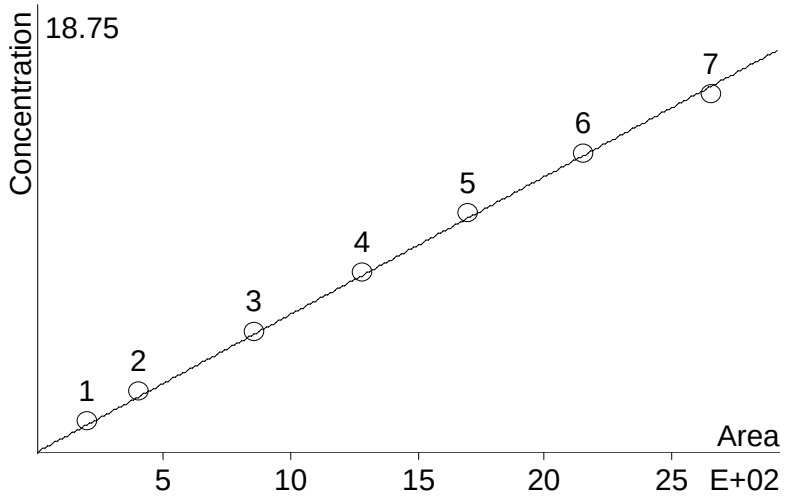
Calibration using ALEXYS data system



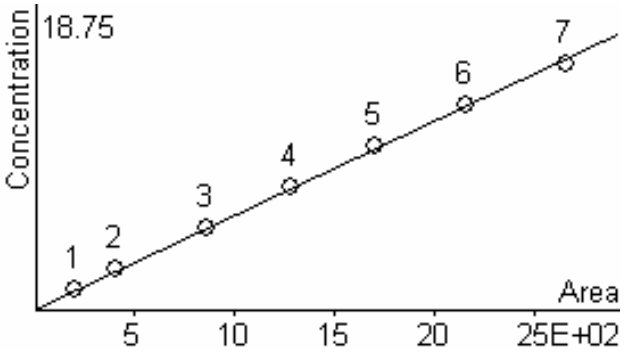
This is not a tutorial…..

- In this presentation we explain what steps are taken when using ALEXYS for calibration.
- For detailed information on how to use the software, we refer to the tutorials on this website.

What does a calibration do for me?

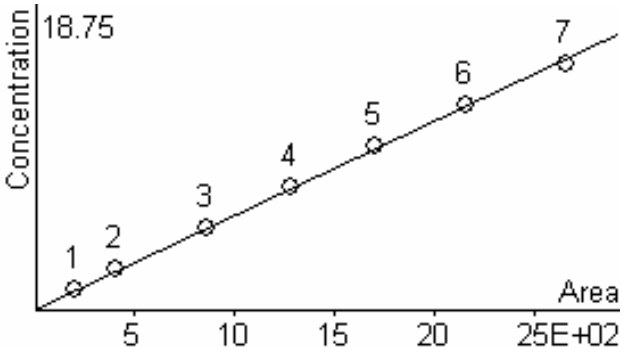
Calibration line

- A calibration line links concentration to a chromatographic peak height (or area). This relationship is found by analysing a number of calibration standards with *known* concentration.



Calibration line

- The calibration information is stored in a method. For ach sample you analyse using this method, the *unknown* concentration of a component is automatically calculated and printed in the results table.



- How does it work?

Calibration procedure

It is a 3 step procedure:

1. Preparing a method
2. Running a queue
3. Reporting results

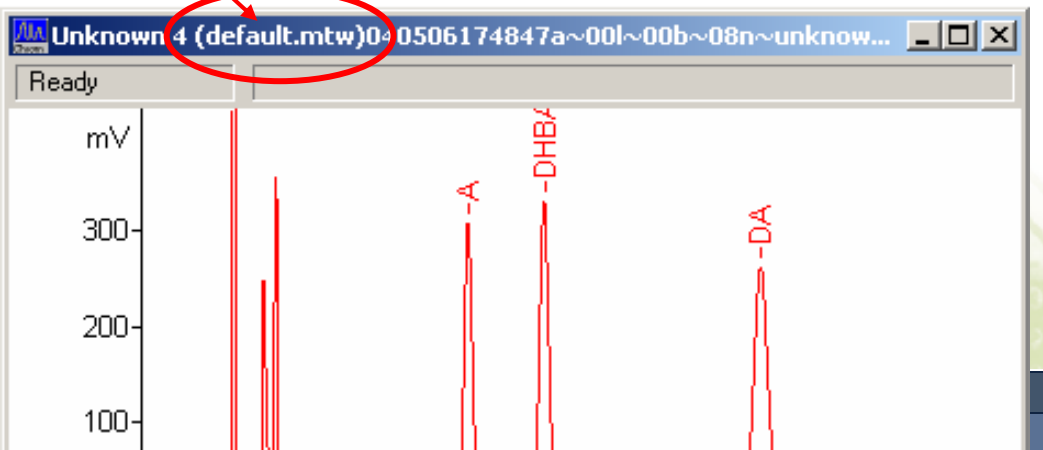
Step 1: preparing a method

- To prepare a method for calibration your input is required. You must create:
  - a peak table
  - a concentration table
  - a sample queue

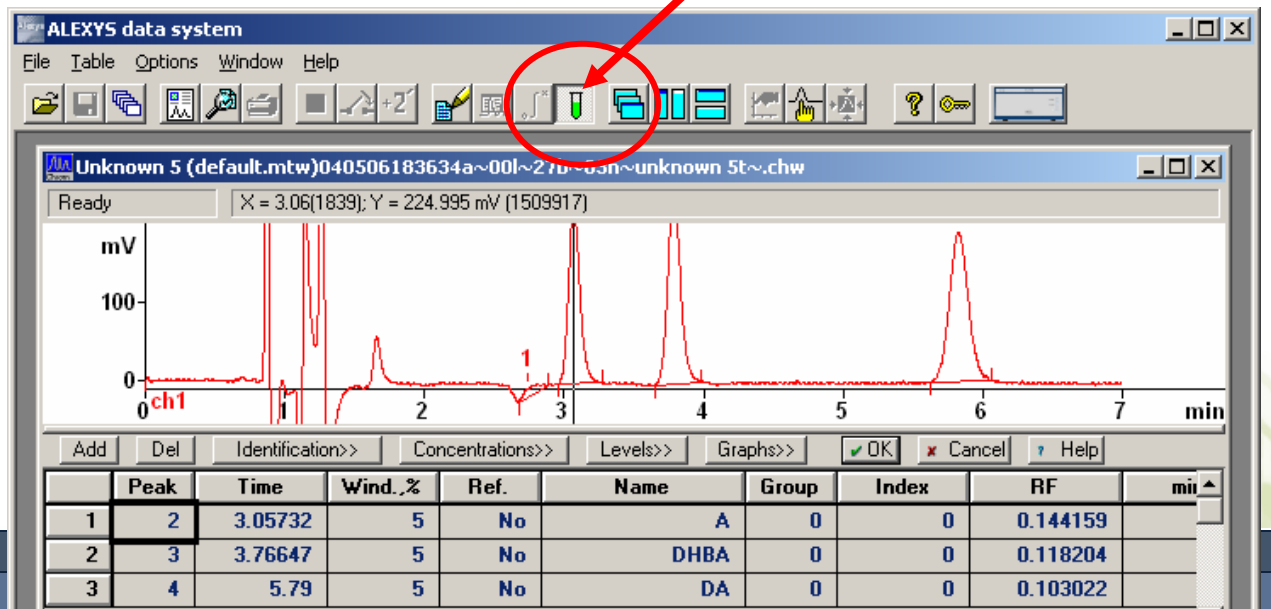
- But first, a test chromatogram is acquired and integrated

- Where do I find my method?

- It all starts by acquiring a first test chromatogram. Calibration and integration settings are entered and stored in the underlying method.



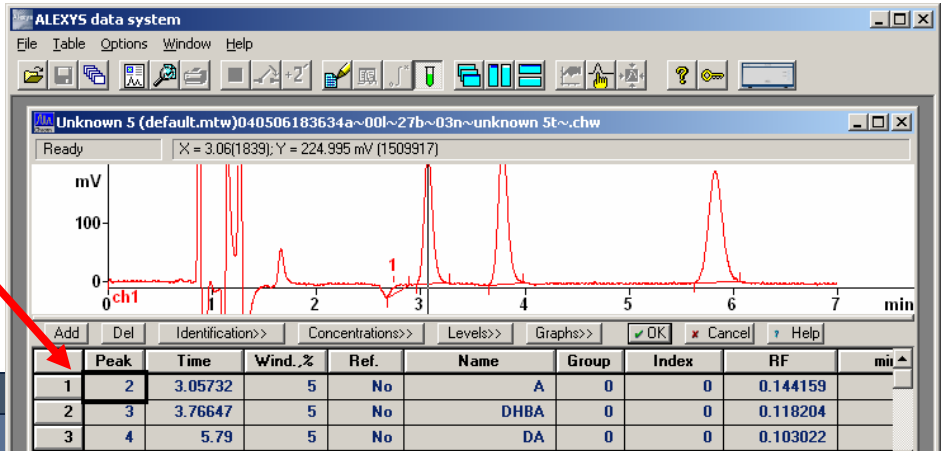
- After integration of a test chromatogram, you can access the peak and concentration tables via the component button.
- The required information is entered and when saving, it is stored in the attached method file.



	Peak	Time	Wind.,%	Ref.	Name	Group	Index	RF
1	2	3.05732	5	No	A	0	0	0.144159
2	3	3.76647	5	No	DHBA	0	0	0.118204
3	4	5.79	5	No	DA	0	0	0.103022

- Why do I need a peak table?

- You have to let ALEXYS know what you are looking for. It can be one or more peaks, to be entered in the peak table (also called component table).



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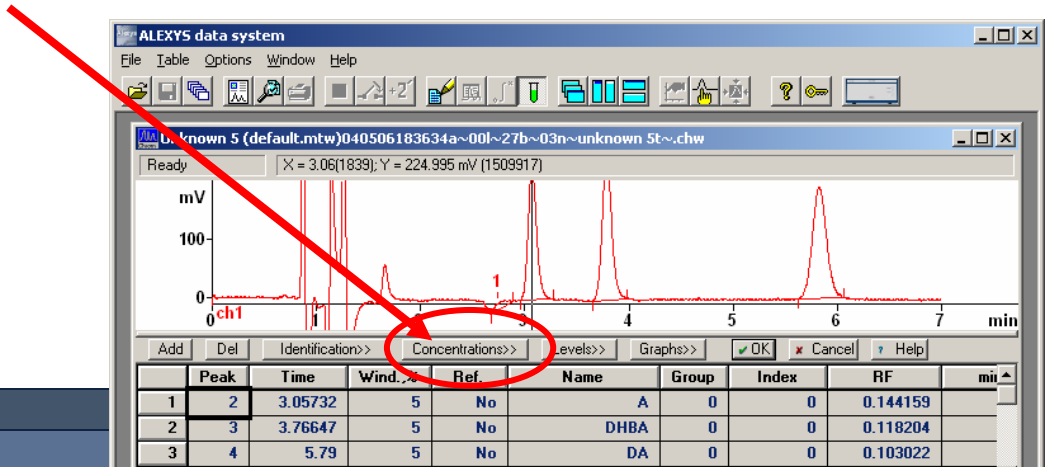
Peak table

- A chromatogram can have multiple peaks with different retention times
- A peak table identifies peaks based on retention time

Peak	Time	Name
1	3.05	A
2	3.76	DHBA
3	5.79	DA

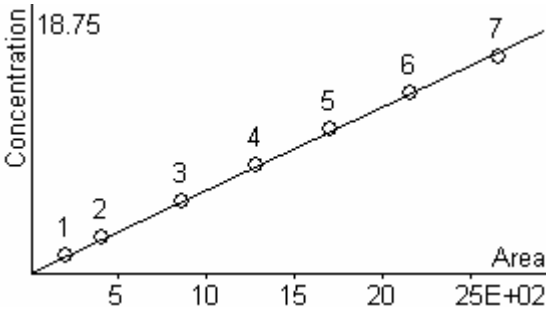
- Why do I need a concentrations table?

- Somehow ALEXYS must know what concentrations you use for calibration. This information must be entered in the concentrations table.



Concentrations table

- Level: each calibration data point is called a ‘level’ in ALEXYS
- Levels are linked to concentrations in the concentrations table
- A level is always unique, duplicates get a new level



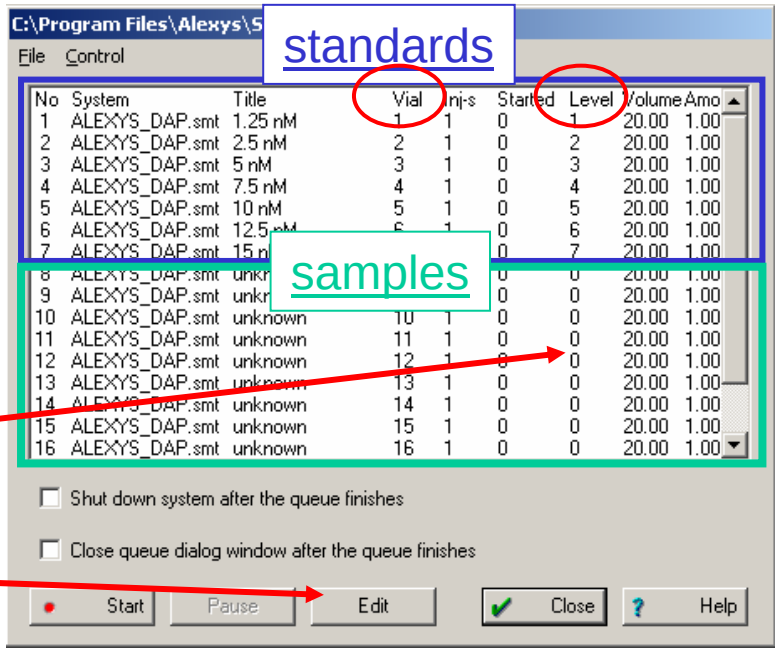
Level	Conc. (nM)
1	1.3
2	2.5
3	5.0
4	7.5
5	10.0
6	12.5
7	15.0

- Why do I need a sample queue?

- The last piece of information ALEXYS needs are the autosampler vial numbers you use for calibration standards and samples. This information, and other information such as injection volume, is entered in the sample queue.

Sample queue

- In a queue an autosampler vial number is assigned to each calibration level and sample
- Samples with unknown concentration have level zero
- ALEXYS has a queue editor that has a number of convenient tools, such as auto fill and auto increment.



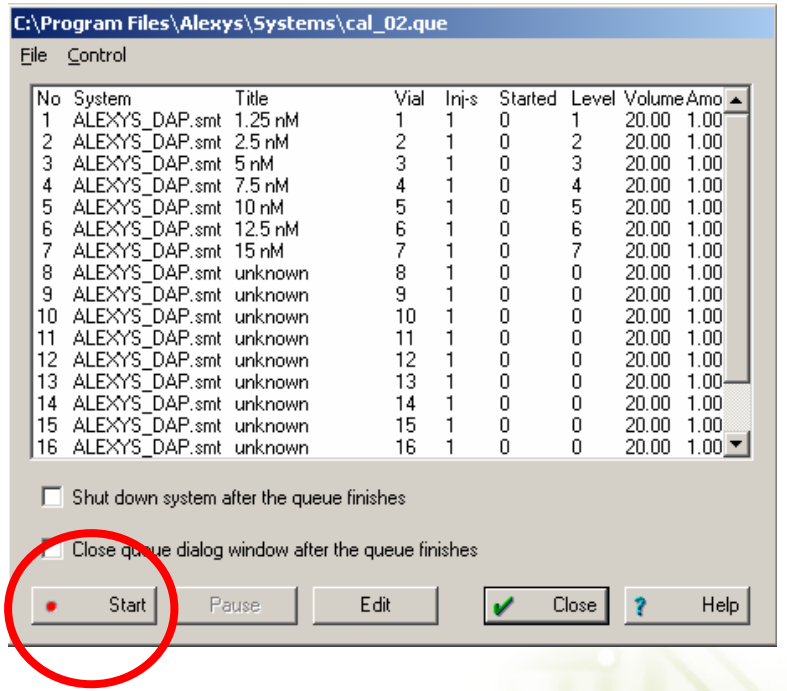
- So, where am I now?

- This is what you did:
  - A test chromatogram has been acquired and used to enter all information in the Method
  - Samples and standards are placed in the autosampler
  - The System (Recorder) has the correct Method, and is stand by

  In other words, you are ready to run!

Step 2: running a queue

- All preparations are done, click ‘Start’ to go!



- So, what happens next?

- As a result of running this queue you will end up with 7 calibration chromatograms and a number of sample chromatograms.

- I like to see the results. Can I get an overlay of chromatograms and the numbers?

- ALEXYS has it all, predefined templates generating instant reports, overlays, statistic results table, and export formats to be used in Excel or Word or any other program you like.

Reports

- Several reporting options are available:
  - Statistics report in a result table
  - Single chromatograms or overlay & settings
  - Calibration line & data
- Exporting data to Excel, Word, Notepad, or as raw data files
- Copy/paste via Windows clipboard

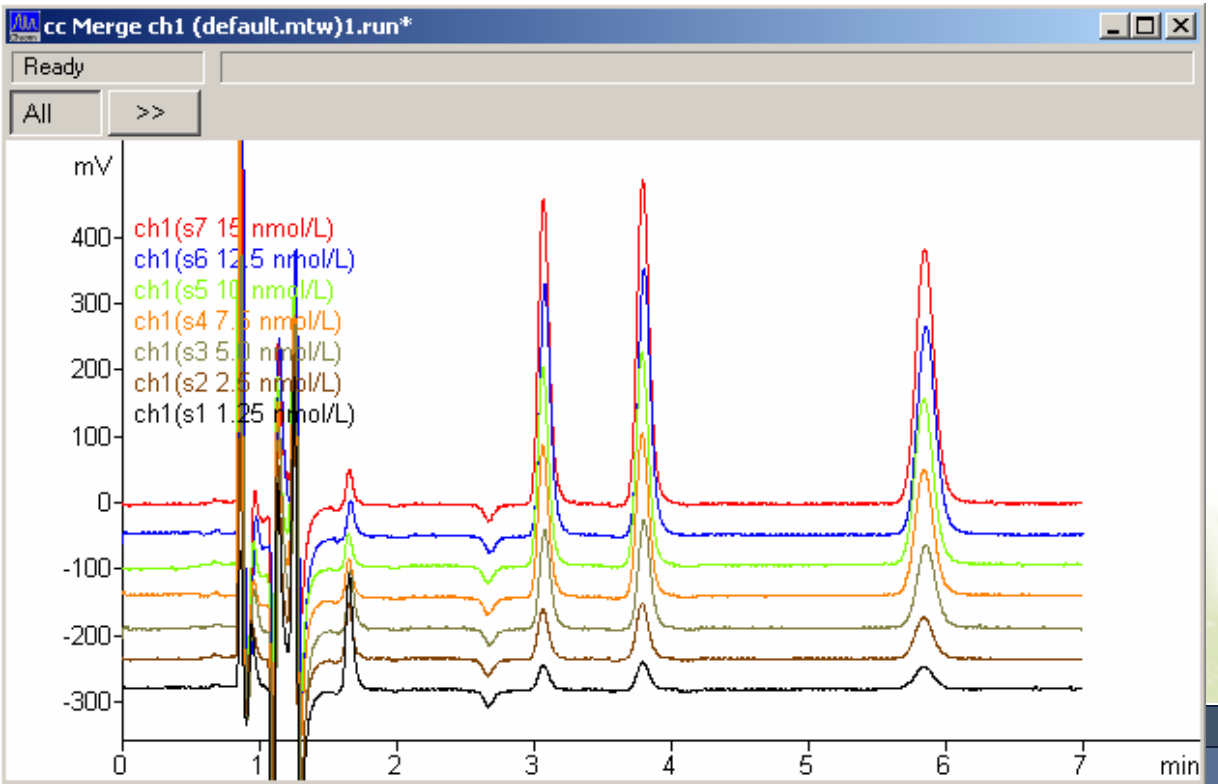
Statistics report

- Reprocess with ‘statistics’ is used to prepare a report table

Component: A						
File	Title	Date & Time	Retention	Height	Area	Conc.
			min	mV	mV*s	nmol/L
1	Unknown 8	6-5-2004 17:16	3.043	41.14	235.76	1.36
2	Unknown 7	6-5-2004 17:24	3.048	81.32	457.79	2.64
3	Unknown 6	6-5-2004 17:32	3.057	154.90	884.05	5.10
4	Unknown 3	6-5-2004 17:40	3.050	233.24	1320.49	7.61
5	Unknown 4	6-5-2004 17:48	3.061	307.60	1740.18	10.03
6	Unknown 5	6-5-2004 18:36	3.069	231.78	1315.75	7.59
7	Unknown 2	6-5-2004 20:20	3.066	75.49	424.73	2.45
8	Unknown 1	6-5-2004 23:07	3.064	36.92	196.98	1.14

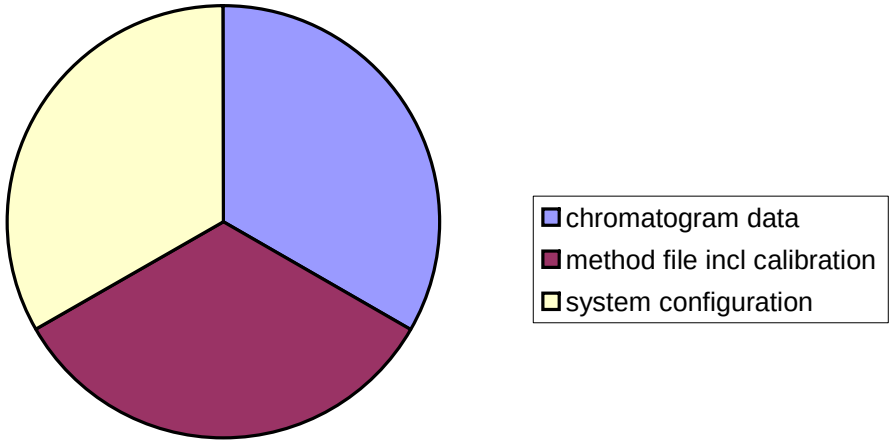
Merge

- Clicking ‘Merge’ is used for chromatogram overlay



Chromatogram

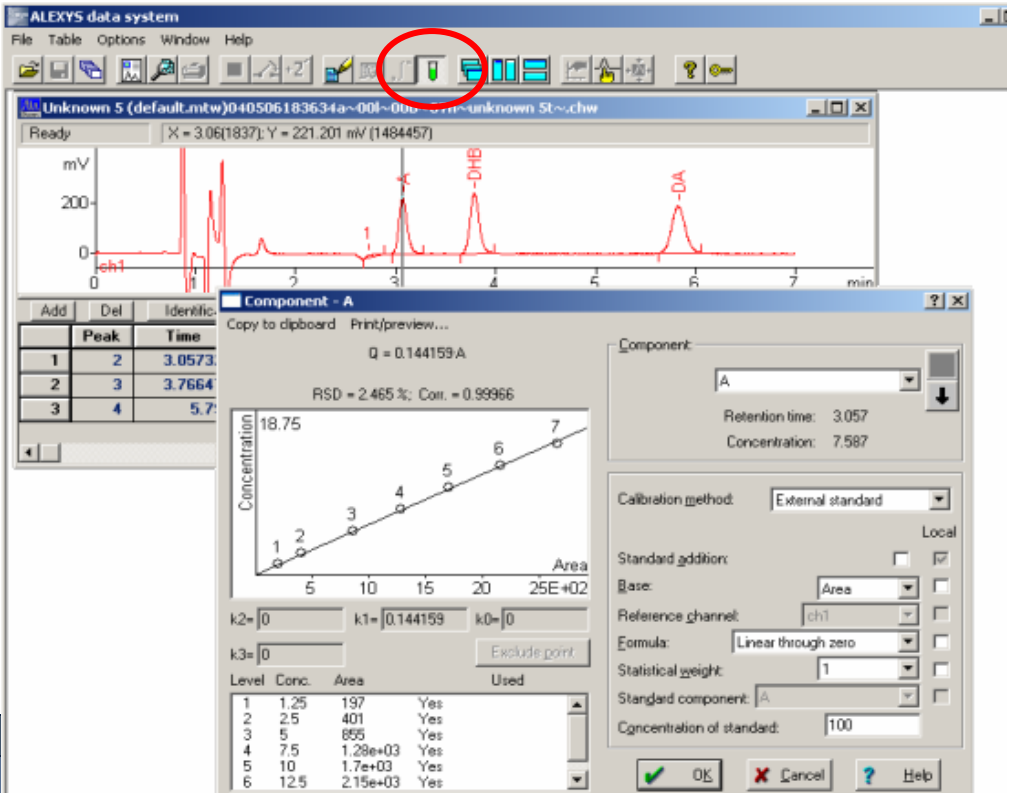
- Calibration results are found in each chromatogram
- An ALEXYS chromatogram contains more than data
  - Data, System, Method (incl. calibration)



Calibration line & data

- Full calibration data stored in each chromatogram that is part of a calibration queue

- Open a chromatogram, via components button and ‘Graph’ button the calibration data is found.



More information….

- For more details on the ALEXYS data system we refer to the manual, and to the ALEXYS screen recordings on this same website.