to improve the peak integration:



 - Make an 'integration interval' with the button that excludes the front peaks, and that includes the important peaks <u>and</u> about 1 min baseline left and right from these peaks.



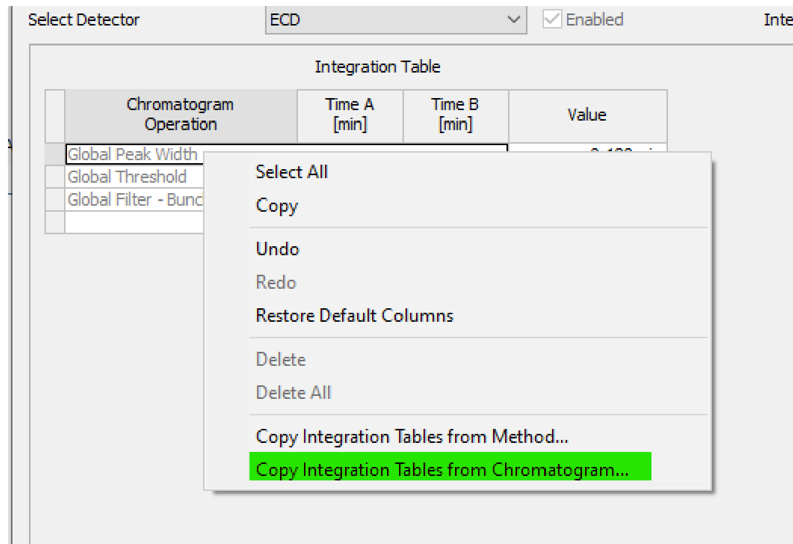
 - The 4 default basic settings can be adjusted using the set of upper buttons. Except for 'Global bunching', all other 3 options can be used to improve the peak integration.
   Tip: when adjusting 'Global threshold', select for example the baseline from 4.3 to 7 min.

For more complex chromatograms, it may be informative to type a few different values in the fields of the Global parameters and see how the peak start and end positioning marks respond.

23. When the integration settings are satisfactory, save and close the chromatogram.

Updating a method with the optimized integration parameters from a chromatogram

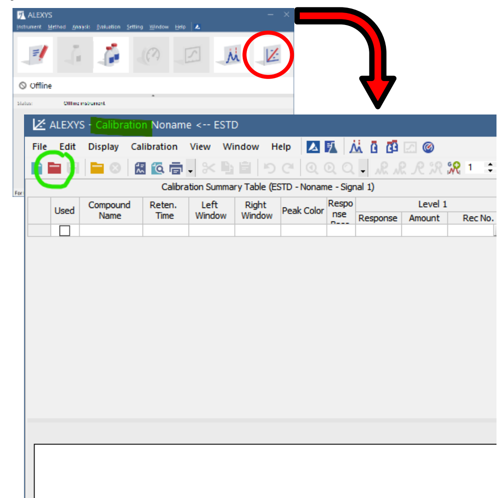
24. Open the method 'yymmdd_INJ' and activate the tab 'Integration'
25. Right-mouse click anywhere in the table and select 'Copy Integration Tables from Chromatogram…'



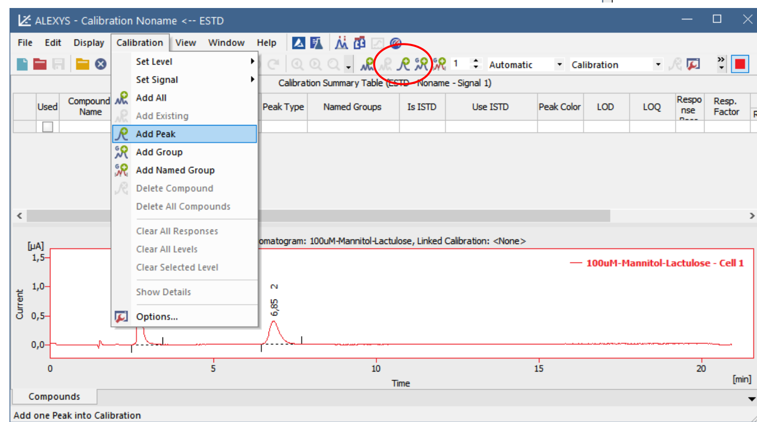
26. Select the chromatogram with the optimized integration table.
27. Close the Method Setup window with the OK/save button.

Optimizing peak identification

The method file does not contain the information about peak names; it only contains a link to a calibration file, which has the table with peak names. The link was already created a few steps ago, but the calibration file still needs to have the correct peak names filled in the table:



28. Open the ‘Calibration Window’.
29. Use the red folder icon (or from the menu *File/Open…*) to open the calibration file ‘Calibration_yymmdd’
30. Use the yellow folder icon (or from the menu *File/Open Standard…*) to open the test injection chromatogram in the Calibration Window.
31. Make an entry line in the peak table for each peak that requires naming, by using the ‘Add peak’ button (or from the menu *Calibration/Add peak*).



32. The automatically generated peak names (in column 'Compound Name') can be renamed. For example, apply the names Glu and Xyl for the example test chromatogram.

33. Set a (light) color for the peaks: this will be helpful later during evaluation of recorded chromatograms.

Step 5 Setting up the calibration levels

Each peak entered in the peak table of the calibration file now has its own tab that can hold the calibration plot. To have the software automatically calculate amounts, the parameters need to be set correctly:

34. In the calibration window activate the 'Calibration options' through the menu (Calibration/Options), and check/adjust the following parameters:
    Calibration options:
    - ☐ Units of Compound: for example, 'μM'
    - ☐ Calibration: Automatic
    - ☐ Mode: Recalibrate
    - ☐ Recalibration: Replace

    Defaults:
    - ☐ Curve fit type: Linear
    - ☐ Origin: Ignore origin

35. Click the button 'Set all now for current signal'

36. Open one of the components tabs, and fill in the concentration values that will be used for calibration, for example:
    - Level 1: 1 μM
    - Level 2: 2 μM
    - Level 3: 5 μM
    - Level 4: 10 μM

    For the other component, the same concentrations are used for calibration. *The values can also be copy-pasted from one tab to the other.*

	Response [nA.s]	Amount [uM]	Resp. Factor	Rec No.		Used
1	######	1,00	0,0001	1	...	☑
2	0,0000	2,00	0,0000	0	...	☐
3	0,0000	5,00	0,0000	0	...	☐
4	0,0000	10,00	0,0000	0	...	☐
5	0,0000	0,00	0,0000	0	...	☐
6	0,0000	0,00	0,0000	0	...	☐
7	0,0000	0,00	0,0000	0	...	☐
8	0,0000	0,00	0,0000	0	...	☐
9	0,0000	0,00	0,0000	0	...	☐
10	0,0000	0,00	0,0000	0	...	☐

Response Base	Area
Peak Type	Ordnr

37. Save and close the updated calibration file.

Step 6 Preparing a sample queue

To run samples using an autosampler, a sequence file is necessary that identifies each injection.

38. Open the 'Sequence Window'

Instrument 1 - Sequence template

File Edit Sequence View Window Help

	Status	Run	Method Name	SV	Sample ID	Sample	Inj.Vol. [µL]	Lvl	Sample Type	File Name
1	⊗	☑	Noname	1			1		Standard	%y%m%o-%H%M_%q_vial%v
2		☐								

Additional explanation for some column headers:

- **SV**: sample vial. The numbered position in the autosampler vial tray
- **Sample type**:
  - a. 'Unknown' for samples
  - b. 'Standard' for the various calibrators
  - c. 'Bypass' for measurement of the baseline (no injection)
  - d. 'Blank' for the blank standard.
- **Lvl**: level. This is the line number in the table containing the calibrator concentrations (see the calibrator file).

Tips for working with the sequence table:

File Name generator:
The code in this field makes unique **file names** for each new chromatogram (and can be adjusted if necessary). We recommend having the date and time tag as first part of the name, to make sure that the chromatograms are listed in timely order (not alphabetically based on their sample name).

When the mouse pointer is hovered over this cell, the projected chromatogram file name will show.

Filling tables:
Use the key combination **[Ctrl]+F** to fill all the underlying cells with the same info as the selected cell. Use 'Fill series' from the context menu to fill a column with incrementing numbers.

39. Insert the following parameters to prepare for 8 analyses:
    - In the field 'Method name' select the method 'yymmdd_INJ.
    - Injection volume: for example, 2 uL
    - Add enough rows by clicking on the empty 'Run' box at the bottom of the list.
    - The first line should only be used for stabilizing and evaluation of the baseline without an injection (Sample type = 'Bypass').
    - Program the analysis of the 4 calibrators (Sample type = 'Standard') from vial positions 6-9. The level value (Lvl=1-4) identifies the various concentrations.
    - Vial positions 1-3 contain 3 samples: a, b and c.

Instrument 1 - Sequence template (MODIFIED)

File Edit Sequence View Window Help

	Status	Run	Method Name	SV	Sample ID	Inj.Vol. [µL]	Sample Type	Lvl	File Name
1		☑	yymmdd_INJ	1		2	Bypass	–	%y%m%o-%H%M_%q_vial%v
2		☑	yymmdd_INJ	2	Cal-1uM	2	Standard	1	%y%m%o-%H%M_%q_vial%v
3		☑	yymmdd_INJ	3	Cal-2uM	2	Standard	2	%y%m%o-%H%M_%q_vial%v
4		☑	yymmdd_INJ	4	Cal-5uM	2	Standard	3	%y%m%o-%H%M_%q_vial%v
5		☑	yymmdd_INJ	5	Cal10uM	2	Standard	4	%y%m%o-%H%M_%q_vial%v
6		☑	yymmdd_INJ	6	a	2	Standard	5	%y%m%o-%H%M_%q_vial%v
7		☑	yymmdd_INJ	7	b	2	Unknown	–	%y%m%o-%H%M_%q_vial%v
8		☑	yymmdd_INJ	8	c	2	Unknown	–	%y%m%o-%H%M_%q_vial%v
9		☐							

40. Save the sequence table under the name ‘yymmdd’ (using todays date tag).

41. The sequence is now in theory ready to run (from the menu *Sequence/Resume* or using the icon.

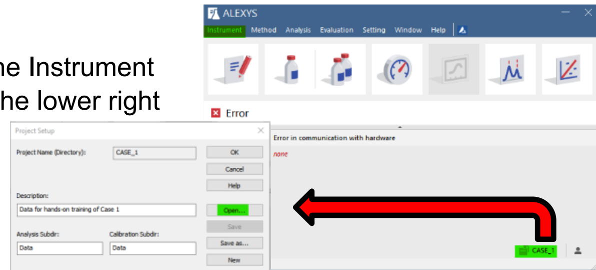
If there was hardware connected to the computer, this sequence would result in analysis of the samples and standards, and automatically processed chromatograms. Without hardware connected, the option to run it is grayed out.

CHAPTER 2

CASE 2: reprocessing of data

This case deals with reprocessing Clarity chromatograms batch-wise, which can be used in case you find out that the post-run method parameters (integration, calibration) were not ideal.

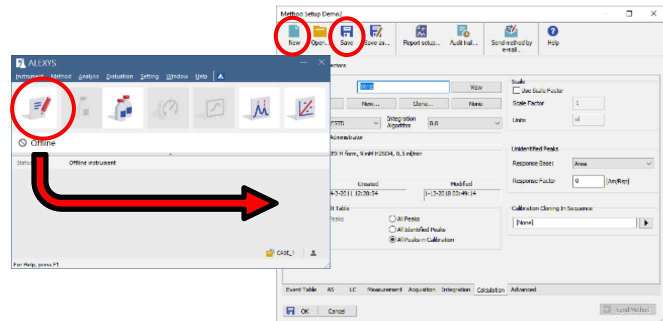
In case you need a set of example calibrator and sample chromatograms to work with, open the Project folder 'CASE 2' (otherwise, apply the following steps to your own chromatograms).



1. Open the Project Setup window (in the Instrument window click on the project name in the lower right corner).
2. Then open Project 'CASE 2'.

Step 1 Create a dedicated reprocessing method

Any existing method can be adjusted and corrected to reprocess chromatograms and thus automatically give processed results in the future, but for this training we will prepare a dedicated method from scratch:

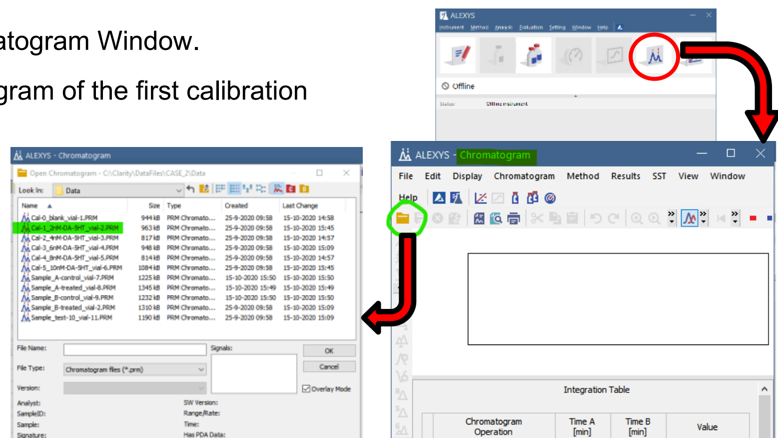


3. Open the Method Setup window (using the icon in the Instrument Window).
4. Create a new method (New button).
5. Save the method with the name 'REPROCESS'.

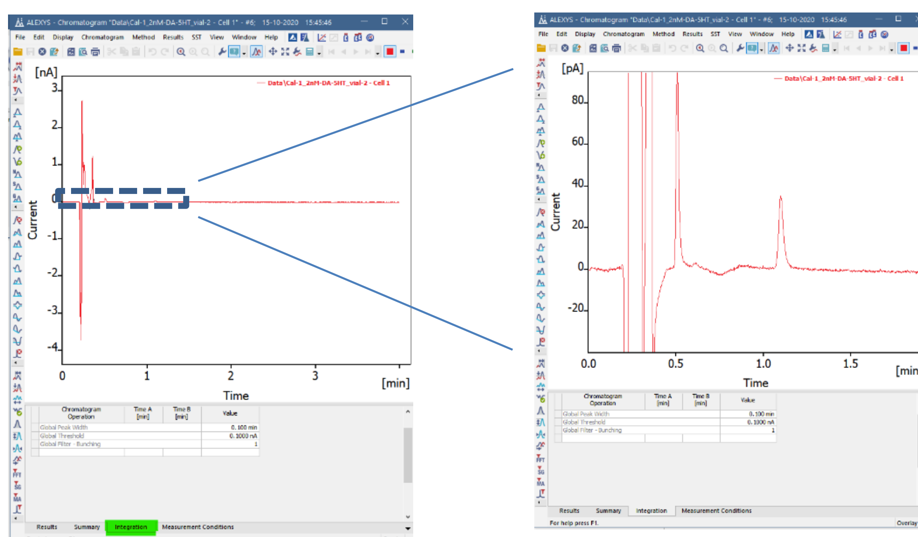
Step 2 Obtain an optimized set of integration parameters

One of the chromatograms can be used as a template to develop the optimized set of integration parameters for the reprocessing method.

6. Activate the Chromatogram Window.
7. Open the chromatogram of the first calibration standard (Cal-1).



8. Zoom in on the relevant part of the chromatogram that contains the peaks (click-draw a box around it).



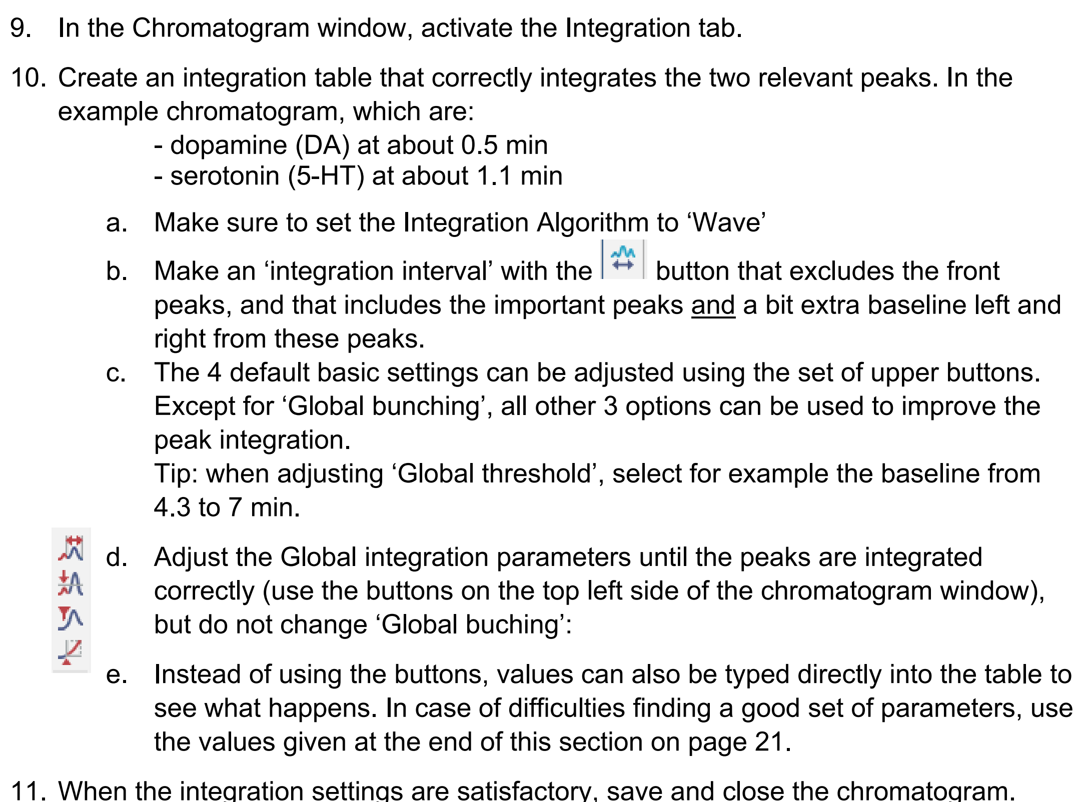
9. In the Chromatogram window, activate the Integration tab.

10. Create an integration table that correctly integrates the two relevant peaks. In the example chromatogram, which are:
    - dopamine (DA) at about 0.5 min
    - serotonin (5-HT) at about 1.1 min

    a. Make sure to set the Integration Algorithm to ‘Wave’

    b. Make an ‘integration interval’ with the button that excludes the front peaks, and that includes the important peaks <u>and</u> a bit extra baseline left and right from these peaks.

    c. The 4 default basic settings can be adjusted using the set of upper buttons. Except for ‘Global bunching’, all other 3 options can be used to improve the peak integration.
    Tip: when adjusting ‘Global threshold’, select for example the baseline from 4.3 to 7 min.



    d. Adjust the Global integration parameters until the peaks are integrated correctly (use the buttons on the top left side of the chromatogram window), but do not change ‘Global buching’:

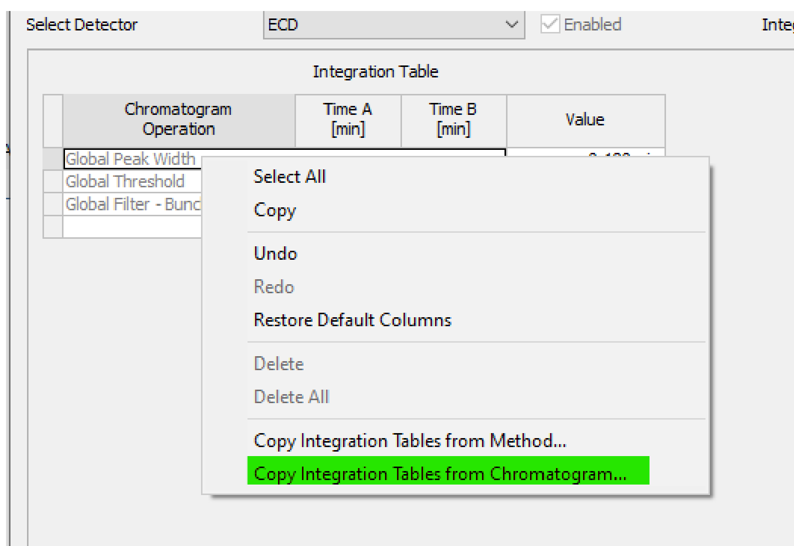
    e. Instead of using the buttons, values can also be typed directly into the table to see what happens. In case of difficulties finding a good set of parameters, use the values given at the end of this section on page 21.

11. When the integration settings are satisfactory, save and close the chromatogram.

Step 3 Update the method with an optimized set of integration parameters

12. Open the method ‘REPROCESS’ and activate the tab ‘Integration’

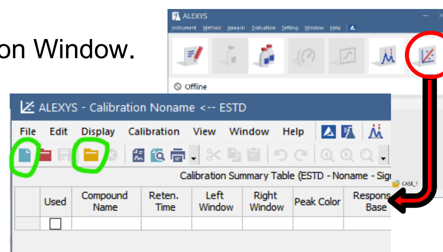
13. Right-mouse click anywhere in the table and select ‘Copy Integration Tables from Chromatogram…’



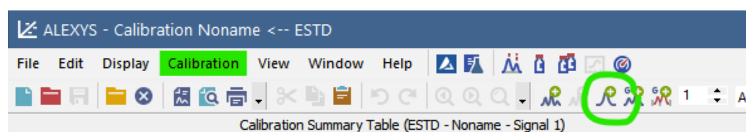
14. Select the chromatogram that contains the optimized integration table.
15. Close the Method Setup window with the OK/save button.

Step 4 Create a calibration file and define peak names

A calibration file contains the table with names that will be assigned to peaks depending on their retention time. To create the peak names table, perform the following steps:



16. From the Instrument Window, open the Calibration Window.
17. Click the blue 'New' button.
18. Click the yellow folder icon 'Open standard' (or though the menu '*File/Open standard*'), and select the calibrator chromatogram that was just integrated (Cal-1).
19. Make an entry line in the peak table for each relevant peak by using the 'Add peak' button for every peak (or through the menu *Calibration/Add peak*).

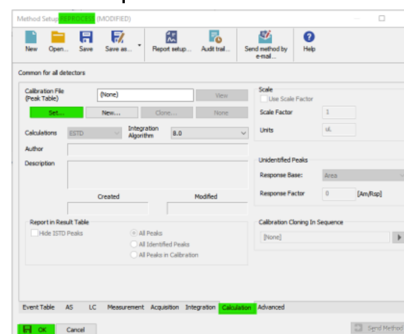


   a. Rename the default given peak names in the column 'Compound Name' to the real peak names. In the example chromatograms it is:
      - dopamine (DA) at about 0.5 min
      - serotonin (5-HT) at about 1.1 min
   b. A light color can be assigned to each peak. This will then be applied to every chromatogram linked to this calibration file and this helps to visually evaluate peak integration.

20. Save the changes to the Calibration file and name it 'Peaks_yymmdd', where yymmdd is todays date format.

Step 5 Linking a calibration file to the reprocessing method

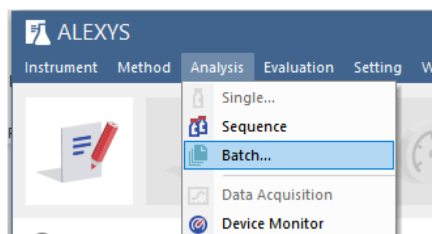
21. Go back to the Instrument Window and open the Method Setup window for the method REPROCESS.
22. Activate the tab ‘Calculation’.
23. Use the ‘Set’ button and select the calibration file ‘Peaks_yymmdd’.
24. Click OK/save to close this window.



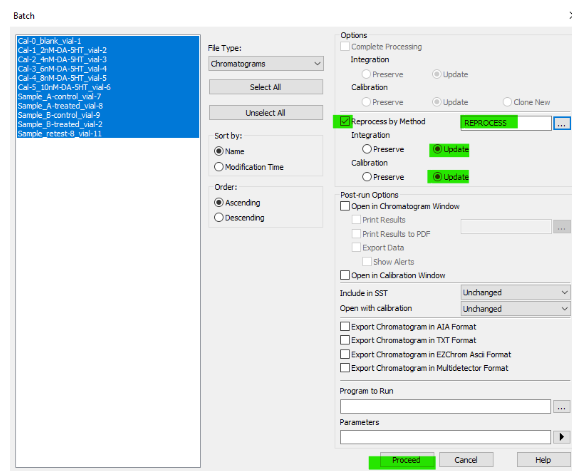
Step 6 Batch-wise reprocessing of chromatograms

In principle, the chromatogram with the optimized integration table could now be ‘Set model’ through the Chromatogram Window menu (*Method/Set model*), and the other chromatograms can receive the integration table one by one from the model through ‘Copy from model’. Connecting the chromatogram to a specific calibration file can also be done manually for each single chromatogram one by one. However, with many chromatograms to be reprocessed this way it is a lot of unnecessary work, as these modifications can also be done batch-wise by applying the optimized settings from the method ‘REPROCESS’. This is how-to:

25. In the Instrument window, open the batch processing window from the menu (*Analysis/Batch…*).



26. Select all the chromatograms that have to be processed.
27. Check the box ‘Reprocess by Method’ and select the method ‘REPROCESS’.
28. Click the “Proceed” button to reprocess all the selected chromatograms.



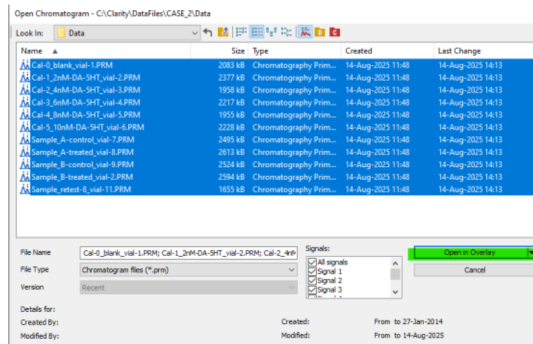
Step 7 Visually inspect and correct all reprocessed chromatograms

Every peak in each chromatogram has to be visually inspected for proper integration after measurement or reprocessing. Manual adjustment of the peak end and start markers can now also be applied to individual chromatograms. If too many peaks need adjustment, then it may be more beneficial to further optimize the integration parameters and Batch-reprocess the chromatograms again. We suggest to start with a general quick evaluation in overlay mode, followed by adjustment of individual chromatograms.

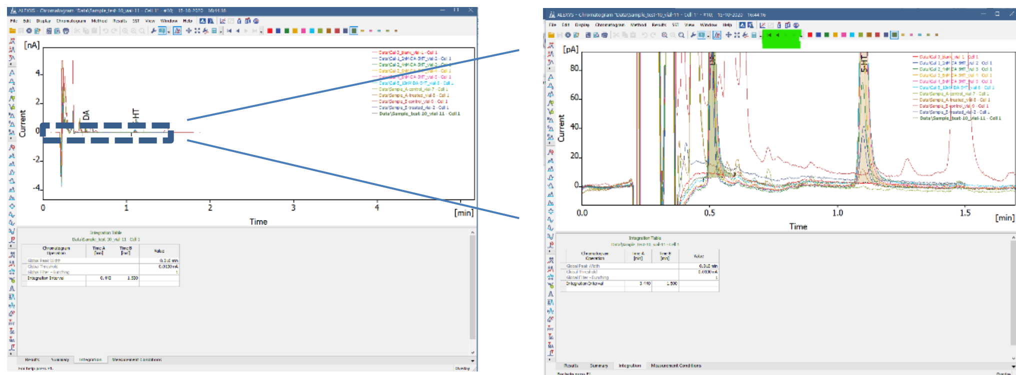
Quick visual evaluation of all chromatograms in overlay mode

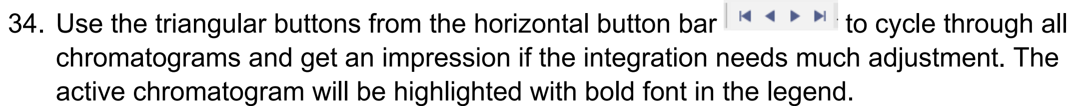
29. Open the Chromatogram window.

30. First, close any displayed chromatograms (though the menu apply *File/Close all*).
31. Open the chromatogram selection panel (yellow folder icon).
32. Select all the chromatograms and Open them in Overlay mode



33. Drag a selection box in the relevant part of the chromatogram to zoom in. The goal is to see the assigned end and start position of the peaks. The light peak coloring helps to make the active chromatogram stand out from the overlay.

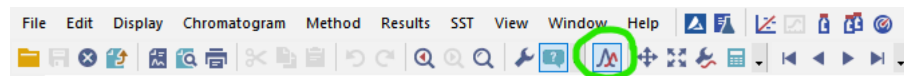


34. Use the triangular buttons from the horizontal button bar  to cycle through all chromatograms and get an impression if the integration needs much adjustment. The active chromatogram will be highlighted with bold font in the legend.

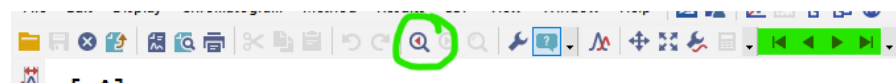
Manual fine-tuning of individual peak integration

Usually, the overlay of all chromatograms contains too many lines to comfortably work on individual chromatograms, so here is how to work efficiently through a list of chromatograms in single display.

35. Close the Overlay view mode with the icon (or through the menu *File/Overlay Mode*)..



36. Note that the active chromatogram will be the one that stayed in display. If you want to start with a different chromatogram, open it with the yellow folder icon.
37. The display will also jump back to full view each time when opening a different chromatogram. To easily jump back to the previous setting of zoom-in, use the magnifier-glass icon with the back arrow inside.



38. The triangle icons can be used to display the next or previous chromatogram in the data folder.

39. The vertical button bar contains a set of icons that are helpful to make adjustments where necessary.

    These are the most commonly used icons for manual integration adjustment:

Icon	Description
	Change the start and end position marker of the peak
	Add a new peak. Use this in case a signal was too small to be recognized
	Use this icon to remove the integration from a part of the baseline.
	These icons change the way that the baseline below the peak is drawn.

    When activated, a helpful text box will appear

    Tip: Hold the [Ctrl] key while activating an icon to make repeated use of it when necessary. The [Esc] button releases the hold.

40. Visually inspect each chromatogram in detail. When in doubt if a peak in a sample is of interest, combine it with a standard in overlay mode.

Step 8 Build the calibration plot

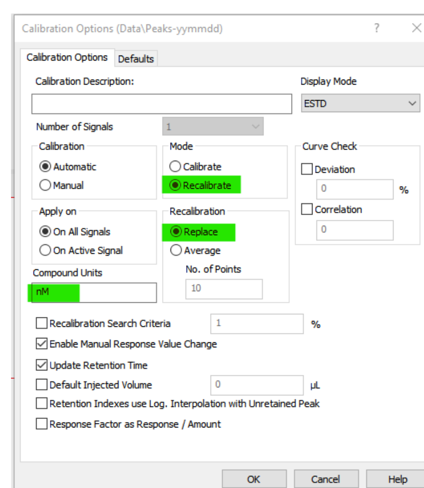
After having batch reprocessed the chromatograms, each one is linked to the calibration file *Peaks_yymmdd*. This file needs to be updated with the information of the calibrator standards to have the software calculate the component amounts in the samples.

Enter the standard concentrations and units in the calibration file

41. Activate the Calibration window and open the calibration file *Peaks_yymmdd*.

42. Open the '*Calibration/Options'* from the menu and check the following settings
    Tab *Calibration options:*
    - ☐ Compound Units: nM
    - ☐ Mode: Recalibrate
    - ☐ Recalibration: Replace

    Tab *Defaults:*
    - ☐ Response base: Area
    - ☐ Curve fit type: Linear

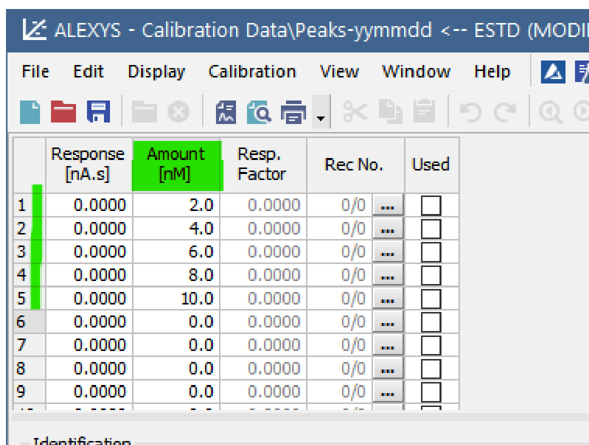


43. Activate the tab of each component, and fill the table with the different concentrations (Amount) of the various calibrator standards.

    Note: the table row numbers are the 'Levels' of the calibrators.

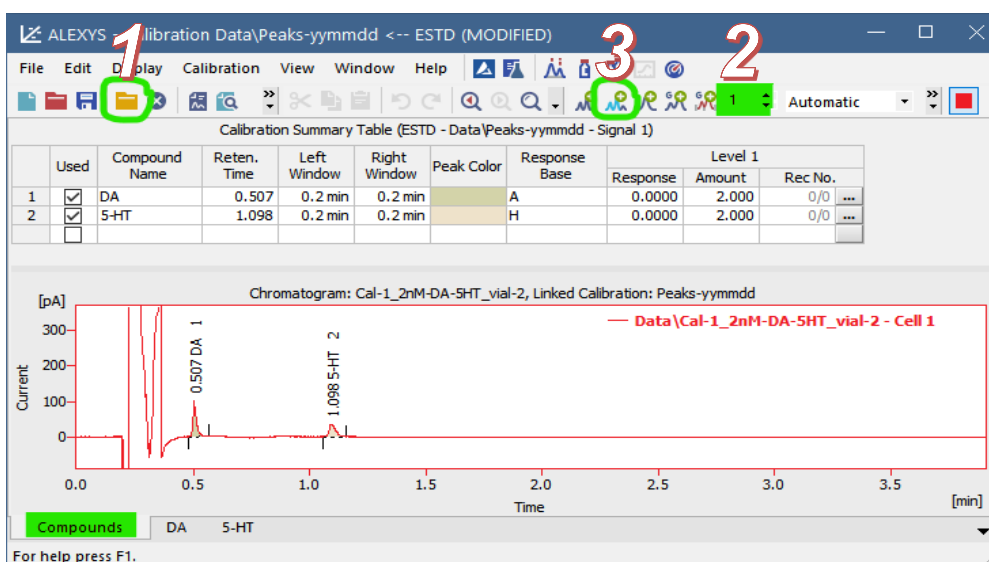
Tip: Once typed, the list can be copy-pasted to the other component tabs (unless they were prepared with different concentration of course).

The calibration file is now ready to receive the data points from the calibrators.



Entering the calibrator data into the calibration plot

44. Activate the 'Compounds-tab in the Calibration Window
45. Entering the calibrator data in the calibration plot is a 3-step process:



1) Open a calibration chromatogram in the Calibration window (use the yellow folder icon, or through the menu: *File/open Standard*)
2) Set the level number associated with the opened standard (use the number icon, or through the menu: *Calibration/set level*)
3) Upload the responses into the calibration plot (use the 'Add Exisitng' icon or through the menu *Calibration/Add Existing*)

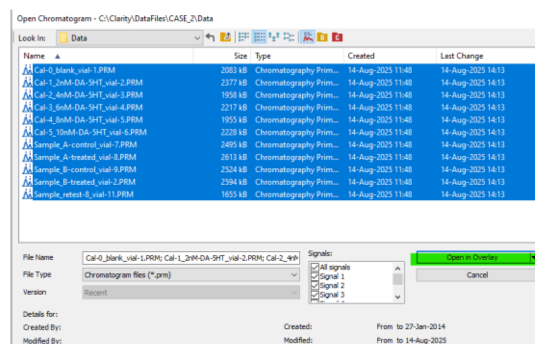
46. Repeat this procedure for each calibration level
47. Check the resulting calibration plot for each component tab.

    After adding the data from all 5 calibrator chromatograms, the calibration curve of the component DA should show a correlation coefficient of at least 0.999 when using the training set. Check/correct the integration and upload the adjusted data if it is any lower.

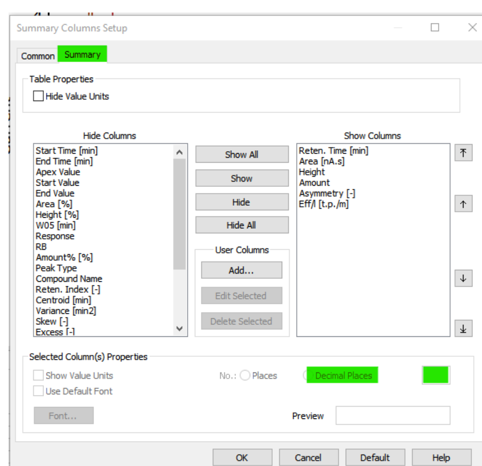
For 5-HT an outlier may be recognizable. For practice purpose, doublecheck the chromatogram that gave this data point (open in chromatogram window), try out some different curve fit types and see how unchecking some data points affects the plot of 5-HT.

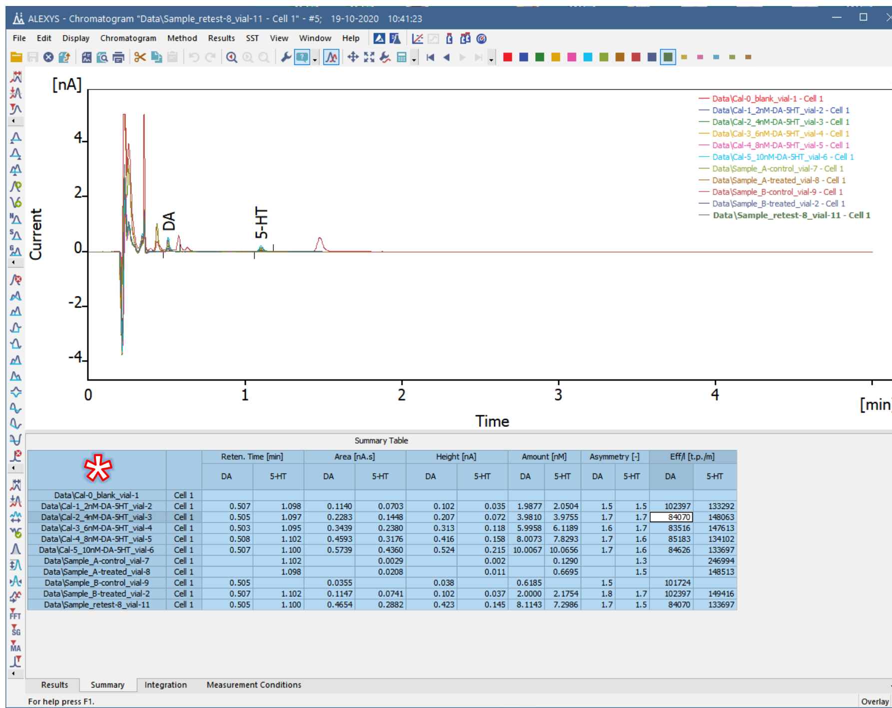
The ‘Unknown’ samples will now automatically have the concentrations of their components calculated on the basis of the linked calibration plot.

Step 9 The data summary table



48. Open the Chromatogram window.
49. Close any displayed chromatograms (though the menu apply *File/Close all*).
50. Open the chromatogram selection panel (yellow folder icon).
51. Select all the chromatograms and open them in Overlay mode
52. Activate the Summary tab
53. Check that the parameters that have to be reported are in the table, and that their number of digits is relevant/enough (hint: look at the *Amount* data). Adjust if necessary (through the menu open ‘*Edit/Setup Columns’,* tab ‘Summary’)





*		Reten. Time [min]		Area [nA.s]		Height [nA]		Amount [nM]		Asymmetry [-]		Eff/l [t.p./m]	
		DA	5-HT	DA	5-HT	DA	5-HT	DA	5-HT	DA	5-HT	DA	5-HT
Data\Cal-0_blank_vial-1	Cell 1												
Data\Cal-1_2nM-DA-5HT_vial-2	Cell 1	0.507	1.098	0.1140	0.0703	0.102	0.035	1.9877	2.0504	1.5	1.5	102397	133292
Data\Cal-2_4nM-DA-5HT_vial-3	Cell 1	0.505	1.097	0.2283	0.1448	0.207	0.072	3.9810	3.9755	1.7	1.7	84070	148063
Data\Cal-3_6nM-DA-5HT_vial-4	Cell 1	0.503	1.095	0.3439	0.2380	0.313	0.118	5.9958	6.1189	1.6	1.7	83516	147613
Data\Cal-4_8nM-DA-5HT_vial-5	Cell 1	0.508	1.102	0.4593	0.3176	0.416	0.158	8.0073	7.8293	1.7	1.6	85183	134102
Data\Cal-5_10nM-DA-5HT_vial-6	Cell 1	0.507	1.100	0.5739	0.4360	0.524	0.215	10.0067	10.0656	1.7	1.6	84626	133697
Data\Sample_A-control_vial-7	Cell 1		1.102		0.0029		0.002		0.1290		1.3		246994
Data\Sample_A-treated_vial-8	Cell 1		1.098		0.0208		0.011		0.6695		1.5		148513
Data\Sample_B-control_vial-9	Cell 1	0.505		0.0355		0.038		0.6185		1.5		101724	
Data\Sample_B-treated_vial-2	Cell 1	0.507	1.102	0.1147	0.0741	0.102	0.037	2.0000	2.1754	1.8	1.7	102397	149416
Data\Sample_retest-8_vial-11	Cell 1	0.505	1.100	0.4654	0.2882	0.423	0.145	8.1143	7.2986	1.7	1.5	84070	133697

In principle, the data in the Summary table can now be selected (click in the table's left upper corner at *) copy-pasted to another program like Excel or Word for further handling.

If you have reprocessed the example set of chromatograms provided in project 'CASE2', have a look and see **what does the data show?**

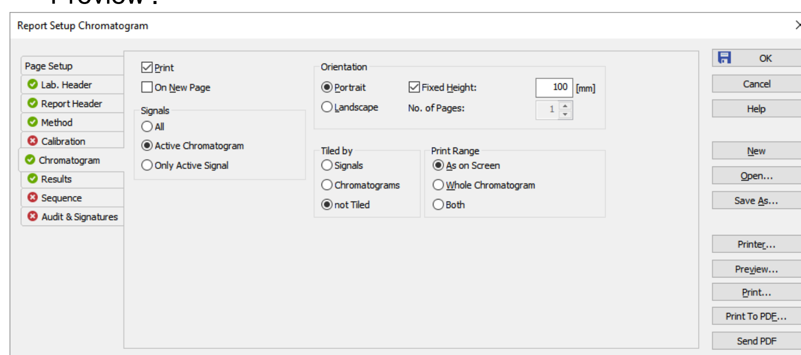
- Did the treatment have an effect?
- Calibrator 4 seems to contain an outlier, and the standard mix was therefore reanalyzed. Was it an instrument hiccup or more likely to be a pipetting error?
- What is wrong with the chromatograms of Sample B?
  Have a look at the chromatogram name and the vial-number code in the name. This example shows why we recommend to have the vial number in the chromatogram name.

Step 10 Creating a report in Clarity

Alternatively, the data and chromatograms can also be printed in a customizable format using a report template.

54. In the Chromatogram Window, open the report setup window (*File/Report set-up*)
55. Open the template 'ALEXYS-chromatogram'
56. Click 'Save as' and rename the file 'CHROM-AND-CAL' before adjusting.

57. Adjust the parameters:
    - ◻ As an exercise, adjust the template to print each chromatogram with the calibration plot that was used for its calculation.
    - ◻ Every change to the template can be checked directly with the button ‘Preview’.



58. The individual chromatogram reports can now in theory be printed.

Box 1

The set of integration parameters for the batch reprocessing set were as follows:

Chromatogram Operation	Time A [min]	Time B [min]	Value
Global Peak Width			0.030 min
Global Threshold			0.0010 nA
Global Filter - Bunching			1
Global Baseline Slope			0.150 nA/min
Integration Interval	0.438	1.380	

These values leave room for some manual tweaking of the sample chromatograms to get familiar with the additional single peak integration adjustment buttons.

CHAPTER 3

CASE 3 Setting up a system configuration from scratch

This case deals with setting up a Clarity system configuration from scratch. The analyzer that is set up as an example will be intended for isocratic LC-ECD measurements. Of course, the principle shown here holds for different configurations as well.

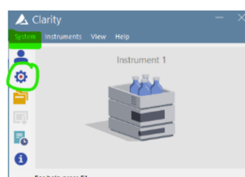
To be able to control all HPLC system components (pump, autosampler and a detector) a so called 'Instrument' has to be defined. This is a three step process:

a. Activate the correct hardware drivers in the Clarity Configuration window.
b. Combine the drivers to define an 'Instrument'.
c. Define the start trigger.

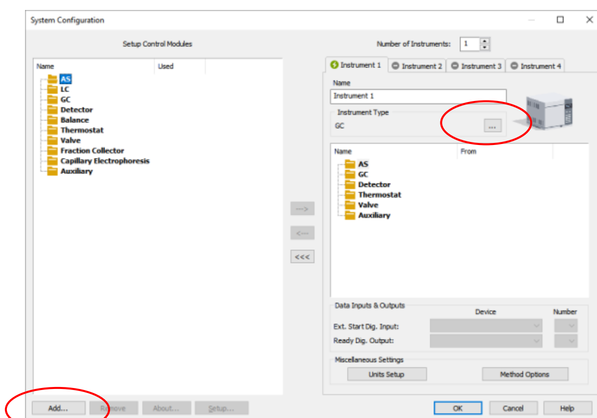
Step 1 Open the Clarity Configuration window

For training purpose, a dedicated 'empty' configuration file can be used. This can be activated as follow:

1. If Clarity was open, close it down completely.
2. Start up the Clarity with the short-cut key 'CASE3', which should be present on the desktop. The Main window opens.
3. Open the Configuration window from the Main window using the icon (or though the menu *System/Configuration*).

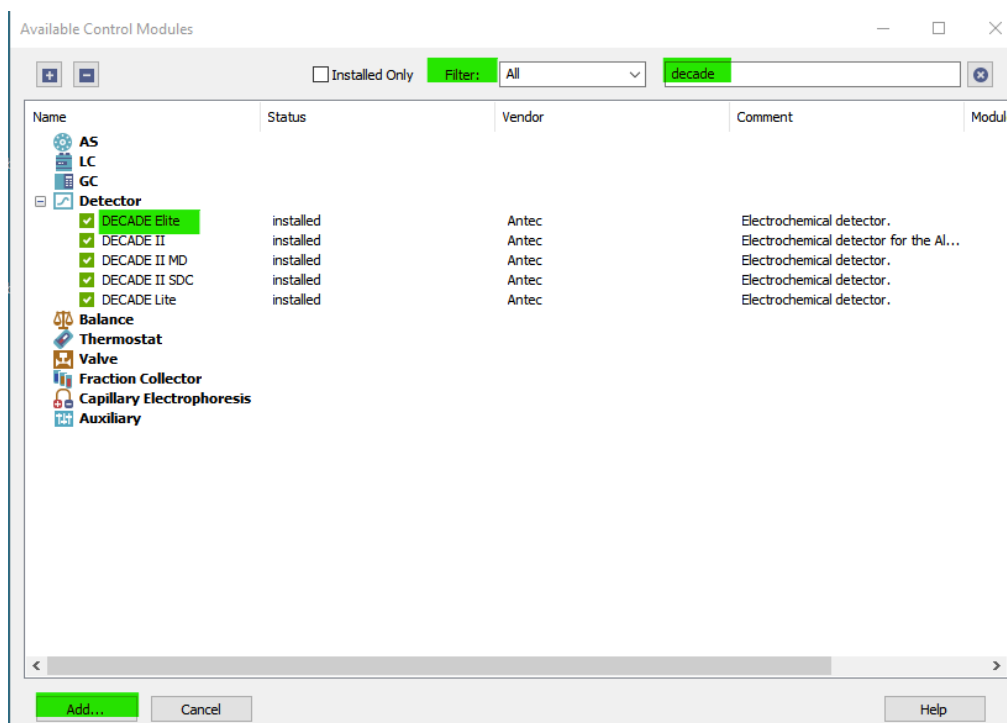


Step 2 Setting up the Control modules of an LC system

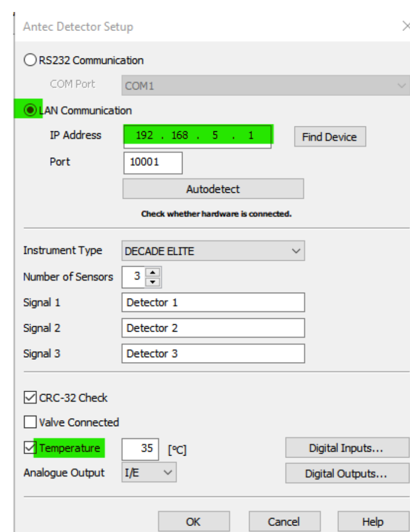


4. Change the Instrument type to 'LC'
5. Click the 'Add' button in the lower left corner. A new window will open that lists all the available drivers for Clarity software.

***Tip:** use the filter option to find the relevant drivers.*



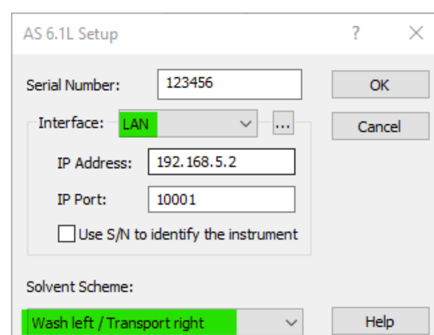
DECADE Elite detector



6. Select and 'Add' the detector 'DECADE Elite'
7. Set the communication protocol to LAN.
8. The default IP address of a detector is 192.168.5.1.
9. Set the oven temperature (to for example 35 °C).
10. Close the Detector set-up windows by clicking the OK button.

Now the DECADE Elite is added to the left side of the configuration window.

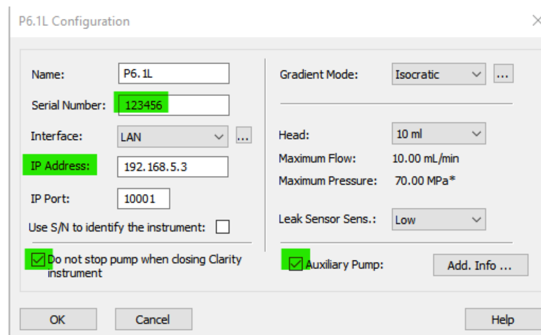
AS 6.1L autosampler



1. Select and 'Add' the autosampler 'AS 6.1L'
2. Set the communication protocol to LAN.
3. The default IP address of an autosampler is 192.168.5.2.
4. Choose the wash and transport orientation
5. Click 'OK' to close the window

P6.1L pump

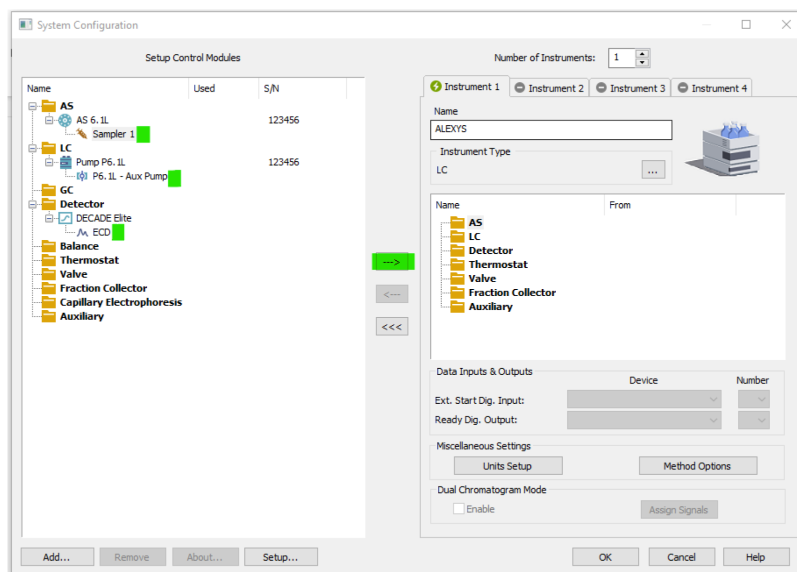
6. Select and ‘Add’ the pump P6.1L
7. Set the communication protocol to LAN.
8. The default IP address of a pump is 192.168.5.3.
9. Type a sham serial number (123456).
10. Check the ‘Auxiliary pump’ check box and the “Do not stop pump when closing Clarity” check box.
11. Click ‘OK’ to close the window



Step 3 Defining the Instrument

Define an instrument by transferring the required control modules to ‘Instrument 1’:

12. Change the instrument name to ‘ALEXYS’
13. Transfer all three relevant control modules to the right side of the System configuration window by drag-drop or by selecting them and using the → button in the middle of the window.



Step 4 Define the start trigger

14. Check/set the ‘Ext. Start Dig. Input’ to AS 110, nr 1 (inject trigger from the autosampler).

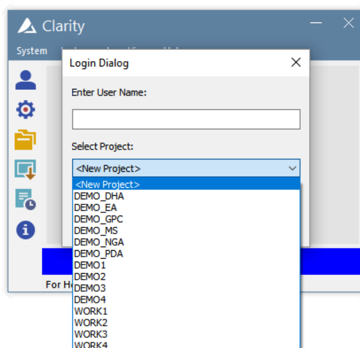
This will trigger the Clarity software to start recording the signal after an injection

15. Click OK to store the new configuration settings, and return to the Clarity main Window.

Step 5 Creating a Project folder for storage of Data and Methods

The Clarity software uses so-called Project folders to keep data, calibration files and methods organized together. To make a new Project:

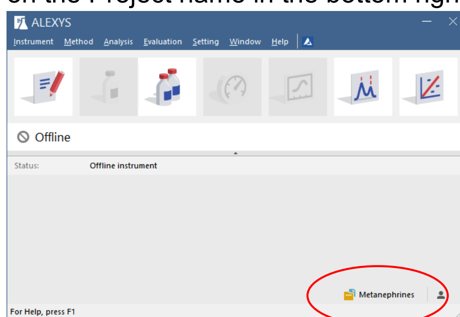
16. Log in to the ‘Instrument’ that was just created.

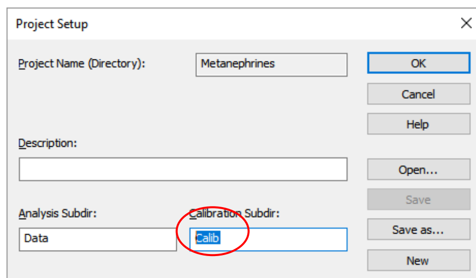


17. In the Login Dialog, select ‘<New project> and give it a name (e.g. Metanephrines)

    Ignore the error messages during log in in case there are no LC system components physically connected to the PC.

18. In the instrument window, open the Project set-up menu (Instrument/Project, or click on the Project name in the bottom right corner).





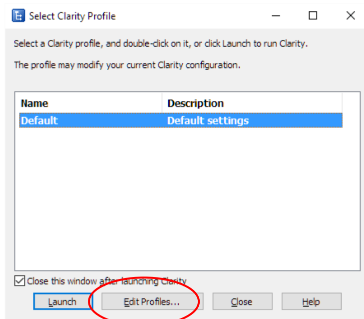
19. Note that the folder structure organizes calibration and data chromatograms in two different folders by default.

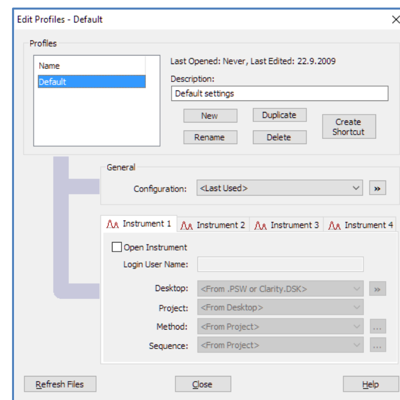
20. Change the Calibration Subdir from ‘Calib’ to ‘Data’. This will result in calibration chromatograms and normal chromatograms to be stored in the same folder. This makes it possible to make overlays directly of standards and samples.

21. Close the ‘Project Setup’ window with the OK button.

Step 6 Making a specific short-cut desktop keys to start Clarity

22. Open the program 'Launch Manager' from the Start menu (this program is installed as part of the Clarity package).
23. Choose 'Edit Profiles'





24. Choose to make a 'New' description and name it 'TEST_Hands-on'
25. Select a Configuration file, check the box 'Open Instrument', select a desktop file (Hands_on_training.dsk), and select the newly made Project folder.
26. Create a shortcut on the desktop for this combination of settings.
27. After closing the Launch Manager, Clarity can be restarted using the new shortcut.

The next step would be to set up the method files and run samples: this is described in chapter CASE 1.

For more supporting information on Clarity we refer to the website of DataApex: www.DataApex.com